Adieu, dit le renard. Voici mon secret. Il est très simple:
On ne voit qu'avec le coeur. L'essentiel est invisible pour les yeux.

Antoine de Saint-Exupery (Le Petit Prince)

Der Mensch sieht, was vor Augen ist, Gott aber sieht das Herz an.

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Sustained-Release Formulations for Compounds Underlying Intestinal Drug Efflux

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Abbreviations

° C	degree(s) Celsius
ABC	ATP-binding cassette
ADP	adenosine diphosphate
AMG	Arzneimittelgesetz (German law on Pharmaceuticals)
approx.	approximately
ATG	atmosphere gauge
ATP	adenosine triphosphate
AWD	Arzneimittelwerk Dresden
BA	bioavailability
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
BBB	blood brain barrier
CL	clearance
C_{max}	maximum plasma concentration
CNS	central nervous system
cp	compare to / with
CR	controlled-release
Da	Dalton
e.g.	example given, for example
EU	European Union
F_a	fraction absorbed
FaSSIF	Fasted-State Simulated Intestinal Fluid
FeSSIF	Fed-State Simulated Intestinal Fluid
FDA	Food and Drug Administration
g	gram(s)
GDR	German Democratic Republic
GI-tract	gastrointestinal tract
GMP	Good Manufacturing Process

h	hour(s)
HPMC	hydroxypropylmethylcellulose
i.e.	which means (from lat.: id est)
IR	immediate release
JPE	Japanese Pharmaceutical Excipients
K_m	Michaelis-Menten constant
l	liter(s)
log P	logarithm of the partition coefficient P
MDR	multi drug resistance
min	minute(s)
mol	mole, $6x10^{23}$ particles
M_r	molecular weight
MRT	mean residence time
N	Newton
n.a.	not available
N.N.	name not (yet) known (from lat.: nomen nescio)
P	partition coefficient
p.o.	peroral, per os (lat.)
P_{eff}	effective permeability
PEI	Paul Ehrlich Institut
P-gp	P-glycoprotein
pH	potentia hydrogenii (lat.)
PharmBetrV	Betriebsverordnung für Pharmazeutische Unternehmer (German ordinance for Pharmaceutical entrepreneurs)
Ph.Eur.	European Pharmacopeia
PIC	Pharmaceutical Inspection Convention
pK_a	coefficient for the acidity of a substance

p.a.	pro analysi
R^2	coefficient of determination
resp.	respectively
SD	standard deviation
SDS	sodium dodecylsulfate
sec	second(s)
SOP	Standard Operating Procedure
$t_{1/2}$	half-life
t_{max}	time until C_{max} is reached
US(A)	United States (of America)
USP	United States Pharmacopeia
V_c / V_D	Volume of distribution
V_{max}	maximum velocity
WHO	World Health Organisation

Chapter I

Chapter I

Introduction and aims of the doctoral thesis

Peroral administration has been the most common route for the application of drugs for decades and continues to be at present time. Obvious advantages of peroral dosage forms are the high patient compliance in combination with a simple, cost-efficient manufacturing process. As a consequence, the design of a peroral dosage form is the first choice for most new chemical entities to be introduced to the market. Besides the development of formulations for newly developed drugs, the optimization of dosage forms for well-established drugs is another goal for the design of new peroral drug products. Such optimizations can include an improvement of bioavailability, a reduction of side-effects or a decrease in the frequency of administration. Generally, the design of new dosage forms should be based on the pharmacokinetic and pharmacodynamic properties of the drug. Information should be provided on the absorption mechanism, which can be a source of insufficient bioavailability. Passive diffusion through biological membranes has been adressed as one important mechanism in drug absorption. Besides passive absorption, active transport processes mediated by transporters and pumps play an important role in the absorption of nutrients from the intestine into the blood circulation. This may as well be applicable for the absorption of drugs, which show affinity to intestinal transporters.

A considerable number of transporters may increase the absorption of drugs, in particular those compounds, for which passive diffusion is too low to be of relevance, due to e.g. a large molecular weight or low partition coefficient. Moreover, a number of transporters are described, that facilitate transport in the opposite direction, by secretion of drug molecules from the interior of enterocytes back into the intestinal lumen. This phenomenon is also refered to as intestinal drug efflux. The best-known efflux pump in the human intestine is P-glycoprotein (P-gp). This transporter has originally been studied due to its high expression in cancer cells, where it acts as a mediator for multi-drug resistance (MDR). Two clinically relevant aspects should be considered for drugs that are substrates of P-glycoprotein: On the one hand,

intestinal secretion may result in a decreased bioavailability, and on the other hand, the transporters mediating the intestinal drug efflux are saturable, which can result in a dose-dependent, non-linear absorption. Such phenomena should be addressed for in the design of oral dosage forms for P-glycoprotein substrates.

I.1 The phenomenon of intestinal drug efflux

The cascade of events determining the systemic availability of drugs following peroral administration has been studied extensively in the past. Although many aspects are well-known today, for some drugs, the process leading to drug absorption and bioavailability needs further examination since it is relatively complex. Some of the mechanisms may involve poor compound solubility in the gastrointestinal fluids, poor permeability across the gastrointestinal epithelium, insufficient stability in some gastrointestinal segments including enzymatic and non-enzymatic degradation, complexation, as well as, in some cases, pronounced hepatic first-pass extraction.
The possible mechanisms involved in the permeation of drugs across the intestinal epithelium have in many cases been well defined. These include paracellular and transcellular pathways of membrane permeation, whereby the contribution of the paracellular pathway to the total transmembraneous drug flux is regarded as being of limited relevance in most cases. The transcellular pathway involves partitioning of drugs into the lipophilic epithelium and diffusion across the membrane, a process which has already been described 100 years ago by the classical works of E. Overton and H. Meyer [Kleinzeller (1999)]. It is also well recognized that forces generated by transport systems intrinsic to the membrane can drive the epithelial transport of several drugs [Tsuji, A. and I. Tamai (1996)]. In these cases, a substance appears to permeate a biological membrane at a different rate than anticipated from its molecular size and hydrophobicity alone, when employing the relationship between permeability and hydrophobicity. Such stoichiometric transport systems are carriers and pumps. Carriers may be involved in three kinds of transport processes: facilitated diffusion, cotransport (symport) and countertransport (antiport). Cotransporters and countertransporters can perform “secondary active transport” by using energy from the downhill transport of one transported substrate to drive the uphill transport of another transported substrate. Pumps are distinguished from carriers by the linkage of transport to an external source of energy, provided by the hydrolysis of a phosphate bond and leading to the generation of ADP from ATP. Pumps perform “primary active transport”.

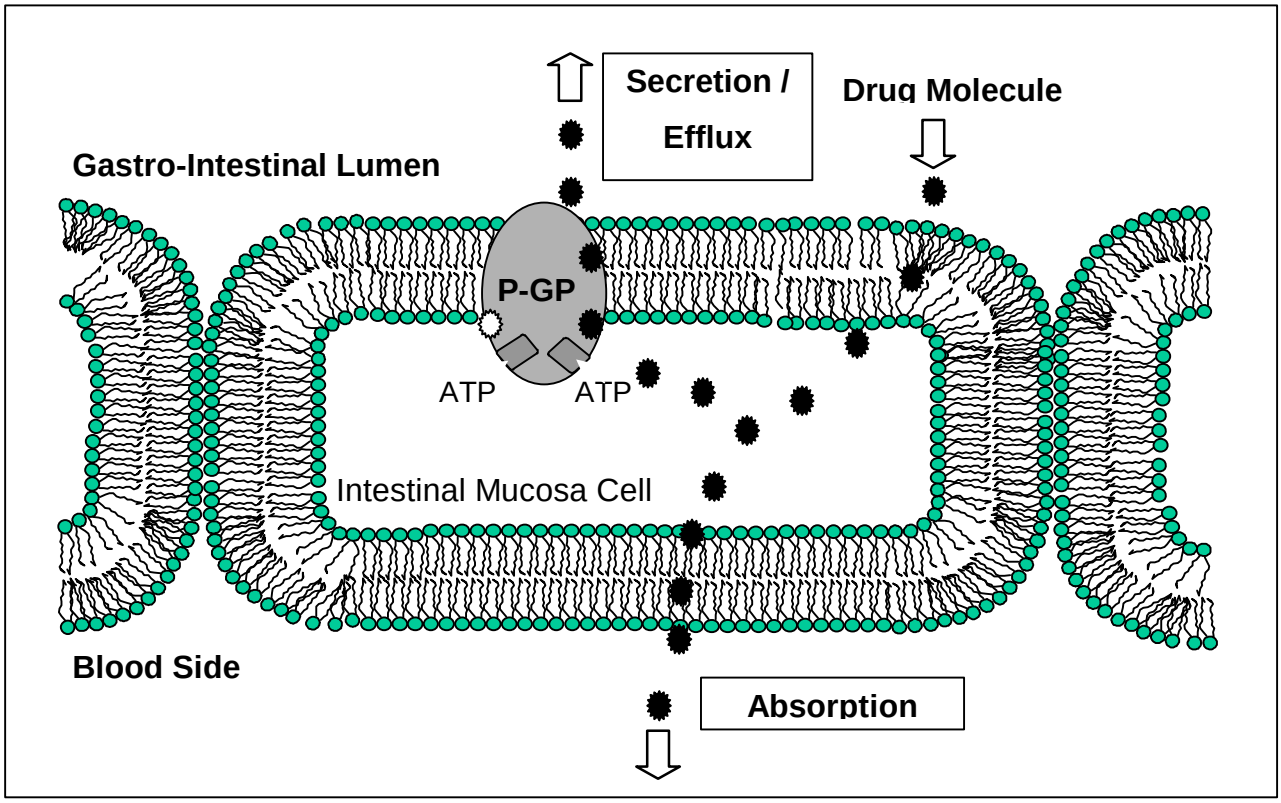


Figure I.1:
Absorption and secretion of a P-glycoprotein substrate in human enterocytes.

Carriers and pumps in the intestinal epithelium may transport substrates from the intestinal lumen to the blood compartment (absorptive transport), other transporters have been discovered which operate in the reverse direction, i.e. from the blood or basolateral side to the luminal or apical side (secretory transport). Both pathways are illustrated in Figure I.1.

Carrier-mediated transport processes are saturable and inhibitable, and may be regulated by a variety of external and internal factors. Saturability of carrier-mediated transport may lead to dose-dependent pharmacokinetics of drugs that are substrates of carriers. This is frequently observed as a deviation from the linear relationship between the drug dose and its systemic exposure. Induction as well as inhibition of carriers involved in drug transport may lead to diminished or enhanced absorption of drugs with affinity for these carriers.

I.2 Carriers involved in intestinal drug efflux

More than 25 years ago, the existence of carriers involved in the secretion of organic molecules from the blood into the intestinal lumen was described for the first time. In 1975, Lauterbach [Lauterbach, F. (1975)] reported of intestinal carrier-mediated secretion of cardiac glycosides and the organic cations tetraethylammonium bromide, N-methylnicotinamide and N-methylscopolamine. It was also pointed out, that sulfanilic acid at low concentrations is preferentially transported in the secretory direction, a process which can be inhibited by toluene sulfonate and metabolic inhibitors. In principle, it has become clear that the intestine, primarily being regarded as an absorptive organ, is as well prepared for elimination of certain organic acids, bases and neutral compounds, depending on their affinity to intestinal carrier systems [Schwenk, M. (1987)]. The reason for that is the fact that several of the transport systems known to mediate efflux in the major clearing organs - liver and kidney - are also expressed in the intestine [Arimori, K. and M. Nakano (1998)].

I.2.1 The intestinal efflux pump P-glycoprotein

The best characterized example for an efflux pump located in the intestine is P-glycoprotein, a glycosylated membrane protein consisting of 1280 amino acids with 12 hydrophobic, helical transmembrane segments, two intracellular ATP binding sites and a molecular weight (M_r) of 170 Da. Its structure is depicted in Figure I.2.

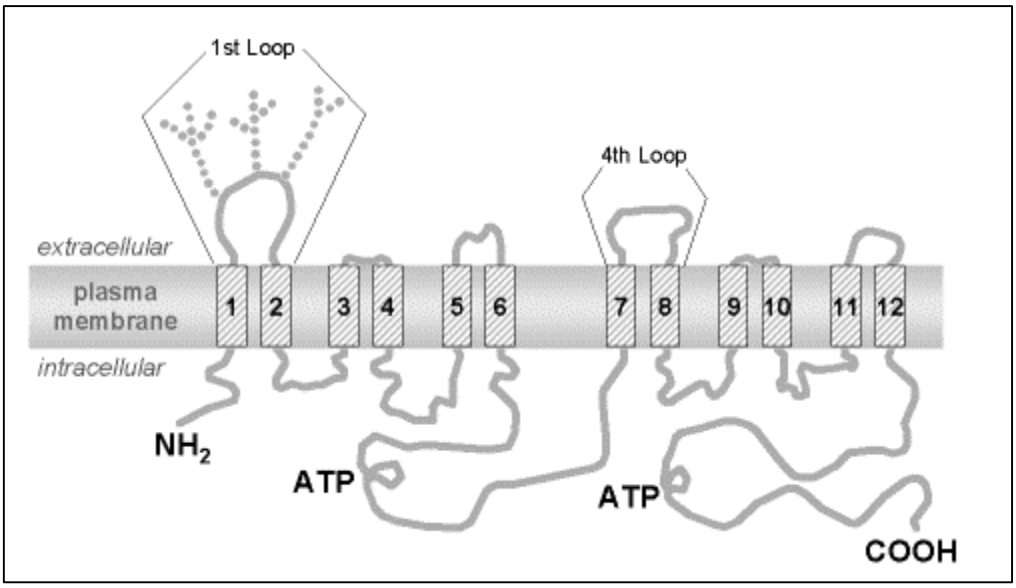


Figure I.2:
Structure of P-glycoprotein.

P-glycoprotein is physiologically expressed in the apical membrane of mucosal cells of the small and large intestine as well as at the luminal membrane of proximal tubular cells in the kidney, the biliary canalicular membrane of hepatocytes, at the blood-brain barrier (BBB), in capillary endothelial cells of testis, the adrenal gland, and the endometrium of the pregnant uterus. P-glycoprotein plays a role in the excretion of toxic substances in the kidneys and in the liver. At the blood-brain-barrier, it prevents entrance of drugs to the central nervous system (CNS). In the intestine the P-glycoprotein mediated efflux can reduce the bioavailability of drugs that are administered perorally. Additionally, P-glycoprotein is discussed as a source for a number of interactions between different P-glycoprotein substrates due to a displacement from the carrier [Gramatté, T. and R. Oertel (1999), Westphal, K., A. Weinbrenner, et al. (2000a), Westphal, K., A. Weinbrenner, et al. (2000b)]. Besides

drug-drug interactions, also interactions with food components, e.g. ingredients of grapefruit juice [Kane, G. C. and J. J. Lipsky (2000), Spahn-Langguth, H. and P. Langguth (2001)] or apricot extract [Deferme, S., R. Mols, et al. (2002)], have been discussed. A recent review on interactions mediated by inhibition and induction of P-glycoprotein was published by Lin [Lin, J. H. (2003)].

Before becoming an issue for biopharmaceutics, research in the area of P-glycoprotein-related drug efflux was to a large part related to its involvement in the development of multidrug-resistance in cancer chemotherapy [Sikic, B. I. (1999)]. P-glycoprotein (Multidrug resistance protein) like the multidrug resistance associated proteins (MRP´s) belong to the family of ATP-binding cassette (ABC) transporters, members of which are also involved in the resistance of plasmodium falciparum to chloroquine and the development of resistance towards antibiotics to prokaryotic as well as eukaryotic cells [Van Bambeke, F., E. Balzi, et al. (2000)]. The biochemistry, pharmacology and structure-activity relationships of P-glycoprotein and its substrates have been described in a number of publications [Ambudkar, S. V., S. Dey, et al. (1999), Chin, J. E., R. Soffir, et al. (1989), Hrycyna, C. A., M. Ramachandra, et al. (1999), Leveille-Webster, C. R. and I. M. Arias (1995), Neuhoff, S., P. Langguth, et al. (2000), Schinkel, A. H., E. Wagenaar, et al. (1995), Seelig, A., X. L. Blatter, et al. (2000), Seelig, A. and E. Landwojtowicz (2000), Wacher, V. J., C. Y. Wu, et al. (1995)].

Presumably due to its very broad substrate “specificity”, P-gp-mediated transport processes have been reported for a variety of drugs including e.g., vincristine, vinblastine, doxorubicin, daunorubicin, etoposide, paclitaxel, cyclosporine A, ketoconazole, verapamil, digoxin, aldosterone, cortisole, dexamethasone, cimetidine, ranitidine, salbutamol, talinolol, celiprolol, domperidone, phenytoin, ondansetron, loperamide, morphine, atorvastatin, fexofenadine, trospium. Clearly it can be seen that P-glycoprotein does not discriminate between substrates carrying different charges, since e.g., trospium, talinolol and cimetidine are either completely (trospium) or in part (talinolol, cimetidine) positively charged at physiological pH, fexofenadine and atorvastatin carry negatively charged carboxyl groups and also neutral compounds may show affinity (e.g., digoxin, cyclosporine A).

I.2.2 Other transporters involved in intestinal drug efflux

The transport of several organic anions is mediated by members of the multidrug resistance protein family (MRP). This family contains at minimum six members (MRP1-MRP6) [Borst, P., R. Evers, et al. (1999)], and has previously been described to show ATP-dependent, primary active transport of various glutathione, sulphate and glucuronide conjugates and organic anions, for example quercetin 4'-β-glucoside [Walgren, R. A., K. J. Karnaky, Jr., et al. (2000)], methotrexate [Bakos, E., R. Evers, et al. (2000)], pravastatin [Yamazaki, M., S. Akiyama, et al. (1997)], etoposide [Wijnholds, J., E. C. deLange, et al. (2000)] and cisplatin following complexation with glutathione [Kool, M., M. de Haas, et al. (1997)]. MRP can even extrude neutral and basic organic compounds if the cells contain normal levels of glutathione [Loe, D. W., R. G. Deeley, et al. (1998), Renes, J., E. G. de Vries, et al. (1999)]. MRP1, MRP4 and MRP5 RNAs are widely distributed in the body, whereas MRP2, MRP3 and MRP6 appear mainly in the liver, kidney and gut [Borst, P., R. Evers, et al. (1999)]. Of these, MRP1 and MRP2, like P-glycoprotein, are expressed at the apical membrane of polarized monolayers, whereas MRP3 is located at the basolateral membrane, transporting compounds from the cell interior to the blood compartment. Significant tissue distribution of MRP1-RNA has been demonstrated in human stomach, duodenum and colon [Kool, M., M. de Haas, et al. (1997)].

Intestinal secretion of phase II metabolites may also be subject to binding and transport via the breast cancer resistance protein (BRCP), which is an ATP-binding cassette “half-transporter” responsible for the cellular extrusion of some compounds such as topotecan. It is located on the luminal membrane of the small intestine [Allikmets, R., L. M. Schriml, et al. (1998), Ross, D. D., W. Yang, et al. (1999), Suzuki, H. and Y. Sugiyama (2000)].

Unlike P-glycoprotein and MRP´s, which are ATP dependent extrusion pumps, the organic cation transporter (OCT) family includes OCT´s which represent an electrogenic import system for organic cations from the aqueous phase of the extracellular fluid into cells. Members of this family currently include OCT1, OCT2, OCT3 and OCTN1 and OCTN2. In the intestine, rOCT1 has been found to be localized at the basolateral membrane of small intestinal enterocytes [Koepsell, H. (1998)] mediating the facilitated transport of substrates into enterocytes. In addition, OCTN2 has been mentioned to be expressed in the intestine [Wu, X., W. Huang, et

al. (1999)] among other tissues such as the kidney, the myocardium, the placenta and in the cortex, hioppocampus, and cerebellum in the brain [Wu, X., W. Huang, et al. (1999)]. rOCT1 accepts a variety of cationic substrates including 1-methyl-4-phenylpyridinium acetate (MPP), N-1-methylnicotinamide (NMN), and choline (type 1 cations). OCTN2 is known as the sodium-dependent carnitine-transporter, which also accepts tetraethylammonium (TEA) as a substrate and which may be inhibited by a variety of organic cations, including desipramine, cimetidine, and clonidine [Wu, X., W. Huang, et al. (1999)]. Recently, it has been shown that several β-lactam antibiotics carrying a quaternary nitrogen atom, e.g., cephaloridine, cefoselis, cefepime and cefluprenam, are able to inhibit OCTN2, leading to carnitine deficiency in patients [Ganapathy, M. E., W. Huang, et al. (2000)].

The intestinal secretion of organic anions has been demonstrated in several laboratories e.g., recently, with β-lactam antibiotics [Saitoh, H., C. Gerard, et al. (1996)], calcein [Fujita, T., H. Yamada, et al. (1997)] and furosemide [Flanagan, S. D. and L. Z. Benet (1999)].

It is known that, in addition to MRP´s, other efflux transporters exist that can mediate intestinal secretory transport of anions. The organic anion polypeptide (OATP) family includes also a prostaglandin transporter (PGT) [Lu, R., N. Kanai, et al. (1996), Tamai, I., J. Nezu, et al. (2000)]. From hydropathy analysis, all of these proteins are predicted to have a twelve-transmembrane domain structure, which is a common structural feature of transporter proteins [Tamai, I., J. Nezu, et al. (2000)]. Of these, OATP-B, OATP-D, OATP-E and PGT have been found to be expressed in the human small intestine and colon by RT-PCR. The range of substrates accepted is rather broad and includes conjugated metabolites of steroid hormones (e.g., estrone-3-sulfate), prostaglandins, bile acids, and anionic drugs. Recently, it was even reported, that the polyspecific human organic anion transporting polypeptide (OATP-A), which is predominantly found in liver and brain tissue, is able to transport type II organic cations such as N-methyl-quinine and N-methyl-quinidine [van Montfoort, J. E., B. Hagenbuch, et al. (1999)], indicating that the substrate specificity of these transporters appears to extend beyond organic anions.

Besides the OATP-family, a possible role of anion exchanger AE2 as the intestinal monocarboxylic acid/anion antiporter and its bi-directional functionality in facilitating also the secretion of monocarboxylic acids from enterocytes to lumen has been discussed [Yabuuchi, H., I. Tamai, et al. (1998)].

Carrier-mediated transport processes of drugs may be further complicated by the fact that substrates exist which show affinities to multiple carrier systems. This has been described for the HMG-CoA reductase inhibitor atorvastatin which is secreted from the enterocytes by P-glycoprotein and taken up by the H^+-monocarboxylic acid cotransporter (MCT) [Wu, X., L. R. Whitfield, et al. (2000)]. Similarly, fexofenadine shows affinity to both, P-glycoprotein and OATP, thus providing an explanation for the interaction of the antihistamine with other organic anions and cations [Cvetkovic, M., B. Leake, et al. (1999)]. A significant number of bisubstrates, that interact with renal contraluminal organic anion and organic cation transport systems, has been published before [Ullrich, K. J., G. Rumrich, et al. (1993)].

I.3 Models for the investigation of intestinal drug absorption and absorption sites

To investigate drug absorption from the gastrointesinal tract, several models have been established. The different approaches can be classified into in vitro, in situ and in vivo models [Lennernas, H. (1998)]. Common in vitro techniques utilize everted gut sacs, Ussing chambers or cell cultures.

As for cell cultures, the Caco-2 cell line, a human colonic adenocarcinoma cell line expressing brush border membrane hydrolases and several transporters including P-glycoprotein [Mohrmann, I., M. Mohrmann, et al. (1986), Inui, K., M. Yamamoto, et al. (1992), Hunter, J., M. A. Jepson, et al. (1993)] is prevalently used [Hunter, J., B. H. Hirst, et al. (1993), Hilgers, A. R., R. A. Conradi, et al. (1990), Artursson, P. and J. Karlsson (1991)]. Although the cell line is derived from colonic tissue, it is applicable for studying drug absorption from the small intestine due its predominating small intestinal cell properties [Hunter, J., B. H. Hirst, et al. (1993)]. However, drawbacks of the model are that it is static, paracellular absorption might be underestimated and very low transport rates compared to the human small intestine are suggested [Barthe, L., J. Woodley, et al. (1999)].

Ussing chambers were introduced by Ussing & Zehran in 1951 for studying the active transport of sodium as a source of electric current in short-circuited, isolated frog skin [Ussing, H. H. and K. Zerahn (1999)]. Later, these chambers were extensively used for the study of ion transport across membranes. The Ussing chamber system uses animal mucosal membranes mounted in diffusion cells [Larsen, E. H. (2002), Tsutsumi, K., S. K. Li, et al. (2003), Soderholm, J. D., L. Hedman, et al. (1998)]. This model can be useful to measure bidirectional transport, but sometimes tissue viability is uncertain [Soderholm, J. D., L. Hedman, et al. (1998), Barthe, L., J. Woodley, et al. (1999)].

Everted gut sacs of rats incubated in tissue culture media are employed in another model for drug absorption studies. Results are reported to have a better reproducibility compared to data obtained in Ussing chamber experiments. Other advantages are the relatively large surface area available for absorption and the presence of mucus and unstirred water layers. However, similar to the Ussing chamber, the tissue viability is limited to approximately 2 hours. [Maurer, H. H. and A. F. Rump (1991), Bouer, R., L. Barthe, et al. (1999), Sharma, P., H. Chawla, et al.

(2002)]. A potential disadvantage of this approach is the presence of the muscularis mucosa, which is usually not removed from everted sac preparations. This might evoke an underestimation of the transport of compounds with a tendency to bind to muscle cells [Le Ferrec, E., C. Chesne, et al. (2001)].

For investigations on the sites of absorption, cell cultures and everted sac models are not suitable [Rouge, N., P. Buri, et al. (1996)]. Ussing chamber experiments can provide limited information on altering permeabilities in different intestinal regions by mounting tissues of specific gastrointestinal regions into the diffusion cells [Ungell, A. L., S. Nylander, et al. (1998)]. However, conclusions from such in vitro experiments on absorption sites in vivo may not be conclusive, especially when active transport processes are involved in drug absorption. For a determination of absorption sites of drugs, in situ and in vivo methods should be favoured.

In situ methods include single-pass perfusion, recirculating perfusion, oscillating perfusion and closed-loop models [Doluisio, J. T., N. F. Billups, et al. (1969), Schurgers, N., J. Bijdendijk, et al. (1986)]. A predetermined gastrointestinal segment of a laboratory animal, such as a rat, rabbit, dog or monkey, is perfused with a drug solution of known concentration. The absorbed fraction of a drug can be determined either by measuring the concentration of the drug and its respective metabolites in the blood or by measuring the amount of drug that has disappeared from the perfusion solution during the perfusion experiment [Lennernas, H. (1998)]. By calculation of permeability values for different perfused segments, in situ perfusion studies can provide information on absorption sites of a drug [Langguth, P., H. P. Merkle, et al. (1994)]. Due to interindividual differences from animal to animal it is recommendable to determine permeability values for all potential absorption sites simultaneously within the same animal [Lu, H. H., J. Thomas, et al. (1992), Hanafy, A., P. Langguth, et al. (2001)]. Perfusion models are also suitable to detect saturable transport mechanisms such as active transport in the absorptive and secretory direction or gut wall metabolism [Sanchez-Pico, A., J. E. Peris-Ribera, et al. (1989)]. However, drawbacks of these animal models are the differences in anatomy and physiology compared to humans. A review on pharmacokinetic differences and similarities between typical laboratory animals and humans was published by Lin [Lin, J. H. (1995)]. Comparisons between perfusion data obtained from jejunum and ileum of rats and humans were reported by Sutcliffe et al. [Sutcliffe, F. A., S. A. Riley, et al. (1988)]. When comparing human in vivo permeabilities in the jejunum with data

obtained from several different preclinical models, Lennernas came to the conclusion that in situ rat perfusion is an appropriate method for predicting human in vivo permeability [Lennernas, H. (1997)].

In vivo methods established for the investigation of drug absorption sites in humans feature the advantage of an optimal presence of all physiological factors with impact on drug absorption. A disadvantage can be that results obtained from in vivo studies do not provide sufficient mechanistic information on the absorption process due to a complex overlapping of numerous factors influencing the drug absorption [Rouge, N., P. Buri, et al. (1996)]. A well established method to gain information on both, the absorption process and the site of absorption, is the human intestinal perfusion method. This technique uses a multiluminal tube which is placed in particular segments of the gastrointestinal tract [Godbillon, J., D. Evard, et al. (1985), Gramatté, T., E. el Desoky, et al. (1994), Gramatté, T. and K. Richter (1994), Gramatté, T., R. Oertel, et al. (1996)]. Similar to the in situ perfusion in laboratory animals, the tube is flushed with a solution of the test drug. The tube is also used to reaspirate the perfusion solution, which can then be analysed in appropriate drug assays. The disappearance of the drug from the solution as well as the drug concentration in blood samples can be used to determine the amount of drug absorbed. To avoid reflux of the drug solution beyond the desired segment and to exclude the contact with enzymes or other secretions of segments distal to the prefused segment, occlusive balloons can be employed [d'Agay-Abensour, L., A. Fjellestad-Paulsen, et al. (1993), Lennernas, H. (1997), Vidon, N., D. Evard, et al. (1985)]. While human perfusion experiments are well established for the detection of regional differences of permeability in the small intestine, a perfusion of the colon is hardly feasible by an intubation from the oral end. This is due to the enormous length of the tube necessary to reach the colon, and the high viscosity of the luminal content that hinders an aspiration of the perfusion solution from this segment.

Another method to examine regional differences in the absorption of a drug is its local instillation to a specific site of the gastrointestinal tract via a catheter [Williams, M. F., G. E. Dukes, et al. (1992), Brockmeier, D., H. G. Grigoleit, et al. (1986a), Brockmeier, D., H. G. Grigoleit, et al. (1986b)]. The site of instillation can be controlled either by endoscopy, fluoroscopy or pH-monitoring. By this means absorption of the drug from regions distal to the site of administration can be excluded [Rouge, N., P. Buri, et al. (1996)]. This method provides only little mechanistic information on the absorption

process, when only the final concentration of the drug in the blood is measured. Conclusions regarding intestinal drug efflux or gut wall metabolism are hardly possible. Another drawback is that an endoscopic localisation of the tube is generally combined with a cleansing of the large intestine, resulting in non-physiological experimental conditions [Gleiter, C. H., K. H. Antonin, et al. (1985)].

The administration of a drug to a specific site of the gastrointestinal tract can also be performed using a high-frequency (HF) capsule. This capsule with a size of 12 mm by 28 mm contains a latex balloon with a dissolved or suspended drug, that is ruptured by a high-frequency signal, as soon as the capsule has reached the desired site of drug administration [Staib, A. H., D. Beermann, et al. (1989), Harder, S., U. Fuhr, et al. (1990), Fuhr, U., A. H. Staib, et al. (1994)]. The location of the capsule during its transit through the gastrointestinal tract is traced by X-ray. This method is also not adequate for mechanistic studies of the absorption process for the same reason described under local instillation via catheters.

Another method used for investigations on the absorption site of drugs is to trace an administered controlled-release dosage form via pharmacoscintigraphy [Kenyon, C. J., F. Brown, et al. (1998), Kenyon, C. J., R. V. Nardi, et al. (1997), Wilding, I. R., J. G. Hardy, et al. (1991), Wilding, I. R., S. S. Davis, et al. (1995)]. The gamma radiation emitted by tracers incorporated within the preparation (e.g. indium-111 [Wilding, I. R., S. S. Davis, et al. (1991)], samarium-153 or erbium-171 [Wilding, I. R., S. S. Davis, et al. (1995)]), without having an apparent impact on the formulation properties, allows one to observe the transit of the controlled-release product. Such studies provide an insight into the fate and integrity of delivery systems and enable the detection of the site of drug release from the dosage form [Wilding, I. R., A. J. Coupe, et al. (2001)]. In combination with measurements of drug concentrations in the blood, regional differences in drug absorption can be demonstrated.

However, all in vivo methods described are not practical for screenings or routine use, since the complex techniques are cost-intensive, time-restrictive, and require approval by ethics commitees [Ungell, A.-L. (1997), Le Ferrec, E., C. Chesne, et al. (2001)].

I.4 Regional differences in drug absorption

In 1985 Brockmeier et al. stated that little was known about the local absorption characteristics along the gastrointestinal tract for most drugs [Brockmeier, D., H. G. Grigoleit, et al. (1985)]. In the meantime, numerous studies have been published using the different techniques desribed in the last section. For several drugs no relevant differences between the observed gastrointestinal segments could be demonstrated: In human perfusion studies, for example, Gramatté et al. found that absorption of paracetamol was similar from the proximal and distal small intestine [Gramatté, T. and K. Richter (1994)]. With the same technique, Delchier et al. found similar absorption rates for nicardipine from the jejunum and ileum [Delchier, J. C., M. Guerret, et al. (1988)]. By means of the local instillation technique, Tay et al. demonstrated a consistent absorption of gepirone throughout all parts of the small intestine [Tay, L. K., F. Dixon, Jr., et al. (1992)]. However, in a number of studies published, more or less distinct site specific differences of drug absorption have been demonstrated. Amongst others, d'Agay-Abensour et al. found in human perfusion experiments that the absorption of 1-deamino-8-D-arginine vasopressin decreased in the order of stomach, duodenum and jejunum > distal ileum > proximal colon [d'Agay-Abensour, L., A. Fjellestad-Paulsen, et al. (1993)]. Barr et al. reported a decreased absorption of amoxicillin from the ileum compared to jejunum and ileum. In colon no amoxicillin absorption could be observed [Barr, W. H., E. M. Zola, et al. (1994)]. Jobin et al. and Godbilon et al. demonstrated that metoprolol was absorbed similarly from jejunum and colon but no absorption was observed from the stomach [Jobin, G., A. Cortot, et al. (1985), Godbillon, J., D. Evard, et al. (1985)]. Ranitidine also shows site dependent differences in drug absorption, as reported by Williams et al. and Gramatté et al. [Williams, M. F., G. E. Dukes, et al. (1992), Gramatté, T., E. el Desoky, et al. (1994)]. Williams et al. reported a similar absorption after local instillation of ranitidine into the stomach and the ileum, but slower absorption when the drug was instilled into the caecum. The results were confirmed and specified by the in vivo human perfusion studies performed by Gramatté et al., who found that the absorption rate of ranitidine decreased with the distance of the perfusion site from the mouth. Other examples of drugs with regional differences in rate and extent of absorption are allopurinol [Schuster, O., M. Haertel, et al. (1985)], benazepril [Chan, K. K., A. Buch, et al. (1994)], ciclosporine [Drewe, J., C. Beglinger, et al. (1992)],

ciprofloxacin [Staib, A. H., D. Beermann, et al. (1989), Harder, S., U. Fuhr, et al. (1990)], glibenclamide [Brockmeier, D., H. G. Grigoleit, et al. (1985)], piretanide [Brockmeier, D., H. G. Grigoleit, et al. (1986a), Brockmeier, D., H. G. Grigoleit, et al. (1986b)], and sumatriptan [Warner, P. E., K. L. Brouwer, et al. (1995)].

The observed regional differences in drug absorption can be associated with different phenomena. Generally, the extent of drug absorption in a particular gastrointestinal segment is determined by the rate of absorption, the available surface area and the transit time through the segment. Due to its large surface area, the small intestine is expected to be the primary absorption site for many drugs [Davis, S. S., J. G. Hardy, et al. (1986)]. However, there are examples of drugs reported in literature that are absorbed from the colon to a relevant extent, e.g. glibenclamide [Brockmeier, D., H. G. Grigoleit, et al. (1985)], theophylline [Yuen, K. H., A. A. Desmukh, et al. (1993)], acetaminophen and phenylpropanolamine [Ishibashi, T., K. Ikegami, et al. (1999)]. In this case the high residence time in the large intestine compared to the small intestine might compensate for the less optimal surface area available for absorption in the large intestine [Waterman, K. C. and S. C. Sutton (2003)].

I.5 Sources of regional differences in drug absorption

Different mechanisms can be involved in the absorption of drugs from the gastrointestinal tract, including passive transcellular diffusion, paracellular diffusion, endocytosis and active transport, both in the absorptive and the secretory direction. For some drugs absorption is mediated by one of these routes exclusively, for others two or more mechanisms overlap.

Some absorption mechanisms provide potential explanations for regional differences in the rate and extent of drug absorption.

I.5.1 Passive diffusion

As for passive diffusion, the pH partition hypothesis [Crevoisier, C. and P. Buri (1976)] suggests a preferred absorption of acidic drugs in proximal regions of the gastrointestinal tract and an increased absorption of basic drugs in more distal regions. This is due to the pH gradient in the gastrointestinal fluids which alters the ratio between the protonated and unprotonated form of acidic and basic drugs. Since an absorption of ionized compounds is assumed to be negligible in most cases, this ratio determines the amount of drug absorbed in a specific region of the gastrointestinal tract. Examples for this phenomenon are the basic β-adrenoceptor antagonists metoprolol (pK_a: 9.5) and oxprenolol (pK_a: 9.7) that are well absorbed from duodenum and jejunum but show no apparent absorption from the acidic milieu of the stomach [Jobin, G., A. Cortot, et al. (1985), Vidon, N., R. Palma, et al. (1986)]. However, these effects are based on pH-dependent differences in the lipid solubility of ionizable drugs and are not due to altered permeabilities of a specific substance in different segments of the gastrointestinal tract. Therefore, regional differences in the absorption of acidic and basic drugs will predominantly follow the rule described above, as long as the solubility and octanol-water partitioning remain sufficiently high over the physiological pH range of the gastrointestinal tract. For many drugs, however, this is not a realistic assumption [Brockmeier, D., H. G. Grigoleit, et al. (1985)].

I.5.2 Paracellular absorption

Another possible source for regional differences in the absorption of drugs is the paracellular route. Instead of crossing the epithelial cells of the gastrointestinal tissues, substances can migrate across pores between the cells to be absorbed. This process is limited by transmembraneous proteins that are located between epithelial cells. These so-called tight junctions decrease the porosity of the epithelial cell layers, such that the paracellular route is only accesible to water and small hydrophilic molecules. This absorption mechanism is assumed to be of minor importance for the absorption of most drugs. However, it is considered to have a relevance for the absorption of hydrochlorothiazide, cimetidine, 5-amino salicylic acid,

small peptides and nucleoside analogues [Zhou, S. Y., N. Piyapolrungroj, et al. (1999), Thwaites, D. T., B. H. Hirst, et al. (1993), He, Y. L., S. Murby, et al. (1996), Lang, V. B., P. Langguth, et al. (1997), Park, G. B. and A. K. Mitra (1992)]. As the junctions between the epithelial cells become progressively tighter from the small intestine to the colon, the paracellular permeability decreases in that direction. This phenomenon is reported as an explanation for the decreasing permeability from jejunum to colon for hydrophilic β-adrenoceptor antagonists such as atenolol [Sasaki, H., Y. Igarashi, et al. (1994)].

I.5.3 Active transport processes

For several drugs carrier-mediated transport is the major mechanism of absorption, for others it provides an additional absorption or secretion pathway. In both cases the saturability of the transporter can result in non-linear pharmacokinetics and dose-dependent absorption. When the transporter carries the drug from the luminal side of the gastrointestinal tract to the blood side, a saturation of the carrier system might result in decreasing absorption with increasing doses, as described for cefatrizine [Yu, L. X. and G. L. Amidon (1998)]. In case of drug efflux carriers, increased absorption is assumed for increasing doses, as found for talinolol [Wetterich, U., H. Spahn-Langguth, et al. (1996)]. For compounds undergoing carrier-mediated active transport in the absorptive direction, differences in the expression of the particular transporters throughout the gastrointestinal tract account for altered absorption from the respective region. When mechanisms apart from active transport play a negligible role in the overall absorption of a drug, any lack of expression of the carrier will result in the occurrence of so-called absorption windows. Absorption windows related to varying carrier expression have been been reported for furosemide [Ritschel, W. A., A. Menon, et al. (1991)], riboflavin [Levy, G. and W. J. Jusko (1966), Klausner, E. A., E. Lavy, et al. (2002)], levodopa [Deleu, D., M. G. Northway, et al. (2002), Klausner, E. A., S. Eyal, et al. (2003)] and several β-lactam antibiotics [Barr, W. H., E. M. Zola, et al. (1994), Sanchez-Pico, A., J. E. Peris-Ribera, et al. (1989), Li, Y. H., K. Ito, et al. (1999), Yu, L. X. and G. L. Amidon (1998), Bretschneider, B., M. Brandsch, et al. (1999), Terada, T., H. Saito, et al. (1997a), Terada, T., H. Saito, et al. (1997b)], such

as amoxicillin, carindacillin, cefadroxil and cefatrizine. These drugs show site-specific absorption in upper parts of the gastrointestinal tract due to a lack of respective carriers (i.e. the amino acid transport systems LAT-2 and b0+ for levodopa [Gomes, P. and P. Soares-da-Silva (2002)], and the peptide transporters PEPT-1 and PEPT-2 for β-lactam antibiotics [Bretschneider, B., M. Brandsch, et al. (1999)]) in lower intestinal segments [Sanchez-Pico, A., J. E. Peris-Ribera, et al. (1989), Ziegler, T. R., C. Fernandez-Estivariz, et al. (2002)].

For active transport processes in the secretory direction (i.e. intestinal drug efflux) no information is available in the literature dealing with a potential correlation between regional differences in the expression of the efflux pump P-glycoprotein within the gastrointestinal tract and regional differences in the absorption of P-glycoprotein substrates. However, opposite to the findings reported for substances transported by carriers from the luminal side to the blood side, the absorption of P-glycoprotein substrates must be assumed to decrease in regions with higher carrier expression. Reports on the distribution of P-glycoprotein are controversial. In a permeation study of the rat intestine Saitoh et al. found indications for a greater efflux in duodenum and jejunum compared to ileum and colon [Saitoh, H. and B. J. Aungst (1995)]. Other rat experiments published by Nakayama et al. suggested the highest efflux in jejunum [Nakayama, A., H. Saitoh, et al. (2000)]. The results from rat studies published by Tamura, et al. and Makhey et al. are in contrast to these findings. Both working groups found that the P-glycoprotein function in ileum and colon was higher than in more proximal parts of the gastrointestinal tract [Tamura, S., A. Ohike, et al. (2002), Makhey, V. D., A. Guo, et al. (1998)]. These findings are in accordance with studies in catfish, reported by Kleinow et al., who observed increasing P-glycoprotein levels from proximal to distal regions of the intestine [Kleinow, K. M., A. M. Doi, et al. (2000)]. Finally, these results are in agreement with the PCR experiments published by Brady et al., who found that mdr1-mRNA levels in rat intestinal tissues increased from duodenum to jejunum and ileum. The highest mRNA levels were reported for the large intestine [Brady, J. M., N. J. Cherrington, et al. (2002)]. The protein expression of P-glycoprotein in micropigs, however, was reported to be higher in proximal parts of the intestine than in more distal parts [Tang, H., Y. Pak, et al. (2002)]. The inconsistent results underline the demand for further investigations in this field.

I.6 Consequences for dosage form design

For several drugs regional differences in drug absorption result in decreased bioavailabilities. Therefore, one argument for the design of new controlled-release (CR) dosage forms can be an optimization of the drug delivery on the basis of the knowledge on preferred absorption sites for a specific drug [Klausner, E. A., S. Eyal, et al. (2003)]. When the occurrence of an absorption window limits the bioavailability of a drug, the goal for the design of an optimized dosage form is to increase the residence time within the gastrointestinal segment of preferred absorption. In literature, dosage forms are described that provide a targeted delivery of a drug to any of the three segments of the gastrointestinal tract, namely the stomach, the small and the large intestine [Rouge, N., P. Buri, et al. (1996)].

I.6.1 Gastroretentive dosage forms

Transit times of dosage forms through the gastrointestinal tract are variable. In case of site-specific absorption the residence times at the prefered absorption sites may be too short for a complete absorption of a drug. In these instances, site-specific controlled-release formulations can improve the bioavailabilty of a drug. For drugs with absorption windows in the upper gastrointestinal tract one approach is to increase the gastric residence time by administration of gastroretentive dosage forms. Such formulations are also suitable to increase the bioavailability of drugs that are hardly soluble in intestinal fluids, but dissolve in the acidic gastric juice, e.g. diazepam, chlordiazepoxide, verapamil or cinnarizine [Sawicki, W. (2002), Machida, Y., K. Inouye, et al. (1989)]. Gastroretentive dosage forms are retained in the stomach. From there, the incorporated drug can be either absorbed directly, when the stomach is the prefered absorption site, or, the dissolving drug in the stomach provides a continuous supply of dissolving drug for the small intestinal absorption sites, when the site of prefered absorption is located in the upper small intestine [Hoffman, A. and D. Stepensky (1999)].

Different approaches have been reported to achieve gastroretentivity of a dosage form. Swelling-controlled [Shalaby, W. S. and K. Park (1990)], buoyant [Hwang, S. J.,

H. Park, et al. (1998), Sawicki, W. (2002), Baumgartner, S., J. Kristl, et al. (2000)], and bioadhesive systems [Lehr, C. M. (1994)] are described. Other preparations described in literature unfold to a size that prevents its passage through the pyloric sphincter [Cargill, R., L. J. Caldwell, et al. (1988), Klausner, E. A., S. Eyal, et al. (2003)]. For example, gastroretentive systems have been developed and tested for verapamil [Sawicki, W. (2002)], furosemide [Menon, A., W. A. Ritschel, et al. (1994)] and levodopa [Klausner, E. A., S. Eyal, et al. (2003),]. Verapamil was a candidate for a gastroretentive dosage form because of its higher solubility in the acidic pH of the stomach, whereas for furosemide and levodopa the low absorbability in lower regions of the gastrointestinal tract caused by the lack of carrier expression gave reason to the development of gastroretentive preparations. However, it is necessary to mention, that gastroretentive formulations are not recommendable for drugs that are well absorbed along the entire gastrointestinal tract and undergo a significant gut wall metabolism, such as nifedipine. The slow and consistent release from the dosage form located in the stomach exposes the drug to a higher degree to metabolic enzymes in enterocytes. The slow drug release leads to a drop of the drug concentration in the gastrointestinal fluids below the saturation level of metabolising enzymes. This can result in an increased first-pass metabolism and a reduced bioavailability [Wilding, I. R., R. A. Sparrow, et al. (1992)].

I.6.2 Drug delivery to the small intestine

A targeted drug release in the small intestine may be achieved by enteric coatings with excipients that dissolve in the upper small intestine. Such coatings may be necessary when a drug is unstable in the gastric juices because of the acidity or the presence of pepsin. Another reason for enteric coatings can be to prevent irritations of the gastric mucosa caused by drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) [Gamst, O. N. (1992), Bechgaard, H., R. R. Brodie, et al. (1982)]. However, absorption from enteric coated tablets is erratic and to a high degree dependent on the gastric residence time [Ritschel, W. A., A. Menon, et al. (1991)]. Therefore, the efficacy of enteric coatings is sometimes unreliable [Davies, N. M. (1999)].
Bioadhesive drug delivery systems have been developed to obtain a prolonged contact with the mucosa of the small intestine and to improve the bioavailability of

drugs with poor absorption in more distal parts of the gastrointestinal tract [Lehr, C. M., J. A. Bouwstra, et al. (1992)]. However, a site specific adhesion of these systems in a particular region of the gastrointestinal tract has not yet been demonstrated.
An example for the design of a controlled-release formulation for an actively transported drug based on the knowledge on absorption windows, was published by Hoffman et al.. In their development of an amoxicillin matrix tablet, designed to minimize the administration frequency, the absorption window in the upper gastrointestinal tract [Barr, W. H., E. M. Zola, et al. (1994)] was taken into account: Compared to typical sustained-release formulations for other drugs, the drug release from these matrix tablets was accelerated (50 % within 3 hours), as a tribute to the higher expression of the peptide carrier in the upper small intestine. [Hoffman, A., H. D. Danenberg, et al. (1998)].

I.6.3 Targeted colon delivery

A targeted delivery to the colon may be advantageous for the local treatment of colonic diseases such as colitis ulcerosa, Crohn`s disease or colon cancer, to prevent an absorption of the drug before the site of action is reached and / or to minimize local adverse effects in more proximal regions of the gastrointestinal tract. Furthermore, a targeted drug delivery to the colon may result in an improved bioavailability for peptides and protein drugs that undergo acidic or enzymatic degradation in the small intestine. For these drugs the colon is thought to be a suitable absorption site due to its lower proteolytic activity [Rubinstein, A., B. Tirosh, et al. (1997), Ishibashi, T., K. Ikegami, et al. (1999), Ritschel, W. A. (1991)]. Additionally, a few drugs are reported to be preferentially absorbed from the colon. Examples for such drugs that are hence candidates for a site-specific drug release in the colon are nisoldipine and dilazep [Rouge, N., P. Buri, et al. (1996)]. There are different approaches to achieve a targeted colon delivery with peroral dosage forms. Physiological specifities such as pH, gastrointestinal transit time and the typical colonic microflora are utilized to release the drug in the colon. Due to high inter- and intraindividual variabilities of the pH along the gastrointestinal tract and the similarities between the pH of the small and the large intestine [Rouge, N., P. Buri, et

al. (1996)], coatings that dissolve at pH values greater than 7.0 are not suitable to ensure a targeted delivery to the colon [Ashford, M. and J. T. Fell (1994)]. Systems featuring a combination of pH- and time-controlled release are assumed to be more effective to prevent an early drug release in the small intestine. Such drug delivery systems can be formulated as capsules or tablets. Ishibashi et al. described a dosage form based on a common hard gelatin capsule, filled with the drug and succinic acid as a pH adjusting agent. The capsule is triple coated with an acid soluble layer based on Eudragit® E, a hydrophilic layer based on hydroxypropyl-methylcellulose and an enteric layer based on hydroxypropyl-methylcellulose acetate succinate. The three layers were designed to prevent a dissolution of the capsule during the transit time through the stomach and the small intestine. By variation of the thickness of the coating layers an onset time of three hours for the drug release after leaving the stomach was achieved, which was in accordance with reported transit times through the small intestine [Davis, S. S., J. G. Hardy, et al. (1986)]. The formulation was assumed to be appropriate for a site-specific release in the colon [Ishibashi, T., K. Ikegami, et al. (1999)]. A similar capsule-based formulation is the Pulsincap system: An enteric coating enables an unaffected passage of the capsule through the stomach. The water soluble cap of the capsule dissolves In the upper small intestine, whereas the water insoluble body of the capsule that is filled with the drug and sealed with a water swellable hydrogel plug does not dissolve. The onset time for the drug release is determined by the water swellable plug [Wilding, I. R., S. S. Davis, et al. (1992), Stevens, H. N., C. G. Wilson, et al. (2002)]. The coated core tablet described by Gazzaniga et al. acts on a similar principle. The drug-containing core is coated with two layers featuring different dissolution properties. The outer enteric layer dissolves in the upper small intestine, the medium layer is water swellable and determines the lag-time until the drug release starts. As for the systems described before this lag-time is adopted to the small intestinal transit time to ensure a site-specific delivery in the colon.

Other approaches utilize the increased number of microorganisms in the colon for drug targeting. In a prodrug approach azo-, glucuronide- or dextran-conjugates of a drug can be synthesized. After oral administration the active drug is released in the colon after cleavage by azoreductases or polysacharidases that are produced by the colonic microflora [McLeod, A. D., D. R. Friend, et al. (1994), Haeberlin, B., W. Rubas, et al. (1993)]. On the other hand, a combination of an outer enteric coating

layer with a second layer that is susceptible to bacterial degradation can be used to deliver drugs to the colon. Such coatings can be based on azo-linked polymers [Van den Mooter, G., C. Samyn, et al. (1995)] or lauric acid dextran esters [Kesslhut, J. F. and K. H. Bauer (1994)]. A more detailed insight into targeted drug delivery to the colon is given in a review published by Yang et al. [Yang, L., J. S. Chu, et al. (2002)].

I.6.4 Dosage form design for drugs undergoing intestinal drug efflux

As described in the last sections, several publications focus on the consequences of carrier-mediated transport of drugs on the design of optimal dosage forms (e.g. β-antibiotics and levodopa). In spite of the large number of P-glycoprotein substrates reported in literature, until today no publication is dealing with the consequences of intestinal P-gp-mediated efflux on dosage form design. Additionally, reports on the distribution of P-glycoprotein throughout the gastrointestinal tract are controversial. A lack of P-glycoprotein expression within a specific segment of the gastrointestinal tract would suggest the development of controlled-release dosage forms targeted to this specific region in order to avoid the absorption-limiting efflux. However, all reports agree, that the efflux pump is expressed throughout the whole intestine, only the findings concerning the ratio of the P-glycoprotein expression between different segments vary. Therefore, P-glycoprotein substrates are no typical candidates for a site-specific drug delivery in the intestine. Rather, the lack of P-glycoprotein expression in the stomach suggests advantageous bioavailabilities for gastroretentive dosage forms, provided that the respective drug can be sufficiently absorbed from the stomach. Another drawback of this suggestion is that the active transport mediated by P-glycoprotein is saturable. Therefore, a rapid exposure of the drug from a rapidly dissolving immediate-release dosage form may result in high drug concentrations in the intestine that exceed the saturation level of the transporter. In consequence, the amount of drug undergoing an intestinal efflux is reduced. On the other hand, the decreased drug release rate from a gastroretentive dosage form results in lower drug concentrations in the gastrointestinal fluids. The dissolved drug leaves the stomach and enters the small intestine where the drug undergoes P-glycoprotein mediated efflux. When the decreased release rate from the gastroretentive dosage form lowers

the concentrations below the saturation level of the transporter, an increased contribution of the drug efflux to the overall absorption can be expected. As a result, the bioavailability for such a dosage form might be decreased instead of increased.
This assumption is similar to the effect described by Wilding et al. who described a decreased bioavailability for a gastroretentive nifedipine formulation. In this case, the saturable process, limiting the bioavailability was gut wall metabolism. The slow drug release lowered the concentrations below the saturation level of the metabolic enzymes, resulting in an increase of the first-pass metabolism in the gut wall and a decrease of the bioavailability [Wilding, I. R., R. A. Sparrow, et al. (1992)].
Another interesting approach for optimized dosage forms of P-glycoprotein substrates with the aim of an increase in bioavailability is the incorporation of P-glycoprotein inhibitors within the formulation. Substances such as PSC 833 (valspodar), VX-710 (biricodar), GF 120918 (elacridar), LY335979 (zosuquidar) or XR 9576 (tariquidar), that have been developed to overcome the multidrug resistance in the therapy of cancer and that have proven a potent ability to inhibit P-glycoprotein in clinical studies [Dantzig, A. H., D. P. de Alwis, et al. (2003)], could be considered for this purpose. A relevant drawback of such an approach is the lack of specifity of most of these substances which can result in an increased toxicity and the occurrence of serious side effects. The inhibition of other active transport proteins might suppress the absorption of vital food compounds or prevent the excretion of toxic substances. In addition, the inhibition of metabolic enzymes has to be considered. An example for the toxic potential of these strong P-glycoprotein inhibitors is reported by Huisman et al.. In studies on the co-administration of saquinavir, ritonavir and elacridar using a mouse model, he reported an increased mortality of the mice under treatment with the P-glycoprotein inhibitor [Huisman, M. T., J. W. Smit, et al. (2003)]. As a safe alternative, plant extracts, e.g. from apricots, grapefruits or green tea, could be employed, that were shown to inhibit P-glycoprotein efflux. Various in vitro and in situ experiments have indicated their potential to increase the absorption of P-glycoprotein substrates [Deferme, S., R. Mols, et al. (2002), Spahn-Langguth, H. and P. Langguth (2001), Jodoin, J., M. Demeule, et al. (2002)]. Moreover, there are reports on P-glycoprotein inhibiting effects of pharmaceutical excipients, such as polyethylene glycols, Cremophor EL® and Tween 80® [Hugger, E. D., B. L. Novak, et al. (2002)]. However, these P-glycoprotein modulating properties were only

demonstrated in in vitro studies with cell cultures, whether these excipients show notable P-glycoprotein inhibiting effects in vivo has not yet been demonstrated.

I.7 Aim of the thesis

The design of gastroretentive controlled-release dosage forms for P-glycoprotein substrates is not considered suitable to decrease the influence of the intestinal drug efflux and improve the bioavailability, as described in the last section. Nevertheless, the general advantages of sustained-release dosage forms, such as better compliance, reduced frequency of administration, reduction of side effects, etc., are undoubted, for P-glycoprotein substrates as well as for many other drugs (cp. introduction to Chapter III).
It was demonstrated in the last sections that specific absorption mechanisms, in particular active transport processes, should be addressed in the design of optimized dosage forms. This also applies to sustained-release dosage forms for P-glycoprotein substrates. In their design, the intestinal drug efflux should be taken into account. However, information on the impact of a sustained drug release on the intestinal secretion does not exist.

The aim of this doctoral thesis was to characterize the influence of the decreased drug release rate from sustained-release dosage forms on the intestinal drug efflux.

To reach this aim,

- the contribution of the intestinal drug efflux to the overall absorption in different segments of the intestine should be determined,

- a sustained-release dosage form for a P-glycoprotein substrate should be developed to enable an evaluation in a human in vivo study, and

- the newly developed sustained-release dosage form should be evaluated in silico.

The variety of different transporters involved in intestinal drug efflux, with their different, in some cases overlapping, substrate-specifities, as pointed out at the beginning of this chapter, complicates the projected investigations. To reduce this complexity, one transporter was selected to focus on in the projected studies. P-gp,

as the most frequently described and best characterized carrier involved in intestinal drug efflux, was chosen for this purpose. All experiments were carried out using a suitable P-gp substrate as model substance.

An in situ rat perfusion model was chosen to determine the contribution of the intestinal drug efflux to the overall absorption. This model enables investigations on the absorption from different intestinal segments, and as an in situ method in living anaesthetized animals, the results are expected to correlate better to the in vivo situation in humans than any in vitro model. Another reason for the choice of the rat perfusion model was that the performance of rat studies is less complicated and cost-intensive than the performance of human in vivo studies, and, contrary to human perfusion studies, experiments on colonic absorption are easily practicable. Since anatomy and physiology of the gastrointestinal tract of rats are similar to humans, which includes the expression of P-glycoprotein, this species was considered adequate for the targeted aim.
Based on the results from perfusion studies, the determination of effective permeabilities of different intestinal regions (jejunum, ileum and colon) should detect potential differences in the absorption of the model compound from different parts of the gastrointestinal tract. A comparison between results from perfusion experiments performed with and without P-glycoprotein inhibitors should enable an estimation of differences in the regional distribution of the expression of the efflux pump P-glycoprotein. Finally, the contribution of the active efflux process to the overall absorption should be evaluated on the basis of the results from rat perfusion studies. Increasing drug concentrations in the perfusion solutions should demonstrate the saturability of intestinal drug efflux combined with a potential concentration-dependence of the intestinal permeability (cp. Chapter II).

The development of a sustained-release dosage form for a drug undergoing intestinal efflux was focused on capsule and tablet formulations. The projected sustained-release preparation was to show a consistent drug release within the gastrointestinal tract over eight hours, without being relevantly affected by altering pH values in different gastrointestinal sections. Furthermore, the sustained-release dosage form should feature adequate technological properties. An overview on formulations for a sustained drug release is presented in the introduction to Chapter III.

Since dissolution media have a pronounced influence on the in vitro drug release, in particular for sustained-release dosage forms, suitable dissolution fluids had to be selected and characterized (cp. Chapter IV).

Another goal was to obtain approval for the production of drug products for an application in humans within a university laboratory. Therefore, a manufacturing process complying with current GMP standards had to be established (cp. Annex I). Additionally, the performance of a human clinical study with the developed sustained-release dosage forms should be prearranged (cp. Annex II).

Finally the newly developed sustained-release dosage form should be evaluated in silico. The term “in silico” testing refers to a simulation of pharmacokinetic parameters (e.g. bioavailability, AUC, C_{max}, t_{max}) employing a computer software program that is able to predict bioavailabilities and other parameters on the basis of well-known characteristics of a drug, in combination with in vitro dissolution data of the particular drug product. An overview on advantages of in silico tests and software programs available is given in the introduction to Chapter V.

I.8 Talinolol as model compound for drugs that are subject to intestinal drug efflux

For the projected studies, a model compound had to be selected. There were several aspects supporting the choice of talinolol, a compound suggested by Spahn-Langguth et al. as a model substance for drugs that are subject to intestinal secretion [Spahn-Langguth, H., G. Baktir, et al. (1998)]. Talinolol is a β_1-specific adrenoceptor antagonist. Its binding to the intestinal efflux pump P-glycoprotein has been proven in vitro and in vivo [Gramatté, T., R. Oertel, et al. (1996), Wetterich, U., H. Spahn-Langguth, et al. (1996)]. The most relevant advantage over other P-glycoprotein substrates for an employment in mechanistic studies is that talinolol undergoes no relevant biotransformation. The metabolic rate of less than 1 % of the administered dose ensures that the intestinal drug efflux can be studied without an interference with biotransformation processes [Oertel, R., K. Richter, et al. (1994), Oertel, R. and K. Richter (1995), Spahn-Langguth, H., G. Baktir, et al. (1998)]. This is of particular importance, since an overlapping substrate specifity has been reported for P-glycoprotein and the CYP3A4 isoform of the cytochrome P 450 enzyme system that is located, amongst others, in human intestinal cells and mediates gut wall metabolism [Schuetz, E. G., A. H. Schinkel, et al. (1996), Wacher, V. J., C. Y. Wu, et al. (1995),]. Additionally, many inducers and inhibitors of P-glycoprotein also modify the CYP3A4-mediated metabolism. Therefore, results from inhibition and induction experiments with substances such as ciclosporine, that undergo both, P-glycoprotein secretion and CYP3A4 metabolism, are difficult to interpret [Hsiu, S. L., Y. C. Hou, et al. (2002)]. A review on the overlapping effects of metabolic enzymes and efflux transporters on the absorption of drugs from the gastrointestinal tract was recently published by Suzuki et al. [Suzuki, H. and Y. Sugiyama (2000)]. In general, overlappings between metabolism and efflux are an obstacle for mechanistic investigations on the drug absorption process, since any effect detected cannot be definitely attributed to either of the two processes. For investigations on the influence of a sustained drug release rate on the intestinal drug efflux, metabolism as additional influencing factor would complicate the interpretation of data obtained, or even make it impossible. For predictions of bioavailabilities and other pharmacokinetic parameters, metabolism means an additional unknown parameter which can hardly be separated from intestinal drug efflux.

Another argument in favour of talinolol as model drug for the projected investigations is its moderate passive membrane permeability which prevents rapid passive absorption of the drug such that an apparent secretion back into the intestinal lumen can not be observed [Spahn-Langguth, H., G. Baktir, et al. (1998)].

Properties of talinolol

The chemical structure of talinolol ((1-(4-cyclohexylureidophenoxy)-2-hydroxy-3-*tert*-butylaminopropane) is depicted in Figure I.3. The basic structure typical for all β-adrenoceptor antagonists is pointed out by the grey frame. Within this basic structure an asymmetrically substituted carbon atom is located, which results in chirality of the molecule. In the figure this carbon atom is marked with a grey star:

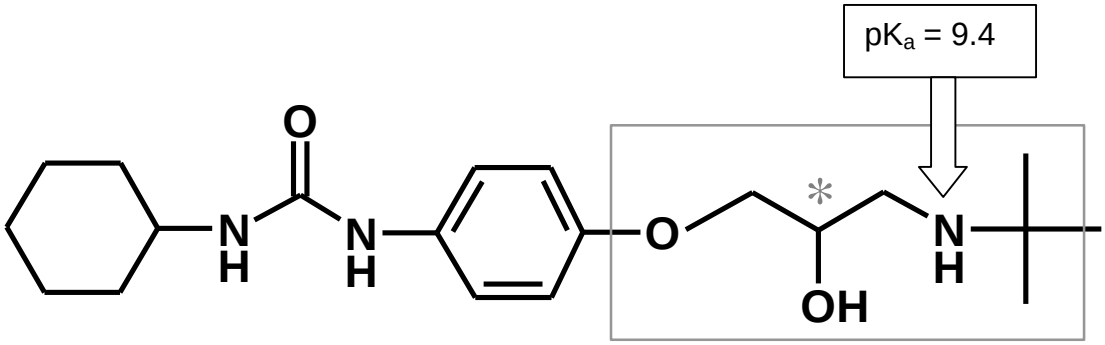


Figure I.3:
Chemical structure of RS-talinolol.

Like acebutolol, atenolol, celiprolol, betaxolol, bisaprolol and metoprolol, talinolol belongs to the class of β_1-specific adrenoceptor antagonists. The specifity of these β-adrenoceptor antagonists is only relative, i.e. they show affinity to both, β_1- and β_2-receptors, with an apparently higher affinity towards β_1-receptors. Talinolol is used in the treatment of arterial hypertension, acute and chronic tachycardic heart arrhythmia and hyperkinetic heart syndrome. Furthermore it is used in prophylaxis of myocardial infarction, as well as after an acute infarction to avoid reinfarction. In the treatment of

coronary heart disease and unstable angina pectoris it is administered in prophylaxis of anginal attacks.

In Tables I.1 to I.3 relevant physico-chemical and pharmacokinetic properties of talinolol are summarized. The basicity of the weak base talinolol refers to a secondary amino functional group. Since the nitrogen of this amino group can be protonated, talinolol features a pH-dependent solubility, which decreases with increasing pH values [Avdeef, A. (2001)]. The log P of 3.2 indicates a moderate lipophilicity, compared to other common β-adrenoceptor antagonists. It is less lipophilic than propranolol (log P: 4.6) and more lipophilic than atenolol (log P: 0.2) [Caron, G., Steyaert, G., et al (1999)].

Talinolol was introduced into clinical practice in 1975 in the former German Democratic Republic (GDR) by AWD (Dresden, Germany) under the trade mark Cordanum. Talinolol is available on the German market in tablet form (immediate release tablets) and as solution for injection. The film-coated Cordanum immediate-release tablets are available with drug contents of 50 and 100 mg RS-talinolol. Recommended daily doses range from 100 to 300 mg, in healthy volunteers safety of the drug was proven up to single doses of 400 mg [Wetterich, U., H. Spahn-Langguth, et al. (1996)]. Composition of the tablets includes magnesium stearate, talcum, sodium carboxymethyl starch, titanium dioxide, polyethylene glycol 35000, potato starch, microcrystalline cellulose, hydroxypropylmethylcellulose, pyrogenic silica (Aerosil) and polyethylene glycol 6000 as excipients, iron oxides are added additionally only in 50 mg tablets. Furthermore, Cordanum is available in Germany as solution for injection in ampoules containing 10 mg RS-talinolol dissolved in 5 ml of solvent.

Table I.1:

Physico-chemical properties of talinolol.

Molecular weight (M.W.)	363.5 Da
pK_a	9.4
Partition coefficient (n-octanol / water, at pH 7.0)	0.74
log P	3.2
Solubility in water (pH 7.0)	0.2 mg/ml
Melting point (M.P.)	143° Celsius

[Wetterich, U., H. Spahn-Langguth, et al. (1996), AWD talinolol product information (2000)]

Table I.2:

Pharmacokinetic properties of talinolol after peroral administration (50 mg).

$AUC_{0\text{-}36h}$	1180 ng•h/ml (SD: 337)
C_{max}	168 ng/l (SD: 47)
t_{max}	3.2 h (SD: 0.8)
Elimination half life ($t_{1/2}$)	11.9 h (SD: 2.4)
Biotransformation	< 1 %
Renal excretion	28.1 % (SD: 6.8) of the administered dose
Extrarenal excretion	40 % (SD: 6.8) of the bioavailable dose
Bioavailability (fasted state)	55 % (SD: 22, dose-dependent)
Bioavailability (fed state)	45 % (dose-dependent)

[Trausch, B., R. Oertel, et al. (1995a), Trausch, B., R. Oertel, et al. (1995b), Oertel, R., K. Richter, et al. (1994), Oertel, R. and K. Richter (1995), Abda Drug Data Base Version 4.2.0 (1998)]

Table I.3:

Pharmacokinetic properties of talinolol after intravenous infusion (30 mg)

Mean residence time (MRT)	11.6 h (range: 8.1 – 20.0 h)
Volume of distribution (V_D)	3.3 l/kg (range: 2.5 – 4.3 l/kg)
Total body clearance (CL_{tot})	4.9 ml/min•kg (SD: 0.6))
Renal clerance (CL_{ren})	2.8 ml/min•kg (SD: 0.7)
Metabolic clearance (CL_{met})	0.017 ml/min•kg (SD: 0.01)
Non-renal clearance (CL_{nonren})	2.1 ml/min•kg (SD: 0.7)
Biliary clearance (Cl_{bil})	0.71 ml/min•kg (range: 0.22 –3.53 ml/min•kg)
Plasma protein binding	60.9 % (range: 50-70 %)

[Trausch, B., R. Oertel, et al. I (1/1995), Trausch, B., R. Oertel, et al. II (5/1995), Terhaag, B., T. Gramatté, et al. (1989), Abda Drug Data Base, Version 4.2.0 (1998)]

Chapter II

Chapter II

Effective permeabilities of talinolol in different regions of the rat intestine

II.1 Introduction

Whereas the affinity of a compound to absorptive carriers may considerably enhance its bioavailability, affinity to secretory carriers may show the opposite effect, i.e., a diminution of the transmembraneous flux in the absorptive direction leading to a reduction of the absorptive intestinal permeability. This may limit the fraction of the administered dose absorbed. In that case, enhancement of absorption and bioavailability is to be expected when higher doses of the same drug are administered (saturation) or in the case of competitive or non-competitive inhibition of the carrier.

To elucidate the relevance of intestinal efflux processes for the absorption of drugs, rat intestinal perfusion studies were performed using talinolol as a model substance [Spahn-Langguth, H., G. Baktir, et al. (1998)]. Talinolol is secreted by a carrier-mediated process from the enterocyte into the intestinal lumen following intravenous or peroral administration. In human small intestinal perfusion studies performed with a modified triple lumen technique [Cooper, H., R. Levitan, et al. (1966), Gramatté, T. (1994), Gramatté, T., E. el Desoky, et al. (1994), Gramatté, T. and K. Richter (1994)] Gramatté et al. found that talinolol absorption is higher in proximal regions of the small intestine than in more distal regions [Gramatté, T., R. Oertel, et al. (1996)]. It is assumed that differences in intestinal drug efflux within the gastrointestinal tract provoke these findings. To elucidate the involvement of P-gp in intestinal drug absorption perfusion studies were performed in different segments of the rat intestine (jejunum, ileum, and colon). Additionally, it was tested whether intestinal secretion was saturable and whether it could be inhibited by co-administering the P-gp modulators vinblastine and rhodamine 123. A further task was to determine the fraction of the intestinal permeability of the drug which is undergoing carrier-mediated efflux. Therefore, coefficients expressing the contribution of the actively secreted and the passively absorbed talinolol were calculated. These coefficients include $P_{eff\ passive}$,

the membrane permeability given by the diffusion of the compound across the membrane, $P_{eff\ max}$, the maximum transport rate for the carrier-mediated process (equivalent to V_{max} in a Michaelis-Menten equation), K_m, the Michaelis-Menten constant, and $P_{eff\ transporter}$, the drug flux resulting from the activity of the carrier, differentiated with respect to the drug concentration.

II.1.2 Dose-dependence of talinolol bioavailability

Due to its affinity to intestinal transporters and the saturability of carrier-mediated transport systems, the dose to plasma concentration ratio of talinolol is expected to be non-linear. A dose-dependence of talinolol absorption was reported by Wetterich et al. [Wetterich, U., H. Spahn-Langguth, et al. (1996)]. To further prove this phenomenon, additional data from clinical studies in humans available from literature were reviewed. The results are presented in Table II.1.

Table II.1:
Compilation of systemic exposure following peroral administration of talinolol in clinical studies in humans.

