PERFORMANCE TESTING OF MEDICATED CHEWING GUMS WITH THE GOAL OF ESTABLISHING IN VITRO IN VIVO CORRELATION

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DECLARATION

I hereby declare that the dissertation titled “Performance testing of medicated chewing gums with the goal of establishing *in vitro - in vivo* correlation” is the result of my own research work and includes nothing which is the outcome of work done in collaboration. Where other sources of information have been used, they have been acknowledged. No portion of work contained in this dissertation has been submitted in support of any application for any other degree or qualification.

Jayachandar Gajendran

Dedicated to My Family

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LIST OF ABBREVIATIONS

API	Active pharmaceutical ingredient
AAPS	American association of pharmaceutical scientists
AUC	Area under the curve
AUMC	Area under first moment curve
BA	Bioavailability
BCS	Biopharmaceutics classification system
BE	Bioequivalence
C_{max}	Concentration maximum
CMC	Chemistry manufacturing and control
CPMP	Committee for proprietary medicinal products
DC	Directly compressible
DCM	Dichloromethane
DE	Dissolution efficiency
DoE	Design of experiments
DRC	Drug resin complex
EMA	European medicines evaluation agency
ER	Extended release
FDA	Food and drug administration
FIP	International pharmaceutical federation
FRA	Fraction dose absorbed
FRD	Fraction dose dissolved
FTIR	Fourier-transform infra-red
GIT	Gastrointestinal tract
GMP	Good manufacturing practice
GRAS	Generally recognized as safe
ICH	International congress on harmonization
IER	Ion exchange resin
IR	Immediate release
IUPAC	International union of pure and applied chemistry
IV	Intravenous
IVIVC	*In vitro -in vivo* correlation
JP	Japanese pharmacopeia
K_a	Absorption rate constant
K_{el}	Elimination rate constant
KN	Kilo newton
LC	Label claim

MA	Marketing authorization
MAT	Mean absorption time
MCG	Medicated chewing gum
MDT	Mean dissolution time
mg	Milligram
min	Minute(s)
µg	Microgram
mL	Milliliter
MRT	Mean residence time
MW	Molecular weight
n/a	Not applicable
ng	Nanogram
N	Newton
NRT	Nicotine replacement therapy
OTC	Over the counter
o/w	Oil-in-water
Ph.Eur	European Pharmacopeia
QC	Quality control
RSD	Relative standard deviation
SD	Standard deviation
SEM	Scanning electron microscope
USP	United States Pharmacopeia
w/o	Water-in -oil

1 INTRODUCTION

1.1 Introduction to buccal drug delivery

Per-oral drug administration has been known for centuries as the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of various pharmaceutical products. Solid dosage forms are popular because of the ease of administration, accurate dosage, self-medication and most importantly the patient compliance (Sreenivas, *et al.* 2005) compared to the parenteral and other drug delivery systems known so far. However the main drawback of this conventional dosage form is the difficulty in swallowing when water is not available and also the bioavailability (BA) concerns due to an extensive first pass metabolism. Based on these reasons, the drug delivery in the oral cavity attracted a great deal of attention. The oropharyngeal/buccal delivery system emerged as an effective alternative drug delivery system.

The need to find a unique drug delivery system compliant to every consumer need without much exploitation is still a dream to the pharmaceutical scientists. With a growing demand to fulfill the consumer needs on the safety and efficacy of the product, great deal of efforts has been spent to develop such a delivery system with a minimal patient incompliance. One such promising system utilizes chewing gum as a platform for the drug delivery to the systemic and local sites. Chewing gums are gaining their importance as an effective carrier/vehicle for the drug delivery at the local and systemic sites due to their “high patient compliance” irrespective of age and gender (Rassing 1994).

Among some of the well-known transmucosal routes that include nasal, sublingual, buccal, rectal, vaginal and ocular, the buccal route is quite optimistic due to its robust nature, relatively fast recovery of oral mucosa after exposure to high drug concentrations and suitability for the sustained drug release (Jacques, *et al.* 1997, Squier, *et al.* 1978, Streisand, *et al.* 1995). Chewing gums are pertinent to such a drug delivery within the buccal cavity.

The consideration of chewing gums as a valuable alternative to the peroral and other conventional routes of drug delivery was not realized until first chewing gums containing nicotine marketed in 1978, though the first patent over the medicated chewing gums (MCG) containing acetyl salicylic acid was issued in United States in 1928.

In 2005, the USP founded the “Mucosal Drug Delivery Advisory Panel” to address the key issues concerning the oropharyngeal drug delivery systems. The major goal of this advisory panel is to provide the key information and awareness of the oro-pharyngeal drug delivery system, organization and call for collaborative trials for comparative and performance studies, standardization and harmonization of the apparatus design for the drug release testing, its

incorporation in the forthcoming pharmacopoeias and the development of monographs for the new and existing marketed products. The consideration of chewing gum as a vehicle for alternative drug delivery, their advantages, disadvantages, regulatory and quality assurance issues are discussed thoroughly in the following sections.

1.1.1 Oral transmucosal drug delivery

Amongst the various routes of drug delivery, per-oral routes are the most preferred by patients and physicians. However, for some drugs, an extensive hepatic clearance and enzymatic degradation in the stomach and small intestine results in poor BA. An alternative drug delivery within the oral cavity would be desirable, and in most instances possess a higher order of patient compliance. Within the oral cavity, the oral mucosa remains a potential site for administration of those drugs which suffer the above said problems.

The oral transmucosal drug delivery can be broadly classified based upon the site of administration (Shojaei 1998); 1) sublingual delivery, which is systemic delivery of drugs through the mucosal membranes lining the floor of the mouth, 2) buccal delivery, which is drug administration through the mucosal membranes lining the cheeks (buccal mucosa) and 3) local delivery, which is drug delivery into the oral cavity.

Drug absorption across the oral mucosa is more rapid because of the rich vascular supply to the mucosa and the lack of a stratum corneum epidermis which is a huge barrier to the transdermal applications. In order to obtain a significant absorption of drug to the systemic circulation, a prolonged exposure of the drug on the mucosal surface is necessary which delimits sublingual and the local drug delivery routes within the oral cavity. The buccal route serves as a potential site for the drug delivery particularly when a sustained drug release is desired together with the higher patient compliance (Shojaei 1998).

1.1.1.1 Oral cavity and buccal mucosa

The mucosal lining of oral cavity has different functions and based on the functional demands within the oral cavity (mastication) they do differ in their structure. The masticatory mucosa that covers gingiva and hard palate are generally subjected to masticatory forces resulting in abrasion and shearing. The mucosal lining of the buccal cavity are elastic to allow for distension. The oral mucosa is composed of an outer layer of stratified squamous epithelium covered with a thin layer of mucus and saliva, followed by lamina propria as a basement membrane and submucosa as the innermost layer (Gandhi, *et al.* 1988). The oral mucosal thickness and the composition of the epithelium vary depending on the site. The epithelium of the buccal mucosa is about 40-50 cell layers thick, which reduces in number with the sublingual epithelium. The buccal mucosa measures 500-800 µm (Harris, *et al.* 1992).

The mucosa of the buccal region is not keratinized (Harris, *et al.* 1992). The degree of keratinization is generally considered as a main barrier for drug absorption due to the presence of neutral lipids like ceramides and acylceramides (Squier, *et al.* 1991, Squier, *et al.* 1996, Wertz, *et al.* 1991). The buccal epithelia contains small amounts of ceramide and neutral polar lipids, mainly cholesterol sulfate and glucosyl ceramides. These epithelia have been found to be considerably more permeable to water than keratinized epithelia (Harris, *et al.* 1992, Squier, *et al.* 1991, Wertz, *et al.* 1991).

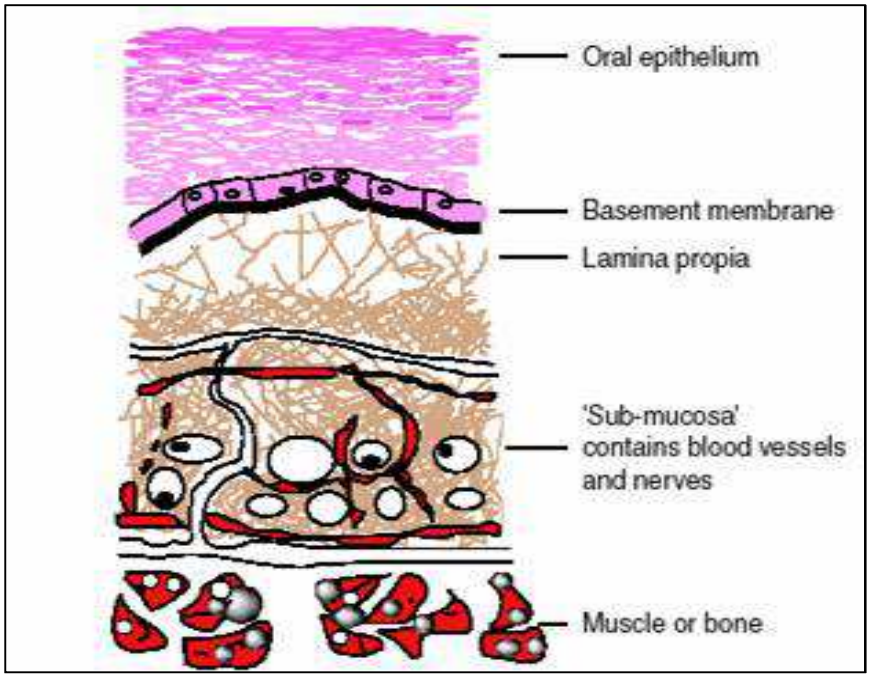


Figure 1-1. Cross section through oral mucosa
Taken from Shojaei 1998

The permeability of buccal mucosa is 4-4000 times greater than that of the skin. The permeation of the drug across the buccal mucosa is influenced by the passive transport which necessarily follow one of the two proposed or both pathways, paracellular and transcellular routes. The selectivity for one among the route depends on the physicochemical properties of the drug substances, namely hydrophilicity and lipophilicity of the drug substance.

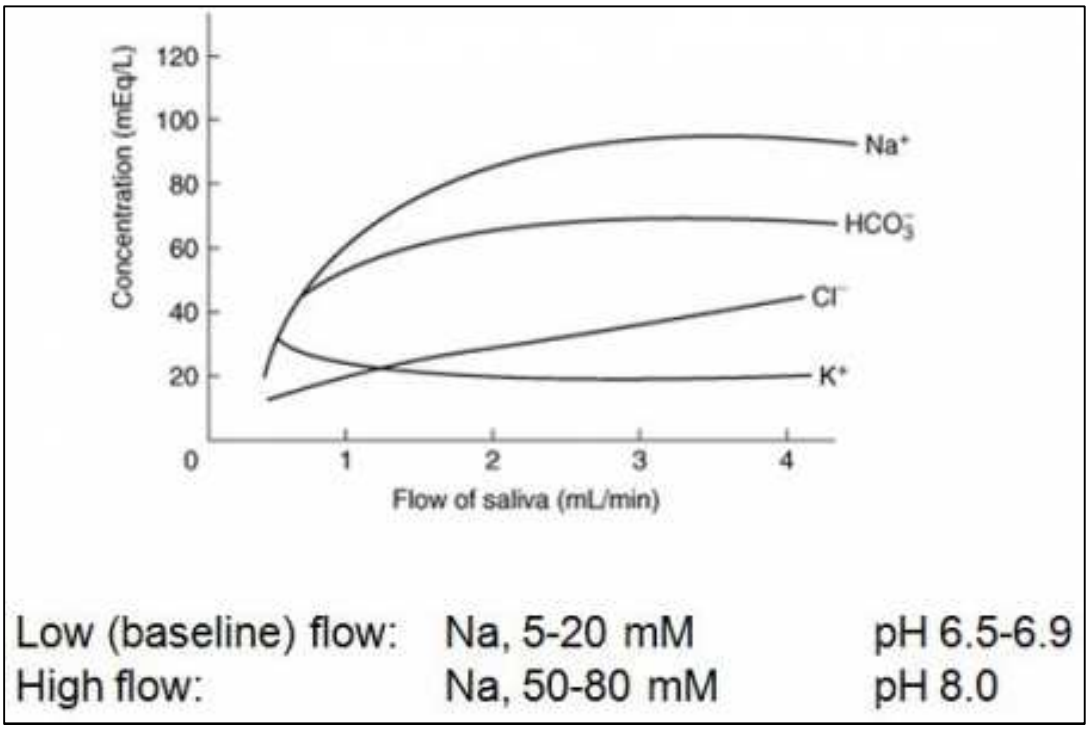


Figure 1-2. Flow of saliva (mL/min)

Taken from Humphrey, et al. 2001. Figure showing salivary composition at different flow rates. The change in the concentration of ions, particularly Na+ with respect to flow rate is very distinct. The information may be useful in determining the suitable concentration of ions in a biorelevant media used during the dissolution testing.

The oral mucosa is richly supplied with the saliva by three major salivary glands, parotid, submandibular and sublingual glands. Numerous other minor salivary glands are also present throughout the oral cavity (Humphrey, *et al.* 2001). Saliva is colorless, viscous and hypotonic (110-220 mOsm/L) liquid primarily composed of water, mucin proteins, mineral salts and amylase (Schenkels, *et al.* 1995). The major ions include sodium, potassium, chlorides and bicarbonates, with a specific gravity of about 1.003 and the pH varies between 7.4 to 6.2 depending on the flow rate and age. Bacterial infections in the oral cavity can reduce the pH to around 3 and 4 (Humphrey, *et al.* 2001). The drug release from the chewable dosage forms is significantly affected by the flow and pH conditions of the oral cavity.

1.2 Drug delivery within the oral cavity

The sublingual mucosa is relatively more permeable to solutes resulting in rapid absorption and onset of action and acceptable bioavailabilities of many drugs. It is also convenient, accessible, and generally well accepted (Harris, *et al.* 1992) for drug delivery. Such systems create a very high drug concentration in the sublingual region before they are systemically absorbed across the mucosa. Comparatively the buccal mucosa is considerably less permeable with poor absorption which accounts for the reduced bioavailabilities. However, the sublingual mucosa is not suitable for a sustained oral transmucosal drug delivery due to the immobile mucosa which is constantly washed by a considerable amount of saliva, hence only appropriate for those drugs with short delivery period requirements with infrequent dosing regimens (Shojaei 1998).

The delayed onset of absorption due to the poor permeability characteristics of the buccal mucosa is quite favored for the sustained release formulations. Similar to any other transmucosal membrane, the buccal mucosa has its limitations as well. One of the major disadvantages associated with buccal drug delivery is the low flux which results in low drug bioavailability. Polar compounds which could easily be ionized at the pH of the mouth are poorly absorbed. In order to be absorbed through the oral mucosa, the active pharmaceutical ingredients (API) released from the dosage forms should be dissolved in the saliva which is also partially swallowed and exposed to the gastric environment. Specialized delivery systems are essential to improve the time of contact of released drug with the oral mucosa to obtain significant blood plasma concentrations. Various compounds have been investigated for their use as buccal penetration enhancers in order to increase the flux of drugs through the mucosa and reviewed thoroughly (Shojaei 1998). Since the buccal epithelium is similar in structure to other stratified epithelia of the body, enhancers used to improve drug permeation in other absorptive mucosae have been shown to work in improving buccal drug penetration (Aungst, *et al.* 1989).

1.2.1 Factors affecting drug absorption

A multitude of foreign objects (foods, liquids, smoke, drugs etc.) comes regularly in contact with oral mucous membranes. A little is known concerning the effects of these substances on oral tissues. Considerable attention has been focused on absorption as it relates to the lower portions of the gastrointestinal tract. But only a less information has been acquired concerning factors that may affect absorption in the oral cavity. In 1900, Overton (Overton 1900) reported that the cell membrane is lipoidal in nature and a variety of organic compounds penetrate cells at rates proportional to their lipid-water partition coefficient. His work laid the foundation for the pH partition hypothesis of drug absorption. Although the lipid solubility of drugs was shown to be an important factor in their absorption through oral mucous membranes, this factor failed to explain how small, lipid-insoluble molecules could penetrate

cell membranes. The authors (Collander, *et al.* 1933) concluded that the cell membrane is a lipid mosaic which contained tiny holes or pores. Nonpolar substances could penetrate this membrane by dissolving in the fat like phase while polar molecules diffuse through the pores if they were small enough. However, the conclusion of Collander and Barlund (Collander, *et al.* 1933) failed to explain the rapid transfer of large lipid-insoluble molecules into cells. Höber 1936 termed this rapid transfer as a physiological permeability. Recent studies concerning the mechanism of gastrointestinal absorption have focused on the processes of active transport and facilitated diffusion rather than on passive diffusion. Briefly, the active transport refers to the passage of a solute across a cell membrane by means of a complex formed with some membrane component or carrier. The complex is formed on one side of the membrane, and then split apart on the other side of the membrane. The carrier then returns to the membrane surface to complex with another molecule. In facilitated diffusion, the solute is not transferred against a concentration gradient. Most of the reports dealing with active transport describe characteristics which distinguish this specialized process from simple diffusion, although specific models of this mechanism have been proposed. As mentioned earlier, only a small amount of information concerning absorption or factors affecting absorption has been obtained from experiments employing oral mucous membranes. Furthermore, the author (Walton 1935, Walton, *et al.* 1977) compared the effectiveness of sublingual doses to subcutaneous doses for a number of drugs administered to dogs and human beings. He concluded from these experiments that drugs that are poorly absorbed sublingually have relatively low oil to water partition coefficients, whereas drugs that are well absorbed have relatively high coefficients. An oil-water (o/w) partition coefficient range of 40-2000 is considered optimal for the drugs to be absorbed sublingually. Thus oil-water distribution coefficient is an important factor in the selective oral absorption of these drugs. Walton also pointed out that relative potency, degree of local vasoconstriction, irritation, and alkalinity or acidity may be significant factors. Additionally Beckett, *et al.* 1967 studied the passive transfer of drugs through buccal membranes.

1.2.1.1 Oral absorption vs. subsequent absorption in the GI tract

For chewable oral dosage forms, the drug release usually begins in the oral cavity and subsequently absorbed through the buccal mucosa to enter into the systemic circulation. Due to the rich vascular supply of the buccal mucosa, adequate concentrations in blood may be reached within minutes of release (Beckett, *et al.* 1967, Gibaldi, *et al.* 1965, Rathbone, *et al.* 1991). Partly, the drug contained in the saliva is swallowed and exposed to the gastric contents, absorbed along the GI tract and metabolized before being available in systemic circulation. As most of the chewable oral products are designed to deliver actives more rapidly in the oral cavity, immediate onset of action is observed for many drugs that are well absorbed through the oral mucosa. Since part of the released drug is also absorbed along the GI tract, in most cases the BA of the drug substance is significantly higher than the solid oral

dosage forms that are poorly absorbed along the GI tract and are subjected to first pass metabolism.

Furthermore, the rate limiting factors for absorption like disintegration process are not involved. The mechanism of drug release of these dosage forms is discussed separately in the following sections. The purpose of formulation of chewable oral dosage forms is to deliver actives within the oral cavity for a local action and/ or for the systemic effect. In the former case, the drug release rate is usually sustained providing adequate concentration within the oral cavity. For example, chewing gums containing chlorhexidine are used to treat against dental caries or chewing gums to reduce the post-operative ileus period after GI surgery have been found to have profound effect (Fitzgerald, *et al.* 2009). Many literatures on the marketed chewable oral products is available and are listed by authors (Aurora, *et al.* 2005, Bandari, *et al.* 2008, Banner, Konapure, *et al.* 2011, Pahwa, *et al.* 2010).

1.2.2 Factors affecting release of active ingredient(s)

1.2.2.1 Contact time

The local or systemic effect is dependent on time of contact of MCG in the oral cavity. In clinical trials chewing time of 30 minutes was considered close to ordinary use (Rassing 1994). An increase in the time of contact between the MCG and the saliva helps in a better interaction. As the drug release from MCG is dependent on mastication (kneading and chewing), penetration of saliva into the gum matrix is necessary for the diffusion of API(s) and subsequent release.