Author, Year of Publication	**Dose [mg]**	**Remarks**	**Number of volunteers**	**Mean AUC [ng ⋅h/ml]**	**SD of AUC** [ng•h/ml]	**SD rel [%]**	**AUC/Dose (SD)**
Wetterich et al., 1996	**25**		12	500	45	9,0	**20,0 (1,8)**
	50		12	1238	127	10,3	**24,8 (2,5)**
	100		12	3282	227	6,9	**32,8 (2,3)**
	400		12	14693	893	6,1	**36,7 (2,2)**
Terhaag et al., 1991	**50**	fasted	11	1374	(range: 944-1882)		**27,5 (n.a.)**
	50	fed	11	776	(range: 300-1151)		**15,5 (n.a.)**
Trausch et al., 1995	**50**		12	1321	382	28,9	**26,4 (7,6)**
Giessmann et al. , 2001	**100**	Capsule fasted	8	2570	795	30,9	**25,7 (8,0)**
	100	Capsule fed	8	2010	541	26,9	**20,1 (5,4)**
Schwarz et al. , 1999	**50**		9	945	188	19,9	**18,9 (3,8)**
Westphal et al., 2000	**100**		10	2970	486	16,4	**29,7 (4,9)**

The ratio of AUC to dose displayed in the last column of the table is a measure for the linearity between the dose administered and the systemic exposure of the drug. If there was no dose-dependence, the values of this ratio should remain constant.
The data are displayed in Figure II.1:

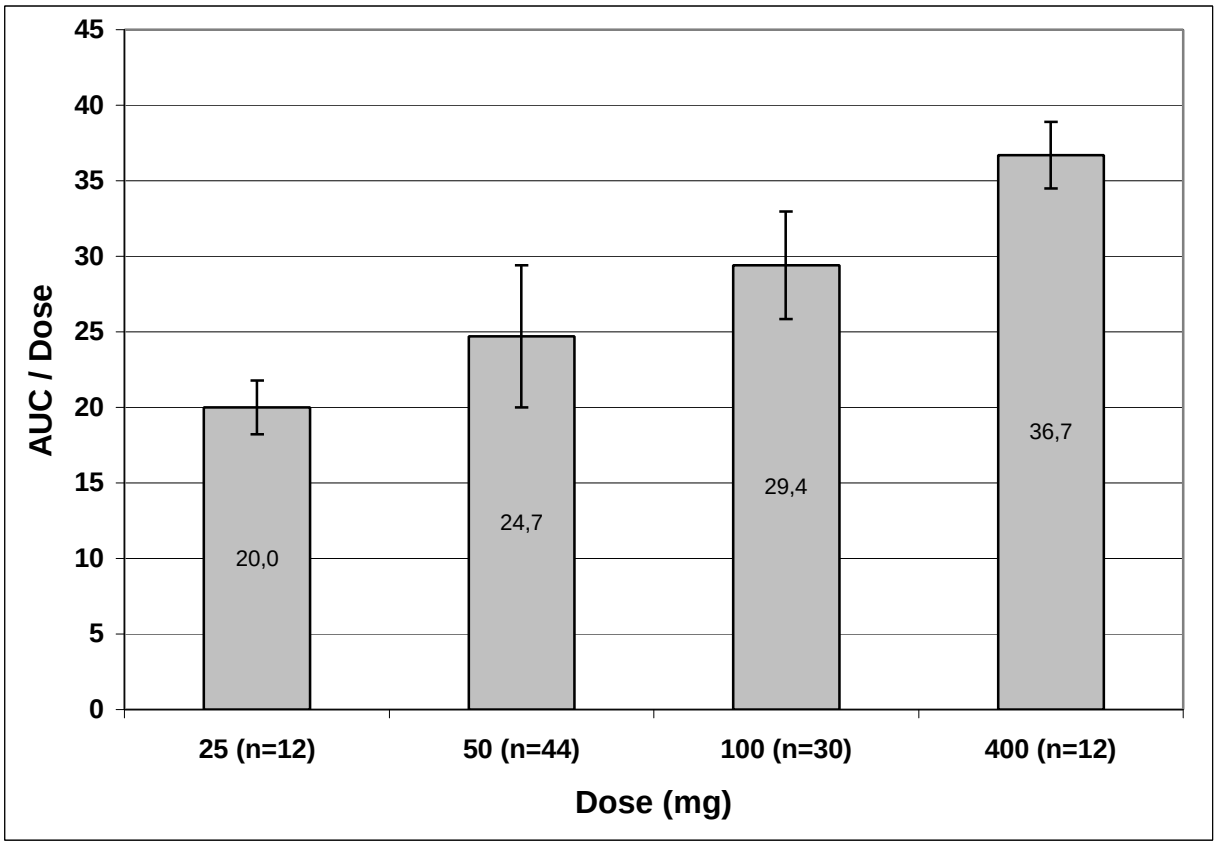


Figure II.1:
Compilation of talinolol clinical studies in humans: AUC / dose ratios versus dose as an indicator for a dose dependent absorption.

It becomes obvious that the AUC increases more than proportional with increasing doses, leading to higher fractions absorbed and hence higher AUC / dose ratios of higher doses. Thus, the absorption of talinolol is non linear and shows the expected dose-dependence. Biotransformation as a potential source for this non-linearity can be excluded, since it accounts for less than 1 % of the administered talinolol dose. Neither is non-linear elimination an adequate explanation, because the elimination half-life ($t_{1/2}$) remains unchanged for increasing doses in the range from 25 to 400 mg [Wetterich, U., H. Spahn-Langguth, et al. (1996)]. Hence, the dose dependence must be due to the saturability of the intestinal efflux process. At higher doses the transporter system responsible for the intestinal secretion is reaching a saturation level, such that further increases in dose, will not lead to increases in secretion. On the other hand, when only passive diffusion processes are governing the absorption, it is expected that the AUC will increase proportionally with dose. A combination of

carrier-mediated and passive transport processes will lead to a disproportionality between dose and exposure at low doses and virtually dose-proportional changes in exposure at higher doses.

II.1.3 Intestinal perfusion studies in rats

Differences between the intestinal permeability in different regions of the intestine as a function of talinolol perfusate concentrations were evaluated using the model of rat intestinal perfusion.

This model was found appropriate for the determination of effective permeabilities with relevance for the absorption in humans [Lennernas, H. (1997), Fagerholm, U., M. Johansson, et al. (1996)]. Although the permeabilities determined by in situ rat perfusion were not equal to the permeabilities found in human studies, the rank order of numerous drugs tested was the same. By means of appropriate scaling factors the rat perfusion model can predict the human in vivo situation very well [Lennernas, H. (1998)]. Cell culture models like the common Caco-2 model could not be used for the projected study, since there is no possibility to distinguish between different intestinal sections.

The perfusions were performed as single-pass studies, i.e. talinolol solutions were pumped through the different sections of the rat intestine, namely jejunum, ileum and colon with a constant flow rate and samples of the solutions having passed the rat intestine were collected in five minute intervals.

The actual experimental setup and a summarizing scheme of the experiment are depicted in Figure II.2.

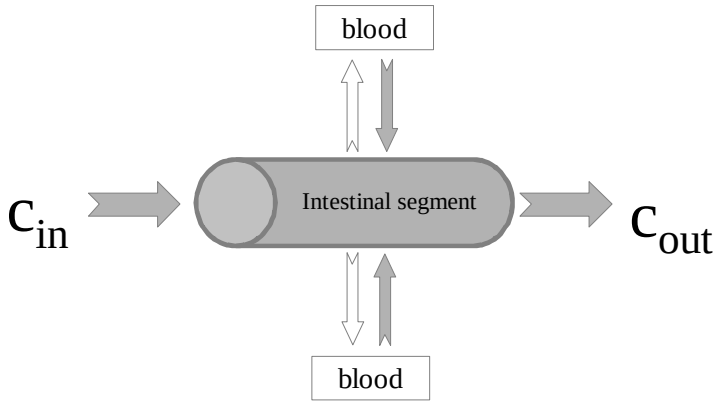


Figure II.2:
Photograph and schematic overview of a single-pass intestinal perfusion experiment in rats.

II.2 Materials and methods

II.2.1 Materials

All chemicals were supplied by E. Merck (Darmstadt, Germany) with the exception of vinblastine, which was purchased from GRY Pharma GmbH (Kirchzarten, Germany), talinolol, which was a grateful gift of AWD Pharma (Dresden, Germany) and the solvents for HPLC analytics, which were purchased from C. Roth (Karlsruhe, Germany).

II.2.2 Animals

Male Wistar rats were purchased from Charles River Germany (Sulzfeld, Germany). When perfusion was performed the rats weighed between 274 and 307g. The animal study protocol was approved by the Committee for Animal Studies of the Martin Luther-University Halle-Wittenberg and the State of Sachsen-Anhalt. The studies complied with governmental requirements for animal studies.

II.2.3 Instrumentation

Intestinal perfusions were performed using an Ismatec MC-MS CA 8/6 peristaltic pump (Glattbrugg-Zürich, Switzerland) with silicone tubes with an inner diameter of 2 mm. For HPLC analytics a computer programmable Beckman HPLC device “System Gold” was used, consisting of a pump module Type 126, an UV-Vis detector module Type 166 and an automatic sampler Type 507.
Graphical, statistical and pharmacokinetic evaluation were performed using S-Plus 2000 (Math Soft, Seattle, USA).

II.2.4 Quantitative analysis of talinolol from intestinal perfusate solutions

The assay developed by Wetterich et al. [Wetterich, U., H. Spahn-Langguth, et al. (1996)] was used with slight modifications for the quantitative analysis of talinolol in the intestinal perfusate samples: Talinolol was isolated by liquid-liquid extraction of 100 µl of each perfusate sample with 2 ml dichloromethane / isopropanol (95:5) after addition of 100 µl 1M sodium hydroxide, which adjusts the pH to values of 12 to 13 and hence leads to a deprotonation of talinolol. Racemic pindolol was used as internal standard. After evaporation of the solvent, the samples were reconstituted in methanol and analysed by HPLC using a 250 x 4 mm ChiraSpher NT column (Merck, Darmstadt, Germany) and ethanol with 0.05 % (v/v) triethylamine added as mobile phase at a flow rate of 0.45 ml/min. The talinolol and pindolol concentrations in the eluate were analysed by measuring the UV-absorption at 245 nm. Talinolol concentrations (i.e. the concentrations of the racemic RS-talinolol) were calculated by addition of the concentrations determined for the R- and the S-enantiomer. Neither vinblastine nor rhodamine 123, the two inhibitors of the intestinal drug efflux tested, did interfere with the assay. The accuracy of the flow rate of the HPLC pump was determined as > 98 %. The limit of quantification was 30 ng/ml for each enantiomer. Interday- and intraday-variabilities were determined in the concentration range from 1 to 200 µg/l. The deviations from the mean were less than 10 %. The coefficients of determination (R^2) determined for the calibration curves were 0.999 or better.

II.2.5 Perfusion of rat small and large intestine

Perfusion studies with vinblastine as P-gp-inhibitor were performed in four rats with each of the following five talinolol concentrations: 0.025, 0.05, 0.1, 0.25 and 0.5 mmol/l. For the studies with rhodamine 123 as P-gp-inhibitor, also four rats were used for each talinolol concentration, however, only the following three concentrations were examined: 0.025, 0.05 and 0.1 mmol/l. The rats were fasted 18 h before the study with free access to water. After inhalation of ether they were anaesthetized by an intraperitoneal injection of 50 mg/kg ketamin and 10 mg/kg rompun®. The intestine was exposed by a four to five cm midline abdominal incision, then tubes were attached to the three different intestinal segments to be perfused

(jejunum, ileum and colon). The lengths of the perfused segments in jejunum were 4.0 to 10.0 cm, in ileum 3.0 to 4.8 cm and in colon 2.0 to 3.0 cm. The average radius of the segments measured in jejunum and ileum was 0.18 cm, while it was 0.20 cm in colon. In order to maintain the rats` body temperatures they were placed on a heating pad at 37°C.

For the studies with vinblastine as inhibitor, 0.025, 0.05, 0.1, 0.25 and 0.5 mmol/l RS-talinolol were dissolved in Tyrode`s buffer, (8.0 g/l NaCl, 1.0 g/l $NaHCO_3$, 1.0 g/l glucose, 0.2 g/l KCl, 0.2 g/l $CaCl_2$, 0.1 g/l $MgCl_2$, and 0.04 g/l $NaH_2PO_4$ dissolved in distilled water). The studies with rhodamine 123 as inhibitor were performed with 0.025, 0.05 and 0.1 mmol/l RS-talinolol solutions also in Tyrode`s buffer. Perfusion solutions were maintained at 37°C and pumped through jejunum, ileum and colon simultaneously at a flow rate of 0.2 ml/min. Outlet perfusate solutions were collected in predefined intervals and frozen at –24°C until analysis. Each rat was perfused for 120 minutes. After an equilibration period of 30 minutes the three intestinal segments were perfused for 30 minutes with the talinolol buffer solutions. Then either 0.1 mmol/l vinblastine or 0.05 mmol/l rhodamine 123 were added to the perfusion solution and after another equilibration period of 30 minutes perfusion was continued for another 30 minutes. Perfusate samples were collected and analysed in five minute intervals.

II.2.6 Net water absorption / secretion during perfusion

Water absorption or secretion processes may falsify the determination of drug permeability. Therefore, water fluxes were monitored and talinolol concentrations were corrected for water fluxes.

Water fluxes were calculated according to equation [1]:

$$\% \text{ Water transport} = \frac{m_{in} - m_{out}}{m_{in}} \cdot 100 \qquad [1]$$

$m_{in}$is the mass of the inlet perfusion solution, m_{out} the mass of the outlet perfusate solution.

II.2.7 Intestinal permeability

Intestinal permeabilities were calculated on the basis of the mixing tank model described by Sinko et al. [Sinko, P. J., G. D. Leesman, et al. (1991)] using equation [2]:

$$P_{eff} = \frac{v \cdot (\frac{c_{in} - c_{out}}{c_{out}})}{2prl} \qquad [2]$$

v is the perfusion flow rate, c_{in} and c_{out} are the drug concentrations in the inlet and outlet perfusion solutions. r represents the radius and l the length of the perfused intestinal segment.

II.2.8 Experimental determination of carrier-mediated and passive components of drug permeability

To demonstrate the influence of the P-gp-mediated active secretion on the overall permeability the results from perfusions with P-gp inhibitors (vinblastine or rhodamine 123) had to be compared with those performed without inhibitor added. As a measure for the transporter influence, the inhibitable P_{eff} fractions were calculated according to equation 3 as differences between effective permeabilities with and without addition of an inhibitor.

P_{eff} difference = P_{eff} in presence of inhibitor – P_{eff} in absence of inhibitor [3]

An advantage of these P_{eff} difference values is that the comparably high variances for P_{eff} values obtained by rat perfusions caused by interindividual variabilities are minimized [Hanafy, A., P. Langguth, et al. (2001)].

II.2.9 Theoretical model describing the concentration-dependence of flux and permeability across membranes for substrates undergoing passive absorption and Michaelis-Menten type carrier-mediated secretion

When describing membrane transport processes, it is important to differentiate between flux (mass transported per unit time and area) and effective permeability coefficients: Flux values are concentration dependent while effective permeabilities are independent on the drug concentration, as long as passive diffusion dominates the transport process. As soon as a saturable component is introduced, e.g. a carrier-mediated process, P_{eff} will change with drug concentration due to the saturation of the carrier at higher substrate concentrations.
Figure II.3 shows the contribution of carrier-mediated and passive transport processes to the overall absorption.

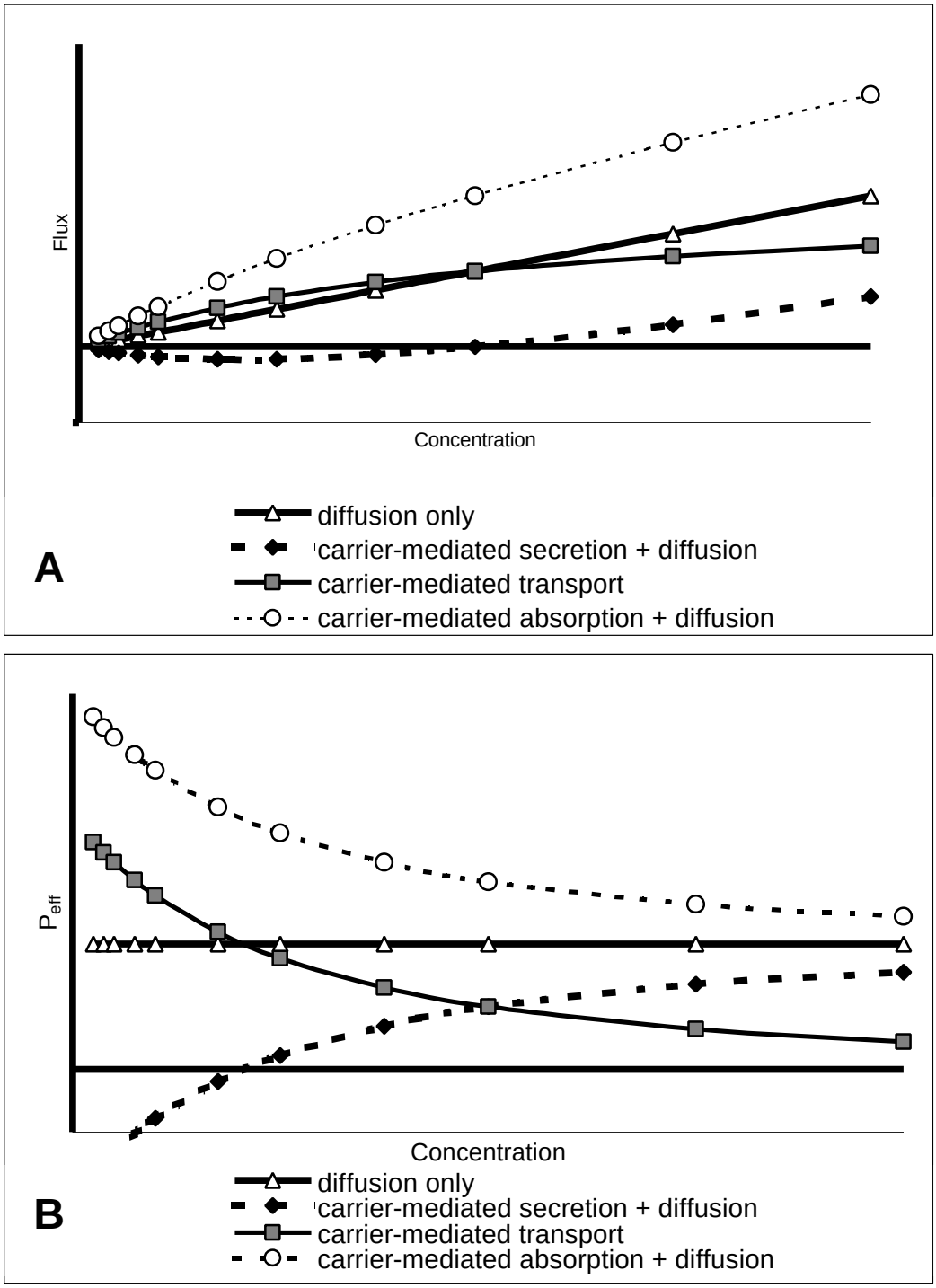


Figure II.3:

Concentration-dependence of drug membrane flux (A) and effective permeability (B) when carrier-mediated transport and passive diffusion processes are operative in intestinal drug transport. The simulations presented have been carried out using equations [4] and [5] and the following values as coefficients:

A) K_m = 0.5 mmol/l; J_{max} = 10 µg/s; $J_{passive}$ = 10 µg·l / s·mmol · c_{Donor}

B) K_m = 0.5 mmol/l; $P_{eff\ max}$ = $10 \cdot 10^{-4}$ cm/s; $P_{eff\ passive}$ = $10 \cdot 10^{-4}$cm/s.

In Figure II.3A fluxes (J) are considered and in Figure II.3B effective permeabilities (P_{eff}).

For the simulations in the figures above demonstrating the concentration-dependence of drug membrane *flux*, a value of 0.5 mmol/l was assumed for K_m.

F_{max}, the maximum drug flux mediated by the carrier was assumed to be 10 µg/s, the passive drug flux was fixed at 10 times the drug concentration in the donor solution.

For the simulation demonstrating the concentration-dependence of drug effective *permeability*, K_m was set to 0.5 mmol/l, $P_{eff\ max}$ was $10 \cdot 10^{-4}$ cm/s and $P_{eff\ passive}$ was $10 \cdot 10^{-4}$ cm/s.

Assumed that membrane permeation of a drug is composed of a carrier-mediated *and* a passive transport process, its total flux (J) is given by equation 4, its total effective permeability (P_{eff},) by equation 5:

$$J = J_{passive} + \frac{J_{\max} \cdot C}{K_m + C} \qquad [4]$$

$$P_{eff} = P_{eff,\ passive} + \frac{P_{eff,\max} \cdot K_m}{(K_m + C)^2} \qquad [5]$$

$J_{passive}$ and $P_{eff\ passive}$ represent the fractions of the total flux or total effective permeability which are due to diffusion. The second term in each equation represents the contribution of the carrier to the overall transport. When fluxes are considered, the carrier-mediated fraction (f(c)) can be expressed by the Michaelis-Menten equation:

$$f(c) = \frac{V_{\max} \cdot C}{K_m + C} \qquad [6]$$

In terms of permeability the Michaelis-Menten equation has to be differentiated with respect to C. The derivation of equation 6 leads to equation 7:

$$f'(c) = V_{\max} \frac{[1 \cdot (K_m + C)] - (C \cdot 1)}{(K_m + C)^2} = \frac{(V_{\max} \cdot K_m)}{(K_m + C)^2} \qquad [7]$$

Equation 7 was used to express the fraction of permeability values which is due to carrier-mediated transport.

Since xenobiotics can be actively transported from the gut lumen to the blood side as well as in the reverse direction, the term in equation 7 may become positive or negative. Consequently, the total effective permeability is composed of a term expressing the fraction of total drug transported by diffusion which is either *enlarged* or *diminished* by the contribution of the carrier-mediated transport process. As far as drug efflux processes are concerned the total transport is passive transport diminished by the contribution of carrier-mediated secretion, leading to total permeabilities that are lower than passive diffusion alone.

Equation 5 was used to estimate the carrier-mediated and passively transported fractions of the total permeabilities that were determined in rat perfusion experiments. The estimations were performed using the S-Plus 2000 software package (Math Soft, Seattle, USA).

II.3 Results

II.3.1 Analysis of talinolol from perfusate samples

A typical chromatogram of talinolol derived from the stereospecific HPLC assay is shown in Figure II.4:

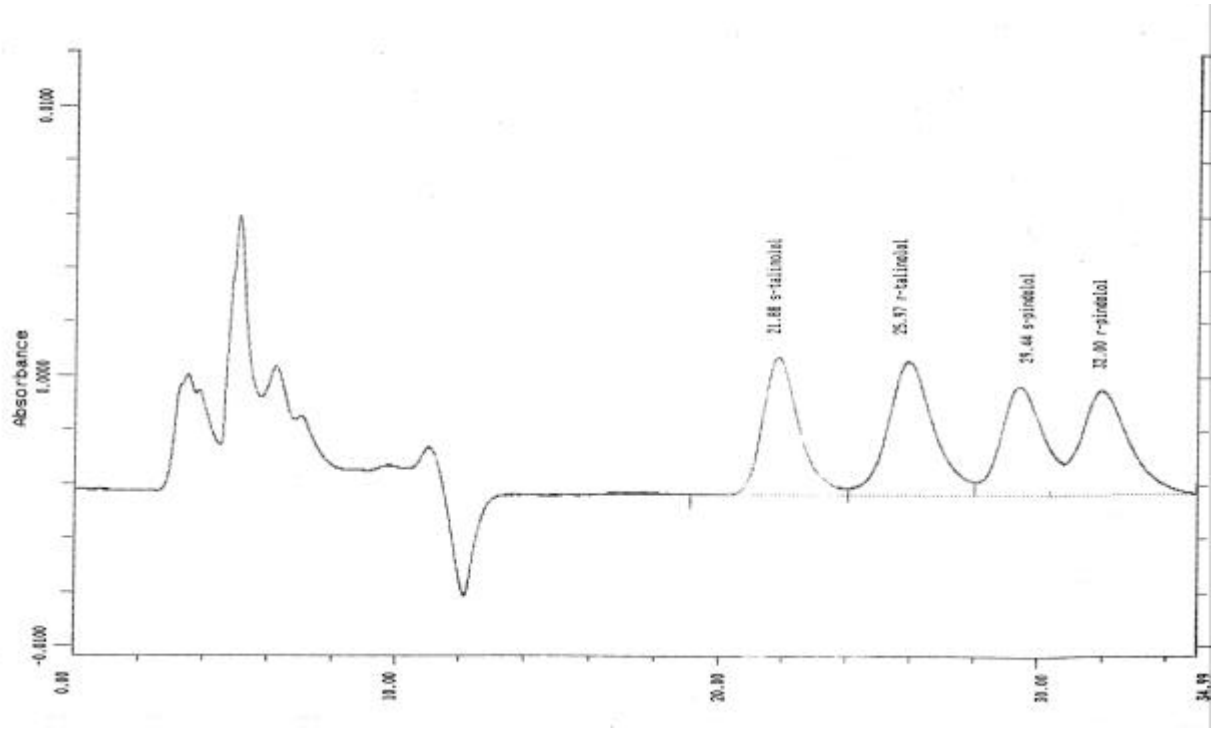


Figure II.4:
HPLC-chromatogram of RS-talinolol (0.025 mM in perfusate buffer) and RS-pindolol (0.08 mM) as internal standard obtained by the stereospecific HPLC assay. UV detection was at 245 nm, the injection volume was 50 µl. Talinolol concentrations were calculated by addition of the peaks for the R- and the S-enantiomer.

Typical outflow concentration patterns from individual animals are demonstrated in Figure II.5:

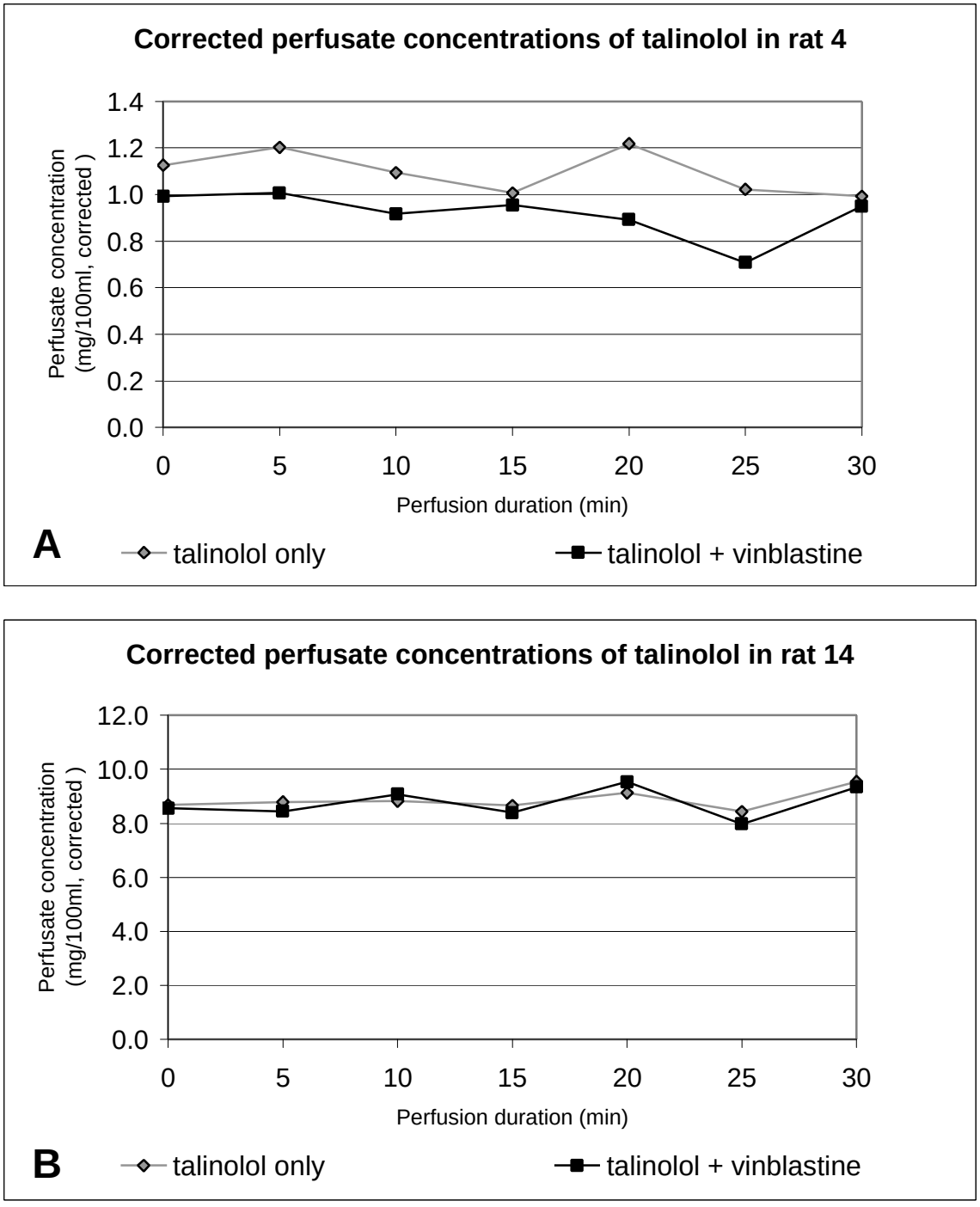


Figure II.5:

Corrected jejunal outflow concentrations of talinolol in single rats with and without co-perfusion of vinblastine as P-gp inhibitor. A: Rat 4 (talinolol perfusion concentration 0.025 mmol/l); B: Rat 14 (talinolol perfusion concentration 0.25 mmol/l).

Figure II.5A shows a distinct difference between the talinolol concentrations in the perfusate with (black line) and without (grey line) vinblastine as P-gp inhibitor.
In contrast in Figure II.5B a difference can not be observed. This matches the expectations, since Figure II.5A points out the perfusion results of a rat, that was perfused with a solution containing only 0.025 mmol/l talinolol, while Figure II.5B displays the results of a rat perfused with a ten times higher concentrated talinolol solution.
In the case of the lower talinolol concentration, P-gp driven secretion of the drug that has already passed the apical membrane of intestinal cells back into the intestinal lumen contributes notably to the overall absorption process, such that an inhibition of the secretion process by adding vinblastine to the perfusion solution leads to a higher total absorption and hence to lower talinolol concentrations in the perfusate.
Contrarily, in case of higher talinolol concentrations in the perfusate, the transporter system responsible for the secretion is saturated, such that the contribution of the secretion process to the overall absorption is neglible and an inhibition of P-gp by vinblastine does not show any remarkable effect on talinolol perfusate concentrations.

In Figure II.6 the effective permeabilities of talinolol in three different intestinal sections (jejunum, ileum and ascending colon) are depicted as function of different perfusion rates. The perfusion rate is the product of the talinolol concentration in the perfusion solution (0.025, 0.05, 0.1, 0.25 and 0.5 mmol/l) and the perfusion flow (0.2 ml/min). Permeabilities are calculated from the perfusate concentrations and are mean values from four or eight rats each.

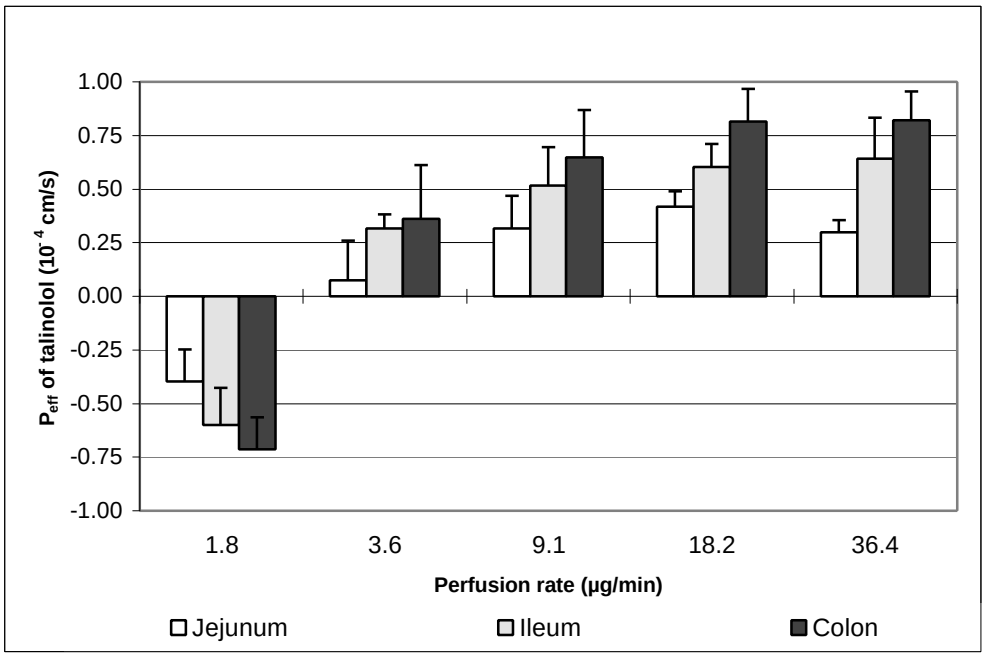


Figure II.6:

Dependence of effective permeabilities of talinolol on the perfusion rate determined by single-pass intestinal perfusion of rat jejunum, ileum and colon (n = 4 - 8).

The figure shows that talinolol permeability is concentration dependent. With increasing talinolol perfusion rates (i.e. an increase in the talinolol concentration in the perfusion fluid) a maximum permeability is approached asymptotically in all segments investigated. At the lowest concentration investigated (0.025 mmol/l), the mean P_{eff} values are negative for all three segments, demonstrating net secretion of talinolol into the intestinal lumen.

Figure II.6 also depicts that total permeabilities of talinolol increase from jejunum to ileum and colon at the higher concentrations where the influence of the efflux process is not as distinct as for the lowest concentration studied. At a perfusion rate of 1.8 µg/min where net secretion is observed in jejunum, ileum, and colon, secretion increases from jejunum to ileum and colon.

II.3.2 Influence of co-administration of P-gp inhibitors on intestinal permeability of talinolol

The effect of vinblastine (100 µmol/l) on the intestinal permeability of talinolol decreases with increasing talinolol concentrations in the perfusion solution. At the lowest concentration examined (0.025 mmol/l) vinblastine addition has a strong influence on talinolol permeability. In jejunum, the effective permeability shifted from $-0.37 \bullet 10^{-4}$cm/s to $-0.03 \bullet 10^{-4}$cm/s after addition of vinblastine. In ileum and colon the shift was even more pronounced ($-0.7 \bullet 10^{-4}$cm/s to $+0.18 \bullet 10^{-4}$cm/s in ileum and $-0.85 \bullet 10^{-4}$cm/s to $+0.11 \bullet 10^{-4}$cm/s in colon). Net secretion was inhibited in all three intestinal segments.

At 0.05 mmol/l the influence of vinblastine is only marginal and at even higher concentrations of talinolol the permeabilities were not influenced relevantly by the addition of vinblastine.

Figure II.7 demonstrates the concentration-dependent effect of vinblastine by outlining the difference values between the effective permeabilities with and without the addition of vinblastine.

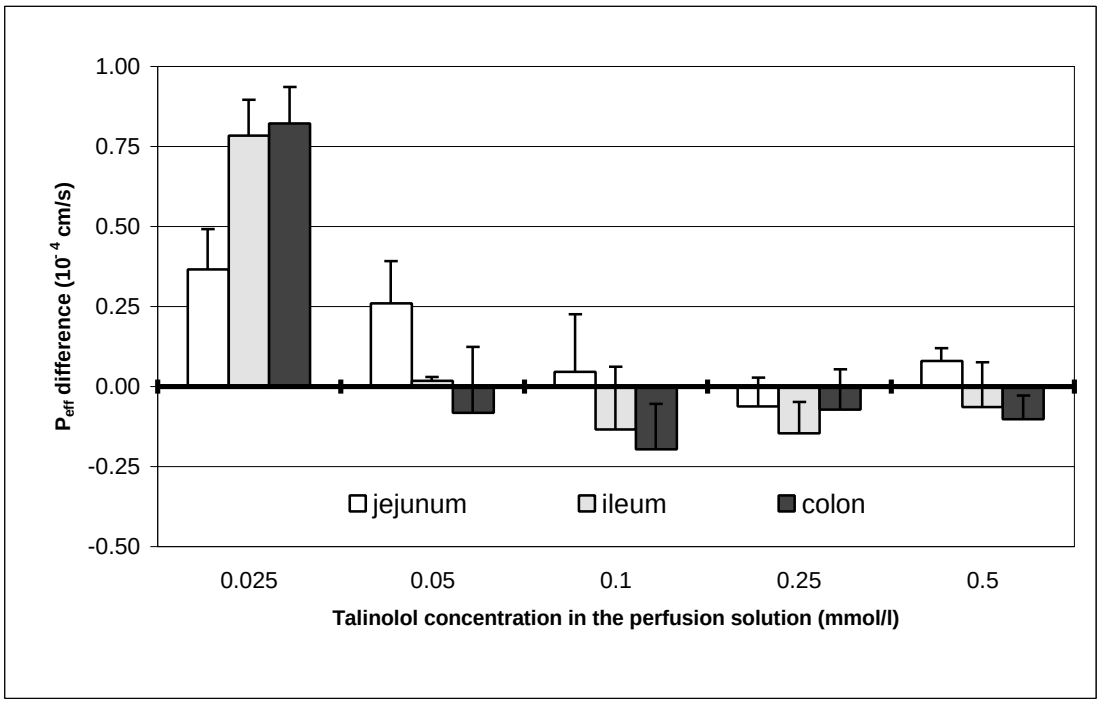


Figure II.7:

The presence of 100 µmol/l vinblastine as P-gp inhibitor in the perfusion solution minimizes the differences in permeability of talinolol between perfusions with and without inhibitor. The effect of vinblastine is clearly dependent on the talinolol concentration in the perfusion solution. Only at low concentrations of talinolol a net secretion is inhibited by the presence of vinblastine, whereas at higher talinolol concentrations the effect of the inhibitor is negligible.

The higher P_{eff} differences at lower talinolol concentrations show evidence for a pronounced effect of vinblastine, while P_{eff} differences close to zero demonstrate no apparent effect of the inhibitor.

Relevant differences between the P_{eff} values with and without inhibitor were only observed at low talinolol concentrations in the perfusate. At higher talinolol concentrations, most probably due to saturation of carrier-mediated secretion by talinolol itself, the effect of vinblastine was negligible.

When rhodamine 123 was added to the perfusion solution as P-gp inhibitor, no significant inhibition was detected, even at low talinolol concentrations. The results are depicted in Figure II.8.

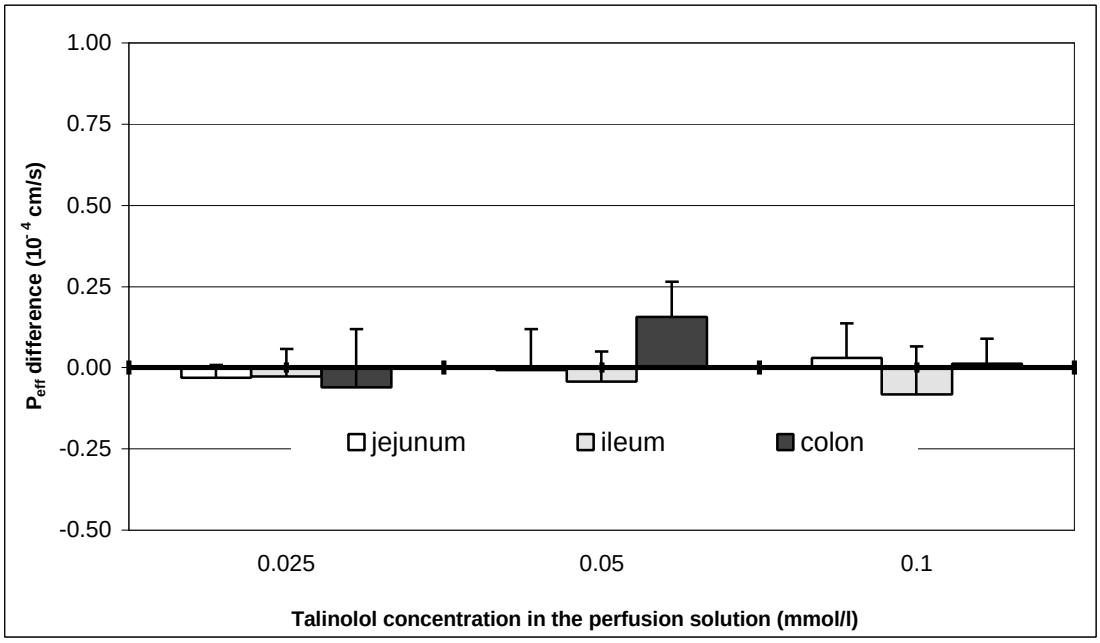


Figure II.8:

The presence of 50 µmol/l rhodamine 123 as P-gp inhibitor in the perfusion solution does not significantly change the differences in permeability of talinolol between perfusions with and without inhibitor.

Co-administration of 50 µmol/l rhodamine 123 did not have a significant influence on talinolol permeabilities, independent on the intestinal region and the talinolol concentration examined. Unlike vinblastine, rhodamine 123 did not inhibit the observed net secretion in the lower talinolol concentration range.

II.3.3 Estimation of parameters for passive and carrier-mediated membrane permeability

The estimates for the carrier-mediated and passive permeability terms for talinolol are given in Table II.2:

Table II.2:

Estimates for the carrier-mediated and passive components of talinolol permeability in different regions of rat intestine using equation 5.

$P_{eff\ passive}$ is the passive permeability, $P_{eff\ max}$ is the maximum transport rate for the carrier-mediated process, K_m is the Michaelis-Menten coefficient, and $P_{eff\ transporter}$ is the drug flux caused by the activity of the transporter, differentiated with respect to the drug concentration c.

	without inhibitor	with vinblastine
Jejunum:		
$P_{eff\ passive}$ [10^4·cm/s]	0.40	0.34
$P_{eff\ max}$ [10^4·cm·l/s·mmol]	0.09	0.09
K_m [mmol/l]	0.02	0.01
$P_{eff\ transporter}$ [10^4·cm/s]		
0.025 mmol/l	0.87	0.66
0.05 mmol/l	0.36	0.21
0.1 mmol/l	0.12	0.06
0.25 mmol/l	0.02	0.01
0.5 mmol/l	0.01	0.00
Ileum:		
$P_{eff\ passive}$ [10^4·cm/s]	0.58	0.80
$P_{eff\ max}$ [10^4·cm·l/s·mmol]	0.24	0.46
K_m [mmol/l]	0.005	0.74
$P_{eff\ transporter}$ [10^4·cm/s]		
0.025 mmol/l	1.27	0.58
0.05 mmol/l	0.37	0.55
0.1 mmol/l	0.10	0.48
0.25 mmol/l	0.02	0.35
0.5 mmol/l	0.00	0.22
Colon:		
$P_{eff\ passive}$ [10^4·cm/s]	0.86	0.80
$P_{eff\ max}$ [10^4·cm·l/s·mmol]	0.17	0.13
K_m [mmol/l]	0.03	0.13
$P_{eff\ transporter}$ [10^4·cm/s]		
0.025 mmol/l	1.72	0.70
0.05 mmol/l	0.81	0.52
0.1 mmol/l	0.31	0.32
0.25 mmol/l	0.07	0.12
0.5 mmol/l	0.02	0.04

In jejunum, for example, at talinolol concentrations of 0.025 mmol/l in the perfusate, the carrier-mediated permeability was approximately twice as high as compared to the passive permeability. When vinblastine was added to the perfusate, a reduction in the contribution of carrier-mediated permeability was noticeable. At 0.1 mmol/l, carrier-mediated secretory permeability of talinolol was merely 1/3 of the absorptive permeability and at 0.5 mmol/l the contribution of the $P_{eff\ transporter}$ to the overall drug flux across the intestine was negligible. These results are in accordance with the predictions of the model as depicted in Figure II.3.

II.4 Discussion and conclusions

The perfusion studies demonstrate that intestinal efflux may have a pronounced influence on drug absorption. Since the efflux system can be saturated, its effect is concentration dependent and may play an important role only at lower substrate concentrations.

Similar to rhodamine 123 an unexpected lack of inhibitor effect was also found for verapamil in human in vivo experiments by Schwarz et al [Schwarz, U. I., T. Gramatté, et al. (1999)] and in in situ intestinal perfusion experiments in rats by Spahn-Langguth et al., although verapamil shows good P-gp inhibiting activity in vitro [Spahn-Langguth, H., G. Baktir, et al. (1998)]. It may be possible that this observation is due to the high inhibitor permeability leading to a rapid absorption of the inhibitor from the perfusate.

For the development of peroral controlled-release dosage forms for a particular drug, it is of considerable importance to be aware of the permeability characteristics of the drug in different segments along the GI tract. For drugs undergoing significant efflux, it is important to realize, whether the contribution of carrier mediated secretion to the overall permeability is similar in the different intestinal segments.

These questions have been addressed for talinolol in a series of experiments using the single-pass perfused rat intestine, as described above.

Compared with P_{eff} values of other ß-adrenoceptor antagonists, the P_{eff} for talinolol is higher than for atenolol (jejunum $0.06 \cdot 10^{-4}$ cm/s, ileum $0.01 \cdot 10^{-4}$ cm/s, colon $0.02 \cdot 10^{-4}$

cm/s), [Fagerholm, U., A. Lindahl, et al. (1997)], which is to be expected. Without the contribution of the secretory carriers the simulations show that talinolol has a higher passive permeability (P_{eff} jejunum $0.40 \cdot 10^{-4}$ cm/s, ileum $0.58 \cdot 10^{-4}$ cm/s, colon $0.86 \cdot 10^{-4}$ cm/s). Considering its physico-chemical properties this behaviour is not unexpected, since the lipophilicity increases from atenolol (log D = -1.8) to talinolol (log D = 0.7) [Fagerholm, U., A. Lindahl, et al. (1997), Narawane, M., S. K. Podder, et al. (1993), Schmidt, J. (1995)].

In in vitro studies with excised intestinal tissues from jejunum, ileum and colon of rats mounted in Ussing-chambers, Ungell et al. [Ungell, A. L., S. Nylander, et al. (1998)] found that permeability coefficients of low permeability drugs with P_{eff} coefficients $<10 \cdot 10^{-6}$ cm/s having negative log D values such as atenolol, decrease in permeability from jejunum to ileum and colon, while an increase in the same order is reported for high permeability drugs ($> 10 \cdot 10^{-6}$ cm/s) with log D values ≥ 0, e.g., for metoprolol and propranolol. The estimation results of the in situ rat perfusion studies presented here correspond well to the in vitro findings of Ungell et al. according to which talinolol would have to be placed into the group of lipophilic/high permeable compounds based on its passive permeability behaviour. However, due to the efficiency of the secretory carrier, this high passive permeability is considerably reduced by efflux mechanisms under non-saturating conditions of the carrier.

Another interesting fact is the tendency towards an increase in P-gp-mediated talinolol secretion in more distal intestinal segments. At the lowest perfusate concentration studied, the $P_{eff\ transporter}$ increased from $0.87 \cdot 10^{-4}$ cm/s in jejunum to $1.27 \cdot 10^{-4}$ cm/s in ileum and $1.72 \cdot 10^{-4}$ cm/s in colon. This might indicate either increased expression levels of P-glycoprotein or alternatively higher activity of secretory carriers in the lower GI-tract.

This observation also implicates that targeting the release of drugs with affinity to P-gp from their dosage form to the lower GI tract, in order to avoid P-gp-mediated secretion, will probably not become a successful strategy for bioavailability enhancement.

A newer source for drug-drug interactions that has been reported a few times is that a concomitant administration of two drugs with affinity to P-glycoprotein can modify the pharmacokinetics of one or both drugs [Wakasugi, H., I. Yano, et al. (1998), Yu,

D. K. (1999), Greiner, B., M. Eichelbaum, et al. (1999), Westphal, K., A. Weinbrenner, et al. (2000), Johne, A., J. Brockmoller, et al. (1999)].

The rat studies support these findings and suggest a potential interaction between vinblastine and talinolol, which might also have a certain clinical relevance.

Chapter III

Chapter III

Development of peroral talinolol controlled-release dosage forms

III.1 Introduction

For acute diseases in most cases the onset of drug action is required to be rapid. In the case of peroral administration for systemic treatment a fast dissolution of the drug from the tablet or capsule is the first prerequisite. Such drug products are termed immediate release (IR) dosage forms.
Drug products in this category include soft gelatin capsules which are filled with solutions or dispersions to ensure rapid drug dissolution. For tablets, disintegration and dissolution rates can be enhanced by the addition of starch, modified starches, polyvinyl pyrrolidon (PVP) or a combination of carbonates with weak acids, e.g. citric acid or tartaric acid [Hartke, K. et al. (2002)].

On the other hand, in several cases rapid dissolution rates may *not* be desirable. For the treatment of many chronic diseases plasma drug levels should be maintained constant. This is hardly achievable with immediate-release dosage forms, except for the cases when the half-life of the drug itself is long.
In all other cases drug elimination leads to a more or less rapid decrease of the plasma levels of the drug and hence a drop below the minimum therapeutic concentration. In order to compensate for the eliminated drug, another dosage form has to be administered if drug action shall endure. At the same time this procedure includes the risk of entering the toxic concentration range and it can result in accumulated plasma peaks instead of constant plasma levels as outlined in Figure III.1.
In order to avoid these fluctuations and to minimize the frequency of dosage form intake for the patient, sustained-release dosage forms have been developed. They release the drug continuously, resulting in more constant plasma levels.

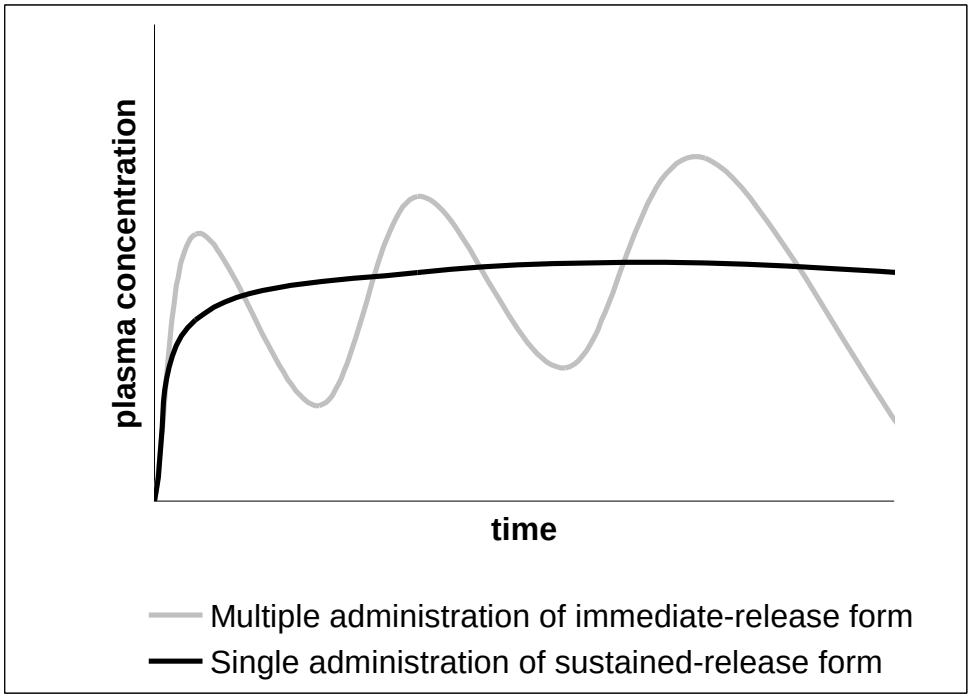


Figure III.1:

Schematic drawing of plasma concentration-versus-time profiles following administration of three immediate-release dosage forms versus one single sustained-release dosage form.

The terms controlled-release (CR) and modified-release dosage forms describe drug products with a modified rate and / or place of drug release compared to immediate-release products.
In addition to the sustained-release profile described above, this includes delayed-release and pulsatile-release profiles, which are depicted in Figure III.2.
For sustained-release dosage forms the terms extended-release, prolonged-release and slow-release dosage forms are also common. Sometimes the term repeat-action-release is used instead of pulsatile-release [Hartke, K. et al. (2002)].

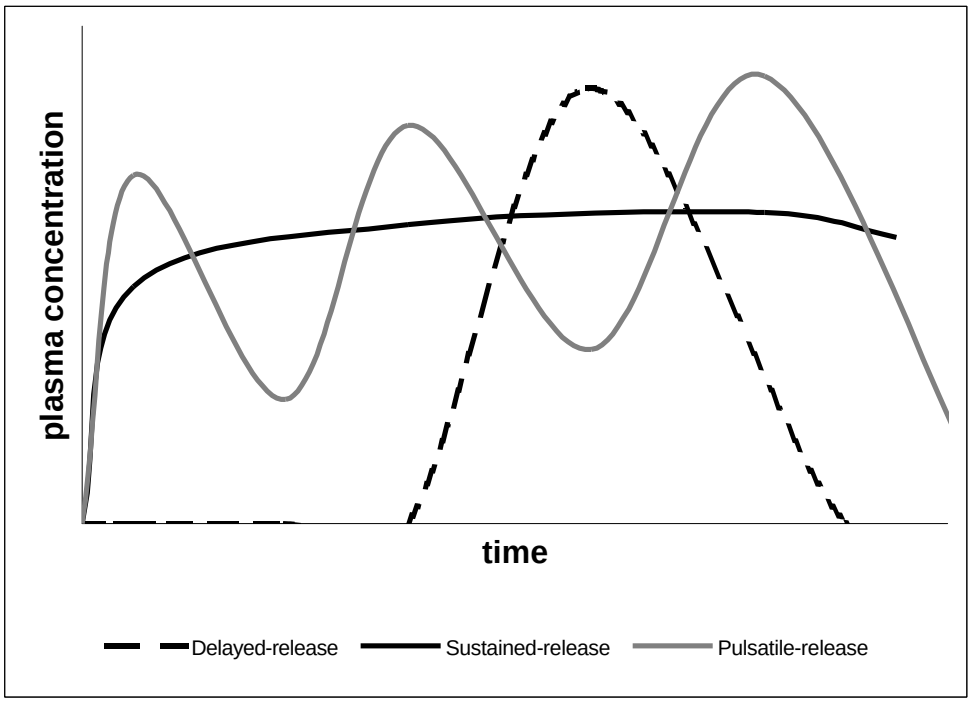


Figure III.2:

Schematic drawing of plasma concentration-versus-time profiles following administration of different types of controlled-release dosage forms: delayed, sustained and pulsatile-release.

A delayed-release profile is for example typical for enteric-coated tablets, that do not release drug within the stomach. Following stomach emptying, they dissolve rapidly in the small intestine. Such a profile is desirable when drugs are instable in the acidic gastric fluid, when they irritate the stomach mucosa or when a local drug action in a certain part of the intestine shall be attained.

Sustained-release dosage forms are useful to maintain drug concentrations in plasma above the minimum effective concentration over a prolonged period of time. In an ideal case, the plasma concentration-versus-time profile following administration of a sustained-release dosage form is similar to the one during an infusion.

In some cases, however, a constant plasma level is not desirable, e.g. when a circadian rhythm has to be imitated. In theses cases, a CR dosage form with a pulsatile-release profile can help to minimize the frequency of drug intake and thus significantly improve the compliance.

III.1.1 The need of a controlled-release dosage form for talinolol

The model compound talinolol is available on the German market as film-coated immediate-release tablets containing 50 or 100 mg of the drug (Cordanum 50 and 100 Filmtabletten) and in 5 ml vials filled with a solution for injection containing 10 mg talinolol (Cordanum Injektionslösung).

A controlled-release dosage form is not available, because talinolol has a mean elimination half life of 11.9 hours [Trausch, B., R. Oertel, et al. (1995)], such that once or twice daily dosing of the IR tablets is sufficient to maintain the plasma concentrations within the therapeutic range.

The recommended daily dosage ranges from 50 to 300 mg, which can be administered either completely in the morning or subdivided to half or three third of the dose in the morning and the residual dose in the evening.

Although no therapeutic benefit for a talinolol controlled-release dosage form is to be expected, its development is useful, in order to investigate the influence of the drug release rate from a dosage form on the intestinal secretion mediated by P-glycoprotein (P-gp) or other secretory transporters.

As discussed more extensively in chapter I, the phenomenon also described as intestinal drug efflux, may influence the fraction of drug absorbed (F_a) and hence the bioavailability (BA).

Since quite a large number of drugs have been reported to be substrate to P-gp, e.g. numerous anti cancer drugs, aldosterone, atorvastatin, celiprolol, cimetidine, cortisole, cyclosporine A, dexamethasone, digoxin, domperidone, fexofenadine, ketoconazole, loperamide, morphine, ondansetron, phenytoin, ranitidine, salbutamol trospium and verapamil, it is important for the development of controlled-release dosage forms for such drugs to know how and how far changes in the drug release from a dosage form may influence the carrier-mediated secretion and consequently the bioavailability of these drugs.

Talinolol is employed as model drug, since it is proven to be substrate to intestinal secretory transporters and its biotransformation is negligible (< 1 % of the administered dose) [Spahn-Langguth, H., G. Baktir, et al. (1998), Trausch, B., R. Oertel, et al. (1995)]. The neglible biotransformation reduces the number of unknown factors with impact on the talinolol blood concentration, such that the factors intestinal

secretion and drug release from the dosage form do not interfere with the factor biotransformation. This is of particular importance since gut metabolism in the intestine mediated by the cytochrome P 450 isoform CYP3A4 often interferes with the intestinal secretion mediated by P-gp, because many drugs metabolized by CYP3A4 are also substrates for P-gp and have the same inducers and inhibitors [Schuetz, E. G., A. H. Schinkel, et al. (1996), Wacher, V. J., C. Y. Wu, et al. (1995)].

The aim of this project was not the development of a CR dosage form for one specific drug which could eventually be brought to the market. Rather, the impact of the drug release rate on intestinal secretion and absorption for P-gp substrates in general should be studied.

The target release rate for the peroral talinolol sustained-release dosage form to be developed was a continuous drug release for approximately eight hours, which should be as much as possible independent of the pH value of the dissolution medium.

III.1.2 Technological aspects

For the development of peroral controlled-release dosage forms a number of different manufacturing methods can be used. Most common dosage forms are tablets and capsules.

As for tablets standard procedures to modify the drug release are coatings [Rhodes, C. T. and S. C. Porter (1998), Fan, T. Y., S. L. Wei, et al. (2001), Leopold, C. S. and D. Eikeler (1998), Parikh, N. H., S. C. Porter, et al. (1993)], multi-layer or core tablets consisting of different powder mixtures [al-Gohary, O. M. and R. S. al-Kassas (2000), Bettini, R., D. Acerbi, et al. (2002), Conte, U. and L. Maggi (1996), Genc, L., H. Bilac, et al. (1999), Krishnaiah, Y. S., R. S. Karthikeyan, et al. (2002), Streubel, A., J. Siepmann, et al. (2000)], matrix systems [Boza, A., I. Caraballo, et al. (1999), Jovanovic, M., G. Jovicic, et al. (1997), Neau, S. H., M. A. Howard, et al. (1999), Sanchez-Lafuente, C., M. Teresa Faucci, et al. (2002)] or oral osmotic systems (OROS) [(Godbillon, J., A. Gerardin, et al. (1985), Good, W., L. J. Leeson, et al. (1985), McInnes, G. T. and M. J. Brodie (1988)].

Hard gelatin capsules can also be coated, but more frequently dissolution is modified by filling hard gelatin capsules with coated or matrix granules and pellets or even small CR tablets. By using mixtures of different filling materials, particular controlled-release profiles can be achieved. Soft gelatin capsules can also be coated but more frequently they are filled with certain liquid or semisolid preparations which lead to a controlled drug release [Bonny, J. D. and M. Kyowa (1995), Edsbacker, S., P. Larsson, et al. (2002), Govender, T., C. M. Dangor, et al. (1997), Green, J. R., A. J. Lobo, et al. (2001), Mitrevej, A., N. Sinchaipanid, et al. (2001), Tu, J., L. Wang, et al. (2001)].

In addition, microspheres and -capsules [Benita, S., A. Hoffman, et al. (1985), Morishita, I., M. Morishita, et al. (1991), Zaghloul, A. A., J. Faltinek, et al. (2001), Zaghloul, A. A., S. R. Vaithiyalingam, et al. (2001)], nanoparticles and -capsules [Friese, A., E. Seiller, et al. (2000), Romero-Cano, M. S. and B. Vincent (2002), Schwarz, C. and W. Mehnert (1999), zur Muhlen, A., C. Schwarz, et al. (1998)] and liposomes [Patel, H. M. (1985), Stenekes, R. J., A. E. Loebis, et al. (2001), Yasui, K., H. Fujioka, et al. (1995)] have been developed to achieve a controlled drug release.

A key factor in the development of controlled-release dosage forms is the choice of release-modifying excipients. A review on controlled-release drug products on the German market showed that various types of Eudragit® (different copolymers of acrylic and methacrylic acid) and ethylcellulose are used most frequently [Arno, E. A., P. Anand, et al. (2002), Fan, T. Y., S. L. Wei, et al. (2001), Ghaly, E. S., J. I. Hernandez, et al. (1992), Lin, K. H., S. Y. Lin, et al. (2001), Lin, S. Y., K. H. Lin, et al. (2001), Majid Khan, G. and J. B. Zhu (1998), Morishita, I., M. Morishita, et al. (1991), Neau, S. H., M. A. Howard, et al. (1999), Sanchez-Lafuente, C., M. Teresa Faucci, et al. (2002), Saravanan, M., K. S. Nataraj, et al. (2002), Zaghloul, A. A., J. Faltinek, et al. (2001), Zaghloul, A. A., S. R. Vaithiyalingam, et al. (2001)].

Therefore, these excipients were used for the development of a controlled-release formulation of talinolol. The choices of the dosage form and the manufacturing process were primarily determined by availability of equipment and practicability of the manufacturing process with regard to GMP conformity. It was decided to focus on coated and matrix granules as filling material for hard gelatin capsules and secondly on matrix tablets.

III.1.3 Properties of excipients used in the formulation of sustained-release dosage forms for talinolol

In the following section, properties of the excipients used to sustain the release rate of talinolol from tablet and capsule formulations are composed [Eudragit® technical bulletin, Röhm (Darmstadt, Germany), Ethocel® product information, Dow (Midland, MI, USA)]:

III.1.3.1 Eudragit polymers

Eudragit® polymers are copolymers of acrylic and methacrylic acid or their esters. They all have the same basic structure, as shown in Figure III.3. Their specific properties are determined by different functional groups R1 to R3 and different ratios acrylic to methacrylic acid on the one hand, and free acids to esters on the other hand.

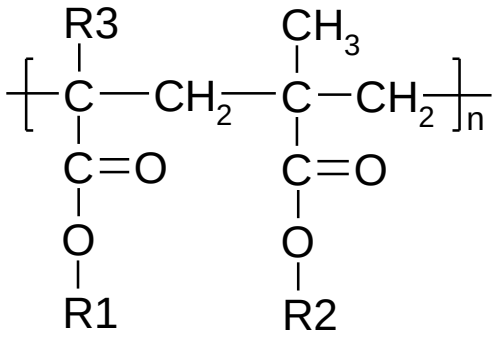
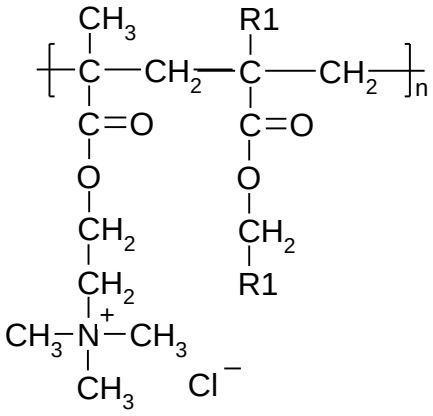


Figure III.3:
Basic structure of Eudragit® polymers.

Eudragit® RS 12.5 (USP/NF)

Eudragit® RS 12.5 is a 12.5 % organic solution of a copolymer of acrylate and methacrylate with 4 to 7 % of quaternary ammonium groups.

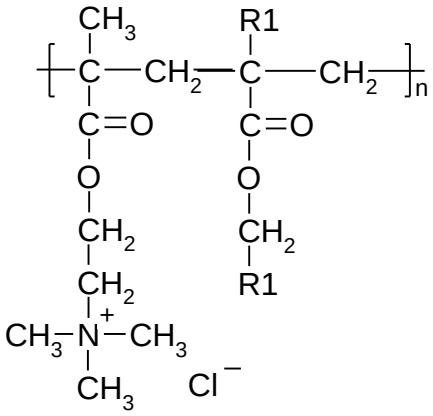


R = H or CH_3

The polymer is insoluble in water, independent of the pH of the solution. Drug release is enabled by swelling of the polymer which enables drugs incorporated in formulations with Eudragit® RS 12.5 to diffuse through the swollen matrix.

Eudragit® RSPO (USP/NF, JPE)

Eudragit® RSPO is also a copolymer of acrylate and methacrylate with 4 to 7 % of quaternary ammonium groups. The chemical structure is identical to Eudragit® RS 12.5, but it is supplied in powder form instead of an organic solution in order to be used for directly compressible matrix formulations.

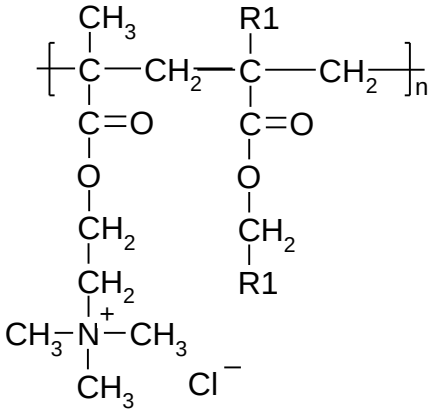


R = H or CH_3

The polymer is insoluble in water, independent of the pH of the solution, but it swells and enables incorporated drugs to be released from the formulation by means of diffusion through the swollen matrix.

Eudragit® RLPO (USP/NF, JPE)

Eudragit® RLPO is a copolymer of acrylate and methacrylate with the same chemical structure as Eudragit® RSPO, but a higher content of quaternary ammonium groups (9 to 12 %).

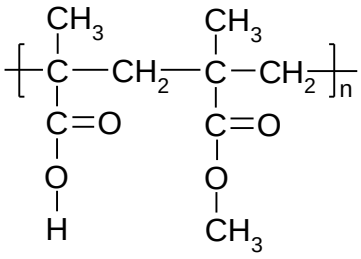


R = H or CH_3

The polymer is insoluble in water, independent of the pH of the solution. Due to the higher content of quaternary ammonium groups it swells more easily than Eudragit® RSPO, which leads to a faster drug release. It is supplied in powder form to be employed in matrix formulations.

Eudragit® L 12.5 (Ph. Eur., USP/NF)

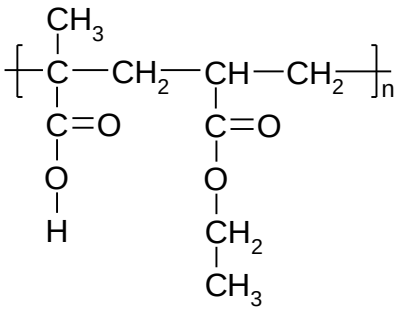
Eudragit® L 12.5 is a 12.5 % organic solution of a copolymer of methacrylic acid and methyl methacrylate.



The ratio of the free carboxyl groups to the ester groups is approximately 1:1.
The free carboxyl groups lead to a solubility above a pH value of 6.0. The polymer can be used for enteric-coated dosage forms.

Eudragit® L 100-55 (Ph. Eur., USP/NF)

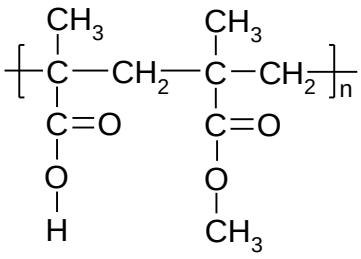
Eudragit® L 100-55 is an anionic copolymer of methacrylic acid and ethyl acrylate.



The ratio of the free carboxyl groups to the ester groups is approximately 1:1.
Due to the altered chemical structure compared to Eudragit® L 12.5 (ethyl acrylate copolymer instead of methyl methacrylate coplymer), the polymer starts to dissolve in water already at pH values of 5.5 and above. It is supplied in spray-dried powder form and can be used for enteric coatings and a targeted delivery in the duodenum or jejunum.

Eudragit® S 100 (Ph. Eur., USP/NF, JPE)

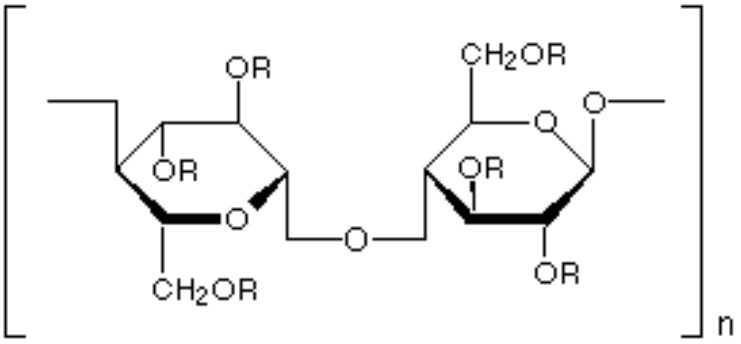
Eudragit® S 100 is an anionic copolymer of methacrylic acid and methyl methacrylate with free carboxyl groups in powder form.



The ratio of the free carboxyl groups to the ester groups is approximately 1:2.
The polymer is soluble in water above pH values of 6.0, but due to the lower content of free carboxyl groups, it dissolves less rapidly than Eudragit® L 12.5 and Eudragit® L 100-55. It can be used for a targeted delivery in the ileum or colon. The site of drug release and the release-rate of drug from the dosage form can be altered by a combination with different Eudragit® L types.

III.1.3.2 Ethocel® (Ph. Eur., USP/NF, JPE)

Ethocel® is ethylcellulose in granule form to be used in controlled-release matrix formulations.



R = H or C_2H_5

The polymer is available in different qualities with different particle sizes. It is insoluble in water, but soluble in ethanol, isopropanol, acetone, methylene chloride and several other organic solvents.

III.2 Materials and methods

III.2.1 Chemicals and other materials

Talinolol was a gift from AWD Pharma (Dresden, Germany). Its purity was determined as 100.1 %. The loss on drying was 0.1 %. The particle size did not exceed 103 µm, 90 % of the particles were smaller than 71 µm. The substance complied in all parameters with the demands of the specifications of the manufacturer (AWD Pharma, Dresden).
The different Eudragit® polymers were received as free samples from Röhm (Darmstadt, Germany), Ethocel® was received as free sample from Dow (Midland, USA), Lipoxol 4000 (polyethylene glycol 4000) was obtained from Sasol (Marl, Germany). Theophylline monohydrate, magnesium stearate, corn starch and mannitol were purchased in Ph.Eur. quality from Caelo (Hilden, Germany). Buffer salts and other chemicals were ordered at Merck (Darmstadt, Germany) and were at least of p.a. grade. The pH test sticks were also received from Merck (Darmstadt, Germany). Empty hard gelatin capsules were ordered from Wepa (Hillscheid, Germany).

III.2.2 Preparation of sustained-release matrix granules

Sustained-release granules were produced by premixing theophylline or talinolol with the particular excipients in a 1000 ml beaker with a stainless steel spoon. After a sufficient amount of liquid was added to the mixing chamber to perform a wet granulation, mixing was continued in an IKA paddle mixer LR 250 (IKA, Staufen, Germany) for 20 minutes. Unless stated otherwise in the results section, the granulation liquid was water. The mixing rate of approximately 120 rpm fluctuated since the viscosity within the chamber varied during the mixing process. The mixture was pressed through a sieve with a mesh size of 2000 µm and subsequently dried in a Binder FED 53 oven (WTB Binder, Tuttlingen, Germany) at 50°C for 24 hours. To destroy larger agglomerates, the dry granules were sieved again through a 2000 µm sieve. To remove the fine powder fraction, a 355 µm sieve was used subsequently.

The bulk densities of the ready-to-fill granules were determined in a 250 ml glass cylinder. The granules were filled into hard gelatin capsules of the sizes 000 (filling volume: 1.37 ml), 0 (0.68 ml) or 3 (0.30 ml) using an Aponorm capsule filling and closure device (Wepa, Hillscheid, Germany) equipped with the suitable inlays for the different capsule sizes. The filling machine allowed to fill 60 capsules at a time. For those instances when less capsules had to be prepared, the superfluous holes in the upper inlay of the apparatus were sealed with adhesive tape.

A formulation number (Form no.) consisiting of 6 numbers and one letter was used to distinguish between different granule formulations. The number refers to the date on which the particular formulation was manufactured for the first time. The first two digits represent the year, the second two digits represent the month plus 1 and the third two digits the manufacturing day plus two for a particular formulation. The following letter is used to distinguish between different formulations manufactured on the same day (e.g.: A refers to the first formulation manufactured at a specific day, B to the second, C to the third formulation, etc.).

III.2.3 Preparation of coated sustained-release granules

To sustain the release rate of talinolol from capsules filled with modified-release granules, granules were film coated with Eudragit® L 12.5.

The organic solution was sprayed onto the granules with a twin fluid spraying nozzle connected to a flexible-tube pump operating with compressed air at a pressure of 1.0 bar (atmosphere gauge ATG) in a copper dragée pan (Erweka, Heusenstamm, Germany) running at a rotational speed of 20 rpm. To accelerate the evaporation of the liquid and to favour the formation of a film around the granules, warm air was blown into the pan.

Each spraying phase (10 to 25 seconds) was followed by a drying phase lasting until the granules ceased sticking together. During the drying phase (1 to 10 minutes) no new polymer was applied to the granule particles. To deagglomerate agglomerated granules, the rotational speed was occasionally increased up to 35 rpm during the drying periods.

The thickness of the film-layer on the granules was varied by changing the ratio between the amount of granule in the pan and the amount of polymer solution or dispersion sprayed onto the granule.
After termination of the spraying process the granules were dried in a Binder FED 53 oven (WTB Binder, Tuttlingen, Germany) at 50°C for 24 hours. The bulk density of the ready-to-fill granules was determined in a 250 ml glass cylinder.
The coated granules were labeled according to the formulation number format described for the matrix granules.

III.2.4 Preparation of sustained-release matrix tablets

Sustained-release matrix tablets were produced by mixing talinolol drug substance with different amounts of the particular excipients in a mortar for five minutes before grinding the mixture to a fine powder with a pestle for another five minutes. The appropriate amount of this powder mixture required to obtain a tablet containing 100 or 200 mg of active drug substance was weighed in 50 ml glass containers on a Mettler AG 285 scale (Mettler Toledo, Greifensee, Switzerland) and then filled into the 13 mm die of a PW 20 GS manual tablet press (Paul Weber, Remshalden-Grumbach, Germany). In those instances when the powders tended to stick to the punch and die, a fine layer of magnesium stearate was applied with a small brush.
Unless stated otherwise, tablets were compressed with a compression force of 40 kN applied for a duration of 30 seconds or 2 minutes in different test series. The tablets were stored in 50 ml plastic containers with screw caps and labeled with the formulation number according to the format described for the matrix granules.

III.2.5 Pharmaceutical characterization

III.2.5.1 Dissolution

In vitro dissolution was tested using “Apparatus 2” of the United States Pharmacopeia (USP) [USP, 23rd edition, <711> “Dissolution”] which is identical to the “Paddle Apparatus” of the European Pharmacopeia (Ph.Eur.) [Ph.Eur., 3rd edition, <2.9.3> “Drug Release From Solid Dosage Forms”]. A Pharma Test PTWS III (Pharma Test Apparatebau, Hainburg, Germany) and an Erweka DT7R (Erweka GmbH, Heusenstamm, Germany) dissolution tester were used.

The apparatuses consist of six glass vessels having a volume of 1000 ml each immersed in a water bath at 37°C ± 0.5°. Stirrers in paddle form are provided as part of the dissolution tester. One (Erweka equipment) or two (Pharma Test equipment) additional glass vessels are provided in order to appropriately heat the dissolution fluids needed to replace the volumes of the samples collected. Both dissolution testers were equipped with Hanson Research Dissoette II automatic samplers.

Each dissolution vessel was filled with 1000 ml of the respective dissolution medium. Rotational speeds of the paddles were 50 or 100 min^{-1} in various phases of product testing. Results are given as mean values of three vessels unless stated otherwise. Standard deviations are outlined in the dissolution profile figures. Occasionally, they are not visible in the figures since they are smaller than the icons of the particular dissolution curves.

For capsules and 100 mg tablets sinkers were used to maintain the dosage forms at the bottom of the dissolution test vessels and prevent their floating. Sinkers were prepared by bowing stainless steel wire (3 cm of length and 1 mm of thickness) in a shape that the particular dosage form could be fixed within.

5 ml samples were collected at the following sampling times: 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h and 24 h. The sample volumes were replaced by pure buffer. At the end of a dissolution test series, the final pH value in each dissolution vessel was measured by means of pH quick test sticks that allow to display differences of 0.2 units in pH.

In no case changes in pH of the dissolution fluids compared to the initial buffers were detected. This may be due to the fact that the concentrations of theophylline and talinolol were generally too low to exceed the buffer capacities.

III.2.5.1.1 Assays

Talinolol and theophylline concentrations were analysed by means of UV spectroscopy using a Lamda 20 UV-VIS spectrophotometer (Perkin Elmer, Überlingen, Germany) at a wavelength of 240 nm for talinolol and 270 nm for theophylline. The samples were diluted such that their absorbances were in the linear range of the Lambert-Beer law.

For talinolol, calibration curves with concentrations from 2 to 20 mg/l were recorded in all different dissolution media employed. The differences between calibration curves obtained from the different media were only marginal. This is displayed in Figure III.4. All curves showed linearity in the concentration range investigated with a R^2 value (coefficient of determination) of at least 0.999.

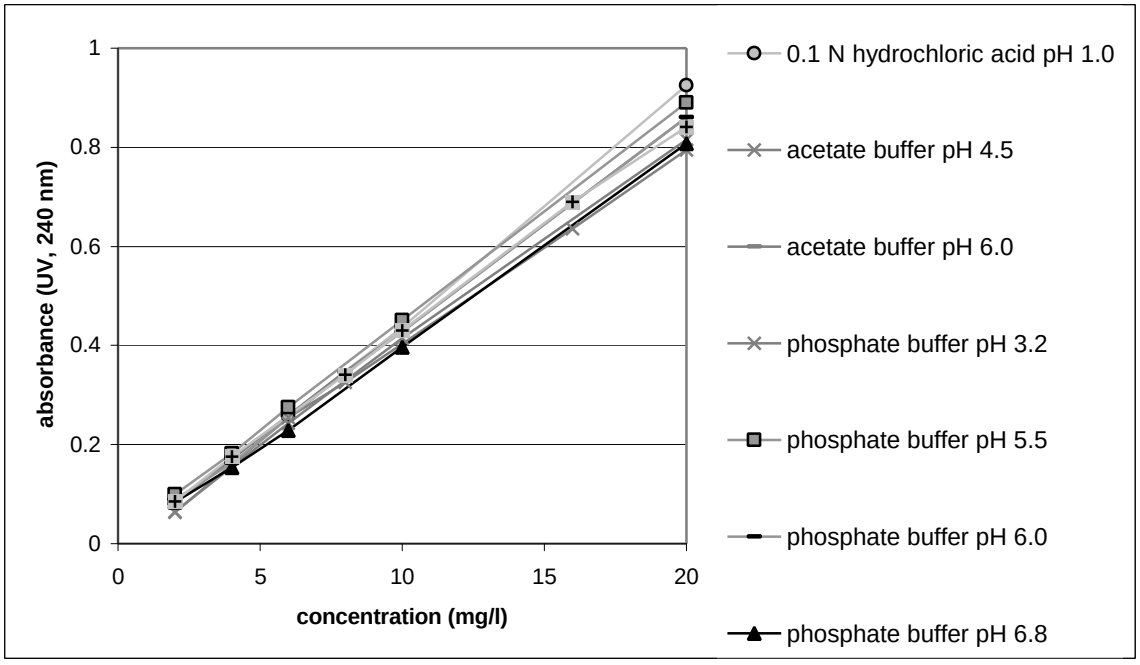


Figure III.4:

Calibration curves for the UV assay of talinolol in different dissolution media in the concentration range between 2 and 20 mg/l.

III.2.5.1.2 Dissolution media

The most frequently used dissolution media were 0.1 N hydrochloric acid (pH 1.0) to mimic the gastric pH in the fasted state, acetate buffer pH 4.5 USP to mimic the pH value in the upper small intestine and phosphate buffer pH 6.8 USP for the imitation of lower small intestinal regions [Efentakis, M. and J. B. Dressman (1998), Russell, T. L., R. R. Berardi, et al. (1993), Youngberg, C. A., R. R. Berardi, et al. (1987)]. To test the drug release at intermediate pH values phosphate buffers with pH values of 3.2, 5.5 and 6.0 were used. Additionally, acetate buffer pH 6.0 and citrate buffer pH 6.8 were employed to test the influence of different buffer salts on the drug dissolution from talinolol dosage forms.

A more detailed discussion on the importance of a careful selection of appropriate media for dissolution testing is presented in chapter IV.

The composition of the buffers used as media in dissolution tests with various talinolol formulations is given in Table III.1.

Table III.1:

Composition of buffered dissolution media deployed to test the drug release from talinolol dosage forms.

Phosphate buffers
pH 3.2: 4 g/l sodium dihydrogen phosphate, 2.5 g/l phosphoric acid.
pH 5.5: 13.1 g/l potassium dihydrogen phosphate, 1.29 g/l sodium monohydrogen phosphate
pH 6.0: 6.8 g/l potassium dihydrogen phosphate, 0.23 g/l sodium hydroxide solution
pH 6.8: 6.8 g/l potassium dihydrogen phosphate, 0.90 g/l sodium hydroxide

Acetate buffers
pH 4.5: 2.99 g/l sodium acetate•$3H_2O$, 1.66 g/l acetic acid
pH 6.0: 10 g/l ammonium acetate, 0.41 ml/l acetic acid

Citrate buffer
pH 6.8: 1.09 g/l citric acid, 0.60 g/l sodium hydroxide.

III.2.5.1.3 Stability of talinolol in dissolution media

The stability of talinolol in various dissolution media was tested by remeasuring calibration solutions containing 4, 6, 10 and 20 mg/l talinolol following their storage in tightly closed, colourless glass flasks at room temperature and normal day light over a time period up to six weeks. The results are presented in Table III.2:

Table III.2:

Stability of talinolol solutions in different dissolution media.

0.1 N HCl	Absorbance						
conc. (mg/l)	at time 0	after 2 weeks	shift (%)	after 5 weeks	shift (%)	after 6 weeks	shift (%)
4	0.1730	0.1836	6.1	0.1655	-4.3	0.1718	-0.7
6	0.2582	0.2702	4.6	0.2531	-2.0	0.2565	-0.7
10	0.4363	0.4466	2.4	0.4283	-1.8	0.4331	-0.7
20	0.9252	0.9341	1.0	0.9168	-0.9	0.9208	-0.5

phosphate buffer pH 3.2	Absorbance				
conc. (mg/l)	at time 0	after 2 weeks	shift (%)	after 6 weeks	shift (%)
4	0.1505	0.1462	-2.9	0.1476	-1.9
6	0.2433	0.2337	-3.9	0.2315	-4.8
10	0.4153	0.4113	-1.0	0.4116	-0.9
20	0.8157	0.8468	3.8	0.8480	4.0

phosphate buffer pH 5.5	Absorbance						
conc. (mg/l)	at time 0	after 2 weeks	shift (%)	after 4 weeks	shift (%)	after 5 weeks	shift (%)
4	0.1738	0.1883	8.3	0.1668	-4.0	0.1630	-6.2
6	0.2574	0.2823	9.7	0.2582	0.3	0.2486	-3.4
10	0.4454	0.4604	3.4	0.4386	-1.5	0.4240	-4.8
20	0.8816	0.8988	2.0	0.8834	0.2	0.8624	-2.2

phosphate buffer pH 6.8	Absorbance				
conc. (mg/l)	at time 0	after 2 weeks	shift (%)	after 6 weeks	shift (%)
4	0.1537	0.1526	-0.7	0.1585	3.1
6	0.2293	0.2392	4.3	0.2382	3.9
10	0.3972	0.3911	-1.5	0.3986	0.4
20	0.8076	0.8154	1.0	0.8059	-0.2

citrate buffer pH 6.8	Absorbance		
conc. (mg/l)	at time 0	after 2 weeks	shift (%)
4	0.1754	0.1777	1.3
6	0.3417	0.3425	0.2
10	0.4306	0.4334	0.7
20	0.8412	0.8403	-0.1

All solutions showed a sufficient stability for the performance of dissolution tests and the use of automatic samplers. Deviations did not exceed ten percent, even at storage times of six weeks. This allows samples drawn automatically overnight to be analysed the next day. All samples were measured within 48 hours, a time period in which considerable degradation could not beobserved.

III.2.5.2 Uniformity of mass and content

Uniformity of tablet and capsule mass and uniformity of drug content were determined according to the methods described in the European Pharmacopeia [Ph.Eur., 3rd edition, <2.9.5>: “Uniformity of mass of single dose preparations” and <2.9.6>: “Uniformity of content of single dose preparations”]. For the uniformity of mass tests, capsules were weighed on a Mettler scale model AG 285 (Mettler Toledo, Greifensee, Switzerland). For the content uniformity tests, drug contents in the capsules were determined by means of UV spectroscopy as described in the dissolution section.

III.2.5.3 Size of tablets

The diameter of all tablets was predefined by the punch and die of the tablet press (13 mm). Tablet thickness varied depending on the compression force and the amount of powder compressed. For the final tablet formulation designated for an in vivo evaluation, the thickness of ten randomly selected matrix tablets was measured with a micrometer (Mitutoyo M 320-25A, Kawasaki, Japan). The mean value and standard deviation were calculated.

III.2.5.4 Crushing strength

The matrix tablets that matched the projected drug release profiles, were characterized in terms of their crushing strength using a Schleuniger tablet crushing strength tester model 6D (Schleuniger - Pharmatron AG, Solothurn, Switzerland). The test was performed with twelve tablets. The mean value and the standard deviation were calculated.

III.2.5.5 Friability

Friability is defined as the loss of mass during the rotation in the test apparatus expressed as percentage of the initial mass. Friability of tablets was tested using a Pharma Test friability tester PTF R (Pharma Test Apparatebau, Hainburg, Germany) complying with the specifications of the European Pharmacopeia. The test was performed according to the method described in the Ph.Eur. [Ph.Eur., 3rd edition, <2.9.7>: “Friablity of uncoated tablets”]. The test was performed three times with 20 tablets each. Only the final tablet formulation that showed appropriate drug release profiles was tested.

III.2.6 Statistical analysis of dissolution data

To investigate the mechanism of drug release, dissolution data of the final formulation was fitted to the square root of time relationship published by Higuchi and the equation of Ritger and Peppas [Higuchi, T. (1963), Ritger, P. L. and N. A. Peppas (1984)]. Both relationships are commonly used to describe drug release kinetics from matrix formulations and to detect release mechanisms, such as diffusion-, erosion-, or swelling-controlled drug release.

Higuchi: $Q = K \bullet t^{½}$

(Q: cumulative drug release from matrix tablets, K: dissolution rate constant, t: time)

Ritger and Peppas: $M_t / M_i = K \bullet t^n$

(M_t: amount of drug released at time t, M_i: amount of drug released at infinity K: release rate constant, n: diffusional exponent, characteristic for the drug release mechanism)

Linear and non-linear regression analysis (Higuchi and Ritger Plots) of the drug release profiles was performed using Prism, version 3.0 (GraphPad Software Inc., San Diego, USA).

III.3 Results and discussion

III.3.1 Granules and hard gelatin capsules

III.3.1.1 Determination of the appropriate capsule size based on bulk density measurements

These tests were performed in a preliminary study with theophylline as active agent instead of talinolol to define the capsule size suitable to accommodate a drug content of 100 or 200 mg per capsule. Theophylline was chosen in replacement of talinolol because it was easy accessible in larger amounts.

The following theophylline granule formulation was prepared:

Eudragit® RSPO	15.0 g
Theophylline monohydrate	60.0 g
Calcium hydrogen phosphate dihydrate	24.3 g
Magnesium stearate	0.7 g

Form. no. 001030A

The bulk density was determined as 520 mg/ml such that 1 ml of the granule refers to 312 mg theophylline since the content of drug in the formulation is 60 %. The granule was filled in capsules of size 3 with a volume of 0.30 ml, resulting in an estimated drug content of 94 mg per capsule. The actual content was determined for 6 capsules spectrophotometrically at a wavelength of 270 nm after the capsule was stirred for 24 hours in 0.1 N HCl on a magnet stirrer to release the drug as completely as possible. The *measured* drug contents were 80.5 mg, 81.4 mg, 80.1 mg, 79.4 mg, 81.8 mg, and 80.8 mg with a mean of 80.7 mg and a standard deviation (SD) of 0.86.
This meant a deviation of the mean content measured from the estimated content of -14.2 %.
To estimate the drug content in the capsules from the capsule weights without the effort of spectrophotometric analysis, three random sample groups of 60 empty capsules were weighed. The masses of 60 empty capsules were determined as 3.03 g, 3.00 g, and 2.97 g, respectively. The mean value of 3.00 g was divided by 60,

the number of capsules per group. This resulted in a mean weight of 50 mg for one capsule shell of size 3 (3.00 g : 60 = 0.05 g).
Then 60 filled capsules were weighed separately to perform a uniformity of mass test. The single weights of the capsules, their means and standard deviations are given in Table III.3.

Table III.3:

Uniformity of mass of theophylline capsules (Form. no. 001030A, size 3).

Nr. 1-10 (mg)	**Nr. 11-20 (mg)**	**Nr. 21-30 (mg)**	**Nr. 31-40 (mg)**	**Nr. 41-50 (mg)**	**Nr. 51-60 (mg)**
179	181	182	180	175	184
174	185	170	166	186	179
165	184	179	183	186	184
172	176	184	173	175	178
178	181	181	181	177	175
188	173	172	176	174	179
179	186	180	184	186	183
186	180	172	187	174	175
173	180	167	180	175	181
180	176	183	175	180	180
Mean 1 - 20: 178.8	SD 1 - 20: 5.7	Mean 21 - 40: 177.8	SD 21 - 40: 6.0	Mean 41 - 60: 179.3	SD 41 - 60: 4.3
Mean (1 - 60): 178.6 mg, SD (1 - 60): 5.3 mg					

The ±10% limits, according to the uniformity of mass test of the European Pharmacopeia [Ph.Eur., 3rd edition, <2.9.5>: “Uniformity of mass of single dose preparations”], allow capsule masses in the range from 161 to 197 mg. All of the 60 measured capsules are thus conform to the specifications of the Ph. Eur. in terms of mass uniformity.

When the mean mass of a capsule shell (i.e. 50 mg) is deducted from the mean total mass of a filled capsule (i.e. 178.6 mg), the average mass of the capsule content is calculated as 128.6 mg. Under the premise of a drug content of 60 % in the granules, the amount of theophylline per capsule is estimated as 77.2 mg. That is a deviation from the spectrophotometrically measured value described above (80.7 mg) of -3.7 % and from the value estimated initially from the bulk density (94 mg) of -17.9 %. These high deviation can be explained by different filling properties between a capsule and a 250 ml glass cylinder. The wide diameter of the glass cylinder used for the determination of the bulk density allows a free flow of the granules and a consistent filling, whereas a capsule with its small diameter can easily be blocked by single granules resulting in larger cavities and thus lower drug contents.

It is thus demonstrated that hard gelatin capsules of size 3 (filling volume 0.30 ml) cannot accommodate a minimum dose of 100 mg of active in the form of controlled-release granules. These findings can be extrapolated to other drugs such as talinolol. A key parameter for the amount of granules that can be accommodated in a capsule, is the total bulk density of the filling material. To this parameter the density of the active substance contributes only to a smaller extent. When the same excipients and same sieves are used, the bulk densities of the resulting granules will be similar independent of the incorporated drug, especially if little amounts of active drug are incorporated in large amounts of excipients. But it is also true for high drug : excipients ratios. In part this is due to the high contribution of between-granule volume to the total bulk density, in part it is also due to the fact that the densities of crystalline drugs only vary to a limited extent.

As a result of these findings, capsules of size 0, accommodating a higher filling volume of 0.68 ml, were selected to be filled with sustained-release granules in the following test formulations with talinolol.

III.3.1.2 Talinolol granule formulations

III.3.1.2.1 Granule formulation with Eudragit® RSPO, calcium hydrogen phosphate and magnesium stearate

The first test formulation with talinolol had a drug content of 40 % and was adopted from a theophylline sustained-release granule formulation [Eudragit® application brochure, Röhm (Darmstadt, Germany)]:

Talinolol	60.0 g
Eudragit® RSPO	60.0 g
Calcium hydrogen phosphate dihydrate	29.3 g
Magnesium stearate	0.7 g

Form. no. 010528A

The powder mixture was kneaded with 65.0 ml of demineralised water and granulated as described in the “materials and methods” section. After drying the granules had a relative humidity of 42.5 % at 20° C. The bulk density of the granules was 0.36 g/ml. The dissolution of 500 mg of granules, containing 200 mg of talinolol, was tested in 0.1 N HCl. The drug product was floating on top of the dissolution medium and talinolol was released very rapidly in the acidic medium (Figure III.7). This was rather unexpected, since the formulation contained as much as 40 % of release-controlling polymer.

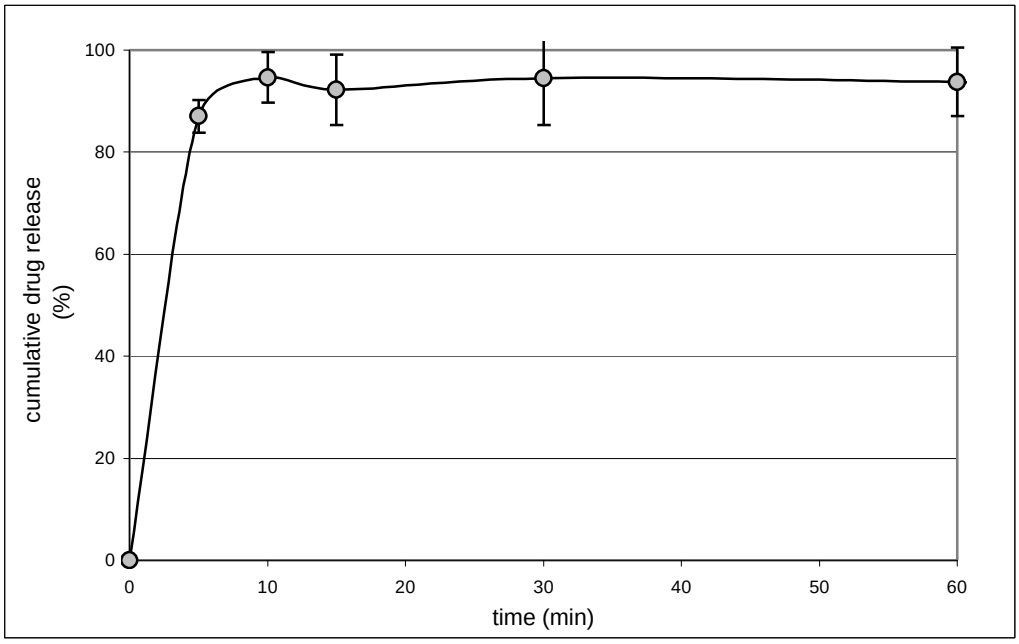


Figure III.7:
Dissolution profile of talinolol controlled-release granules formulated with 40 % Eudragit® RSPO.
Dissolution conditions: 0.1 N HCl, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 6.

III.3.1.2.2 Granule formulation with Eudragit® RSPO and Eudragit RS 12.5

To achieve a better control over the drug release, 30 g of the initial test granules (form. no. 010528A) were re-granulated with 70 g of Eudragit® RS 12.5 (12.5 % organic Eudragit® RS solution). After a drying period of 96 hours at 45° C the granules were sieved and the size-fraction from 355 µm to 2000 µm was filled in capsules of size 0 (0.68 ml).
Dissolution of talinolol from these capsules (form. no. 010623A) was tested in 0.1 N HCl and is presented in Figure III.8.

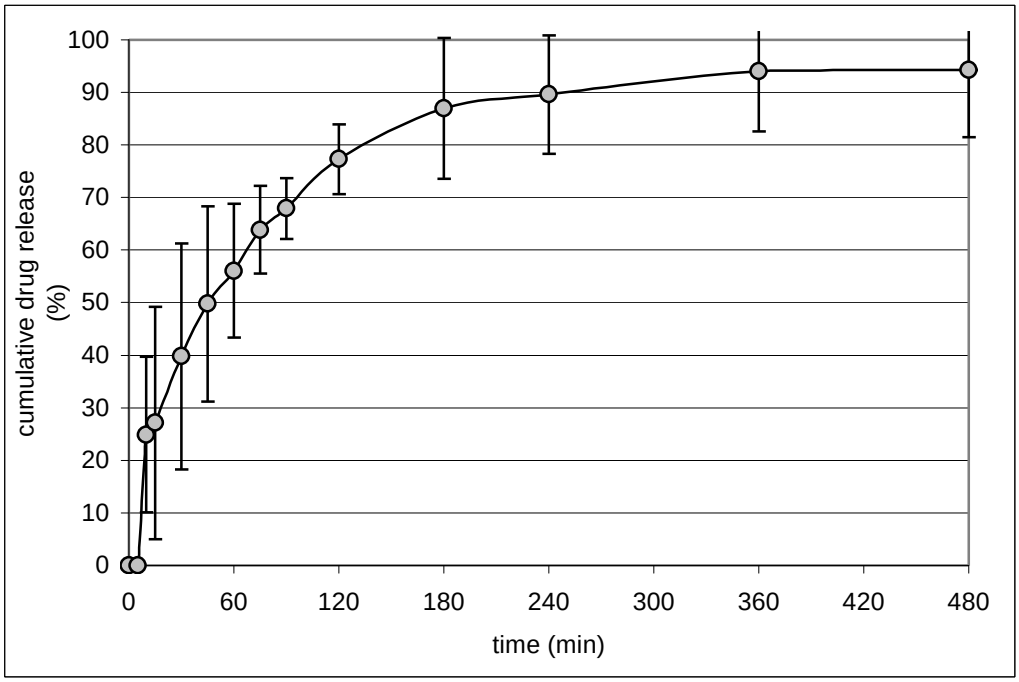


Figure III.8

Dissolution profile of talinolol sustained-release granules filled into hard gelatin capsules. Granulation with 40 % Eudragit® RSPO was followed by a second granulation with 70 % Eudragit RS 12.5.

Dissolution conditions: 0.1 N HCl, paddle apparatus 50 rpm, 37°C, means ± SD, n = 3.

It was observed that control over the drug release was from zero to four hours.

On the other hand the granules were hard to handle since the product was resin-like. Larger particles could hardly be crushed, a diminution of larger particles by pressing them through a sieve was not possible. A consistent filling of capsules was barely feasible.

High variations of the granule particle size led to particular differences in the release rates between different capsules tested. This is indicated by the high standard deviations in the amounts dissolved at given times. Because of these problems, wet granulations with organic solutions of Eudragit® were not followed further.

III.3.1.2.3 Granule formulation with Eudragit® RSPO, corn starch and gelatin

In another attempt to decrease the dissolution velocity, 50 g of the initial test granules (form. no. 010528A) were re-granulated with 10 grams of Eudragit® RSPO, 20 g corn starch and 40 g of a 6% aqueous gelatin solution. In this case, the dissolution should be decelerated by the increased Eudragit® RSPO fraction in the formulation and by the stronger bonds within the granules as a result of the glue effect of gelatin. Drying was for 24 hours at 40° C. Thereafter the granules were sieved.

To additionally test the impact of the granule particle size on the dissolution profile, two different sieve fractions, namely the fractions with diameters between 355 µm and 2000 µm (formulation number 010624B, 14 capsules) and with more than 2000 µm (formulation number 010624A, 42 capsules), were filled in capsules of size 0 (0.68 ml).). Their mass uniformity was tested (Table III.4), as well as their dissolution in 0,1 N HCl and phosphate buffer pH 6.8 (Figure III.9).

Table III.4:

Mass Uniformity of talinolol capsules (Form. no. 010624A and B, size 0)

	lot 010624A (1-20)	**lot 010624B (1-14)**
	286.1 mg	294.6 mg
	287.1 mg	284.9 mg
	273.2 mg	288.5 mg
	279.1 mg	305.0 mg
	290.1 mg	296.2 mg
	297.6 mg	279.6 mg
	300.9 mg	301.0 mg
	298.1 mg	303.8 mg
	297.2 mg	290.5 mg
	307.3 mg	300.1 mg
	313.3 mg	306.3 mg
	278.9 mg	318.0 mg
	314.3 mg	291.6 mg
	309.0 mg	302.9 mg
	295.6 mg	-)*
	300.4 mg	-)*
	291.5 mg	-)*
	282.8 mg	-)*
	274.9 mg	-)*
	289.9 mg	-)*
mean	293.4 mg	297.4 mg
S.D.	12.2 mg	10.0 mg
upper limit (mean + 10 %)	322.7 mg	327.1 mg
lower limit (mean - 10 %)	264.0 mg	267.6 mg
maximum value	314.3 mg	318.0 mg
minimal value	273.2 mg	279.6 mg

)*:only 14 capsules filled

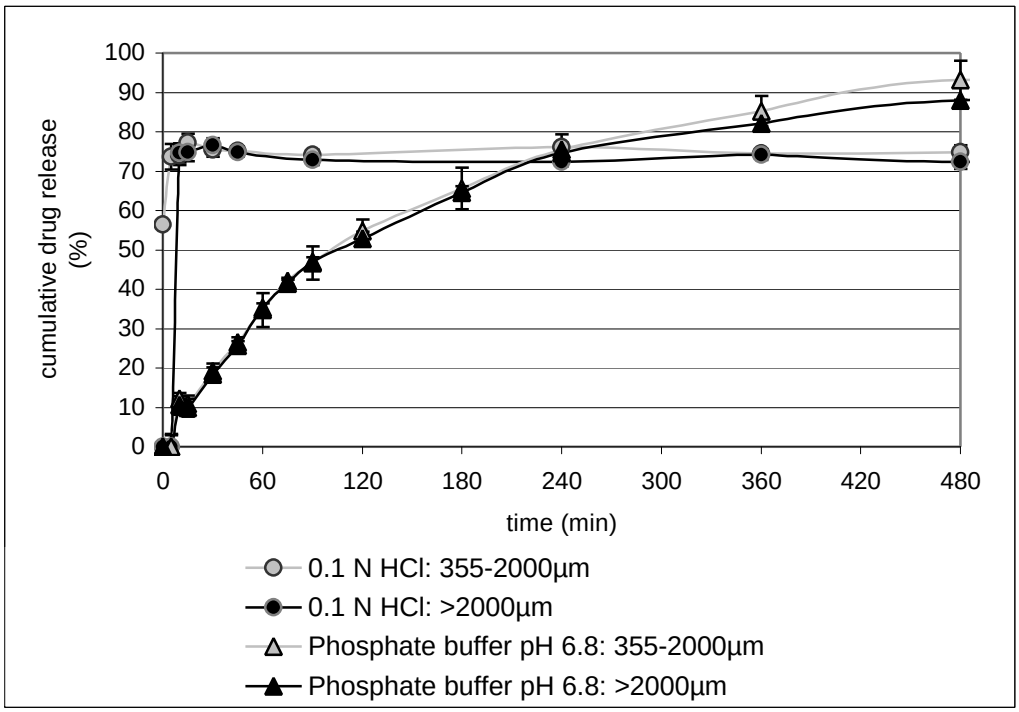


Figure III.9:

Dissolution profiles of talinolol sustained-release granules formulated with Eudragit® RSPO, corn starch and gelatin. The granules were filled in hard gelatin capsules.
Dissolution conditions: 0.1 N HCl and phosphate buffer pH 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

These results show that, for this particular formulation, the impact of the granule particle size on the dissolution behaviour is negligible. The dissolution curves show no relevant diferences - neither in the acidic range, where dissolution of talinolol is favoured by its ionisation, nor in the neutral pH range, where the intrinsic dissolution of the drug is slower. In principle, diffusion of a drug through a granule matrix is dependent on the particle size, since the diffusion distance is longer in larger granules. In addition, according to the Noyes-Whitney equation, dissolution velocity is also influenced by the particle size as the specific surface area increases when particles become smaller. On the other hand, it must be realized that in the acidic range the dissolution is so rapid that the method has no discriminatory power to detect an influence of the particle size. Therefore, dissolution was also tested at neutral pH where the dissolution rate is slower and hence the impact of factors with

influence on dissolution profiles should be more obviously detectable. Unexpectedly, also in the neutral pH range, no differences were recognised.

However, the formulation was not appropriate, since the control of drug release in the acidic medium was completely dissatisfactory.

III.3.1.2.4 Granules coated with Eudragit® L 12.5

The insufficient control of drug release in 0.1 N HCl should be improved by coating the granules with an organic Eudragit® L 12.5 solution.
Therefore 20.0 g of the initial test granules (form. no. 010528A) were coated with an Eudragit® L 12.5 solution as described in the “Materials and Methods” section. A problem during the preparation of these coated granules was that they tended to stick to the pan and to each other, thus forming large agglomerates. Consequently, the drying and the spraying times could not be fixed in advance but had to be adopted in dependence on the sticking behaviour of the granule.
Following the spraying process, the coated granule weighed 25.4 grams, which means a mass increase of 27 % or a coating weight of 270 mg/g. The dissolution of the coated granules (form. no. 010715A), filled in capsules of size 0, was tested (Figure III.10).

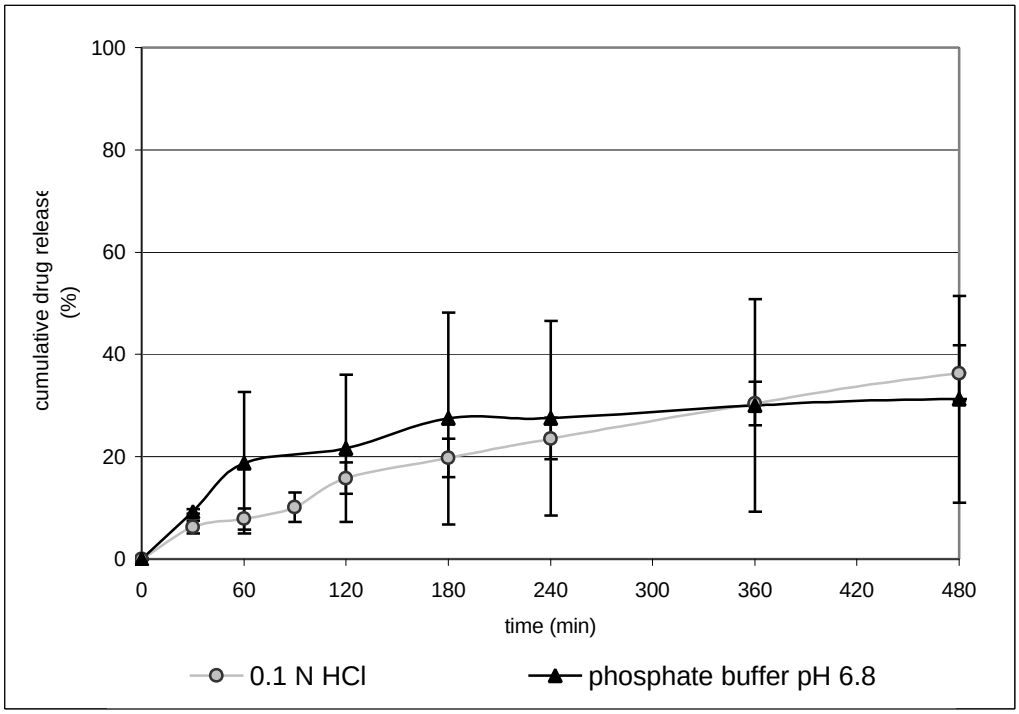


Figure III.10:

Dissolution profiles of talinolol sustained-release capsules filled with Eudragit® L 12.5 coated granules.
Dissolution conditions: 0.1 N HCl and phosphate buffer pH 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

Similar to the granules prepared by wet granulation with the organic Eudragit® solution RS 12.5 (form. no. 010623A), variations in the amounts dissolved at given times between capsules were extraordinarily high. In this case, the variances in the neutral pH range were higher than in the acidic medium. A possible explanation for the high variabilities are the resin-like consistency of the granules and their irregular shapes that lead to an inconsistent filling of the capsules.
Furthermore, for this formulation dissolution was not complete within eight hours. For sustained-release peroral formulations, a control of drug release beyond eight to twelve hours is not advisable, due to the fact that in case of rapid intestinal transit times relevant parts of the dose may not have been dissolved and may be excreted with the faeces. In order to accelerate the drug release and achieve a complete dissolution within eight hours, the spraying time and thus the thickness of the Eudragit® film around the granules could be reduced.

However, due to the additional problems with the standardisation of the spraying process combined with a low homogeneity within one lot and an insufficient lot-to-lot conformity caused by inconsistencies of the capsule contents, methods apart from film-coating were favoured in the further development of an appropriate talinolol sustained-release formulation.

III.3.1.2.5 Granule formulation with ethylcellulose

In another granule formulation, ethylcellulose was tested as release-sustaining excipient. The composition of the granule was as follows:

Ethylcellulose (Ethocel®)	5.0 g
Acetone	55.0 g
Talinolol	5.0 g
Form. no. 010728A	

Ethylcellulose was dissolved in acetone, then talinolol powder was added in small amounts and finely dispersed. The mixture was stored in a fume hood for 15 minutes. During this time the acetone had evaporated to a large extent and the consistency of the formulation was suitable to be granulated through a sieve. After drying, the granule was filled in capsules of size 0 (0.68 ml).
The masses of six empty capsule shells and the total masses of these six capsules after filling were determined. The granule mass for one capsule was calculated as difference between the total mass and the mass of the shell. The mass of drug per capsule was calculated as mass of the granule multiplied by 0.5, according to the fraction of active ingredient in the formulation after evaporation of acetone. The results of the mass uniformity test are presented in Table III.5.

Table III.5:

Mass uniformity and drug content of talinolol capsules (form. no. 010728A).

mass of capsule shell in mg (tara)	**total mass of filled capsule in mg (brutto)**	**mass of granule in mg (netto)**	**mass of drug in mg**
106	200	94	47
107	168	61	31
96	181	85	43
95	192	97	49
99	180	81	41
100	168	68	34
mean: 101	**mean: 182**	**mean: 81**	**mean: 41**
S.D.: 5	**S.D.: 13**	**S.D.: 14**	**S.D.: 7**

The number of capsules produced was not sufficient to perform a mass uniformity test according to the European Pharmacopeia [Ph.Eur., 3rd edition, <2.9.5>: “Uniformity of mass of single dose preparations”]. For this purpose, a number of 20 units of dosage forms would have been necessary. The observed variations and deviations from the mean values displayed in Table III.5 demonstrate that the filling of the capsules was not homogeneous.

The dissolution of talinolol from these capsules was tested in 0.1 N hydrochloric acid and a phosphate buffer pH 6.8. The results are presented in Figure III.11.

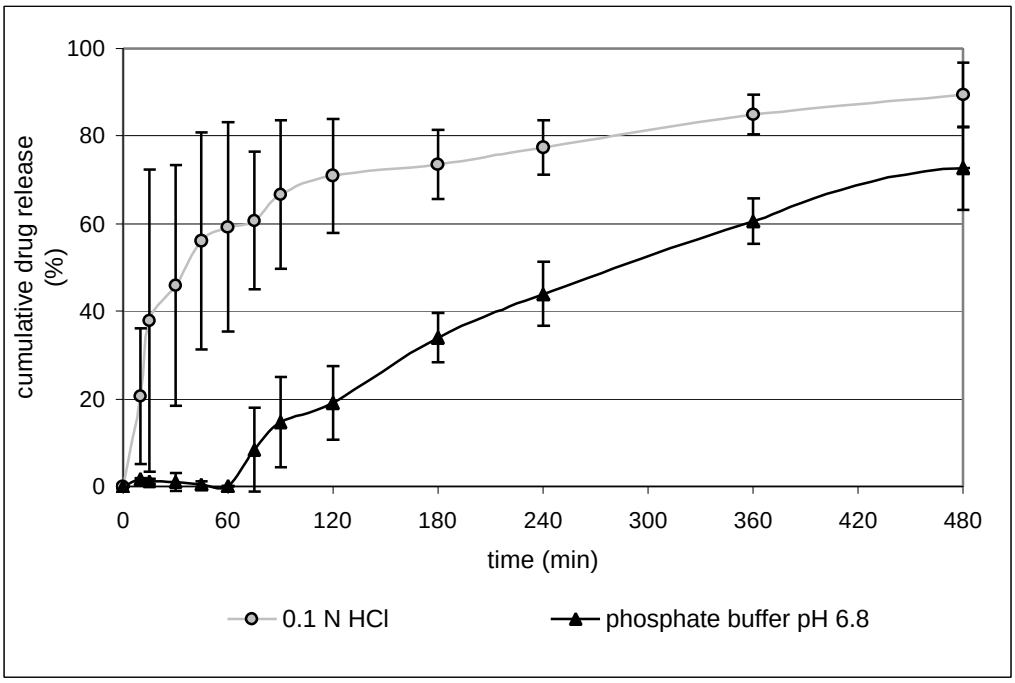


Figure III.11:

Dissolution profiles of talinolol sustained-release granules filled in hard gelatin capsules. Formulation: 50 % ethylcellulose, 50 % talinolol.
Dissolution conditions: 0.1 N HCl and phosphate buffer pH 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

The profiles explicitly differed between the two tested pH values: While approximately 60 % of the drug dissolved in hydrochloric acid within the first hour, a lag-time of one hour was observed in the phosphate buffer at pH 6.8, followed by a period of consistent drug release over several hours. With a drug release of approximately 70 %, dissolution was not complete in the phosphate buffer after eight hours. As indicated by the high standard deviations, drug release varied distinctively between single capsules tested, especially in the acidic medium. One reason for the variations in the time period between three and eight hours can be the inconsistent filling of the capsules as previously described for other granule formulations filled into capsules. However, this does not explain the higher variations in hydrochloric acid compared to the phosphate buffer in the time period up to three hours. These variations must be caused by differences in drug release. In part this can be due to differences in the lag-time, i. e. the time until the capsule shell opens and releases the granules. Another reason might be differences in the shape and size of the granules causing

different diffusion properties. These lead to a more or less rapid release of the drug incorporated in a granule. This effect can be observed especially at intermediate drug release rates as detected for the ethylcellulose-based granules in hydrochloric acid within the first three hours. When the drug release is more rapid, no matrix effects can be observed. The granules disintegrate and dissolve rapidly and no diffusion of the drug through the matrix is recognizable. On the other hand, when the drug release is slower, as detected for the same granules in the phosphate buffer, the amount of drug released within a particular time intervall is smaller and consequently detectable variations due to different diffusion properties are not so pronounced.

III.3.2 Influence of the pH on talinolol dissolution

III.3.2.1 Dissolution study with capsules containing pure talinolol dry substance

For all granule formulations dissolution of talinolol was more rapid at pH 1.0 (hydrochloric acid) than at pH 6.8 (phosphate buffer). This could be due to dissolution properties of the active drug itself or it could be caused by other excipients in the formulation. To investigate the pH-dependence of the dissolution of the pure active drug substance, capsules of size 0 (0.68 ml) were filled with 200 mg talinolol each, without any further excipients added. A capsule filling machine could not be used, because this requires a higher volume of filling material. Instead, a funnel was employed to fill each single capsule manually with 200 mg of exactly weighed talinolol.

Dissolution of these capsules was tested in 0.1 N hydrochloric acid and in three different phosphate buffers with pH values of 3.2, 5.5 and 6.8. The results are presented in Figure III.12.

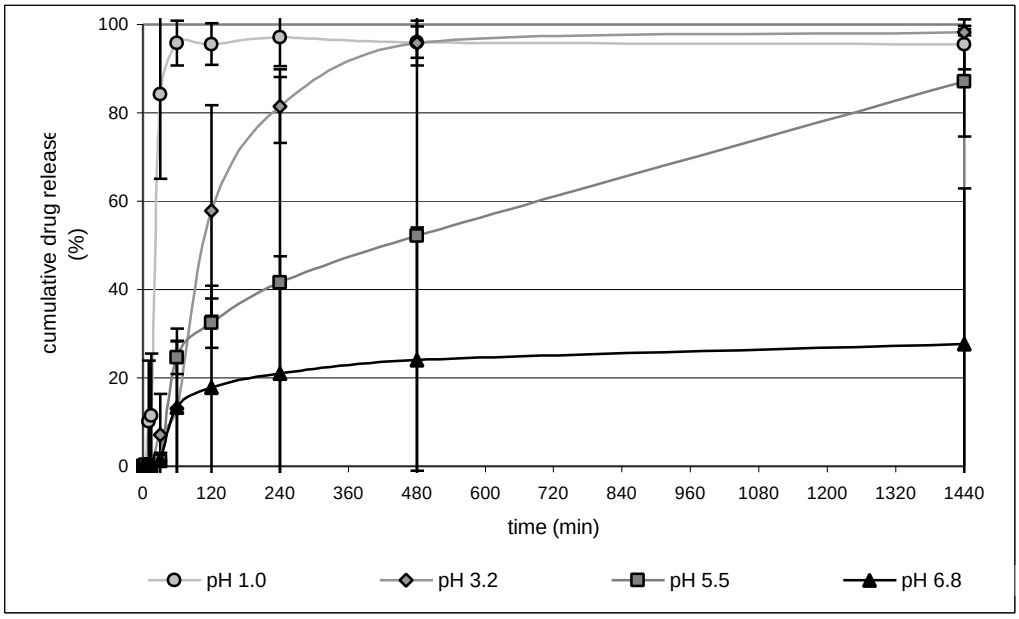


Figure III.12:

Dissolution profiles of capsules filled with 200 mg of pure talinolol.
Dissolution conditions: 0.1 N HCl and phosphate buffers pH 3.2, 5.5 and 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

The dissolution rate is decreasing with increasing pH values. This is not unexpected, since the solubility of talinolol increases with decreasing pH values, caused by the basic nitrogen (cp. Figure I.2) that is protonated to a higher extent at lower pH values. Furthermore, due to the lack of any excipients increasing the wettability of the talinolol powder, the drug can form agglomerates when it is exposed to the dissolution medium. The low wettability of these agglomerates can explain the unexpectedly slow drug release rates observed in all dissolution media tested. It is assumed that the formation of irregular drug agglomerates largely contributes to the high variability of talinolol dissolution from the different units.

III.3.2.2 Dissolution study with talinolol immediate-release tablets (Cordanum 100)

To additionally demonstrate the strong influence of the pH value of the dissolution medium on talinolol dissolution, tests with immediate release tablets with a drug content of 100 mg available on the German market (Cordanum 100), were performed at pH 1.0 and pH 6.8. The results are presented in Figure III.13.

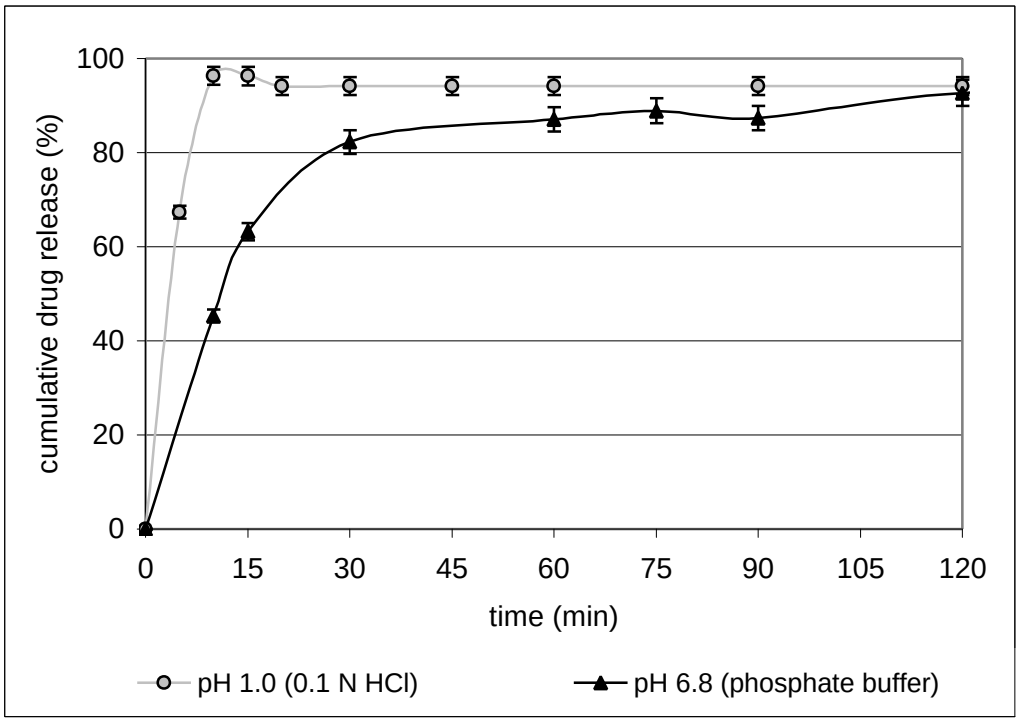


Figure III.13:

Dissolution profiles of immediate-release talinolol tablets with a drug content of 100 mg (Cordanum® 100).
Dissolution conditions: 0.1 N HCl and phosphate buffer pH 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

The results demonstrate that drug release from immediate-release tablets also is pH-dependent. At pH 1.0 talinolol is released considerably faster from Cordanum tablets than at pH 6.8. Opposite to the capsules filled with pure talinolol, the drug release is complete within approximately two hours. This can be explained by excipients of the Cordanum tablets such as potato starch and carboxymethyl starch which facilitate

disintegration, improve the wettability of talinolol and prevent the formation of hardly soluble agglomerates.

III.3.2.3 Dissolution study with tablets formulated with talinolol and corn starch

In another study to demonstrate a pH-dependent dissolution for talinolol, immediate-release tablets were prepared containing 85 % talinolol and 15 % corn starch. Dissolution of talinolol from these tablets was tested in five different media from pH 1.0 to pH 6.8. The results are given in Figure III.14.

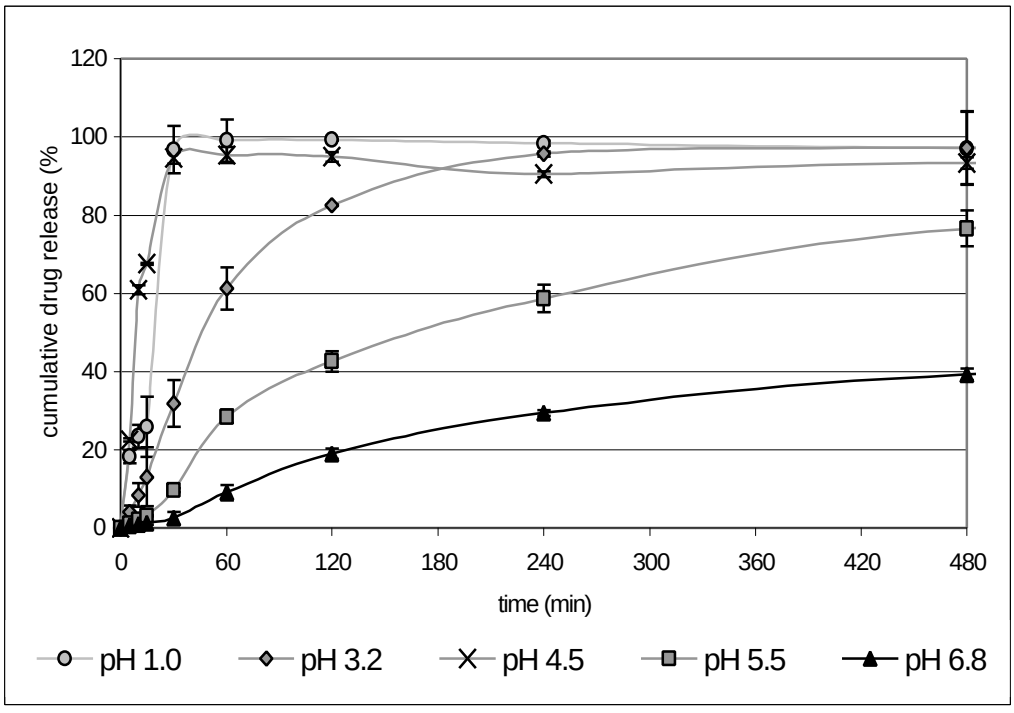


Figure III.14:

Dissolution profiles of talinolol immediate-release tablets.
Formulation: 85 % talinolol, 15 % corn starch. Dissolution conditions: paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

Again, a pH-dependence of the talinolol dissolution is obvious. The drug release rate increases with decreasing pH values.

However, at pH 4.5 the drug release was faster and higher than expected. An obvious difference towards the other buffers was, that for pH 4.5 an acetate buffer was used, whereas for pH values of 3.2, 5.5, and 6.8 phosphate buffers were employed. In spite of the higher pH value, the drug dissolved faster in this acetate buffer than at pH 3.2 in a phosphate buffer. Definitely, dissolution in this acetate buffer was rather similar to dissolution in 0.1 N hydrochloric acid at pH 1.0. The reason for this is that in phosphate buffers talinolol forms structurally different crystals with other physico-chemical properties than in acetate buffers. This aspect is described and discussed in more detail in chapter IV.

III.3.3 Final discussion on hard gelatin capsule preparations

None of the formulations tested, neither with matrix nor with coated granules as filling material for hard gelatin capsules, showed the desired dissolution behaviour.
Three major problems emerged:

1) Dissolution differed between the acidic (pH 1.0) and the neutral pH range (pH 6.8). All tests demonstrated that drug release was considerably faster in acidic versus neutral pH dissolution media. Even when excipients were used which claim to yield pH- independent drug release profiles, the properties of the active drug predominated in the present case.

2) Size and shape of the granules showed a high variability. By separation into different sieve classes variations in size could be reduced but not completely eliminated. Together with the irregular shapes of the granules this caused variations in dose uniformity and drug release properties as well. Inhomogeneities in the drug content were caused by an inconsistent filling of the capsules. Variations in the drug release rate were due to differences in the diffusion and dissolution properties between differently sized and shaped granules.

3) The volumetric dosing of the capsules provoked the problem that a specified drug content could never be exactly achieved. Since only a limited number of defined capsule sizes is available and the granules were rather voluminous, capsules with a drug content of 100 or even 200 mg could not be prepared with the typically used capsule sizes. Capsules of the largest size available (000) have a filling volume of 1.37 ml. Preliminary tests and calculations had shown that even these capsules could hardly be filled with a granule containing 100 mg talinolol. This was due to the need of a relatively high percentage of excipients for a sufficient control of the drug release. Furthermore, capsules of size 000 are hard to swallow because of their size.

Since exact dosing and a good within-lot and lot-to-lot homogeneity are important requirements for the sustained-release dosage form projected, and the technical equipment and methods available showed only limited possibilities to improve these parameters, the production of matrix tablets was assumed to be more appropriate as

method for the manufacturing of dosage forms with a sustained talinolol release than formulations with granules and capsules.

III.3.4 Matrix tablets

III.3.4.1 Powder losses during the manufacturing process of matrix tablets

As an alternative to the capsule formulations, matrix tablets were prepared. During the compression process of tablets particular amounts of powder get lost. These losses can be compensated by weighing an adequate amount of powder additionally to the amount theoretically needed to compress one tablet. To determine the powder losses during the compression process of matrix tablets, 20 sample tablets with a labelled mass of 500 mg were weighed. The differences between the labelled and the measured masses were determined. The results are presented in Table III.6.

Table III.6:

Masses of differently formulated tablets demonstrating losses during the compression process.

Theoretical mass (mg)	Measured mass (mg)	Difference (mg)
500	497	3
500	498	2
500	488	12
500	485	15
500	493	7
500	497	3
500	500	0
500	492	8
500	496	4
500	496	4
500	496	4
500	500	0
500	495	5
500	490	10
500	495	5
500	490	10
500	495	5
500	495	5
500	496	4
500	495	5

The medium loss was 5.6 ± 3.8 mg (mean ± SD). For a total tablet mass of 500 mg this means a mean deviation from the labelled mass of 1.1 %. The maximum deviation was 15 mg, i.e. 3 %. Although these deviations comply with the requirements of the European Pharmacopeia in terms of mass uniformity [Ph.Eur., 3rd edition, <2.9.5>: “Uniformity of mass of single dose preparations”], the fact that all deviations are negative suggests that the deviations are due to losses during the

tablet compression process. To compensate for these losses, the total tablet mass to be compressed was increased by 1 % for the production of matrix tablets. For example, 505 mg of a powder mixture were compressed to obtain a 500 mg tablet.

III.3.4.2 Parameters with potential influence on the drug release from matrix tablets

III.3.4.2.1 Compression force

Dissolution tests with identically formulated matrix tablets compressed with different compression forces were performed in order to investigate the impact of the compression force on dissolution profiles. The tablets used for these tests consisted of 40 % talinolol, 40 % Eudragit® S 100 and 20 % Eudragit® RSPO. Compression forces of 15 and 40 kN were compared. In both cases the compression time was 30 seconds. Dissolution was tested in 0.1 N hydrochloric acid. Within this medium the drug release rate was expected to be most susceptible to a decrease in compression force. In preliminary experiments, insufficient amounts of release-controlling excipients in tablet formulations resulted in instable matrices that eroded rapidly. The most pronounced accelerations of talinolol release from such instable matrices, that can also be expected when compression forces are too low, were observed in 0.1 N hydrochloric acid, due to the high solubility and rapid dissolution of talinolol in this medium.

Figure III.15 compares the dissolution of talinolol from tablets compressed with compression forces of 15 kN and 40 kN.

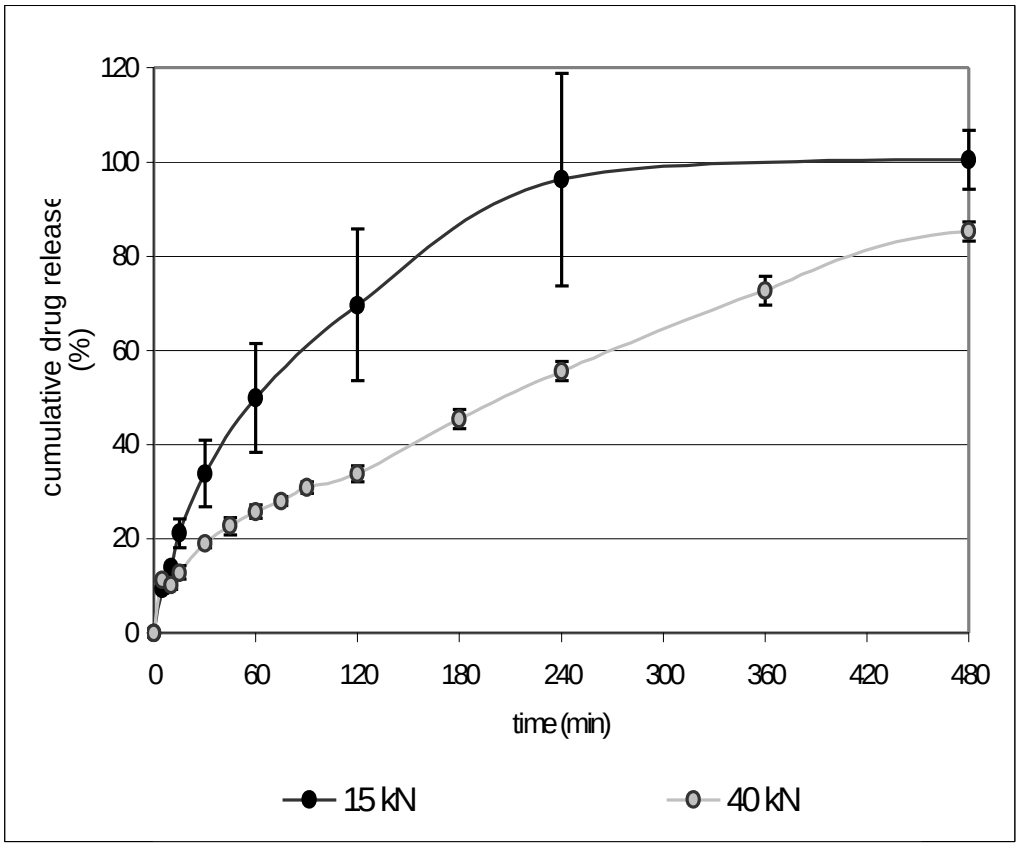


Figure III.15:

Influence of the compression force on the dissolution of sustained-release talinolol matrix tablets.

Formulation: 40 % Eudragit® S 100, 20 % Eudragit® RSPO, 40 % talinolol (drug content 200 mg). Dissolution conditions: 0.1 N HCl, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

As expected, dissolution was accelerated by a lower compression force. A force of 15 kN for a compression time of 30 seconds was not sufficient to generate a non-erodible matrix with a consistent release of talinolol over 8 hours. After 4 hours 96.3 % of the drug were already dissolved and the tablets were disintegrated. The need for higher compression forces to achieve stable matrices with sufficient release-control was also underlined by the high variations in the release profiles between single tablets compressed at 15 kN. These high variations can at least in part be

explained by the differences in the erosion and disintegration of the tablets manufactured with the lower compression force. This is in accordance with studies of sustained-release matrix tablets formulated with hydroxypropylmethylcellulose (HPMC) reported by Velasco et al. and Kabanda et al. [Velasco, M. V., J. L. Ford, et al. (1999), Kabanda, L., R. A. Lefebvre, et al. (1994)]. They found that low compression forces can lead to burst effects that are due to erosion and disintegration of the matrices. However, Velasco et al. also reported that the influence of the compression force on drug release becomes neglectable as soon as the compression force is sufficient to achieve a stable, non-disintegrating matrix. For the specific HPMC tablets in their test series, a compression force as low as 6 kN was adequate to achieve that aim. Apart from the differences in the composition of the matrix, the different shape of the HPMC matrix tablets causes the need for higher compression forces for the talinolol matrix tablets: The tablets tested by Velasco et al. were only 6 mm in diameter and were thicker than the talinolol tablets. Thus, the matrix was more compact with a relatively smaller surface and less tendencies towards disintegration and erosion. In another study with HPMC sustained-release tablets Nokhodchi et al. investigated the influence of different compression forces between 5 and 20 kN on the porosity of the matrix [Nokhodchi, A., J. L. Ford, et al. (1996)]. They reported that the porosity decreases with increasing compression forces until a minimum value is reached. It can be assumed that this minimum porosity and hence a particular minimum compression force is necessary to achieve stable matrices that show an appropriate control of the drug release.

According to these findings, all test formulations for talinolol matrix tablets were compressed with a force of 40 kN.

III.3.4.2.2 Compression time

To examine the influence of the compression time on the drug release from matrix tablets, tablets were compressed from the same powder mixture for 30 seconds and for 2 minutes. The powder mixture consisted of 60 % Eudragit® S 100 and 40 % talinolol.

The differences in drug release at pH 6.8 between the matrix tablets with different compression times are presented in Figure III.16.

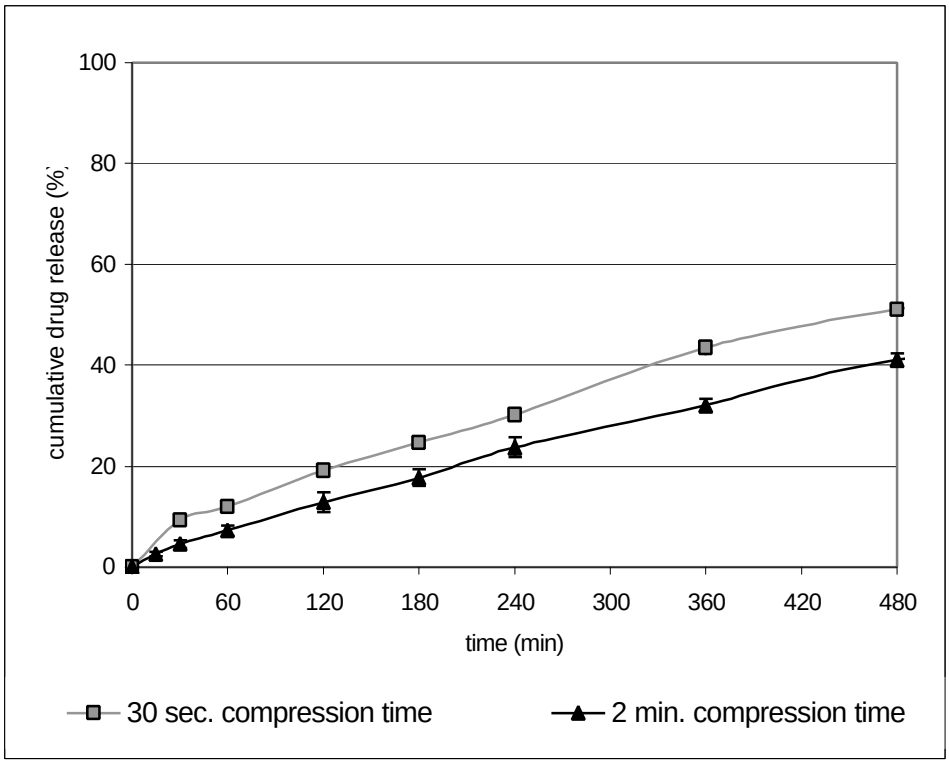


Figure III.16:

Influence of the compression time on the dissolution of talinolol from sustained-release matrix tablets.

Formulation: 60 % Eudragit® S 100, 40 % talinolol (drug content: 200 mg). Dissolution conditions: phosphate buffer pH 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3. For a compression time of 30 seconds standard deviations were smaller than sizes of the rectangles.

The amount of drug released from the tablets compressed for two minutes was approximately 5 to 10 % lower at all time points. It can be concluded that longer compression times lead to a slower dissolution. However, this effect was not very pronounced. Due to the advantageous time-saving effect of shorter compression times, matrix tablets were compressed for 30 seconds, unless stated otherwise.

III.3.4.2.3 Rotational speed of the paddles in the dissolution apparatus

To test the influence of the stirring velocity in the dissolution vessels on the drug release profiles, dissolution tests with matrix tablets consisting of 40 % talinolol, 40 % Eudragit® S 100 and 20 % Eudragit® RSPO were performed. The drug release profiles from tests with paddle rotational speeds of 50 and 100 rounds per minute were compared. 0.1 N HCl was selected as dissolution medium, since in this medium matrix tablets are due to be most susceptible to changes, also with respect to rotational speed. The results are presented in Figure III.17.

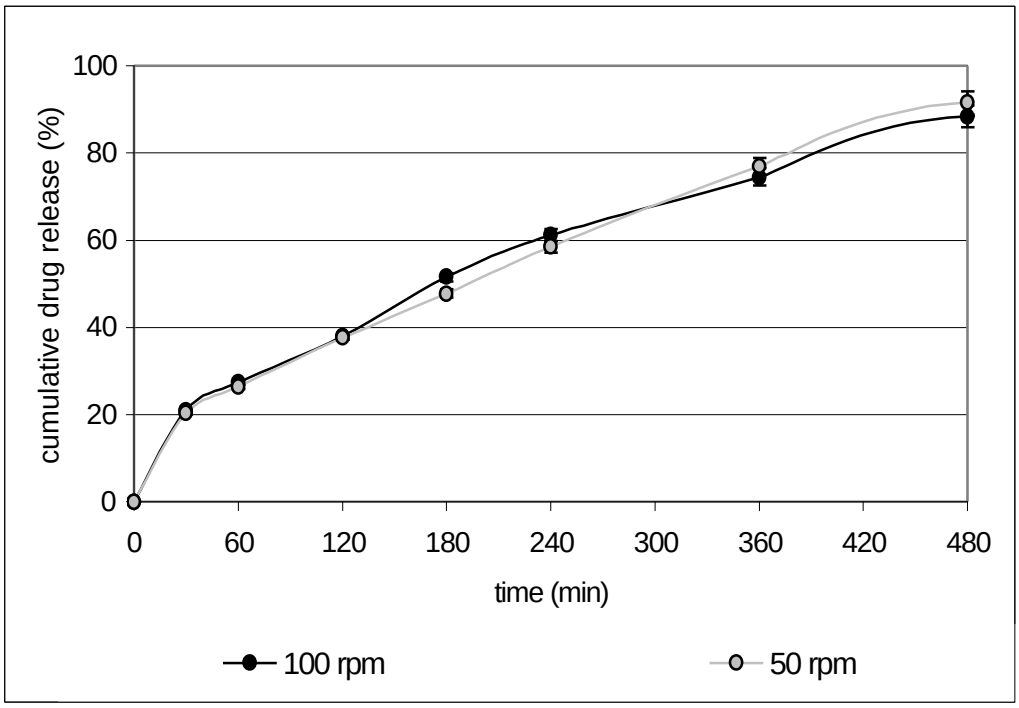


Figure III.17:

Influence of the rotational speed on the dissolution of talinolol from sustained-release matrix tablets.
Formulation: 40 % Eudragit® S 100, 20 % Eudragit® RSPO, 40 % talinolol. Dissolution conditions: 0.1 N HCl, paddle apparatus, 37°C, means ± SD, n = 6.

Drug release from this specific matrix was not notably influenced by the higher rotational speed and the modified hydrodynamics. Furthermore, the release of talinolol from single dosage forms did not show increased variations. This proved that stable and robust matrices that are relatively resistant to abrasion can be produced by direct compression with the equipment and the release-sustaining excipients employed. A relative independence of the drug release from the rotational speed demonstrates the stability of a matrix [Majid Khan, G. and J. Bi Zhu (1998)].

III.3.4.3 Matrix tablets formulated with Eudragit® RSPO, Eudragit® S 100, Eudragit® RLPO and Ethocel®

To investigate the influence of different release-sustaining excipients on the talinolol release from matrix tablets, powder mixtures for direct compression of tablets with Eudragit® RSPO, Eudragit® S 100, Eudragit® RLPO and ethylcellulose (Ethocel®) were prepared. The ratio between active drug and the particular release-sustaining excipient was 40 : 60 in all four tablet formulations tested. The composition of the powder mixtures is given in Table III.7.

Table III.7:
Composition of powder mixtures for talinolol sustained-release matrix tablets formulated with different release-sustaining polymers.

Form. no.	Talinolol		Eudragit® RSPO		Eudragit® S 100		Eudragit® RLPO		Ethocel®	
011008A	5.0 g	40 %	7.5 g	60 %	-	-	-	-	-	-
011013A	5.0 g	40 %	-	-	7.5 g	60 %	-	-	-	-
011014A	5.0 g	40 %	-	-	-	-	7.5 g	60 %	-	-
011008B	5.0 g	40 %	-	-	-	-	-	-	7.5 g	60 %

Due to the drug content of 40 % in all four formulations, 500 mg of each powder was needed to achieve a talinolol content of 200 mg talinolol per dosage form. The tablets were compressed with a force of 40 kN for 2 minutes. The results of dissolution tests in different media are presented in Figures III.18 to III.21.

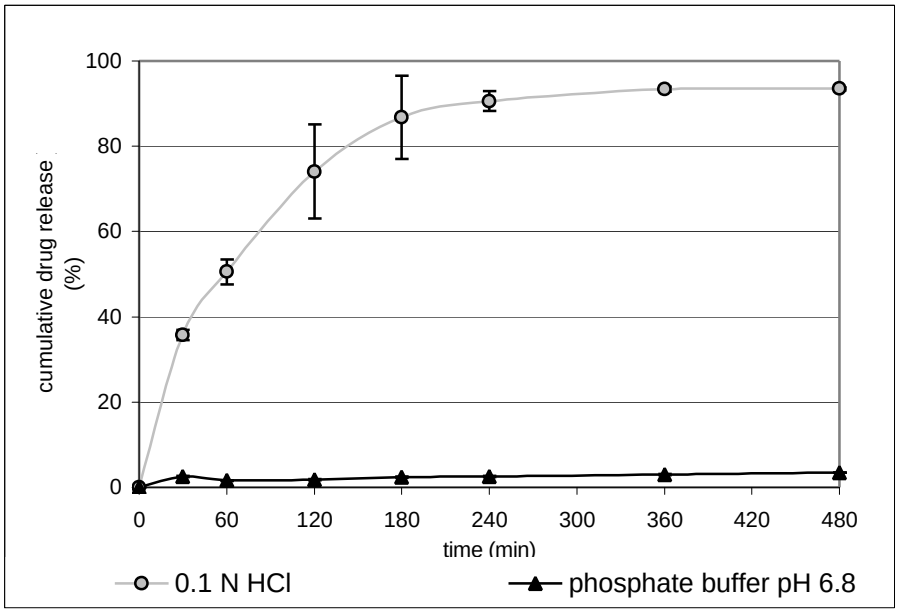


Figure III.18:

Talinolol release from sustained-release matrix tablets with a drug content of 200 mg. Formulation: 60 % Eudragit® RSPO, 40 % talinolol. Dissolution conditions: 0.1 N HCl and phosphate buffer pH 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3, form. no. 011008A. The standard deviations were in part smaller than the triangles and circles.

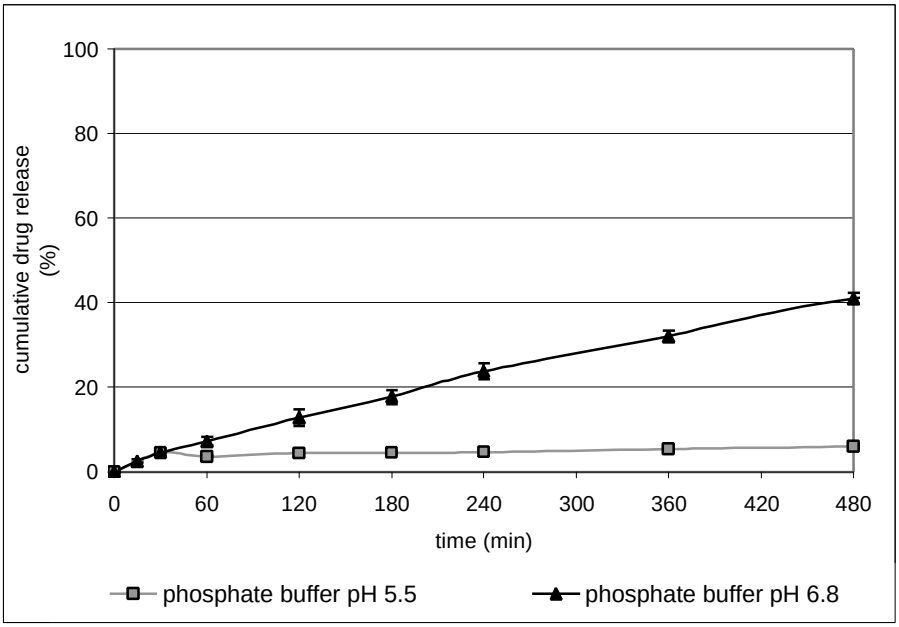


Figure III.19:

Talinolol release from sustained-release matrix tablets with a drug content of 200 mg. Formulation: 60 % Eudragit® S 100, 40 % talinolol. Dissolution conditions: phosphate buffers pH 5.5 and pH 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3. Standard deviations were in part smaller than sizes of triangles and rectangles.