1.2.2.2 Physicochemical properties of active ingredient

A physicochemical property of active ingredient plays a very important role in release of drug from MCG. The saliva soluble ingredients will be immediately released within a few minutes whereas lipid soluble drugs are released first into the gum base and then released slowly (Rassing 1994). The gum matrix in general is lipophilic in nature. The degree of lipophilicity depends on the amount present in a formulation. Therefore, hydrophilic active(s) and excipients are released much faster than the lipophilic components.

1.2.2.3 Inter individual variability

The chewing frequency and chewing intensity which affect the drug release from MCG may vary from person to person (inter-individual variability). The release of API from the MCGs is voluntarily controlled by the chewing rate and force applied. Therefore the variability associated with the release *in vivo* is reflected in the plasma concentration-time profiles. In order to obtain reproducible results *in vitro* from the MCGs, the European Pharmacopoeia (Ph.Eur.) recommends an in-vitro release testing methodology with 60 cycles/minute as a chewing rate for proper release of active ingredient(s) (Rassing 1994).

1.2.2.4 Formulation factors

Composition and amount of gumbase also affects the release rate of active ingredient(s). The gumbase (for e.g., Styrene-butadiene or poly-isobutylene) is generally lipophilic in nature. An increase in the ratio of the gumbase to the other excipients will result in an increase in the release of a hydrophilic drug substance in the gum formulation or will reduce the release of a lipophilic drug substance. Several factors have been shown to affect release of drugs from chewing gums. The major determinants include the chewing time, chewing rate, aqueous solubility of the drugs, and the composition of the chewing gum (Christrup, *et al.* 1988, Faraj, *et al.* 2007, Jensen, *et al.* 1988, Na, *et al.* 2005, Pedersen, *et al.* 1990, 1991, Rassing 1994).

1.2.2.4.1 Chewing time and rate

A self-reporting questionnaire technique was developed to determine the length of chewing time. The mean chewing time per piece of gum was found to be 36 min. The rate at which a

subject chews the gum also affects the amount of drug released. (Christrup, *et al.* 1986, Rassing 1994,Bruun, *et al.* 1978, Kassem, *et al.* 1973, Lingstroem, *et al.* 2005, Yang, *et al.* 2004, Yoshii, *et al.* 2007).

1.2.2.4.2 Aqueous solubility

The release of water soluble drugs (aqueous solubility > 1:10) in general is about 75% or more during the first 5 min of chewing and 90% or more during 15 min of chewing at a rate of 60 chews/min (Andersen, *et al.* 1990). Drugs with aqueous solubility between 1:10 and 1:300 demonstrate up to 60% release during 10 min of chewing and between 50% and 90% when the gum is chewed for 15 min. For the poorly water soluble drugs, it is expected to be small (less than 5%) even if the gum is chewed for 30 min (Rassing 1994).

1.2.2.4.3 Composition of chewing gum

The influence of gumbase amount on drug release has been investigated using salicylamide as model drug. When salicylamide was incorporated into a chewing gum, which contained a relatively large percentage of gumbase, the release after 30 min of chewing was significantly lower (25.6%) compared to a gum in which less gumbase was present (52%). A nicotine containing chewing gum (Nicorette®, Leo) has been marketed as an aid to circumvent smoking (Roussel). When nicotine is incorporated into an ordinary gum composition, the release occurs rapidly. Such a release profile is however undesirable for its clinical use which requires that the release from a nicotine chewing gum formulated as smoking substitute should be uniform and last for at least 20 min. In addition, the released nicotine should produce a “feeling of smoking”. Lichtneckert, *et al.* 1975 described a method utilizing tobacco alkaloids bound to a cation exchanger containing functional carboxylic or sulfonic groups. This provided a desired release behavior of nicotine.

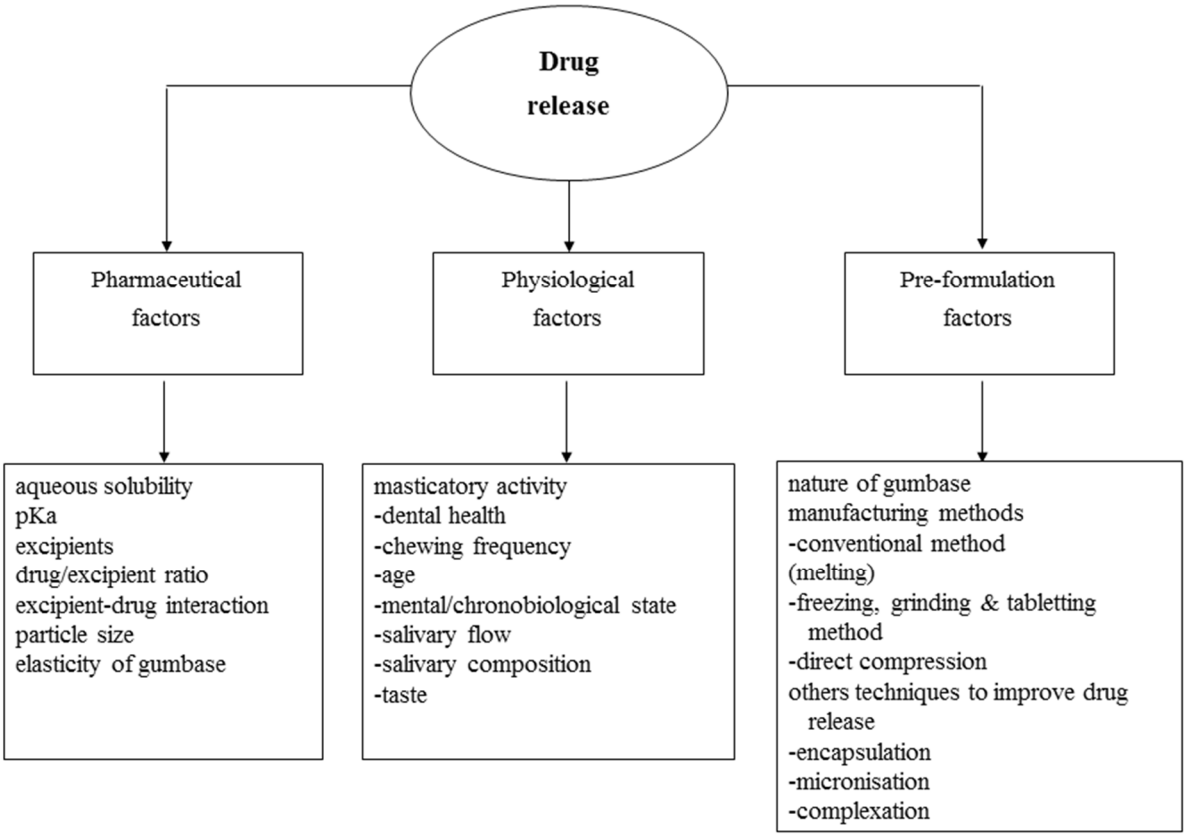


Figure 1-3. Summary of factors affecting drug release from chewing gum formulations

1.3 Dosage form taxonomy

The USP's dosage form taxonomical classification provides a clear understanding and application of dosage forms. The purpose is to systematically organize and categorize the pharmaceutical dosage forms (Brown, *et al.* 2011).

Medicinal drug products are administered in the body by one of the five routes of drug administration: oral, topical/dermal, mucosal, parenteral, and inhalations. For each route of the drug administration, two types of tests are proposed;

a) Product quality tests
b) Product performance test

The product quality tests include identity, strength, uniformity of dosage units, purity, etc., whereas a product performance test in most cases constitutes a drug release test, analogous to a dissolution test. The five routes of drug administration with their intended sites of drug release and examples are shown in the following Table 1-1.

Often the products with such new technologies (novel dosage forms) face difficulties to categorize themselves in the existing pharmaceutical dosage form taxonomy. In order to accommodate all the technological aspects of the dosage forms under one roof, the USP has initiated an ad-hoc advisory panel to revise its existing general chapters on pharmaceutical dosage forms. The intent of such categorization is to provide users an easy understanding and user friendly solution to link dosage forms with their corresponding monographs or to their therapeutic use. The current recommended system of categorization for pharmaceutical dosage forms is based on different tiers.

The first tier is according to the route of administration by which the drug substance is delivered to provide the therapeutic efficacy. These include all drug products delivered to the Gastro Intestinal (GI) tract, Topical or Mucosal route and so on.

The second tier is based on the general type of dosage form and physicochemical properties (solids, semisolids, liquids etc). Furthermore, the dosage forms are further grouped in one of many subsections in the second tier. For e.g., the solid dosage forms could be formulated as powders, beads, tablets, granules etc. The second tier facilitates downward expansion into the third tier.

The third tier is based on the type of release pattern of the drug substance and performance characteristics of the dosage form. With such a hierarchy, the dosage forms can be grouped and identified by their physical and chemical characteristics. Continuous efforts have been

made to harmonize the pharmaceutical dosage forms in the widely acceptable pharmacopeias such as United States Pharmacopeia (USP), European Pharmacopeia (Ph. Eur.) and the Japanese Pharmacopeia (JP). The schematic representation of the classification system adapted by the USP is given in Table 1-1.

Table 1-1 shows the taxonomical classification adopted by the USP to characterize dosage forms based on the route of delivery, physical state of the dosage form and the release pattern. MCGs are generally classified as solid or semi-solid preparations, primarily absorbed through mucosal membranes, buccal mucosa in particular. The drug release is either conventional or modified.

Table 1-1. Dosage form taxonomy

Route of administration	Intended site of release	Dosage form examples
Oral	Gastro-intestinal (GI)	Solid dosage forms Tablets, Capsules, Disintegrating tablets Oral/dispersible Chewables (tablets and gums) Liquid filled capsules Powders, Granules Solid solutions, dispersions Liquid dosage forms
Topical/dermal	Skin	Transdermal delivery system (patches) Semi-solid dosage forms Gels, Creams, lotions and ointments
Mucosal (Local or systemic)	Oral	Chewing gums Thin dissolvable films (wafers)
	Ophthalmic	Implants Liquids Suspensions
	Rectal	Suppositories
	Intrauterine	Devices
	Otic	Liquids
	Vaginal, Urethral	Suppositories Semi solids Thin dissolvable films (wafers)
Parenteral	Bodily tissues and fluids	Microparticulate systems Subcutaneous liposomes Intramuscular drug-eluting stents Implants
Inhalation	Nasal cavity	Aerosols (solutions and suspensions)
	Lung	Powders, Liquids

taken from Brown, et al. 2011.

1.3.1 Medicated chewing gum as pharmaceutical dosage form

Medicated chewing gums are solid or semi-solid pharmaceutical dosage forms and contain one or more active pharmaceutical ingredients (API) and water-soluble/insoluble excipients blended with a water insoluble gumbase (USP 2015). The drug product is intended to be chewed in the oral cavity for a specific period of time, after which the insoluble gumbase is discarded. MCGs are defined by the Ph. Eur.(Ph.Eur. 2014) and the guidelines for pharmaceutical dosage forms issued in 1991 by the Committee for Medicinal Products for Human Use (CPMP) as "solid single dose preparations with a base consisting mainly of gum that are intended to be chewed but not swallowed, providing a slow steady release of the medicine contained".

Table 1-2 (Gajendran, *et al.* 2008) shows the classification of dosage forms for transmucosal delivery and the recommendation of the associated safety tests which is a performance test likely required for the corresponding dosage form.

Table 1-2. Classification of dosage forms for transmucosal drug delivery and safety test requirements

Dosage form (Solid)	**Activation by patient needed**	**Safety test to be included**
Platelets	no	no
Lozenges	n/a	n/a
-melting	no	no
-dissolving	yes	yes
Soft gelatin capsules	yes	yes
Chewable tablet	yes	yes
Medicated gums	yes	yes
	no	no
adhesive tablet patch (buccal/sublingual)	no	yes

n/a – not applicable

1.4 Manufacturing process

The art of manufacturing of MCGs have come a long way since the introduction of gums containing acetyl salicylic acid (Rassing 1994). The chewing gums as a vehicle for drug delivery was recognized when nicotine containing gum were marketed worldwide and proved as an effective nicotine replacement therapy (NRT). Since then, several drug substances have been tested in gum formulations and the release characteristics were investigated. With undue advantages and recent developments in manufacturing, the gum components are tailored to customer requirements to deliver drug substances based on their physicochemical properties.

Many technologies have been investigated by the scientist to provide a simple, cost effective method to manufacture chewing gums without compromising the quality of the end product. Among the various techniques used, most common methodologies recognized are described briefly in the following section (Athanikar, *et al.* 2001, Chaudhary, *et al.* 2012, Cherukuri Subraman, *et al.* 1988, Mochizuki, *et al.* 1976, Pedersen, *et al.* 1990).

1.4.1 Conventional method (Extrusion)

This is the most common method employed currently, which comprises mixing of gumbase and other components in a mixer. Components of gumbase are softened / melted and placed in a kettle mixer to which sweeteners, syrups, active ingredients and other excipients are added at a definite time. Precisely, the gumbase is usually heated between 60°C and 70°C to a molten semi-fluid state during the mixing and blending process. The molten mixture is extruded to a definite geometry and cut to single units after cooling. The gums may be coated further to improve the organoleptic properties. Advantages of this method include homogeneity of the actives during the blending and mixing, and better control over the release characteristics. However, thermo-labile substances pose stability related problems during the melting process and the amount of moisture contained in the final product set limitations to widely utilize this method.

Another problem would be to control the mass and uniformity of the dosage unit as the API's are distributed in the semi-solid gum matrix. Knowledge on "know-how" of mixing and blending is crucial to attain the texture, uniformity of API(s) and other excipients in the matrix. Furthermore, the manufacturing technology is not easily adaptable to a conventional manufacturing pharmaceutical industry.

1.4.2 Direct compression method

This method involves the use of directly compressible (DC) gumbases. The DC gumbase is a pre-formulated mixture containing the gum base as a dry free flowing powder together with all the necessary excipients. The process comprises physical blending of the dry components of the formulation with the API until a homogenous mixture is obtained. In many cases, the

gumbases are pre-tailored with other excipients which are not limited to the flavors, sweeteners, softening agents etc. Gliding agents like magnesium stearate and talc may be added to improve the flow properties. The resulting final blend is usually compressed into a tablet and coated when required. The method is advantageous for drug substances that are hygroscopic and pose stability concerns with heat treatments. Often crumbling of the dosage form is observed during initial mastication of the gum, resulting in a burst release behavior (Morjaria, *et al.* 2004) and is difficult to establish homogeneity of the blend during mixing procedure.

By this method, the limitations of melting, blending and extruding can be avoided. The pre-tailored gumbase matrix is available from multi-source manufacturers that can be compacted into a gum tablet using conventional tableting machines available. It is manufactured under cGMP conditions and complies with Food Chemicals Codex (FCC) specifications as well as with FDA. Therefore they are classified by the FDA as "Generally regarded as safe" (GRAS).

One frequent problem often encountered with the application of DC gumbases is the stickiness due to the heat developed which is more observed particularly for large batches of manufacturing. Various methods including using of different lubricants and glidants were investigated to overcome this problem.

1.5 Quality evaluation of chewing gum formulations

The nature and type of performance tests used to characterize dosage forms administered to the oral cavity differ significantly and thus demand different release testing apparatus (Gajendran, *et al.* 2008). For many dosage forms given orally but not limited to mucoadhesive buccal tablets, chewable tablets, and sublingual preparations, product performance tests are adapted from existing procedures and are well characterized in the United States Pharmacopeia (USP). Unlike chewable tablets, medicated gums are not supposed to be swallowed and may be removed from the site of application without resort to invasive means. Moreover, medicated gums require an active and continuous masticatory activity for activation and continuation of drug release.

MCGs are dosage forms given orally for both local and systemic effect, and so far no performance test has been indicated in the USP (USP 2015).

1.5.1 Pharmacopeial description and requirements

In the Ph. Eur., a general monograph on medicated chewing gums (Ph.Eur. 2014) and a monograph describing the apparatus for dissolution testing of medicated chewing gums (Ph.Eur. 2014) has been adopted. The monograph on nicotine polacrilex gum was incorporated in the USP (USP 2015) in late 90's but the apparatus for the dissolution testing of medicated chewing gums is not yet recognized.

In Ph. Eur. medicated chewing gums have been separately categorized under solid dosage forms. However, medicated chewing gums could also be defined as both solid and semisolid preparations (Gajendran, *et al.* 2008) based on the art of manufacturing described earlier in this chapter. Table 1-3 shows the product quality tests associated with the chewing gum preparations described in Ph. Eur. in general and specifically for nicotine polacrilex resins and gums in the USP. In addition to the product quality tests, additional testing specific to the product may be performed to ensure the final quality of the finished product.

Table 1-3. Summary of product quality tests

Dosage Form	Nature/Physical characteristics	Patient Activation	Safety Test Requirement	Product Quality Tests
Medicated gums	Solid/semi-solid	Yes	Yes	Assay Identification Uniformity of dosage units, content, and mass

1.5.2 Rationale for *in vitro* release tests

In vitro performance testing of solid and semi-solid dosage forms has been widely recognized as one series of tests used in the routine quality control (QC) purposes and in the early phases of formulation selection and development. Various apparatuses for testing the performance of drug products *in vitro* have already been adapted and harmonized in the existing major pharmacopeias.

General monographs on apparatus and product quality tests have already been indicated for MCGs in the Ph. Eur (Ph.Eur. 2014), but no monograph specific to the product has been incorporated. This delineates the fact that the apparatus, performance tests and the accompanying quality control tests are not part of the public standard and their lack of commercial availability calls for an evaluation of the comparative performance of commercially available apparatuses in terms of their suitability, robustness, discriminative power and reproducibility in the GMP environment. This would eventually lead to the standardization and incorporation of an alternative drug release testing apparatus and development of monographs in the Ph. Eur.

In vitro dissolution/drug release testing of the dosage forms has been widely used in the pharmaceutical industry;

- in quality control (QC) to assess the differences in quality of the products within the technological range of manufacturing variables
- to assess the differences in the bioavailabilities, the surrogate parameter of the therapeutic efficacy
- to evaluate the robustness as a parameter of drug product related safety
- to identify the critical manufacturing variables in formulation development
- to estimate the *in vivo* performance of the dosage form

Additionally, the purpose of the *in vitro* drug release/dissolution testing is to provide an accurate estimate of drug release rates from a given drug product. Besides, the *in vitro* drug release/dissolution finds its application in the biopharmaceutical characterization of the dosage form from early phases of product development to the end product design and throughout the product's life cycle as a measure of performance and stability. It can also serve as a surrogate for a bioequivalence (BE) study and as a predictor of *in vivo* performance.

The development of a suitable and bio-relevant drug release testing method is a primary requirement to ensure discriminatory power of the method, which could be directly linked either to the chemistry, manufacturing and control (CMC) of the drug product or the biological parameter (C_{max}, T_{max}, AUC) of the product. It should aim to be sensitive enough to

detect differences in product formulation or manufacturing changes, and robust enough to detect such differences only when they are biologically relevant. The ultimate goal of the test is to generate information that can provide an insight into the mechanism by which the drug is being released from the dosage form and provide data to facilitate the rational and rapid research, optimization and development of a dosage form.

As far as the apparatus for the dissolution/drug release testing is concerned, the purpose is to simulate the relevant *in vivo* conditions as close as possible to *in vivo* physiology. By this way, the dissolution/drug release from a dosage form under a set of standardized set of conditions will be used to predict performance characteristics and physico-chemical integrity of the dosage unit.

This holds true for the MCGs as well. In case of gum products, the basic principle in release testing is a simple masticatory movement employed to simulate the chewing action on a piece of gum placed in a small chewing chamber containing a known volume of buffer solution at a given temperature (Ph.Eur. 2014). The drug release rate is influenced by the chewing rate and angle, which provides the necessary shear force to expose new gum surfaces and is a requisite for further drug release. The mechanism and kinetics of drug release from chewing gums have not yet been completely understood due to the complexity of the formulation itself. The transition from the inactive gum to the active dosage form is influenced by;

- Mechanical forces
- Temperature
- Wettability and water permeation rate

As a general rule, under sink conditions, the rate at which the drug is released is directly proportional to the chewing frequency and aqueous solubility of drug substance and is inversely proportional to the mass of the gumbase.

Currently, the USP monograph for nicotine polacrilex gums does not contain a drug release test. Much effort has been spent describing the *in vitro* release kinetics of special dosage forms, including medicated chewing gums (Möller, *et al.* 1999, Siewert, *et al.* 2003, Yang, *et al.* 2004). Due to the complexity of the release mechanisms involved, researchers proposed minimal requirements for experimental settings with respect to the site of release and absorption.

The performance tests must, however, be able to detect the influence of critical manufacturing variables, discriminate between different degrees of product performance, and to some extent describe the biopharmaceutical quality of finished products. Besides the product quality tests,

the drug release tests can provide useful information about the characteristics of the product itself, which includes but is not limited to the influence of the composition of the gum and other excipients on drug release, a main tool required primarily during product screening and development, and to some extent the product performance *in vivo*.

1.5.3 *In vitro* drug release testing justification

Dissolution is defined as the process by which a solid substance enters in the solvent to yield a solution and is controlled by the affinity between the solid substance and the solvent (Gajendran, *et al.* 2005). It is an important property of a dosage form that is a necessary prerequisite to drug absorption and one that contributes to the rate and extent of drug availability to the body.

1.5.3.1 Mechanical forces

Generally, the drug release from chewable dosage forms is initiated by masticatory activity within the oral cavity. The masticatory forces could be simply interpreted in terms of external forces required to break, rupture, or to knead the dosage form to influence the dissolution/ drug release. For instance, in case of medicated chewing gums, the release rate is controlled by the chewing frequency, whereas in case of chewable capsules, rate of dissolution of capsule shell determines the exposure of the liquid contents to the oral cavity. The mechanism of drug release from the chewable dosage forms vary significantly and are dependent on the nature and type of dosage form. Wetting followed by erosion and diffusion are the primary stages involved for release from most of the chewable oral products. Mechanical kneading is additionally required by the chewing gums to expose the new surface areas for further drug release (Gajendran, *et al.* 2008, Gajendran, *et al.* 2009).

For the mechanical kneading of medicated gums in the oral cavity, the required forces are induced by the combination of masseter muscle and the angular jaw movement. Many investigators have demonstrated the enormous amount of force exerted during the human mastication which is particularly sufficient to knead the gums. An average human biting force corresponds to 700 N (Hajian 2004, Kleber, *et al.* 1981, Nakata 1998). This may depend on age, gender and the oral health of the subject. A comparable force of this magnitude is adequate to induce the *in vitro* release testing of the gums which requires a special attention and an apparatus. Many mechanical chewing simulators have been designed and investigated (Christrup, *et al.* 1986, Pharmeuropa 2008). Methods to determine the appropriate chewing force have been proposed and widely published (Castelo, *et al.* 2008, Goldmann, *et al.* 2008, Kohyama, *et al.* 2004, Steiner, *et al.* 2009). The primary objective of these investigations is to demonstrate a comparable *in vitro* method by which the characteristics of the drug product be evaluated or the influence of masticatory force on the dentures.

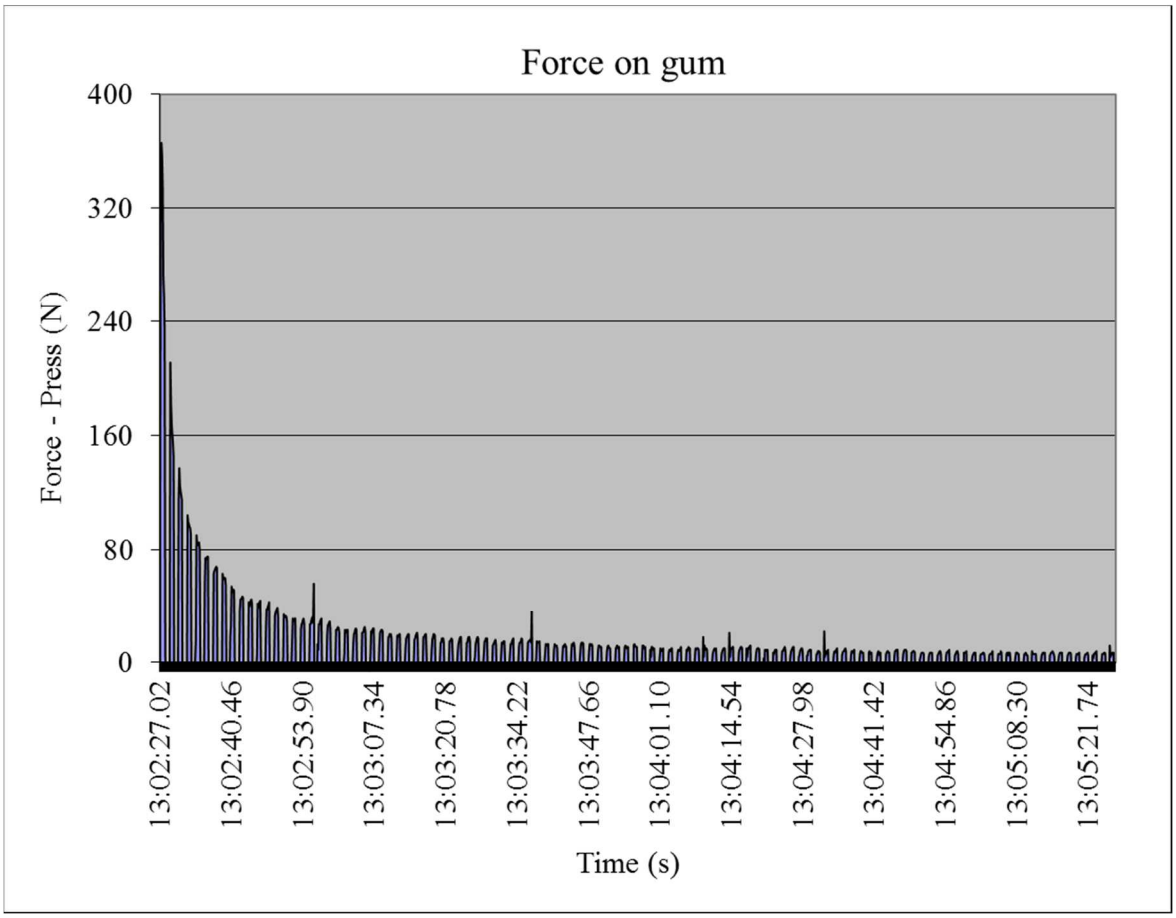


Figure 1-4. Figure showing the force measured on a chewing machine during the simulation of chewing action to test the release of actives from medicated gums

Data supplied by FIA, Sweden (unpublished). The figure shows the force measured on the surface of the upper chewing jaw in the Ph. Eur. Apparatus B during mastication with nicotine based gum product. The force in newton (N) applied on the gum was measured for a period of about 3 min continuously.

1.5.4 Justification of masticatory action

Unlike conventional solid oral dosage forms, the chewable dosage forms require additionally masticatory forces for drug release. As far as the MCGs are concerned, the drug substance is well blended into a molten gumbase and the release is not expected as long as the integrity of the dosage form is maintained. Since *in vitro* release testing is a measure of performance of the dosage form, mastication is a prerequisite for these dosage forms. Even accidental swallowing of the dosage form results in a negligible amount of drug released in the GI tract which is of no therapeutic significance. Safety after accidental swallowing of the dosage form can be demonstrated using the compendial methods under physiological test conditions. Oral dosage forms other than medicated gums for which the oral cavity is the primary site of absorption should be scientifically justified with respect to safety in case of accidental swallowing. Because these dosage forms are formulated to release its components even after accidental swallowing, the therapeutic efficacy, safety and compliance will be in jeopardy, which is not the case for medicated gums.

1.6 *In vitro in vivo* correlation

Acceptable release of an API from the dosage form and its subsequent availability in solution ensures adequate concentration of drug substance at the site of absorption to provide therapeutic efficacy. Besides from a quality control perspective, assessing the *in vivo*-performance by an *in vitro* release testing helps in waiving expensive and time consuming clinical trials and the *in vitro* tests serve as a potential surrogate for the bioequivalence studies.

A simple and cost effective method of dissolution/drug release testing employs a mixing mechanism where the drug product is stirred in a defined volume of media at a constant temperature. The idea is to simulate the conditions in the stomach where usually the mixing is expected after the administration of the dosage form. In most of the cases, the rate of appearance of the API in solution is correlated to the rate of its appearance in blood plasma. Various methods have already been proposed by the authors to treat the data using mathematical models and to demonstrate the relationship between the *in vitro* and *in vivo* data.

Correlations between *in vitro* and *in vivo* data (IVIVC) are often used during pharmaceutical development in order to reduce development time and optimization of the formulation. A good correlation is a tool for predicting *in vivo* results based on *in vitro* data. Various definitions of *in vitro–in vivo* correlation have been proposed by the International Pharmaceutical Federation (FIP), the USP working group and regulatory authorities such as the FDA or EMA. The FDA (FDA 1997) defines IVIVC as "a predictive mathematical model describing the relationship between an *in vitro* property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant *in vivo* response, e.g., plasma drug concentration or amount of drug absorbed. As stressed in this definition, IVIVC is more an *in vitro–in vivo* relationship than a strict correlation.

1.6.1 Biorelevant release testing

The development of a suitable biorelevant *in vitro* drug dissolution/release method is useful in predicting the *in vivo* dissolution/release behavior of the drug product. The validity of the biorelevant dissolution/release method can be demonstrated by establishing a meaning relationship between an *in vivo* biological parameter (C_{max}, AUC etc.) and the *in vitro* parameter (% release, $T_{50,}$ MDT $_{in\ vitro}$ etc). The biorelevant method could also be used as a valuable indicator to test the batch to batch consistency of the drug product.

Since drug absorption depends on the dissolution and solubilization of the drug under physiological conditions as well as its permeability across the mucosa, the physicochemical characteristics of the drug substance, overall composition of the drug product, wettability,

disintegration and de-aggregation characteristics are among the important factors that can influence the dissolution of drugs and hence the biological response.

In order to develop a biorelevant drug release method for MCGs, physiological factors within the oral cavity have to be considered. Some of these factors such as masticatory activity and/ frequency, composition of saliva can be easily simulated *in vitro* by adjustments of the apparatus setup and the dissolution media. Others like dental health, salivary flow are difficult to be simulated *in vitro* and therefore has to be later on verified with the *in vivo* findings.

1.6.2 Method of *in vitro in vivo* correlation

A biorelevant drug release testing methodology is a crucial step to derive a data which is expected to be reflective of the *in vivo* physiology. Developing an IVIVC is a dynamic process that starts from the early phases of drug product design and development and proves its usefulness in the drug product life cycle.

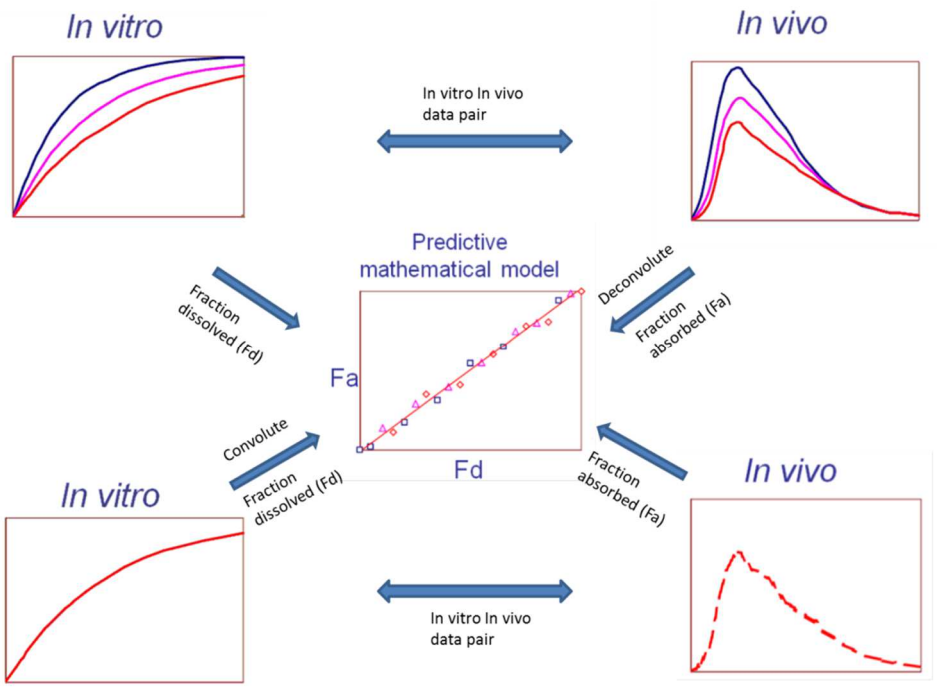


Figure 1-5. Schematic overview of the steps involved for establishing IVIVC

Illustration taken and modified from Kumar, et al. 2013. The in vivo profile (upper right) can be de-convoluted and compared to that of in vitro data (upper left) or the in vitro data (lower left) can be convoluted suitably to compare with the in vivo data (lower right). In both the cases, the fraction of dose absorbed (Fa) is correlated to the fraction of the dose dissolved (Fd).

The Figure 1-5 shows various domains of the *in vitro* and *in vivo* parameters that are theoretically related to the mathematical models to establish the relationship. The FDA and the drug authorities worldwide accept the levels of different correlations detailed below in the order of their acceptance and importance.

Bases on the type of data used to establish the relationship, three main levels that are defined by the FDA are as follows;

1.6.2.1 Level A correlation

The level A correlation is a point to point correlation between the *in- vitro* parameters (e.g., % dissolution, cumulative amount dissolved /released over time) and the in-vivo biological parameter (e.g., cumulative fraction of dose absorbed "FRA"). The level A correlation utilizes the complete data set generated and considered as the most robust and desirable among the other correlation levels. In such a correlation, the *in vitro* dissolution and *in vivo* input curves may be directly superimposable or may be made to be superimposable by the use of a scaling factor. Nonlinear correlations have also been demonstrated by investigators however it is not a common practice. Independent of the method used to establish the level A IVIVC, the model should predict the entire *in vivo* time course from the *in vitro* data. In this context, the model refers to the relationship between *in vitro* dissolution of an extended release (ER) dosage form and an *in vivo* response such as plasma drug concentration or amount of drug absorbed.

1.6.2.2 Level B correlation

A level B correlation uses the principles of statistical moment analysis (Podczeck 1993). The mean *in vitro* dissolution time ($MDT_{in\ vitro}$) is compared either to the mean residence time (MRT) or to the mean *in vivo* dissolution time ($MDT_{in\ vivo}$). The MDT itself is a measure of the rate of the dissolution process; the higher the MDT, the slower the release rate. A level B correlation does not uniquely reflect the actual *in vivo* plasma level curve, because a number of different *in vivo* curves will produce similar mean residence time (MRT) values, therefore is not a preferred method with higher priority.

1.6.2.3 Level C correlation

A level C correlation is established using single parameters derived from the clinical data (e.g., AUC, C_{max}, and T_{max}) and the *in vitro* data ($t_{50\%}$, $t_{80\%}$). A level C correlation does not reflect the complete shape of the plasma concentration-time curves. In addition to these three levels, a multiple level C is also described. A multiple level C correlation relates one or several pharmacokinetic parameters of interest to the amount of drug dissolved at several time points of the dissolution profile. For the establishment of a correlation as described in the FDA guidance, various parameters can be used as presented in the Table 1-4.

1.6.2.4 Multiple-level C correlation

A multiple level C correlation relates one or several pharmacokinetic parameters of interest (C_{max}, AUC, or any other suitable parameters) to the amount of drug dissolved at several time

points of the dissolution profile. A multiple point level C correlation may be used to justify a biowaiver provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. A relationship should be demonstrated at each time point at the same parameter such that the effect on the *in vivo* performance of any change in dissolution can be assessed. If such a multiple level C correlation is achievable, then the development of a level A correlation is also likely. A multiple Level C correlation should be based on at least three dissolution time points covering the early, middle, and late stages of the dissolution profile.

Table 1-4. Summary of parameters used for establishing different levels of correlation

Level	***In vitro***	***In vivo***
A	Dissolution curve	Input (absorption) curves
B	Statistical moments: MDT	Statistical moments: MRT, MAT, etc
C	Disintegration time, time to have 10, 50, 90% dissolved, dissolution rate, dissolution efficiency (DE)	C_{max}, T_{max}, Ka, T_{10}, T_{50}, T_{90} (Time to 10, 50, 90% absorption), AUC (total or cumulative)

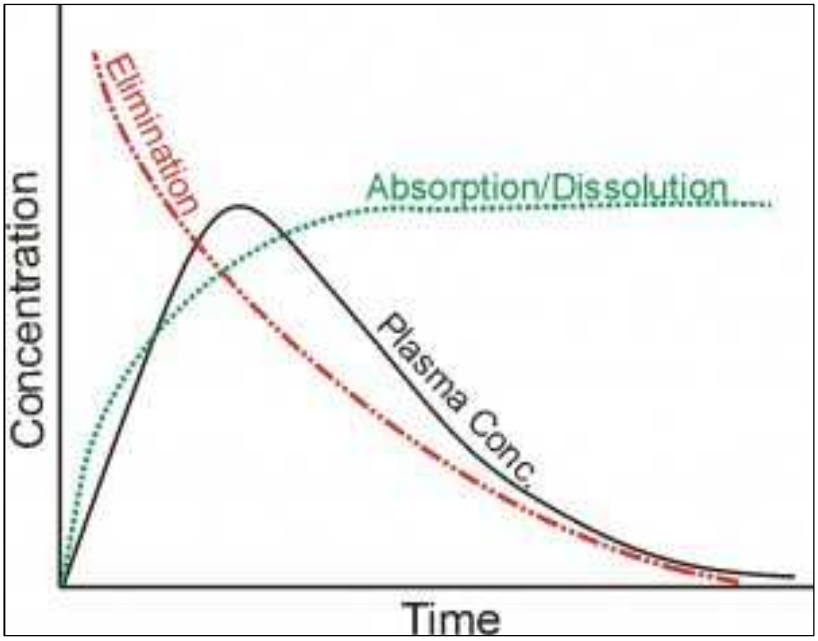


Figure 1-6. Figure showing the typical plasma concentration time curve along with the elimination curve and fraction absorbed/dissolution curve

Figure taken from Qureshi 2010.

The Figure 1-6 shows the profile of plasma concentration time curve following oral administration, and the elimination curve usually better predicted following the IV dose and the absorption curve which are usually obtained from the de-convolution of the plasma concentration curve. The *in vitro* dissolution curve is usually compared directly to that of the absorption curve or convoluted to superimpose the plasma concentration curve.

The techniques for demonstrating IVIVC are generally confined to the conventional and modified dosage forms particularly the oral dosage forms where dissolution is the rate limiting step for absorption. An IVIVC is more likely for the biopharmaceutics classification system (BCS) class 1 substances (IVIVC is expected if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlation) and can be extended to BCS class 2 substances provided the *in vivo* dissolution is the rate limiting step in drug absorption.

The MCGs present a different release approach compared to other dosage forms. The solubility characteristic of the drug substance is highly affected by the gum matrix composition. MCG is generally an inactive form of the drug product and activated by the masticatory action in the presence of a solvent system namely the dissolution/drug release medium. A major portion of the dissolved drug in the saliva is usually absorbed in the oral cavity followed by subsequent absorption along the GI tract representing a very large absorption window with/without any difference in absorption rates. Such situations often make the estimation of the PK parameters tedious and less reproducible. The primary objective of the clinical study is to determine the fate of the drug substance in the body and to predict the *in vivo* dissolution behavior of the dosage form from the given PK data. Since the site of application and release is the oral cavity, the dosage form is always accessible at any time point during mastication. Determining the amount of drug substance yet to be released from the dosage, one can interpret and predict the *in vivo* dissolution / release behavior of the dosage form. This in turn will provide valuable information about the product behavior *in vivo*. In this part of the study, such an attempt to study the performance of the product *in vivo* (chew-out study) was conducted on the commercially available products and the principles of establishing the IVIVC were attempted. It was then later used to verify the *in vitro* drug release testing methodology developed for the quality control (QC) purposes.