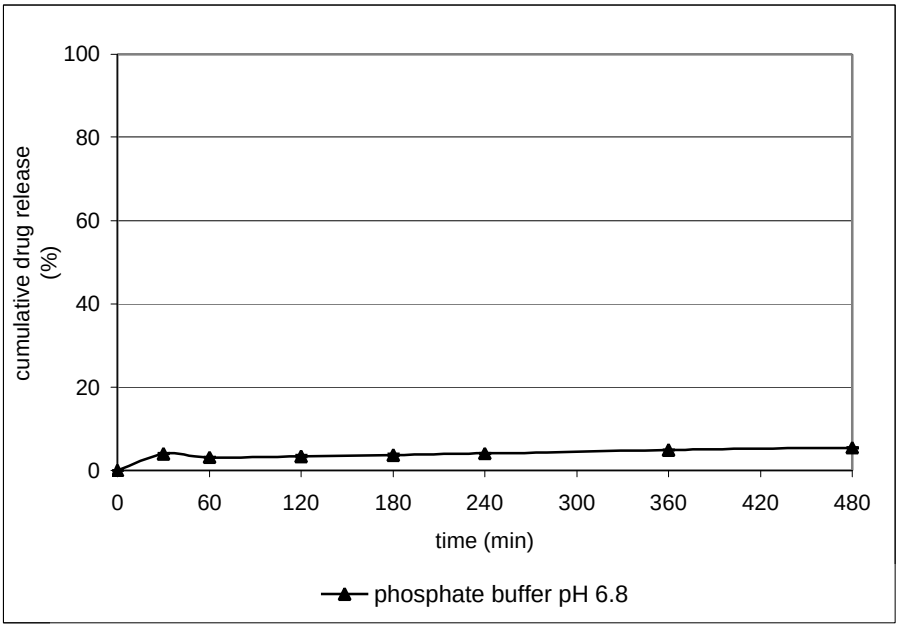


Figure III.20:

Dissolution profile of sustained-release matrix tablets with a drug content of 200 mg. Formulation: 60 % Eudragit® RLPO, 40 % talinolol. Dissolution conditions: phosphate buffer pH 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3. Standard deviations were smaller than sizes of triangles.

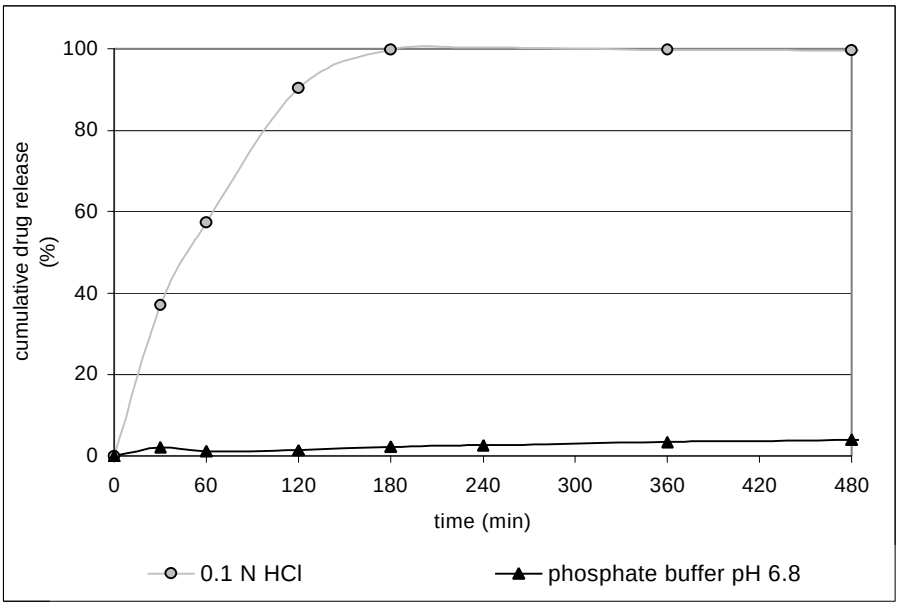


Figure III.21:

Talinolol release from sustained-release matrix tablets with a drug content of 200 mg. Formulation: 60 % Ethocel®, 40 % talinolol. Dissolution conditions: 0.1 N HCl and phosphate buffer pH 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3. Standard deviations were smaller than sizes of triangles and circles.

None of the four formulations matched the required dissolution properties. From the formulation using Eudragit® RSPO as single release-controlling excipient talinolol dissolved too fast at acidic pH. At pH 6.8 the drug release was less than 5 % within eight hours. The formulation with Eudragit® S 100 only released 40 % talinolol over eight hours at pH 6.8 and less than 10 % at pH 5.5 in the same time interval. Similarly, less than 10 % were released from the formulation with Eudragit® RLPO at pH 6.8. Finally, the tablets based on Ethocel® showed dissolution profiles similar to those of the formulation with Eudragit® RSPO. Drug release was relatively fast in the acidic milieu and at pH 6.8 nearly no release was observed.
However, the standard deviations in the figures demonstrate that the within-batch variability of the drug release was significantly reduced compared to the capsule and granule formulations.

III.3.4.4 Matrix tablets formulated with Eudragit® S 100 and corn starch

In preliminary studies the drug release from matrix tablets formulated with Eudragit® S 100 was too slow at pH 5.5 and pH 6.8. In order to accelerate the talinolol dissolution rate, the fraction of the release-sustaining polymer in the matrix was reduced. A reduction of the percentage of Eudragit® S 100 from 60 % to 50 % (form. no. 011016B) accelerated the dissolution rate of talinolol in phosphate buffer of pH 6.8. After eight hours, 69.0 % of the active drug were released. However, the decreased Eudragit® S 100 fraction did not lead to an appropriate acceleration of the drug release in phosphate buffer of pH 5.5. In this medium, drug release after eight hours was merely 9.1 %.

III.3.4.5 Influence of pore formers on the dissolution of talinolol matrix tablets

To increase drug release from matrix tablets showing incomplete dissolution, hydrophilic substances can be incorporated in the matrix in order to form pores which promote a more rapid dissolution of the active ingredient [Majid Khan, G. and J. Bi Zhu (1998), Lapidus, H. and N. G. Lordi (1966), Bauer, K. H., K.-H. Frömming, et al. (1993)]. Pore formers are biological inactive water soluble excipients that form channels in the polymer matrix. These channels formed by dissolution of the water soluble substances incorporated in the matrix facilitate the movement of water into the matrix, the solubilization of the active drug, and the movement of the dissolved drug out of the matrix [Kim, J. E., S. R. Kim, et al. (2000)]. Examples of excipients employed for that purpose are lactose, glucose, mannitol, polyethylene glycol, microcrystalline cellulose, albumin and starch [Majid Khan, G. and J.-B. Zhu (1999), Kim, J. E., S. R. Kim, et al. (2000)].

In preliminary tests corn starch was selected as pore former in combination with Eudragit® S 100 as release-sustaining polymer. Dissolution tests showed that corn starch strongly influences the drug release from talinolol matrix tablets. In phosphate buffer of pH 5.5, more than 90 % of the drug were dissolved within 15 minutes from a formulation consisting of 33 % talinolol, 50 % Eudragit® S 100, and 17 % corn starch. These findings combined with the observation that drug release from the formulation with 50 % talinolol and 50 % Eudragit® S 100 without corn starch was too slow, initiated the manufacturing of matrix tablets with corn starch contents of less than 17 %. Tablets containing 1 %, 2 %, and 5 % of the pore former were prepared, in order to achieve drug release profiles with intermediate dissolution rates. In order to investigate the dissolution of a pure mixture of talinolol and corn starch, one formulation was tested without any release-sustaining polymer added (Formulation number 011124A: 85 % talinolol, 15 % corn starch). Table III.8 gives an overview over the composition of the formulations tested based on Eudragit® S 100 and corn starch.

Table III.8:
Composition of powder mixtures for direct compression of talinolol sustained-release matrix tablets formulated with Eudragit® S 100 and corn starch.

Form. no.	Talinolol		Eudragit® S 100		Corn starch		Mass per tablet
011021A	2.40 g	33.3 %	3.60 g	50.0 %	1.2 g	16.7 %	0.600 g
011023A	4.95 g	49.5 %	4.95 g	49.5 %	0.1 g	1 %	0.404 g
011023B	4.90 g	49.0 %	4.90 g	49.0 %	0.2 g	2 %	0.408 g
011023C	4.75 g	47.5 %	4.75 g	47.5 %	0.5 g	5 %	0.421 g
011124A	4.25 g	85.0 %	-	-	0.75 g	15 %	0.235 g

The different total masses per tablet as shown in Table III.8 arise from the demand to achieve a content of 200 mg talinolol per tablet.
Dissolution of these tablets was tested in media of different pH values (pH 1.0, 3.2, 5.5, and 6.8). The profiles are displayed in Figures III.22 to III.25. Standard deviations were in part smaller than the particular icons of the dissolution curves.

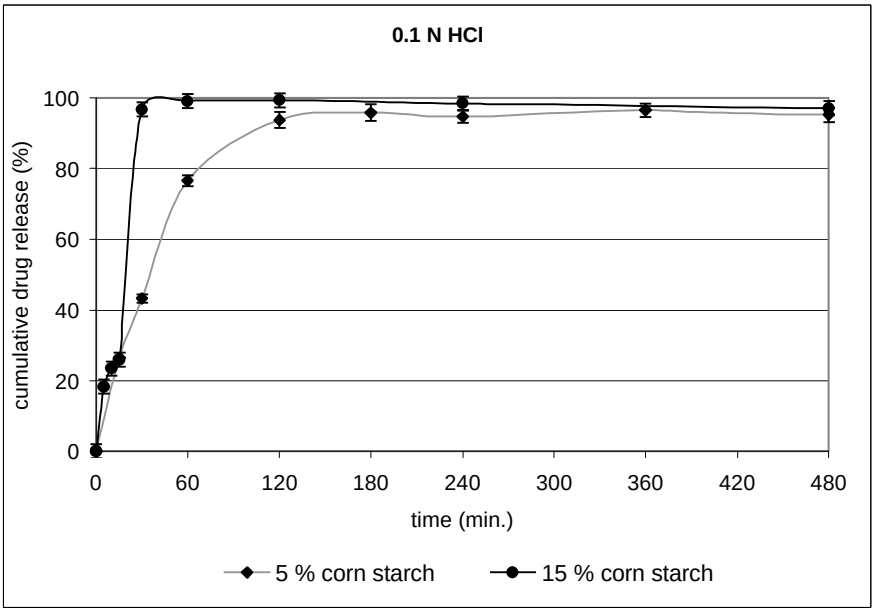


Figure III.22:

Talinolol release from sustained-release matrix tablets with a drug content of 200 mg. Formulations: 47.5 % Eudragit® S 100, 5 % corn starch, 47.5 % talinolol / 15 % corn starch, 85 % talinolol. Dissolution conditions: 0.1 N HCl (**pH 1.0**) paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

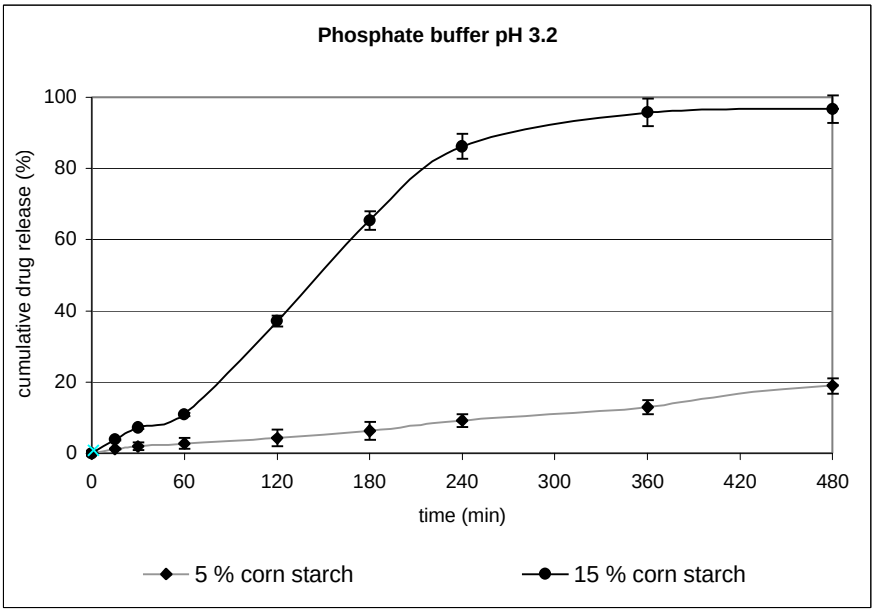


Figure III.23:

Talinolol release from sustained-release matrix tablets with a drug content of 200 mg. Formulations: 47.5 % Eudragit® S 100, 5 % corn starch, 47.5 % talinolol / 15 % corn starch, 85 % talinolol. Dissolution conditions: phosphate buffer **pH 3.2,** paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

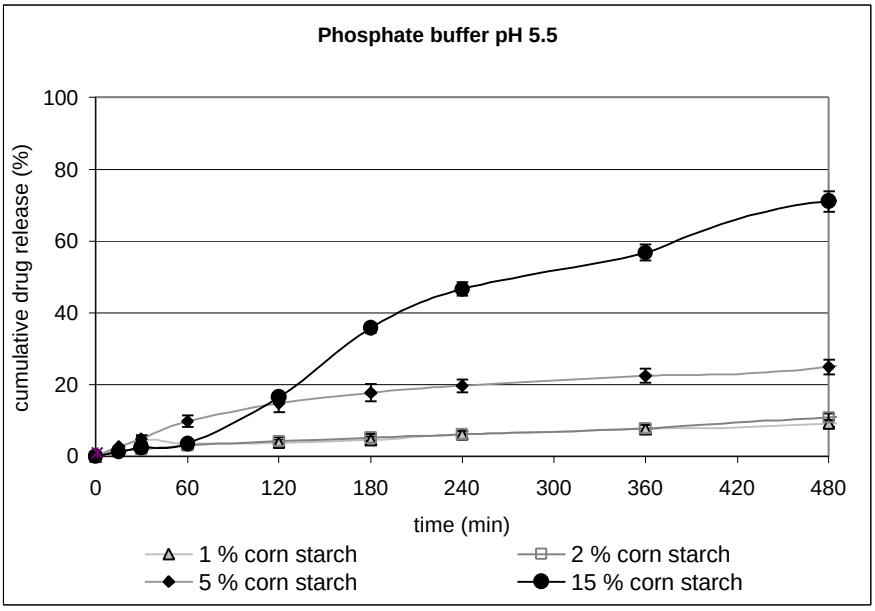


Figure III.24:

Talinolol release from sustained-release matrix tablets with a drug content of 200 mg. Formulations: 49.5 % Eudragit® S 100, 1 % corn starch, 49.5 % talinolol / 49 % Eudragit® S 100, 2 % corn starch, 49 % talinolol / 47.5 % Eudragit® S 100, 5 % corn starch, 47.5 % talinolol / 15 % corn starch, 85 % talinolol. Dissolution conditions: phosphate buffer **pH 5.5,** paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

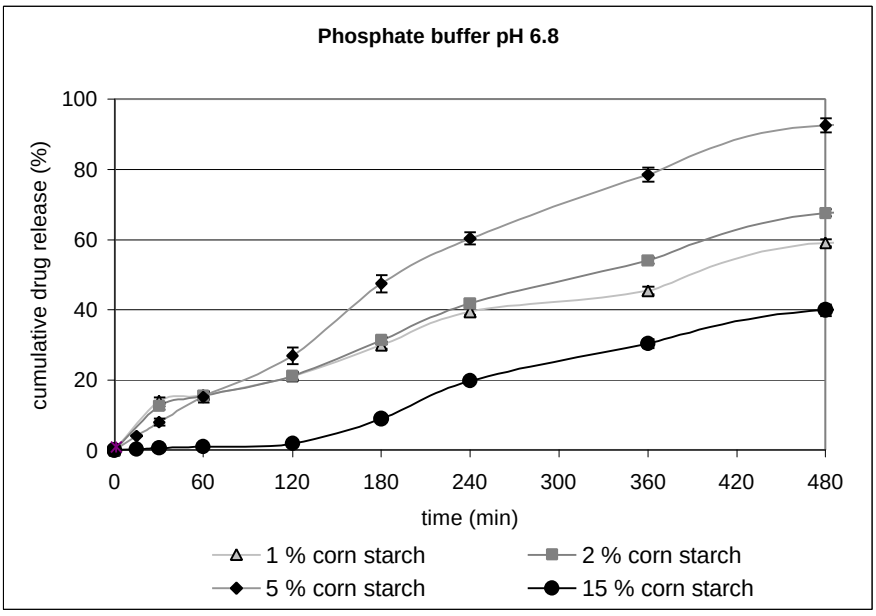


Figure III.25:

Talinolol release from sustained-release matrix tablets with a drug content of 200 mg. Formulations: cp. Figure III.24. Dissolution conditions: phosphate buffer **pH 6.8,** paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

In hydrochloric acid at pH 1.0 the formulation with 5 % corn starch, which showed the most appropriate dissolution behaviour in phosphate buffer of pH 6.8, failed. The drug release was nearly 80 % within the first hour. As expected, the tablets consisting of corn starch and talinolol only, without any Eudragit® added, dissolved even faster. More than 90 % of the drug was released within 30 minutes. As the drug release properties of the formulations containing only 1 and 2 % corn starch were inferior to the one containing 5 % corn starch at pH values of 5.5 and 6.8, these formulations were not tested at pH values of 1.0 and 3.2.

Also at pH 3.2 the dissolution of talinolol from tablets containing only drug and corn starch was too rapid (more than 85 % within four hours), whereas the formulation containing Eudragit® S 100 and 5 % corn starch demonstrated inadequate drug release (less than 50 % and less than 20 % release within eight hours).

At pH 5.5 the drug release from all formulations was too slow and incomplete within eight hours. Even the tablets containing only talinolol and corn starch without any release-sustaining excipient added showed a drug release of merely 70 % after eight hours.

At pH 6.8 the tablets containing 5 % corn starch consistently released more than 90 % of the drug within eight hours, which matched the requested profile. The dissolution profiles of the tablets containing only 1 and 2 % corn starch were not adequate but still rather close to the requested profile with a consistent release of 60 to 70 % over eight hours. Solely the drug release from the tablets containing only talinolol and corn starch was much too slow and incomplete. In spite of the high percentage of corn starch, less than 40 % of the drug were released after eight hours. This unexpected effect can be explained by the decreased solubility of talinolol at pH 6.8 in combination with the lack of the release-sustaining polymer. Eudragit® S 100 swells and dissolves at this pH value and hence facilitates the intrusion of buffer and the wetting of the drug. Since the formulation with a corn starch content of 15 % did not contain any of this polymer, the lack of these two effects led to a decreased drug release rate.

Summarizing the results for the four test formulations it can be stated that the main problem was the strong pH influence on the dissolution. When the drug release profile was adequate at one particular pH, it was either too slow or too fast at one of the other pH values.

Drug release independent of the pH could not be achieved with any of the formulations containing Eudragit® S 100 and corn starch. Thus adequate release profiles were not expected from other formulations with the same ingredients in different ratios, since any increase of the starch fraction would lead to rapid drug release in the acidic pH-range. On the other hand, increases in the amount of Eudragit® S 100 could decelerate talinolol dissolution in the acidic pH-range, but would at the same time cause decelerations at higher pH values, resulting in an incomplete drug release after eight hours, in particular at pH 3.2 and pH 5.5.

III.3.4.6 Matrix tablets formulated with a combination of Eudragit® RSPO and Eudragit® S 100

The desired dissolution profiles of sustained-release talinolol matrix tablets should be as much as possible independent of the pH of the dissolution medium. Formulations with either Eudragit® RSPO or Eudragit® S 100 did not match this aim. Therefore, matrix tablets containing a combination of both polymers were prepared.
The following thoughts guided this formulation selection:

1) Eudragit® S 100 as anionic polymer delays the drug release in 0.1 N HCl. Due to its insolubility in the acidic pH range, it prevents the formulation from releasing the drug rapidly in the stomach. At pH values larger than 6.5 the polymer dissolves.

2) Eudragit® RSPO ensures constant, pH-independent drug release.

3) Talinolol itself as a weak base dissolves more rapidly in the acidic pH range than at the physiological pH values of lower intestinal regions. This rapid release should be compensated by the addition of a polymer with acidic functional groups (Eudragit® S 100).

In Table III.9 the test formulation containing a mixture of the two release-sustaining polymers Eudragit® S 100 and Eudragit® RSPO is presented.

Table III.9:
Composition of powder mixture for the direct compression of talinolol sustained-release matrix tablets. Eudragit® S 100 and Eudragit® RSPO are employed as release-sustaining excipients.

Form. no.	Talinolol		Eudragit® S 100		Eudragit® RSPO		Mass per tablet
020306B	10.0 g	40 %	10.0 g	40 %	5.0 g	20 %	0.500 g

The dissolution profiles of talinolol in 0.1 N hydrochloric acid, phosphate buffers pH 3.2, 5.5 and 6.8 and an acetate buffer pH 4.5 are shown in Figure III.26.

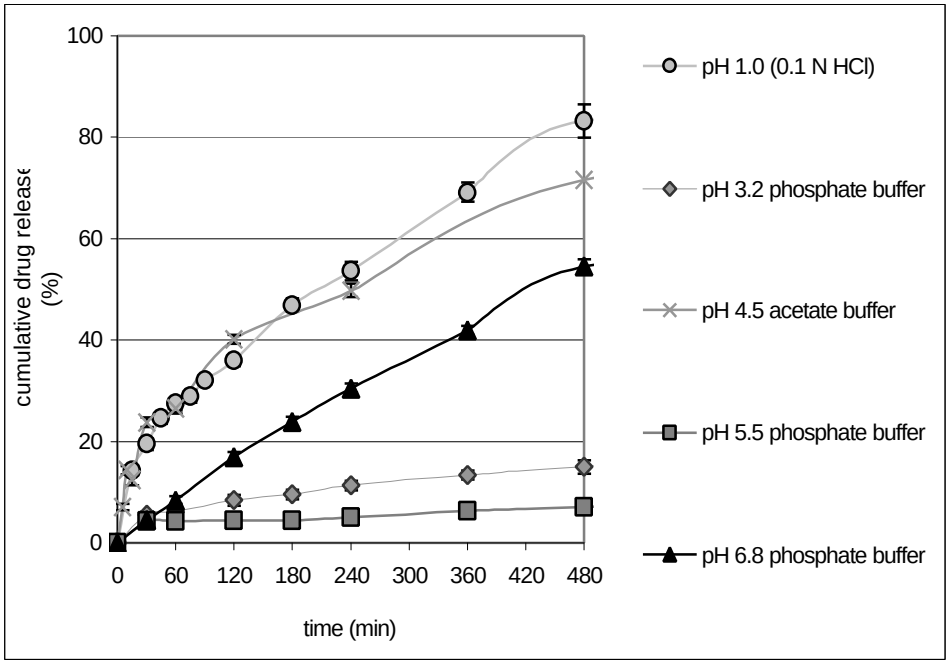


Figure III.26:

Talinolol release from sustained-release matrix tablets with a drug content of 200 mg. Formulation: 40 % Eudragit® S 100, 20 % Eudragit® RSPO, 40 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffers pH 3.2, 5.5, and 6.8, paddle apparatus, 50 rpm, 37°C, means ± SD, n = 3.

In the three most commonly used media, i. e. 0.1 N HCl, acetate buffer pH 4.5 and phosphate buffer pH 6.8, the tablets showed a relatively linear and constant drug release of 54.5 to 83.2 % within 8 hours, but in phosphate buffers of pH 3.2 and 5.5 dissolution was much too slow.

III.3.4.7 Matrix tablets formulated with Eudragit® L 100-55

In order to moderately accelerate the dissolution of talinolol at pH values of 4.5 and 6.8, Eudragit® L 100-55 was tested as alternative polymer. It slows down the drug release in the acidic range similar to Eudragit® S 100. However, Eudragit® L 100-55 dissolves and releases the drug already at pH values of appoximately 5.5, while Eudragit® S 100 as well as Eudragit® L 100 clearly dissolve and release the drug only at pH values above 6.5.

To determine the appropriate fraction of this excipient necessary for achieving the projected dissolution profiles, three tablet formulations with different amounts of Eudragit® L 100-55 were prepared. In the second formulation corn starch was added as pore former. Table III.10 gives an overview of the test formulations.

Table III.10:

Composition of directly compressible powder mixtures for 200 mg talinolol sustained-release matrix tablets formulated with Eudragit® L 100-55. In formulation no. 020421C corn starch is employed as pore former.

Form. no.	Talinolol		Eudragit® L 100-55		Corn starch		Mass per tablet
020421B	2.0 g	60 %	1.3 g	40 %	-	-	0.333 g
020421C	2.0 g	39 %	3.0 g	59 %	0.1 g	2 %	0.500 g
020421A	2.0 g	33.3 %	4.0 g	66.6 %	-	-	0.600 g

Dissolution behaviour of the three tablet formulations was tested in 0.1 N HCl, acetate buffer pH 4.5 and phosphate buffer pH 6.8, the media that were found most suitable for dissolution tests with talinolol in previous investigations. Additionally, dissolution was tested in phosphate buffer pH 4.5 to allow a comparison between the dissolution in a phosphate buffer and an acetate buffer of the same pH.

Furthermore, dissolution tests were performed in Tris (i. e. Tris-(hydroxymethyl)-aminomethane) buffer of pH 6.8 to investigate whether dissolution in this buffer differs markedly from that in phosphate buffer of pH 6.8. Tris buffer is commonly used as physiological buffer in particular in cell culturing and biochemical experiments. Figures III.27 to III.31 depict the drug release profiles for these formulations and buffer systems, respectively.

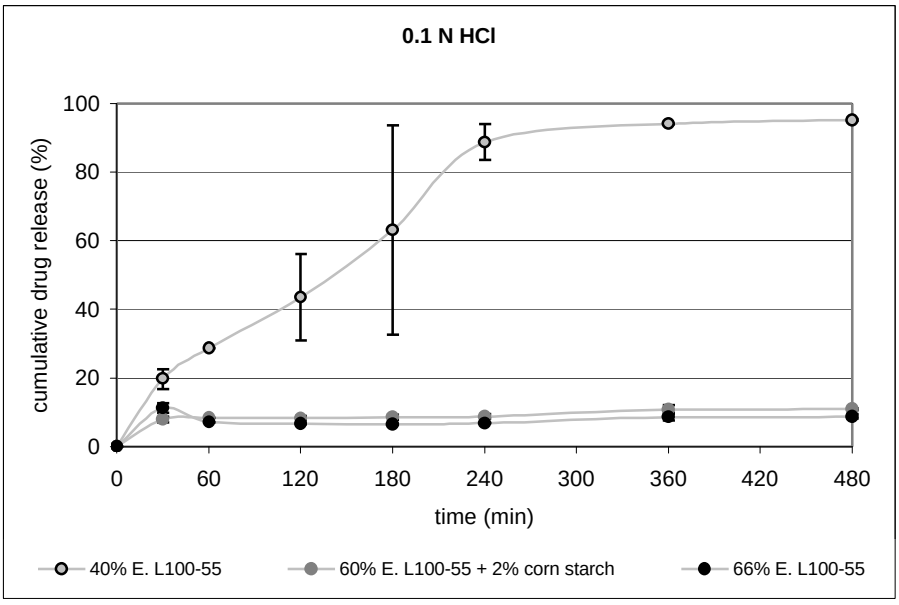


Figure III.27:

Dissolution of talinolol from sustained-release matrix tablets with a drug content of 200 mg.

Formulations: 40 % Eudragit® L 100-55, 60 % talinolol / 60 % Eudragit® L 100-55, 2 % corn starch, 38 % talinolol / 66 % Eudragit® L 100-55, 34 % talinolol. Dissolution conditions: 0.1 N HCl, paddle apparatus, 37°C, means ± SD, n = 3.

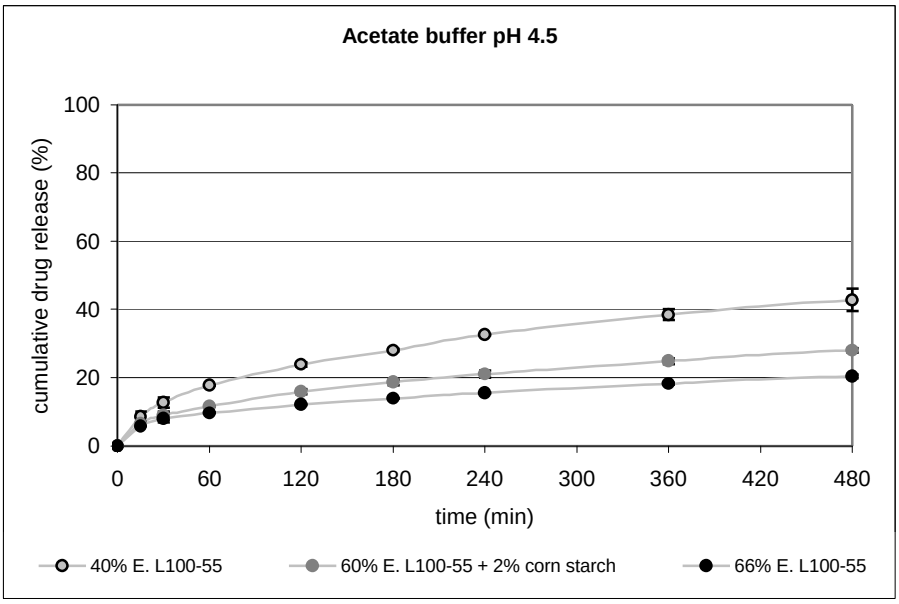


Figure III.28:

Dissolution of talinolol from sustained-release matrix tablets with a drug content of 200 mg.

Formulations: 40 % Eudragit® L 100-55, 60 % talinolol / 60 % Eudragit® L 100-55, 2 % corn starch, 38 % talinolol / 66 % Eudragit® L 100-55, 34 % talinolol. Dissolution conditions: acetate buffer pH 4.5, paddle apparatus, 37°C, means ± SD, n = 3.

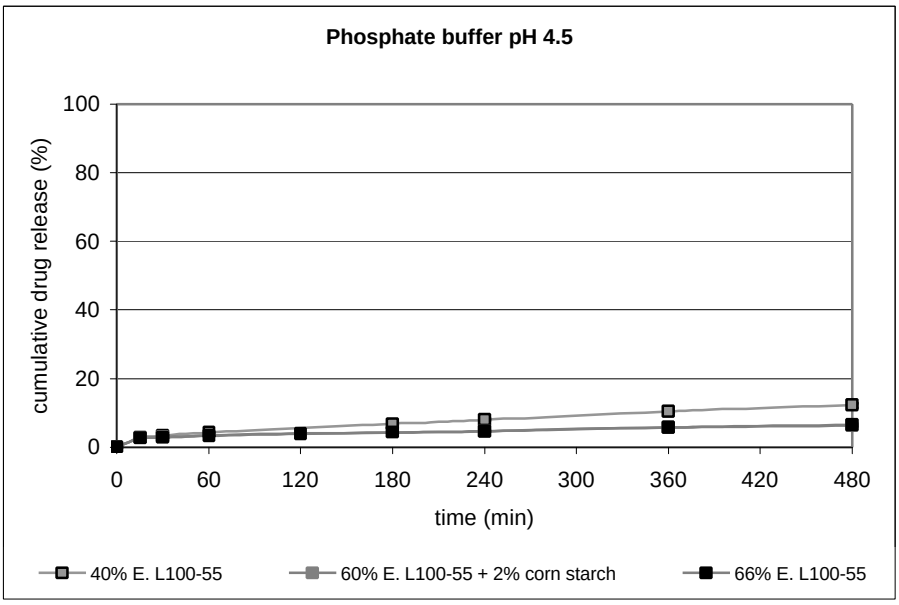


Figure III.29:

Dissolution of talinolol from sustained-release matrix tablets with a drug content of 200 mg.

Formulations: cp Figure III.28. Dissolution conditions: phosphate buffer pH 4.5, paddle apparatus, 37°C, means ± SD, n = 3.

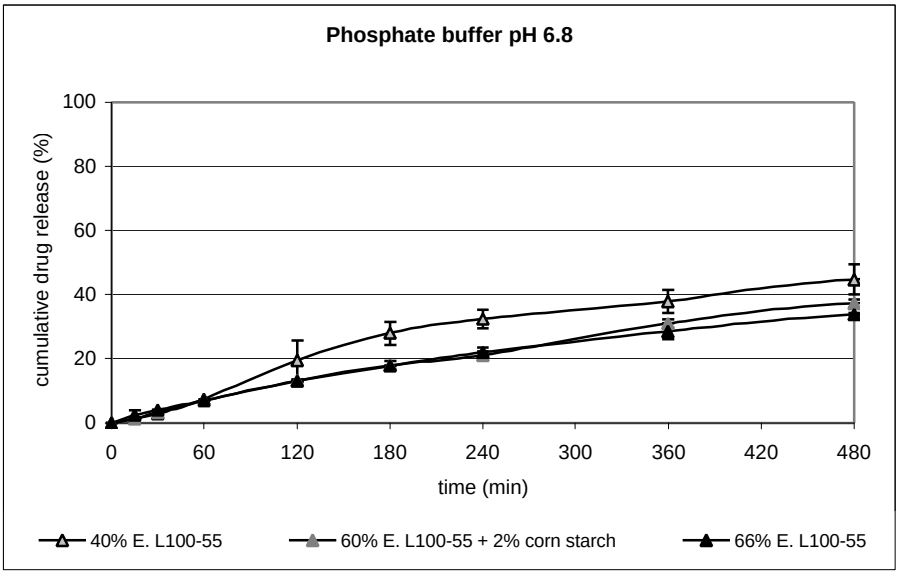


Figure III.30:

Dissolution of talinolol from sustained-release matrix tablets with a drug content of 200 mg.

Formulations: 40 % Eudragit® L 100-55, 60 % talinolol / 60 % Eudragit® L 100-55, 2 % corn starch, 38 % talinolol / 66 % Eudragit® L 100-55, 34 % talinolol. Dissolution conditions: phosphate buffer pH 6.8, paddle apparatus, 37°C, means ± SD, n = 3.

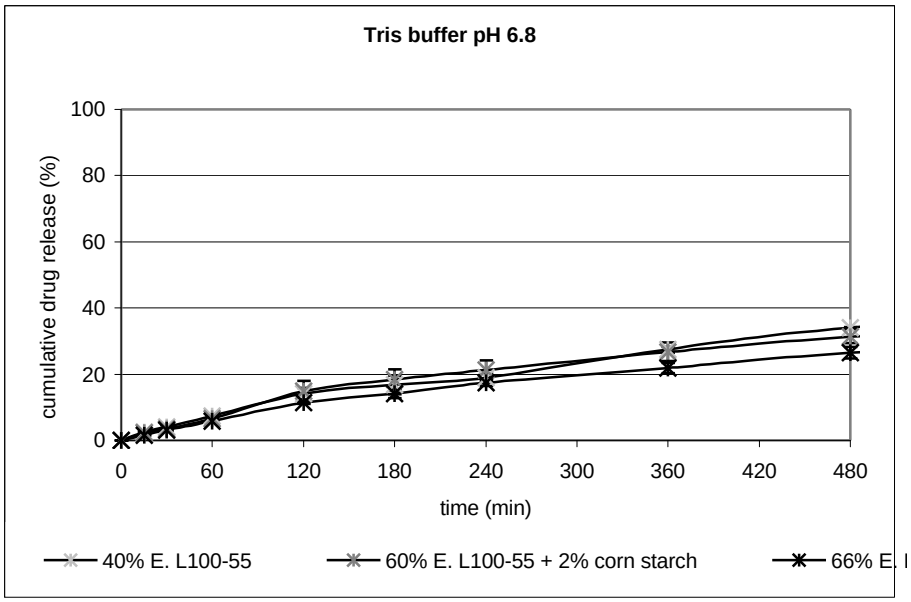


Figure III.31:

Dissolution of talinolol from sustained-release matrix tablets with a drug content of 200 mg.

Formulations: cp Figure III.30. Dissolution conditions: Tris buffer pH 6.8, paddle apparatus, 37°C, means ± SD, n = 3.

The impact of changes in the formulation on dissolution profiles was most discriminating for hydrochloric acid as dissolution medium, as can be seen in Figure III.27. Changes of the formulation had neglible impact on the dissolution of talinolol in phosphate buffers pH 4.5 and 6.8, in Tris buffer pH 6.8 and in acetate buffer pH 4.5 as well. In hydrochloric acid a reduction of the fraction of Eudragit® L 100-55 led to a faster dissolution. For example, the cumulative drug release after 4 hours raised from 6.7 and 8.7 % for the formulations containing 60 and 66 % Eudragit® L 100-55 up to 88.7 % for the formulation containing only 40 % of this polymer.

In general, none of the three formulations tested matched the desired release properties. Except for the formulation containing only 40 % Eudragit® L 100-55, that showed a too rapid dissolution at pH 1.0, drug release was too slow and incomplete after 8 hours in all dissolution media tested.

Furthermore, distinct differences between the dissolution profiles in acetate and phosphate buffers at pH 4.5 were detected, whereas a switch from phosphate buffer pH 6.8 to a Tris buffer of the same pH did not lead to relevant changes of the dissolution profiles of the formulations tested.

III.3.4.8 Matrix tablets formulated with Eudragit® L 100-55 and different pore formers (corn starch, PEG 4000, glucose, and mannitol)

Corn starch, polyethylene glycol 4000 (PEG 4000), glucose, and mannitol were tested as hydrophilic pore formers to increase the dissolution rates of talinolol from tablets formulated with Eudragit® L 100-55.

III.3.4.8.1 Corn starch

Corn starch was successfully employed previously to accelerate talinolol release in formulations with Eudragit® S 100. Table III.11 presents four test formulations with different fractions of corn starch in combination with Eudragit® L 100-55.

Table III.11:
Composition of directly compressible powder mixtures for talinolol sustained-release matrix tablets employing Eudragit® L 100-55 as release-sustaining excipient and corn starch as pore former.

Form. no.	Talinolol		Eudragit® L 100-55		Corn starch		Mass per tablet
020517A	2.0 g	45 %	2.0 g	45 %	0.4 g	10 %	0.444 g
020517B	2.0 g	40 %	2.0 g	40 %	1.0 g	20 %	0.500 g
020520A	2.0 g	50 %	1.5 g	37.5 %	0.5 g	12.5 %	0.400 g
020615A	1.9 g	34 %	3.0 g	54 %	0.6 g	11 %	0.588 g

Figures III.32 to III.35 give an overview of the drug release properties of the four tablet formulations in 0.1 N hydrochloric acid, acetate buffer pH 4.5 and phosphate buffer pH 6.8. Due to inappropriate dissolution properties at pH 4.5 and pH 6.8, dissolution of talinolol from the second and the fourth formulation (form. no. 020517B and form. no. 020615A) was not tested in hydrochloric acid.

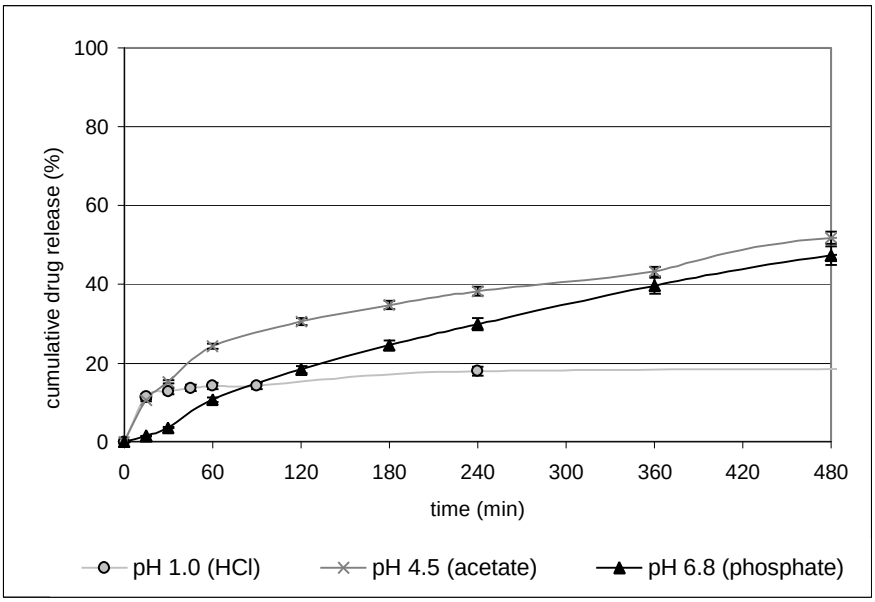


Figure III.32:

Dissolution of talinolol from sustained-release matrix tablets with a drug content of 200 mg.

Formulation: 45 % Eudragit® L 100-55, 10.0 % corn starch, 45 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8, paddle apparatus, 37°C, means ± SD, n = 3.

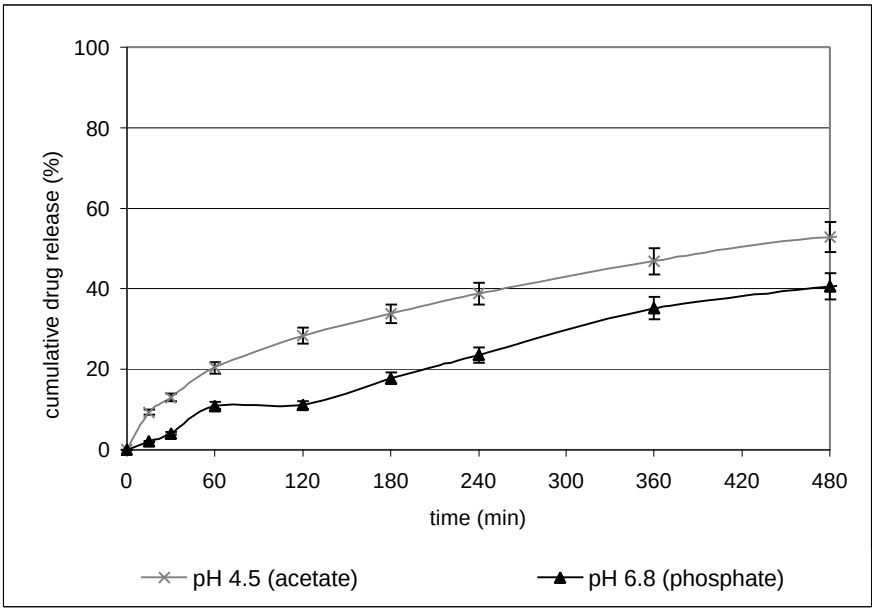


Figure III.33:

Dissolution of talinolol from sustained-release matrix tablets with a drug content of 200 mg.

Formulation: 40 % Eudragit® L 100-55, 20.0 % corn starch, 40 % talinolol. Dissolution conditions: cp. Figure III.32.

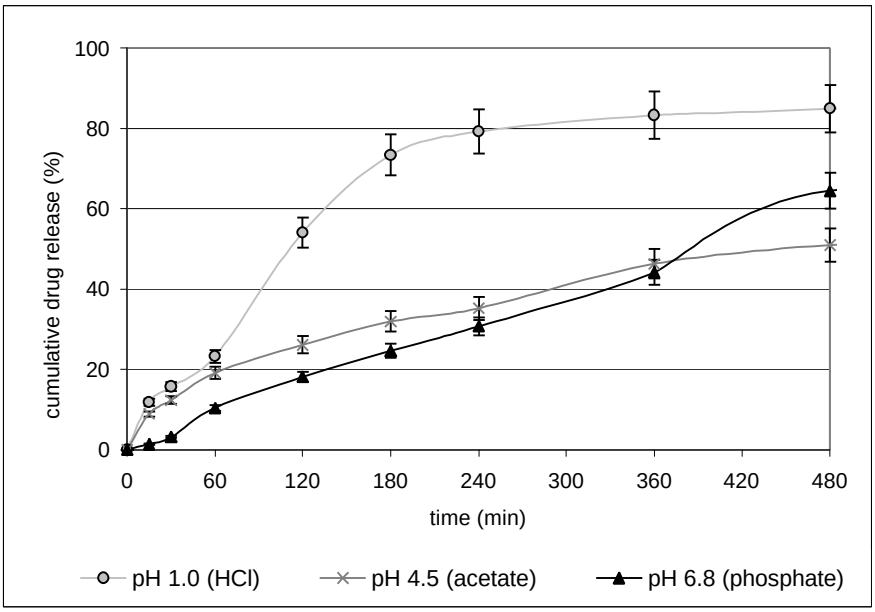


Figure III.34:

Dissolution of talinolol from sustained-release matrix tablets with a drug content of 200 mg.

Formulation: 37.5 % Eudragit® L 100-55, 12.5 % corn starch, 50 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8, paddle apparatus, 37°C, means ± SD, n = 3.

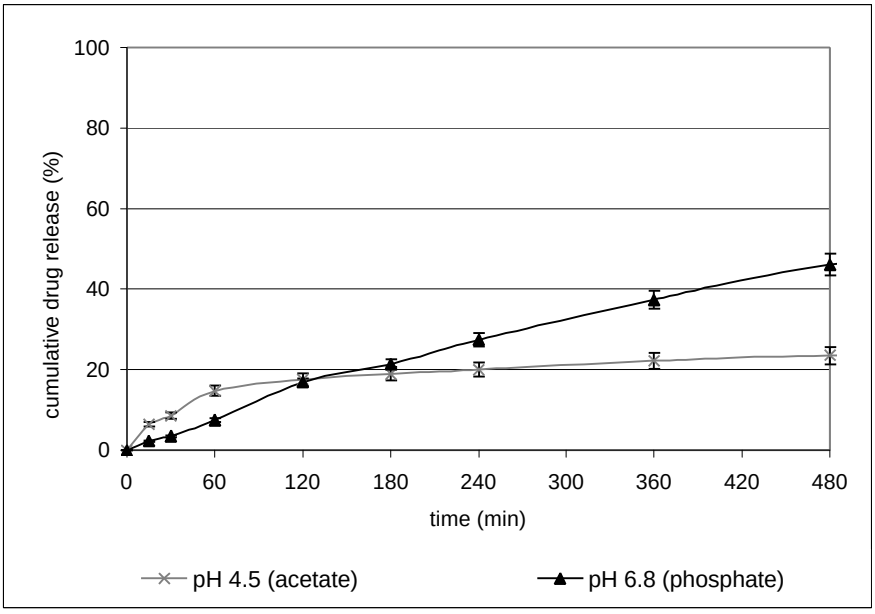


Figure III.35:

Dissolution of talinolol from sustained-release matrix with a drug content of 200 mg.

Formulation: 54.5 % Eudragit® L 100-55, 11.0 % corn starch, 34.5 % talinolol. Dissolution conditions: cp. Figure III.34, 0.1 N HCl was not tested.

None of the drug release profiles of the four test formulations was appropriate. Dissolution of talinolol from the formulation consisting of 12.5 % corn starch and 37.5 % Eudragit® L 100-55 (form. no. 020520A) was too fast. In hydrochloric acid, 73.4 % talinolol were dissolved after 3 hours. On the other hand, the formulations with higher release-sustaining Eudragit® fractions (forms. no. 020517A, 020517B, and 020615A) released the drug too slowly and incompletely. In all media tested drug release was lower than 60 % after 8 hours.

III.3.4.8.2 Polyethylene glycol 4000

A formulation containing 33 % talinolol, 50 %Eudragit® L 100-55, and 16.6 % PEG 4000 (Lipoxol 4000) (form. no. 020430C) was prepared to test the pore-forming properties of PEG 4000 in matrix tablets. According to disslution data published by Breitkreutz who investigated the influence of PEG 400 and PEG 4000 on Eudragit® based film coatings in modified-release tablets [Breitkreutz, J. (2000)], an acceleration of the drug release would have been expected. In his study, an addition of polyethylene glycol to acidic dissolution and disintegration media caused an acceleration of disintegration and dissolution of the active drug from tablets film-coated with Eudragit®. In spite of these findings, talinolol release from the matrix formulation tested was too slow and not complete within eight hours in all three media tested (24.8 % cumulative drug release in 0.1 N HCl, 23.1 % in acetate buffer pH 4.5, and 34.3 % in phosphate buffer pH 6.8 after eight hours).

III.3.4.8.3 Glucose

The drug release from a formulation employing glucose monohydrate (Cerestar CL) as hydrophilic pore former in matrix tablets formulated with Eudragit® L 100-55 (45 % talinolol, 45 % Eudragit® L 100-55, 10 % glucose, form. no. 020617B) was tested in 0.1 N hydrochloric acid, acetate buffer pH 4.5 and phosphate buffer pH 6.8. Similar to the results from the tablets employing polyethylene glycol 4000 as pore former (form. no. 020430C), dissolution was too slow and incomplete. In all three test media, less than 60 % of the drug were dissolved after 8 hours.

III.3.4.8.4 Mannitol

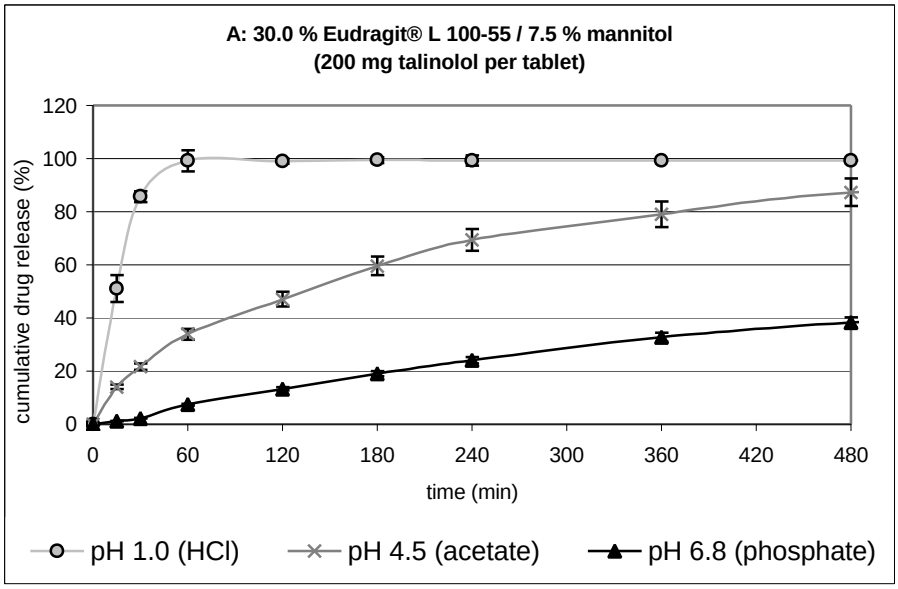
The hydrophilic six carbon sugar alcohol mannitol was successfully tested to accelerate the drug release from matrix formulations by Kim et al. [Kim, J. E., S. R. Kim, et al. (2000)]. To investigate its influence on the drug release from talinolol matrix tablets formulated with Eudragit® L 100-55, different formulations with varying mannitol fractions were prepared. Table III.12 shows the composition of the powder mixtures.

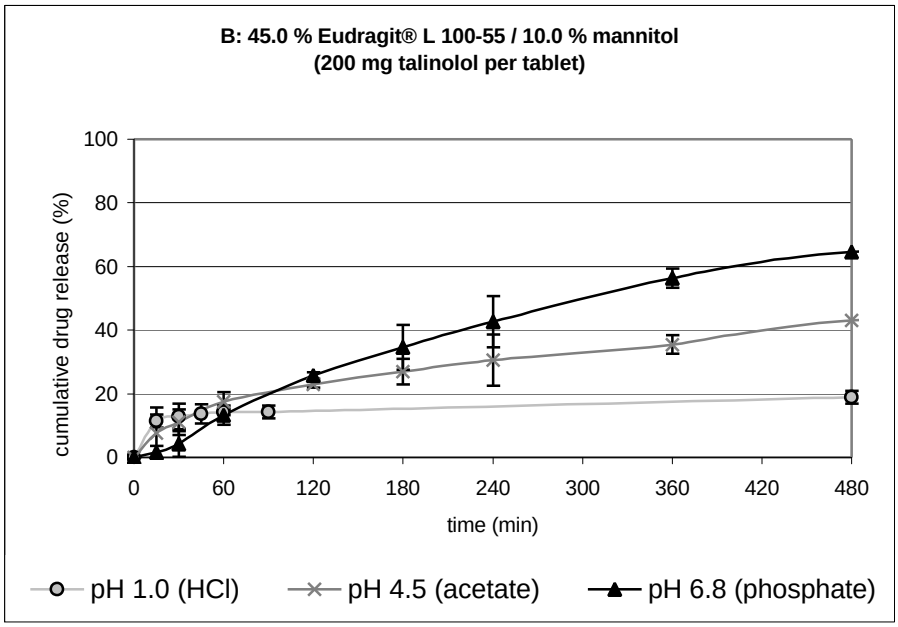
Table III.12:

Composition of powder mixtures for 200 mg talinolol sustained-release matrix tablets formulated with Eudragit® L 100-55 and mannitol.

	Form. no.	Talinolol		Eudragit® L 100-55		Mannitol		Mass per tablet
A	020706B	2.0 g	62.5 %	0.96 g	30 %	0.24 g	7.5 %	0.320 g
B	020617A	2.0 g	45 %	2.0 g	45 %	0.44 g	10 %	0.444 g
C	020726A	10.0 g	50 %	7.0 g	35 %	3.0 g	15 %	0.400 g

Dissolution of the tablets was tested in 0.1 N hydrochloric acid, acetate buffer pH 4.5 and phosphate buffer pH 6.8. The results are presented in Figure III.36.


A: 30.0 % Eudragit® L 100-55 / 7.5 % mannitol
(200 mg talinolol per tablet)
cumulative drug release (%)
time (min)
pH 1.0 (HCl)
pH 4.5 (acetate)
pH 6.8 (phosphate)


B: 45.0 % Eudragit® L 100-55 / 10.0 % mannitol
(200 mg talinolol per tablet)
cumulative drug release (%)
time (min)
pH 1.0 (HCl)
pH 4.5 (acetate)
pH 6.8 (phosphate)

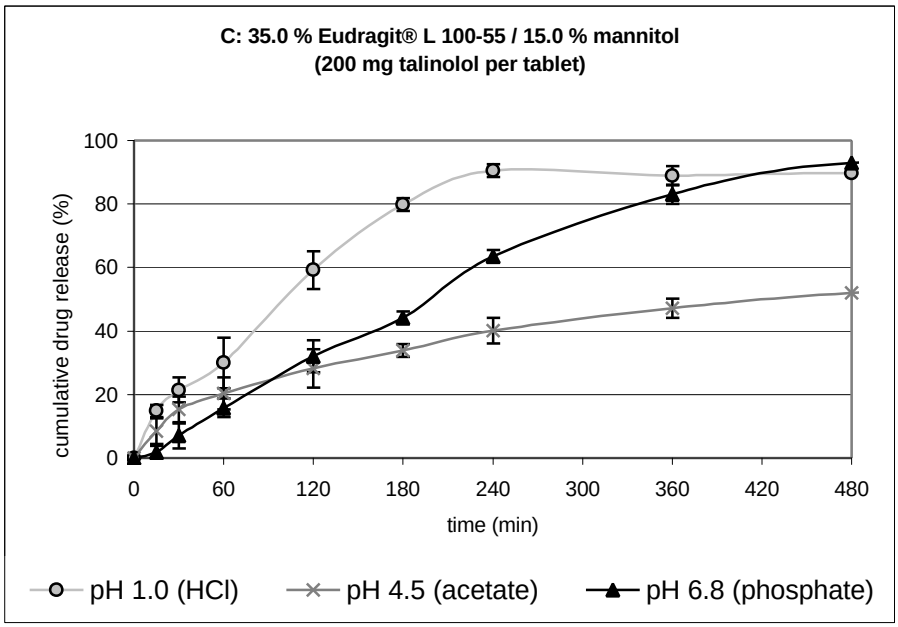

C: 35.0 % Eudragit® L 100-55 / 15.0 % mannitol
(200 mg talinolol per tablet)
cumulative drug release (%)
time (min)
pH 1.0 (HCl)
pH 4.5 (acetate)
pH 6.8 (phosphate)

Figure III.36 (on previous page):

Dissolution of sustained-release matrix tablets with a drug content of 200 mg. Formulations: A: 30 % Eudragit® L 100-55, 7.5 % mannitol, 62.5 % talinolol / B: 45 % Eudragit® L 100-55, 10 % mannitol, 45 % talinolol / C: 35 % Eudragit® L 100-55, 15 % mannitol, 50 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8, paddle apparatus, 37°C, means ± SD, n = 3.

Figure III.36 A shows that a portion of 30 % Eudragit® L 100-55 was not sufficient to achieve a suitable control over the drug release at pH 1.0. After 30 minutes, the complete dose of talinolol was already dissolved.

On the other hand, Figure III.36 B outlines that tablets formulated with a combination of 45 % Eudragit® L 100-55 and 10 % mannitol (formulation B, form. no. 020617A) release the drug too slowly. In all three dissolution media the cumulative drug release was lower than 65 % after 8 hours.

As expected, the reduced Eudragit® content (35 % Eudragit®) in formulation C (form. no. 020726A) in combination with the increased mannitol fraction (15 % mannitol) compared to formulation B (form. no. 020617A) lead to an acceleration of the drug release in all three test media (Figure III.36 C). The effect can be observed most explicitly at pH 1.0: Here the drug release was faster than in the two other media, whereas for formulation B it was just the other way round. This can be explained by the different Eudragit® contents. In the acidic pH range, the drug release from formulation B (form. no. 020617A) is slow due to the high fraction (45 %) of Eudragit® L 100-55 that is insoluble at pH values below 5.5. The high polymer concentration enables the formation of a stronger polymer network than in formulation C where the polymer content is lower (35 %) [Krajacic, A. and I. G. Tucker (2003)]. The drug release is accelerated, since the polymer network in this matrix is not that strong. At pH 6.8, this effect plays a minor role since the polymer swells and dissolves in this pH range and the release-sustaining properties of Eudragit® L 100-55 are not that pronounced. Therefore, at a pH value of 6.8, the lower Eudragit® content in formulation C (form. no. 020726A) leads to a less pronounced acceleration of the drug release than at a pH value of 1.0.

However, when compared with formulations A and B, the acceleration of the dissolution of talinolol from formulation C is still obvious at pH 6.8, whereas the

release profile at pH 4.5 differs only marginally from the one of formulation B. After 8 hours the cumulative drug release at that pH was incomplete for both formulations (43.0 % for formulation B versus 52.1 % for formulation C).

III.3.4.9 Considerations on the appropriate talinolol dose per tablet

Dissolution tests during the development phase of sustained-release dosage forms and further tests on the influence of different dissolution media on talinolol solubility and dissolution (cp. chapter IV) demonstrated the strong impact of changes in the composition of dissolution media on the in vitro dissolution of talinolol. Hence it has to be expected that in vivo dissolution might differ explicitly from in vitro results. By testing two different doses in equal formulations with the same in vitro dissolution behaviour in a clinical human study, both, dissolution effects and the influence of intestinal secretory transporters, can be studied.

The development of matrix tablets yielding identical dissolution of active substance but containing different doses of the drug is difficult, because size and shape of the release-controlling matrix change as soon as the composition is modified. Dissolution is influenced by such changes due to altered diffusion properties of drug within the matrix.

Independent of any changes in the formulation, the radius (r) of the tablet remains the same as long as the same punch is used for the compression of the matrix tablet. However, the height (h) of the tablet changes with any alterations in the tablet formulation. The tablet becomes thinner with decreasing tablet masses and thicker with increasing tablet masses. As an example to illustrate the influence of tablet size and shape on the drug release from matrix tablets, Figure III.37 shows two tablets (A and B) consisting of the same powder mixture. For tablet A two times the amount of powder were compressed compared to tablet B. Therefore, tablet A has approximately twice the thickness of tablet B. The black dots indicate drug molecules in the center of the particular tablet. For these molecules, the shortest possible diffusion distance to the surface of the tablet is twice higher in tablet A. Assuming a constant diffusion rate of the drug through both matrices, tablet B can be expected to release the entire drug incorporated more rapidly than tablet A, although both tablets consist of the same powder mixture.

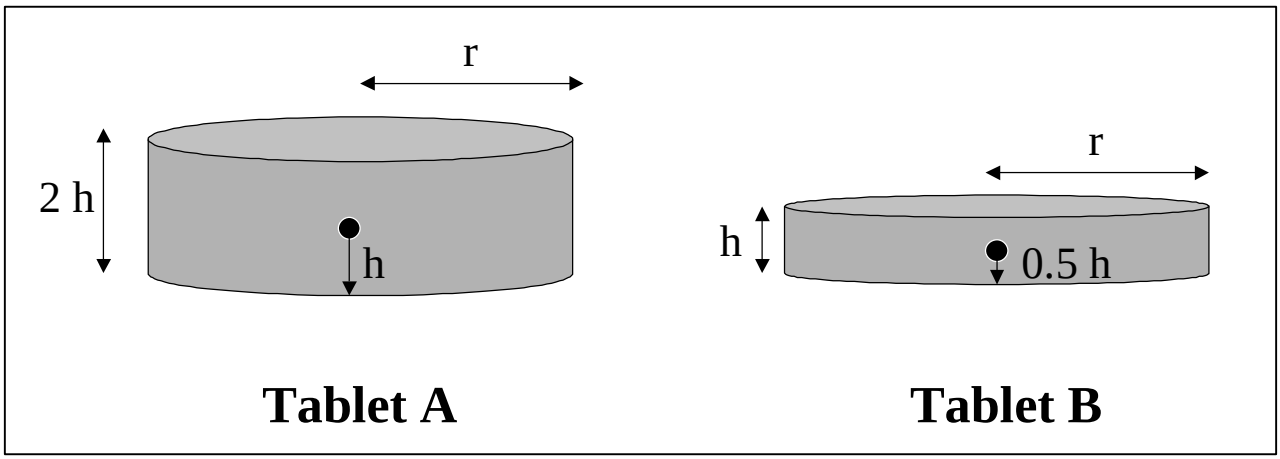


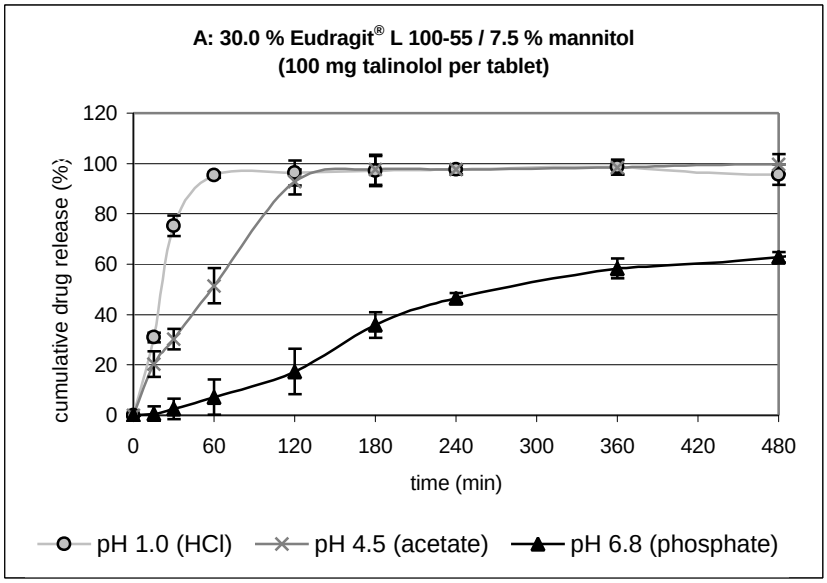
Figure III.37:
Scheme demonstrating the influence of tablet size and shape on the drug release from matrix tablets. Tablets A and B, both with the same radius r, consist of the same powder mixture. Since tablet A contains the double amount of powder compared to tablet B, tablet A has approximately two times the height of tablet B. The black dots indicate drug molecules in the center of the tablets. The arrows describe the shortest possible diffusion distance for these molecules through the particular matrix. For tablet B, this distance is half as long as for tablet A.

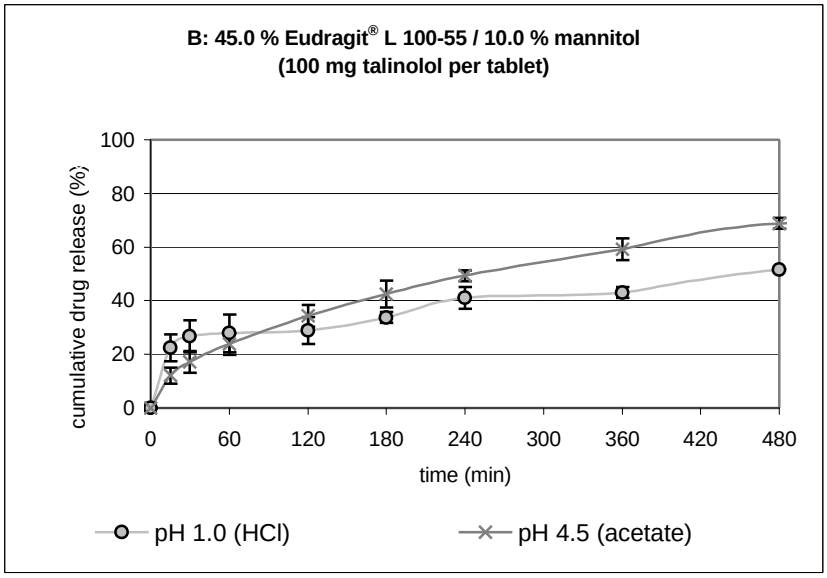
An alternative to the administration of two different tablets containing different drug doses is to administer one tablet in the first phase of the clinical study and two tablets of the same type during a second phase. Thus, the dose is doubled without changes in the dissolution profiles caused by altered matrix size and shape. A disadvantage that has to be accepted with this approach is the fact that the two tablets when administered together may not show identical passage times through the gastrointestinal tract. The residence times in the different gastrointestinal regions might vary between the two dosage forms even when administered together. In particular, differences in gastric emptying could lead to variable passage times of the tablets through the gastrointestinal tract.
The administration of two tablets containing 200 mg of active drug each would exceed the normal therapeutic dose range of talinolol. Therefore, a dose of 100 mg talinolol per matrix tablet would be more appropriate.

III.3.4.10 Talinolol matrix tablets with a drug content of 100 mg

As a consequence of the considerations on the appropriate dose for talinolol sustained-release dosage forms, matrix tablets with a talinolol content of 100 mg were compressed. The same powder mixtures containing Eudragit® L 100-55 and mannitol (forms. no. 020706B, 020617A, and 020726B) were used as for the previously manufactured 200 mg talinolol tablets. The resulting total tablet masses for the formulations A, B, and C were 160 mg, 222 mg, and 200 mg, respectively. Due to the lower weight of the tablets containing only 100 mg talinolol in combination with their rather large diameter, the dosage forms did not sink to the bottom of the dissolution vessels but floated. This phenomenon made it necessary to use sinkers in order to maintain the tablets at the bottom of the vessels.

In Figure III.38 the dissolution profiles of talinolol matrix tablets formulated with different fractions of Eudragit® L 100-55 and mannitol (cp. Table III.12) containing 100 mg talinolol are presented. Dissolution tests were performed using the same dissolution media as for the tablets containing 200 mg talinolol (0.1 N hydrochloric acid, acetate buffer pH 4.5, phosphate buffer pH 6.8).


A: 30.0 % Eudragit® L 100-55 / 7.5 % mannitol
(100 mg talinolol per tablet)
cumulative drug release (%)
time (min)
pH 1.0 (HCl)
pH 4.5 (acetate)
pH 6.8 (phosphate)


B: 45.0 % Eudragit® L 100-55 / 10.0 % mannitol
(100 mg talinolol per tablet)
cumulative drug release (%)
time (min)
pH 1.0 (HCl)
pH 4.5 (acetate)

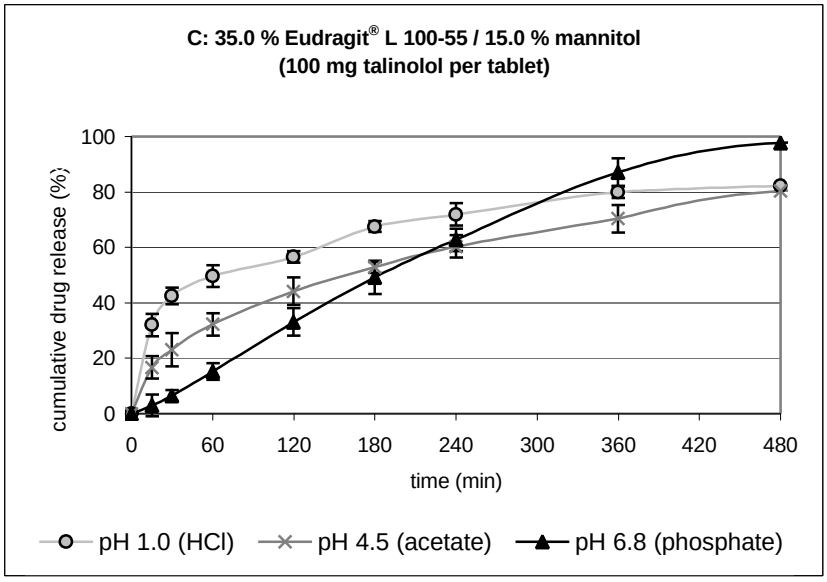
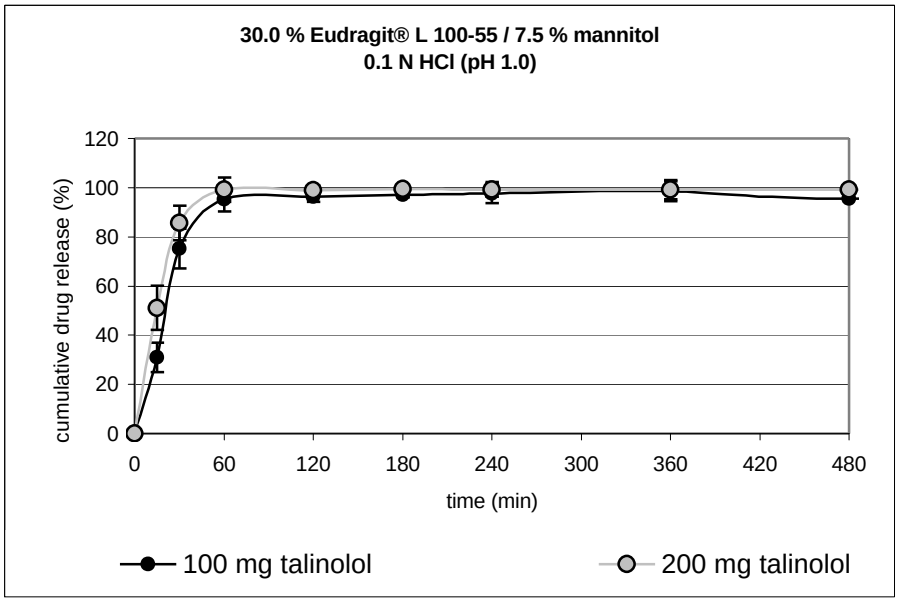
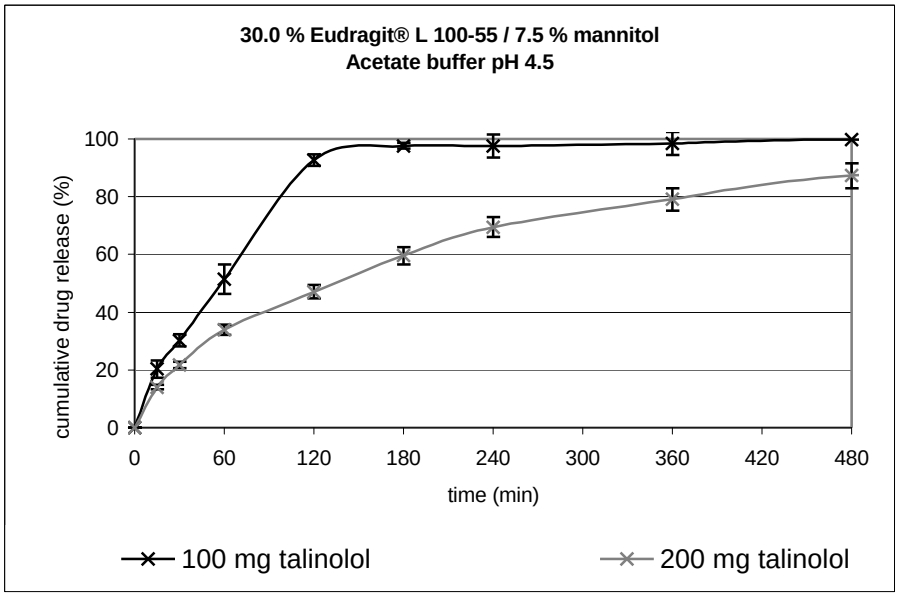

C: 35.0 % Eudragit® L 100-55 / 15.0 % mannitol
(100 mg talinolol per tablet)
cumulative drug release (%)
time (min)
pH 1.0 (HCl)
pH 4.5 (acetate)
pH 6.8 (phosphate)

Figure III.38 (on previous page):
Dissolution of sustained-release matrix tablets with a drug content of 100 mg. Formulations: A: 30 % Eudragit® L 100-55, 7.5 % mannitol, 62.5 % talinolol / B: 45 % Eudragit® L 100-55, 10 % mannitol, 45 % talinolol / C: 35 % Eudragit® L 100-55, 15 % mannitol, 50 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8, paddle apparatus, 37°C, means ± SD, n = 3.

The altered tablet thickness resulted in the expected changes of the dissolution profiles: The thinner matrix with a diminished diffusion distance caused an accelerated drug release rate.
Exemplarily, the dissolution profiles of tablets containing 100 mg and 200 mg talinolol formulated with 30 % Eudragit L 100-55 and 7.5 % mannitol (Formulation A) are compared in Figure III.39.