2 AIMS OF THE THESIS

2.1 Aims of the thesis and dissertation outline

The MCGs have been gaining its importance as an alternative to many conventional dosage forms. However, much less information has been published so far about this dosage form and understanding the quality control aspects of the dosage form is not widely explored.

The objectives of this dissertation are divided into two aspects to understand the MCGs with respect to formulation development and assessment of product quality in combination with specialized tools namely the chewing (masticatory) apparatus.

The following were the objectives;

a) To develop a suitable *in vitro* drug release testing methodology for medicated chewing gum products.

b) To evaluate comparatively the chewing apparatuses described in the European Pharmacopoeia (apparatus A and apparatus B) for bio-relevant *in vitro* release testing.

c) To propose an alternative chew-out (spit-out) study model and assess the robustness of the model to verify the in- vitro drug release testing methodology (IVIVC).

d) Attempt to establish an IVIVC using the literature reported/acquired clinical study data.

e) To propose a standardized *in vitro* testing methodology and to assess the interchangeability of the apparatus for a given gum product.

The applicability of the *in vitro* drug release method during the development of medicated chewing gums was assessed. A highly water soluble drug substance, nicotine hydrogen tartrate salt was used as a model drug to be incorporated into the gum matrix. The feasibility of manufacturing chewing gum using a tableting technique was evaluated. For this purpose, the nicotine salt was loaded onto a cation exchange resin and coated using a pH independent polymer, Eudragit RS 100 prior to tablet compression. The release of nicotine was characterized based on the extent of coating, type and the composition of gum matrix formulated. The purpose was to demonstrate the possibility to modify the release characteristic of a drug substance and to establish methods to assess the biopharmaceutical quality of the manufactured nicotine chewing gums using the direct compression (DC) technique.

3 MATERIALS

3.1 Selection of drug products for the study

Currently only very few chewing gum products are available in the market worldwide and still most of them are in the development phase. In order to understand the basic mechanism of release of API from the chewing gums and to describe their release kinetics, drug products which are already having the marketing authorization (MA) in Europe and North America were considered. The objective is to have standardized drug products which have been approved in the market based on the clinical data supplied and well investigated. As far as the chewing gums are concerned, no *in vitro* performance tests as a compendial requirement is indicated in the well-known pharmacopoeias. No standardized apparatus was available to test the *in vitro* performance when first nicotine containing chewing gums were manufactured and marketed. The product approval was primarily based on the clinical data submitted to the regulatory authorities and the performance of the products was assessed from the *in vivo* data.

In order to test the suitability of the apparatus described in the Ph. Eur. and the one described in the Pharmeuropa (Ph.Eur. 2014), multi-source chewing gum products were the first choice and the test methodology was extended to other MCGs available in the European and North American market. For the comparative performance studies, nicotine containing chewing gums mainly indicated for the smoking cessation were chosen for the feasibility trial, because of the;

- availability from multiple manufacturers around the world (multi-source product)
- conventional manufacturing processes with a comparable product appearance
- established robust analytical techniques using HPLC/UV detection

The drug products selected and the composition of the gum products are given in the Table 3-1.

Table 3-1. Drug products for *in vitro* drug release feasibility study

Innovator/Generic name	Active pharmaceutical ingredient (API)	Batch	Pharm. manufacturer / Country
Nicorette ® 2 mg [Fresh mint]	Nicotine as Nicotine polacrilex	II896A	Pfizer Consumer Healthcare, Germany
Nicorette ® 2 mg [Classic]	Nicotine as Nicotine polacrilex	LL644	Pfizer Consumer Healthcare, Germany
Nicorette ® 4 mg [Classic]	Nicotine as Nicotine polacrilex	ZNG9010	Pfizer Consumer Healthcare, Germany
Nicotinell® 2 mg [mint]	Nicotine as Nicotine polacrilex	B000034771	Novartis Consumer Healthcare, Switzerland
Rockstar Energy Gum [Iced mint]	Caffeine [40 mg]	4222430_A	Rockstar Inc, United States of America
Superpep® forte Reise Kaugummi Dragees	Dimenhydrinate	6036518	Hermes Arzneimittel GmbH, Germany

Among the products listed in the Table 3-1, the nicotine based chewing gums are available in Germany and in the United States as over the counter (OTC) product. The Dimenhydrinate containing chewing gums are marketed as an OTC product under the trade name “Superpep” and are also available in Europe as Travel gum. Caffeine containing chewing gum is currently available in the US market.

3.2 Physicochemical properties of marketed chewing gum formulations

The physicochemical characteristics of all the investigated gum products are given in the following sections.

3.2.1 Nicotine containing chewing gum formulations

Among all the marketed chewing gum products, nicotine based chewing gums are well recognized for the nicotine replacement therapy. In the gum product, nicotine is present as a nicotine polacrilex, a weak cationic resin usually bound to nicotine in the ratio of 1 to 4 (nicotine to resin). The USP contains an assay monograph for nicotine polacrilex and nicotine polacrilex gum (USP 2015).

3.2.1.1 Nicotine

Nicotine is a colorless to pale yellow, oily liquid, miscible with water in its base form, very hygroscopic in nature, turns to brown on exposure to air or light and develops odor of

pyridine. As a nitrogenous base, nicotine forms salts with acids that are usually solid and water soluble. It is also a naturally occurring alkaloid with a strong bitter taste.

3.2.1.1.1 Chemical structure

It is a bicyclic compound with a pyridine cycle and a pyrrolidine cycle. The IUPAC name is 3-(1-Methyl-pyrrolidinylyl) pyridine, ß-Pyridyl-α-N-methyl pyrrolidine. It has as molecular weight of 162.23.

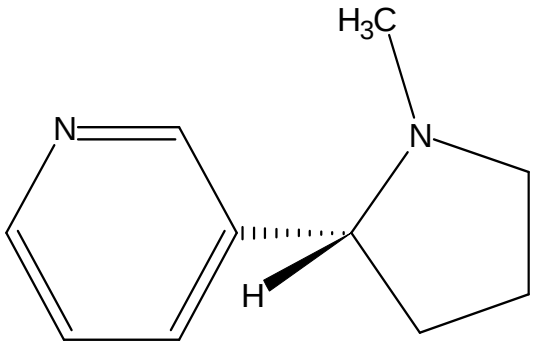


Figure 3-1. Chemical structure of nicotine

3.2.1.2 Solubility

Nicotine in its base form is miscible with water below 60 °C forming hydrates. It is very soluble in alcohol, chloroform and ether (USP 2015).

3.2.1.3 Physicochemical data

pKa (1) = 8.02, pKa (2) = 3.12 (Merck-Index 2006)
Melting point: 79 °C
Density: 1.01 g/cm³

3.2.1.4 Nicotine hydrogen tartrate

Nicotine hydrogen tartrate appears as a white to off white crystalline powder and is a salt of nicotine and tartaric acid. It is generally used in the preparation of formulations for nicotine replacement therapy due to its high solubility and stability characteristics compared to nicotine alkaloid. It is also used as an analytical reference standard for the quantification of nicotine from pharmaceutical preparations.

3.2.1.4.1 Solubility

It is highly soluble in solvents such as water (50 mg/mL), methanol, acetonitrile, chloroform and petroleum ether (USP 2015).

3.2.1.4.2 Chemical structure

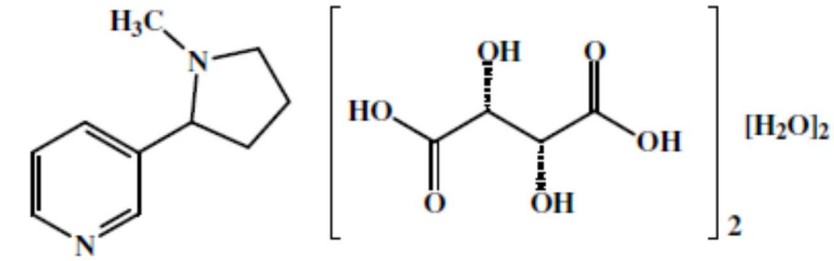


Figure 3-2. Chemical structure of nicotine hydrogen tartrate

3.2.1.5 Nicotine polacrilex resin

Nicotine polacrilex is a weak carboxylic cation-exchange resin prepared from methacrylic acid and di-vinyl benzene, in complex with nicotine. Polacrilex resin is a white powder with a fine particle size. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of nicotine (C10H14N2), calculated on the anhydrous basis (USP 2015).

3.2.1.5.1 Chemical structure

The chemical structure of nicotine polacrilex resin is shown in Figure 3-3.

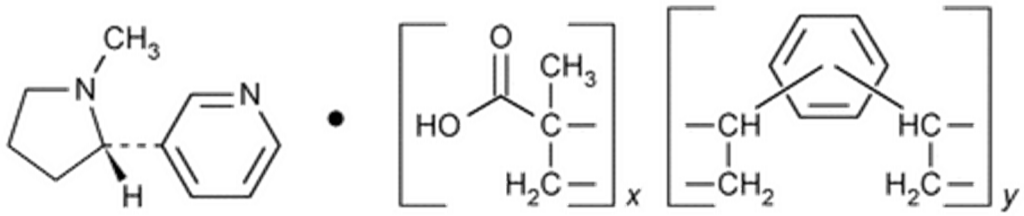


Figure 3-3. Chemical structure of nicotine polacrilex resin

3.2.1.5.2 Solubility

The polacrilex resin is practically insoluble in water and in most of the organic solvents. The resin contains ionizable groups distributed along its polymer backbone. Weak cation exchange resins contain H+ ions for exchange and behave as free acids below pH 4. The degree of dissociation of weak acids [-COOH] depends on the pH of the solution.

3.2.2 Caffeine containing chewing gum formulations

The chewing gum from Rockstar contains caffeine, taurine, inositol, nicotinamide, pantothenic acid, riboflavin, pyridoxine and cyanocobalamin. The registered trade name is Rockstar Energy Gum Iced Mint. The chewing gum has ergogenic properties.

3.2.2.1 Chemical structure

Caffeine has the IUPAC name 1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione and the formula $C_8H_{10}N_4O_2$. Its molecular weight is 194.19 g/mol.

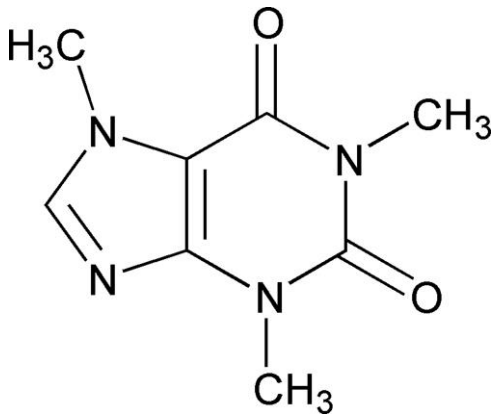


Figure 3-4. Chemical structure of caffeine

3.2.2.2 Solubility

Caffeine is sparingly soluble in water and ethanol, and slightly soluble in chloroform. 1 g of caffeine dissolves in 46 mL of water (Merck-Index 2006). Since caffeine is an extremely weak base with a pKa of 0.6, solubility is expected to be pH independent within the physiological range.

3.2.2.3 Physicochemical data

The following physicochemical data have been reported for caffeine (Merck-Index 2006).
pKa: 0.6
Melting point: 238°C
Density: 1.23 g/mL
Appearance: White or almost white crystalline powder

3.2.3 Dimenhydrinate containing chewing gum formulations

Dimenhydrinate chewing gums which is well known as Travel gum is primarily used for symptoms against travel sickness and to prevent and relieve motion sickness due to its antiemetic and antihistaminic agent properties. It is primarily a H_1-antagonist, but also possesses an antimuscarinic effect and is usually available over the counter (OTC).

3.2.3.1 Chemical structure

Chemically, dimenhydrinate consists of two chemical moieties, namely diphenhydramine and 8-cholorotheophylline, approximately in a molar ratio of 1:1, diphenhydramine slightly higher than the other component.

Dimenhydrinate has the IUPAC name "8-chloro-1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-7-ide;[2-(diphenylmethoxy) ethyl] dimethylazanium. The USP states that dimenhydrinate contains not less than 53.0 percent and not more than 55.5 percent of diphenhydramine ($C_{17}H_{21}NO$), and not less than 44.0 percent and not more than 47.0 percent of 8-chlorotheophylline ($C_7H_7ClN_4O_2$), calculated on the dried basis. The chemical structure of dimenhydrinate hydrochloride is given in the Figure 3-5.

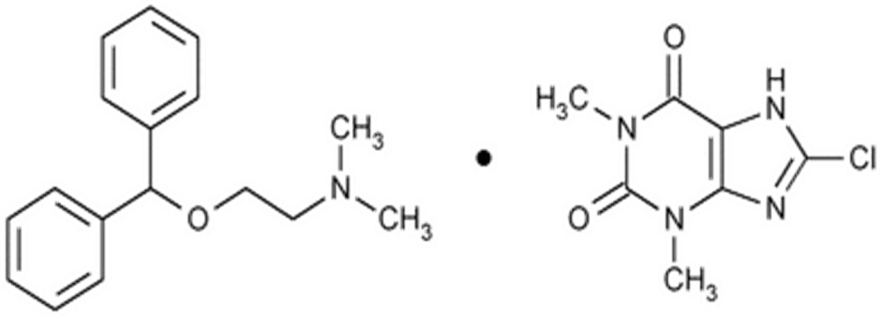


Figure 3-5. Chemical structure of dimenhydrinate
Taken from USP 2015.

3.2.3.2 Solubility

Dimenhydrinate is poorly soluble in water. The aqueous solubility ranges from 0.1-1 g/100 mL at 22 ºC and the reported solubility in an aqueous medium is approximately about 3mg/mL (Shahrzad, *et al.* 2005). Dimenhydrinate is soluble in 1 in 2 parts of alcohol and 1 in 2 parts of chloroform and sparingly soluble in ether.

3.2.3.3 Physicochemical data

Dimenhydrinate appears as white or almost white crystalline powder. The following physicochemical data have been reported.

Log P (Octanol: Water): -0.39 (Meylan, *et al.* 1995), experimental logP 2.67 (ALOGPS), predicted logP 3.65 (ChemAxon Molconvert), predicted, Melting point: 204.5 (SRC Physprop database).

Table 3-2. Composition of the investigated chewing gum products

Function of excipient/active(s)	Nicorette 2mg Fresh mint	Nicorette 2mg Classic	Nicorette 4mg Classic	Nicotinell 2mg Mint	Rockstar Gum Iced mint	Superpep forte Travel gum
Active pharmaceutical ingredient (API)	Polacrilin-nicotine	Polacrilin-nicotine	Polacrilin-nicotine	Polacrilin-nicotine	Caffeine	Dimenhydrinate
Gumbase	Styrene-butadiene (polyisobutylene)	Styrene-butadiene (polyisobutylene)	Styrene-butadiene (polyisobutylene)	Styrene-butadiene (polyisobutylene)	Styrene-butadiene (polyisobutylene)	Styrene-butadiene (polyisobutylene)
Anti-oxidant	Butylated hydroxyl toluene (BHT)	Butylated hydroxyl toluene (BHT)	Butylated hydroxyl toluene (BHT)	Butylated hydroxyl toluene (BHT)	Butylated hydroxyl toluene (BHT)	-
Sweetening agent, Humectant, Emollient	Acesulfam-Potassium, Xylitol	Sorbitol, Glycerol	Sorbitol powder, Sorbitol 70%,	Sorbitol, Saccharin, Sodium saccharin, Acesulfame potassium, Mannitol	Maltitol Syrup, Sorbitol, Sucralose, Aspartame, Acesulfame potassium	Sorbitol, Saccharose, Aspartame, Saccharin sodium, Glucose Syrup
Coating agent	Carnauba wax	-		Carnauba wax	Resinous Glaze, Carnauba wax	Methyl, Butyl methacrilate-copolymer
Coloring agent	Titanium dioxide	-	Quinoline yellow (E104)	Titanium dioxide	Titanium dioxide	Titanium dioxide
Emulsifying agent, Binder	Gum Arabic	-	-	-	-	-
Diluent, Lubricant, Anti-caking agent	Magnesium oxide	Talc	Talc	Calcium carbonate, Talc	-	Dextrin, Calcium carbonate, Talc, Macrogol 35000, Montanglycol wax, Magnesium oxide, Magnesium stearate, Silicon dioxide (micronized)
Flavoring agent	Levomenthol, Peppermint oil	Haverstroo flavor	Haverstroo flavor	Levomenthol, Peppermint oil, Eucalyptus oil	-	Peppermint flavor, Levomenthol

Function of excipient/active(s)	**Nicorette 2mg Fresh mint**	**Nicorette 2mg Classic**	**Nicorette 4mg Classic**	**Nicotinell 2mg Mint**	**Rockstar Gum Iced mint**	**Superpep forte Travel gum**
Alkalizing agent	Sodium carbonate, Sodium hydrogen-carbonate	Sodium carbonate, Sodium bicarbonate	Sodium carbonate	Sodium carbonate, Sodium hydrogen - carbonate	-	-
Plasticizer, Antimicrobial agent	-	-	Glycerol 85%	Glycerol, Xylitol	Glycerine	
Binding agent	-	-	-	Gelatin	Gum Arabic	Povidone K 25
Emulsifier/solubilizer	-	-	-	-	Soy lecithin	-
Suspending agent	-	-	-	-	-	Cocoa butter
Buffering agent	-	-	-	-	-	Potassium dihydrogen phosphate
Polishing, Stabilizing agent	-	-	-	-	-	Yellow wax
Other therapeutic agents	-	-	-	-	Taurine, Inositol, Niacinamide, Calcium Pantothenate, Riboflavine, Cyanocobalamine, Pyridoxine hydrochloride	-

3.3 Reagents and chemical substances

The reagents and chemicals used for the preparation of buffer solution, in the manufacturing of chewing gum formulations are listed in the table below. All the chemicals and reagents used for the analyses are of at least analytical grade and the excipients used for chewing gum formulations are accepted as “generally regarded as a safe (GRAS)”.

Table 3-3. Reagents and chemical substances used for preparation of solutions

Substance	Specification	Origin/Provider
Deionized Water	Ph. Eur. 8.0	PHAST, Germany
Methanol (MeOH)	for HPLC	VWR, Germany
Hydrochloric acid (HCl): 32%, 1m	analytical grade	VWR, Germany
Sodium hydroxide pellets (NaOH)	pellets extra pure	VWR, Germany
Sodium chloride (NaCl)	analytical grade	VWR, Germany
Potassium dihydrogen phosphate (KH_2PO_4)	analytical grade	VWR, Germany
Purified water	Ph. Eur. 8.0	PHAST, Germany
Acetonitrile	gradient grade	VWR, Germany
n-Heptane	for analysis	VWR, Germany
Calcium chloride ($CaCl_2$)	for analysis	VWR, Germany
Phosphoric acid (H_3PO_4)	85%, analytical grade	VWR, Germany
Chloroform	for analysis	VWR, Germany
Dichloromethane	for analysis	VWR, Germany

Table 3-4. Chemical substances used for the formulation studies

Component name	Purpose	Batch / Lot	Supplier / Country
All in One Gum SF Cool	Gumbase for tabletting	NA	Cafosa, Spain
All in One Gum SF Extra	Gumbase for tabletting	NA	Cafosa, Spain
Amberlite IRP 64	Resin for nicotine loading	03329BJ	Sigma Aldrich, Germany
Amberlite IRP69	Resin for nicotine loading	08207CH	Sigma Aldrich, Germany
Ethyl cellulose	Coating of DRC	MKAA1638	Sigma Aldrich, Germany
Eudragit L 100	Coating of DRC	B070803075	Evonik, Germany
Eudragit RS100	Coating of DRC	E080608143	Evonik, Germany
Eudragit S100	Coating of DRC	B071005090	Evonik, Germany
HPC (average Mw ~80,000, 20 mesh particle size)	Binding agent for granulation	20293996	Sigma Aldrich, Germany
HPMC (~15 mPa, 2 % in H_2Oat 25 °C)	Binding agent for granulation	078K0022	Sigma Aldrich, Germany
Magnesium stearate	Excipient for tabletting	10132396	Sigma Aldrich, Germany
Microcrystalline cellulose Type 102	Excipient for tabletting	V09-0103	Sigma Aldrich, Germany
PEG 10000	Impregnation of DRC	NA	Sigma Aldrich, Germany
PEG 4000	Impregnation of DRC	NA	Sigma Aldrich, Germany
PEG 6000	Impregnation of DRC	NA	Sigma Aldrich, Germany
PG Nutra PEPP TA	Gumbase for tabletting	NA	Gum Base Co, Italy
PG Nutra TA	Gumbase for tabletting	NA	Gum Base Co, Italy
Pharmagum C	Gumbase for tabletting	05H042	SPI Pharma, Germany
Pharmagum M	Gumbase for tabletting	144F-090	SPI Pharma, Germany
Phosphatidyl choline	Excipient for coating DRC	K37661508928	VWR, Germany
Polyvinyl alcohol hydrolyzed (MW 89000-98000)	External aq. medium for coating DRC	S95603901	VWR, Germany
Prosolv Easytab	Excipient for tabletting	4381301	JRS Pharma, Germany
Polyvinyl pyrrolidine (PVP) K15	Binding agent for granulation	1369964	Sigma Aldrich, Germany
PVP K25	Binding agent for granulation	1345233	Sigma Aldrich, Germany
Sorbitab SD250	Excipient for tabletting	0705101018	J.T. Baker, Germany
Talc	Excipient for tabletting	80170	Sigma Aldrich, Germany
Tween 20	Surfactant for coating	09I080522	VWR, Germany
Vinylec K	Excipient for tabletting	1160193	Sigma Aldrich, Germany
Vivapur Type 105	Excipient for tabletting	6610501226	JRS Pharma, Germany

Among the excipients listed, the powdered gumbase contains Butylated hydroxyl toluene as main component preformulated with sugars and polyols. The powdered gumbase or the DC

gumbase powder was provided by SPI Pharma, Gumbase co and Cafosa. Similarly the excipients, Vivapur which is microcrystalline cellulose and the Prosolv Easytab containing mixture of colloidal silicon dioxide, sodium starch glycolate, microcrystalline cellulose and sodium stearyl fumarate (SSG), were provided by JRS Pharma. Rest of the excipients was bought from the chemical suppliers in Germany.

3.4 Reference standard substances

The reference standard substances were purchased from the local suppliers in Germany. The list of all the reference standards used for the entire study is given in Table 3-5.

Table 3-5. Chemical references substances (CRS) used for quantification

Reference compound	Synonym (IUPAC)	Mol. formula	Purity [%]	Mol. Wt./ # Batch	Supplier/ Country
Nicotine hydrogen tartrate	(−)-1-Methyl-2-(3-pyridyl) pyrrolidine	$C_{10}H_{14}N_2$. $2C_4H_6O_6$	100	462.41/ # 098K0676	Sigma Aldrich, Germany
Dimenhydrinate	(2-Benzhydryloxyethyl) dimethylammonium-8-chlor-1,3- dimethyl-3,7-dihydropurin-2,6-dione	$C_{17}H_{21}NO$. $C_7H_7ClN_4O_2$	99.0	469.96/ # 0907	Sigma Aldrich, Germany
Caffeine	1,3,7-trimethyl-1H-purine-2,6 (3H,7H)-dione 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione	$C_8H_{10}N_4O_2$	99.6	194.19/ # 1395937	Sigma Aldrich, Germany

For the purpose of quantification and analysis, nicotine tartrate salt was used due to its high solubility and stability characteristics compared to its alkaloid counterpart which is a weak base. Similar is the case of Dimenhydrinate, which is a salt of diphenhydramine and 8-Chlorotheophilline. The quantification was done by taking into consideration of both the peaks obtained in the chromatogram.

3.5 Analytical instruments and laboratory supplies

The analytical instruments and the other consumables used for the analysis, preparation and transfer of sample and standard solutions are listed in Table 3-6 - Table 3-9.

Table 3-6. Analytical instruments for the analysis and quantification

Analytical Instrument / Type (system)	Purpose	Provider / Country
Degasser / series 200 Vacuum degasser	HPLC analysis	Perkin Elmer / Germany
Pump / series 200 pump	HPLC analysis	Perkin Elmer / Germany
Auto sampler / series 200	HPLC analysis	Perkin Elmer / Germany
Diode array/UV-Detector-series 200/785A	HPLC analysis	Perkin Elmer / Germany
PC for device control / series Dell Optiplex GX110/ GXa	HPLC analysis	Dell Computers / Germany
Chromatographic data system TurboChrom version 6.1.2.0.1: D19	HPLC analysis	Perkin Elmer / Germany
Interface / Series 900	HPLC analysis	Perkin Elmer / Germany
Column oven / Series 200	HPLC analysis	Perkin Elmer / Germany
Column oven / series Jet stream 2 Plus	HPLC analysis	Jasco, Germany
Waters Alliance HPLC system	HPLC analysis	Waters Corporation, United States
Empower Software	For analysis HPLC data	Waters Corporation, United States
Certified volumetric pipettes	Preparation of sample and standard solutions	Brand, Hirschmann, Germany
Certified volumetric flasks	Preparation of sample and standard solutions	Brand, Hirschmann, Germany
Analytical balance/ AX205DR/M	Preparation of standard and sample solutions	Mettler Toledo, Germany
Analytical balance/ CP34001S-OCE	Preparation of dissolution medium	Sartorius AG, Germany
Ultrasonic Sonorex Digital 10 P	Preparation of standard solutions	Bandelin AG, Germany
Acrodisc LC-13mm PVDF filters	Filtration of placebo sample solutions	Pall AG, Germany

Table 3-7. Equipment for the preparation of the media for the solubility determination

Analytical Instrument/Type	Use	Provider
Balance AC211S	Preparation of media	Sartorius, Germany
Analytical balance AX205 Delta Range	Preparation of reference standard solution and samples	Mettler Toledo, Germany
pH meter Multi-Seven	pH-measurement	Mettler Toledo, Germany
Magnetic stirrer MR3001K	Preparation of media and mobile phase	Heidolph, Germany
Mechanical shaker	Preparation of sample solution	Edmund Bühler, Germany
Screwcap vials (25 mL)	Preparation of sample solution	VWR, Germany
Rotanta RP 4300	Centrifugation of sample solution	Hettich, Germany
Filters (13 mm, 25 mm PVDF syringe filter)	Filtration of dissolution samples	Whatman, Germany

Table 3-8. Instruments used for manufacturing and analysis of pre-formulated gum components and directly compressed chewing gum formulations

Instrument (Type)	Purpose	Provider
Tablet machine, K0, Single punch	Manufacturing chewing gums by direct compaction	Korsch, Germany
Tablet punch and Dye EK0, Rounded-Flat surface, diameter 18 mm (D18)	Compaction of chewing gum mixture (tableting)	Korsch, Germany
Fourier transform infrared spectroscopy (FTIR), Perkin Elmer 1720	Analysis	Perkin Elmer Germany
Turbula mixer	Mixing DC powder and other excipients	Willy A Bachofen AG, Switzerland
“Home Depot” apparatus	Compressing chewing gum formulations	PHAST GmbH, Germany
Malvern Master Sizer, Hydro 2000S	Measurement of particle size	Malvern, Germany

Table 3-9. Apparatus used for the *in vitro* drug release testing

Apparatus description	Purpose	Manufacturer/Provider, Country
Ph. Eur.8.0, 2.9.25 (apparatus A)	Drug release testing	Heide Hansen Maskinfabrik, Horsens, Denmark
Ph. Eur. 8.0, 2.9.25 (apparatus B)	Drug release testing	AB FIA / Erweka, Sweden, Germany
USP 38/NF 33apparatus 2 (paddle) with sinkers (DT 6)	Dissolution/drug release testing	Erweka, Germany

4 METHODS

4.1 Scientific rationale for *in vitro* drug release determination

In vitro dissolution/drug release testing for the pharmaceutical dosage forms has become a vital tool to test the quality of most of the drug products. Numerous literatures have already been published (Azarmi, *et al.* 2007, Gajendran, *et al.* 2008, Gajendran, *et al.* 2012, Möller, *et al.* 1999, Morjaria, *et al.* 2004, Shah, *et al.* 2006, Siewert, *et al.* 2003) to describe the importance of dissolution drug release testing for the pharmaceutical dosage forms. However, owing to the multitude of new products and technologies incorporated, performance testing has always become a challenge to set specifications for product quality. Such novel products demand a special apparatus to test the performance owing to their design and /or release mechanism.

As far as the chewing gums are concerned, the USP 38/NF 33 contains a monograph for nicotine polacrilex gums which however does not include a release test. Recently, much effort has been spent describing the *in vitro* release kinetics of special dosage forms, including medicated chewing gums (Möller, *et al.* 1999, Siewert, *et al.* 2003, Yang, *et al.* 2004). Due to the complexity of the release mechanisms involved, researchers proposed minimal requirements for experimental settings with respect to the site of release and absorption. The performance tests, however, must be able to detect the influence of critical manufacturing variables, discriminate between different degrees of product performance, and to some extent, describe the biopharmaceutical quality of finished products.

Besides the product quality tests, drug release tests can provide useful information about the characteristics of the product itself, which includes but is not limited to the influence of the composition of the gum and other excipients on drug release, a main tool required primarily during product screening and development, and to some extent the product performance *in vivo*.

4.2 Current status of drug release/dissolution testing apparatus

The current status of all the dissolution testing apparatus described in the USP and Ph. Eur. are given below in Table 4-1 and Table 4-2. Some of the apparatus designs have already been harmonized in the ICH guidelines. The USP still does not include the chewing apparatus for the performance testing; however, it contains monographs for the nicotine polacrilex gums and resins. Product quality tests like assay, identity are part of the compendial requirements and appear as monographs. In case of Ph. Eur., both the apparatuses described earlier appear as monographs in chapter 2.9.25 and the general guidelines to perform the dissolution/drug release testing has been described. No product specific monographs for MCGs appear in the USP 38/NF 33 and Ph.Eur. 8.0.

Table 4-1. List of compendial apparatus described in the Ph. Eur. 8.0

Dosage form	Apparatus for dissolution/ drug release testing
Solid dosage forms	Apparatus 1 (Basket) Apparatus 2 (Paddle) Apparatus 3 (Reciprocating cylinder) Apparatus 4 (Flow-through cell)
Transdermal patches	Disk assembly method Cell method Rotating cylinder method
Special dosage forms (chewing gums, suppositories, granulates)	Chewing apparatus A (Ch. 2.9.25) Chewing apparatus B (Ch. 2.9.25) Apparatus 4 (Flow-through cell)

Table 4-2. List of compendial apparatus in the USP 38-NF 33

Name	Type of dosage form	Operating principle
Apparatus 1 (Basket)	Tablets, capsules etc	rotating basket / stirring
Apparatus 2 (Paddle) 2	Tablets, suspensions	rotating paddle / stirring
Apparatus 3 (Reciprocating cylinder)	Special solid dosage forms-chewables, swellable & modified release	reciprocation
Apparatus 4 (Flow-through cell)	Solids, beads, suspensions, implants, powders, granules & creams	continuous solvent flow
Apparatus 5 (Paddle over disk)	Transdermal patches	rotating stirrer
Apparatus 6 (Rotating cylinder)	Transdermal patches	rotating cylinder
Apparatus 7 (Reciprocating holder)	Transdermal patches/ solids	reciprocation

4.3 Apparatus for release testing of MCGs

Many apparatus designs have been proposed to test the release of actives from the MCGs. Notable designs include simulated masticatory movements to chew the gums (Christrup, *et al.* 1986, Kvist, *et al.* 1999).

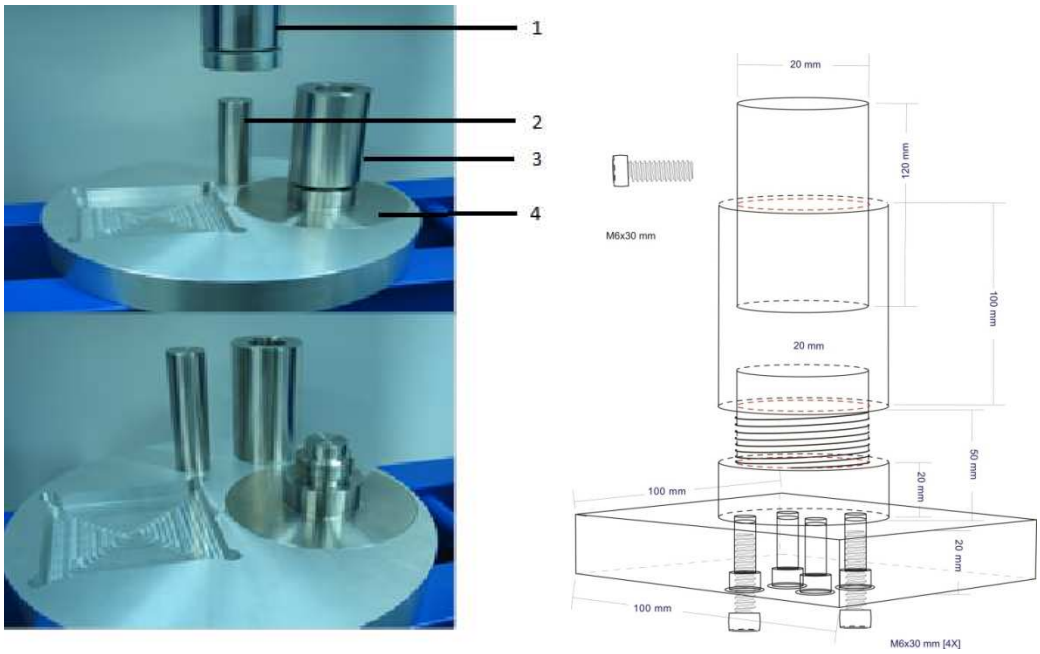
The USP 38-NF 33 and the Ph. Eur. 8.0 contain monographs for the dissolution/ drug release testing apparatus which is generally applicable to most of the dosage forms with a minor or no modifications. For the development of new dissolution/drug release methods for MCGs, a better approach would be to test the feasibility of the existing apparatus in the pharmacopoeias. Even for medicated gums, a test method was developed to utilize the existing USP paddle apparatus (apparatus 2) together with the sinker and evaluated for its suitability for the release testing. Owing to the different geometry of the marketed MCGs, prior modification of the gums was required to provide a uniform surface area during the dissolution/drug release testing. The construction and description of the apparatus used are discussed in the following sections.

4.3.1 Non-compendial method: "Home Depot"

The objective was to develop an apparatus by which the existing chewing gum products can be transformed to a uniform shape and size. The gum products with a larger surface area will facilitate better interaction between the drug release medium and the product, thereby enhancing the release of active(s) from the surface. The initial concept was to use existing compendial USP apparatus to test the performance without a need for a new apparatus. Methods described under the general chapter dissolution testing were used with minor modification in the apparatus setup. The modifications allow the dissolution testing of medicated gum products. The apparatus can be successfully employed as a means of measuring and evaluating the safety/fate during accidental swallowing of the drug product.

4.3.1.1 Construction

The construction of "Home Depot" apparatus is shown in Figure 4-1. The design is primarily based on the existing wood's apparatus intended for the intrinsic dissolution testing (USP 2015). The apparatus consists of a flat surfaced punch, a base plate and a chamber. The mounted and the un-mounted form of the apparatus are shown in. The apparatus can be operated additionally with a table top press to compress the gums with a suitable predetermined force read directly from a calibrated gauge in the press.



(a) (b)

Figure 4-1. "Home depot" apparatus developed by Phast for compression of chewing gums

(*a*). *Schematic representation of the Home depot method showing a modified form of the intrinsic dissolution testing apparatus ("Home Depot") with a punch and dye style to compress chewing gums to a uniform shape and thickness. Figure shows the fabricated home depot method in mounted (above) and un-mounted (below) position. Components, 1) Hydraulic piston (to compress gums), 2) Punch (fits into dye chamber to press gums, 3) Dye chamber (together with base plate (4) holds the chewing gum to be compressed), 4) Base support Plate (mounting system).*

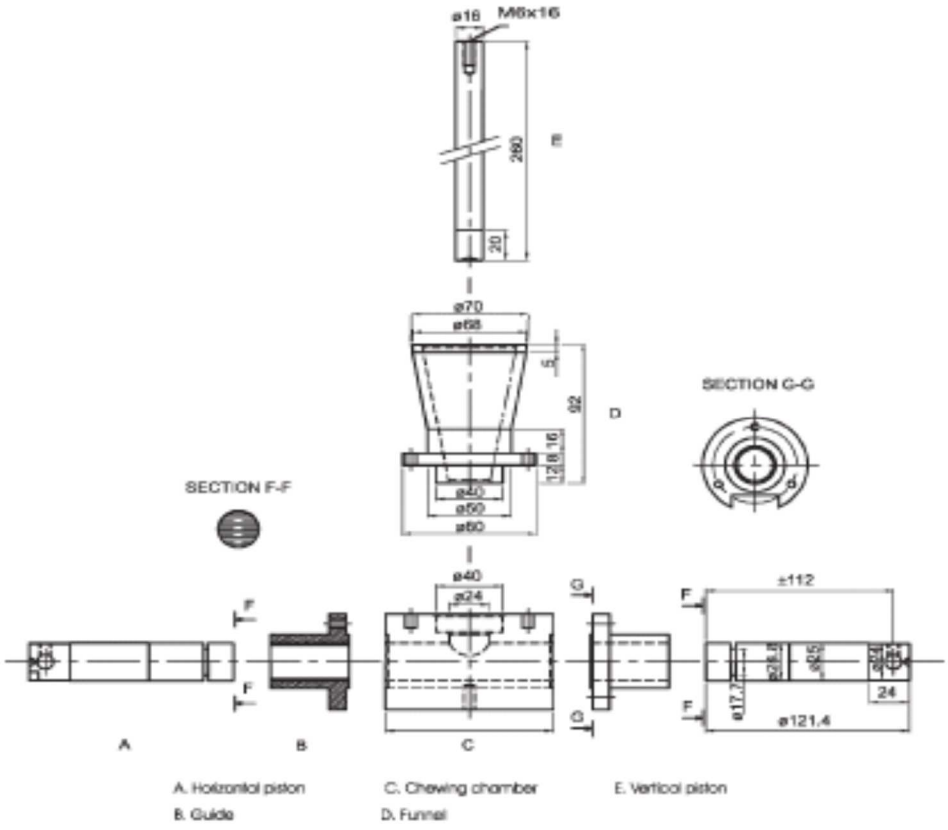
4.3.2 Compendial apparatus

The apparatus for the *in vitro* drug release testing of MCGs has been incorporated in the Ph. Eur. in the year 2000. However, the apparatus design was not completely standardized. In 2008, an alternative chewing apparatus was described in the supplementary edition of the Ph. Eur., "Pharmeuropa" (Pharmeuropa 2008). Both the apparatus described below had attained a compendial status as of 2014 and the USP is on the way to investigate the suitability of the apparatus to be included in the future.