30.0 % Eudragit® L 100-55 / 7.5 % mannitol
0.1 N HCl (pH 1.0)
cumulative drug release (%)
time (min)
100 mg talinolol
200 mg talinolol


30.0 % Eudragit® L 100-55 / 7.5 % mannitol
Acetate buffer pH 4.5
cumulative drug release (%)
time (min)
100 mg talinolol
200 mg talinolol

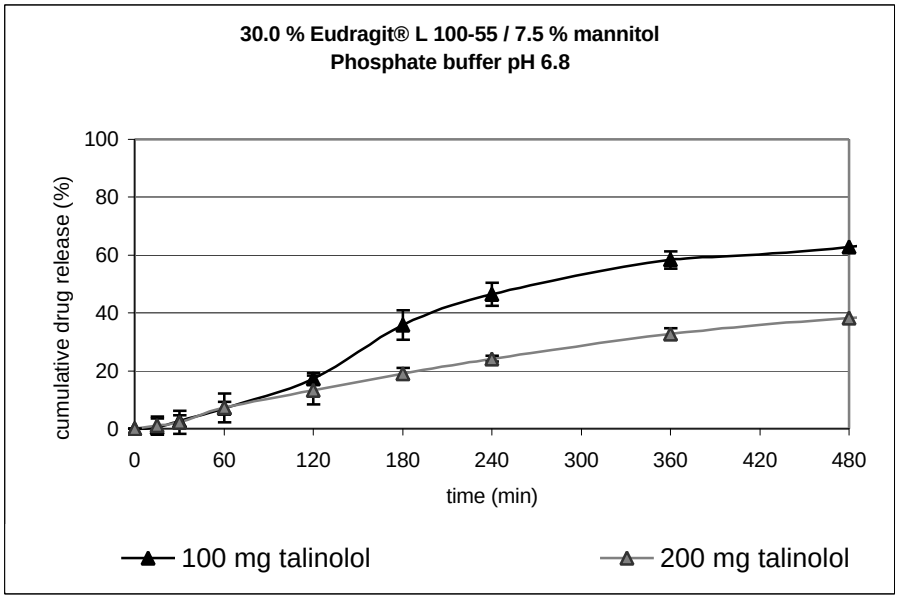

30.0 % Eudragit® L 100-55 / 7.5 % mannitol
Phosphate buffer pH 6.8
cumulative drug release (%)
time (min)
100 mg talinolol
200 mg talinolol

Figure III.39 (on previous page):

Comparison of talinolol dissolution from sustained-release matrix tablets containing 100 mg and 200 mg talinolol, both.
Formulation: 30 % Eudragit® L 100-55, 7.5 % mannitol, 62.5 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8, paddle apparatus, 37°C, means ± SD, n = 3.

At pH 1.0 no apparent differences between the dissolution of talinolol from 100 mg versus 200 mg could be observed. Due to the rapid drug release from both tablets the discriminatory power gets lost. At pH values of 4.5 and 6.8, apparent differences between the cumulative drug release of 100 mg and 200 mg tablets were detected. For example, at pH 4.5 approximately 45 % of the talinolol dose were released from the tablets containing 200 mg after two hours whereas more than 90 % of the incorporated dose were dissolved from the tablets containing 100 mg within the same time interval. The differences are so pronounced that not only the previously described phenomenon of decreased diffusion distances for the thinner tablets containing 100 mg talinolol (cp Fig. III.37) may play a role. In addition, it must be assumed that abrasion and superficial matrix erosion are relatively higher for the thinner 100 mg talinolol tablets. Since the radius for the 100 mg and 200 mg tablets is equal, the surface area differs only slightly between the two dosage forms. This means that the working surface for abrasion processes is similar for both tablets, though drug content is doubled for the 200 mg tablets. Consequently, relative to the total amount of drug molecules incorporated, more drug molecules are located in marginal regions in the 100 mg tablets and are thus exposed to abrasion of outer layers of the matrix. This leads to a more rapid release of talinolol from the thinner tablets containing only 100 mg talinolol.

III.3.4.11 Formulation optimization of talinolol matrix tablets with a drug content of 100 mg

The dissolution properties of the matrix tablets formulated with 35 % Eudragit® L 100-55 and 15 % mannitol (form. no. 020726A2) and a talinolol content of 100 mg presented in Figure III.36 (Formulation C) were rather close to the required criteria for an appropriate talinolol sustained-release dosage form. In all three test media 80 to 100 % of the drug were released continuously over 8 hours, though the dissolution profiles were not identical.

However, the burst effect with a drug release rate of more than 50 % within the first hour in hydrochloric acid, demanded an optimization of the formulation. Apparently, the reason for the burst effect, that was even more pronounced for formulation A with a polymer content of merely 30 %, is that a polymer fraction of 35 % or less is not sufficient to obtain a stable, non-eroding matrix. This assumption is supported by the findings of Boza et al. who postulated the existence of a percolation treshold, i.e. a minimum amount of polymer necessary to obtain stable matrices [Boza, A., I. Caraballo, et al. (1999)]. Below these concentrations the insoluble polymer forms only finite clusters, without a coherent structure. In consequence, when drug molecules dissolve within such a matrix, the tablet tends to disintegrate. Since burst effects were observed when formulations contained less than 40 % Eudragit® L 100-55, a threshold similar to the one reported for Eudragit® RSPO (i. e. between 40 and 50 % [Boza, A., I. Caraballo, et al. (1999)]) can be assumed for this polymer.

To optimize the drug release, further formulations had to be tested. Based on the results from dissolution tests with the formulations presented in Table III.12 (A - C), two more powder mixtures containing Eudragit® L 100-55 and mannitol were prepared (D and E).

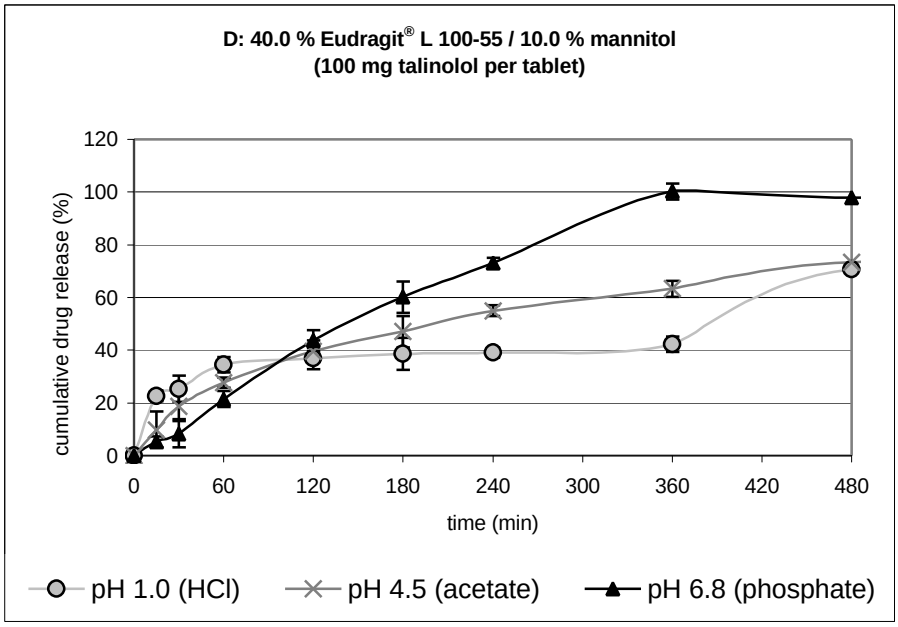
Compared to formulation B (Eudragit content: 45 %) the drug release rate had to be accelerated and compared to formulations A (Eudragit content: 30 %) and C (Eudragit content: 35 %) it had to be decelerated in the acidic pH range. Therefore, an intermediate Eudragit content of 40 % was chosen for the new test formulations, combined with relatively high mannitol fractions to prevent incomplete drug release, in particular at pH 6.8.

The composition of the formulations is given in Table III.13.

Table III.13:
Composition of powder mixtures for 100 mg talinolol sustained-release matrix tablets formulated with Eudragit® L 100-55 and mannitol.

	Form. no.	Talinolol		Eudragit® L 100-55		Mannitol		Mass per tablet
D	020623A	2.0 g	50 %	1.6 g	40 %	0.4 g	10 %	0.200 g
E	020623B	2.0 g	40 %	2.0 g	40 %	1.0 g	20 %	0.250 g

Dissolution of talinolol from these formulations in 0.1 N hydrochloric acid, acetate buffer pH 4.5, and phosphate buffer pH 6.8 is shown in Figure III.40.



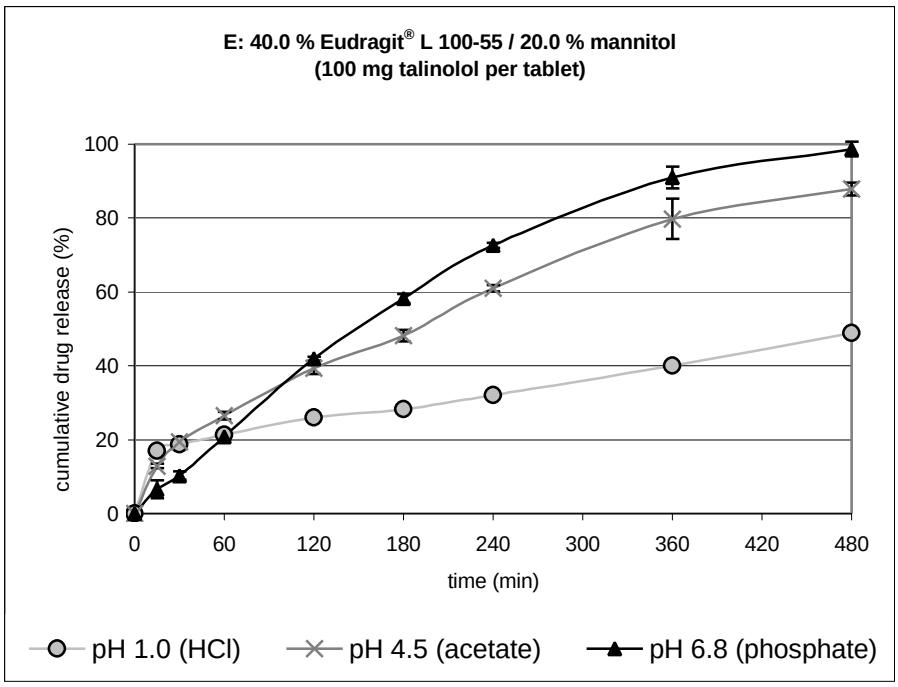


Figure III.40:

Dissolution of sustained-release matrix tablets with a drug content of 100 mg. Formulations: 40 % Eudragit® L 100-55, 10 % mannitol, 50 % talinolol / 40 % Eudragit® L 100-55, 20 % mannitol, 40 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8, paddle apparatus, 37°C, means ± SD, n = 3.

Formulation D with a higher content of Eudragit® and a lower mannitol fraction compared to formulation C did not show an improved dissolution behaviour. At pH 6.8 the complete drug was released after 6 hours, whereas drug release at the lower pH values was incomplete after 8 hours.

At pH 4.5 and pH 6.8, the dissolution profiles of formulation E with both, a higher Eudragit® and a higher mannitol content compared to formulation C, were similar to those of formulation C with a continuous drug release within eight hours, whereas at pH 1.0 only approximately 50 % were dissolved after eight hours.

In spite of the incomplete dissolution in the acidic pH, formulation E is more appropriate for an in vivo investigation than formulation C. According to Lindahl et al. who have reported a median pH of 1.8 in the human stomach in the fasted state [Lindahl, A., A. L. Ungell, et al. (1997)], hydrochloric acid is a relevant dissolution medium to mimic the gastric residence time of the matrix tablet. The time the tablet remains in the stomach can vary [Davis, S. S., J. G. Hardy, et al. (1986)]. Average residence times for solid substances are reported to be 30 to 40 minutes in the fasted state [Wagner, J. G. (1971)]. Therefore, in in vitro dissolution tests in 0.1 N hydrochloric acid, the drug release rate within the initial period is decisive for the decision on an appropriate sustained-release formulation. Due to the burst effect described previously for formulation C with a drug release of more than 50 % within the first hour at pH 1.0, this formulation is inappropriate. On the other hand, dissolution of talinolol from formulation E is adequate at pH 1.0 within the first two hours (20.9 % after one hour, 25.9 % after two hours).

After leaving the stomach, the tablet enters the upper small intestine. For jejunum, Mahe et al. reported an average physiological pH of 5.1 under fasted conditions [Mahe, S., J. F. Huneau, et al. (1992)], Lindahl et al. reported a relevantly higher mean pH value of 7.1 for the same intestinal segment in the fasted state [Lindahl, A., A. L. Ungell, et al. (1997)]. However, both working groups suggest pH values far beyond 1.0 for the upper small intestine. Since an appropriate drug release from the matrix tablets of formulation E was shown at pH values of 4.5 and 6.8, their incomplete dissolution at pH 1.0 after 8 hours in vitro is not relevant for the in vivo situation, because the tablets do not reside at an acidic pH for long time periods.

Hence, it was decided to use formulation E (40 % Eudragit® L 100-55 and 20 % mannitol) to manufacture 100 mg talinolol matrix tablets for the projected in vivo study in humans. More detailed information about this clinical study investigating the

influence of a decelerated drug release rate from the sustained-release matrix tablet on the P-glycoprotein mediated secretion in the gastrointestinal tract is given in Annex II.

III.3.4.12 Summary of talinolol matrix formulations

In Table III.14 the formulations of all matrix tablets tested and the cumulative drug release from these tablets after one and eight hours in three relevant dissolution media (0.1 N HCl, acetate buffer pH 4.5 or phosphate buffer pH 5.5, and phosphate buffer pH 6.8) are composed.

Table III.14 (continued on the following page):
Composition and drug release properties of talinolol sustained-release matrix tablets. The drug content per tablet was 200 mg, unless stated otherwise. 0.1 N HCl (pH 1.0), acetate buffer pH 4.5 or phosphate buffer pH 5.5, and phosphate buffer pH 6.8 were used as dissolution media. The fractions of the components in the formulations and the amounts of drug released are rounded for better clarity.
Within the table, italicized bold font indicates that the dissolution of talinolol from the particular matrix formulation was too rapid; normal font indicates that the dissolution was too slow; underlined font indicates that the drug release in the particular dissolution medium was in an acceptable range.

Active drug	**Release-sustaining excipients**				**Pore former**	**Drug release at**					
Talinolol	**Eudragit® RSPO**	**Eudragit® S 100**	**Eudragit® RLPO**	**Ethocel®**	**Corn starch**	**pH 1.0**		**pH 5.5**		**pH 6.8**	
						1 h	**8 h**	**1 h**	**8 h**	**1 h**	**8 h**
40 %	60 %					***50 %***	***>90 %***	-		<5 %	<5 %
40 %		60 %				-		<10 %	40 %	<10 %	<10 %
40 %			60 %			-		-		<5 %	<10 %
40 %				60 %		***60 %***	***>90 %***	-		<5 %	<5 %
50 %		50 %				-		<10 %	10 %	15 %	70 %
33 %		50 %			17 %	-		***>90 %***	***>90 %***	-	
50 %		49 %			1 %	-		<5 %	10 %	15 %	60 %
49 %		49 %			2 %	-		<5 %	10 %	15 %	70 %
48 %		47 %			5 %	***75 %***	***>90 %***	10 %	25 %	15 %	>90 %
85 %					15 %	***>90 %***	***>90 %***	5 %	70 %	<5 %	40 %
40 %	20 %	40 %				30 %	>80 %	<10%[1]	10 %[1]	10 %	55 %

Active drug	Rel. sust. excip.	Pore formers				Drug release at					
Talinolol	Eudragit® L 100-55	Corn starch	PEG 4000	Glucose	Mannitol	pH 1.0		pH 4.5		pH 6.8	
						1 h	8 h	1 h	8 h	1 h	8 h
60 %	40 %					30 %	>90 %	20 %	45 %	<5 %	10 %
67 %	33 %					<10 %	10 %	10 %	20 %	<5 %	10 %
33 %	50 %		17 %			10 %	25 %	15 %	25 %	10 %	35 %
45 %	45 %			10 %		20 %	40 %	20 %	45 %	10 %	55 %
39 %	59 %	2 %				<10 %	10 %	10 %	30 %	<5 %	15 %
45 %	45 %	10 %				15 %	20 %	25 %	50 %	10 %	50 %
34 %	54 %	11 %				-		15 %	20 %	<10 %	45 %
50 %	38 %	12 %				20 %	>80 %	20 %	50 %	10 %	65 %
40 %	40 %	20 %				-		20 %	55 %	10 %	40 %
63 %	30 %				7 %	***>90 %***	***>90 %***	***35 %***	***>80 %***	10 %	40 %
45 %	45 %				10 %	15 %	20 %	15 %	40 %	15 %	60 %
50 %	35 %				15 %	30 %	>90 %	20 %	50 %	20 %	>90 %
63 %[2]	30 %[2]				7 %[2]	***>90 %***	***>90 %***	***50 %***	***>90 %***	<10 %	60 %
50 %[2]	40 %[2]				10 %[2]	***35 %***	***70 %***	25 %	70 %	20 %	>90 %
45 %[2]	45 %[2]				10 %[2]	30 %	55 %	25 %	70 %	-	
50 %[2]	35 %[2]				15 %[2]	***50 %***	***>80 %***	30 %	80 %	20 %	>90 %
40 %[2]	40 %[2]				20 %[2]	20 %	50 %[3]	25 %	>80 %	20 %	>90 %

[1] drug release tested at pH 4.5.

[2] 100 mg talinolol content per tablet.

[3] cp. discussion on the relevance of an incomplete drug release in 0.1 N HCl.

III.3.4.13 Pharmaceutical characterization of 100 mg talinolol sustained-release tablets formulated with 40 % Eudragit® L 100-55 and 20 % mannitol

Apart from drug release tests, the tablet formulation selected for an in vivo evaluation (Formulation E, form. no. 020623B) was characterized in terms of tablet thickness, uniformity of mass, crushing strength and friability.

III.3.4.13.1 Tablet thickness

The average thickness of the tablets was 1.29 mm. Table III.15 presents the results of the single measurements.

Table III.15:
Thickness of ten randomly selected 100 mg sustained-release tablets (40 % talinolol, 40 % Eudragit® L 100-55, 20 % mannitol).

Thickness	
1.30 mm	**Mean:** 1.29 mm
1.30 mm	
1.15 mm	**Standard deviation (S.D.):** 0.077 mm
1.28 mm	
1.40 mm	**Highest value measured:** 1.40 mm
1.34 mm	
1.27 mm	**Lowest value measured:** 1.15 mm
1.30 mm	
1.19 mm	
1.38 mm	

III.3.4.13.2 Uniformity of mass

In Table III.16 the results of the uniformity of mass test are presented. The tablets complied with the demands of the monograph of the European Pharmacopeia.

Table III.16:
Uniformity of mass test of talinolol sustained-release tablets formulated with 40 % Eudragit® L 100-55 and 20 % mannitol with a drug content of 100 mg. The theoretical total mass of one tablet was 250 mg.

Mass of one tablet	
251 mg	**Mean:** 250.5 mg
252 mg	
251 mg	**Standard deviation (S.D.):** 2.58 mg
251 mg	
252 mg	**Highest value measured:** 255 mg
255 mg	
248 mg	**Lowest value measured:** 244 mg
249 mg	
252 mg	**Acceptable range according to Ph.Eur.:** 250.5 mg ± 18.8 mg (± 7.5 %) 231 mg - 269 mg
249 mg	
253 mg	
255 mg	
250 mg	
249 mg	
244 mg	
252 mg	
247 mg	
250 mg	
250 mg	
249 mg	

III.3.4.13.3 Crushing strength

The results of the measurements of the crushing strength are presented in Table III.17.

Table III.17:
Crushing strength of 100 mg talinolol sustained-release tablets formulated with 40 % Eudragit® L 100-55 and 20 % mannitol. The values given represent the force at which the tablets crushed.

Crushing strength	
106 N	**Mean:** 96 N
86 N	
93 N	**Standard deviation (S.D.):** 12.1 N
94 N	
78 N	**Highest value measured:** 118 N
78 N	
118 N	**Lowest value measured:** 78 N
91 N	
104 N	
96 N	
101 N	
108 N	

The crushing strength of the tablets was sufficient for the projected purpose. All tablets measured resisted at least a force of 78 N before crushing. Crushing strength reported for tablets in literature vary to a large extent. This is in part due to the different instruments used for the measurement of the crushing strength. Additionally the crushing strendth is strongly influenced by the composition of the particular tablets and the compression force applied during their manufacturing process.

Friability

The friability test according to the European Pharmacopeia was performed three times. The results are composed in Table III.18.

Table III.18:
Friability tests performed with twenty 100 mg talinolol sustained-release tablets formulated with 40 % Eudragit® L 100-55 and 20 % mannitol. The drum of the friability tester rotated 100 times (4 minutes at 25 rpm). The test was performed three times.

Test No.	Initial mass	Final mass	Difference	Friability
1	5.030 g	4.975 g	0.055 g	1.09 %
2	5.133 g	5.088 g	0.045 g	0.88 %
3	5.102 g	5.080 g	0.022 g	0.43 %

Friability is defined as the loss of mass during the performance of the test expressed as percentage of the initial mass. The mean friability obtained from the three tests performed was 0.8 %. According to the European Pharmacopeia, a friability of up to 1 % is acceptable. [Ph.Eur., 3rd edition, <2.9.7>: “Friablity of uncoated tablets”]. Friabilities of tablets reported in literature are in a similar range or higher [Danckwerts, M. P. and J. G. van der Watt (1995), Ferrero, C. and M. R. Jimenez-Castellanos (2002), Ebube, N. K., A. H. Hikal, et al. (1997)]. The high friability in the first of the three tests is not due to the abrasion of small powder particles, but was caused by the crushing of one bigger pieces from the border of one of the twenty tablets. Therefore, an adequate friability of the tablets can be stated.

III.3.4.14 Final discussion on matrix tablets

Among different approaches for oral sustained-release dosage forms for commercial marketing, matrix tablets have been gaining increasing attention because of the simple and low-cost manufacturing process [Ferrero, C. and M. R. Jimenez-Castellanos (2002)]. Colombo et al. reported in the year 2000 that the majority of all oral drug delivery systems were matrix-based [Colombo, P., R. Bettini, et al. (2000)]. In the developmental phase of a talinolol sustained-release dosage form, apparent advantages of matrix tablets, when compared to granule formulations tested, were a constant within-lot and lot-to-lot homogeneity as well as a good mass and content uniformity. The manufacturing process was easy to handle and compliance with GMP standards was achieveable.

Embedding a drug within an insoluble matrix is a convenient way of controlling the drug release. In such a system, drug release is preceded by the penetration of the dissolution medium into the porous matrix to dissolve the drug, followed by diffusion of the dissolved molecules out of the matrix. Drug molecules located on the surface of the matrix will be dissolved first. Upon exhaustion of the surface, the drug depleted zone will increase progressively as the solid drug front recedes into the matrix [Azarmi, S., J. Farid, et al. (2002)], as illustrated in Figure III.41.

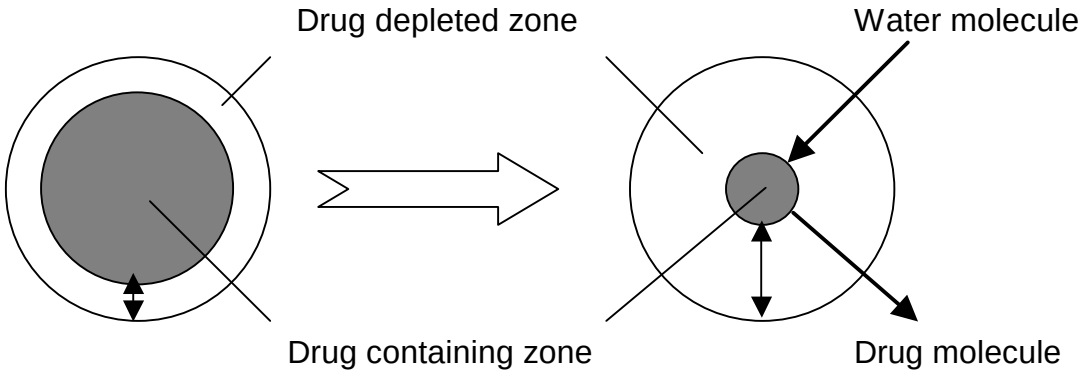


Figure III.41:
Drug release from a non-eroding matrix tablet. The left tablet represents the beginning diffusion from the matrix. In the right tablet the drug depleted zone has increased notably. The black arrows indicate the increase of the diffusion distance for water and drug molecules [Modified from Pather, S. I., I. Russell, et al. (1998)].

Due to the elongated diffusion distances for the penetration of water and drug molecules throughout the matrix, drug release from the tablet becomes progressively slower with time. In 1963 Higuchi published a theoretical analysis of the dissolution of drugs from matrices [Higuchi, T. (1963)]. He found a linear relationship between the cumulative drug release from matrix tablets (Q) and the square root of time ($t^{½}$):

$$Q = K \bullet t^{½} \qquad [1]$$

In this equation the slope K is the dissolution rate constant. The Higuchi relationship is applicable in praxis as long as drug release from the dosage form is strictly driven by Fickian diffusion. When disintegration, erosion or swelling occur, deviations from the relationship can be observed. In this context, erosion means the slow removal of particles from the surface of a tablet. The process is similar to disintegration, except that erosion is slow and occurs only at surfacial layers of the tablet [Pather, S. I., I. Russell, et al. (1998)].

Another deviation from the theoretical considerations of Higuchi observed in practical dissolution tests with matrix formulations is that the Higuchi equation assumes a y-intercept of zero in drug release versus root of time profiles, whereas the immediate dissolution of drug molecules located at the surface of a matrix can result in non-zero y-intercepts [Katikaneni, P. R., S. M. Upadrashta, et al. (1995)].

Figure III.42 presents a Higuchi plot (Cumulative drug release versus square root of time profile) of the talinolol matrix tablet formulation selected for the application in a human in vivo study.

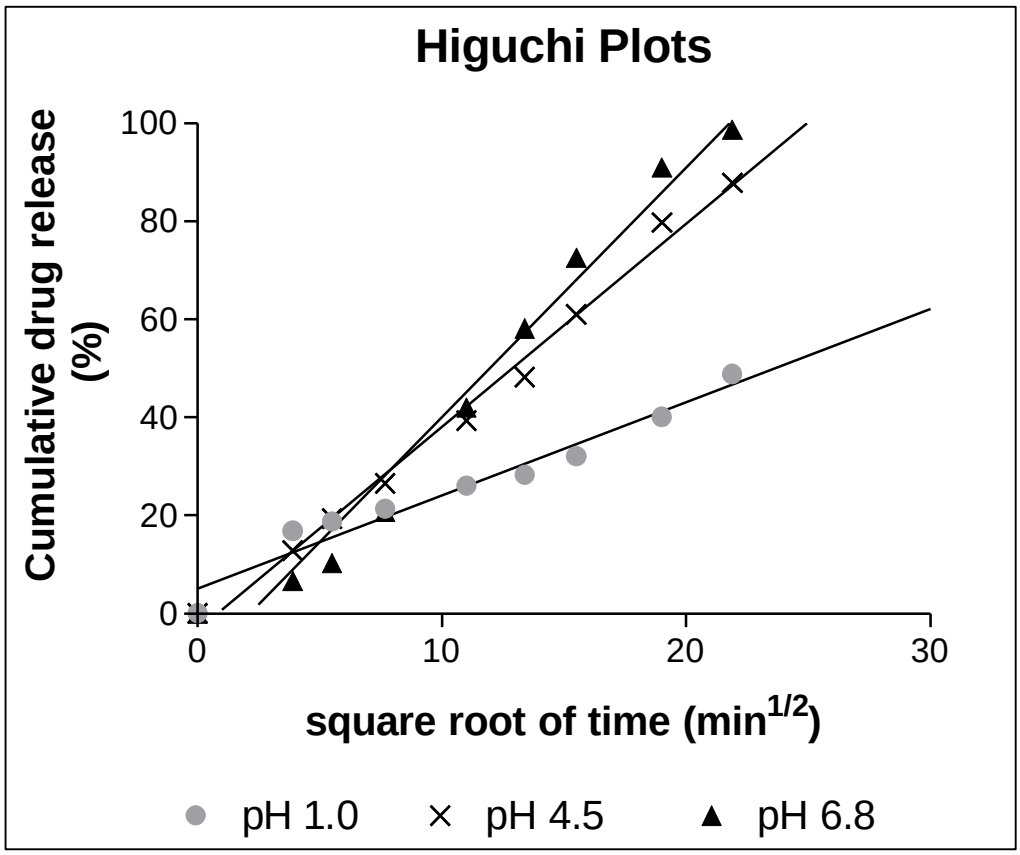


Figure III.42:

Cumulative drug release versus square root of time profile derived from dissolution studies with 100 mg talinolol sustained-release matrix tablets. The lines show the best fits obtained by linear regression analysis.
Formulation: 40 % Eudragit® L 100-55, 20 % mannitol, 40 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8, paddle apparatus, 37°C, means, n = 3, standard deviations were omitted to maintain clarity.

The Higuchi plots indicate that the dissolution rates are similar in acetate buffer pH 4.5 and phosphate buffer pH 6.8. The calculated dissolution rate constants (K) were in a similar range (4.1 and 5.1, rerspectively). The apparently lower dissolution rate constant of merely 1.9 in 0.1 N HCl is an expression of the slower dissolution in this medium. However, on the basis of drug release data from 0 to 60 min only (i.e. $t^{1/2}$ = 0 - 7.7 $min^{½}$), a higher dissolution rate constant of 3.1 is obtained. These findings reemphasize that in the initial dissolution period, which is of particular relevance for the in vivo situation, adequate dissolution of talinolol occurs in 0.1 N HCl.
A linear regression analysis of the drug release data can be used as indicator for the release mechanism from the matrix. If the drug is released from the matrix by Fickian

diffusion alone, the relationship between square root of time and cumulative drug release is linear [Higuchi, T. (1963)]. The involvement of other release mechanisms apart from diffusion results in non-linear Higuchi plots. For the drug release profiles of the talinolol test formulation dedicated for an in vivo evaluation the goodness of fit to the Higuchi equation varied dependent on the pH of the dissolution test medium. For pH 4.5 and 6.8 coefficients of determination (R^2) of 0.9919 and 0.9743 indicate a good fit to the Higuchi equation, wheras at pH 1.0 the fit was not that good (R^2 = 0.9522). The explanation for this is that an initial phase with a relatively high drug release rate is followed by a phase in which the decrease of the release rate is more pronounced than proposed by the theoretical considerations of Higuchi.
When factors apart from diffusion contribute to the drug release from a matrix, the model described by Ritger an Peppas [Ritger, P. L. and N. A. Peppas (1984)] is in many cases more appropriate for fitting dissolution data than the Higuchi model:

$$M_t / M_i = K \bullet t^n \qquad [2]$$

In this equation M_t and M_i are the amounts of drug released at time t and infinity, K is the release rate constant and n is the diffusional exponent. This exponent n is characteristic for the mechanism of the drug release. With an n value of 0.5, this equation is equal to the square root model described by Higuchi, which means that drug release from the matrix is driven by Fickian diffusion. For n > 0.5, an anomalous non-Fickian drug diffusion occurs, that can be due to swelling of the matrix. For n = 1, non-Fickian, case II, erosion-controlled or zero-order release kinetics can be observed [Majid Khan, G. and J.-B. Zhu (1999), Colombo, P., R. Bettini, et al. (2000)].

To describe the release kinetics from the sustained-release talinolol matrix tablet developed, the dissolution data was fitted to the equation of Ritgers and Peppas. The results are presented graphically and numerically in Figure III.43 and Table III.15.

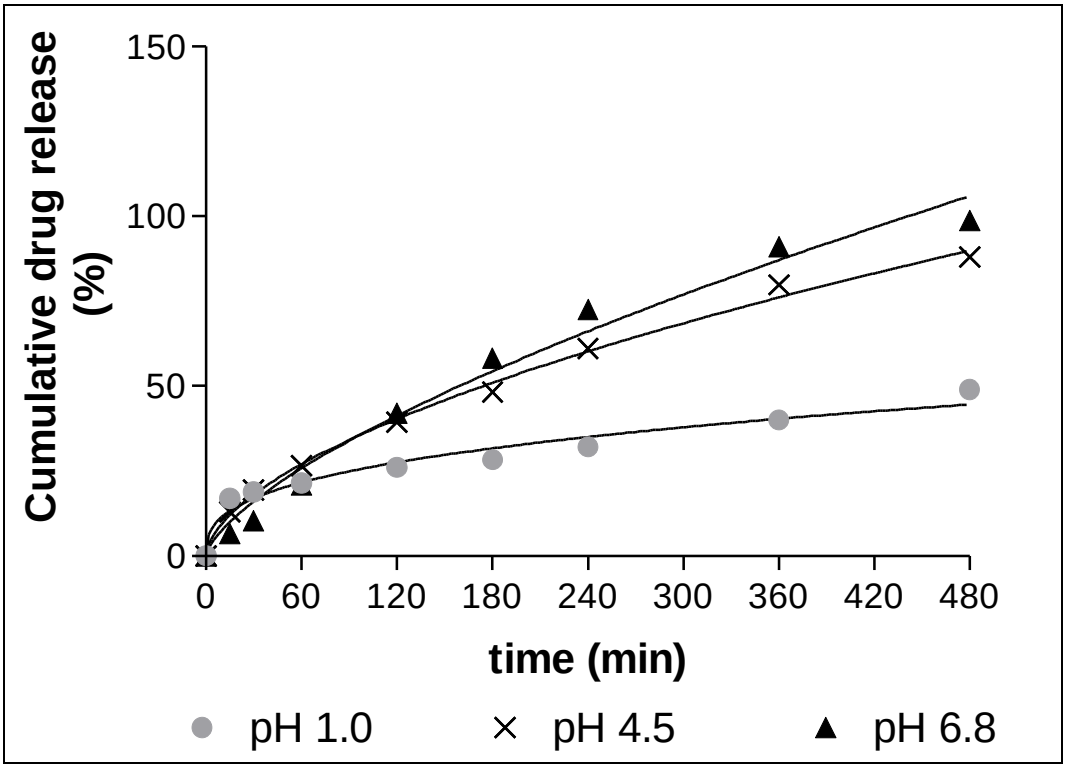


Figure III.43:

Talinolol release from sustained-release matrix tablets. The lines illustrate the best fit to the model of Ritgers and Peppas [Ritger, P. L. and N. A. Peppas (1984)]. M_i, i.e. the amount of drug released at infinity, is assumed to be 100 %.

Formulation: 40 % Eudragit® L 100-55, 20 % mannitol, 40 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8, paddle apparatus, 37°C, means, n = 3, error bars were omitted for the sake of clarity.

Table III.19:

Model parameters for the drug release characteristics from talinolol sustained-release matrix tablets. Dissolution data was fitted to the model published by Ritger and Peppas [Ritger, P. L. and N. A. Peppas (1984)]. K is the release rate constant, n is the diffusional exponent, which is characteristic for the mechanism of the drug release. M_i, i.e. the amount of drug released at infinity, is assumed to be 100 %.
Formulation: 40 % Eudragit® L 100-55, 20 % mannitol, 40 % talinolol. Dissolution conditions: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8, paddle apparatus, 37°C, means ± SD, n = 3.

Dissolution medium	Parameter	Best-fit value (± S.D.)	R^2
pH 1.0	K	0.052 (± 0.01)	0.9639
	n	0.35 (± 0.04)	
pH 4.5	K	0.025 (± 0.003)	0.9960
	n	0.56 (± 0.02)	
pH 6.8	K	0.016 (± 0.005)	0.9827
	n	0.68 (± 0.06)	

The estimated values for the diffusional exponent n vary in media with different pH values. This is in accordance with observations of other working groups, that also found altered values for n at different pH values [Rao, V. M., K. Engh, et al. (2003)]. These differences can be explained by the altered solubility of the drug that also can alter the release mechanism from matrix formulations [Tahara, K., K. Yamamoto, et al. (1996)]. At pH 4.5 the estimated parameter n is close to 0.5, which means that the drug release from the matrix is predominantly controlled by diffusion and follows the release kinetics described by Higuchi. This assumption is in accordance with the results of the linear regressions presented in Figure III.42, where the best coefficient of determination was found for the fit of the dissolution data at pH 4.5 (R^2 = 0.9919). At a pH value of 6.8, the parameter n increases to 0.68. This increase can be due to two different changes in the release mechanism: Tahara et al. reported, that a

decreased solubility, as observed for talinolol at this pH value, can lead to an erosion-controlled release mechanism [Tahara, K., K. Yamamoto, et al. (1996)]. A second explanation for the increased value of n at pH 6.8 can be a shift from diffusion-controlled towards swelling-controlled drug release [Majid Khan, G. and J.-B. Zhu (1999)]. Since Eudragit L 100-55 as release-sustaining excipient, swells at pH values of 5.5 and above, swelling controlled release kinetics can be assumed. This assumption is supported by the fact that 0.68 as estimated value for n is not close to 1.0, the value suggested for formulations with an erosion-controlled drug release [Colombo, P., R. Bettini, et al. (2000)]. The low value calculated for n at pH 1.0 can be explained by the slow drug release in the time window between two and eight hours. The higher dissolution rate in the initial phase is assumed to be due to the dissolution of talinolol from superficial layers of the matrix tablet [Pather, S. I., I. Russell, et al. (1998)]. In the following period, medium infiltration into the matrix and drug diffusion through the matrix are hindered by the polymer that is insoluble at that pH. This results in the pronounced decrease of the drug release rate in 0.1 N HCl.

III.4 Conclusions

The production of directly compressible matrix tablets was evaluated as an appropriate method to sustain the drug release of talinolol. The solubility of talinolol is strongly dependent on the pH value, which results in pronounced alterations of the dissolution of talinolol in media of different pH values. A sustained-release matrix tablet formulation with a talinolol content of 100 mg was developed. The drug release rate from these tablets can be assumed to be similar during the passage of the gastrointestinal tract, in spite of differing pH values. The matrix tablets formulated with 40 % talinolol, 40 % Eudragit® L 100-55 as release-sustaining polymer and 20 % mannitol as hydrophilic pore former showed a consistent drug release of more than 80 % within eight hours in acetate buffer of pH 4.5 and phosphate buffer of pH 6.8. At pH 1.0, the drug release within the initial two hours, which are relevant for the in vivo situation, was 26 %.

The technological characterization of the newly developed matrix tablets in terms of uniformity of mass, crushing strength, and friability demonstrated adequate properties.

The mechanisms of drug release from the matrix tablets were investigated by fitting the dissolution data to the models described for matrix systems by Higuchi and Ritger and Peppas. It was detected that the mechanism of drug release was pH-dependent. At pH 1.0 free dissolution was hindered by Eudragit ® L 100-55 which is insoluble at that pH value. At pH 4.5, the drug release was predominated by Fickian diffusion through the matrix and at a pH value of 6.8, talinolol dissolution was influenced by the swelling of the release-sustaining polymer (Eudragit® L 100-55), resulting in deviations from the linear Higuchi relationship.

Chapter IV

Chapter IV

Considerations on in vitro dissolution test methods

IV.1 Introduction

In dissolution tests performed during the development of talinolol sustained-release dosage forms described in chapter III a pronounced influence of different dissolution media on talinolol solubility and dissolution was detected. These observations gave reason for further examinations on this topic.

In addition to the incomplete and variable bioavailability of talinolol (Trausch et al. reported an absolute bioavailability of 55 %, with a 95 % confidence interval ranging from 36 to 69 % after administration of 50 mg talinolol in an immediate release formulation in the fasted state [Trausch, B., R. Oertel, et al. (1995)]), plasma concentration time curves frequently display ”double peak phenomena” rather than one single C_{max} value, indicating discontinuities in drug absorption profiles [Wetterich, U., H. Spahn-Langguth, et al. (1996)]. Food diminishes the bioavailability of talinolol even further. The AUC values in the fed state decrease to 43.5 % [Terhaag, B., H. Sahre, et al. (1991)] and 21.8 % [Giessmann, T., M. Zschiesche, et al. (2001)] of the AUC values in the fasted state, respectively.

Biotransformation does not contribute to the peculiarities of the drug input process, since it is lower than 1 % for talinolol [Oertel, R., K. Richter, et al. (1994), Oertel, R. and K. Richter (1995)]. As described in chapter I, one reason is that talinolol is substrate to the efflux pump P-glycoprotein (P-gp) in the intestine [Gramatté, T., R. Oertel, et al. (1996), Wagner, D., H. Spahn-Langguth, et al. (2001)]. As such, the drug molecules are secreted back into the intestinal lumen before reaching the basolateral membrane of the enterocytes and hence the blood circulation [Suzuki, H. and Y. Sugiyama (2000), Preiss, R. (1998), Hunter, J., B. H. Hirst, et al. (1993)]. Furthermore, intestinal secretion is to a smaller extent a clearance pathway for talinolol from the systemic circulation [Wetterich, U., H. Spahn-Langguth, et al. (1996)]. Since it is discussed that the expression of carriers varies between individuals and between different regions of the gastrointestinal tract [Wagner, D., H. Spahn-Langguth, et al. (2001), Ungell, A. L., S. Nylander, et al. (1998), Fagerholm, U., A. Lindahl, et al. (1997)], the variable bioavailability of talinolol is explainable.

Physicochemical matters as a source of variability in the absorption of talinolol have not been addressed thus far. In particular, solubility and dissolution issues have not been reported. For the weak base talinolol (pK_a 9.4) the solubility is high in acidic media, but quite poor at pH values above four. Thus it can be assumed, that the drug, when administered as immediate-release dosage form, will dissolve completely in the stomach but may precipitate again when entering the intestine. Since the intestinal pH values are subject to considerable inter- and intraindividual fluctuations in particular after ingestion of a meal [Dressman, J. B., G. L. Amidon, et al. (1998)], the rate and extent of dissolution can also be expected to fluctuate, resulting in diminished bioavailabilities with high variabilities. This chapter deals with the aqueous solubility of talinolol and its in vitro dissolution behaviour. Results are discussed with respect to an in vivo relevance of the in vitro data.

The dissolution of talinolol immediate and controlled-release tablets was tested in several media, such as 0.1 N HCl, acetate buffers pH 4.5 and 6.0, phosphate buffers pH 3.2, 5.5, 6.0, 6.8, citrate buffer pH 6.8, TRIS buffer pH 6.8 and buffers supplemented with sodium dodecylsulfate, cholic acid, Tween 80, Cremophor RH 40 or cetylpyridinium chloride as surfactants. By adding 0.4 to 1.25 % sodium chloride to the dissolution medium a salt effect was investigated.

It was found that pH, ionic strength and surface tension of the dissolution medium had a pronounced influence on its dissolution profiles. However, there were conspicuities that could not be explained by these parameters alone: The dissolution profiles differed completely when the type of the buffer was changed although the other parameters were kept constant.

The underlying mechanism was detected in the formation of different talinolol crystal structures when different buffers were used. It was attempted to characterize the modifications with the aid of light microscopy, fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) and x-ray powder diffraction (XRPD).

In addition, solubility tests were performed to demonstrate differences in the physicochemical properties of the talinolol crystal forms investigated.

IV.2 Materials and methods

IV.2.1 Chemicals and other materials

Talinolol pure substance and Cordanum tablets containing 100 mg talinolol were generous gifts from AWD Pharma, Dresden, Germany. Eudragit® polymers were received in powder form as samples from Roehm (Darmstadt, Germany). The buffer salts and other chemicals were purchased from Merck (Darmstadt, Germany), Grüssing (Filsum, Germany) and Caelo (Hilden, Germany) and had at least p.a. or Ph.Eur. quality.

IV.2.2 Solubility studies

200 mg talinolol were weighed in 30 ml glass containers each with screw caps, and, depending on the expected solubility, 10 to 20 ml of the test solvent were added. The samples were shaken vigorously by hand and then by a thermostatically controlled shaker (GFL 3032, Ges. für Labortechnik, Burgwedel, Germany) with 185 rpm at a temperature of 37°C for 30 h until an equilibrium was reached. The samples were filtered through a 0.45µm filter, diluted approximately with the particular test solvent so that the absorbance was in the range between 0.2 and 0.8 and analysed with a UV-Vis Spectrophotometer Perkin Elmer Lamda 20 (Perkin Elmer GmbH, Überlingen, Germany). When buffers were used as solvents the final pH values were measured after saturation with talinolol. Since the buffer capacities were in most cases exceeded by the high concentrations of the basic talinolol, the measured pH values were in most cases higher than the initial buffer pH values. Comparisons between the solubilities in different buffers were performed formulated with the final pH values. Adjustment of ionic strengths was performed by diluting the solutions showing the higher ionic strengths with demineralised water. The common method to achieve higher ionic strengths by adding sodium chloride was desisted since it was found that an addition of sodium chloride by itself influenced the solubility.

IV.2.3 Preparation of controlled-release dosage forms

The drug release was controlled by addition of Eudragit® S 100, a 1:2 methacrylic acid - methyl methacrylate copolymer, and Eudragit® RS PO, an ethyl acrylate - methyl methacrylate - trimethyl ammonioethyl methacrylate chlorid copolymer, both in powder form suitable for direct compression (cp. Chapter V). The mixtures were vigorously ground with the drug substance talinolol. Then controlled-release matrix tablets containing 100 or 200 mg of talinolol were compressed on a PW 20 GS tablet press (Paul Weber, Remshalden-Grumbach, Germany) in a 13 mm die with a compression force of 40 kN.

IV.2.4 Dissolution studies

The dissolution of talinolol tablets was tested on a Pharma Test PTWS III (Pharma Test Apparatebau, Hainburg, Germany) and an Erweka DT7R (Erweka GmbH, Heusenstamm, Germany) dissolution tester complying with USP standards (Apparatus II, paddles) and equipped with Hanson Research Dissoette II automatic samplers. The water bath temperature was 37° ± 0.5° C, rotational speed 50 min^{-1}, volume of dissolution media 1000 ml. The final pH values in the dissolution vessels after 24 hours were measured in order to detect an overload of the buffer capacities. Contrary to the solubility studies, changes in the pH of the buffer after the tests were not detected on any occasion, due to the fact that the talinolol concentrations reached in these studies were much lower than in the solubility studies.

The amount of drug released after specific times was analysed by means of UV spectroscopy with a Lamda 20 UV-VIS Spectrophotometer (Perkin Elmer, Überlingen, Germany) at a wavelength of 240 nm.

IV.2.5 Preparation of talinolol crystal forms

1.3 g of talinolol was mixed with 50 ml of the different dissolution media (water and miscellaneous buffer solutions) in 100 ml tubes with screw caps. The suspensions were shaken at 37°C in a thermostatically controlled shaker (GFL 3032, Ges. für Labortechnik, Burgwedel, Germany). Thereafter the samples were centrifuged at 5000 min^{-1} for 5 minutes and the supernatant was removed. The powders were dried in an oven (B28, BTW Binder, Tuttlingen, Germany) for 72 hours at 40°C. Then these samples were observed under a light-microscope and analysed by FT-IR, DSC and XRPD.

IV.2.6 Light microscopy

The crystal structures of the different talinolol salts and modifications were observed under a Hund Wilovert S (Hund, Wetzlar, Germany) light microscope equipped with a Kodak Digital Science DC 120 Zoom Digital Camera. Digital photographs of the crystals were taken with a magnification factor of 1:500.

IV.2.7 Fourier Transform Infrared Spectroscopy (FT-IR)

3 mg of each sample was vigorously ground in an agate mortar and triturated with 250 mg of dried, finely powdered potassium bromide. Disks of 13 mm diameter were compressed with a compression force of 30 kN. Spectra were recorded between wavenumbers of 400 and 4000 cm^{-1} with a Nicolet Impact 400 FT-IR Spectrometer (Nicolet, Madison, USA). To exclude influences of impurities of pure buffer salts used for the preparation of the dissolution media, spectra of these salts were also recorded and compared with the different talinolol spectra.

IV.2.8 Differential Scanning Calorimetry (DSC)

Thermograms were measured with a differential scanning calorimeter Mettler DSC 30 connected to a TC 11 TA Processor (Mettler, Greifensee, Switzerland). The instrument was calibrated with 6.55 mg indium in a temperature range between 20 and 600°C at a heating rate of 10°C/min (Onset temperature: 155.8°C, peak temperature: 156.6°C). Between 1.2 and 1.8 mg of the powdered samples were exactly weighed and their thermal behaviour was studied in sealed pans with a pinhole under nitrogen purge. Thermograms were taken between 40 and 300°C at a heating rate of 10°C/min.

IV.2.9 X-Ray Powder Diffraction (XRPD)

X-ray powder diffractograms were recorded on a Siemens D8 x-ray diffractometer working in the reflection mode using CuK_{α} radiation with a wavelength of 1.54 nm. The measurements were carried out using 40 kV voltage and 20 mA current. 1 g of the finely ground sample powders were packed into standard sample holders and measured at room temperature under the following conditions: start angle 3° 2θ, end angle 40° 2θ, step 0.02° 2θ, step time 5.0 s.

IV.3 Results

IV.3.1 Solubility in aqueous media

The general dependence of the solubility of a monobasic drug such as talinolol from the pH of the medium is [Avdeef, A (2001)]:

$$s = s_0 \cdot (1+10^{(pKa-pH)})$$

with s as the solubility at a certain pH and s_0 as the intrinsic solubility, i.e. the theoretical solubility of the uncharged substance. The theoretical solubility – pH profile for talinolol referring to this equation is shown in Figure IV.1, taking 9.4 as pK_a and 200 mg/l as solubility at pH 7.0.

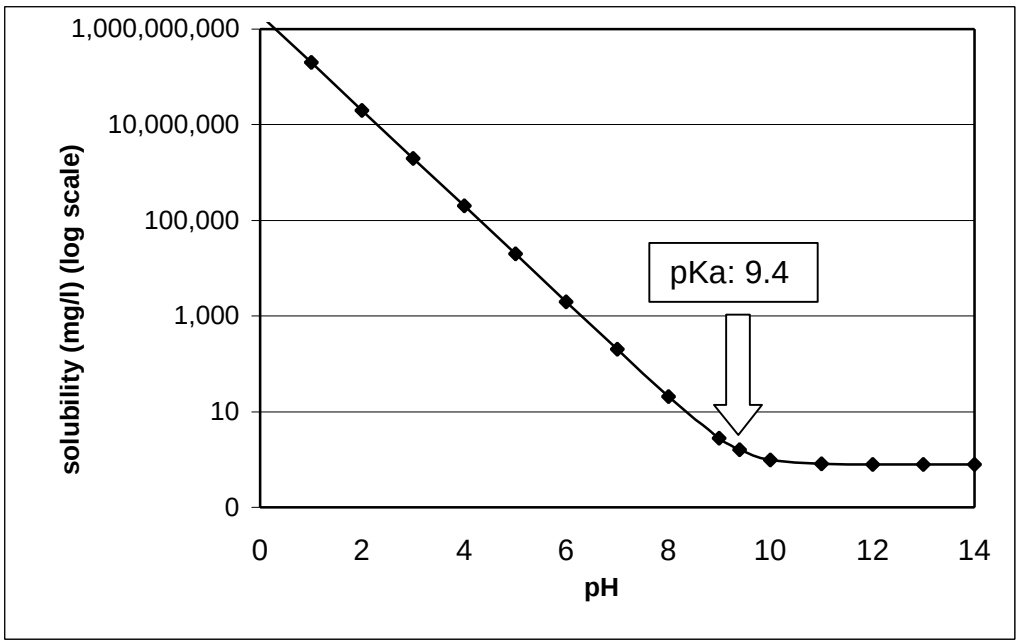


Figure IV.1:
Calculated pH - solubility profile of talinolol, a monobasic compound with a pK_a of 9.4.

However, this profile could not be observed in practice as the influence of the pH was superimposed by other factors that have a more pronounced impact on talinolol solubility. Variation of the ionic strength of a certain buffer system, for example, led to distinct changes which is outlined in Table IV.1:

Table IV.1:
Impact of the buffer concentration and ionic strength on talinolol solubility at different pH values (means ± SD, n = 3).

Medium	Molarity (mol/l)	Ionic strength	Final pH	Solubility (mg/l)
Pure water	-	0	10.0	143
Acetate buffer pH 4.5	0.132	0.132	10.0	*)
	0.265	0.265	7.5	5478
	0.529	0.529	6.5	44994
	1.058	1.058	5.0	66700
Phosphate buffer pH 6.8	0.044	0.105	9.0	532
	0.087	0.211	8.5	446
	0.175	0.422	8.0	343
	0.349	0.843	7.5	199

*) No clear solution after centrifugation

It was remarkable that the solubility in the acetate buffer increased with higher acetate concentrations while it decreased with higher phosphate concentrations. Table IV.1 already indicates that a change of the buffer system strongly influenced the talinolol solubility. Independent from all other factors the solubility in acetate buffers was always higher than in phosphate buffers. In Figure IV.2 talinolol solubilities in different dissolution media based on acetate, phosphate or citrate buffers are displayed. The media differed in their ionic strengths, their final pH values and also their surface tensions since sodium dodecylsulfate and cholic acid as surfactants have been added to some of them.

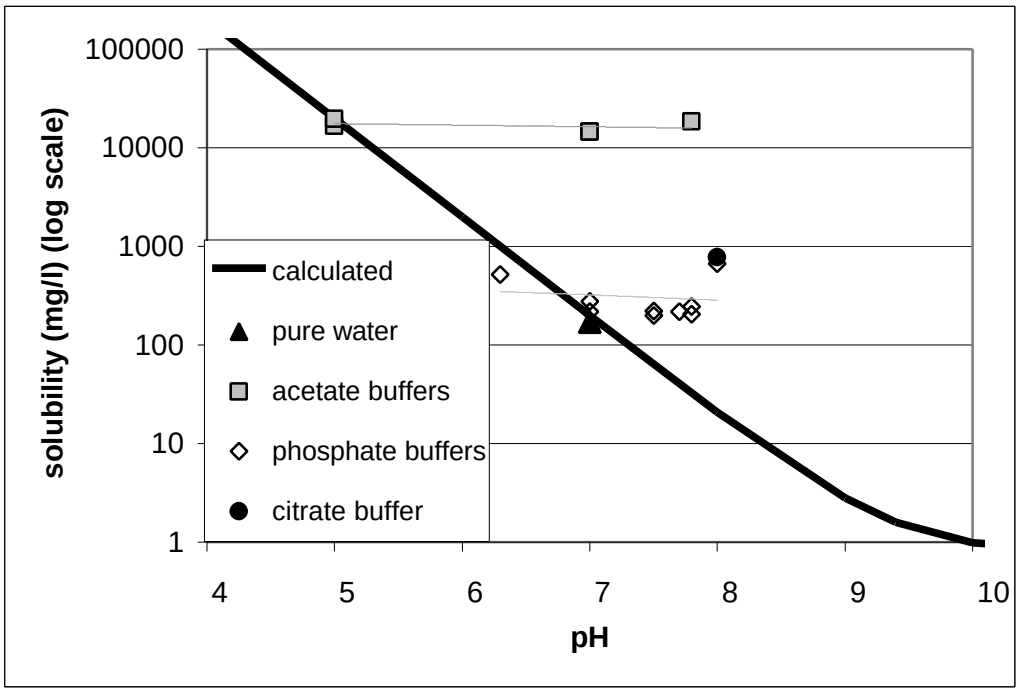


Figure IV.2:

Solubilities of talinolol in different dissolution media based on phosphate buffers (white diamonds), acetate buffers (grey squares), a citrate buffer (black circle) and pure water (black triangle) (Mean values of n = 3).

The bold line indicates the theoretically calculated solubility at certain pH values without respect to any other influencing factors. The chart shows that all the solubilities in phosphate buffers as well as all those in acetate buffers are located within similar regions, independent of the pH, the ionic strength and the addition of the surfactants. This demonstrates the predominant impact of the choice of the buffer type on the solubility. For example, talinolol solubility in a phosphate buffer at a final pH of 7.0 was about 5 times lower than in an acetate buffer of the same pH and ionic strength. The impact of the addition of different concentrations of sodium dodecylsulfate and cholic acid as surfactants to the diverse buffers was not as distinct as the choice of the buffer type. Depending on the concentration of the surfactant, the talinolol solubility did not always increase as it would be expected. For sodium dodecylsulfate (SDS) the talinolol solubility decreased when less than 0.75 % SDS were added, only with SDS concentrations above 1 %, i.e. about four times higher than the critical micelle concentration (CMC) of 0.23 %, a pronounced improvement of the solubility could be achieved, which is shown in Figure IV.3.

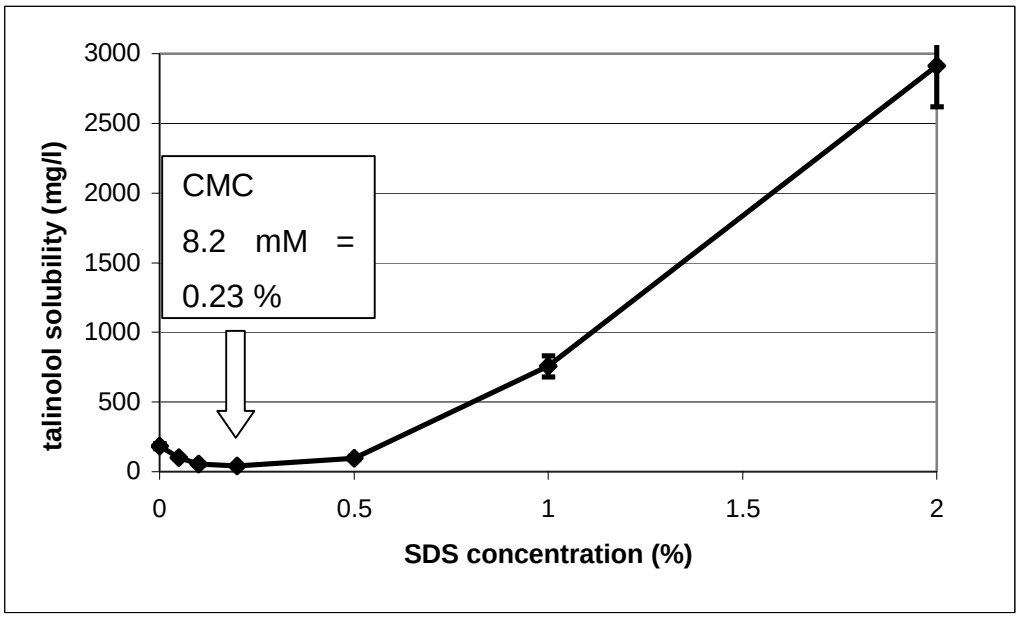


Figure IV.3:

Influence of the addition of sodium dodecyl sulphate (SDS) on the talinolol solubility in a phosphate buffer pH 6.8 (Mean values of n = 3 with indicated standard deviations).

Within the physiological concentration range the addition of sodium chloride (NaCl) led to an increase of the talinolol solubility, e. g. in a 0.9 % NaCl solution the solubility was nearly 40 % higher than in pure water as outlined in Figure IV.4.

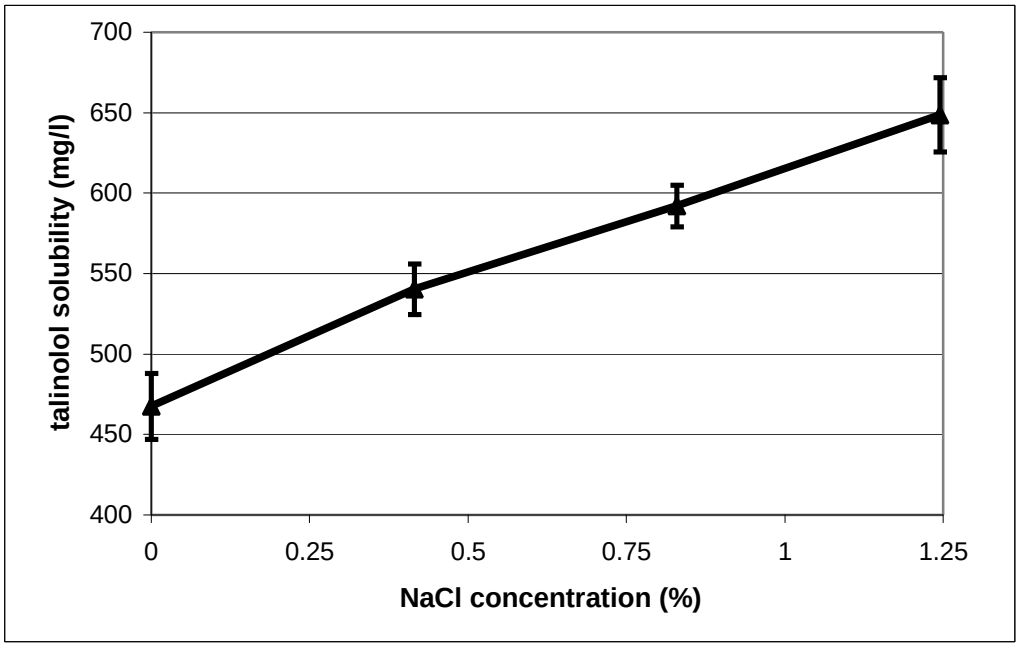


Figure IV.4:

Influence of the addition of sodium chloride (NaCl) on the talinolol solubility in a phosphate buffer pH 6.8 (Mean values of n = 3 with indicated standard deviations).

IV.3.2 Dissolution

To detect the impact of the buffer system on the dissolution of talinolol tablets, dissolution tests with Cordanum 100 mg were performed at pH 6.0 in acetate buffer and phosphate buffer of the same ionic strength, and at pH 6.8 in citrate buffer and phosphate buffer of the same ionic strength, respectively. Figure V.5 shows that the dissolution in acetate buffer was faster at pH 6.0 (A) and at pH 6.8 it was faster in citrate buffer (B) as compared to the phosphate buffers at the respective pH value.

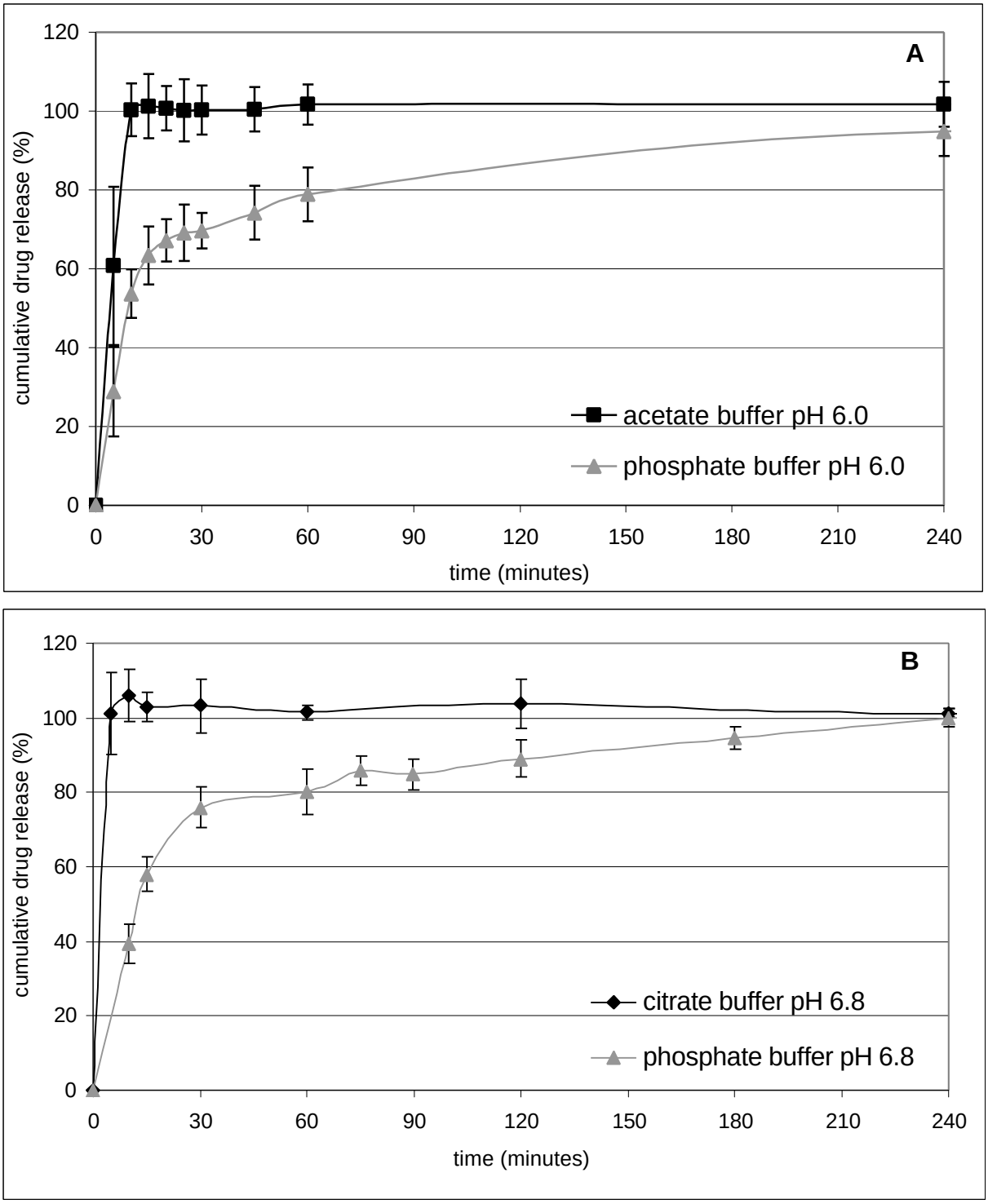


Figure V.5:

Dissolution profiles of talinolol immediate release tablets (Cordanum 100 mg) in

A) acetate versus phosphate buffer at pH 6.0

B) citrate versus phosphate buffer at pH 6.8

(Dissolution conditions: paddle apparatus, 50 rpm, 37°C, 1000 ml, , means ± SD, n = 3).

For controlled-release matrix tablets consisting of 40 % talinolol, 40 % Eudragit® S 100 and 20 % Eudragit® RSPO the results of the dissolution experiments in several different media with and without addition of surfactants are outlined in Table IV.2.

Table IV.2:

Dissolution of talinolol sustained-release matrix tablets in different media.

(Mean values of n = 3, standard deviations in brackets)

(Formulation: Talinolol 40 %, Eudragit® S 100 40 %, Eudragit® RSPO 20 %)

Medium	Dissolution after 1 hour % (S.D.)	Dissolution after 2 hours % (S.D.)	Dissolution after 4 hours % (S.D.)	Dissolution after 8 hours % (S.D.)	Dissolution after 24 hours % (S.D.)
pH 1.0, 0.1 N HCl	25.4 (1.3)	33.7 (1.3)	56.0 (1.8)	85.6 (2.0)	95.6 (1.9)
pH 3.2, phosphate buffer	5.4 (0.6)	7.4 (0.8)	10.5 (1.0)	15.2 (0.9)	27.8 (1.3)
pH 4.5 acetate buffer	26.5 (3.2)	40.1 (3.9)	49.8 (4.1)	71.6 (4.9)	97.7 (3.0)
pH 5.5 phosphate buffer	3.7 (0.4)	4.2 (0.5)	5.1 (0.5)	7.0 (0.7)	13.6 (2.5)
pH 6.8 phosphate buffer	7.7 (0.8)	15.6 (1.2)	30.0 (2.4)	59.4 (6.3)	84.4 (4.4)
pH 6.8 citrate buffer	3.6 (0.2)	6.1 (0.4)	7.5 (0.4)	10.7 (0.4)	20.3 (1.2)
pH 6.8 TRIS buffer	5.3 (0.2)	8.6 (0.5)	13.5 (1.6)	19.4 (1.8)	32.2 (2.0)
pH 6.8 phosphate buffer +0.1% sodium dodecylsulfate	10.4 (0.6)	13.2 (0.8)	15.3 (0.9)	17.8 (2.1)	17.6 (1.8)
pH 6.8 phosphate buffer +0.43% cholic acid	24.4 (0.4)	26.1 (0.4)	27.4 (0.7)	35.4 (0.8)	57.3 (8.6)
pH 6.8 phosphate buffer +1.0% cholic acid	24.4 (0.7)	26.7 (0.7)	27.0 (1.7)	36.4 (0.3)	40.9 (0.9)
pH 6.8 phosphate buffer +1.0% Tween 80	10.9 (1.0)	11.2 (2.6)	12.3 (0.4)	14.3 (0.5)	22.5 (4.7)
pH 6.8 phosphate buffer +1.0% Cremophor RH 40	4.3 (0.1)	5.4 (0.2)	6.3 (0.3)	19.0 (4.6)	76.7 (4.8)
pH 6.8 phosphate buffer +0.5% cetylpyridinium chloride	5.7 (1.9)	14.1 (2.1)	20.7 (2.7)	33.9 (4.7)	90.4 (3.0)

Composition of buffers:

1) Phosphate buffer pH 3.2: 4 g/l sodium dihydrogen phosphate, 2.5 g/l phosphoric acid.
2) Acetate buffer pH 4.5: 2.99 g/l sodium acetate $\bullet 3H_2O$, 1.66 g/l acetic acid
3) Phosphate buffer pH 5.5: 13.1 g/l potassium dihydrogen phosphate, 1.29 g/l sodium monohydrogen phosphate
4) Phosphate buffer pH 6.8: 6.8 g/l potassium dihydrogen phosphate, 0.90 g/l sodium hydroxide
5) Citrate buffer pH 6.8: 1.09 g/l citric acid, 0.60 g/l sodium hydroxide.
6) TRIS buffer pH 6.8: 6.0 g/l tris (hyroxymethyl) aminomethane, 47.5 ml/l 1N hydrochloric acid

When the buffer type is altered, the dissolution profiles change significantly. As demonstrated for the immediate release tablets, the influence of the buffer type on the dissolution of the sustained-release tablets also exceeds the pH influence by far. In spite of a pH difference of 3.5, dissolution in an acetate buffer pH 4.5 is nearly as fast as in 0.1 N hydrochloric acid. Furthermore, compared to a phosphate buffer pH 6.8, dissolution is decelerated in citrate and TRIS buffers of the same pH. Surfactants modified the dissolution of talinolol in different ways. Cholic acid in concentrations of 0.43 and 1.0 % led to an increased dissolution in the first hours, thereafter the dissolution rate decreased so that the amount of drug released after 24 hours was significantly lower than without an surfactant added. Cremophor RH 40 and Tween 80 at concentrations of 1.0 % and sodium dodecylsulfate of a concentration of 0.1 % decreased both rate and extent of drug release from the matrix tablets. Only cetylpyridinium chloride (0.5 %) accelerated the dissolution and increased the amount of drug released after 24 hours.

Furthermore, it was demonstrated in phosphate buffer at pH 6.8 that under addition of sodium chloride (NaCl) rate and extent of talinolol dissolution increased. The higher the NaCl concentration the faster was the dissolution in the observed concentration range up to 1.25 % NaCl.

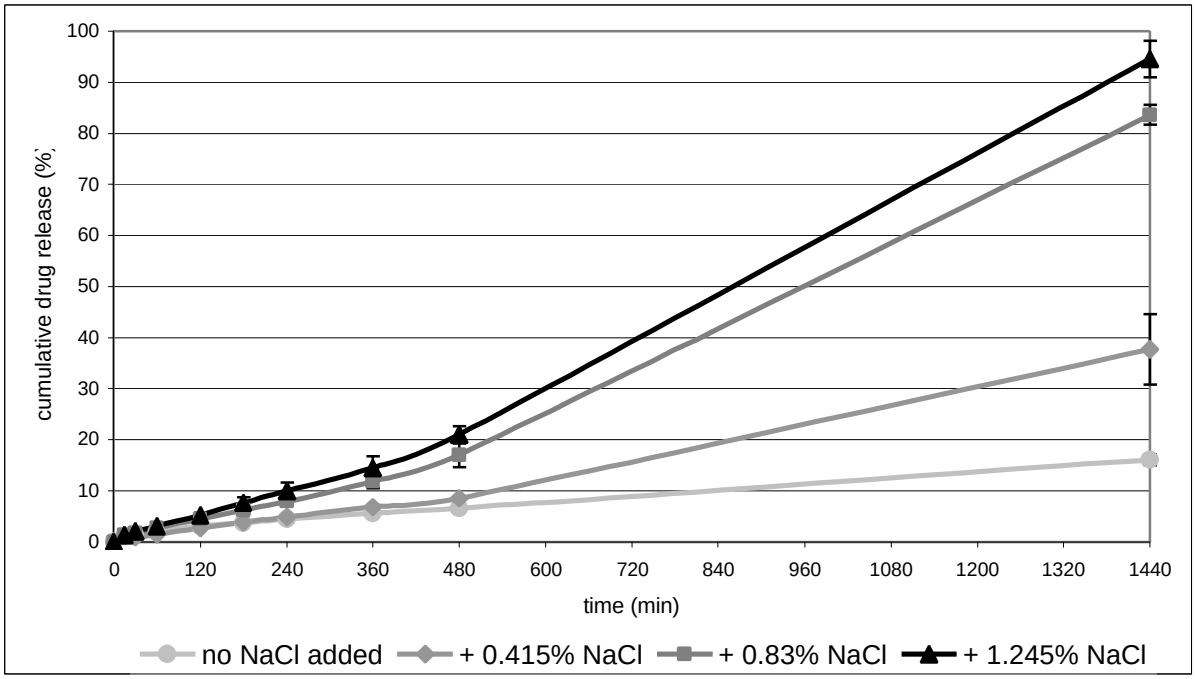


Figure V.6:

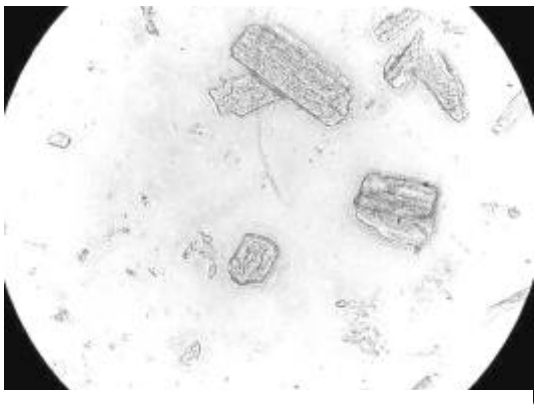
Dissolution profiles of talinolol sustained-release tablets in a phosphate buffer at pH 6.8 under addition of different amounts of sodium chloride.