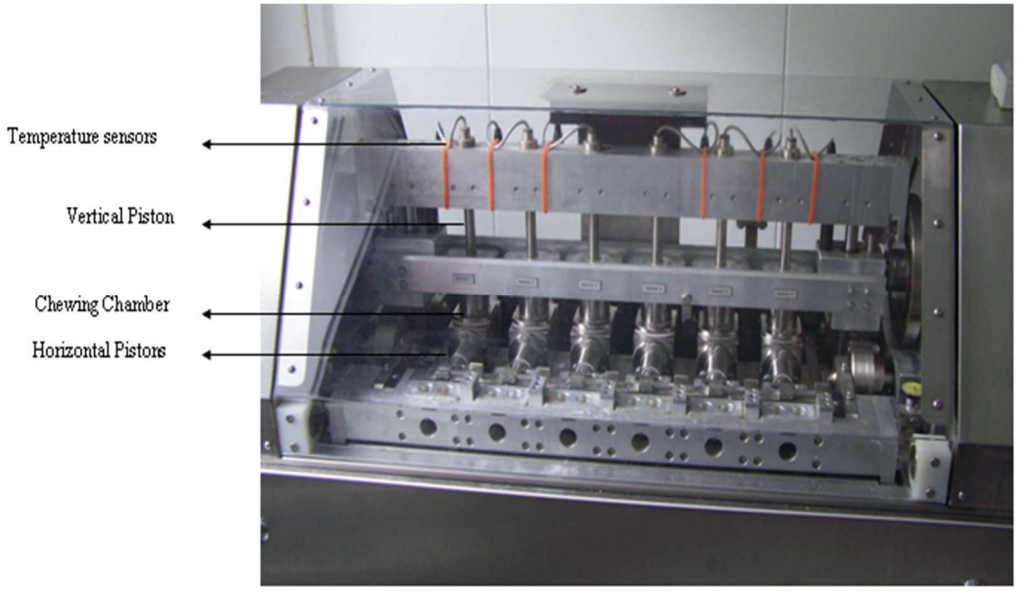
4.3.2.1 Apparatus A. Chewing gum apparatus, compendial—*Ph. Eur.*

The chewing apparatus for medicated chewing gums was adopted by Ph. Eur. in 2000 (Ph.Eur. 2000). The construction of the apparatus is shown in the Figure 4-2. The chewing apparatus comprises a chewing chamber, two horizontal pistons, and a third vertical piston (tongue). The vertical piston operates alternatively with the two horizontal pistons and ensures the gum stays in the right place between mastication. If necessary, it is feasible to construct the machine so that at the end of the chew the horizontal pistons rotate around their own axes in opposite directions to each other to obtain maximum chewing. The working procedure of this chewing apparatus is described in Ph. Eur. (Ph.Eur. 2014). However, the

drug release data generated from the Ph. Eur. apparatus A is not widely available or published elsewhere in public domains (Gajendran, *et al.* 2014).



(a)



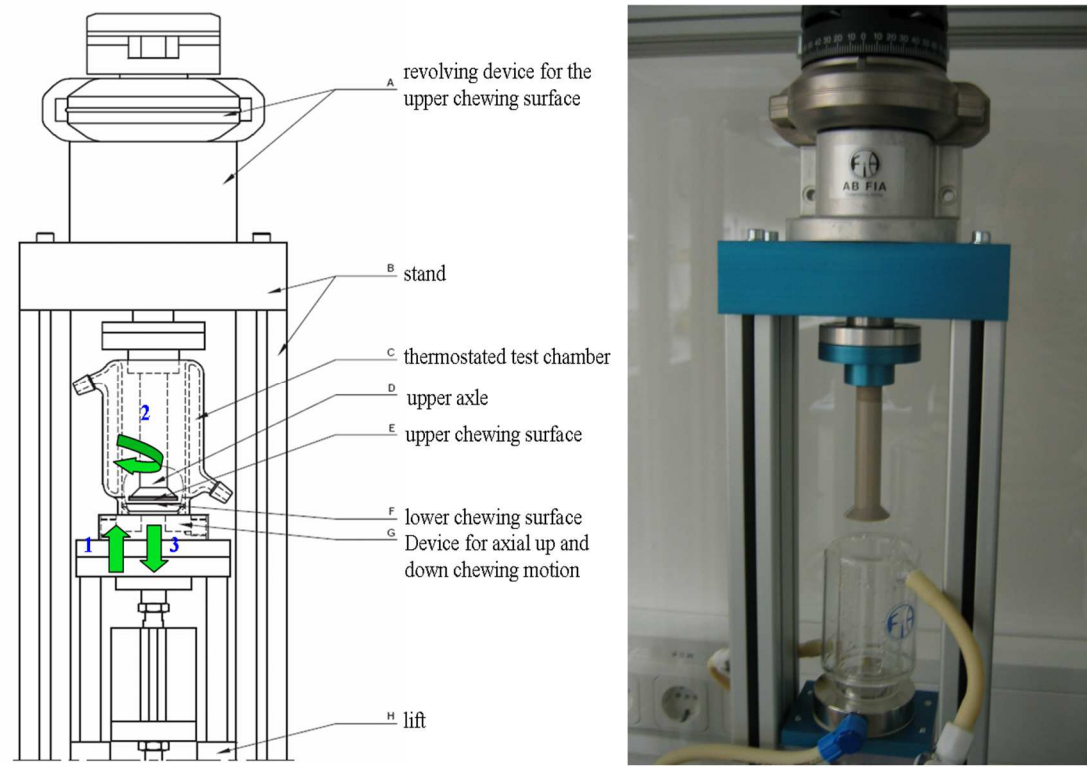
(b)

Figure 4-2. Apparatus A for the drug release testing of medicated chewing gums

a) *Schematic representation of apparatus A with the acceptable dimensions of the parts of the apparatus, b) Construction of apparatus B with 6 modules of chewing chamber with the temperature probes running in each chamber*

4.3.2.2 Apparatus B. Chewing gum apparatus, compendial—*Ph. Eur*

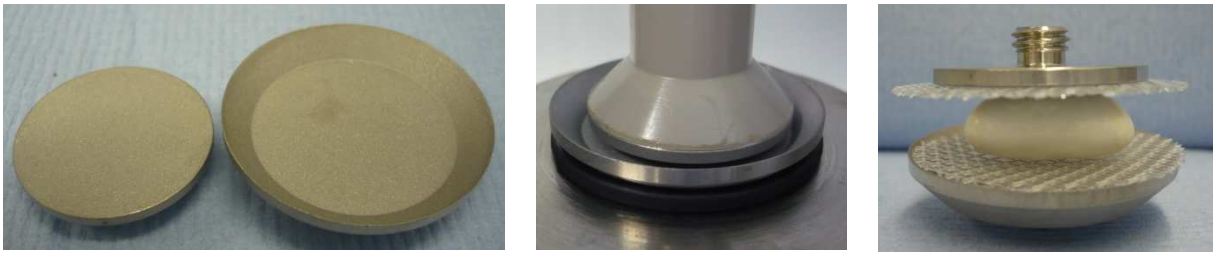
One of the other compendial apparatus commercially available was designed by Wennergren (Kvist, *et al.* 1999, Kvist, *et al.* 2000). The schematic representation of the Wennergren chewing apparatus is shown in the following Figure 4-3. The chewing procedure consists of reciprocations of the lower surface in combination with a shearing (twisting) movement of the upper surface that provides mastication of the chewing gum and at the same time adequate agitation of the test medium. The upper jaw has a flat surface that is parallel to the central part of the lower surface. The small brim of the lower surface is angled upwards (45 degrees) so that the lower surface functions as a small bowl with a flat bottom (Figure 4-4). This bowl prevents the chewing gum from sliding during mastication. Investigations on the performance of the chewing apparatus with multiple drug products were published by the authors (Kvist, *et al.* 2000). The influence of different operational parameters of the chewing gum apparatus on drug release have also been carefully investigated (Kvist, *et al.* 2000).



(a) (b)

Figure 4-3. Apparatus B for the drug release testing of medicated chewing gums

a) Schematic representation of the apparatus B described in the Ph. Eur. and in the Pharmeuropa. Green arrows with numbers indicate the operational sequence of the apparatus. b) Construction of single module of chewing apparatus in a mounted position. Picture shows the glass test chamber with the inlet and outlet for pre-tempered water flow.



(a) (b) (c)

Figure 4-4. Un-mounted parts of the chewing apparatus

a) Figure showing the upper and the lower (angled brim) chewing jaw. b) Mounted position of the chewing jaws in the apparatus. Position represents actual chewing distance set between the chewing jaws for the drug release testing. c) Visual representation of the chewing gum placed between the nylon nets positioned between the chewing jaws before the start of the chewing procedure.

Both the apparatus described have been investigated and reported (Kvist, *et al.* 1999, Pharmeuropa 2008). The results show that these apparatus can provide strong mechanical forces that influence drug release and can prove to be a useful tool for drug release testing of MCGs both in quality control as well as in product development. To some extent, relevance to *in vivo* behavior has been demonstrated using both the apparatus (Kvist, *et al.* 1999, Rider, *et al.* 1992).

4.4 Preparation of buffer solutions

The buffer solutions described herein are used for the extraction of API from the gum formulations and for the solubility investigations. The buffers solutions listed are either part of the compendia described in the Ph. Eur., and the USP (USP 2015) or developed separately in order to provide better characterization of the biopharmaceutical quality of the product. The composition of all the buffer solutions used for the study is given in the Table 4-3 – Table 4-6.

Table 4-3. Composition of compendial (USP/Ph.Eur) buffer solutions

Index / Name	Composition/ preparation
0.01M HCl	98 mL HCl 32 % ad 10 L purified water
0.05M Acetate buffer pH 4.5	18.0 g Sodium Acetate and 16.8 g glacial acetic acid ad 10 L purified water, pH adjusted with glacial acetic acid
Phosphate buffer pH 5.5	68.1 g KH_2PO_4 and 1.4 g NaOH ad 10 L purified water, pH adjusted with H_3PO_4 85 %
Phosphate buffer pH 6.8	68.1 g KH_2PO_4 and 9.0 g NaOH ad 10 L purified water, pH adjusted with 1N NaOH
Phosphate buffer pH 7.4	68.1 g KH_2PO_4 and 15.6 g NaOH ad 10 L purified water, pH adjusted with 1N NaOH

Table 4-4. Composition of extraction buffer solutions

Index / Name	Composition/ preparation
0.04M Phosphate buffer pH 2.5 (extraction solution)	3.4 g KH_2PO_4 ad 1000 mL purified water, pH adjusted with H_3PO_4 85 %
0.02M Phosphate buffer pH 4.4	2.72 g KH_2PO_4 ad 1000 mL purified water, pH adjusted with 1N NaOH

Table 4-5. Composition of the simulated saliva pH 6.2

Substance	Quantity mM/L
KH_2PO_4	12
NaCl	40
$CaCl_2$	1.5
NaOH (1N)	to pH 6.2

The dissolution medium was prepared using the corresponding quantities listed in the table. The simulated saliva was filtered using the 0.45µm Pall membrane filters prior to drug release testing/preparation of the standard solutions.

Table 4-6. Composition of dissolution media for solubility investigations

Medium	Composition	Quantity
Simulated gastric fluid pH 1.2, SGFsp (USP 38)	HCl 32 % NaCl purified water adjusted to	70 mL 20.0 g 10.0 L
0.05 M Phosphate buffer solution, pH 4.5	KH_2PO_4 purified water adjust to	68.05 g 10.0 L
0.05 M Phosphate buffer solution, pH 5.8	KH_2PO_4 NaOH purified water adjust to	68.05 g 1.44 g 10.0 L
0.05 M Phosphate buffer solution, pH 6.8	KH_2PO_4 NaOH purified water to	68.05 g 8.96 g 10.0 L
0.05 M Phosphate buffer solution, pH 7.4	KH_2PO_4 NaOH purified water to	68.05 g 15.64 g 10.0 L

4.5 Method of pharmaceutical analysis for gum formulations

Prior to the feasibility study, analytical methods were developed and validated to quantify the API in the chewing gum formulations. The developed methods were generally used for one or more of the following;

- assay and content uniformity testing
- quantitation of active (s) contained in chew-out study samples
- *in vitro* drug release and stability testing

4.5.1 Nicotine based chewing gum formulations

The quantification of nicotine released from the chewing gums was performed using the HPLC-UV technique. The methods described below were primarily used either for the quantification during *in vitro* drug release testing (method A and B) and content uniformity/ assay testing purposes (method C). Additionally, method A is useful for determining nicotine concentration from the flavored (mint) gum formulations. In most of the situations, the flavored components are released along with the actives and separation of the chromatographic peaks is important to quantify the nicotine without any interferences.

4.5.1.1 Method A for *in vitro* release samples

Stationary phase:	Xterra RP 18, 150 x 4.6 mm, 5 µm
Mobile phase:	Methanol/0.04M KH_2PO_4 buffer pH 6.5, 2/98 (V/V)
Flow rate:	1.2 mL / min
Column temp:	30°C
Sample temp.:	ambient (approx. 25 °C)
Injection vol.:	50 µL
Detection:	UV, 260 nm
Run time:	5 min
Ret. time Nicotine:	approx. 4.0 min

4.5.1.2 Method B alternative method for *in vitro* release samples

Stationary phase:	XBridge RP 18, 150 x 3.9 mm, 3.5 µm
Mobile phase:	Methanol / 0.04M KH_2PO_4 buffer pH 6.5, 15/85 (V/V)
Flow rate:	1.0 mL/min
Column temp.:	30°C
Sample temp.:	ambient (approx. 25 °C)
Injection vol.:	20 µL
Detection:	UV, 260 nm
Run time:	5 min
Ret. time Nicotine:	approx. 2.1 min

4.5.1.3 Method C for content uniformity / assay testing

Stationary phase:	Xterra RP 18, 150 x 3.9 mm, 5 µm
Mobile phase:	Acetonitrile / 0.04M KH_2PO_4 buffer pH 4.5, 2/98 (V/V)
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Sample temp.:	ambient (~25 °C)
Injection vol.:	20 µL
Detection:	UV, 260 nm
Run time:	5 min
Ret. time Nicotine:	approx. 2.1 min

4.5.2 Dimenhydrinate based chewing gum formulations

The analysis of drug concentration of dimenhydrinate, 8-chlorotheophylline and diphenhydramine in different buffer solutions was carried out sequentially according to the method described in section 4.5.2.1.

4.5.2.1 Method for *in vitro* drug release, assay and solubility investigations

Stationary phase:	Lichrospher 60 RP-Select B, 125 x 4 mm, 5 µm
Mobile phase:	Methanol/KH_2PO_4 buffer pH 2.6, 33/67 (v/v)
Flow rate:	1.0 mL/min
Column temp.:	25 °C
Sample temp.:	Ambient (approx. 25 °C)
Injection volume:	20 µL
Detection:	UV, 220 nm
Run time:	20 min
Ret. time 8-Chlorotheophylline:	approx. 4.3 min
Ret. time Diphenhydramine:	approx. 15.5 min

4.5.3 Caffeine based chewing gum formulations

The quantification of caffeine present in the *in vitro* release samples was carried out according to the method described in section 4.5.3.1.

4.5.3.1 Method for *in vitro* release samples

Stationary phase:	Symmetry C18, 75 x 4.6 mm, 3.5 µm, 100°A
Mobile phase:	Acetonitrile/water (10/90, v/v)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Sample temp.:	Ambient (approx. 25 °C)
Injection vol.:	10 µL
Detection:	UV, 244 nm
Run time:	6 min
Ret. time caffeine:	approx. 3 min

4.6 Stability testing procedure

The stability testing of *in vitro* release samples from the nicotine containing gum formulations were additionally performed. The purpose was to ensure the stability of the samples during transport from one site to the other prior to the HPLC analysis. The initial *in vitro* feasibility study was performed at two different laboratories located in Germany and Denmark, owing to the availability of the two apparatus at the respective sites. The *in vitro* release samples simulated in Denmark was frozen to -5.0 °C and transported to Germany for further analysis. The stability was tested on samples generated from Nicotinell and Nicorette 2 mg dosage strengths and stored at -5.0 °C and on lab table for the period of 15 days and investigated at regular intervals. The analysis was performed using the method established earlier.

4.7 Method of solubility investigations

The solubility of nicotine and caffeine is already reported in the literature (Caudle, *et al.* 2001, Jessen, *et al.* 2003, Potard, *et al.* 1999). Nicotine salt is considered to be highly soluble and caffeine exhibits pH independent solubility behavior with an aqueous solubility of 21.74 mg/mL measured at room temperature. The highest dosage strength available for nicotine and caffeine chewing gum is 4 mg and 40 mg respectively. Based on the solubility data given, the drug release testing volume of 40 mL is sufficient to maintain the sink conditions for both the API's. However, the dimenhydrinate is a poorly soluble substance and the salt form enhances its solubility characteristics. Due to these reasons, the solubility of dimenhydrinate in various buffer solutions was investigated. Since the volume of buffer solution used in the chewing apparatus for release testing is limited to 40 mL, it was necessary to evaluate whether the given volume would be sufficient to dissolve the highest dosage strength (20 mg) and to ensure the dissolution / drug release is not limited by the API's solubility characteristics. For this purpose, 30 mg of each of dimenhydrinate, diphenhydramine hydrochloride and 8-chlorotheophylline were accurately weighed in duplicate in a screw-cap vial and suspended using 20 mL buffer solution of different pH values covering the complete physiological range. Each suspension was shaken (400 angular rotations/min) over 24 h at room temperature. When the sediment at the bottom of the screw-cap vial was observed, aliquots of the samples were carefully withdrawn and subjected to centrifugation at 4200 rpm for 10 min at 22°C. Then the samples were suitably diluted with the respective buffer solutions to bring the concentration theoretically equivalent to 100 % of the standard solution.

The drug concentration in the clear supernatant was determined using HPLC method described earlier in the section 4.5.2. If the complete amount of drug was dissolved, the solubility of the drug was assumed to be higher than the concentration corresponding to the amount dissolved. Theoretically, the solubility > 0.5 mg/mL was predicted sufficient to

conclude that the solubility would not be the rate limiting step during the drug release testing of MCGs containing 20 mg of dimenhydrinate in 40 mL of the dissolution medium.

4.8 Assay and content uniformity testing

An assay method to determine the amount of nicotine present in the chewing gum has already been described in the USP. The method involves use of n-hexane to disperse and dissolve the gum matrix, followed by phase separation and analysis of nicotine present in the organic phase. The procedure was found to be more complex and reproducibility of the results had to be scrutinized. Furthermore, unlike other gum formulations, the nicotine present in the gum formulation is bound to ion exchange resin. The release of nicotine is usually an ion exchange process or otherwise the resin particles have to be dissolved to release the nicotine ions. Besides, the described HPLC method in the USP involves ion exchange chromatography, where the surface properties of the column is changed using the modifiers which render the column unusable for normal chromatographic applications. Due to the disadvantages associated with the compendial procedure, a separate methodology to extract the available nicotine in the gum formulation was developed and quantified using the developed HPLC method described in section 4.5.1.

4.8.1 Single step liquid-liquid extraction

A single step liquid-liquid extraction (LLE) was developed in house to quantify nicotine. The method involves dispersing and dissolving the gum matrix in a suitable organic phase. Later, the undissolved nicotine resinates from the organic phase was extracted using an aqueous buffer solution. The method was found to be acceptable and reproducible. The advantage of this method is that it could be performed well in a QC laboratory, and the method can be extended not only to test the content uniformity but also to quantify the nicotine present in the cud (remaining) gums obtained during the *in vivo* chew out studies.

4.8.1.1 Methodology

10 pieces of nicotine containing chewing gums from the same batch were individually weighed. Each gum was cut into small pieces and each transferred separately to 40 mL glass stoppered conical flasks. To this, 10.0 mL of n-Heptane was added and sonicated for 20 min at a temperature below 30 °C. The samples were cooled to room temperature and 20 mL extraction buffer solution (0.02 M phosphate buffer pH 4.4) was pipetted into the samples dissolved in n-Heptane solution. The sample solutions were shaken mechanically for 5 min and stirred vigorously for 30 min. After stirring, the sample solutions were kept aside without disturbing until the phases separated. From this, 5 mL of the aqueous phase was carefully pipetted without disturbing the insoluble components and filtered using 0.45 µm Acrodisc LC-13 mm PVDF membrane filters. The first 2 mL of the filtrate was discarded prior to filling of the HPLC vials. The samples were analyzed in duplicate. The results were reported

as the mean and individual recovery from 10 pieces of chewing gum calculated using an external standard calibration method. In order to quantify the amount of nicotine present in the samples, an external standard calibration curve was constructed using five different concentrations of nicotine hydrogen tartrate solution.

4.9 *In vitro* drug release testing

4.9.1 Non-compendial method: "Home Depot"

The *in vitro* drug release studies from the chewing gum formulations were initially performed using the compendial apparatus described in the pharmacopeias. The goal was to investigate the release using conventional/compendial methods rather than using a specialized apparatus. Additionally, the test would serve as a tool to establish the safety of the dosage form during accidental swallowing of the dosage form.

4.9.1.1 Test methodology

Nicotine based gum samples (Nicotinell and Nicorette) of 2 mg dosage strength were chosen for the study. The geometry of the gum formulations (multi-source) is different from each other. Since the hydrodynamics of the medium in the vessel has no significant role in the disintegration of the dosage form, it is assumed that the drug release can only be influenced by the diffusion of the active in the gum matrix after wetting and permeation of the drug release medium. In order to better evaluate different test products under similar test conditions, the gum formulations were subjected to a specific force of 5 KN for 10 s in the "home-depot" apparatus. By this way, the chewing gums of different geometries were transformed to uniform shape and size. The surface area of the gum formulations after transformation was approximately 11.39 cm^2. The surface modified gums and the dissolution testing is shown in Figure 4-5 and Figure 4-6.

The parameters of *in vitro* drug release methodology are given in section 4.9.1.1.1. The chewing gums of uniform surface area were placed inside the sinker and the test was performed like a conventional dissolution. Samples were withdrawn at suitable intervals, filtered and analyzed using a previously validated HPLC-UV method.

4.9.1.1.1 Method parameters

Apparatus:	USP Paddle <2>
Medium:	Simulated saliva pH 6.2
Volume [mL]:	250
Temperature [°C]:	37± 0.5
Rotation [rpm]:	75
Sinker:	Stainless steel closed
Filtration:	0.45 µm PVDF filter
Vol. replacement:	Yes (with freshly prepared medium)

Figure 4-5. Chewing gums of uniform surface area after pressed with "Home Depot" method

Upper row representing Nicotinell 2 mg mint and below is the Nicorette 2mg freshmint. Average surface area exposed to the drug release medium is approx. 11.39 cm^2.

Figure 4-6. Conventional dissolution tests with MCGs

Picture showing the dissolution testing of nicotine based chewing gums using the USP paddle apparatus with n=3 units for two different chewing gums containing nicotine as active pharmaceutical excipient. The gums are placed in the sinker before introducing them into the test vessel.

4.9.2 Chewing apparatus: Compendial

In order to evaluate the suitability of the release testing apparatus, a feasibility study plan was developed and implemented. The apparatus parameters expected to have strong influence on the drug release characteristics from the chewing formulations were identified and combination of these parameters was considered for the study design. The initial study was performed using the multisource nicotine based products using both the apparatus described in the Ph. Eur. and further testing was done using Apparatus B.

4.9.2.1 General method parameters for release testing

Apparatus:	Medicated gum apparatus (Apparatus A and B)
Number of units:	one piece of gum
Dissolution medium:	artificial saliva pH 6.2
Volume of buffer:	40 mL
Temperature:	37 °C ± 0.5 °C
Sampling time:	2, 5, 10, 15, 20, 30, 45 and 60 min
Sampling vol.:	3 mL (discard first 1 mL)
Filtration:	0.45 µm Acrodisc LC-13 mm PVDF membrane filter

The study design adopted for the feasibility study is given in the following tables. The *in vitro* drug release testing was performed on nicotine containing chewing gums according to the apparatus parameters given in Table 4-7 - Table 4-8.

Table 4-7. Operational settings for apparatus A

Setup	Distance between horizontal pistons [mm] at its closest position within the chewing chamber	Chewing frequency [strokes/min]	Distance from vertical piston to bottom [mm] of chewing chamber
1	0.3	40	3
2	0.3	60	3
3	0.5	40	3
4	0.5	60	3
5	0.5	40	6
6	0.5	60	6
7	0.7	40	3
8	0.7	60	3

It is generally assumed that the apparatus A and apparatus B variables given in the Table 4-7 and Table 4-8 might have an influence on the release of the active(s) from chewing gum formulations.

Table 4-8. Operational settings for apparatus B

Setup	Distance between upper and lower chewing jaw at its closest position [mm]	Chewing frequency [strokes/min]	Twisting angle of jaws [°]
1	1.4	40	20
2	1.4	60	40
3	1.4	40	20
4	1.4	60	40
5	1.6	40	20
6	1.6	60	40
7	1.6	40	20
8	1.6	60	40
9	1.8	40	20
10	1.8	60	40
11	1.8	40	20
12	1.8	60	40

4.9.2.2 Operational parameters of apparatus A

Vertical chewing distance

The vertical chewing distance defines the distance (mm) between the vertical chewing piston and the surface of the chewing chamber at its closest position during mastication (2 distances were selected, 3 mm and 6 mm). The smallest and default distance adjustment is 3 mm. Theoretically the smaller the chewing distance, larger will be the force exerted on the gum surface resulting in a larger surface area. This will enable more interaction of the medium with the chewing gum matrix, subsequently more drug release into the medium.

Horizontal chewing distance

The vertical chewing distance defines the distance (mm) between the two horizontal pistons within the chewing chamber at their closest position during mastication (3 distances have been selected, 0.3mm, 0.5 mm and 0.7 mm). The default distance is 0.5 mm. Additionally, two other values representing lowest and highest possible distances were chosen to study their influence. Like the vertical chewing distance, the horizontal chewing distance was also assumed to change the surface of the gum formulation impacting drug release.

The combination of these factors together with the chewing frequency, which is directly proportional to the drug release rate, was investigated to understand the importance of these factors in the development of *in vitro* drug release methodology.

4.9.2.3 Operational parameters of apparatus B

The major apparatus B variables that might have an influence on the release of active(s) include;

Chewing distance

The chewing distance defines the distance between the chewing jaws during mastication within the chewing chamber. The default chewing distance is 1.6 mm. Chewing distances of 1.4 mm and 1.6 mm were also chosen for evaluation. It is expected that a chewing distance reduction in chewing distance increases the surface area and drug release as well.

Twisting angle

Another parameter which is not a characteristic of apparatus A is the twisting angle. The twisting angle defines the preset angle to which the upper chewing jaw can twist during mastication such that the surface area of a chewing gum can be renewed for drug release. In case of apparatus B, the expansion of the gum surface and its renewal proceed concomitantly during each stroke. The default twisting angle is 20 °. Additionally, 40° twisting angle was included in the study to evaluate the influence on drug release.

It is generally assumed that the combination of smaller chewing distance, larger twisting angle and higher chewing frequency accelerate the release of active(s) present in the gum formulations. The data generated from the drug release tests were later statistically treated to evaluate the significant factors that might influence the release characteristics of the API from the gum matrix.

4.10 Chew-out study

The purpose of the chew out study in general was to obtain/extract information about the *in vivo* dissolution behavior and to assess the product characteristics. For most dosage forms, it is not possible to estimate the *in vivo* dissolution behavior directly and complex mathematical treatments are necessary to interpret the data obtained from the plasma concentration time or urinary excretion data. Depending on the pharmacokinetic and –dynamic nature of the dosage forms, reliability of the extracted data has to be scrutinized. On the other hand, medicated chewing gums present a different strategy, where the dosage form is still accessible at any time directly even after the administration in the oral cavity. The illustration of the chew-out process compared to the classical bioavailability approach is shown in the figure below.

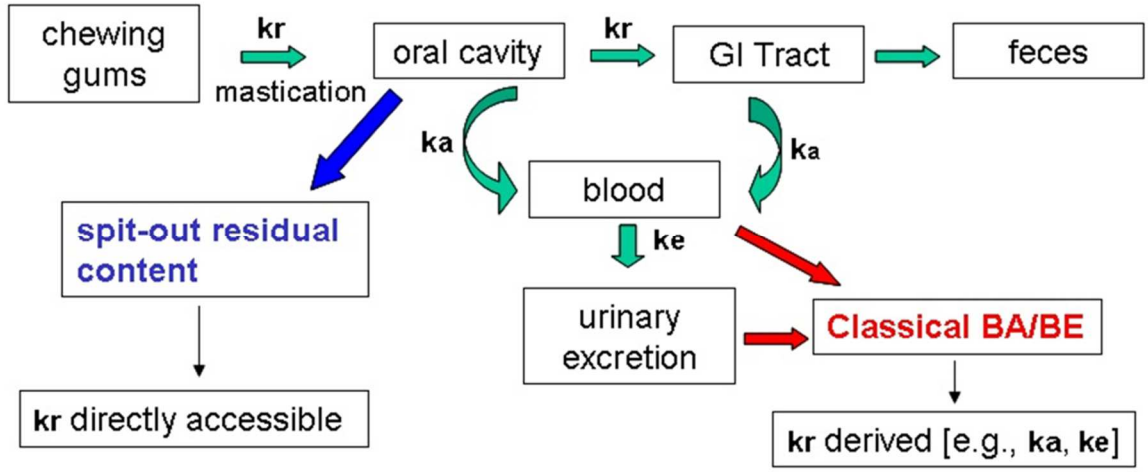


Figure 4-7. Schematic representation of the *in vivo* chew out model vs. the classical BA model
K_r: release rate constant, K_{el}: elimination rate constant, K_a: absorption rate constant
(Taken from Gajendran et. al. 2012)

As shown in the Figure 4-7, the drug product can be directly removed and analyzed for the amount of drug remaining which is a direct indicator of the performance *in vivo*. For this study purpose, trained volunteers chewed a nicotine based gum product of 2 mg dosage strength for a specific period of time. The chew rate was controlled using a metronome set to 40 acoustic signals per minute. After a specific chew out time, the cud (remaining) gum was removed from the oral cavity and was frozen to - 80°C. The frozen gum piece was then finely ground using a mortar and pestle. Quantification procedure described in the assay/content method was used to determine the nicotine content.

4.11 Formulation of nicotine chewing gums by direct compression technique

The main objective of formulating a new chewing gum is to explore the possibilities of utilizing the manufacturing technologies currently available. In case of medicated chewing gums, the conventional methods of manufacturing is considered to be a complex procedure involving a number of techniques not just limited to melting, blending, hot melt extrusion, drying and packaging. Due to presence of moderate humidity in the final product, the shelf life of the product has to be carefully evaluated that delimits the use of conventional techniques to incorporate many pharmaceutically active substances (APIs) in chewing gums.

Recently, manufacturing MCGs using a conventional tabletting technique was explored. The gumbases are available as a dry powdered mixture to which an API and other excipients can be directly mixed and compressed to a gum product (tablet) of suitable size and shape.

The techniques of manufacturing and optimizing the product release behavior have not yet been completely investigated and explored. Various research activities are on the way to extend this platform for a more efficient and economical drug delivery (Conway 2003, Morjaria, *et al.* 2004). Our primary objective was to develop a nicotine based chewing gum formulation by a direct compression technique and to investigate the influence of various factors that might have an influence on the performance of the product.

In this study, techniques to characterize the raw materials, excipients and to optimize a product with desired release characteristics have been investigated. The nicotine release from resinate was optimized using the design of experiments (DoE) and was finally incorporated into the gumbase. The optimized nicotine containing gum product may exhibit its usefulness in the qualification of the existing *in vitro* drug release testing apparatus.

For the described objective, a literature search was initially performed to explore the attempts by investigators to develop chewing gum formulations using a direct compression technique (Conway 2003, Fertin-Pharma 2003, Fritz 2003, Jessen, *et al.* 2003, Lingstroem, *et al.* 2005, Madhav, *et al.* 2009, Maggi, *et al.* 2005, Morjaria, *et al.* 2004, Noehr-Jensen, *et al.* 2006, Ochoa, *et al.* 2008, SPIPharma, Woodford, *et al.* 1981, Yang, *et al.* 2004, Yoshii, *et al.* 2007). There were only few literatures published on this topic. In one single study, the authors have attempted to manufacture gums by incorporating the nicotine polacrilex into the pre-formulated gumbase which is commercially available (Morjaria, *et al.* 2004). The product performance and texture analysis of the gum was evaluated against the commercially available products. The results have indicated that the release of the highly water soluble nicotine in the gum product was rapid and reached 70% of label claim within 10 min of mastication (Figure 4-8).

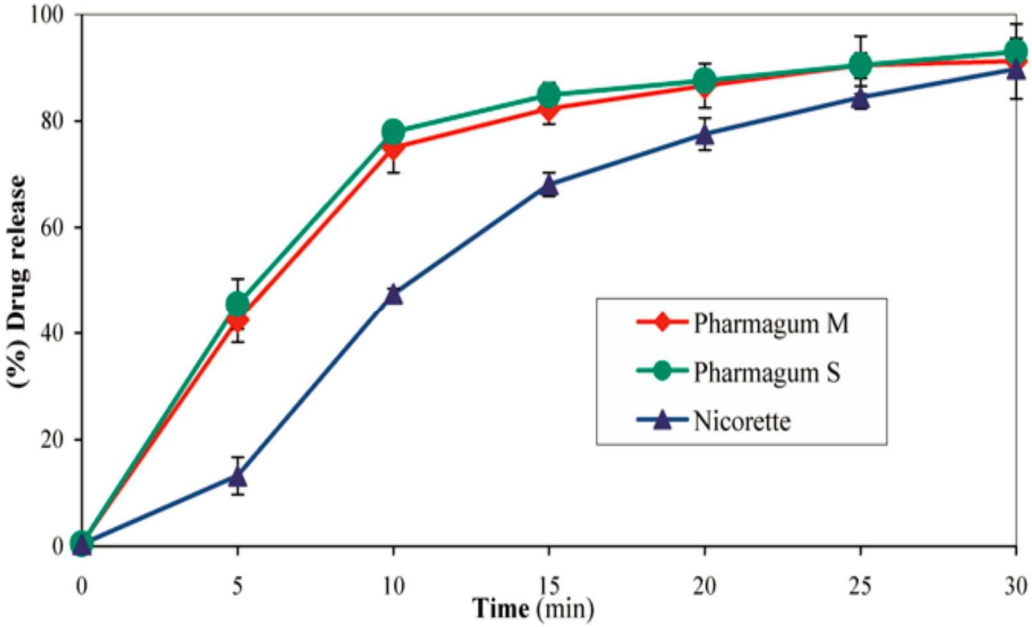


Figure 4-8. Release of nicotine from a commercially available gum and a directly compressed chewing gum formulation

Taken from Conway 2003

This is a most common scenario with a highly water soluble drug in a lipophilic gumbase. Studies have already shown that the part of the active(s) released in the salivary fluid is swallowed and partially absorbed which in most cases metabolized by the gastro-intestinal (GI) and hepatic enzymes, resulting in a poor bioavailability.

One of the aims is to control the release of nicotine from the gum formulation and to optimize its release characteristics to a desired value. For this, various excipients have been carefully considered during the formulation design.

The purpose of excipients in the formulation is to aid any of the following objectives;

- controlled release of the active ingredient from the gum formulations
- aid wet granulation to increase the processability (flowability, compaction etc.)
- coating of the active and or the granulate itself
- optimize the sensitivity of the formulation to the critical operational parameters of the drug release testing instrument.

4.11.1 Ion exchange resin for loading nicotine

Nicotine is a naturally occurring alkaloid and is extracted mostly from tobacco plants. Nicotine as an alkaloid is extremely volatile and unsuitable to be used as an active ingredient in the solid dosage forms. Alternatively, nicotine salts available as nicotine bitartrate or nicotine hydrogen tartrate is readily water soluble and stable, suitable for oral administration.

Since the aqueous solubility of nicotine is rapid, incorporation of nicotine salt in chewing gums results in rapid release within minutes of chewing. It is shown Figure 4-9 that the release of nicotine salt was rapid and independent of the formulation variables.

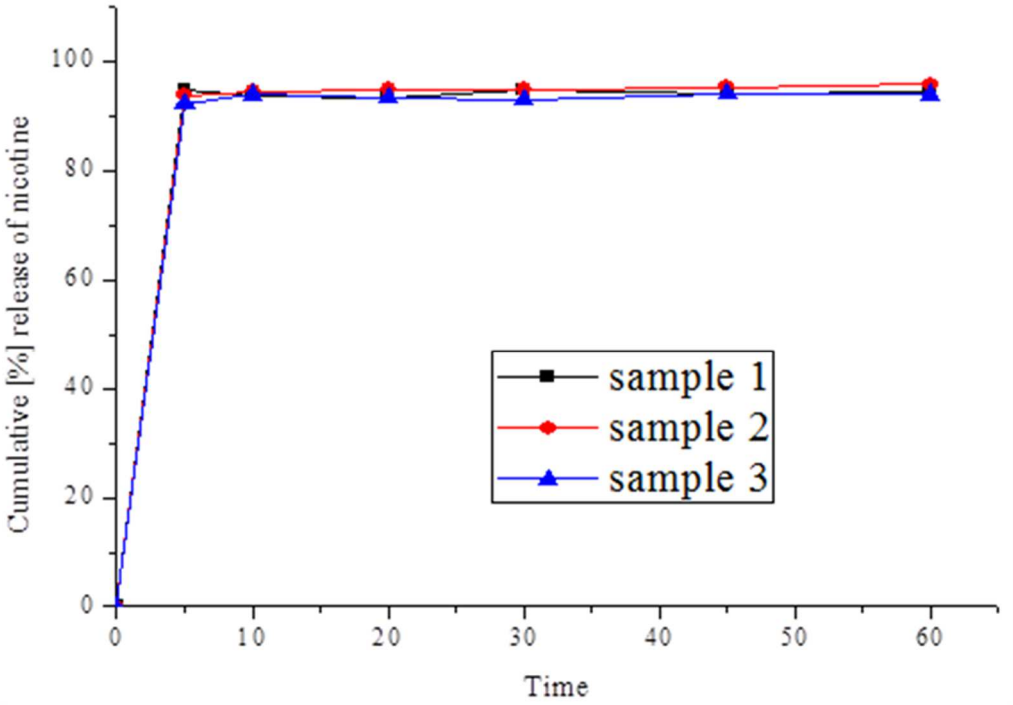


Figure 4-9. Release of nicotine from directly compressed chewing gum formulation

In vitro drug release data generated from Ph.Eur. apparatus B, using 40 mL of artificial saliva pH 6.2 maintained at 37 °C with the following apparatus setup; chewing distance 1.4mm; twisting angle 20° and chewing frequency 40 strokes/min.

One of methods to overcome the disadvantages associated with highly water soluble substance exhibiting immediate release is to utilize the ion exchange resins (IER). Ionizable drug candidates are ideal for utilizing the IER. The selection of IER should be based on the ion group necessary for exchange. The method used to load nicotine and the subsequent evaluation procedures are discussed in the sections 4.11.2 - 4.11.8 (Atyabi, *et al.* 1996, Bodamer, *et al.* 1953, Dowex, Halder, *et al.* 2006, Hughes 2011, Jeong, *et al.* 2008, Singh, *et al.* 2007, Sriwongjanya, *et al.* 1997, 1998).

4.11.2 Preparation of Amberlite IRP64 and IRP 69 resins for loading

Prior to the loading step, the resin particles were washed, activated and dried. The washing and cleaning steps include, initial washing with demineralized water, followed by washing with ethanol 95% and 50% in demineralized water. Then the washing was continued using 1N HCl, followed by water and 1N NaOH. The activated resin was finally washed using demineralized water until the pH of the supernatant was neutral. The resin particles were then dried in the hot air oven maintained at 60 °C for 12h.

4.11.3 Mechanical sieving of resins

Accurately weighed 40 g of Amberlite IRP 69 (Hughes 2011) resin was grounded well using a mortar and pestle. In order to improve the texture and feel of the final gum product, resin particles of 40 µm - 80 µm were chosen for the study. The particles were separated using mechanical sieving prior to loading. For this purpose, 40 g of Amberlite IRP 69 resins was weighed accurately and sieved mechanically using an 80 µm and 40 µm sieve. The retained particles > 80 µm in 0.08 mm sieve and particles < 40 µm passed through 0.04 µm are discarded. The retained particles in 0.04 mm (40 µm to 80 µm) sieve were chosen for the study.