(Dissolution conditions: paddle apparatus, 50 rpm, 37°C, 1000 ml, means ± SD, n = 3).

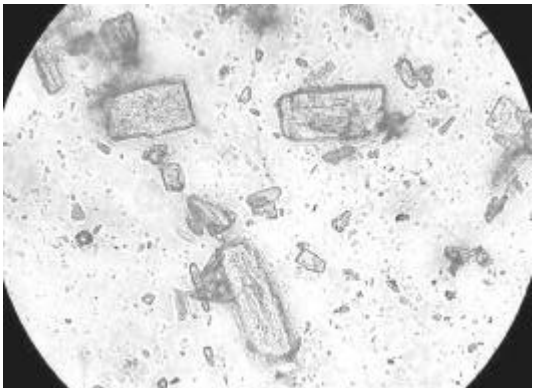
(Formulation: Talinolol 40 %, Eudragit® S 100 40 %, Eudragit® RSPO 20 %)

IV.3.3 Crystal structures

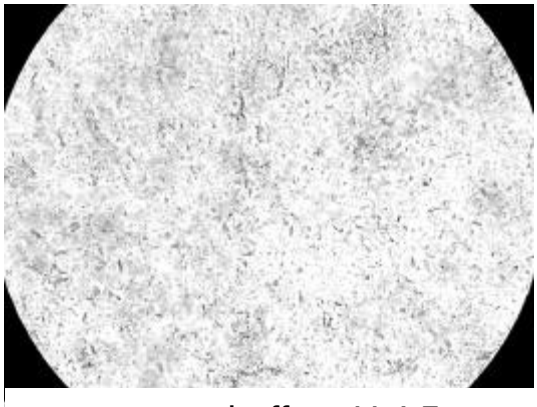
Under a light microscope the shape and the mean size of the crystals obtained following crystallization from pure water and from phosphate buffers at pH 6.0 and 6.8 were similar. Under all three conditions cubic crystal structures were observed, whereas the shape of the powders obtained following precipitation in acetate buffers pH 4.5 and 6.0 was different: their crystal size was considerably smaller and no cuboids were observed as can be seen in Figure IV.7.



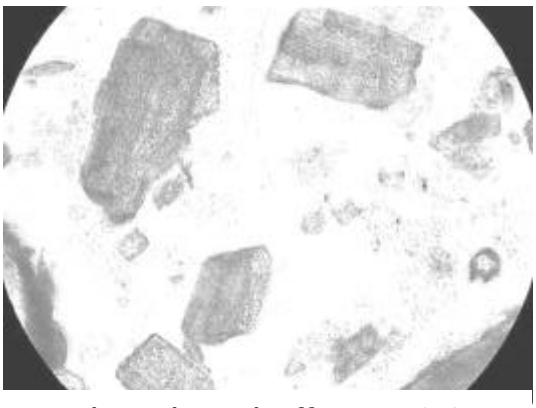
pure water



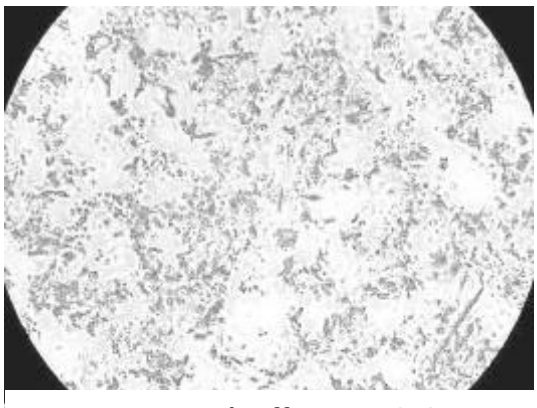
phosphate buffer pH 4.5



acetate buffer pH 4.5



phosphate buffer pH 6.8



acetate buffer pH 6.0

Figure IV.7:

Crystal shapes of talinolol modifications or salts obtained by precipitation in different dissolution media under a light microscope (Magnification 1:500).

IV.3.4 Fourier Transform Infrared Spectroscopy

The spectra showed no significant differences between talinolol as received from AWD Pharma and the drug substance precipitated from water, phosphate buffer pH 6.8 and acetate buffer pH 4.5. The spectra are composed in Figure IV.8.

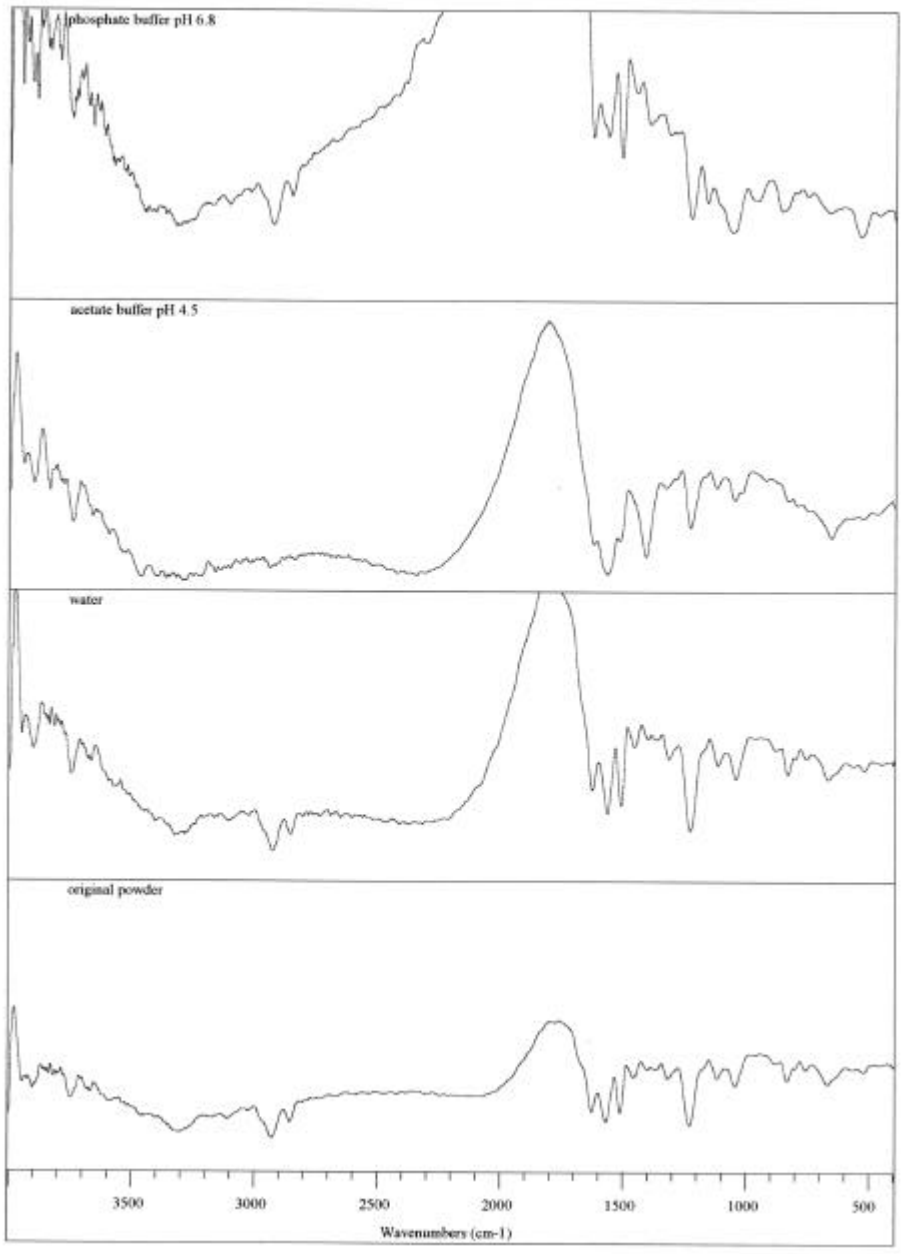


Figure IV.8:
FT-IR spectra of talinolol modifications or salts obtained by precipitation in different dissolution media in the following order (up to down): phosphate buffer pH 6.8, acetate buffer pH 4.5, pure water, original powder.

The main peaks remained unchanged, only some peak ratios differed slightly. Impurities of remaining buffer salts were detected by comparison of the different talinolol spectra with those of the pure buffer components. For example, the loss of the triplet between 1500 and 1600 cm^{-1} in the IR-spectrum of talinolol that was observed following crystallization from the acetate buffer was caused by impurities of sodium acetate. The latter showed only one broad peak in this area and also the peak at 1400 cm^{-1} in this spectrum, that did not occur in the other spectra, had its origin in sodium acetate left-overs. The peak between 500 and 600 cm^{-1} in the spectrum of talinolol precipitated out of the phosphate buffer was due to traces of sodium dihydrogen phosphate, that were not removed completely.

IV.3.5 Differential Scanning Calorimetry

Unlike the FT-IR spectra, the DSC spectra showed significant differences for the test samples: Pure talinolol and the powder re-crystallised from water had nearly identical spectra showing a pair of two peaks, the first one between 126 and 131°C and the second one between139 and 152°C. That is in the range of the melting point of talinolol reported in literature (143°C). The samples crystallized out of acetate and phosphate buffers showed only one peak. This single peak was in the range between 156 and 163°C for acetate and between 200 and 204°C for phosphate, respectively. The spectra are presented in Figure IV.9.

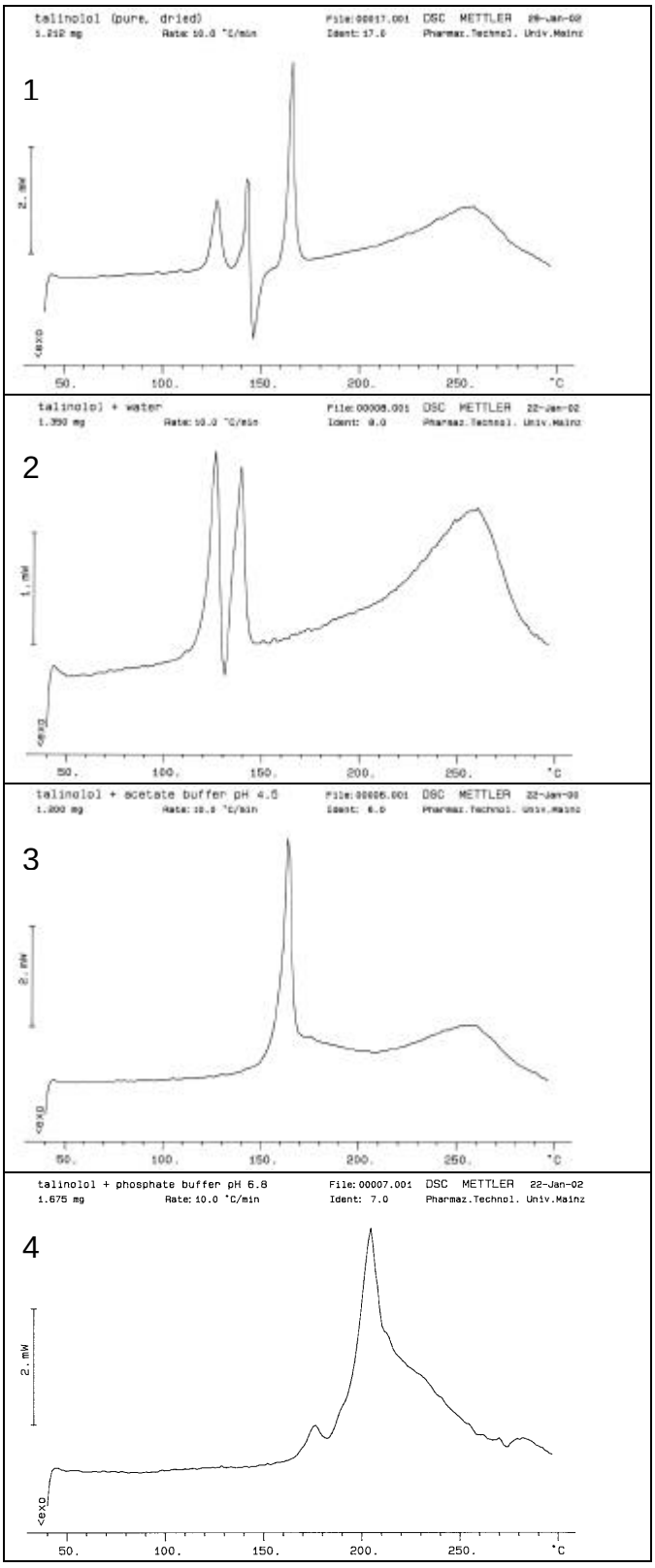


Figure IV.9:

DSC spectra of talinolol modifications or salts obtained by precipitation in different dissolution media in the following order: 1) original powder, 2) pure water, 3) acetate buffer pH 4.5, 4) phosphate buffer pH 6.8.

IV.3.6 X-Ray Powder Diffraction

The x-ray diffractograms of untreated talinolol and talinolol powder crystallized from purified water were almost identical, while crystallisation from phosphate and acetate buffer led to specific changes in the talinolol diffractograms, proving differences in the crystal structures of talinolol precipitated in the different dissolution media. The spectra are composed in Figure IV.10.

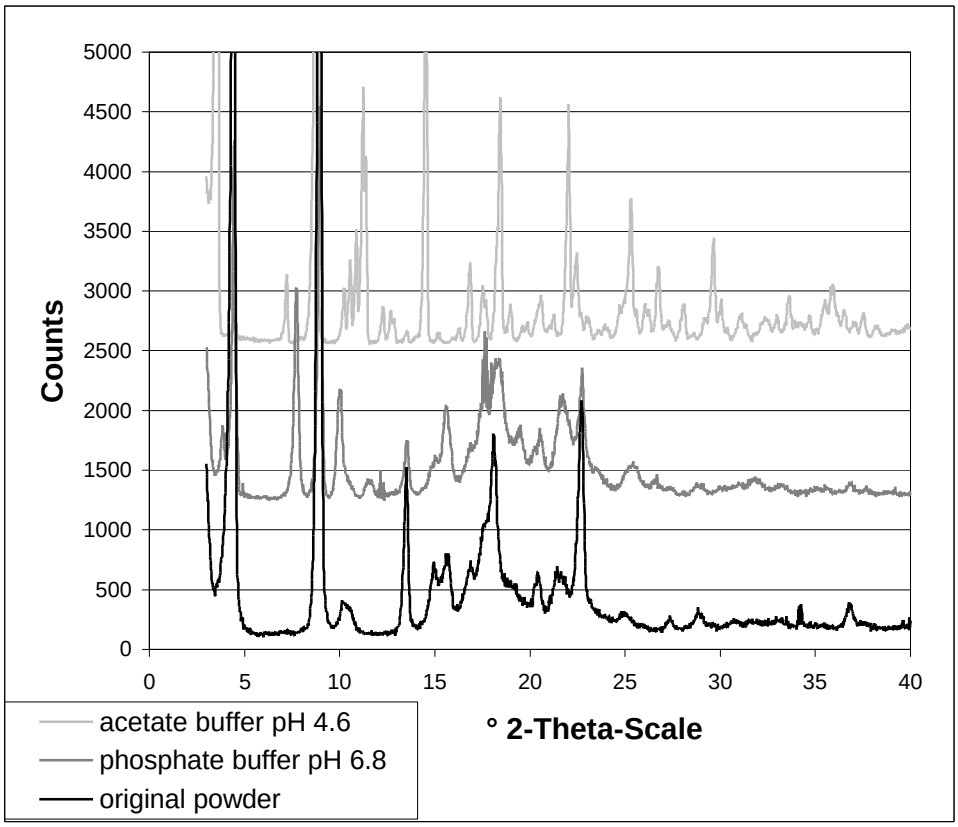


Figure IV.10:
XRPD spectra of talinolol modifications or salts obtained by precipitation in different dissolution media (black line: original powder, dark grey line: phosphate buffer pH 6.8, light grey line: acetate buffer pH 4.5).

IV.4 Discussion and conclusions

The results reemphasize that media used for dissolution tests have to be chosen with care. The effect – discovered in the studies - that different media may lead to different crystal structures of solutes with varying physicochemical properties may not be restricted to the studied compound talinolol but likewise may play an important role for the dissolution of other drugs as well.

For various immediate-release formulations, drug dissolution tests in media simulating the gastric fluid are appropriate, since many drugs completely dissolve before leaving the stomach and show little tendencies towards precipitation in the intestine. For such drug products, 0.1 molar hydrochloric acid or simulated gastric fluid (USP), a solution of 0.2 % sodium chloride and 0.32 % pepsin in hydrochloric acid, can be used.

If dissolution in middle and lower parts of the gastrointestinal tract needs to be simulated, as for the characterization of controlled-release products, acetate buffers with a pH of 4.5 are commonly used to simulate the pH of the upper small intestine in the fasted state. Phosphate buffers of pH 6.8 and 7.4 as well as the simulated intestinal fluid of the USP, based on a phosphate buffer pH 7.5 to which 1 % pancreatin is added, are common for the lower intestinal regions.

To simulate the physiological appearance of surfactants in the gastrointestinal fluids and particularly for dissolution tests with formulations containing low-solubility drugs, either 0.1 to 2 % sodium dodecylsulfate or the more physiological cholic acid or sodium cholate can be added to the buffer solutions at concentrations of 0.1 to 1 %.

To improve the in vivo relevance of dissolution tests two simulated intestinal fluids have been proposed, that mimic the presence of surfactants and enzymes in the intestine [Dressman, J. B., G. L. Amidon, et al. (1998), Galia, E., E. Nicolaides, et al. (1998)]: One for the simulation of the fasted state (FaSSIF, Fasted-State Simulated Intestinal fluid) and one for the simulation of the fed state (FeSSIF, Fed-State Simulated Intestinal fluid). FaSSIF is based on a phosphate buffer pH 6.5, whereas FeSSIF basically consists of an acetate buffer with a pH of 5.0. This switch from phosphate to acetate has no in vivo counterpart and might lead to misinterpretations for drugs that – like talinolol - form different salts or modifications with different physicochemical properties: When dissolution tests are performed in FaSSIF and FeSSIF to investigate the influence of the ingestion of food on the drug release, changes in the dissolution profiles may hence not only be due to the differences in lecithin and cholate concentrations and different pH values which shall imitate

the fasted and fed state, but can also be caused by the change of the buffer type from phosphate to acetate.

With respect to the type of buffer used, none of the media discussed above reflects the actual composition of the intestinal fluids in vivo correctly. Carbonate buffers, that physiologically regulate the pH in the intestine are inapplicable for in vitro tests because of the evaporation of carbon dioxide and the resulting changes in the pH of the dissolution media.

Apart from the buffer type, the effect of sodium chloride on the solubility and dissolution rate o ftalinolol show that the concentrations of other ionic constituents that appear physiologically in the gastrointestinal fluids such as sodium, potassium or calcium chloride may also influence both the solubility of drugs and the dissolution of drug products, which could, at least partly, be caused by changes in the crystal structures of the drugs as well.

With respect to the in vivo relevance, the investigations on talinolol dissolution show that, in addition to P-gp-mediated intestinal secretion [Spahn-Langguth, H., G. Baktir, et al. (1998), Gramatte, T., R. Oertel, et al. (1996)], differences in the composition of gastrointestinal fluids may also contribute to the relatively poor and variable bioavailability of talinolol. It can be assumed that the decrease in bioavailability after ingestion of a meal may also be due to changes in the composition of the gastrointestinal fluids.

Since the in vitro dissolution tests showed that up to a specific level the addition of surfactants can diminish or at least decelerate talinolol dissolution, it may well be possible that the physiological surfactants such as bile salts that are secreted after ingestion of food could be held responsible for the decrease in bioavailability as well. An explanation for both, the in vitro and the in vivo phenomenon, could be a complexation between talinolol and the surfactants as it is reported for pafenolol, another beta-adrenoceptor antagonist with a similar chemical structure [Lennernas, H. and C. G. Regardh (1993a, b and c)].

The findings presented in chapter IV were submitted as paper and accepted for publication in “Drug Development and Industrial Pharmacy” in revised form in March 2003.

Chapter V

Chapter V

In silico evaluation of talinolol sustained-release tablets

V.1 Introduction

In chapter III the development of sustained-release talinolol matrix tablets with a modified drug release rate was described. The final formulation showed a rather consistent drug release over a period of approximately eight hours in the respective dissolution media.

To investigate the interaction between the P-gp-mediated drug efflux in the GI-tract and the decelerated drug release, in silico tests were projected.

The term in silico refers to the estimation of certain parameters with the aid of computer-based software programs. Terstappen and Reggiani give an overview of the different areas in drug discovery, in which in silico approaches are used [Terstappen, G. C. and A. Reggiani (2001)]. Among other methods, computer-based programs are employed for the discovery of new drug targets, the analysis of gene-expression, the search for new lead substances and the prediction of physico-chemical properties of drugs such as solubility and lipophilicity [Engkvist, O. and P. Wrede (2002), Huuskonen, J., J. Rantanen, et al. (2000), Peterson, D. L. and S. H. Yalkowsky (2001), Tetko, I. V., V. Y. Tanchuk, et al. (2001), Tetko, I. V., V. Y. Tanchuk, et al. (2001)]. In addition, several approaches regarding the in silico modeling of the pharmacokinetic behaviour of drugs in the human body have been reported [Beresford, A. P., H. E. Selick, et al. (2002), Butina, D., M. D. Segall, et al. (2002), Smith, D. A. and H. van de Waterbeemd (1999)]. These approaches are based on the ADME model, a model which describes the route of a drug through the human body in four steps: absorption (A), distribution (D), metabolism (M) and excretion (E).

For perorally administered drugs, absorption means the transition of dissolved drug molecules from the lumen of the GI-tract into the systemic circulation. The second step describes the distribution of the drug to different compartments, including blood, brain, fat and body water. Metabolism is the process of chemical alteration of a drug.

This occurs in particular in the liver, but also in other organs, e.g. in the gut wall. Finally, excretion involves the removal of the drug and its metabolites from the body via urine, faeces or other routes of elimination.

However, for a simulation of the bioavailability of peroral sustained-release drug products an additional step representing the liberation of the drug from the dosage form must be added to the ADME model. With the implementation of this process, the model is useful for in silico predictions of bioavailability, C_{max}, t_{max}, AUC and other pharmacokinetic parameters after administration of peroral immediate-release and sustained-release dosage forms. Table V.1 presents examples of widely used simulation software programs for different applications.

Table V.1:
Commercially available simulation software focusing on different aspects in drug development and pharmaceutical research.

Product	**Company**	**Description / Focus**
PhysioLab	Entelos (Menlo Park, CA, USA)	Models of asthma, obesity, and AIDS
IDEA	Lion Biosciences (San Diego, CA, USA	Simulation of metabolism and pharmacokinetics of drugs
WinNonLin, WinNonMix	PharSight (Mountain View, CA, USA)	Clinical trial modeling and variance analysis
GastroPlus	Simulations Plus (Lancaster, CA, USA)	Simulation of the absorption of drugs from the human gastrointestinal tract

A more detailed description of six different software programs for in silico predictions of ADME and pharmacokinetics and an evaluation of these programs is reported by Boobis et al. [Boobis, A., U. Gundert-Remy, et al. (2002)].

Of outstanding importance for the goodness of predictions performed in silico is the model the simulation software uses for gastrointestinal absorption. Drug absoption from the gastrointestinal tract can be very complex. It is influenced by numerous factors that can be classified into the categories physiochemical, physiological and formulation-related. Due to this complexity, simple models often fail to characterize the absorption process exactly. Models suggested for the simulation of the gastrointestinal tract in the past are the dispersion model [Ho, N. F., W. I. Higuchi, et al. (1972)], homogenous [Sinko, P. J., G. D. Leesman, et al. (1991)] and heterogenous [Kalampokis, A., P. Argyrakis, et al. (1999a), Kalampokis, A., P. Argyrakis, et al. (1999b)] tube model and compartmental absorption and transit (CAT) model [Yu, L. X. and G. L. Amidon (1999), Yu, L. X., J. R. Crison, et al. (1996)]. However, for simulations of the absorption of drugs undergoing active transport, including both, uptake and efflux processes, a model may not focus on passive diffusion alone, but must provide simulation steps for the transporters involved. The advanced compartmental absorption and transit (ACAT) model used by the software program Gastro Plus® is able to simulate such active transport processes [Agoram, B., W. S. Woltosz, et al. (2001)]. In addition, the ACAT model accounts for factors such as variations in pH along the gastrointestinal tract, physicochemical parameters of the drug molecule that affect dissolution and absorption, variations in effective permeability and structure in the intestine, physical formulation properties, and saturable first-pass extraction and biotransformation.

For a simulation of the bioavailability of talinolol sustained-release tablets formulated as described in chapter III, both, formulation properties and intestinal drug efflux mediated by P-glycoprotein must be considered. The ACAT model is appropriate for such simulations, since simulation steps for both, the sustained drug release and the drug efflux, can be included. Therefore, Gastro Plus® was selected for the in silico evaluation of the talinolol sustained-release matrix tablets developed.

V.2 Materials and methods

V.2.1 Computer hardware and software

The simulations were performed on a Siemens Xpert Pentium III computer (500 MHz) using the Gastro Plus® software. The program was developed at Simulations Plus (Lancaster, California, USA) under the direction of Michael B. Bolger in collaboration with Gordon L. Amidon. Besides common estimations of pharmacokinetic parameters this software package allows simulations of non-linear kinetics and non-standard absorption patterns. The program enables predictions of rate and extent of drug absorption from the gastrointestinal tract. Transporter and metabolism data can be included in these predictions.

The program allows to consider modified release of the drug from the dosage form as well as intestinal drug efflux in its predictions of pharmacokinetic parameters. Both are necessary for reliable simulations of the bioavailability of the talinolol sustained-release matrix tablets developed.

Only a few basic parameters are initially required to run a simulation with Gastro Plus®. However, there is a large number of input parameters that are not essential for a simulation but can enhance the quality of the results. The more properties of a drug and its particular dosage form are included in a simulation, the more valid and reliable results can be expected.

V.2.2 Input parameters for simulations of talinolol IR and CR dosage forms

V.2.2.1 Compound properties

Figure V.1 displays the basic compound parameter input screen of the Gastro Plus® program. The parameters available for talinolol are filled in. When no information was available concerning a certain input parameter, default settings proposed by the program were used.

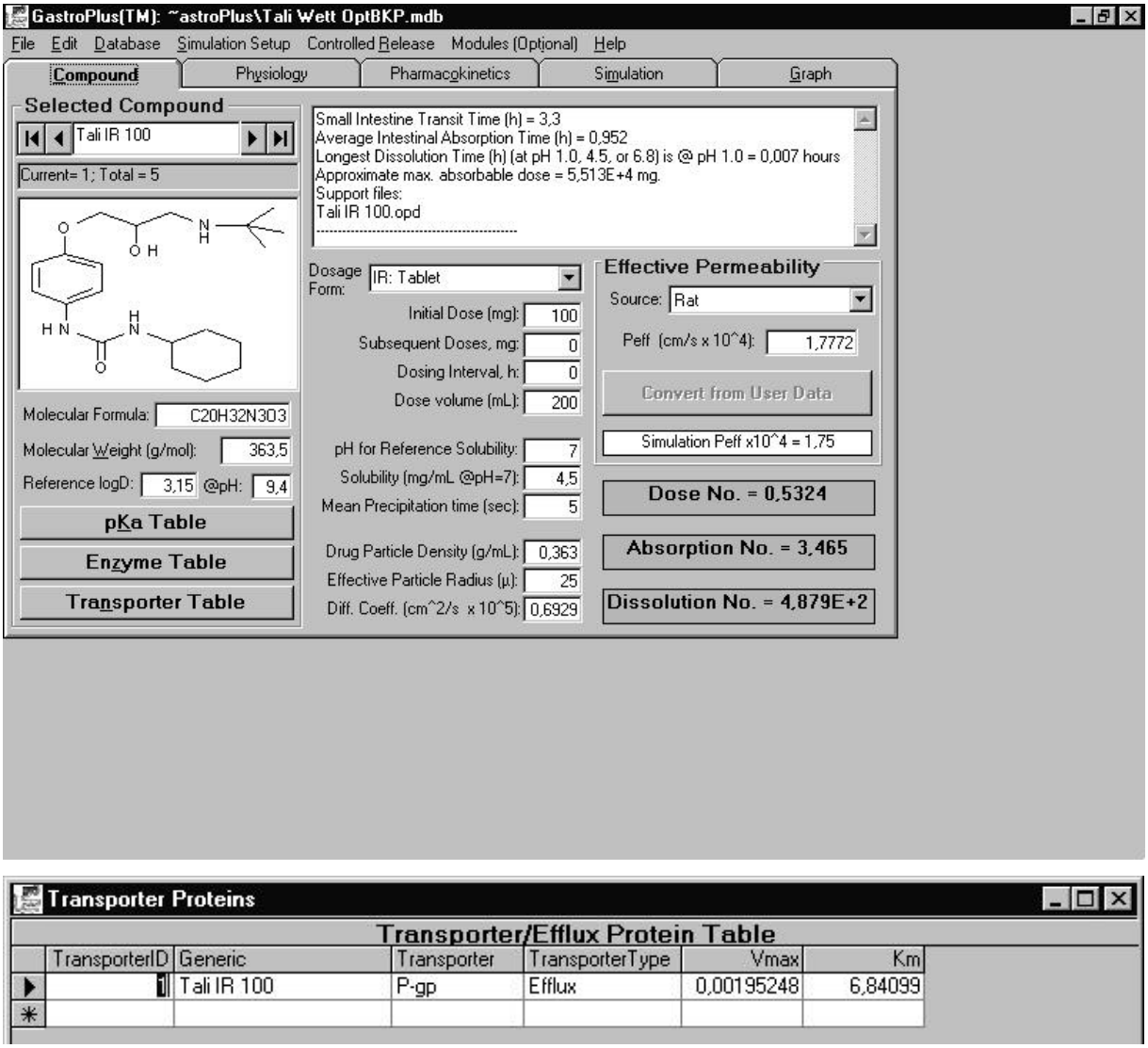


Figure V.1:

Basic compound parameter input screen and transporters / efflux proteins table of the pharmacokinetic program Gastro Plus®. The parameters available for an immediate-release tablet containing 100 mg talinolol and optimized values for P_{eff}, K_m and V_{max} are entered.

Table V.2 displays the parameters entered in the basic compound parameter input screen of the Gastro Plus® software package.

Table V.2:
Parameters used in computer simulations with different talinolol dosage forms with the pharmacokinetic software program Gastro Plus®.

Drug properties:	
Molecular formula	$C_{20}H_{32}N_3O_3$
Molecular weight (M_r)	363.5 g/mol
Reference logD	3.14 at pH 9.4
Solubility at pH 7.0	4.5 mg/ml

Dosage form properties:	
Dosage form	IR Tablet CR Integral Tablet i.v. Bolus
Initial dose	p.o.: 25 mg, 50 mg, 100 mg, 400 mg i.v.: 30 mg
Subsequent doses	none
Dose volume)*	200 ml
Mean precipitation time)*	Default (5 sec)
Drug particle density)*	Default (0.363 g/ml)
Effective particle radius)*	Default (25 µm)
Diffusion coefficient)*	Default ($0.6929 \bullet 10^{-5}$ cm^2/s)

)*: only used in simulations for peroral dosage forms

V.2.2.2 Model of the physiology of the gastrointestinal tract

The “Physiology” screen displayed in Figure V.2 allows to define physiological parameters of the gastrointestinal tract such as pH, P_{eff} or transit times for particular regions. However, default physiological conditions are proposed by the program for both, the fasted and fed state. In all simulations with varying talinolol dosage forms, default conditions of the fasted state proposed by the software program were used, except for the P-gp distribution. As a result of the perfusion studies in different regions of the rat intestine (cp. chapter II) the scaling factors for the P-gp distribution were adopted. Increasing expressions of P-gp from jejunum to ileum and colon were reflected by scaling factors increasing from 1 for the jejunum to 3 for the ileum to 5 for the colon (cp. Figure V.2). As the transporter effect in the duodenum was not tested in the rat studies and reports in literature are controversial and propose higher as well as lower P-gp expressions than in more distal intestional regions [Saitoh, H. and B. J. Aungst (1995), Tang, H., Y. Pak, et al. (2002), Brady, J. M., N. J. Cherrington, et al. (2002), Tamura, S., A. Ohike, et al. (2002), Makhey, V. D., A. Guo, et al. (1998)], a medium scaling factor of 3 was selected for the duodenum.

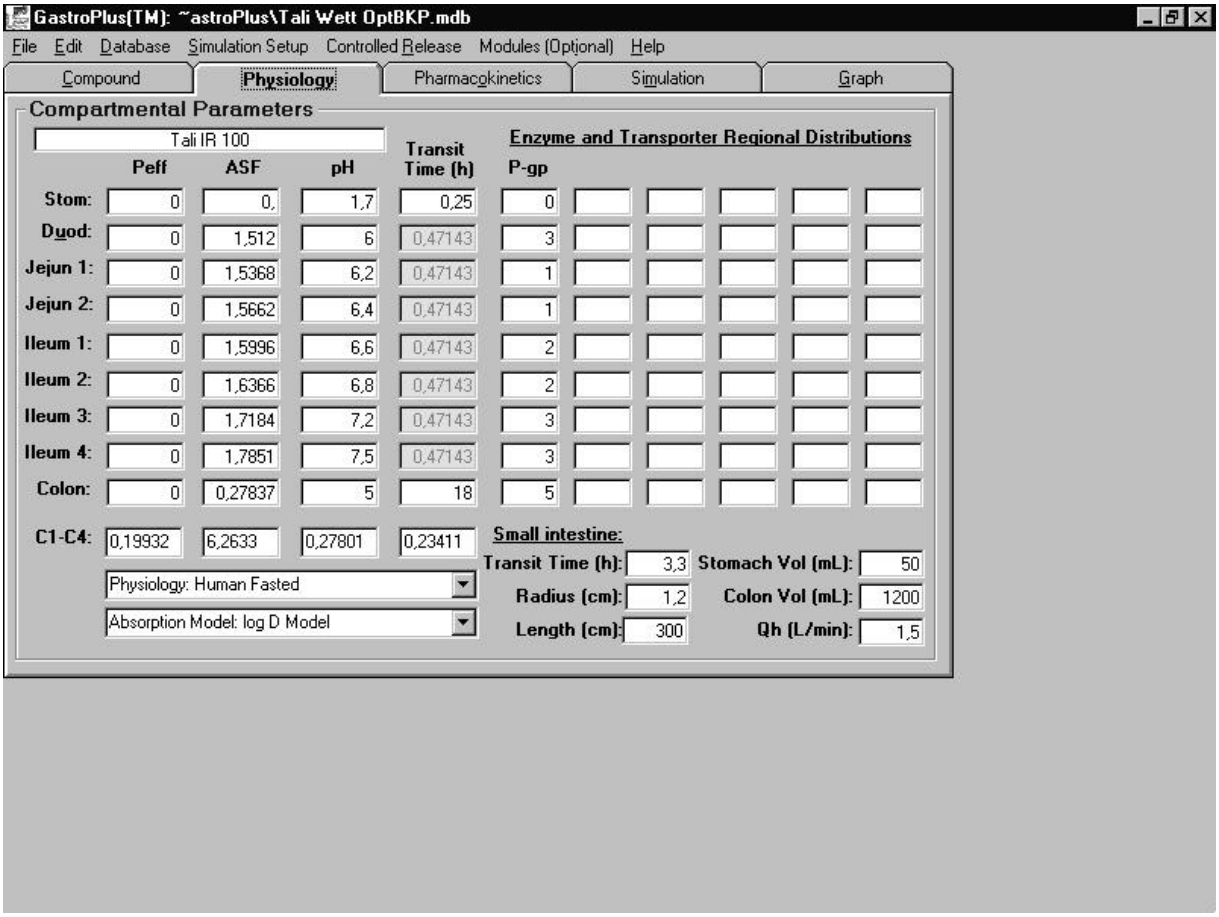


Figure V.2:

Physiological compartmental parameters for the fasted state in humans proposed by the Gastro Plus® simulation program. Scaling factors for the P-gp distribution were adopted on the basis of the results from rat perfusion studies presented in chapter II.

V.2.2.3 Pharmacokinetic properties of the compound

Additional pharmacokinetic parameters can be employed for Gastro Plus® simulations, as displayed in Figure V.3. The most adequate compartment model for the simulations was selected on the basis of data from a human study with 30 mg talinolol administered intravenously. For predictions of bioavailability and plasma profiles, clearance (CL) and the volume of the central compartment (V_c) are of particular importance, since they determine the elimination rate of a drug. Assuming a one-compartment model, the elimination rate constant k_e can be calculated as:

$k_e = CL / V_c$ [1]

The clearance (CL) is determined taking into consideration the fraction that is bioavailable (F_b), multiplied by the administered dose (D) and divided by the area under the curve (AUC) of the plasma concentration-versus-time profile:

$CL = F_b \cdot D / AUC$ [2]

Since the values reported in literature for clearance and volume of the central compartment of talinolol vary considerably, these parameters were estimated using the optimization module of Gastro Plus®. Additionally, preliminary simulations demonstrated that the P_{eff} values derived from the rat perfusion studies led to underestimated plasma levels when used as input for simulations in humans. Therefore this parameter was also subject to an optimization step. Constraints were set to assure that the estimated values for CL (0.01 to 10 l/h/kg), V_c (0.01 to 50 l/kg) and P_{eff} (0.01 to $2 \cdot 10^{-4}$ cm/s) lie within a reasonable range.

The optimization module of Gastro Plus® can calculate the most probable value for any unknown parameter on the basis of oral plasma concentration-versus-time or other in vivo data. The optimization is based on the search for the minimum sum of squared errors (SSE). The program calculates the square of the deviations between all calculated and observed values using one specific value for the parameter to be optimized and sums them up. This procedure is repeated numerous times employing altering values for the parameter to be optimized. Finally, the value leading to the lowest sum of squared errors is proposed as optimized parameter.

For the optimization of CL, V_c, P_{eff} and the P-gp transporter parameters K_m and V_{max}, data from a human in vivo study with different doses of talinolol (25 mg, 50 mg, 100 mg, and 400 mg p.o. and 30 mg i.v.) administered as immediate-release dosage forms and bolus injection published by Wetterich et al. were used [Wetterich, U., H. Spahn-Langguth, et al. (1996)]. The original data were taken from the doctoral thesis of U. Wetterich [Wetterich, U. (1995)].

Due to the fact that the biotransformation of talinolol accounts for less than 1 % [Oertel, R., K. Richter, et al. (1994), Oertel, R. and K. Richter (1995)], the parameter for the first-pass extraction was set to 0 %. The unbound fraction in plasma was

calculated as the difference between 100 % and the fraction bound to plasma proteins (i.e. 55 % [Trausch, B., R. Oertel, et al. (1995)]):

$F_{unbound}$= 100 % - 55 % = 45 %.

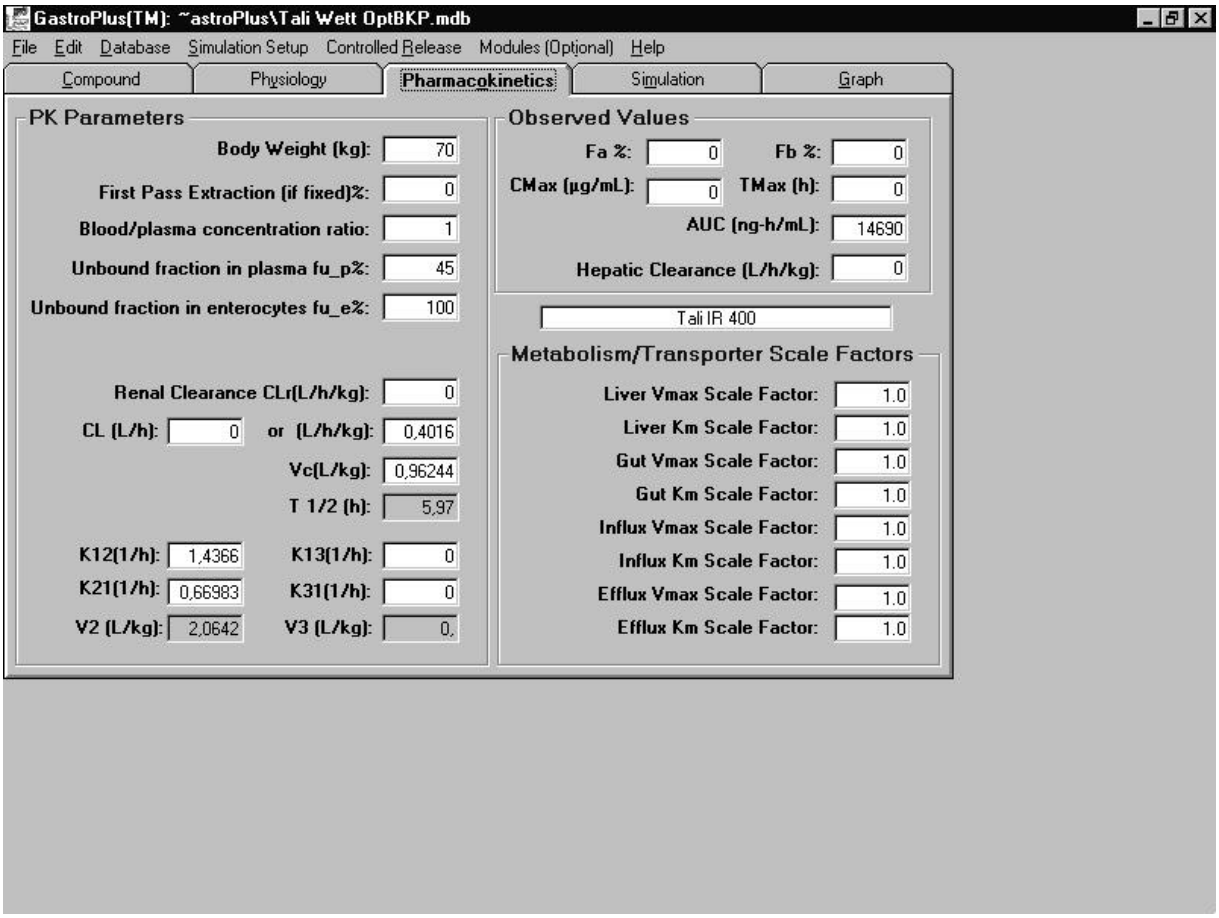


Figure V.3:

Pharmacokinetic screen of the Gastro Plus® simulation program showing the pharmacokinetic data available for talinolol and the optimized parameters suggested by Gastro Plus®.

V.2.2.4 Drug release profile of controlled-release dosage forms

The dissolution profile entered for the sustained-release tablet developed (cp. Chapter III) in the “controlled-release data file” is displayed in Figure V.4. Since different drug release profiles for different pH values or different intestinal regions could not be entered, a profile with a rather consistent release of 90 % of the drug over an eight hour period was selected as input for the computer simulations with CR

dosage forms. The profile was used as an average of the profiles obtained in in vitro dissolution tests at pH values of 1.0, 4.5 and 6.8.

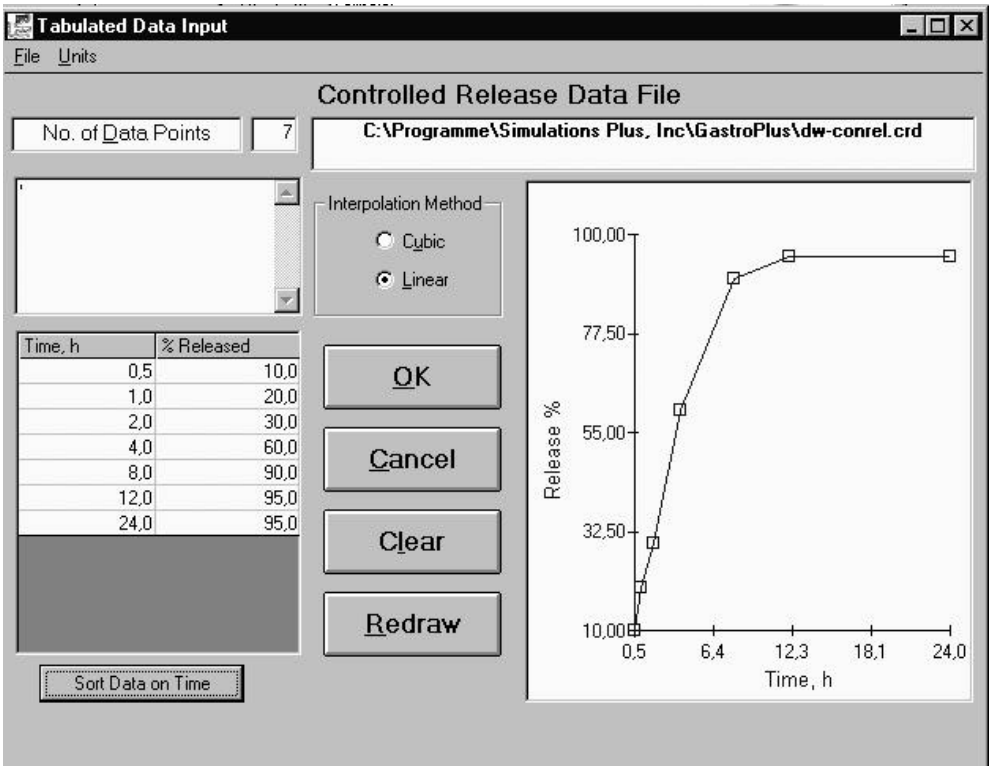


Figure V.4:

The controlled-release data input screen of the Gastro Plus® software program showing a drug release similar to the release from the newly developed talinolol sustained-release dosage form described in Chapter III.

Figure V.5 displays the in vitro dissolution properties of the sustained-release tablets developed at different pH values, along with the drug release profile used in the computer simulations with Gastro Plus®.

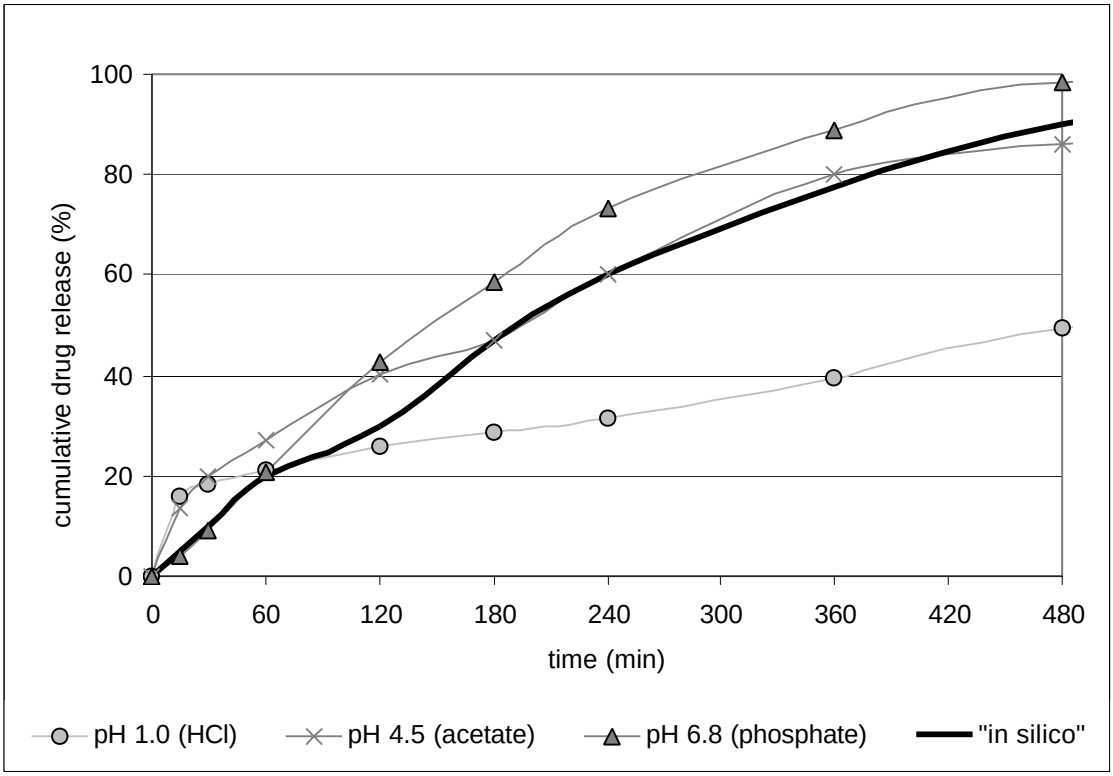


Fig. V.5:

Comparison between dissolution profiles obtained in vitro in dissolution media of different pH values and the drug release profile used for in silico simulations of talinolol sustained-release dosage forms.

Gastro Plus® enables the simulation of different controlled-release dosage forms, e.g. multiple unit controlled-release (“dispersed”), single unit controlled-release (“integral tablet”) or gastroretentive dosage forms (“gastric release”). For the in silico evaluation of the newly developed talinolol sustained-release matrix tablets (cp. Chapter III) the mode “controlled-release integral tablet” was selected, since the properties of this dosage form (non-disintegrating single unit CR tablet) were typical for matrix tablets.

V.2.3 Evaluation of the software

To evaluate the Gastro Plus® software program, simulations were performed for talinolol immediate-release tablets containing 25, 50, 100 and 400 mg talinolol and an intravenous bolus injection of 30 mg talinolol. The results were compared with the original data published by Wetterich [Wetterich, U. (1995), Wetterich, U., H. Spahn-Langguth, et al. (1996)] The simulations should demonstrate the capability of the software program to simulate intestinal efflux effects. Thereafter, bioavailability and plasma concentration-versus-time profiles were estimated for sustained-release tablets with a drug content of 100 and 200 mg.

V.3 Results and discussion

V.3.1 Selection of an adequate compartment model

The compartment model for all simulations was selected on the basis of human plasma data after an intravenous bolus of 30 mg talinolol. The data were fitted to a one-, two- and three-compartment model. The results are displayed in Figure V.6

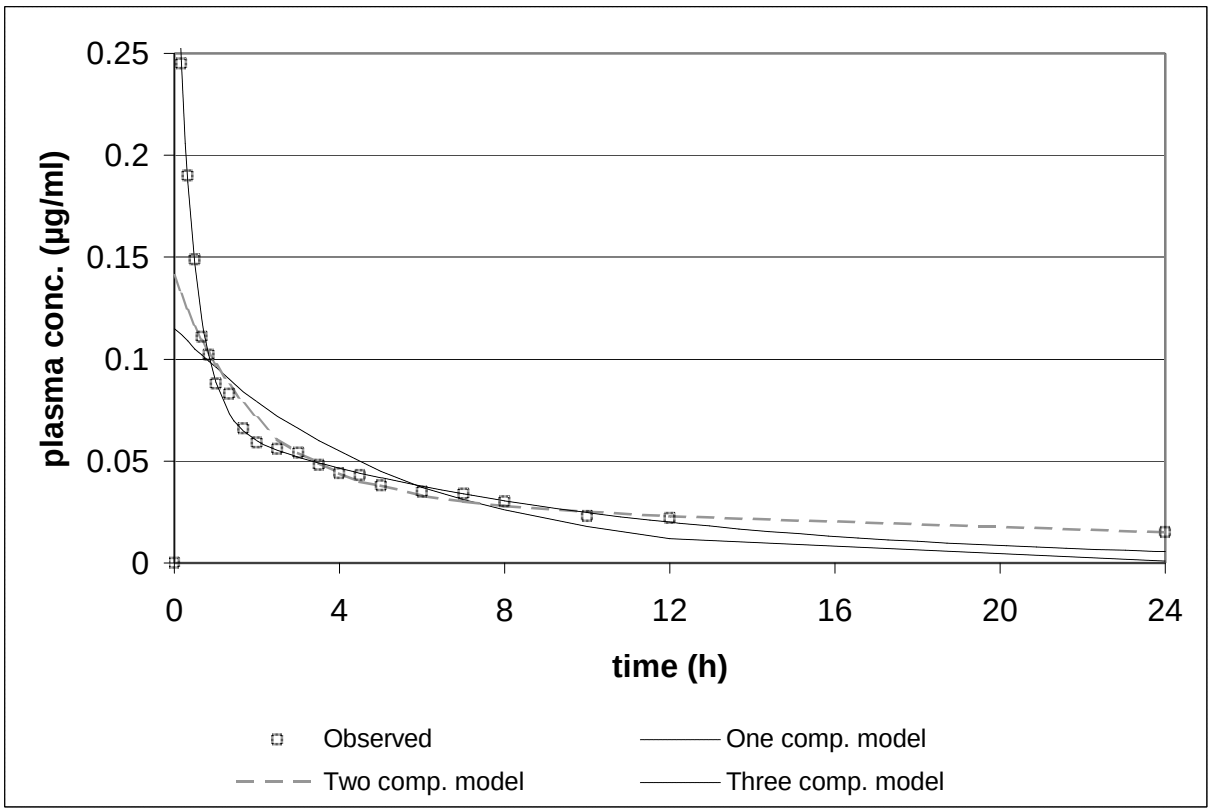


Figure V.6:
Fit of plasma data from a human study with 30 mg talinolol administered as i.v. bolus to one-, two-, and three-compartment models.

Figure V.6 illustrates that the three-compartment model provides the best fit of the data. For the one-compartment model deviations were rather distinct, whereas the two-compartment model shows only minor deviations within the first four hours. Two

different initial distribution phases can be observed, arguing for the three-compartment model.

However, when two models lead to similar fits the less complicated model generally should be preferred. For predictions of peroral dosage forms the initial distribution phase that is described better in the fit to the three-compartment model can be neglected, because the drug release from the dosage form and the absorption process have a stronger impact on the plasma levels in the initial phase. Therefore the implication of a third compartment is not expected to improve the predictions, but complicates the model and the calculations notedly. Consequently, the two-compartment model was selected for the following simulations.

Compared to the simple one-compartment model, the assumption of a two-compartment model requires three further parameters, k_{12}, k_{21} and V_2. K_{12} and k_{21} describe the exchange rate between the two compartments: k_{12} determines the flow rate from the central compartment 1 to compartment 2 and k_{21} determines the flow rate from compartment 2 back to the central compartment 1. V_2 represents the volume of the second compartment. The intercompartmental exchange rate constants k_{12} and k_{21} and the volume of the second compartment V_2 were estimated using the optimization module of the Gastro Plus® software program. K_{12} and k_{21} were predicted as 1.4366 h^{-1} and 0.66983 h^{-1}, for V_2 a value of 2.0642 l/kg was calculated.

V.3.2 Optimization of clearance, volume of central compartment and effective permeability

For the clearance (CL) and the volume of the central compartment (V_c) the optimization suggested 0.402 l/h/kg and 0.962 l/kg, respectively. The value for the clearance lies within the range reported by Wetterich et al. (0.253 to 0.811 l/h/kg), whereas the value suggested for the volume of the central compartment is higher than reported (0.2 to 0.5 l/kg) [Wetterich, U. (1995)]. As for the effective permeability, the optimization estimated an approximately three times higher value than suggested by the rat studies (1.777 instead of 0.58). These deviations can be explained by apparent differences between the two species. Although Gastro Plus® tries to adopt

rat data to the human physiology by means of a scaling factor, such adaptions may not result in perfectly matching permeability values.

V.3.3 Optimization of the transporter parameters K_m and V_{max}

Preliminary simulations demonstrated that the transporter parameters K_m and V_{max} (Michaelis-Menten parameters) have a pronounced influence on the in silico results. This is due to the fact that these parameters determine the extent of the intestinal drug efflux as well as the saturation level of the efflux pump.

In order to obtain good correlations between in silico estimations and in vivo data, appropriate values for K_m and V_{max} had to be selected. The optimization performed using the Gastro Plus® optimization module suggested 6.841 µg/ml for K_m and 0.00195 mg/s for V_{max}. These results are realistic considering potential talinolol concentrations in the gastrointestinal fluids after peroral administration of 25 mg to 400 mg talinolol.

For the interpretation of the parameter optimization results, it is important to keep in mind the meaning of the parameters K_m and V_{max}. Figure V.7 shows the general Michaelis-Menten kinetic model including the definition of the parameters K_m and V_{max}.

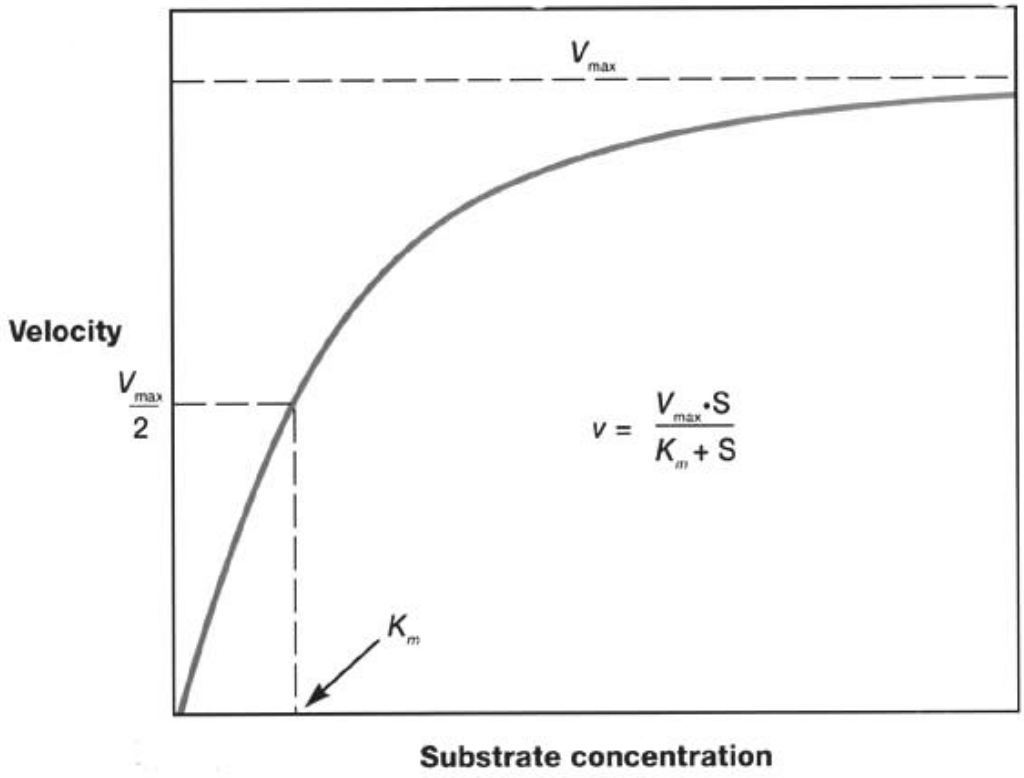


Figure V.7:
Characteristic saturation curve for processes with Michaelis-Menten kinetics including definitions of the parameters K_m and V_{max}.

Primarily, this model was set up to describe the kinetics of enzymatic reactions. For lower substrate concentrations, the velocity of an enzymatic reaction between enzyme and substrate increases rapidly. The increase decelerates with increasing substrate concentrations and approximates a maximum velocity level. The turnover of the substrate is limited by the saturability of the enzyme.

V_{max} is the maximum velocity of the enzymatic reaction. K_m is the substrate concentration when the velocity is at half maximum ($V_{max}/2$).

The kinetic model can be applied for transporter-mediated reactions, since the phenomenon of saturation is homologous. In this case, V_{max} describes the maximum transport velocity and K_m describes the substrate concentration when the transport velocity is of half of its maximum. Thus, the two parameters are a good measure to characterize the properties of transport proteins: High V_{max} values refer to transporters with high turnover rates and low K_m values indicate that a transporter rapidly reaches its saturation level. Since actual values are not directly measurable,

V_{max} and K_m can only be estimated for the P-gp-mediated secretion in the human intestine.

V.3.4 Simulations with immediate-release dosage forms containing 25 mg, 50 mg, 100 mg, and 400 mg talinolol

Figure V.8 displays the results screen for simulations performed with Gastro Plus®. The route of the administered dosage form through the different regions of the gastrointestinal tract with respect to the sites of dissolution and absorption can be observed during a simulation. In Figure V.8 the absorption of talinolol from an immediate-release tablet containing 100 mg talinolol is simulated using the optimized parameters for k_{12}, k_{21}, CL, V_c, V_2, P_{eff} and the transporter parameters K_m and V_{max}.

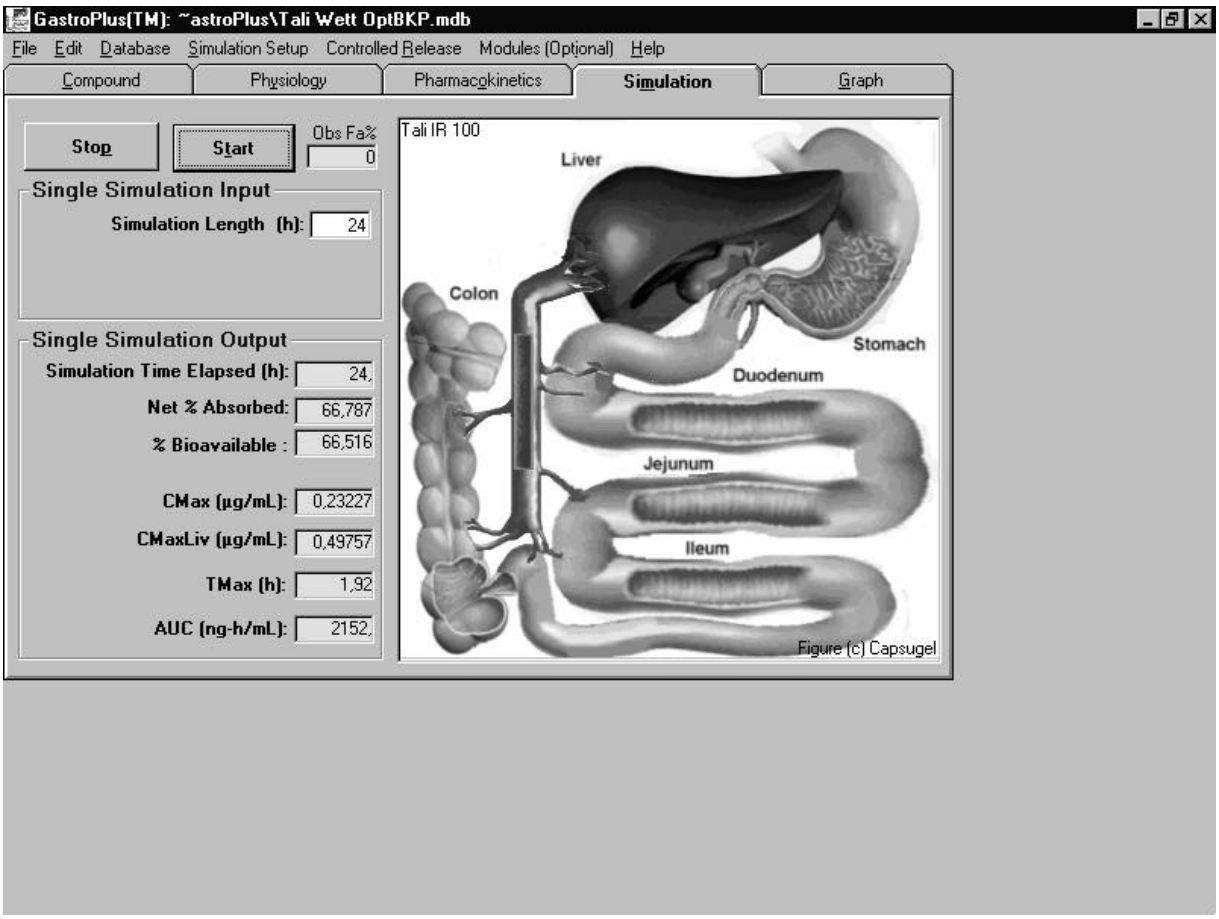
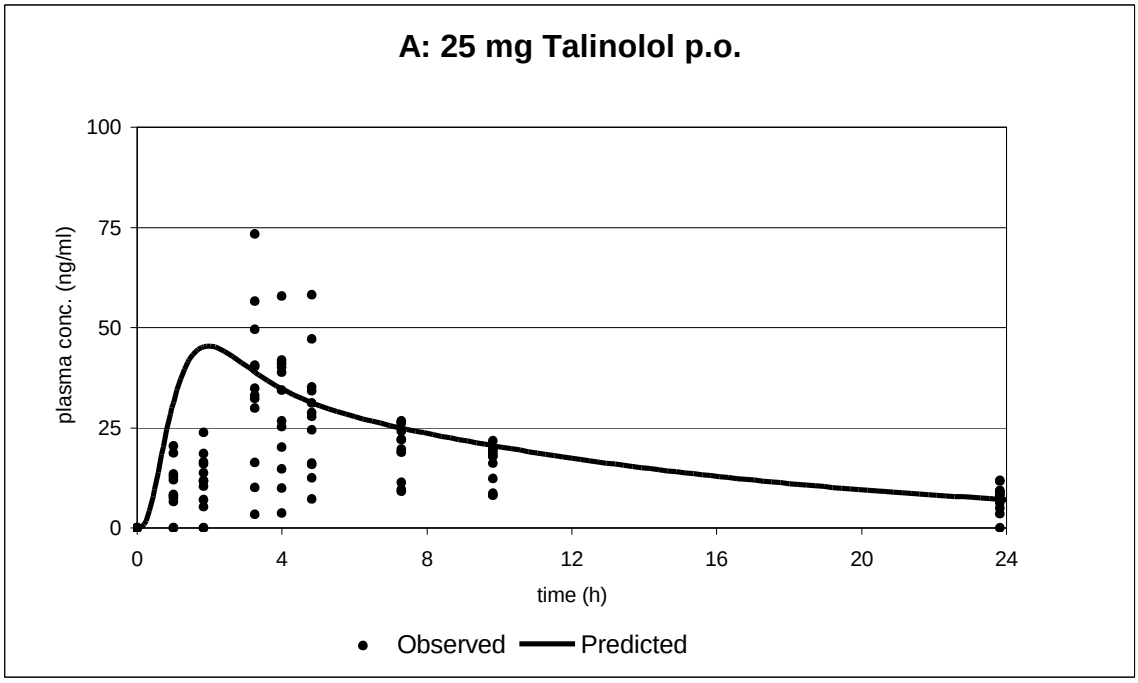
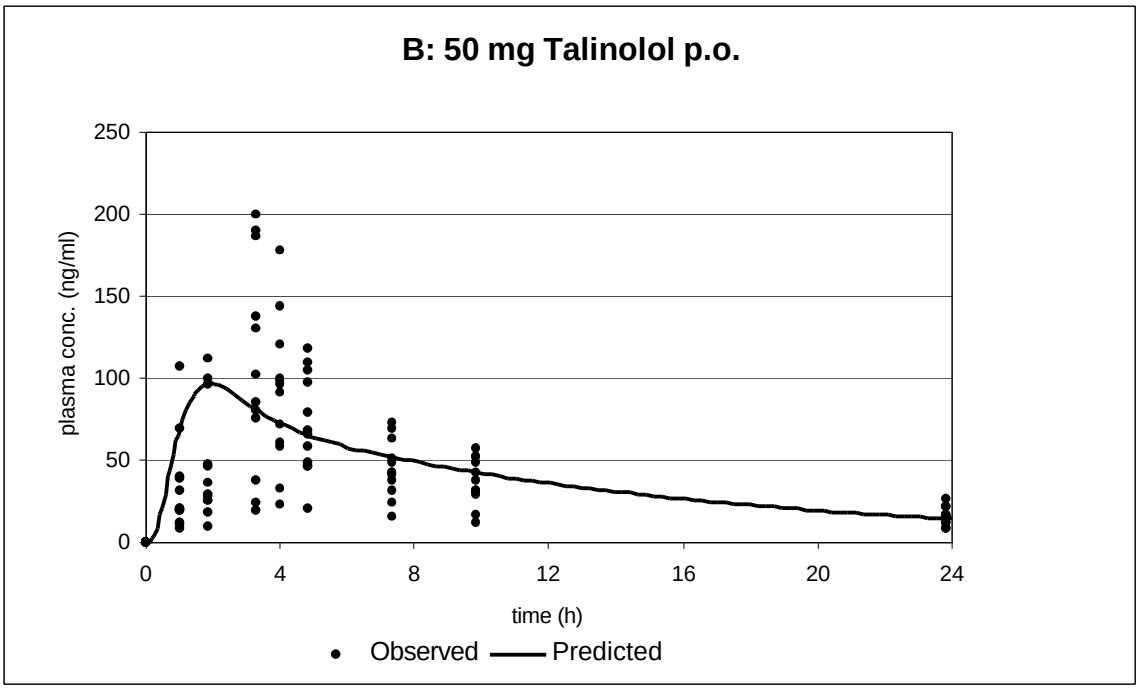
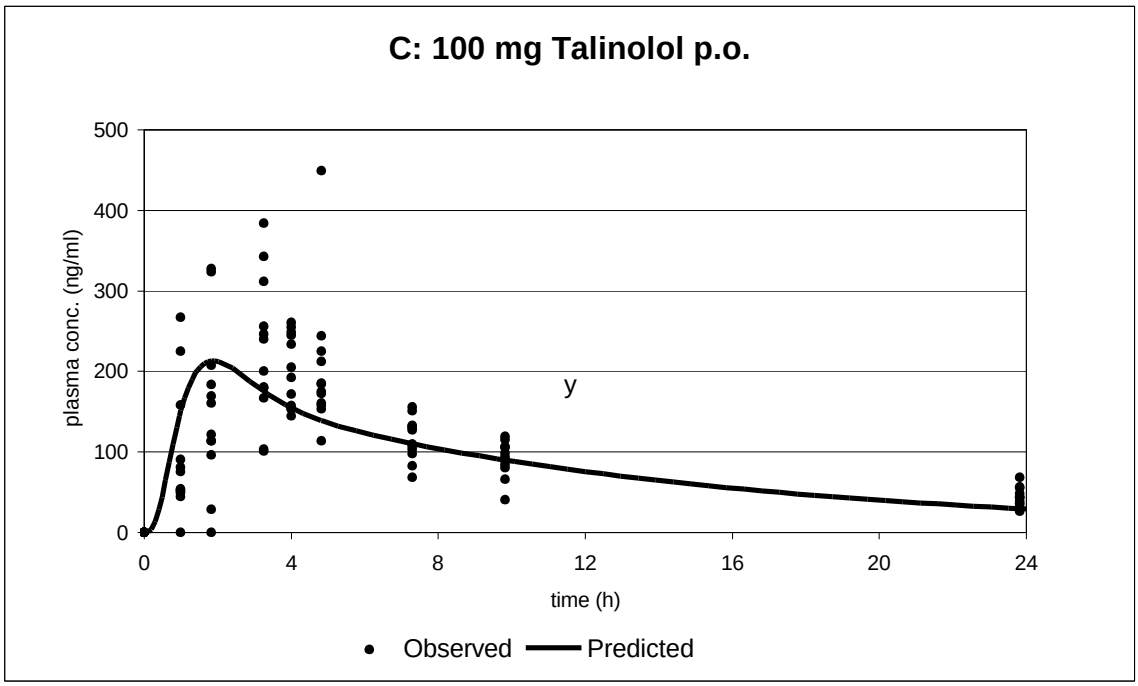


Figure V.8:
Simulation results screen of the Gastro Plus® program, showing the simulation of the absorption of a 100 mg talinolol immediate-release tablet.

The capability of the Gastro Plus® program to simulate intestinal drug efflux related effects had to be demonstrated in order to show that the program was suitable to predict the bioavailability and other pharmacokinetic parameters of talinolol sustained-release tablets. In vivo, an overproportional increase of AUC and bioavailability with increasing doses was observed due to a saturation of the intestinal efflux pump P-gp (cp. Chapter II) [Wetterich, U., H. Spahn-Langguth, et al. (1996)]. This phenomenon should be simulated in silico by the Gastro Plus® software. Simulations for immediate-release dosage forms containing 25 mg, 50 mg, 100 mg, and 400 mg of talinolol were compared with the plasma concentration-versus-time curves observed in vivo. The results are given in Figures V.9 A - D.


A: 25 mg Talinolol p.o.
plasma conc. (ng/ml)
100
75
50
25
0
0
4
8
12
16
20
24
time (h)
Observed
Predicted


B: 50 mg Talinolol p.o.
plasma conc. (ng/ml)
250
200
150
100
50
0
0
4
8
12
16
20
24
time (h)
Observed
Predicted



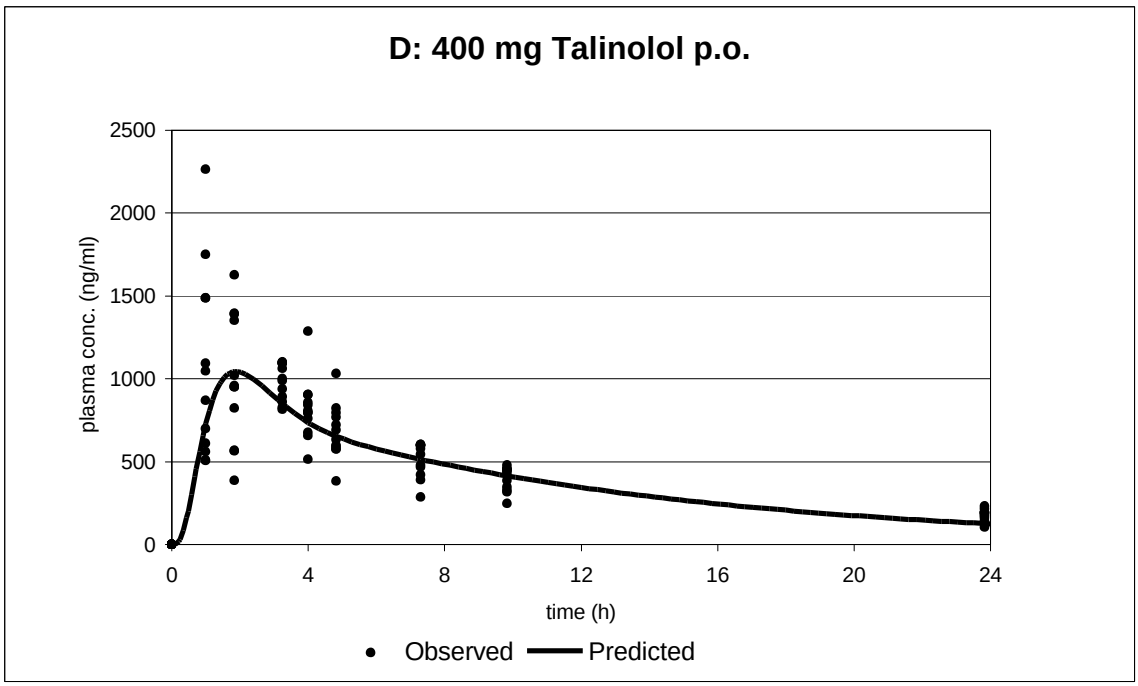


Figure V.9 A to D:

Comparison between predicted and observed plasma levels after peroral administration of talinolol immediate-release dosage forms with drug contents of 25 mg (A), 50 mg (B), 100 mg (C) and 400 mg (D). The plasma concentrations displayed as black dots were observed in an in vivo study in 12 healthy volunteers. The predictions were performed using the Gastro Plus® software.

The simulation results demonstrate acceptable predictions of the in vivo plasma profiles. Most of the predicted plasma concentrations lie in the range between the minimum and maximum plasma levels obtained in the in vivo study. The predicted values are similar to the mean values obtained from the 12 plasma concentration-versus-time curves.

The results given in Table V.3 demonstrate that it is possible to simulate dose dependent absorption. Bioavailability relevantly increases with higher doses and the AUC-dose ratio increases notably. These findings are important to demonstrate that the program is capable of simulating effects caused by intestinal drug efflux. As the program can simulate the P-gp effect for immediate-release dosage forms, it can also be assumed to predict P-gp effects for sustained-release dosage forms reliably. The deviations between the AUC-dose ratios calculated from the in silico simulations and from the in vivo data are not astonishing, since the absorption and elimination process in vivo is so complex that the model used in silico can not consider all factors exactly.

Table V.3:

Simulation results for immediate-release formulations containing 25 mg, 50 mg, 100 mg, and 400 mg talinolol. The increases in bioavailability and the AUC / dose-ratio demonstrate a dose dependence caused by the saturability of the intestinal drug efflux. For comparison AUC / dose-ratios (Means ± S.D.) observed in vivo are added [Wetterich, U., H. Spahn-Langguth, et al. (1996)].

Dosage Form	**Bioavailability (%)**	**AUC (ng h/ml)**	**AUC / Dose**	**AUC / Dose In vivo study**
IR 25 mg	63.2	560	22.4	20.0 ± 6.32
IR 50 mg	65.4	1160	23.2	24.8 ± 8.8
IR 100 mg	68.8	2440	24.4	32.8 ± 7.9
IR 400 mg	78.0	11100	27.8	36.7 ± 7.7

V.3.5 Simulations for talinolol controlled-release dosage forms

Simulations of bioavailability and plasma concentration-versus-time curves for sustained-release dosage forms containing 100 mg and 200 mg talinolol were performed using the optimized parameters evaluated in the simulations with different doses of talinolol immediate-release dosage forms. To demonstrate the pronounced effect of intestinal drug efflux, the simulations were repeated omitting the influence of the intestinal efflux pump P-gp (CR 100 / 200 mg Integral Tablet - P-gp). For these estimations the transporter data set was deleted. All other parameters were kept constant. The estimation results are presented in Table V.4. For better visualization of the differences between IR and CR dosage forms, simulation results for 100 mg and 200 mg IR tablets are presented together with the results for the CR formulations.

Table V.4:

Simulation of basic pharmacokinetic parameters (bioavailability, C_{max}, t_{max}, and AUC) for controlled-release dosage forms containing 100 and 200 mg talinolol performed with the Gastro Plus® software. The simulation results for the CR formulations without consideration of P-gp as mediator of an intestinal secretion (Integral Tablet - P-gp) underline the pronounced effect of the intestinal drug efflux. For comparison, simulated parameters of the according immediate-release tablets are added in italics.

Dosage Form	**Bioavailability (%)**	**C_{max} (ng/ml)**	**t_{max} (h)**	**AUC (ng h/ml)**
IR 100 mg Tablet	*68.8*	*232.3*	*1.9*	*2442*
CR 100 mg Integral Tablet	**37.4**	**64.1**	**3.6**	**1322**
CR 100 mg Integral Tablet - P-gp	75.7	144.8	3.8	2689
IR 200 mg Tablet	*73.2*	*516.5*	*1.9*	*5198*
CR 200 mg, Integral Tablet	**40.9**	**149.6**	**3.7**	**2892**
CR 200 mg, Integral Tablet - P-gp	75.7	289.5	3.8	5379

The simulation results showed the expected effect of a decrease in bioavailability for controlled-release dosage forms. As for the 200 mg controlled-release formulation, bioavailability decreased from 73.2 % for the immediate-release dosage form to 40.9 % for the sustained-release tablet. The results for the 100 mg dosage form were

similar. The biovailability of 68.8 % estimated for the IR tablet was reduced to 37.4 % for the CR tablet.

The lower values for the CR dosage forms can be explained by differences in the contribution of the P-gp-mediated drug efflux to the overall absorption. In CR drug products the talinolol release from the dosage form is decelerated. Thus, the drug concentrations in the GI tract are maintained below the saturation level of the efflux pump. As a result, the amount of talinolol secreted back into the gastrointestinal lumen by P-gp increases. This leads to a decrease of the overall absorption and to reduced bioavailabilities.

In addition, the Gastro Plus® simulations assumed higher P-gp expression levels in lower gastrointestinal regions, especially in the colon, which is in accordance with the results of the intestinal rat perfusion studies presented in chapter II and intestinal P-gp distribution data from rats reported in the literature [Tamura, S., A. Ohike, et al. (2002), Makhey, V. D., A. Guo, et al. (1998)]. This also factors into the increased drug efflux, since the decelerated dissolution of the drug from CR dosage forms displaces the drug release to lower regions of the GI tract. This in turn leads to diminished estimates for the bioavailability.

The decrease in bioavailability goes in parallel with a reduction of the maximum plasma concentration (C_{max}) for the CR formulations to less than one third of the C_{max} of the IR dosage forms. Furthermore, the time until this maximum plasma concentration is reached (t_{max}) is approximately doubled after administration of the CR tablet. Generally, decreases in C_{max} and increases in t_{max} are not uncommon for CR dosage forms and can also be observed for other controlled-release dosage forms that are not subject to intestinal drug efflux.

Figure V.10 compares the plasma concentration-versus-time profiles simulated for 100 mg talinolol immediate-release and controlled-release tablets as displayed by the Gastro Plus® program.

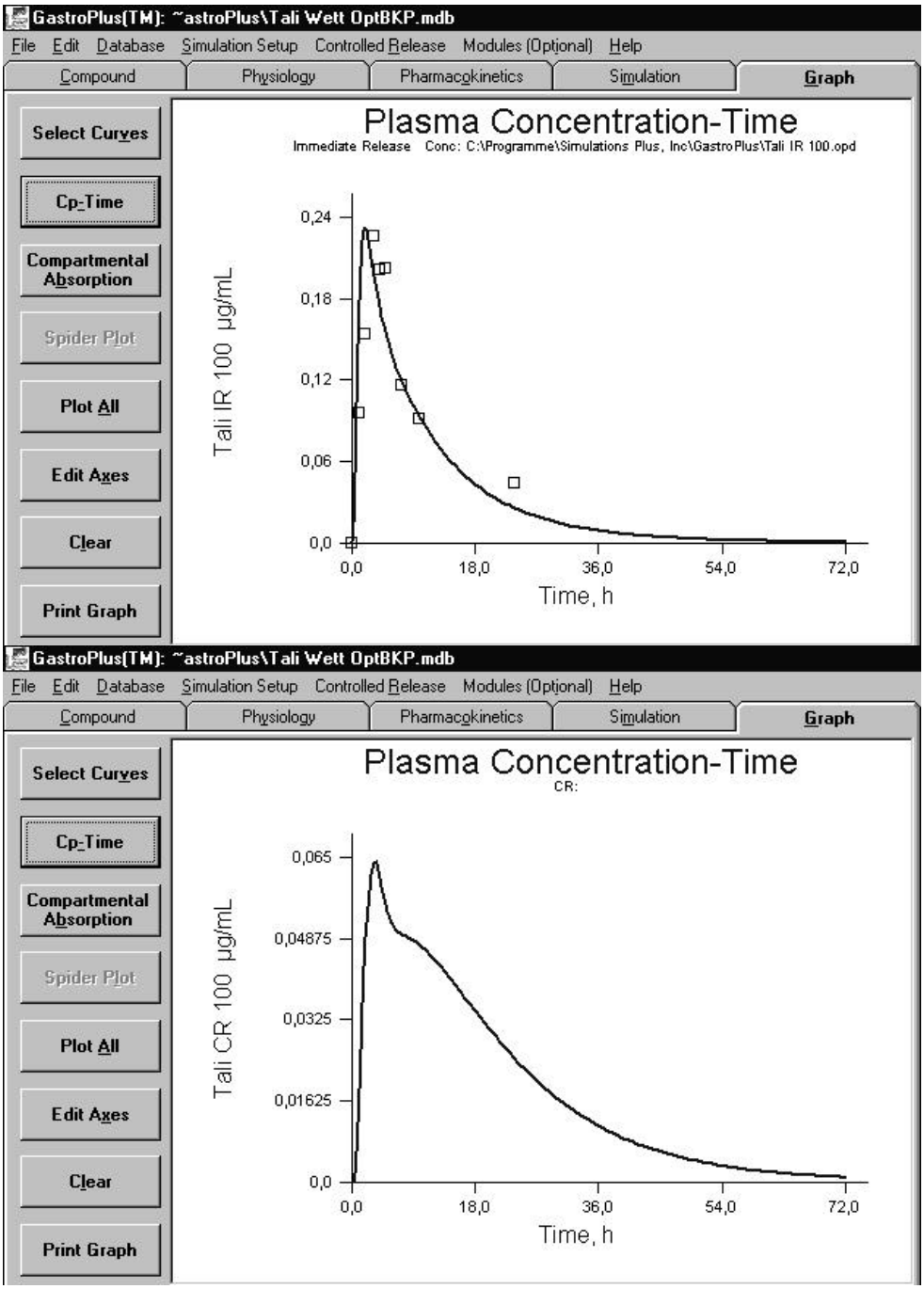


Figure V.10:

Plasma concentration-versus-time profiles after administration of a 100 mg talinolol immediate-release and a 100 mg talinolol controlled-release tablet simulated by Gastro Plus®. The black squares in the upper profile indicate plasma concentrations observed in vivo after administration of a 100 mg IR dosage form.

The simulated plasma profile after administration of the CR formulation shows three phases. In the first phase (0 – 4 h) the drug concentration in the blood is persistently and rapidly increasing. In the following phase (4 – 6 h) a rapid decrease can be observed. Finally, in the third phase (> 6 h) the decrease of the plasma levels is decelerated.

These phases can be explained by the changes in the ratio between release of the drug from the dosage form, absorption, distribution and elimination: Within the first phase, drug release and absorption markedly exceed distribution to the second compartment and elimination, provoking the steep rise of the talinolol plasma concentrations. In the second phase, the drug release rate from the tablet decreases due to the longer diffusion distances from the inner regions of the matrix tablet. Additionally the drug is notably distributed into the second compartment. In combination with a more or less unchanged absorption and elimination this leads to the rapidly decreasing plasma concentrations. In the third phase, this decrease is decelerated by the redistribution of drug from the second compartment.

V.4 Conclusions

Computer simulations are a helpful tool to estimate the in vivo absorption of drugs. For simulations with drugs exhibiting non-linear absorption patterns, it is necessary to utilize software programs that are able to employ estimation steps that simulate the particular mechanisms responsible for the non-linearities. Gastro Plus® is considered to be an appropriate program to simulate the absorption of drugs that undergo carrier-mediated transport or intestinal biotransformation. These observations are in accordance with the assessment of Parrott and Lave, who found that Gastro Plus® was a powerful tool in the predictions of intestinal absorption in humans [Parrott, N. and T. Lave (2002)]. In their assessment, Gastro Plus® was compared to IDEA®, another commercially available simulation program described in literature by Grass et al [Grass, G. M. (1997), Norris, D. A., G. D. Leesman, et al. (2000)]. According to Parrott et al., the advantages of Gastro Plus® include the possibility to easily adjust model parameters and to integrate available in vitro or in vivo data to increase the reliability of the predictions. In a test series employing 28 drugs, 70 % of the drugs were classified correctly in terms of their fractions absorbed [Parrott, N. and T. Lave (2002)].

For simulations of pharmacokinetic parameters of talinolol the P-gp-mediated intestinal drug efflux plays an important role. In this case, Gastro Plus® simulations can be optimized by employing data on the transporter properties on the one hand and the distribution of the transporter throughout the gastrointestinal tract on the other hand. Data on the distribution of P-gp in different sections of the gastrointestinal tract is reported from rats [Wagner, D., H. Spahn-Langguth, et al. (2001), Tamura, S., A. Ohike, et al. (2002), Makhey, V. D., A. Guo, et al. (1998)], catfish [Kleinow, K. M., A. M. Doi, et al. (2000)], and micropigs [Tang, H., Y. Pak, et al. (2002)]. However, the conclusions are controversial. Rat data suggests increasing P-gp levels in the ileum and colon, which is in accordance with the observations in catfish. However, in micropigs P-gp expression is reported to be higher in proximal parts of the small intestine as compared to distal portions.

As long as reliable in vivo data from humans are not available, neither for the transporter parameters, K_m and V_{max}, nor for the distribution of P-gp in the gastrointestinal tract, simulations are to some extent uncertain.

Overall, it can be concluded that in silico estimations can not completely replace in vivo tests. Even complex models are not able to reflect the in vivo situation completely. A clinical study in humans is useful to confirm the estimates of the simulations.

Therefore, prearrangements for a clinical study with the newly developed sustained-release tablets (cp. chapter III) are in progress. The study protocol and the request for approval of the study by the local ethics committee are attached in Annex II.

Finally, a comparison between the results of the in vivo study and the in silico simulations will determine the quality of the in silico predictions. Furthermore, an in vivo evaluation of talinolol controlled-release dosage forms can be helpful to further optimize parameters (e.g. K_m and V_{max} for the P-gp efflux) used as input for the in silico predictions and thus minimize uncertainties of in silico simulations for P-gp substrates in the future.

Summary

Intestinal drug efflux mediated by transporters located in the gastrointestinal tract, such as P-glycoprotein (P-gp), is a known source of incomplete and variable bioavailability, as well as of interactions with other drugs and food components. However, there are no publications, which deal with arising consequences for the design of new peroral formulations. The aim of this doctoral thesis was to point out that the occurrence of intestinal secretion has to be accounted for in the development of controlled-release dosage forms.

For this purpose, effective permeabilities of the model compound talinolol were determined for different intestinal segments (jejunum, ileum and colon) employing an intestinal perfusion model in rats. Increasing passive permeabilities were detected from jejunum to ileum and colon. Perfusion experiments with P-gp inhibitors added to the perfusion solutions suggested an increasing role of the P-gp mediated drug efflux in more distal parts of the gastrointestinal tract (jejunum < ileum < colon).

Furthermore, a sustained-release formulation was developed for the model drug talinolol. The matrix tablet releases the drug rather consistently within approximately eight hours, without being relevantly affected by changing pH values within the gastrointestinal tract. In this context, it was demonstrated that the selection of the type of buffer used as media in dissolution tests had a pronounced influence on the drug release. The formation of different talinolol crystal structures in different buffers was detected as source for this unexpected phenomenon.

The newly developed sustained-release matrix tablets were evaluated in silico, i.e. with the aid of a suitable computer software program with which pharmacokinetic parameters and plasma levels can be predicted. Amongst others, the use of Gastro Plus®, the software employed, enables to simulate both, a modified drug release from the dosage form and a saturable intestinal drug efflux mediated by transporters such as P-gp.

The interplay between decelerated drug release from the dosage form and intestinal drug efflux led to a considerably decreased bioavailability of the model drug talinolol from sustained-release tablets in the in silico predictions compared to immediate-release dosage forms. This decrease in bioavailability can be explained by the slower exposure of the intestinal lumen to the drug. As a consequence, the saturation level

of the efflux pump P-gp, which is exceeded by the rapid exposure to the drug after administration of immediate-release dosage forms, is no longer reached. This leads to an increased impact of the secretion compared to passive absorption, which, finally, results in a decreased bioavailability.
To pursue an evaluation of these in silico results in vivo, preparations were made to test the newly developed talinolol sustained-release tablets in vivo. Therefore, a permission for the manufacturing of solid peroral dosage forms (“Herstellungserlaubnis”) at the University was granted, to be allowed to manufacture sustained-release tablets within a suitable room at the University in compliance with GMP (Good Manufacturing Practice) standards. Furthermore, following a request to the local ethics committee, a positive vote for a human study with talinolol immediate-release and sustained-release tablets was obtained from this ethics committee.

The results of this doctoral thesis lead to the conclusion that the bioavailability of drugs that are substrate to intestinal secretory transporters may be apparently diminished in sustained-release dosage forms. This demands that the influence of intestinal exsorptive transporters such as P-gp has to be considered in the development and design of controlled-release dosage forms.

Zusammenfassung

Die Sekretion von Arzneistoffen aus Darmzellen zurück ins Darmlumen, die durch im Gastrointestinaltrakt lokalisierte Transporter wie P-Glykoprotein (P-GP) vermittelt wird, stellt eine bekannte Quelle sowohl für unvollständige und variable Bioverfügbarkeiten, als auch für Interaktionen mit anderen Arzneimitteln und Nahrungsbestandteilen dar. Dennoch liegen bisher keine Veröffentlichungen vor, die sich mit daraus resultierenden Konsequenzen für die Entwicklung neuer peroraler Darreichungsformen befassen. Ziel der vorliegenden Arbeit war es, deutlich zu machen, dass dem Auftreten von intestinalen Sekretionsphänomenen bei der Entwicklung von Retardarzneimitteln Rechnung getragen werden muss.

Dazu wurden anhand eines Rattendarmperfusionsmodells effektive Permeabilitäten für den Modellarzneistoff Talinolol in unterschiedlichen Darmabschnitten (Jejunum, Ileum und Colon) bestimmt. Die effektive passive Permeabilität stieg vom Jejunum über das Ileum zum Colon an. Versuche unter Zusatz von P-GP-Hemmern in den Perfusionslösungen deuteten auf eine stärkere Rolle der intestinalen Sekretion in tieferen Darmabschnitten hin (Jejunum < Ileum < Colon).

Des weiteren wurde eine Retardmatrixtablette für den Modellarzneistoff Talinolol entwickelt, die den Wirkstoff über ca. acht Stunden relativ unabhängig von den wechselnden pH Werten im Gastrointestinaltrakt freigibt. Dabei wurde gezeigt, dass die Auswahl der Pufferart für die Prüfflüssigkeiten entscheidenden Einfluss auf die Wirkstofffreisetzung hat. Als Ursache für diesen unerwarteten Effekt konnte die Ausbildung unterschiedlicher Talinolol-Kristallstrukturen in verschiedenen Puffern ausgemacht werden.

Die neu entwickelten Retardmatrixtabletten wurde in silico, das heißt mit Hilfe eines geeigneten Computersoftwareprogrammes, mit dem pharmakokinetische Parameter sowie Blutspiegelkurven vorhergesagt werden können, evaluiert. Unter Verwendung des Computerprogramms Gastro Plus® war es möglich, sowohl veränderte Wirkstofffreigabegeschwindigkeiten aus einer Arzneiform als auch eine sättigbare intestinale Sekretion durch Transporter wie P-GP zu simulieren.

Die Computersimulationen mit schnell- und langsamfreisetzenden Talinololzubereitungen ergaben eine verringerte Bioverfügbarkeit bei verlangsamter Wirkstofffreigabe. Die erniedrigte Bioverfügbarkeit kann dadurch erklärt werden, dass

die verringerte Wirkstofffreigabegeschwindigkeit zu einem langsameren Anfluten des Arzneistoffs und damit zu niedrigeren Arzneistoffkonzentrationen in den intestinalen Flüssigkeiten führt. Dies hat zur Folge, dass die Sättigungsgrenze des sekretorischen Transporters P-GP, die beim raschen Anfluten des Wirkstoffs nach Gabe schnellfreisetzender Arzneiformen überschritten wird, nicht mehr erreicht wird. Dadurch steigt der Einfluss der Sekretion im Vergleich zur passiven Absorption an und die insgesamt in den Blutkreislauf absorbierte Menge an Arzneistoff sinkt.
Um eine Bestätigung dieser Hypothese in vivo zu ermöglichen, wurden im Rahmen dieses Dissertationsprojekts die Voraussetzungen dafür geschaffen, die entwickelte Talinolol-Retardformulierung in vivo zu testen. Dazu wurde eine Herstellungserlaubnis von der zuständigen Landesbehörde erhalten, um die Retardtabletten in einem geeigneten Herstellungsraum an der Universität unter GMP (Good Manufacturing Practice) konformen Bedingungen produzieren zu können. Außerdem wurde das Einverständnis der zuständigen Ethik-Kommission für eine klinische Studie mit schnell- und retardiert-freisetzenden Talinololtabletten erhalten.

Für die Entwicklung von Retardarzneiformen ergibt sich aus der vorliegenden Arbeit, dass beim Auftreten von intestinaler Sekretion die Bioverfügbarkeit entsprechender Retardpräparate entscheidend absinken kann. Daher sollte bei Arzneistoffen, für die Retardarzneiformen entwickelt werden sollen, der Einfluß intestinaler sekretorischer Transporter wie P-GP unbedingt berücksichtigt werden.

Annex I

Annex I

Production of clinical trial formulations according to the rules of Good Manufacturing Practice (GMP) in university laboratories

AI.1 Introduction

The manufacture of dosage forms to be used either for the treatment of diseases or the testing of dosage forms to be used in clinical trials requires a prescription by an authorized medical doctor which is subsequently filled by a pharmacist in a public or hospital pharmacy. Alternatively, the drug product may be manufactured by a pharmaceutical manufacturer (“Pharmazeutischer Unternehmer”) under the conditions and rules of the principles of Good Manufacturing Practice. A pharmaceutical manufacturer must have been approved and - in most cases - inspected by the regulatory authorities of his country of origin and / or by the authorities of the country the pharmaceutical product shall be exported to. Since a university laboratory cannot be accredited as a public or hospital pharmacy, the only feasible alternative in order to produce formulations to be evaluated in clinical trials is to seek for regulatory approval as pharmaceutical manufacturer and to obtain official permission of manufacture (“Herstellungserlaubnis”) for defined dosage forms in the university laboratories.

The permission implies that its holder assures that all products manufactured at his site comply with the three basic requirements for pharmaceuticals: *quality*, *efficacy* and *safety*.

Efficacy and *safety* of a drug substance have to be proven in clinical studies. In the present project, efficacy and safety of the investigated drug talinolol have been shown in several clinical studies. The drug has been approved by regulatory authorities [de Mey, C., V. Schroeter, et al. (1995), Fachinformation Cordanum, AWD Pharma Dresden, (2001)].

Quality has to be ensured by the manufacturer for all drug products produced at his manufacturing sites.

Figure A.1 points out different aspects of quality, efficacy and safety of drug products and describes the respective responsibilities. The duties of the authorities include the surveillance of all parties involved in any process of the development, the manufacturing and the dispensary of drug products.

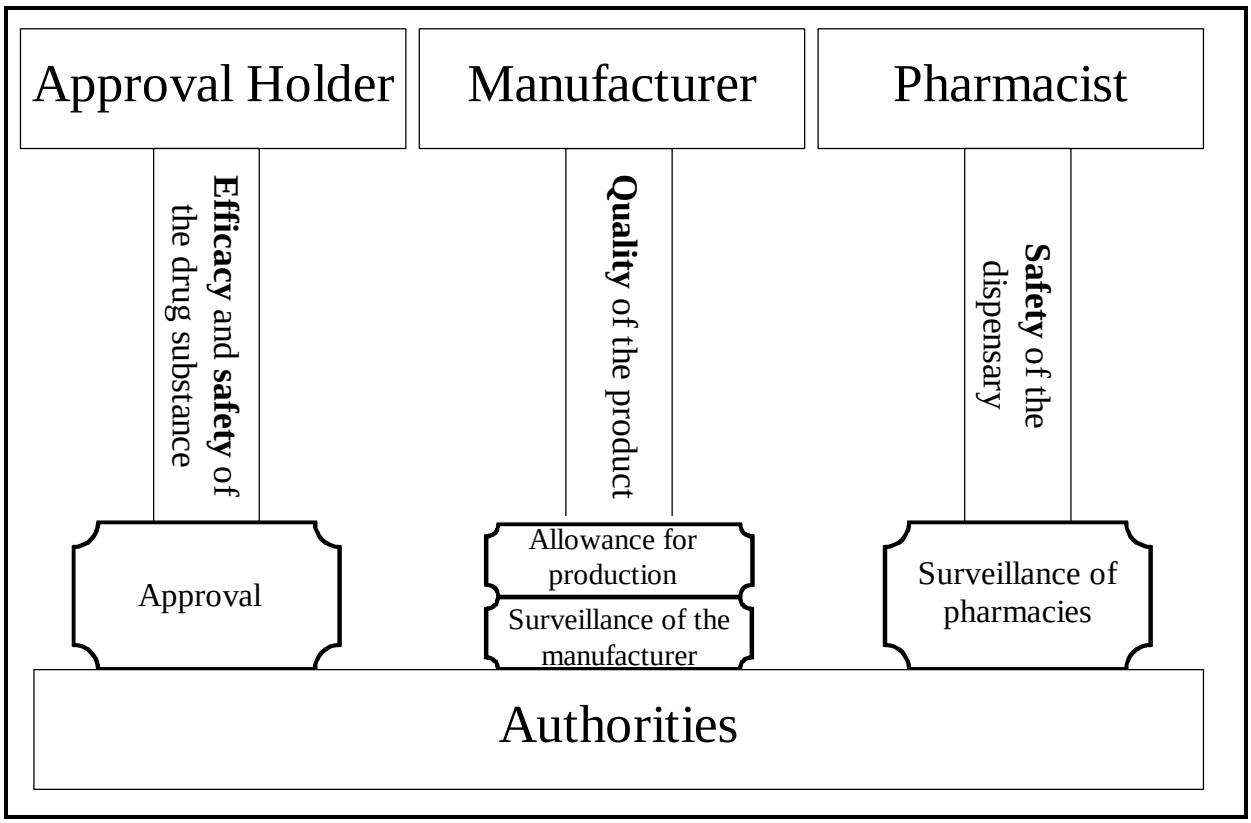


Figure A.1:
Different aspects of quality, efficacy and safety of pharmaceuticals and responsibilities of parties involved in the processes.

AI.2 Regulatory aspects of manufacture and handling of drug products in Germany

A regulatory basis for the production and handling of pharmaceutical products in Germany is the *German law on pharmaceuticals* (*Arzneimittelgesetz, AMG*) passed in 1976. After fundamental revisions the version published December 11, 1998 is still valid with several modifications, the latest being published August 21, 2002.
The law is structured in 18 sections with 98 paragraphs and several additional transition regulations [Paul-Ehrlich-Institut, 10. Arzneimittelgesetz, (2002), www.pei.de/downloads/10amg.pdf].
Of special interest in this context are the second, the third and the eighth section.
The second section including paragraphs 5 to 12 is dealing with requirements for drug products, including rules for their labelling.
In the third section (§§ 13 to 20a) regulations for the production of pharmaceuticals are published and section eight (§§ 54 to 55a) includes the aspects of quality assurance and quality control of drug products.

In paragraph 13 it is determined that - apart from pharmacies, hospitals and veterinaries – an institution which intends to produce pharmaceuticals will have to have a particular permission from the responsible authorities.
In the case of the University of Mainz, for example, the responsibility lies with the Department of Social Affairs, Youth and Provision, a Division of the Federal Administration of Rheinland-Pfalz (Landesamt für Soziales, Jugend und Versorgung, Schießgartenstraße 6, D-55116 Mainz).

Paragraph 14 describes the requirements mandatory to receive this permission:

- A production manager and a quality control manager, both with certain expertises (cp. paragraph 15), as well as a marketing manager, need to be nominated.
- The production and quality control manager both must demonstrate reliability and personal integrity by an official certificate ("Polizeiliches Führungszeugnis") stating that they did not seriously violate German laws previously.
- The production and quality control manager have to fulfill their duties permanently.
- Production and control laboratories have to fulfill certain standards.

The expertises needed for the position of a production manager and a quality control manager are outlined in paragraph 15:

- They need to be either approbated pharmacists by training or, in case they have obtained a university degree in chemistry, biology, human medicine or veterinary medicine, they have to demonstrate additional university training on twelve particular pharmacy-related topics, e.g. pharmaceutical chemistry, microbiology, pharmacology and pharmaceutical technology.
- They need to demonstrate practical experience during a minimum of two years in the production or quality testing of pharmaceuticals.

Paragraph 54 authorizes the German Ministry of Health to enact ordinances that assure the proper handling and the required quality of drugs and drug products.
According to this paragraph the O*rdinance for Pharmaceutical Entrepreneurs* (*Betriebsverordnung für Pharmazeutische Unternehmer, PharmBetrV*), including 20 paragraphs, was enacted in 1985. It is still valid today with several modifications [Bundesgesundheitsministerium, Betriebsverordnung für Pharmazeutische Unternehmer, (1998), www.bmgesundheit.de/downloads-gesetze/arzneimittel/pharma/pharma.pdf]. It is of vital importance for anyone who wants to produce pharmaceuticals.

The following list presents an insight into the contents of this ordinance:

§1a Quality assurance system
§2 Staff
§3 Appearance, size and equipment of rooms
§ 4 Hygienic requirements
§ 5 Production
§ 6 Quality Testing
§ 8 Storage
§10 Packaging
§ 11 Labelling
§ 12 Production and quality testing by order
§ 14 Reclamations
§ 15 Documentation
§ 15a Self-inspection

In essence, this ordinance implements the basic principles of the *Good Manufacturing Practice (GMP) Guidelines* into German law.

Already in 1968 the World Health Organisation (WHO) published GMP guidelines in order to implement basic quality standards in the production of pharmaceuticals worldwide. The GMP guidelines were revised and published by the WHO in 1992 [WHO, GMP Guidelines, (1992), www.who.int/medicines/organization/qsm/activities/qualityassurance/gmp/gmpcover.html].

Also the European Union (EU) has published their own version of GMP guidelines in 1989. In addition, the Pharmaceutical Inspection Convention (PIC), an international federation of authorities, dealing with the supervision of the production of drug products, released the same EU-GMP guidelines with identical wording in the same year. The underlying reason for publishing the identical guideline by two different organisations was to broaden the basis for GMP rules since the PIC also includes several non EU member states. The WHO and the EU / PIC versions of these guidelines do not have the same wording, but the basic principles are identical, they are so-called “consonant”.

The current member states of the European Union (EU) and the Pharmaceutical Inspection Convention (PIC) are summarized in Table A.1:

Table A.1:

Comparison of the current member states of the EU and the PIC.

EU member states	**PIC member states**
Austria	Australia
Belgium	Austria
Denmark	Belgium
Finland	Canada
France	Czech Republic
Germany	Denmark
Greece	Finland
Ireland	France
Italy	Germany
Luxembourg	Greece
Portugal	Hungary
Spain	Iceland
Sweden	Ireland
The Netherlands	Italy
United Kingdom	Liechtenstein
	Malaysia
	Norway
	Portugal
	Romania
	Singapore
	Slovak Republic
	Spain
	Sweden
	Switzerland
	The Netherlands
	United Kingdom

A guideline which is released by an international organisation, such as the WHO or PIC; represents initially only a recommendation without being legally binding for the member states. However, as soon as a guideline is being translated into laws and ordinances of a state, its content becomes legally binding within that particular state. Ordinances usually are more precise than laws and can be enacted by the administration whereas laws need to be enacted by the parliament as legislative. All ordinances need to be authorised by a corresponding law.

The upper part of Figure A.2 describes the general procedure of implementing international guidelines into national laws and ordinances, the special case of implementing GMP into German law is shown below.

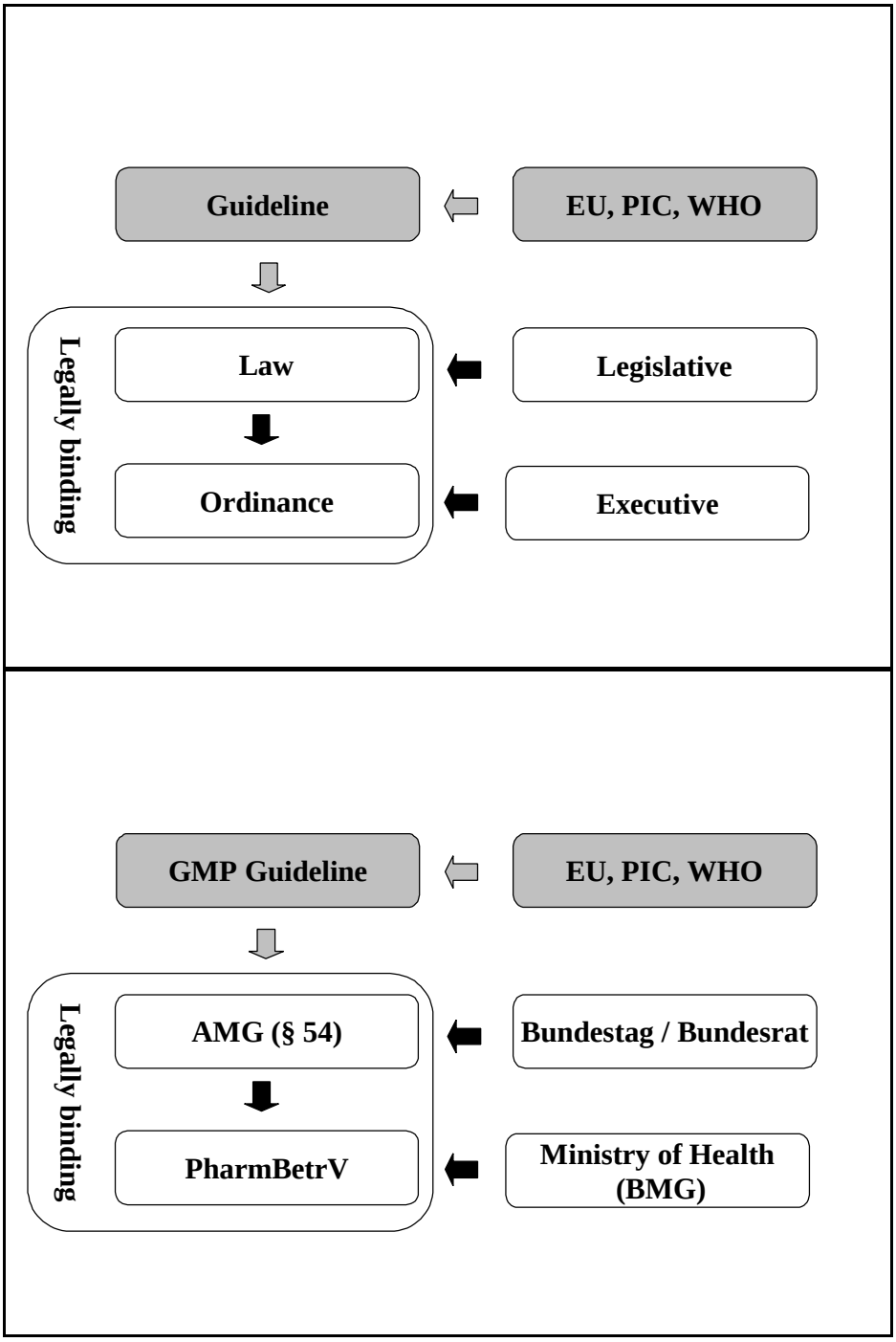


Figure A.2:
The pathway during which a guideline is implemented into legally binding laws and ordinances in general (upper figure) and with respect to the GMP guidelines (lower figure).

Apart from *guidelines*, the European Union can also release directly legally binding *regulations* that need no further implementation in national laws (e.g. Commission Regulation 1964/2002/EC on marketing standards for olive oil, Commission Regulation 1937/2002/EC on a procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin), decisions that are binding for those to whom they are addressed (i.e. any or all EU member states, enterprises or individuals) without the need of an implementation into national legislation (e.g. Commission Decision 2002/75/EC on the import from third countries of star anise), and *directives* that have to be implemented in national laws within a specified time limit (e.g. Commission Directive 2002/67/EC on the labelling of foodstuffs containing quinine, and of foodstuffs containing caffeine, Commission Directive 2002/82/EC laying down specific purity criteria on food additives other than colours and sweeteners). There are also EU guidelines which are not implemented in national law and are as such not legally binding. However, since they describe the current state of the scientific knowledge that authorities refer to during inspections they need to be followed unless one has convincing justifications not to observe these guidelines.

Table A.2 lists the index of the PIC / EU GMP guideline, which consists of 9 chapters [Eudralex, EU GMP guidelines, (2002), http://pharmacos.eudra.org/F2/eudralex/vol-4/home.htm].

Table A.2:
Index of the PIC / EU GMP guideline.

Chapter 1:	Quality assurance system
Chapter 2:	Staff
Chapter 3:	Rooms and equipment
Chapter 4:	Documentation
Chapter 5:	Production
Chapter 6:	Quality control
Chapter 7:	Production and quality testing by order
Chapter 8:	Reclamations and products recall
Chapter 9:	Self-inspection

A comparison with the paragraphs of the ordinance for pharmaceutical entrepreneurs (PharmBetrV) shows that all topics of the chapters of the GMP guideline are also handled in this ordinance.
In addition to the basic GMP guideline several *annexes* dealing with specific drug products and particular issues were published by EU and PIC. Table A.3 lists all currently published annexes [Eudralex, EU GMP guidelines, (2002), http://pharmacos.eudra.org/F2/eudralex/vol-4/home.htm].

Table A.3:

Annexes to the basic GMP guideline published by EU and PIC.

Annex 1:	Manufacture of sterile medicinal products
Annex 2:	Manufacture of biological medicinal products for human use
Annex 3:	Manufacture of radiopharmaceuticals
Annex 4:	Manufacture of veterinary medicinal products other than immunological veterinary medicinal products (Not yet adopted by PIC)
Annex 5:	Manufacture of immunological veterinary medicinal products (Not yet adopted by PIC)
Annex 6:	Manufacture of medicinal gases
Annex 7:	Manufacture of herbal medicinal products
Annex 8:	Sampling of starting and packaging materials
Annex 9:	Manufacture of liquids, creams and ointments
Annex 10:	Manufacture of pressurised metered dose aerosol preparations for inhalation
Annex 11:	Computerised systems
Annex 12:	Use of ionising radiation in the manufacture of medicinal products
Annex 13:	Manufacture of investigational medicinal products
Annex 14:	Manufacture of products derived from human blood or human plasma
Annex 15:	Qualification and validation
Annex 16:	Certification by a qualified person and batch release (Not yet adopted by PIC)
Annex 17:	Parametric release
Annex 18:	Good manufacturing practice for active pharmaceutical ingredients
Furthermore:	GMP guide for blood establishments
	GMP guide for active pharmaceutical ingredients

Of particular interest for an implementation of GMP in university laboratories is Annex 15, which deals with basic principles of qualification and validation processes.

AI.3 Basic GMP principles

One fundamental issue of GMP is ***documentation***. Every work that has been performed has to be documented, otherwise it is regarded as if it had not occurred.
A close documentation is vital for the following reasons:

- Retraceability of all elements of the production and quality control process.
- Quality assurance of production, storage, labelling and laboratory controls.
- Control of the personell involved in the production and quality control process.
- Motivation of the staff.
- Proof that the production and all related processes were performed in accordance to GMP rules and other regulations.
- Standardisation of the production and quality control processes.
- Ensure a regular education and training of all persons involved in production, quality control, cleaning or maintenance processes.

An important module in the field of documentation is the so-called SOP. SOP stands for *S*tandard *O*perating *P*rocedure and means a document that exactly describes an operating sequence in written form. Such operating sequences can be, for example, the handling of an instrument, a certain step in the production of pharmaceuticals or a cleaning and sanitizing procedure.
Such SOPs should exist for all important steps connected with the production and quality testing of a drug product. In one SOP the appearance of all SOPs and their general structure should be settled.

Two further crucial aspects connected to GMP are ***qualification*** and ***validation***.
Qualification means that all devices used for the production of pharmaceuticals need to be qualified for their usage.
One differentiates between Design Qualification (DQ), Installation Qualification (IQ), Operational Qualification (OQ), Performance Qualification (PQ) and Maintenance Qualification (MQ).

- *Design Qualification* means to establish that equipment meets all requirements for the predetermined process concerning technical as well as GMP and regulatory aspects.
- *Installation Qualification* means to provide documented evidence that equipment was installed according to its specifications.
- *Operational Qualification* means to provide documented evidence that equipment performs according to vendor claims and purchase specifications.
- *Performance Qualification* means to provide documented evidence that a system performs as designed and reproducibility of results is ensured. This can also be called *Process Qualification*.
- *Maintenance Qualification* means to provide documented evidence of regular maintenance, calibration and performance testing.

“To provide documented evidence” means that the person who has performed a particular task documents everything he or she does. Certificates for standards and references have to be attached to the document.
Performance qualification of a scale, for example, means that a person tests, whether the scale determines weights correctly and reproducibly. It is necessary to measure a *series* of weights to cover the whole mass range of the scale. To proof reproducibility, the same weights must be weighed several times. The weights used for this test series need to be reference weights, their exact weight must be certified by a bureau of standards. Documented evidence for the proper function of the scale is provided by setting up a document, in which the person who performed the qualification of the scale, writes down all weighings he did. Deviations between the measured values and the labelled values of the reference weights have to be reported and, if the deviations do not succeed specified values, it can be stated that the scale is qualified. The date of the qualification has to be reported within the document. All

inscriptions in the qualification document must be confirmed by the signature of the respective person. Certificates for the reference weights must be attached to the document.

Figure A.3 visualizes the life-cycle of an instrument with respect to the necessity of qualification:

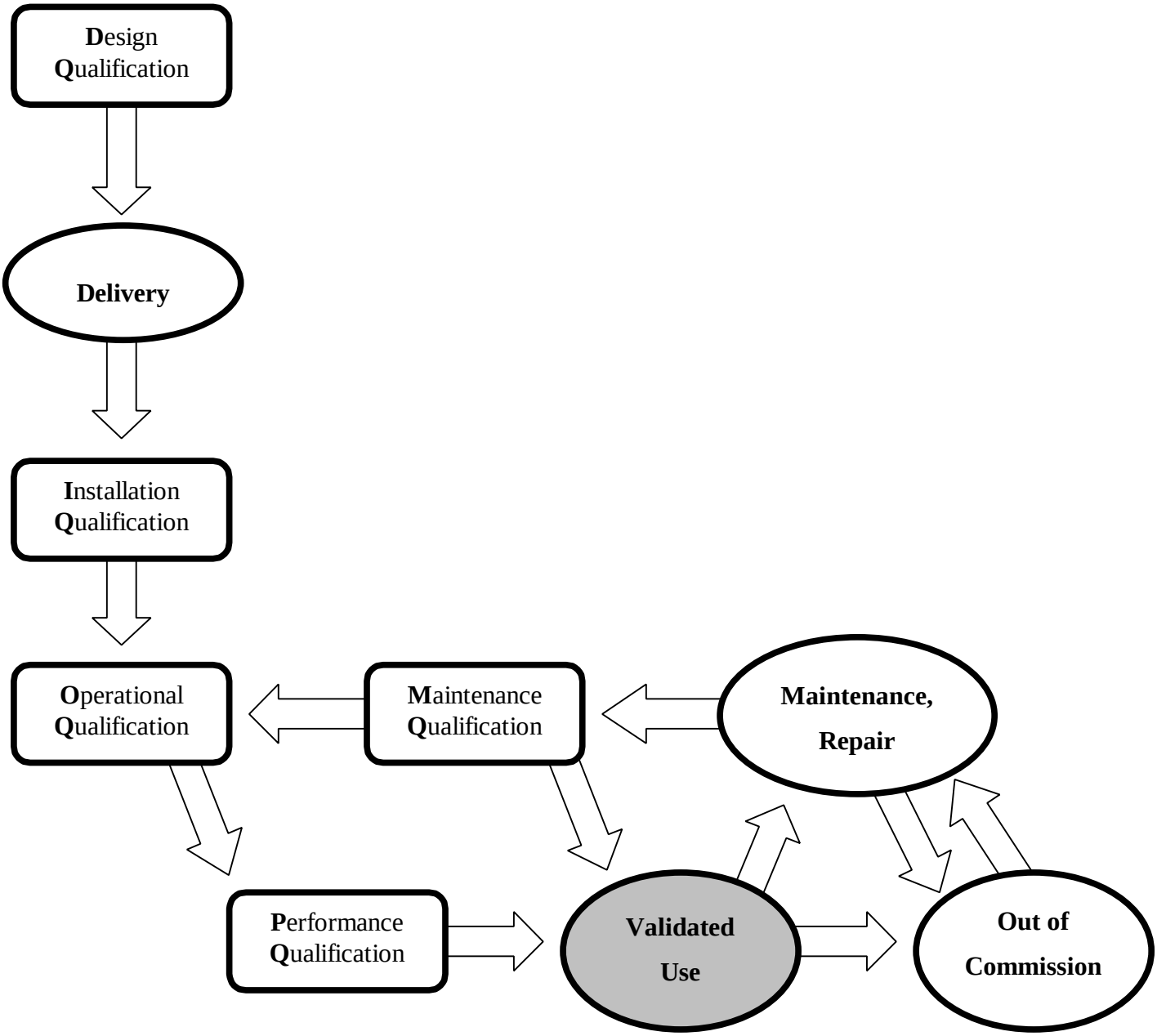


Figure A.3:

The life-cycle of an instrument demonstrating the different steps of qualification.

After a particular time of validated use the instrument has to be maintained. As long as the instrument is still working within its specifications it can be used again after a maintenance qualification, otherwise it is taken out of commission. In addition, malfunction of the instrument can be a reason to take the instrument out of commission, otherwise it has to be repaired. If successful repair is possible, the instrument can be used again after a maintenance qualification, an operational qualification and a performance qualification. In case of irreparability, the instrument has to be taken out of commission.

Whereas qualification refers to equipment, validation mainly refers to procedures, which includes the *handling* of equipment.

Validation generally means to provide documented evidence that a process consistently leads to reliable results.
For the production process of pharmaceuticals the following definition issued by the American Food and Drug Administration (FDA) is more precise [FDA, Guideline on general principles of process validation, (1987), www.fda.gov/cdrh/ode/425.pdf].
Validation means “establishing documented evidence which provides a high degree of assurance that a specific process will constantly produce a product meeting its predetermined specifications and quality attributes.”
Inherent and implicit in this definition of validation is the concept of the proper interpretation and applications of the GMP principles to each system or subsystem, so that the facility, when fully operational, will consistently meet GMP requirements. Validation applies to systems, processes, and programs that support the manufacturing process. To exceed GMP requirements means that minimum standards, described in the GMP guidelines, are *more than fulfilled*, e.g. when a peroral drug product is manufactured under sterile conditions or when calibration or education intervals are shorter than required.

Another term in this context important for the handling of measuring instruments is *calibration*.
Calibration means the determination of the deviation of the value indicated by a measuring instrument from the correct value. For this purpose, an object whose values are exactly known, a so-called standard, is measured with the instrument to be calibrated, and the deviation of the reading from the known value is determined. The result and the respective uncertainty of measurement are recorded in a calibration certificate.

Furthermore GMP makes high demands on the *premises* and *personnel*.
Premises must be located, designed, constructed, adapted and maintained to minimize build-up of dirt, dust and cross-contamination. Effective cleaning has to be permitted and any adverse effects on quality need to be eliminated.

Personnel has to be present in sufficient numbers to ensure that the quality of a drug product is not endangered by an overburdened staff. People involved in any step of the process of producing pharmaceuticals need to be qualified for their jobs, they need to be educated in how to act conforming with GMP principles and need to meet the hygienic requirements.
An organisation scheme should exist pointing out the tasks and responsibilities of each staff member.

Closely connected to premises and personnel is the aspect of *hygiene*.
Hygiene is vital for the production of microbiologically unobjectionable drug products. All hygienic measurements which have to be taken must be listed in a hygiene plan, in which each task is described, as well as the frequency of its execution and the persons responsible for it. Examples for such hygienic measurements are cleaning and sanitizing of floors and instruments, hand washing and disinfection or waste removal.

AI.4 Precise steps for the implementation of GMP in university laboratories

AI.4.1 General considerations

In order to implement GMP principles and practices in selected laboratories at the Institute of Pharmacy of the University of Mainz and to receive a permission for the production of defined pharmaceuticals, several measurements had to be taken.

Initially a decision had to be taken on the type of dosage forms intended to be produced. Different requirements exist for various groups of dosage forms, such that authorities grant permissions only restricted to one or more particular groups of dosage forms.
These groups are generally based on the aggregate state of the dosage form, such that subdivisions into solid, liquid, and semi-solid dosage forms are performed. Furthermore, the production and handling of some pharmaceuticals requires special attention with respect to safety and quality such as antibiotics, anti-cancer drug products or injectibles. Therefore for these groups special permissions are needed.
Since it was planned to develop and produce peroral controlled-release dosage forms, it was aimed at an allowance for the production of solid peroral pharmaceuticals, which includes the permission for the production of powders, granules, tablets and capsules.

AI.4.2 Documentation-related measures

AI.4.2.1 Room concept

For the elaboration of a suitable room concept, rooms had to be defined for the preparation and quality control of ingredients and raw materials, for the actual production of the pharmaceuticals and for the quality control of the finished product. A flow chart had to be created outlining the material flow during these processes.

Figure A.4 depicts this chart in a generalised version for four imaginary rooms A, B, C, and D:

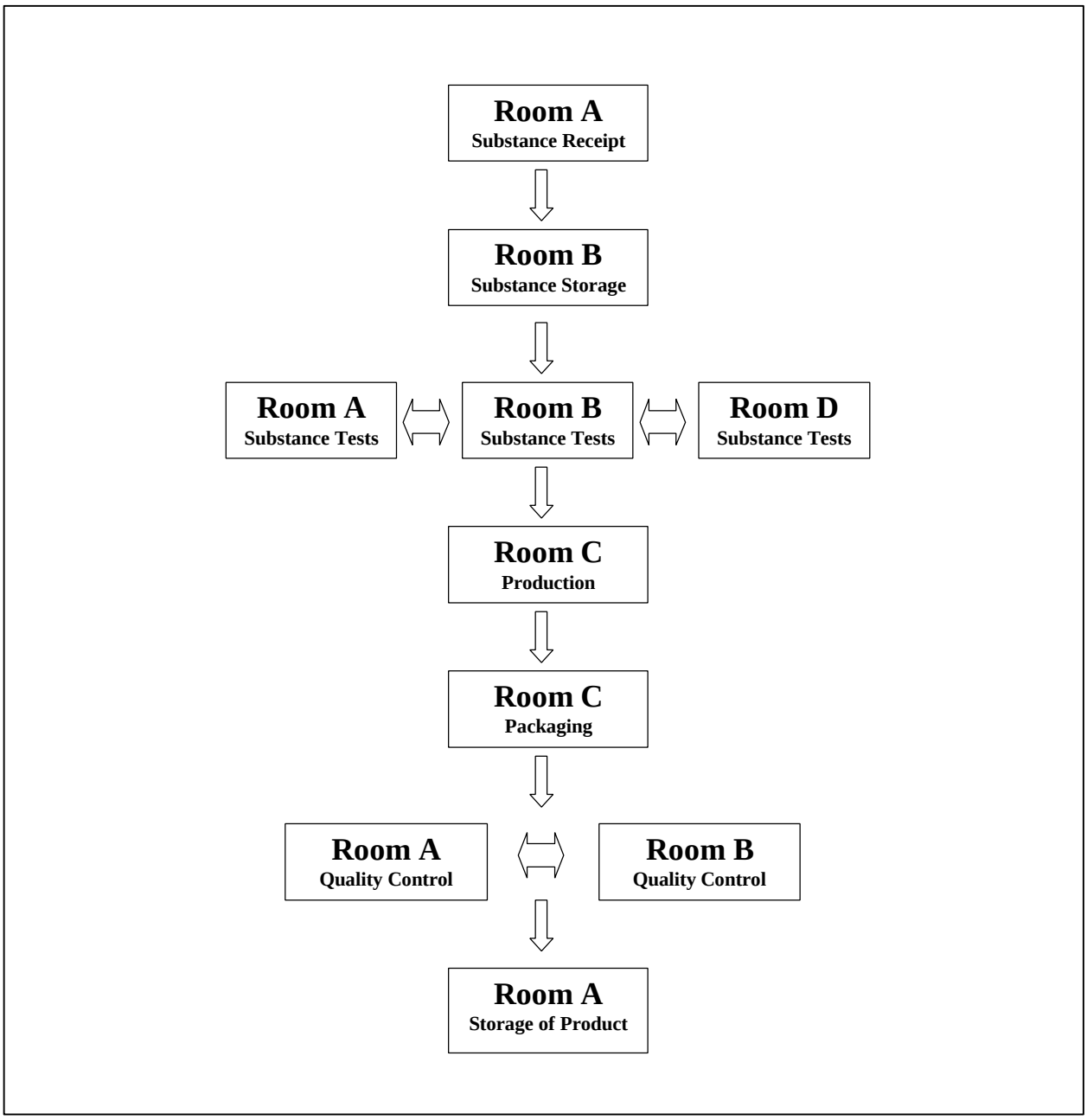


Figure A.4:
Material flow during the production and quality control of pharmaceuticals at the University of Mainz, Institute of Pharmacy.

All raw materials, intermediate products and final products have to be transported between the rooms outlined in Figure A.4 in a sealed box. A label must display the date of the sealing and the name of the person that sealed the box. To exclude opening and relocking of the seals, sealing pliers pressing the letters “JGU” for Johannes Gutenberg-University on the seal are used. These pliers are locked up in a steel locker inaccessible to unauthorized persons.

AI.4.2.2 Personal structure

An organigram describing the personal structure of all people involved in the production and quality control processes had to be created. It is displayed in Figure A.5 in anonymous form :

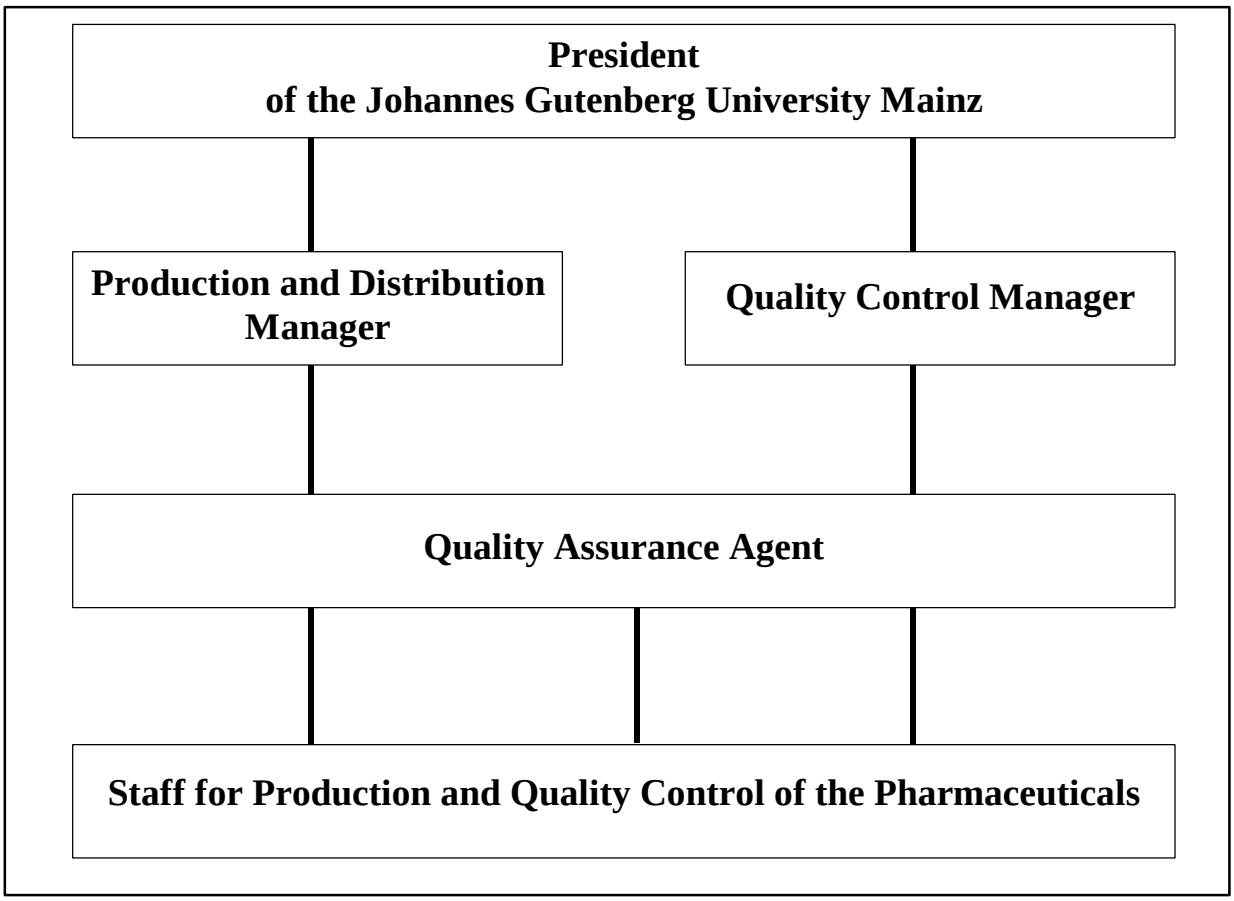


Figure A.5:
Structure of personnel involved in production and quality control of pharmaceuticals at the Institute of Pharmacy of the Johannes Gutenberg- University Mainz.

Key positions in this chart are the production manager and the quality control manager that need to be independent of each other. The position of the distribution manager was fulfilled by the production manager as well, which complies with the regulations [AMG § 14 (2)].
Apart from the quality control manager according to AMG §§ 14 and 15, a quality assurance agent had to be appointed. He / She is responsible for the implementation and realisation of a quality assurance system according to PharmBetrV § 1a.
For a production company, an insurance would have to be named covering costs arising from damages in connection with the produced pharmaceuticals. The university, however, is a public body and therefore self-liable for losses, damages

and risks associated with the production of pharmaceuticals. Consequently, the president of the Johannes Gutenberg-University, being its representative, is pointed out at the head of the personal structure chart.

AI.4.2.3 Standard Operating Procedures (SOP`s)

Standard Operating Procedures (SOP´s) had to be written for all critical steps in the production and quality control of the manufactured products. The SOP`s were marked with letters and numbers to arrange them in proper order.
SOP`s dealing with the handling of instruments and devices were labelled with the letter “G” (abbreviation of the German term “Geräte”) plus a two digit number characteristic for the sequence of their appearance.
SOP`s with an initial letter “R” describe cleaning procedures, whereas an initial letter “V” characterizes SOP`s associated with the production and quality control process of pharmaceuticals. “R” refers to the German term for cleaning “Reinigung”. “V” stands for “Verfahren”, the German word for processes or procedures.

Table A.4 lists the titles of currently available SOP`s and their identification codes:

Table A.4:
Titles of currently available SOP`s at the Institute of Pharmacy, University of Mainz and their identification codes.

Procedures

Identification Code	**English title**	**German original title**
V01	Generation of SOP´s	Erstellung von SOP`s
V02	Position and tasks of the quality assurance agent	Qualitätssicherungsbeauftragter
V03	Keeping instrument log books	Führen von Gerätebüchern
V04	Goods receipt	Wareneingang Wirk- und Hilfsstoffe
V05	Quarantine storage of new substances	Quarantänelagerung Wirk- und Hilfsstoffe
V06	Reception inspection	Eingangskontrolle Wirk- und Hilfsstoffe
V07	Prearrangements for the production of pharmaceuticals	Vorbereitung auf die Arzneimittelherstellung
V08	Production of pharmaceuticals	Arzneimittelherstellung
V09	Packaging and labelling	Verpackung und Kennzeichnung
V10	Quality control	Qualitätsprüfung
V11	Release of tested substances	Arzneimittelfreigabe durch Kontrollleiter
V12	Storage of drug products	Lagerung der fertigen Arzneimittel
V13	Dispensing of the finished product	Abgabe an Prüfarzt
V14	Alarm-plan for adverse events	Alarmplan

Instruments

Code	English title	German original title
G01	Dissolution tester	Dissolutionstester PharmaTest PTW S III
G02	Laboratory blender	Laborreaktor IKA LR 250
G03	Scale	Waage Sartorius Typ 2254

Cleaning

Code	English title	German original title
R01	Cleaning of the laboratory blender	Laborreaktor-Reinigung (IKA LR 250)
R02	Cleaning of glass ware and sieves	Glasgeräte-, Siebe-, Reibschalen-Reinigung
R03	Cleaning of drying ovens	Trockenschrank Reinigung
R04	Extraordinary cleaning of the production room	Generalreinigung nach Praktikumsende

All SOP`s are stored in a lockable steel locker together with a list of all available SOP`s. SOP`s that are needed on site to allow SOP-compliant operations are copied and positioned at the specific sites. Such copies need to be authorised and itemised in the list mentioned above.

Like all other documents, the SOP`s have a headline, which includes the address of the Institute of Pharmacy and the University-logo (Figure A.6).

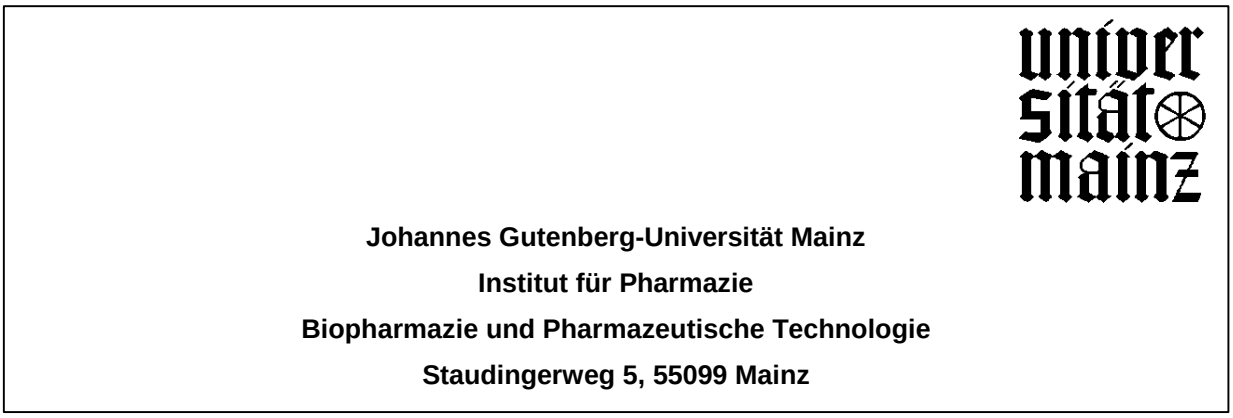


Figure A.6:
Headline of GMP-related documents of the Institute of Pharmacy, University of Mainz.

In the footnote the title, version number, dates of compilation, displacement, revision by the quality assurance agent, release by the production or quality control manager and page number in the form x (i.e. current page number) of y (i.e. total number of pages) are given respectively. The particular form of numbering the pages shall confirm that no pages have been removed or are missing for whatever reason. The revision by the quality assurance agent and the approval for release by the production or quality control manager have to be documented by their signatures.
A translation of the original German version of such a footnote into the English language is shown in Figure A.7.

Version number **2**	set up 08/13/01	by N.N.
Replaces version number 1	set up 02/24/01	by N.N.
Revised	08/15/01	by N.N.: _____
Approved	08/18/01	by N.N.: _____
SOP V 02		Page 5 of 10

Figure A.7:
Example of a footnote for SOP`s and other GMP-documents. The original German version was translated into the English language.

An example of a complete SOP in the original German language is attached in the Appendix to this chapter.

AI.4.2.4 Manufacturing and testing instructions

The production, packaging and testing procedures for the determination of quality of the manufactured products have to be specified in manufacturing and test instructions in order to ensure reproducible working conditions and that the resulting products show uniform quality. The instructions include the instruments and devices to be used, as well as specifications for the duration of certain processes, e.g. grinding or mixing times.
The compliance with these instructions must be documented in manufacturing, packaging and testing protocols, in which all steps of these processes are listed. In addition, the personnel carrying out the procedures have to be pointed out. The

correct performance of each step needs to be documented by the person involved. The person has to certify this in writing by stating the date and signature.
An example manufacturing *instruction* as well as a manufacturing *protocol* for talinolol controlled-release tablets, both in German, can be found in the Appendix to this chapter.

AI.4.3 Personnel and education

Only qualified personnel may be recruited for the production and testing of pharmaceuticals. All members involved in the process need to be listed in a separate document including their qualifications, areas of responsibility and their signatures. Together with this document, copies of certificates proving the qualification of the personnel employed are stored.

Semi-annual GMP educations were introduced in order to assure that persons in contact with rooms and instruments for the production and quality control of pharmaceuticals are informed about the basic GMP rules as well as the specific requirements in conjunction with the production and testing of pharmaceuticals intended for human use at the Institute of Pharmacy, Johannes Gutenberg-University Mainz.
The participants of these educational training sessions have to be listed as well as the topics of the particular educational exercise. The attendance of the participants needs to be confirmed by their signature.
Besides the personnel directly involved in the manufacture and quality control of pharmaceuticals, the cleaning personnel for the production and packaging rooms needs to be educated regularly on the specific cleaning requirements for these rooms and the documentation of the cleaning process.

AI.4.4 Specific requirements for the production and packaging room

The room for the production and packaging (Room C in Figure A.4) consists of an anteroom and the actual production and packaging room, which are seperated by a glass sliding door. The function of the anteroom is to act as a buffer zone between the actual production and packaging room and the outside atmosphere. During the transfer of material or personnel, this helps to keep the production and packaging area free from infiltrating dirt.

Particular requirements with respect to the constructive elements of the room include a coverage of the ceiling with flat, non porous material that can be cleaned with water and a coverage of the ceiling lamp with a plastic chip guard to prevent particulate material of the neon lamp from contaminating the drug product in case of lamp malfunction (ex- or implosion).

According to the hygiene plan and the SOP “Prearrangements for the production of pharmaceuticals”, which are both posted in the anteroom, persons who intend to enter the actual production and packaging room have to change clothes and fulfil the required sanitizing procedures within the anteroom.
Clothing requirements include a clean lab coat, a disposable facemask, a cap, gloves and shoe covers. For the storage of street clothes a locker was positioned in the anteroom.
Dispensers for hand disinfection solutions and soap were installed at the wash basin.

Only authorized persons are allowed to enter the production and packaging room, consequently it has to be kept locked unless somebody is working inside. In addition, a sign at the door has to point out that unauthorized persons may not enter. Another sign indicates whether a production of pharmaceuticals takes place in the room at the moment or not.

Authorized persons entering this room have to pay attention that the main entrance door as well as the intermediate sliding door are closed directly after passing through.

In addition, the windows have to be kept closed at all times. Appropriate signs at the doors and windows are pointing this out. It must be documented in the production protocol that the windows were definitely closed during the entire production and packaging process of pharmaceuticals. This also includes the preceding disinfection process.

Persons performing work in this production and packaging area have to document their presence with name, date, performed activities and signature in a room log book, which is positioned in the anteroom of the production and packaging area. The pages of this book must be numbered in order to prevent pages from being unintentionally or intentionally removed.

For the production and packaging room a hygiene plan had to be set up, which describes all cleaning and sanitizing procedures. The plan describes methods, intervals, cleaning material and responsibilities for the different processes, e.g. hand washing, disinfection of technical devices or the cleaning of the floor.
A copy of the original hygiene plan in German language is attached in the Appendix to this chapter.

The hygienic status of the room including surfaces of instruments, furniture, walls and windows is monitored regularly by means of surface sampling tests with Rodac (Replicate Organism Detection And Counting) Plates (Becton Dickinson, Franklin Lakes, USA) or Hygicult microbial indicators (Orion Diagnostica, Espoo, Finland).
Rodac plates are surface contact plates containing trypticase soy agar and polysorbate 80. They are recommended for the detection of microorganisms on non-porous surfaces. The microbiological status can be monitored by counting colony forming units on the plates after a specified time of incubation.
Hygicult consists of plastic hinged slides covered on both sides with an agar growth medium. Each slide is stored in its own sterile vial. Sampling and inoculation are done by pressing the slide onto the surface to be tested, by transferring the sample directly from sterile swabs or by dipping the slide into fluids. Following incubation, the microbiological status of raw materials, production facilities or finished products can

be determined by comparing the colony density on the agar slide against the density shown on a model chart provided with the Hygicult kit.

AI.4.5 Analytical instruments and test apparatus

For all instruments employed in the production and quality control process, log books are established. In these log books all maintenance, repair and calibration work is documented as well as all measurements or tests performed. The recordings need to include the date and the name of the person fulfilling a particular task.

Analytical instruments such as UV-Vis spectrophotometer, IR spectrometer and HPLC device must be calibrated.
The temperature of the water bath of the dissolution tester is calibrated against a thermometer certified by a German bureau of standards. In accordance with the instructions of the European Pharmacopeia, the position of the water bath and the rotational speed and immersion depth of the paddles are calibrated regularly as well.

Scales are maintained by a service agent of the manufacturing company annually. Monthly and before every use for the production of pharmaceuticals they are tested for accuracy using a set of standard weights.

AI.5 Summary

To assure the quality of pharmaceuticals the manufacturer has to follow a number of rules and regulations, amongst which the GMP rules are of particular importance. The implementation of these basic rules of Good Manufacturing Practice at a university laboratory is described in this chapter.

In addition to a number of constructional and technical measures, appropriate documentation had to be established. Following several discussions with representatives of the local authorities the allowance for the production of solid oral dosage forms was granted on April 19, 2001. It is since then possible to produce tablets and capsules to be used in clinical studies in the designated university laboratories. A copy of the allowance certificate is attached in the Appendix to this chapter.

AI.6 Appendix to Annex I

The following documents appear in this Appendix, all in the original German format:

- Example of an SOP (SOP V 01: SOP on how to set up SOP`s).
- Hygiene Plan for the production and packaging room.
- Manufacturing *instruction* for the production of talinolol controlled-release tablets containing 100 mg talinolol (HV 001).
- Manufacturing *protocol* for the production of talinolol controlled-release tablets containing 100 mg talinolol (HP 001).
- Certificate permitting the production of solid oral dosage forms at the Institute of Pharmacy of the Johannes Gutenberg-University, Mainz, from the authorities of Rheinland-Pfalz (copy, without attachments).

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SOP Standardarbeitsanweisung

SOP V 01

Titel:

SOP über die Verfahrensweise bei der Erstellung von Standardarbeitsanweisungen (SOP`s)

Version-Nr. **1**	erstellt am 13.02.01	von D. Wagner
Ersetzt Version Nr. -	erstellt am -	von -
Geprüft am	_____	von A. Braun _____
Freigegeben am	_____	von P. Langguth _____

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Biopharmazie und Pharmazeutische Technologie
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SOP über die Verfahrensweise bei der Erstellung von Standardarbeitsanweisungen (SOP`s)

Zielsetzung:

Sicherstellung einer ordnungsgemäßen Erstellung und Aufbewahrung von Standardarbeitsanweisungen (SOP`s).

Standardarbeitsanweisungen, im Folgenden als SOP`s bezeichnet, sind für alle wichtigen Verfahrensschritte bei der Herstellung und Prüfung von Arzneimitteln, sowie die dafür benötigten Geräte zu erstellen.

Die Kopfzeile besteht aus der Anschrift des Institutes für Pharmazie und dem Logo der Johannes Gutenberg-Universität Mainz, die Fußzeile weist das Erstelldatum, das Datum der letzten Änderung, das Datum der Prüfung sowie das der Freigabe der SOP aus und die jeweils durchführende Person aus. Seitenzahlen werden entsprechend dem Schema „Seite x von y“ in Bezugnahme auf die Gesamtseitenzahl des Dokumentes angegeben Auf einem Deckblatt ist der Titel der SOP ersichtlich, die Zielsetzung der SOP wird am Beginn der SOP kurz umrissen. In den Texten der

Version-Nr. **1**	erstellt am 13.02.01	von D. Wagner
Ersetzt Version Nr. -	erstellt am -	von -
Geprüft am	_____	von A. Braun _____
Freigegeben am	_____	von P. Langguth _____

SOP sind die durchzuführenden Tätigkeiten oder Benutzungsanweisungen klar und eindeutig zu formulieren.

Jede SOP erhält eine Nummer, anhand derer sie – zusammen mit der entsprechenden Versionsnummer – eindeutig identifizierbar ist.

Alle erstellten SOP`s werden zentral in einem Aktenschrank gelagert. Soweit erforderlich befindet sich am Ort der Nutzung eines entsprechenden Gerätes bzw. der Durchführung der beschriebenen Tätigkeit ein Duplikat der entsprechenden SOP.

Änderungen einer SOP bedürfen einer erneuten Prüfung durch den Qualitätssicherungsbeauftragten sowie die Freigabe durch den Herstellungs- oder Kontrollleiter. Wurde eine SOP überarbeitet, so ist dies im Änderungsindex, der jeder SOP angefügt ist, unter Angabe des Änderungsgrundes und des Gültigkeitsbeginns der neuen SOP zu dokumentieren. Nicht mehr gültige SOP`S werden nicht entsorgt, sondern in einem Ordner mit der Aufschrift „Archivierung nicht mehr gültiger SOP`s“ katalogisiert.

Version-Nr. **1**	erstellt am 13.02.01	von D. Wagner
Ersetzt Version Nr. -	erstellt am -	von -
Geprüft am	_____	von A. Braun _____
Freigegeben am	_____	von P. Langguth _____

Johannes Gutenberg-Universität Mainz
Institut für Pharmazie
Biopharmazie und Pharmazeutische Technologie
Staudingerweg 5, 55099 Mainz

SOP Änderungsindex

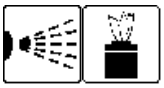
Versions-Nr.	Grund der Änderung / Außerkraftsetzung	gültig ab

Nicht mehr aktuelle SOP`s befinden sich im Ordner „Außer Kraft gesetzte SOP`s“.

Version-Nr. **1**	erstellt am 13.02.01	von D. Wagner
Ersetzt Version Nr. -	erstellt am -	von -
Geprüft am	_____	von A. Braun _____
Freigegeben am	_____	von P. Langguth _____

Johannes Gutenberg-Universität Mainz
Institut für Pharmazie
Biopharmazie und Pharmazeutische Technologie
Staudingerweg 5 55099 Mainz

Hygieneplan für Arzneimittelherstellungsbereiche

Zu behandelnder Bereich	Zeitpunkt der Hygiene-maßnahme	Art der Behandlung	Desinfektions- und Hilfsmittel	Betroffene Personen
Hände  *Desinfektion*	Vor Arbeitsbeginn, nach Toilettengang und vor und nach der Einnahme von Nahrungsmitteln.	Desinfizieren	Händedesinfektionsmittel ***Sterilium***	An der Herstellung und Prüfung von Arzneimitteln beteiligte Personen
 *Reinigung*		Reinigen	Fließend warmes Wasser, hautschonendes Reinigungsmittel	dito
 *Pflege*	Bei Bedarf	Pflegen (Eincremen)	Hautpflegemittel ***Baktolan Lotion***	dito
Einrichtungsgegenstände und Arbeitsflächen *Desinfektion* 	Im Herstellungsbereich vor Arbeitsbeginn und nach Arbeitsende	Desinfektionsreinigung durch Besprühen und Wischen.	Desinfektionsmittel **Einwegpapiertücher Sprühdesinfektion** ***Quartamon med*** oder ***Isopropanol 70%***	dito
Arbeitsgeräte 	Nach Benutzung.	Abwischen, ggf. Spülen	Warmes Wasser mit geeignetem Reinigungsmittel, Einwegpapiertücher, bei stärkeren Verschmutzungen: Spülmaschine	dito
Fußböden 	Zweimal wöchentlich sowie nach besonderen Verschmutzungen	Abwischen	Desinfizierendes Reinigungsmittel ***Bucazid S G 467 (100ml / 10l Wasser)***	Beauftragtes Reinigungs-personal
Abfallentsorgung	Nach Bedarf.	Sammeln. Spitze, scharfe und zer-brechliche Gegenstände werden in speziellen Behältern entsorgt.	Abfallbeutel, spezielle Behälter für verletzungsgefährdenden Abfall	Beauftragte(r) Mitarbeiter(in)

Merke:

Bei der Arzneimittelherstellung Einmalhandschuhe, Kopfhaube, Mundschutz, Überschuhe und Arbeitskittel tragen !

Bei Desinfektionsmaßnahmen sind Konzentration und Einwirkzeit zu beachten!

Alle Hygienemaßnahmen sind im ausliegenden Raumtagebuch zu dokumentieren!

Alle Personen, die in diesem Raum arbeiten und nicht zum Arbeitskreis Prof. Langguth gehören, müssen sich im ausliegenden Anwesenheitsbuch eintragen!

Version-Nr. **1**	erstellt am 13.02.01	von D. Wagner
Ersetzt Version Nr. -	erstellt am -	von -
Geprüft am	_____	von A. Braun _____
Freigegeben am	_____	von P. Langguth _____

Johannes Gutenberg-Universität Mainz
Institut für Pharmazie
Biopharmazie und Pharmazeutische Technologie
Staudingerweg 5, 55099 Mainz

Herstellvorschrift

HV 001

Talinolol Retard-Matrix-Tabletten 100 mg

Version 1	erstellt am 20.06.02	von D. Wagner
Letzte Änderung vom	-	-
Geprüft am	_____	von A. Braun _____
Freigegeben am	_____	von P. Langguth _____

1. Zielsetzung

Diese Herstellungsanweisung beschreibt den Herstellvorgang von Talinolol Retard-Matrix-Tabletten, mit einem Wirkstoffgehalt von 100 mg. Die Tabletten werden aus einer Pulvermischung direkt verpresst.

2. Zusammensetzung

Für 500 Tabletten sind folgende Rohstoffe in entsprechender Menge bereitzustellen und zu verwenden:

Pos. Nr.	**Rohstoff**	**% Anteil**	**g pro Tablette**	**g (gesamt)**	**Funktion**
1	Talinolol	50 %	0,1	50,0	Wirkstoff
2	Eudragit® L 100-55	35 %	0,07	35	Retardierhilfsstoff: Matrixbildner
3	Mannitol	15 %	0,03	15	Porenbildner für Matrix
Summe:		**100 %**	**0,2**	**100,0**	

Die Bereitstellung und der Transport der benötigten Substanzen in den Herstellungsraum hat in einer verschlossenen Box zu erfolgen. Durch eine

Versiegelung dieser Box ist sicherzustellen, dass Manipulationen an den Ausgangssubstanzen ausgeschlossen sind.

3. Herstellung

3.1.Rohstoffeinwaage / Einwaagekontrolle

Zu verwendende Geräte:
Waage Mettler PG 503 – SDR (Einstellung: 0,01 g Genauigkeit)
3 Kristallisierschalen aus Glas, 250 ml
Edelstahllöffel
Die Einwaagen sind nach dem Vier-Augen-Prinzip von einer zweiten Person zu kontrollieren und gegenzeichnen zu lassen. Die Einwaage der Substanzen erfolgt in die Kristallisierschalen.

3.2. Herstellung einer Pulvermischung

Zu verwendende Geräte:
Reibschale (25 cm Durchmesser), Kartenblatt (Kunststoff), Pistill,

Die Substanzen Pos. 1 bis 3 werden in der Reibschale vereinigt und zunächst mit dem Kartenblatt durchmischt. Anschließend wird die Pulvermischung mit dem Pistill verrieben.

- Mischzeit Pos. 1 bis 3 mit Kartenblatt: 7 min.
- Mischzeit Pos. 1 bis 3 mit Pistill: 7 min.

Die Pulvermischung ergibt eine theoretische Ausbeute von 483 Tabletten a 200 mg Gesamtgewicht bei einer Einwaage von 207 mg Pulver pro Tablette, wobei die 7 mg, die die 200 mg überschreiten, einen Zuschlag für Staubverluste darstellen.

3.3. Verpressen der Pulvermischung zu Tabletten

Zu verwendende Geräte:
Waage Mettler PG 503 – SDR (Einstellung: 0,001 g Genauigkeit)
Tablettenhandpresse Paul Weber PW 20 GS
Kristallisierschalen 50 ml, Glas

Von der Pulvermischung werden jeweils 207 mg in Kristallisierschalen eingewogen, die Pulvermischung wird dann in die Matrize der Tablettenpresse überführt. Pulverreste, die nicht in die Vertiefung der Matrize sondern auf die obere Matrizenfläche gelangt sind, werden mit einem Kunstoffkartenblatt vorsichtig in die Vertiefung gestrichen. Dann wird jede Tablette einzeln bei einer Presskraft zwischen 40 und 45 kN 30 s lang verpresst.

3.4. Inprozesskontrollen

Stichprobenweise wird die Masse einzelner Tabletten nach dem Pressvorgang auf der Waage Mettler PG 503 – SDR (Einstellung: 0,001 g Genauigkeit) überprüft.
Außerdem werden alle Tabletten optisch auf Risse und Unebenheiten der Oberfläche geprüft.

4. Reinigung und Reinigungskontrolle

Nach Gebrauch sind alle Geräte, die bei der Arzneimittelherstellung verwendet wurden, gründlich entsprechend den jeweiligen Reinigungsanweisungen zu reinigen. Die Reinigung ist zu protokollieren und optisch zu überprüfen (Akzeptanzkriterium: optisch keine sichtbaren Rückstände erkennbar). Für die Reibschale und das Pistill ist einmalig eine Reinigungsvalidierung durchzuführen, bei der das letzte Waschwasser spektrophotometrisch auf den Restgehalt an Wirkstoff (Talinolol) geprüft wird.
Gereinigte Geräte sind mit einem Aufkleber mit der Aufschrift „gereinigt“ zu versehen. Der Aufklebende hat auf diesem Aufkleber das Datum des Anbringens und sein Namenszeichen zu notieren.
Gereinigte Geräte sind ab dem Reinigungsdatum sieben Tage lang zur sofortigen Benutzung freigegeben, werden sie erst zu einem späteren Zeitpunkt wieder verwendet, ist eine erneute Reinigung vor einer Benutzung erforderlich.

Liste der beigefügten Anlagen:

Titel und Thema der Anlage	Beigelegt von

Johannes Gutenberg-Universität Mainz
Institut für Pharmazie
Biopharmazie und Pharmazeutische Technologie
Staudingerweg 5, 55099 Mainz

Herstellprotokoll für klinische Prüfmuster

Präparat: Talinolol Retard-Matrix-Tabletten 100 mg

Herstellprotokoll

HP 001

Talinolol Retard-Matrix-Tabletten 100 mg

Johannes Gutenberg-Universität Mainz
Institut für Pharmazie
Biopharmazie und Pharmazeutische Technologie
Staudingerweg 5, 55099 Mainz

Herstellprotokoll für klinische Prüfmuster

Präparat: Talinolol Retard-Matrix-Tabletten 100 mg

Die Herstellung erfolgt gemäß Herstellvorschrift HV 001 für Talinolol Retard-Matrix-Tabletten 100 mg. Die Pulvermischung ergibt eine theoretische Ausbeute von 483 Tabletten a 200 mg Gesamtgewicht bei einer Einwaage von 207 mg Pulver pro Tablette. Dabei stellen die 7 mg, die die 200 mg überschreiten, einen Zuschlag für Staubverluste dar.

Datum der Herstellung: _____

Chargen-Bezeichnung (Ch.-B.): _____

1. Herstellung

1.1. Vorbereitung

Fenster und Glasschiebetür geschlossen?

ja / nein _____

Namenszeichen

Kittel, Handschuhe, Mundschutz, Kopfhaube und Überschuhe angelegt?

ja / nein _____

Namenszeichen

1.2. Rohstoffeinwaage für Pulvermischung

Zu verwendende Waage	Letzte Kalibrierung
Mettler PG 503-SDR (0,01g)	

Vorgeschriebene Waage verwendet: _____

Datum, Unterschrift

Herstellprotokoll für klinische Prüfmuster

Präparat: Talinolol Retard-Matrix-Tabletten 100 mg

Pos. Nr.	Rohstoff	Ch.-Bez.	Einwaage Soll (g)	Einwaage Ist (g)	Unterschrift Wiegender	Unterschrift Kontrollierender
1	Talinolol		50,0			
2	Eudragit® L 100-55		35,0			
3	Mannitol		15,0			
		Summe:	100,0			

1.3. Mischvorgang

Zu verwendende Geräte:
Reibschale (25 cm Durchmesser)
Kartenblatt (Kunststoff)
Pistill

Vorgeschriebene Geräte verwendet: _____

Datum, Unterschrift

	Soll	Ist
Mischzeit Pos. 1 bis 3 (Kartenblatt)		
Mischzeit Pos. 1 bis 3 (Pistill)		
Produktionsdatum		
Ausgeführt von		

1.4. Verpressen der Pulvermischung zu Tabletten

Zu verwendende Geräte
Waage Mettler PG 503 – SDR (Einstellung: 0,001 g Genauigkeit)
Tablettenhandpresse Paul Weber PW 20 GS
Kristallisierschalen 50 ml, Glas

Vorgeschriebene Geräte verwendet: _____

Datum, Unterschrift

	Soll	Ist
Anzahl zu verpressender Tabletten		
Pulvermasse pro Tablette	207 +/- 1 mg	
Masse fertige Tablette	200 +/- 7 mg	
Produktionsdatum		
Ausgeführt von		

2. Inprozesskontrollen:

Stichproben Tablettenmassen:

Zu verwendende Waage	Letzte Kalibrierung
Mettler PG 503-SDR (0,001g)	

Vorgeschriebene Waage verwendet: _____

Datum, Unterschrift

3. Ausbeuteberechnung:

Masse pressfertige Pulvermischung: _____ g

Anzahl verpresste Tabletten: _____

Anzahl verpresste Tabletten • 207 mg = - _____ g

Rest nichtverpresste Pulvermischung - _____ g

Verluste (Staub u. ä.): _____ g

Soll < 5 % Ist: _____ %

Begründung bei Abweichung:

_____

_____

_____

_____

Anzahl verpresste Tabletten: _____

davon Bruch: - _____

davon sonstiger Ausschuss: - _____

Zur Verpackung und Freigabe geeignete Tabletten: _____

Bemerkungen, besondere Vorkommnisse:

_____

_____

_____

_____

4. Reinigung und Reinigungskontrolle:

4.1. Reinigung der Geräte nach Gebrauch:

Gerät Nr.	Gerät	Gereinigt am	Gereinigt von
1			
2			
3			
4			
5			
6			
7			
8			

4.2. Optische Prüfung der Geräte nach Reinigung:

Gerät Nr.	Geprüft am	Geprüft von
1		
2		
3		
4		
5		
6		
7		
8		

(Akzeptanzkriterium: optisch keine sichtbaren Rückstände erkennbar)

Herstellprotokoll für klinische Prüfmuster

Präparat: Talinolol Retard-Matrix-Tabletten 100 mg

Nachreinigung erforderlich: ja / nein)*

für Geräte Nr. _____

Anforderung erfüllt: ja / nein)*

für Geräte Nr._____

)* : Nichtzutreffendes streichen

Geräte freigegeben zur weiteren Verwendung:

Prüfer:_____ Datum / Unterschrift: _____

Die o.g. Arzneimittel wurden gemäß der entsprechenden Herstellanweisung und entsprechend § 5 PharmBetrV hergestellt.

Datum: _____

Unterschrift Herstellungsleiter: _____

Liste der beigefügten Anlagen:

Titel und Thema der Anlage	Beigelegt von

Landesamt für Soziales, Jugend und Versorgung

HERSTELLUNGSERLAUBNIS

Der

JOHANNES GUTENBERG – UNIVERSITÄT MAINZ,
Institut für Pharmazie
Biopharmazie und Pharmazeutische Technologie
55099 Mainz

erteile ich gemäß § 13 Abs.1 des Gesetzes über den Verkehr mit Arzneimitteln (Arzneimittelgesetz - AMG) in gültiger Fassung die Erlaubnis zur Herstellung von

Humanarzneimitteln zur klinischen Prüfung

in der Betriebsstätte

Staudinger Weg 4, 55099 Mainz
gemäß des vorliegenden Lageplanes (Anlage 2)

Die Erlaubnis erstreckt sich ausschließlich auf die in

- Anlage 1 aufgeführten Herstellungstätigkeiten und Darreichungsformen
- Anlage 2 Lageplan SB III Pharmazie # 2411 vom 10.11.1993
- Anlage 3 aufgeführten mit der teilweisen Prüfung der Arzneimittel beauftragten Betriebe

Die Anlage 4 ist Bestandteil der Erlaubnis.

Az: R 55 M / 167-1501/02
Mainz, 19.04.2001

Gudrun Bach
Apothekerin, Reg.-Angestellte

Landesamt für Soziales, Jugend und Versorgung 53 Rheinland-Pfalz

Anlagen:
Anlage 1: Herstellungstätigkeiten
Anlage 2: Lageplan
Anlage 3: Beauftragte Betriebe für die Prüfung gem. § 14 Abs. 4 AMG
Anlage 4: Herstellungs,- Kontroll- und Vertriebsleiter, Stufenplanbeauftragter

Annex II

Annex II

Preparations for a human in vivo study with talinolol sustained-release tablets

To evaluate in vivo the in silico results for talinolol absorption from the sustained-release matrix tablets developed (cp. Chapters III and V), a clinical study in humans was projected. The study should be performed with six healthy volunteers. One additional volunteer is included in the study as a surrogate for possible drop outs.
The study will be performed in three periods according to a cross over design, in which each volunteer should swallow the three different following dosage schemes with intermediate wash out phases of at least one week:
- one talinolol IR tablet with a drug content of 100 mg,
- one talinolol CR tablet with a drug content of 100 mg and
- two talinolol CR tablets with a drug content of 100 mg each.

The study protocol has been submitted for approval at the local ethics committee of Rheinland-Pfalz. Copies of the study protocol and the request to the ethics committee are attached in the original German format.

Attachments

(in German original format)

- Request for approval of a clinical study with talinolol IR and CR dosage forms as submitted to the ethics committee of Rheinland-Pfalz.

- Study protocol for a clinical study with talinolol IR and CR dosage forms.

Names, adresses and telephone numbers were partly omitted for privacy reasons.

Antrag an die Ethik-Kommission bei der Landesärztekammer Rheinland-Pfalz

Allgemeine Angaben

1. Datum der Antragstellung:

12. November 2002

2. Titel des Forschungsvorhabens:

Untersuchung des Einflusses einer retardierten Freigabe auf die Absorption und Bioverfügbarkeit intestinal sezernierter Arzneistoffe anhand von Talinolol als Modellsubstanz

3. Verantwortlicher Studienleiter (LKP):

[Name, Funktion, Adresse]

4. Verantwortlicher Sponsor:

Eigeninitiierte und -finanzierte Studie der Universität

Initiator: Prof. Dr. P. Langguth, [Funktion, Adresse]

5. Handelt es sich um eine multizentrische Studie?

Nein

6. Sonstige Teilnehmer:

Prüfarzt:

[Name, Funktion, Adresse]

Wissenschaftliche Betreuung:

Prof. Dr. Peter Langguth, Apotheker, [Funktion, Adresse]

7. Ort(e) der Durchführung:

Institut für Pharmazie, Universität Mainz, [Adresse]

I. Medizinische Klinik und Poliklinik, Universität Mainz, [Adresse]

8. Finanzierung:

a) Eigenfinanzierte Studie der Universität

b) Die Probanden erhalten auf Wunsch ein Honorar von 300 €.

9. Wurde die Arzneimittelprüfung der zuständigen Behörde angezeigt?

Wird nach positivem Votum der Ethik-Kommission dem BfArM in Bonn und dem Landesamt für Soziales, Jugend und Versorgung in Trier gemeldet.

10. Wurde das Forschungsvorhaben bereits einer Ethik-Kommission vorgelegt?

Nein

Begründung des Forschungsvorhabens

1. Ziel des Vorhabens:

Ziel der geplanten Studie ist es, die Pharmakokinetik und insbesondere Absorption und Bioverfügbarkeit bei durch P-Glykoprotein intestinal sezernierten Arzneistoffen am Beispiel des ß-Adrenozeptorenantagonisten Talinolol zu untersuchen. In dieser Studie soll der Einfluss der Freigabegeschwindigkeit auf die Pharmakokinetik nach peroraler Applikation untersucht werden.

2. Gründe für die Durchführung, Problemdarstellung:

Für die zukünftige Entwicklung retardierter Arzneiformen mit Wirkstoffen, die an intestinale Carrier wie P-GP binden, ist es wichtig, den Einfluss einer veränderten Freigabegeschwindigkeit auf die Absorption und Bioverfügbarkeit des Arzneistoffes zu kennen. Talinolol hat sich als geeignete Modellsubstanz für P-GP Substrate erwiesen (vgl. Lit. 4) und wird deshalb in dieser Studie verwendet.

3. Stand der Wissenschaft:

Die Beteiligung intestinaler Carrierproteine am Absorptionsprozess ist für zahlreiche Arzneistoffe nachgewiesen. Dabei können Arzneistoffe sowohl in resorptiver Richtung (z.B. einige Cephalosporine, Penicilline, ACE-Hemmer) als auch in sekretorischer Richtung (z.B. verschiedene Betablocker und Antiarrhythmika sowie Ofloxacin, Ciprofloxacin, Ciclosporin) transportiert werden. Für die Bioverfügbarkeit haben carriervermittelte Absorptions- und Sekretionsprozesse insofern Bedeutung, als intestinal absorbierte Substanzen zum Teil sehr effizient vom Darmlumen zur Blutseite durch die Darmwand transportiert werden können, während Sekretionsprozesse den gegenteiligen Effekt, d.h. einen effizienten Transport von der Blut- zur Darmseite, bewirken. Damit führen Sekretionsprozesse im Allgemeinen zu einer Verringerung des absorbierten Anteils einer Dosis und der Bioverfügbarkeit.

Bei der geplanten Studie soll der Einfluss der Freigabegeschwindigkeit aus der Formulierung auf die Absorption und Bioverfügbarkeit des Betablockers Talinolol, einer Modellsubstanz für carriervermittelte Sekretionsprozesse, beim Menschen untersucht werden.

Dabei ist zu erwarten, dass durch eine verzögerte Freigabe des Wirkstoffs dessen Konzentration im Darm gegenüber einer rasch freisetzenden Zubereitung

(Fertigarzneimittel Cordanum) verändert sein wird. Als Folge ist zu erwarten, dass der durch Carrier sezernierte Anteil des Wirkstoffs bei den Retardzubereitungen erhöht sein wird und damit die Bioverfügbarkeit mehr oder weniger stark und in Abhängigkeit von der Freigabegeschwindigkeit absinken wird.

4. Pharmakologisch-toxikologische Eigenschaften des verwendeten Arzneistoffs

Siehe Fachinformation Cordanum und vgl. Lit. 1

Literatur:

(1) de Mey, C., V. Schroeter, R. Butzer, P. Jahn, K. Weisser, U. Wetterich, B. Terhaag, E. Mutschler, H. Spahn-Langguth, D. Palm and et al., 1995, Dose-effect and kinetic-dynamic relationships of the beta-adrenoceptor blocking properties of various doses of talinolol in healthy humans, J Cardiovasc Pharmacol 26, 879.

(2) Gramatté, T. and R. Oertel, 1999, Intestinal secretion of intravenous talinolol is inhibited by luminal R-verapamil, Clin Pharmacol Ther 66, 239.

(3) Gramatté, T., R. Oertel, B. Terhaag and W. Kirch, 1996, Direct demonstration of small intestinal secretion and site-dependent absorption of the beta-blocker talinolol in humans, Clin Pharmacol Ther 59, 541.

(4) Spahn-Langguth, H., G. Baktir, A. Radschuweit, A. Okyar, B. Terhaag, P. Ader, A. Hanafy and P. Langguth, 1998, P-glycoprotein transporters and the gastrointestinal tract: evaluation of the potential in vivo relevance of in vitro data employing talinolol as model compound, Int J Clin Pharmacol Ther 36, 16.

(5) Wetterich, U., H. Spahn-Langguth, E. Mutschler, B. Terhaag, W. Rosch and P. Langguth, 1996, Evidence for intestinal secretion as an additional clearance pathway of talinolol enantiomers: concentration- and dose-dependent absorption in vitro and in vivo, Pharm Res 13, 514.

(6) Wagner, D., H. Spahn-Langguth, A. Hanafy, A. Koggel, P. Langguth, 2001, Intestinal drug efflux: formulation and food effects, Adv Drug Deliv Rev 50 Suppl. 1, 13

Allgemeine Planung

1. Zusammenfassende Darstellung des Prüfablaufs:

Die Studie ist auf 6 Probanden ausgelegt. Zur Kompensation möglicher Drop-Outs werden jedoch 7 freiwillige Probanden in die Studie eingeschlossen. Diese erhalten zunächst eine Einmaldosis von 100 mg Talinolol in Form einer schnellfreisetzenden Cordanum 100 mg Tablette. Daraufhin werden über einen Zeitraum bis zu 48 h nach der Applikation des Arzneimittels Blutproben von jeweils 7 ml aus einer Unterarmvene zu folgenden definierten Zeitpunkten entnommen: 0; 1; 2; 3; 4; 5; 7; 10; 13; 24; 33; 48 Stunden. Die entnommenen Blutproben werden bei 600 g zentrifugiert, dann wird das Plasma abgetrennt und bei –22° C bis zur Analyse eingefroren. Die Analyse der Plasmaproben auf Talinolol erfolgt mittels eines validierten HPLC Verfahrens. Nach einer Wash Out Phase von mindestens acht Tagen wird denselben Probanden eine rezepturmäßig hergestellte Talinolol Retardtablette, die ebenfalls 100 mg Wirkstoff enthält, verabreicht. Um das in vivo Freisetzungsverhalten der Retardtabletten nachvollziehen und von P-GP verursachten Effekten abgrenzen zu können, werden nach einer erneuten mindestens achttägigen Wash Out Phase zwei derselben rezepturmäßig hergestellten Retardtabletten mit jeweils 100 mg Wirkstoffgehalt gleichzeitig verabreicht. Die Probennahme nach Gabe der retardierten Arzneiformen erfolgt ebenso wie nach Einnahme der schnellfreisetzenden Tabletten. Des weiteren werden in allen drei Phasen der Studie von den Probanden über 48 h Sammelurinproben in definierten Intervallen gesammelt.

2. Geplanter Beginn:

Voraussichtlich Sommer 2003
voraussichtliche Dauer: ca. 4 Wochen

3. Gesunde Probanden

Es handelt sich um eine Untersuchung an gesunden Probanden, die ambulant durchgeführt wird. Jeder Proband wird innerhalb von ca. vier Wochen dreimal zwei Tage mit der Studie beschäftigt sein.

4. Patienten

Es werden keine Patienten in die Studie miteinbezogen.

5. Kategorisierung der Untersuchung und der Prüfsubstanz

Es handelt sich um eine für Forschung und Wissenschaft wichtige pharmakokinetische Prüfung, bei der ein seit über 20 Jahren als Tablette auf dem deutschen Markt befindlicher, gut verträglicher Arzneistoff verabreicht wird, so dass unter Einhaltung der Ausschlusskriterien das Risiko für die Probanden als gering eingestuft werden kann.

6. Studienart

Es handelt sich um eine offene Studie mit drei parallelen Gruppen und einem Cross over-Design.

7. Auswahl der Probanden

Es werden gesunde Probanden rekrutiert. Hinsichtlich des Geschlechts bestehen keine Einschränkungen, das Alter der Probanden ist auf 18 bis 50 Jahre festgelegt, die Probanden sollen Angehörige der kaukasischen Rasse sein und keine Arzneimittel mit Wirkung auf das Herz-Kreislaufsystem einnehmen.

In einer Voruntersuchung der Probanden wird neben Blut- und Urinuntersuchungen auch ein Elektrokardiogramm (EKG) aufgenommen.

Ausschlusskriterien sind:

ein PQ-Intervall im Ruhe-EKG von mehr als 220 ms

eine Herzfrequenz in Ruhe von weniger als 45 min^{-1}

ein systolischer Blutdruck von weniger als 100 mm Hg liegend

Neigung zu orthostatischer Dysregulation, Ohnmacht und Blackouts

klinisch relevante Erkrankungen des Herz-Kreislauf-Systems

Asthma (sowohl aktiv als auch in der Anamnese).

Es werden keine Probanden eingeschlossen, bei denen Zweifel an der Einsichtsfähigkeit bestehen.

Voraussetzung für die Teilnahme an der Studie ist das schriftliche Einverständnis des Probanden. Über den Zweck der Studie und die eventuell damit verbundenen Risiken wird der Proband in Form eines Informationsblattes schriftlich aufgeklärt.

8. Behandlung

Jeder Proband erhält drei Medikationen, jeweils mit Talinolol als Wirkstoff:

a) eine schnellfreisetzende 100 mg Tablette

b) eine retardierte 100 mg Tablette

c) zwei gleichzeitig einzunehmende retardierte 100 mg Tabletten.

Zwischen den drei Medikationen wird eine Wash Out Phase von mindestens acht Tagen eingehalten. Es werden Blut- und Sammelurinproben bis zu 48 Stunden nach Einnahme des Medikamentes genommen.

Die Compliance wird durch den späteren Nachweis des Arzneistoffs im Blut belegt.

Sollten wider Erwarten schwerwiegende unerwünschte Wirkungen oder Komplikationen auftreten, werden diese der Ethik-Kommission gemeldet. Sollten diese ursächlich auf die Studienteilnahme zurückzuführen sein, werden die betroffenen Probanden von der Studie ausgeschlossen. Sollten bei mehr als 2 der 7 Probanden schwerwiegende Komplikationen auftreten, wird die gesamte Studie abgebrochen.

9. Feststellung der Wirksamkeit

Entfällt

10. Statistik und Auswertung

Die Ergebnisse werden entsprechend den datenschutzrechtlichen Bestimmungen in anonymisierter Form protokolliert, gespeichert und ausgewertet.

Zwischenauswertungen sind nicht vorgesehen.

11. Ethisch - rechtliche Aspekte

Bestehende Gesetze und Richtlinien bezüglich der Herstellung der Arzneimittel, der Durchführung der Studie und des Datenschutzes werden eingehalten.

Das Forschungsvorhaben dient der wissenschaftlichen Grundlagenforschung und der zukünftigen Entwicklung retardierter peroraler Arzneiformen mit Wirkstoffen, die intestinal sezerniert werden.

In der Studie wird ein Arzneistoff getestet, der seit über 20 Jahren auf dem deutschen Markt etabliert ist und gut vertragen wird. Dies haben klinisch-pharmakologische Studien gezeigt (vgl. II: Literatur (1)). Die Gefahr eines Dose Dumpings, die allgemein bei Retardzubereitungen besteht, ist bei dieser Studie nicht

relevant, da die in den Retardformulierungen enthaltene Dosis die der schnellfreisetzenden Tablette nicht übersteigt.

Durch Einhaltung der Ausschlusskriterien ist sichergestellt, dass das Risiko für die an der Studie teilnehmenden Probanden gering ist. Unerwünschte Wirkungen, die über die in der Fachinformation für Cordanum 100 mg Tabletten erwähnten hinausgehen, sowie besondere Komplikationen sind nicht zu erwarten.

Seltene unerwünschte Wirkungen sind auf Grund der geringen Probandenzahl und der nur dreimaligen Einnahme des Medikamentes mit entsprechenden Wash Out Phasen kaum zu erwarten.

Bei wider Erwarten auftretenden schwerwiegenden unerwünschten Wirkungen oder Komplikationen bei einzelnen Probanden werden die entsprechenden Probanden von der weiteren Teilnahme an der Studie ausgeschlossen und ärztlich behandelt.

Als schwerwiegende unerwünschte Ereignisse werden neben dem Tod des Probanden alle Krankheitsbilder definiert, die eine stationäre Behandlung bedingen oder bleibende Schädigungen erwarten lassen. Solche Ereignisse werden der zuständigen Ethik-Kommission innerhalb von 24 Stunden nach Bekanntwerden durch den Prüfarzt gemeldet. Durch die regelmäßigen Blutentnahmen sind die Probanden während der Studienphasen regelmäßig unter ärztlicher Kontrolle, so dass eventuell auftretende Komplikationen umgehend erkannt werden und Gegenmaßnahmen ergriffen werden können.

Um das Risiko unerwünschter Wirkungen zu minimieren werden die Probanden verpflichtet, an den Versuchstagen keine schweren körperlichen Arbeiten zu verrichten und besondere Anstrengungen zu vermeiden.

Eine Probandenversicherung nach §§ 40 / 41 ist abgeschlossen worden, die Deckungsbestätigung ist beigefügt.

Der Leiter der Klinischen Prüfung ist über die rechtlichen Bestimmungen des AMG und über die GCP Richtlinien der ICH informiert.

Ähnliche Forschungsvorhaben mit Talinolol in retardierten Arzneiformen sind unseres Wissens nach bisher nicht durchgeführt worden.

Die Probanden erhalten eine schriftliche Probandeninformation und werden darüber hinaus mündlich über Zweck, Art und Umfang der Studie sowie mögliche Risiken, die mit einer Teilnahme an der Studie verbunden sind, aufgeklärt.

Es werden keine Probanden eingeschlossen, bei denen Zweifel an der Einsichts- oder Einwilligungsfähigkeit bestehen.

Ein Probandenausweis wird nicht ausgestellt.

Eine offizielle Mitteilung an die Hausärzte der Probanden über deren Teilnahme an dieser Studie ist nicht vorgesehen.

Der Prüfarzt verfügt über die notwendige fachliche und persönliche Qualifikation, Erfahrung, Zeit und Einrichtung, um dieses Forschungsvorhaben durchzuführen (CV ist beigefügt).

Mainz, den _____

_____

Probandeninformation und Einwilligungserklärung

Für die Studie:
Untersuchung des Einflusses einer retardierten Freigabe auf die Absorption und Bioverfügbarkeit intestinal sezernierter Arzneistoffe anhand von Talinolol als Modellsubstanz

Die Aufnahme von Arzneistoffen aus dem Magen-Darm-Trakt ins Blut geschieht auf unterschiedliche Art und Weise. Im einfachsten Fall durchdringt der gelöste Arzneistoff die Magen- bzw. Darmwand passiv, d. h. ohne Beteiligung von Transportproteinen. Nicht wenige Arzneistoffe werden jedoch auch aktiv transportiert, d. h. es existieren spezifische Transportermoleküle, die die Substanz gezielt ins Blut schleusen. Wiederum andere Arzneistoffe werden von speziellen Transportern nach einer passiven Aufnahme in Zellen der Darmwand wieder in das Innere des Magen-Darm-Traktes zurückgeschleust, so dass der Anteil an Arzneistoff, der letztlich ins Blut gelangt, verringert wird. Ein solcher Arzneistoff ist das in der Studie verwendete Talinolol, ein ß-Blocker, der insbesondere bei Bluthochdruck und Angina pectoris eingesetzt wird. Um überhaupt die Wand des Magen-Darm-Traktes durchdringen zu können, muss jeder Arzneistoff zunächst in Lösung gehen. Die Geschwindigkeit, mit der dies geschieht, lässt sich durch Hilfsstoffe, die einer Tablette zugesetzt werden, verändern. Durch eine Verzögerung der Freisetzung von Talinolol aus der Tablette wird auch der Einfluss des Transporters auf die Aufnahme ins Blut verändert. Für die zukünftige Entwicklung neuer Retardarzneimittel (d.h. Arzneimittel mit verlängerter bzw. verzögerter Wirkung) ist es wichtig, zu wissen, wie sich dieser Einfluss auswirkt, und dazu ist es notwendig diese Studie durchzuführen.
Dazu sind zwei unterschiedliche Arzneiformen einzunehmen, die sich in der Geschwindigkeit, mit der der Wirkstoff Talinolol in Lösung geht, unterscheiden. Um den Lösungsvorgang des Wirkstoffes im Magen-Darm-Trakt genauer charakterisieren zu können, wird von der langsam den Wirkstoff freigebenden Tablette neben einer Einmaldosis von 100 mg durch Gabe einer Tablette auch noch eine Einzeldosis von 200 mg in Form von zwei 100 mg Tabletten verabreicht. Jeder Proband erhält somit jeweils drei mal eine Medikation, wobei zwischen diesen drei Verabreichungen jeweils mindestens eine Woche liegt. Nach jeder Einnahme werden

zu 12 definierten Zeitpunkten Blutproben innerhalb von 48 h aus einer Armvene entnommen sowie der Urin gesammelt.

Das mit der Teilnahme an der Studie verbundene Risiko ist gering, da sich der Wirkstoff Talinolol in Tablettenform unter dem Handelsnamen Cordanum seit über 20 Jahren auf dem deutschen Markt befindet und allgemein gut vertragen wird. Da mit einer Blutdrucksenkung zu rechnen ist, kann es jedoch vorübergehend zu Müdigkeit, Schlappheitsgefühlen oder Schwindel kommen. Außerdem können die für die Cordanum Tabletten beschriebenen Nebenwirkungen auftreten. Eine Kopie des entsprechenden Beipackzettels wird jedem Probanden ausgehändigt.

Um das Risiko unerwünschter Wirkungen zu verringern sind an den Versuchstagen nach Einnahme der Prüfmedikation schwere körperliche Arbeiten sowie größere Anstrengungen zu vermeiden. Da eine Beeinträchtigung der aktiven Teilnahmefähigkeit am Straßenverkehr möglich ist, wird außerdem empfohlen, 12 h nach Einnahme der Prüfmedikation ohne Begleitung kein Kraftfahrzeug zu führen.

Die erhobenen Krankheitsdaten werden Dritten nicht zugänglich gemacht, d.h. die wissenschaftliche Verwertung und ggf. eine Veröffentlichung der Daten erfolgt ausschließlich in anonymisierter Form.

Für Schäden, die auf die Teilnahme an der Studie zurückzuführen sind, und für die kein anderer haftbar ist, wurde eine Probandenversicherung mit einer Schadenshöchstsumme von 500.000 Euro pro Proband bei folgender Versicherung abgeschlossen:

Ecclesia Versicherungsdienst GmbH

[Adresse, Telefonnummer, Police-Nummer]

Die Obliegenheiten des Probanden sind als Anhang beigefügt.

Der Versicherungsschutz erlischt, wenn ausdrücklich gegen Anweisungen zuwider gehandelt wird.

Ich erkläre hiermit, dass ich über Ziel, Ablauf und Durchführung des Forschungsvorhabens aufgeklärt worden bin, dass ich die Erläuterungen verstanden habe und meine Fragen zur Zufriedenheit beantwortet wurden. Ich weiß, dass ich auch weiterhin jederzeit Fragen stellen kann. Ich wurde auch darüber aufgeklärt, dass ich ohne persönliche Nachteile und ohne Angabe von Gründen nicht an der Studie teilzunehmen brauche. Außerdem kann ich meine Teilnahme an der Studie jederzeit ohne Angabe von Gründen durch schriftlichen oder mündlichen Widerruf

dieser Einwilligungserklärung ebenfalls ohne persönliche Nachteile abbrechen. Einem Einblick in die Originalakte und einer eventuellen Weitergabe anonymisierter Daten aus wissenschaftlichen Gründen stimme ich zu.
Die Versicherungsbedingungen der für diese Studie abgeschlossenen Probandenversicherung sind mir bekannt.
Eine Kopie der Probandeninformation und Einwilligungserklärung wurde mir ausgehändigt.

Name des aufklärenden Arztes: _____

Ich werde innerhalb der letzten 60 Tage vor Beginn der Studie nicht an einer anderen Studie teilgenommen haben und bestätige, dass meine Angaben zur Einnahme von Medikamenten und Drogen der Wahrheit entsprechen.
Ich werde jeweils 12 h vor Einnahme der Prüfmedikation sowie bis 48 h danach keinen Alkohol zu mir nehmen.

Ich bin bereit, freiwillig an der Studie teilzunehmen.

Mainz, den _____ _____ _____

Proband Arzt

Einwilligungserklärung zum Datenschutz (AMG)

Bei wissenschaftlichen Studien werden persönliche Daten und medizinische Befunde über Sie erhoben. Die Weitergabe, Speicherung und Auswertung dieser studienbezogenen Daten erfolgt nach gesetzlichen Bestimmungen und setzt vor Teilnahme an der Studie folgende freiwillige Einwilligung voraus:

1. Ich erkläre mich damit einverstanden, dass im Rahmen dieser Studie erhobene Daten / Krankheitsdaten auf Fragebögen und elektronischen Datenträgern aufgezeichnet und ohne Namensnennung weitergegeben werden können an

a) Mitarbeiter des Institutes für Pharmazie der Johannes Gutenberg-Universität, [Adresse] als Auftraggeber der Studie zur wissenschaftlichen Auswertung
b) an die zuständige Überwachungsbehörde (Landesamt, Trier) oder Bundesoberbehörde (Bundesinstitut für Arzneimittel und Medizinprodukte, Bonn) zur Überprüfung der ordnungsgemäßen Durchführung der Studie.

2. Außerdem erkläre ich mich damit einverstanden, dass ein autorisierter und zur Verschwiegenheit verpflichteter Beauftragter des Auftraggebers, der zuständigen Überwachungsbehörde oder der Bundesoberbehörde in meine beim Prüfarzt vorhandenen personenbezogenen Daten Einsicht nimmt, soweit dies für die Überprüfung der Studie notwendig ist. Für diese Maßnahme entbinde ich den Prüfarzt von der ärztlichen Schweigepflicht.

Name des Probanden:_____

_____ _____

Datum Unterschrift des Probanden

Allgemeine Versicherungsbedingungen
für klinische Prüfungen von Arzneimitteln
(Probandenversicherung)

Abschnitt 14 II: Obliegenheiten des Versicherten

(1) Während der Dauer der klinischen Prüfung darf sich die versicherte Person einer anderen medizinischen Behandlung nur nach Rücksprache mit dem klinischen Prüfer unterziehen. Dies gilt nicht in einem medizinischen Notfall; der klinische Prüfer ist von einer Notfallbehandlung unverzüglich zu unterrichten.

(2) Eine Gesundheitsschädigung, die als Folge der klinischen Prüfung eingetreten sein könnte, ist dem Versicherer unverzüglich anzuzeigen.

(3) Der Versicherte hat alle zweckmäßigen Maßnahmen zu treffen, die der Aufklärung der Ursache und des Umfanges des eingetretenen Schadens und der Minderung dieses Schadens dienen.

(4) Auf Verlangen des Versicherers ist der behandelnde Arzt - als solcher gilt auch ein Konsiliararzt oder ein gutachterlich tätiger Arzt- zu veranlassen, einen Bericht über die Gesundheitsschädigung und, nach Abschluss der ärztlichen Behandlung, einen Schlussbericht zu erstatten; außerdem ist dafür Sorge zu tragen, dass alle etwa weiter noch von dem Versicherer geforderten Berichte des behandelnden Arztes geliefert werden. Alternativ kann der Versicherte den behandelnden Arzt von der ärztlichen Schweigepflicht entbinden, damit der Versicherer die vorab genannten Berichte direkt beim Arzt anfordern kann.

(5) Die behandelnden Ärzte, auch diejenigen, von denen der Versicherte aus anderen Anlässen behandelt oder untersucht worden ist, und die Sozialversicherungsträger sowie andere Versicherer, wenn dort die

Gesundheitsschädigung gemeldet ist, sind zu ermächtigen, dem Versicherer auf Verlangen Auskunft zu erteilen.

(6) Hat der Versicherungsfall den Tod zu Folge, so ist dies unverzüglich anzuzeigen, und zwar auch dann, wenn eine Meldung nach Abs. (2) bereits erfolgt ist. Dem Versicherer ist das Recht zu verschaffen, eine Obduktion durch einen von ihm beauftragten Arzt vornehmen zu lassen.

Untersuchung des Einflusses einer retardierten Freigabe auf die Absorption und Bioverfügbarkeit intestinal sezernierter Arzneistoffe anhand von Talinolol als Modellsubstanz

Prüfplan

Synopsis

Titel:

Untersuchung des Einflusses einer retardierten Freigabe auf die Absorption und Bioverfügbarkeit intestinal sezernierter Arzneistoffe anhand von Talinolol als Modellsubstanz

Projektleiter:

Prof. Dr. P. Langguth

Multizenterstudie:

nein

Leiter der klinischen Prüfung:

[Name, Adresse]

Voten anderer Ethik-Kommissionen:

nein

Prüfzentren:

Institut für Pharmazie, Universität Mainz, [Adresse]
I. Medizinische Klinik und Poliklinik, Universität Mainz, [Adresse]

Prüfarzt:

[Name, Adresse]

Sponsor:

eigenfinanzierte Studie der Universität
Initiator: Prof. Dr. P. Langguth, [Adresse]

Studientyp / Studiendesign:

offene randomisierte Cross over-Studie in 3 Parallelgruppen

Zeitplan:

ca. 4 Wochen, voraussichtlich Sommer 2003

Probandenanzahl:

7

Geschlecht:

männlich oder weiblich

Alter:

18 bis 50 Jahre

Probandenversicherung:

Ecclesia Versicherungsdienst GmbH

[Adresse, Telefonnummer, Police-Nummer]

Verantwortlichkeiten und Anschriften:

Leiter der Klinischen Prüfung (gemäß § 40 AMG):

[Name, Funktion, Adresse, Telefonnummer]

Prüfzentrum:

I. Medizinische Klinik und Poliklinik, Universität Mainz

[Adresse]

Institut für Pharmazie, Universität Mainz

[Adresse]

Prüfarzt:

[Name, Funktion, Adresse, Telefonnummer]

Initiator:

Prof. Dr. P. Langguth

[Funktion, Adresse, Telefonnummer, private Telefonnummer]

Kurzfassung des Projekts

Titel:

Untersuchung des Einflusses einer retardierten Freigabe auf die Absorption und Bioverfügbarkeit intestinal sezernierter Arzneistoffe anhand von Talinolol als Modellsubstanz

Projektleiter:

Prof. Dr. P. Langguth, Professor für Biopharmazie und Pharm. Technologie

1. Wissenschaftlicher Hintergrund

Ziel der geplanten Studie ist es, die Pharmakokinetik, insbesondere die Absorption und Bioverfügbarkeit bei durch P-Glykoprotein (P-GP) intestinal sezernierten Arzneistoffen am Beispiel des ß-Adrenozeptorenantagonisten Talinolol zu untersuchen. Weitere Beispiele für P-GP Substrate sind die Arzneistoffe Vincristin, Vinblastin, Doxorubicin, Paclitaxel, Ciclosporin A, Ketoconazol, Verapamil, Digoxin, Dexamethason, Cimetidin, Ranitidin, Salbutamol, Celiprolol, Phenytoin, Morphin und Loperamid.

In dieser Studie soll der Einfluss der Freigabegeschwindigkeit auf die Pharmakokinetik nach peroraler Applikation untersucht werden.
Für die zukünftige Entwicklung retardierter Arzneiformen mit Wirkstoffen, die an intestinale Carrier wie P-GP binden, ist es wichtig, den Einfluss einer veränderten Freigabegeschwindigkeit auf die Absorption und Bioverfügbarkeit des Arzneistoffes zu kennen. Talinolol hat sich als geeignete Modellsubstanz für P-GP Substrate erwiesen (vgl. Lit. 4) und wird deshalb in dieser Studie verwendet.

2. Studiendaten

Studienziel:

Ziel der geplanten Studie ist es, die Pharmakokinetik und insbesondere Absorption und Bioverfügbarkeit bei durch P-Glykoprotein intestinal sezernierten Arzneistoffen am Beispiel des ß-Adrenozeptorenantagonisten Talinolol zu untersuchen. In dieser Studie soll der Einfluss der Freigabegeschwindigkeit auf die Pharmakokinetik nach peroraler Applikation untersucht werden.
Es handelt sich um eine Experimentalstudie von wissenschaftlichem Interesse.

Studiendesign:

Es handelt sich bei dem geplanten Vorhaben um eine offene Studie mit drei parallelen Gruppen und einem Cross over-Design.
Anzahl der Probanden: 7. Die Studie ist auf 6 Probanden ausgelegt, zur Kompensation etwaiger Drop-Outs wird ein siebter Proband in die Studie eingeschlossen.

Primäre Zielgrößen:

Konzentrationen des Arzneistoffs in Blut und Urin.

3. Probanden

Anzahl der Probanden:

7

Durchführung der Studie:

Voraussichtlich Anfang 2003
voraussichtliche Dauer: ca. 4 Wochen

Einschlusskriterien:

- Männliche und weibliche gesunde Probanden
- Alter: 18 bis 50 Jahre
- Keine regelmäßige Medikamenteneinnahme

Ausschlusskriterien:

- Teilnahme an einer anderen klinischen Prüfung innerhalb der letzten 3 Wochen
- gleichzeitige Teilnahme an einer anderen klinischen Prüfung
- Schwangerschaft oder Stillzeit
- Suchtkranke
- Bekannte Unverträglichkeit / Überempfindlichkeit gegen den Wirkstoff oder Hilfsstoffe
- Q-Intervall im Ruhe-EKG von mehr als 220 ms
- Herzfrequenz in Ruhe von weniger als 45 min-1
- Systolischer Blutdruck von weniger als 100 mm Hg liegend
- Neigung zu orthostatischer Dysregulation, Ohnmacht und Blackouts
- Klinisch relevante Erkrankungen des Herz-Kreislauf-Systems

Asthma (sowohl aktiv als auch in der Anamnese)

Medizinisches Screening:

- Persönliche Daten, Klinische Anamnese
- Allgemeine Untersuchung
- Blut- und Urinuntersuchungen
- Elektrokardiogramm (EKG)

4. Prüfmedikation

Pharmakologisch-toxikologisches Gutachten

Entfällt, da Vergleichspräparat bereits seit 1975 am Markt und Retardtestpräparat in gleicher Dosierung und Arzneiform vorliegt.

Pharmakologisch-toxikologische Angaben siehe Fachinformation Cordanum 100 Tabletten

Medikation A:

- Handelsname: Cordanum 100 Tabletten
- Substanzname: Talinolol 100 mg
- Zubereitungsform: Tabletten
- Hersteller: AWD Pharma, Dresden

Medikation B:

- Talinolol 100 mg Retardtabletten
- Substanzname: Talinolol 100 mg
- Zubereitungsform: Tabletten
- Hersteller: Universität Mainz, Institut für Pharmazie

5. Versuchsdurchführung / Studienablauf

- 3 Prüfungsphasen
- (d. h. dreimalige Verabreichung einer Prüfmedikation jeweils mit nachfolgender Blut- und Urinprobennahme bis 48 Stunden nach Einnahme)
- zwischen den Prüfphasen mindestens 8 tägige Wash Out Phase
- Blutproben jeweils 7 ml

1. Prüfungsphase:

Vor Prüfungstag:

- in den letzten 24 Stunden vor Einnahme der Prüfmedikation kein anstrengender Sport
- in den letzten 24 Stunden vor Einnahme der Prüfmedikation kein Alkohol oder andere Medikamente
- ab dem Vortag der Einnahme der Prüfmedikation 22 Uhr kein Essen mehr

Am Prüfungstag:

- nur standardisiertes Essen und Getränke zu sich nehmen!
- keine anstrengenden Tätigkeiten
- über den Tag verteilt mindestens 1,4 Liter Flüssigkeit (Mineralwasser, Orangensaft oder Cola) trinken

8:15 Uhr:	Eintreffen im Prüfraum
	Leerprobe Urin
	Verweilkatheter legen
	Leerprobe Blut (1.1)
8:30 Uhr:	Einnahme der Prüfmedikation:
	Proband 3 und 4 : eine Cordanum 100 mg Tablette
	Proband 5 und 6 : eine Talinolol 100 mg Retardtablette
	Proband 1 und 2 : zwei Talinolol 100 mg Retardtabletten
	(Einnahme jeweils mit 200 ml Mineralwasser)
9:30 Uhr:	Blutprobe (1.2)
anschl.	Standardfrühstück
10:30 Uhr	Blutprobe (1.3)
11:30 Uhr:	Blutprobe (1.4)
12:30 Uhr:	Blutprobe (1.5)
anschl.	Mittagessen
13:30 Uhr:	Blutprobe (1.6)
15:30 Uhr:	Blutprobe (1.7)
18:30 Uhr:	Blutprobe (1.8)
18:45 Uhr:	Verweilkatheter rausnehmen
anschl.	Abendessen
21:30 Uhr:	Blutprobe (1.9)

Am folgenden Tag:

8:30 Uhr:	Blutprobe (1.10)
17:30 Uhr:	Blutprobe (1.11)

Am übernächsten Tag:

8:30 Uhr:	Blutprobe (1.12)

2. Prüfungsphase:

Vor Prüfungstag:

- in den letzten 24 Stunden vor Einnahme der Prüfmedikation kein anstrengender Sport
- in den letzten 24 Stunden vor Einnahme der Prüfmedikation kein Alkohol oder andere Medikamente
- ab dem Vortag der Einnahme der Prüfmedikation 22 Uhr kein Essen mehr

Am Prüfungstag:

- nur standardisiertes Essen und Getränke zu sich nehmen!
- keine anstrengenden Tätigkeiten
- über den Tag verteilt mindestens 1,4 Liter Flüssigkeit (Mineralwasser, Orangensaft oder Cola) trinken

8:15 Uhr:	Eintreffen im Prüfraum
	Leerprobe Urin
	Verweilkatheter legen
	Leerprobe Blut (2.1)
8:30 Uhr:	Einnahme der Prüfmedikation:
	Proband 3 und 4 : eine Cordanum 100 mg Tablette
	Proband 5 und 6 : eine Talinolol 100 mg Retardtablette
	Proband 1 und 2 : zwei Talinolol 100 mg Retardtabletten
	(Einnahme jeweils mit 200 ml Mineralwasser)
9:30 Uhr:	Blutprobe (2.2)
anschl.	Standardfrühstück
10:30 Uhr	Blutprobe (2.3)
11:30 Uhr:	Blutprobe (2.4)
12:30 Uhr:	Blutprobe (2.5)
anschl.	Mittagessen
13:30 Uhr:	Blutprobe (2.6)
15:30 Uhr:	Blutprobe (2.7)

18:30 Uhr: Blutprobe (2.8)

18:45 Uhr: Verweilkatheter rausnehmen

anschl. Abendessen

21:30 Uhr: Blutprobe (2.9)

Am folgenden Tag:

8:30 Uhr: Blutprobe (2.10)

17:30 Uhr: Blutprobe (2.11)

Am übernächsten Tag:

8:30 Uhr: Blutprobe (2.12)

3. Prüfungsphase:

Vor Prüfungstag:

- in den letzten 24 Stunden vor Einnahme der Prüfmedikation kein anstrengender Sport
- in den letzten 24 Stunden vor Einnahme der Prüfmedikation kein Alkohol oder andere Medikamente
- ab dem Vortag der Einnahme der Prüfmedikation 22 Uhr kein Essen mehr

Am Prüfungstag:

- nur standardisiertes Essen und Getränke zu sich nehmen!
- keine anstrengenden Tätigkeiten
- über den Tag verteilt mindestens 1,4 Liter Flüssigkeit (Mineralwasser, Orangensaft oder Cola) trinken

8:15 Uhr: Eintreffen im Prüfraum
Leerprobe Urin
Verweilkatheter legen
Leerprobe Blut (3.1)

8:30 Uhr: Einnahme der Prüfmedikation:
Proband 3 und 4 : eine Cordanum 100 mg Tablette

Proband 5 und 6 : eine Talinolol 100 mg Retardtablette
Proband 1 und 2 : zwei Talinolol 100 mg Retardtabletten
(Einnahme jeweils mit 200 ml Mineralwasser)

9:30 Uhr: Blutprobe (3.2)
anschl. Standardfrühstück

10:30 Uhr Blutprobe (3.3)
11:30 Uhr: Blutprobe (3.4)
12:30 Uhr: Blutprobe (3.5)
anschl. Mittagessen

13:30 Uhr: Blutprobe (3.6)
15:30 Uhr: Blutprobe (3.7)
18:30 Uhr: Blutprobe (3.8)
18:45 Uhr: Verweilkatheter rausnehmen
anschl. Abendessen

21:30 Uhr: Blutprobe (3.9)

Am folgenden Tag:
8:30 Uhr: Blutprobe (3.10)
17:30 Uhr: Blutprobe (3.11)

Am übernächsten Tag:
8:30 Uhr: Blutprobe (3.12)

- insgesamt 36 Blutentnahmen a 7 ml
- Gesamtblutentnahme 252 ml innerhalb von >/= 3 Wochen

Die Compliance wird durch den späteren Nachweis des Arzneistoffs im Blut belegt.

Unerwünschte Ereignisse, Abbruchkriterien:

Sollten wider Erwarten schwerwiegende unerwünschte Wirkungen oder Komplikationen auftreten, werden diese der Ethik-Kommission gemeldet. Sollten diese ursächlich auf die Studienteilnahme zurückzuführen sein, werden die betroffenen Probanden von der Studie ausgeschlossen. Sollten bei mehr als 2 der 7 Probanden schwerwiegende Komplikationen auftreten, wird die gesamte Studie abgebrochen.

Zu dokumentieren sind Angaben zu Art, Zeitpunkt des Auftretens und Dauer des unerwünschten Ereignisses, Intensität, Häufigkeit, die getroffene Maßnahme, der Zusammenhang mit der Medikation, der Schweregrad und ob das Symptom bereits bei Beginn der Untersuchung bekannt war.

Sollten ernste Komplikationen auftreten, erhält der Proband eine Therapie durch einen Spezialisten. In solchen Fällen ist die Ethik-Kommission darüber unverzüglich zu berichten. Sollte dieses Ereignis im Zusammenhang mit der Versuchsdurchführung stehen, ist der Prüfarzt verpflichtet, den Probanden sofort aus der Studie auszuschließen. Behandlung und eventuell notwendige Laboruntersuchungen erfolgen nach Ermessen des Prüfarztes.

Interim Analysen:

Eine Zwischenauswertung ist nicht vorgesehen.

Es handelt sich um eine offene Studie mit sechs Probanden in drei parallelen Gruppen und einem Cross over-Design.

6. Ethische und rechtliche Belange

Rechtliche Grundlagen:

Bei der Durchführung der Studie sind neben der Deklaration von Helsinki folgende Richtlinien und Gesetze zu beachten:

- Grundsätze für die ordnungsgemäße Durchführung der klinischen Prüfung von Arzneimitteln (Bundesanzeiger Nr. 243 vom 30.12.1987 S. 16617 ff
- ”Note for Guidance on Good Clinical Practice” (GCP) der ICH, gültig ab 17.1.1997; Internet source: http://www.eudra.org/emea.html

- Bekanntmachung der Neufassung des Arzneimittelgesetzes (AMG) 19. Oktober 1994. Bundesgesetzblatt, Teil I, 1994:3018-67), geändert durch das 8. Gesetz zur Änderung des AMG vom 7.9.1998

Die Studie wird nach erfolgtem Votum der Ethikkommission bei der Bundesoberbehörde (BfArM in Bonn), sowie der zuständigen Landesbehörde (Landesamt für Soziales, Jugend und Versorgung in Trier) angemeldet.

Votum der Ethik-Kommission:

Voraussetzung der klinischen Prüfung ist die Einhaltung der oben aufgeführten Richtlinien und Gesetze. Der Beginn und die Durchführung der Studie sind an das Votum der Ethik-Kommission gebunden. Im Falle des Auftretens schwerwiegender Ereignisse , bei einem Studienabbruch sowie bei Prüfplanänderungen wird die Ethik-Kommission umgehend informiert.

Versicherung:

Eine Probandenversicherung gemäß AMG ist abgeschlossen, ein Nachweis darüber befindet sich im Anhang.
Die allgemeinen Versicherungsbedingungen werden den Probanden erläutert und auf Wunsch zugänglich gemacht.

Leiter der klinischen Prüfung / des Forschungsvorhabens:

Der Leiter der klinischen Prüfung nach AMG bestätigt durch Unterschrift des Prüfplans, dass er ein approbierter Arzt mit mindestens zweijähriger Erfahrung in der Durchführung klinischer Studien ist. Die Mitteilung der ‘Arzneimittelkommission der deutschen Ärzteschaft - Anforderungsprofil des Leiters der Klinischen Prüfung’ ist zu beachten (Deutsches Ärzteblatt 94, Heft 41 vom 10.10.1997, S. C 2012).
Der Leiter der klinischen Prüfung kontrolliert den ordnungsgemäßen Verlauf des Projekts. Er muss während der gesamten Prüfdauer erreichbar sein.

Prüfarzt:

Durch Unterzeichnung des Prüfplans bestätigt der Prüfarzt, dass er den Prüfplan gelesen und verstanden hat sowie gemäß Prüfplan arbeiten wird.
Der Prüfarzt gewährleistet die Vertraulichkeit aller Informationen.

Archivierung und Datenschutz:

Alle im Rahmen der klinischen Prüfung anfallenden Daten unterliegen dem Datenschutz. Personenbezogene Daten (außer Geburtsdatum, Alter, Geschlecht) werden vom Leiter der klinischen Prüfung bzw. dem Prüfarzt nicht weitergegeben. Die Weitergabe von Prüfbögen und die Datenspeicherung zur Auswertung erfolgt nur mit anonymisierten Daten des Probanden. Eine Zuordnung der persönlichen Daten zu den Studien-Daten darf nur der Prüfarzt vornehmen. Nach Abschluss der Studie werden die Prüfungsunterlagen gemäß den Bestimmungen des Datenschutzes bzw. des AMG verwaltet.

7. Unterschriften

_____

LKP

_____

Prüfarzt

_____

Initiator

8. Literatur

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Removed in the ArchiMed version for privacy reasons.

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Talinolol conc.						
0,9 mg/100ml	Rat	1	2	3	4	**mean of 1-4**
	Jejunum	-0.00002	0.00001	-0.00009	-0.00007	-0.42
	+ rho 123	-0.00002	-0.00001	-0.00008	-0.00005	-0.43
	Ileum	0.00000	-0.00003	-0.00008	-0.00009	-0.50
	+ rho 123	-0.00002	0.00000	-0.00012	-0.00011	-0.63
	Colon	0.00005	0.00010	-0.00019	-0.00018	-0.57
	+ rho 123	-0.00005	0.00006	-0.00020	-0.00013	-0.77
1,8 mg/100ml	Rat	5	6	7	8	**mean of 5-8**
	Jejunum	0.00002	-0.00007	0.00004	-0.00003	-0.10
	+ rho 123	-0.00001	-0.00001	0.00003	0.00002	0.07
	Ileum	0.00004	0.00003	0.00008	-0.00001	0.34
	+ rho 123	0.00000	0.00004	0.00006	0.00002	0.27
	Colon	-0.00006	0.00008	0.00019	0.00004	0.62
	+ rho 123	-0.00007	0.00007	0.00013	0.00008	0.52
3,6 mg/100ml	Rat	9	10	11	12	**mean of 9-12**
	Jejunum	0.00007	-0.00003	0.00003	0.00005	0.31
	+ rho 123	0.00007	0.00000	0.00006	0.00001	0.35
	Ileum	0.00007	-0.00005	0.00011	0.00016	0.74
	+ rho 123	0.00005	-0.00008	0.00014	0.00007	0.44
	Colon	0.00004	-0.00007	0.00014	0.00023	0.83
	+ rho 123	-0.00006	0.00001	0.00014	0.00018	0.66

Original data of rat perfusion study with different talinolol concentrations and rhodamine 123 (rho 123) as P-gp inhibitor (cp. Chapter II).

Talinolol conc.						
0,9 mg/100ml	Rat.	1	2	3	4	**mean of 1-4**
	Jejunum	-0.00010	-0.00001	0.00001	-0.00005	-0.37
	+ vinblastine	-0.00008	0.00007	-0.00001	0.00000	-0.03
	Ileum	-0.00015	-0.00003	0.00000	-0.00010	-0.70
	+ vinblastine	-0.00010	0.00008	0.00013	-0.00004	0.18
	Colon	-0.00027	-0.00004	0.00006	-0.00010	-0.85
	+ vinblastine	-0.00017	0.00010	0.00016	-0.00005	0.11
1,8 mg/100ml	Rat	5	6	7	8	**mean of 5-8**
	Jejunum	0.00008	-0.00006	0.00004	0.00004	0.25
	+ vinblastine	0.00004	0.00000	0.00005	0.00005	0.33
	Ileum	0.00005	0.00002	0.00002	0.00002	0.29
	+ vinblastine	0.00005	0.00003	0.00003	0.00003	0.33
	Colon	0.00004	0.00002	0.00007	-0.00009	0.10
	+ vinblastine	0.00003	0.00003	0.00003	0.00002	0.28
3,6 mg/100ml	Rat	9	10	11	12	**mean of 9-12**
	Jejunum	0.00011	0.00000	0.00004	-0.00003	0.33
	+ vinblastine	0.00004	0.00002	0.00005	0.00004	0.36
	Ileum	0.00009	0.00000	0.00007	-0.00004	0.30
	+ vinblastine	0.00007	0.00005	0.00000	0.00003	0.38
	Colon	0.00007	0.00010	0.00001	0.00000	0.47
	+ vinblastine	0.00004	0.00005	0.00005	0.00004	0.45

Original data of rat perfusion study with different talinolol concentrations and vinblastine as P-gp inhibitor (cp. Chapter II).

Talinolol conc.						
9,1 mg/100ml	Rat	13	14	15	16	**mean of 13-16**
	Jejunum	0.00006	0.00002	0.00004	0.00005	0.42
	+ vinblastine	0.00002	0.00002	0.00002	0.00008	0.36
	Ileum	0.00008	0.00003	0.00002	0.00011	0.60
	+ vinblastine	0.00005	0.00002	0.00005	0.00007	0.46
	Colon	0.00008	0.00011	0.00001	0.00013	0.82
	+ vinblastine	0.00008	0.00005	0.00000	0.00016	0.74
18,2 mg/100ml	Rat	17	18	19	20	**mean of 17-20**
	Jejunum	0.00006	0.00002	0.00003	0.00001	0.30
	+ vinblastine	0.00005	0.00003	0.00005	0.00002	0.38
	Ileum	0.00009	0.00012	0.00000	0.00005	0.64
	+ vinblastine	0.00002	0.00011	0.00002	0.00008	0.58
	Colon	0.00008	0.00014	0.00005	0.00007	0.82
	+ vinblastine	0.00004	0.00016	0.00004	0.00005	0.72

Original data of rat perfusion study with different talinolol concentrations and vinblastine as P-gp inhibitor (cp. Chapter II).

0.1 N HCl						
	absorption	dilution factor	conc. (mg/l)	drug released (%)	mean	S.D.
15min-1	0.2239	3	16.1	16.1	16.9	0.8
15min-2	0.2456	3	17.7	17.7		
15min-3	0.2362	3	17.0	17.0		
30min-1	0.2517	3	18.2	18.2	18.8	0.6
30min-2	0.2588	3	18.7	18.7		
30min-3	0.2687	3	19.4	19.4		
1h-1	0.2896	3	21.0	21.0	21.4	0.4
1h-2	0.2948	3	21.4	21.4		
1h-3	0.2995	3	21.7	21.7		
2h-1	0.3549	3	25.9	25.9	26.0	0.9
2h-2	0.3684	3	26.9	26.9		
2h-3	0.3458	3	25.2	25.2		
3h-1	0.391	3	28.6	28.6	28.3	0.3
3h-2	0.388	3	28.4	28.4		
3h-3	0.3821	3	27.9	27.9		
4h-1	0.43	3	31.5	31.5	32.0	0.5
4h-2	0.4421	3	32.4	32.4		
4h-3	0.4358	3	32.0	32.0		
6h-1	0.5372	3	39.6	39.6	40.1	0.5
6h-2	0.5487	3	40.4	40.4		
6h-3	0.5478	3	40.4	40.4		
8h-1	0.666	3	49.2	49.2	48.9	0.6
8h-2	0.6532	3	48.3	48.3		
8h-3	0.6674	3	49.3	49.3		
24h-1	0.3286	11	89.6	89.6	89.7	0.9
24h-2	0.3324	11	90.7	90.7		
24h-3	0.3257	11	88.8	88.8		

Drug release from the final talinolol sustained-release formulation in 0.1 N HCl (cp. Chapter III).

Acetate buffer pH 4.5						
	absorption	dilution factor	conc. (mg/l)	drug released (%)	mean	S.D.
15min-1	0.1909	3	13.6	13.6	12.9	0.6
15min-2	0.1802	3	12.8	12.8		
15min-3	0.1747	3	12.4	12.4		
30min-1	0.2726	3	19.7	19.7	19.4	0.3
30min-2	0.2684	3	19.4	19.4		
30min-3	0.2642	3	19.1	19.1		
1h-1	0.3722	3	27.2	27.2	26.5	1.1
1h-2	0.3698	3	27.0	27.0		
1h-3	0.3457	3	25.2	25.2		
2h-1	0.5448	3	40.1	40.1	39.4	1.6
2h-2	0.5102	3	37.5	37.5		
2h-3	0.5487	3	40.4	40.4		
3h-1	0.6388	3	47.2	47.2	48.2	1.5
3h-2	0.6428	3	47.5	47.5		
3h-3	0.6748	3	49.9	49.9		
4h-1	0.8093	3	60.0	60.0	61.0	0.9
4h-2	0.8325	3	61.7	61.7		
4h-3	0.8259	3	61.2	61.2		
6h-1	1.079	3	80.2	80.2	79.8	5.5
6h-2	1.1452	3	85.2	85.2		
6h-3	0.9987	3	74.2	74.2		
8h-1	0.3151	11	85.9	85.9	87.9	1.7
8h-2	0.3257	11	88.8	88.8		
8h-3	0.3263	11	89.0	89.0		
24h-1	0.3409	11	93.0	93.0	93.9	1.9
24h-2	0.3521	11	96.1	96.1		
24h-3	0.3392	11	92.6	92.6		

Drug release from the final talinolol sustained-release formulation in acetate buffer of pH 4.5 (cp. Chapter III).

Phosphate buffer pH 6.8						
	absorption	dilution factor	conc. (mg/l)	drug released (%)	Mean	S:D:
15min-1	0.0636	3	4.0	4.0	6.6	2.4
15min-2	0.1258	3	8.7	8.7		
15min-3	0.1039	3	7.1	7.1		
30min-1	0.1328	3	9.2	9.2	10.3	1.2
30min-2	0.1452	3	10.2	10.2		
30min-3	0.1633	3	11.5	11.5		
1h-1	0.2878	3	20.9	20.9	20.8	0.8
1h-2	0.2963	3	21.5	21.5		
1h-3	0.2755	3	19.9	19.9		
2h-1	0.5759	3	42.5	42.5	42.0	0.5
2h-2	0.5632	3	41.5	41.5		
2h-3	0.568	3	41.9	41.9		
3h-1	0.7893	3	58.5	58.5	58.2	1.2
3h-2	0.7695	3	57.0	57.0		
3h-3	0.7999	3	59.3	59.3		
4h-1	0.9873	3	73.3	73.3	72.6	0.7
4h-2	0.9758	3	72.5	72.5		
4h-3	0.9691	3	72.0	72.0		
6h-1	0.3256	11	88.8	88.8	91.0	2.9
6h-2	0.3301	11	90.0	90.0		
6h-3	0.3454	11	94.3	94.3		
8h-1	0.3602	11	98.3	98.3	98.7	2.0
8h-2	0.355	11	96.9	96.9		
8h-3	0.3695	11	100.9	100.9		
24h-1	0.376	11	102.7	102.7	103.0	2.7
24h-2	0.3874	11	105.8	105.8		
24h-3	0.3677	11	100.4	100.4		

Drug release from the final talinolol sustained-release formulation in phosphate buffer of pH 6.8 (cp. Chapter III).

List of publications, lectures and poster presentations

D. Wagner, H. Spahn-Langguth, A. Hanafy, A. Koggel, P. Langguth (2001):
Intestinal drug efflux: formulation and food effects.
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D. Wagner (2002):
Talinolol: Ursachen variabler intestinaler Absorption und Bioverfügbarkeit
Lecture at the Meeting of the Boehringer Ingelheim Advisory Board, Heiligkreuzthal, February, 28., 2002.

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Nonlinear Model for intestinal absorption of actively and passively transported drugs using talinolol as model compound.
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March, 23. - 25., 2001
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Poster at the Molecular Biopharmaceutics Meeting of the Drug Delivery Foundation, Honolulu, January, 22. - 24., 2003.

Curriculum Vitae - Daniel Wagner

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