Table 4-9. Mechanical sieving of resins for loading nicotine

Sample	Batch	Test Sieve 1 (VWR)	Test Sieve 2 (VWR)
Amberlite IRP 69 (Sigma Aldrich)	MKBB1426	0.04mm DIN ISO 3310-1 (Sr.nr. 20091228)	0.08mm DIN ISO 3310-1 (Sr.nr.3408248)

4.11.4 Loading of nicotine onto IER

The resins under investigation were tested for their suitability in terms of loading efficiency. About 1 g of each resin was accurately weighed and transferred to a 25 mL conical flask. 10 mL of 0.01M HCl containing 250.09 mg and 499.97 mg nicotine was pipetted to each flask and mechanically stirred for 24 h. Samples were set aside and centrifuged at 11000 g for 10 min before the aliquots are decanted. The aliquots were suitably diluted to bring the expected concentration within the established analytical range and measured using the method established for quantification of nicotine from gum formulation. The resinates are further washed using 0.01M HCl once and twice with demineralized water and centrifuged at 17000 G for 5 min. The solution is decanted and dried at 45 °C for 12-14 h in a hot air oven.

4.11.5 Loading based on particle size

The Amberlite IRP 69 resin obtained commercially from Rohm & Haas contain resin particles of different sizes ranging from 10 µm to 150 µm. Therefore it was necessary to determine the effect of particle size distribution (PSD) on loading of nicotine onto the resin. Additionally, the equilibration time required for loading was also evaluated. For this purpose, 10 mL of 0.01M HCl containing 25 mg/mL of nicotine (available as nicotine hydrogen tartrate salt) was pipetted into a separate glass flask and to this accurately weighed 1 g of the Amberlite IRP 69 resin was added and stirred mechanically. The experiment was performed in triplicate for each of the particle size ranges (< 40 µm, 40-80 µm, > 80 µm). Samples were withdrawn, filtered and analyzed using the HPLC method previously established for the nicotine based gums. The amount of nicotine lost during the sampling was taken into consideration and amount lost directly correspond to the amount loaded onto the resin. Initial concentration of

the solution was measured at t=0 and the reduction of nicotine concentration in the solution is measured at regular intervals and interpreted as the amount loaded per mg of resin.
The following Table 4-10 lists the amount of substances used for batch production of nicotine resinate namely, drug resin complex (DRC).

Table 4-10. Evaluation of batch loading of nicotine onto Amberlite IRP 69 resins

Sample	Batch	Weight [g]	Theoret. Nicotine [mg of nicotine/mg of resin]	Vol. of HCl [ml] for loading
NHT (Sigma Aldrich)	SLBC2533V	21.38 (eq. 7.5 g Nic)	0.250	300
Amberlite IRP 69 (Sigma Aldrich)	MKBB1426	30.01		

NHT: Nicotine hydrogen tartrate, Nic.: Nicotine

4.11.6 Determination of loading efficiency

The objective of this study was to optimize and achieve a resin to nicotine loading ratio of 1:4. Theoretically, Nicotine hydrogen tartrate equivalent to 1 part of nicotine was loaded onto 4 parts of resin. The amount of nicotine present in the supernatant at the end of loading time point, which is approx. 12 h, was evaluated. The loading efficiency of nicotine is calculated by the formula,

$$Loading\ efficiency\ [\%] = \frac{Measured\ nic./mg\ of\ resin}{Theoretical\ nic./mg\ of\ resin} \times 100$$

4.11.7 Impregnation of DRC using polyethylene glycol 6000

It was observed that the nicotine contained in the resins was released rapidly into the buffer during the *in vitro* release testing experiments. Burst release is a common phenomenon observed for all DRCs during the release testing. The release of nicotine from the resin complex is largely influenced by diffusion and ion exchange process, which in turn is accelerated by the water uptake rate. By controlling the water uptake, the DRC retains their size and increases the time needed for the water to permeate, the drug release could be effectively reduced. Another added advantage of DRC pretreatment with PEG 6000 (Pisal, *et al.* 2004) is that it helps the polymer film coat to remain intact during an encapsulation and drying procedure.

For the purpose of impregnation, accurately weighed 8 g of DRC was transferred to the beaker containing 16 mL of 2% w/v PEG 6000. The DRC was soaked in the PEG solution for 1 h and the supernatant was discarded and the resin dried at 45 °C for 24 h until the water content is below 5%. The supernatant was additionally analyzed for dissolved nicotine.

4.11.8 Evaluation of particle size by laser diffraction

Particle size measurements using laser diffraction technique were performed using the wet module Hydro 2000S- Malvern Master Sizer 2000. Fraunhofer measurement model was used to estimate the particle size distribution and the average particle size. Since swelling was observed for resin particles when suspended in an aqueous medium, sunflower oil was used as a carrier medium. Prior to the sample analysis, the sunflower oil was degassed using VTS E1 Vacuum Dryer at 200 mbar for 10 min. Initially, the system was flushed using isopropanol and ethanol, followed by sunflower oil. The system was equilibrated using the dispersion medium at 3000 rpm until stable laser intensity was observed. The system was aligned to record the background. The refractive index (RI) of sunflower oil (1.47) given in the literature (Ariponnammal 2012) was used for particle size estimation. The samples were introduced carefully in the dispersion medium previously filled in the chamber. The obscuration of the samples was kept between 6 -10% volume. The samples were analyzed for 2 min consisting of both the blue and red light laser measurements.

4.11.9 Characterization of DRC by *in vitro* drug release testing

The *in vitro* drug release testing was performed for the resins loaded with nicotine. Conventional method of testing (USP <2> paddle apparatus) was employed to test the release of the active. About 110 mg [1:4 loaded] and 50 mg [1:2 loaded] of DRCs containing 25 mg of active nicotine was weighed individually in a small plastic cells generally used for the dissolution testing of nano-particulate suspensions. The cells were dropped into the dissolution vessel at the respective time points. This prevents the floating of particles during the initial testing period. As the resin particles absorb drug release medium with time, they usually tend to get dispersed in the medium.

4.11.9.1 *In vitro* drug release methodology

The following release testing parameters were used to test the rate of nicotine release from the DRCs.

Sample:	DRC Complex PEG-6000 treated
Apparatus:	USP paddle apparatus <2>
Temperature:	37 °C ± 0.5 °C
Dissolution medium:	Artificial saliva pH 6.2
Rotation:	50 rpm and 100 rpm
Filter:	0.45µm cannula filter
Volume [mL]:	500
Sample volume [mL]:	3
Infinity point:	24 h at 250 RPM

4.12 Coating and micro-encapsulation of DRC

Various methods of coating DRC's have been reported in the literatures (Halder, et al. 2006, Liu, et al. 2007). The methods commonly used for a small batch size are discussed briefly in the following sections.

4.12.1 Agitation and filtration

Methods of coating of resinates have been widely investigated (Halder, et al. 2006, Liu, et al. 2007). This method involves the preparation of DRC slurry in a solvent system in which Eudragit L100 is completely soluble. The solvent system is a mixture of acetone (10 mL) and 0.1M HCl (1 mL) containing PEG 4000 and Tween 20. PEG 4000 and Tween 20 act as a plasticizer and wetting agent for the DRCs. The slurry was stirred using the orbital shaker for 30 min and was filtered under vacuum using 1 µm glass fiber filter (GFF). The residue (coated DRC) was isolated and dried in hot air for 48 h maintained at 45 °C. The coated DRC was then assessed for the nicotine release characteristics. The composition and the corresponding amounts for the slurry are summarized in section 4.11.

4.12.2 Solvent evaporation

The preparation of DRC / polymer slurry is same as that of the procedure described in the agitation and filtration method. However the slurry was transferred to the round bottom flask and agitated by rotation for 1 h in the water bath maintained at 45 °C or until all the organic phase was evaporated. The dispersion was then filtered under vacuum using 1 µm glass fiber filter (GFF) and the residue was dried and stored for further analysis.

4.12.3 Encapsulation and solvent evaporation

A simple cost effective coating of the nicotine resin complex (DRC) was attempted. Suitable polymer [Eudragit L 100] was dispersed in an organic solvent [dichloromethane: acetone] containing Polyethylene glycol [PEG 4000] and DRC. PEG reduces the swelling of the resins and act as a plasticizer for the film coating. The dispersion was transferred to the aqueous phase containing polyvinyl alcohol [0.25 % w/v] which acts as a stabilizer. The dielectric constant of Dichloromethane is 9.1 and the aqueous PVA solution is 2.0, which is suitable for forming the microencapsulated particles.

4.12.3.1 Preparation of DRC dispersed internal polymer phase

The internal polymer phase was prepared by dissolving Eudragit L100 in a solvent system consisting of equal portions of dichloromethane (DCM) and acetone. Previously, the PEG and tween 20 were dissolved in acetone prior to mixing with DCM. The polymer dispersion was prepared by suspending the weighed amount of DRC in the polymer solution. The dispersion was mechanically stirred for 20 min or until the dispersion was homogenous.

4.12.3.2 Preparation of external aqueous phase

For the preparation of the external aqueous phase, 1% w/v solution of polyvinyl alcohol solution (PVA) was prepared by dissolving PVA in demineralized water. The solution was heated upto 80 °C and mechanically stirred until all the PVA particles were dissolved. The solution was cooled to room temperature and was diluted suitably with demineralized water to achieve 0.25% w/v PVA solution to be used as an external aqueous phase.

4.12.3.3 Coating DRC using pH dependent polymer Eudragit L 100

For the coating process, 100 mL of the external aqueous phase was transferred to a beaker and stirred mechanically at 600 rpm using magnetic stirrer. To this aqueous phase, the internal polymer phase consisting of DRC was added and the stirring was continued for 1h to ensure complete evaporation of dichloromethane and acetone from the solvent system. The coated DRC was then filtered under vacuum using 10 µm cellulose acetate filter and the residue in the filter was washed with water and dried at 45 °C for 12 h in a hot air oven. The dried DRCs were sieved using a 40 µm filter to remove the agglomerates. The coated DRC samples were tested for the release characteristics.

Table 4-11. Composition of excipients used for different coating techniques

Composition	**Amounts used for different methods of coating DRCs**		
	Agitation and Filtration	**Solvent Evaporations**	**Encapsulation and Solvent Evaporation**
IRP69-Nicotin-Komplex	1000 mg	1000 mg	1000 mg
Eudragit L 100	1000 mg	1000 mg	1000 mg
PEG 4000	70 mg	70 mg	70 mg
Tween 20	40 mg	40 mg	40 mL
Acetone	10 mL	10 mL	5 mL
Dichloromethane	-	-	5 mL
0.01M HCl	1 mL	1 mL	-
Polyvinyl alcohol (PVA)	-	--	100 mL (0.25% w/v)

In vitro drug release on the coated DRC samples were performed using the USP <2> paddle method described earlier. Since the Eudragit L100 dissolved rapidly at above pH 6.0, acetate buffer pH 4.5 was used to evaluate the release of nicotine from the coated DRCs.

4.12.3.4 Coating DRC using pH independent polymer Eudragit RS 100

Several studies have demonstrated the usefulness of Eudragit RS100 polymers in controlling the rate of release of the pharmaceutically active substances. Our goal is to utilize a simple approach to coat the DRC and to investigate the effect of coating in terms of release and optimize the release characteristics of nicotine from the coated DRC which can later be used as an API in the chewing gum formulations.

4.12.3.4.1 Eudragit RS 100

EUDRAGIT® RS 100 is a copolymer of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups. The ammonium groups are present as salts and make the polymers permeable.

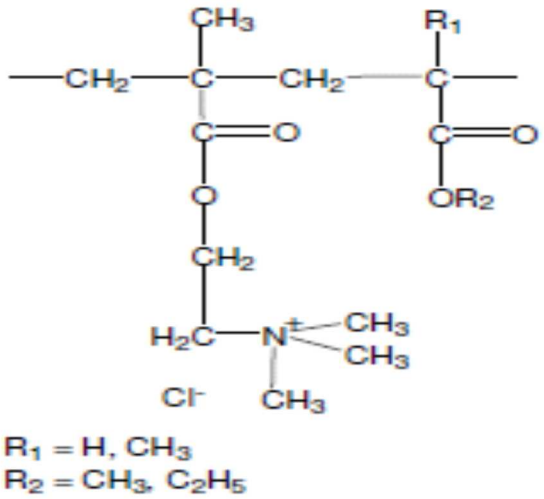


Figure 4-10. Structure of Eudragit RS 100

4.12.3.4.2 Microencapsulation method

The methods of encapsulation have already been widely investigated for various purposes (Cuna, *et al.* 2000, Halder, *et al.* 2006, Junyaprasert, *et al.* 2008, Liu, *et al.* 2007, Sriwongjanya, *et al.* 1997, Torres, *et al.* 1998). The microencapsulation method can be initiated by the formation of oil-in- water (O/W) emulsion and subsequent evaporation of the organic phase and separation by vacuum filtration. In case of O/W method, the resin particles are suspended in a polymer containing solvent system followed by emulsification of organic phase using an aqueous phase usually the liquid paraffin containing surfactants. The resulting emulsion was mechanically stirred at a controlled temperature to ensure complete evaporation of the organic phase. The coated microcapsules will be separated by vacuum filtration.

For the initial feasibility studies, O/W method was chosen. The composition of the formulations is given in Table 4-12. Accurately weighed resin particles of the core to coat ratio given in the table was suspended in a solution containing dissolved Eudragit RS 100 and phosphatidyl choline as a stabilizer. The suspended resin particles were then emulsified by transferring the contents to 150 mL of 0.25% w/v PVA solution. The emulsion was allowed to stir at 600 rpm for 1h in a rotating round bottom flask heated externally by a water bath maintained at 30 °C. After the complete evaporation of dichloromethane, the microcapsules were recovered by vacuum filtration and washed using 200 mL of deionized water and dried at 50 °C in oven for 24 h.

Table 4-12. Microencapsulation of DRC using Eudragit RS 100

Composition	**F1**	**F2**	**F3**
DRC [mg]	250	250	250
Eudragit RS100 [mg]	100	300	500
Phosphatidyl Choline [mg]	12.5	12.5	6
Dichloromethane [mL]	10	6	6
Polyvinyl alcohol [0.25% w/v]	150	150	150

The coated DRCs were tested for their release characteristics.

4.13 Design of Experiments (DoE)

The primary goal of the study is to optimize the coating efficiency by which a well-controlled release of nicotine from the resin matrix is achieved. The coating also provides the stability of the DRC at lower pH and retards the release of the nicotine. The amount of polymer, wetting agents and the total volume of the organic solvent do affect the coating efficiency.

In order to investigate the influence of different concentrations of Eudragit RS100, lecithin and dichloromethane (DCM) on the coating and subsequent release of nicotine from the coated DRCs, a "Box Behnken-surface response methodology" given in the design expert software was employed. The design requires 3 levels within a numerical factor and can be extended to 10 different factors. This procedure creates designs with desirable statistical properties but, most importantly, with only a fraction of the experiments required for a three-level factorial. Because there are only three levels, the quadratic model was considered to be appropriate.

The complete design was limited to 17 experimental runs with 5 center points per block. The cube model of the study is shown in the Figure 4-11.

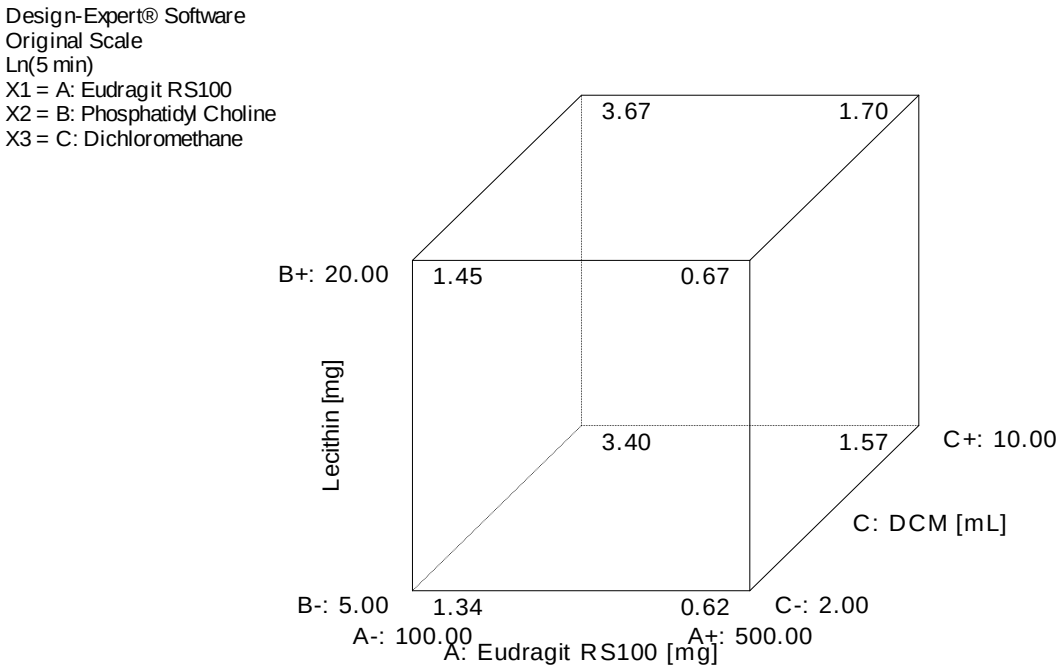


Figure 4-11. Illustration of the proposed model for DoE experiments

The components and the corresponding amounts used for the study is shown along the axis

The proposed design for the current study is summarized in the Table 4-13. The corresponding coded values for the amounts are also given for reference.

Table 4-13. Summary DoE for coating of drug resin complex

Run	A: Amt. of Eudragit RS 100 [mg]	A: Eudragit RS 100 coded value	B: Amt. Phosphatidyl choline [mg]	B: Phosphatidyl choline coded value	C: Vol. of DCM [mL]	C: Vol. of DCM coded value	Amt. of DRC weighed [mg]
1	500	1	12.5	0	10	1	250.59
2	100	-1	5	-1	6	0	250.59
3	300	0	5	-1	10	1	250.64
4	300	0	12.5	0	6	0	249.72
5	300	0	5	-1	2	-1	251.43
6	300	0	12.5	0	6	0	251.17
7	100	-1	12.5	0	2	-1	249.97
8	500	1	5	-1	6	0	249.19
9	500	1	20	1	6	0	249.80
10	300	0	20	1	10	1	250.95
11	100	-1	12.5	0	10	1	250.12
12	300	0	20	1	2	-1	250.79
13	300	0	12.5	0	6	0	250.41
14	300	0	12.5	0	6	0	251.04
15	500	1	12.5	0	2	-1	251.89
16	300	0	12.5	0	6	0	250.76
17	100	-1	20	1	6	0	250.43

There were totally 17 different combinations/designs generated by the design expert for the proposed model. The DRC's were coated according to the method described earlier using the O/W emulsification method. The coated DRCs produced were vacuum filtered, washed, dried and stored for further analysis.

4.13.1 Evaluation of drug leaching during coating

The release of drugs bound to ion exchange resin is controlled by diffusion process. The feasibility of the coating method could be verified by the amount of nicotine released to the external aqueous phase during the coating procedure. In this study, 0.25% w/v polyvinyl alcohol has been used as an external aqueous phase which is immiscible with the internal organic phase “dichloromethane”. For the purpose of determining the amount of nicotine released into the external phase, about 3 mL of PVA solution was removed from the solution containing coated resin samples and filtered using a 0.45 µm filter before filling into the HPLC vials. The nicotine present in the samples was quantified using a previously established and validated HPLC-UV method.

4.14 Fourier transform infra-red analysis

The Fourier transform-infrared (FTIR) spectroscopy utilizes various types of measurement method, such as the diffuse reflection method and attenuated total reflection method. The selection of the measurement method depends on the type of sample available. All the samples used for this study were in the powder form. The classical method utilizing the potassium bromide (KBr) pellet was used to prepare the samples (Breunig, *et al.* 2005). The purpose of this method was to evaluate the compatibility of various excipients and their interaction during the microencapsulation process.

4.14.1 Methodology

The concentration of the sample in KBr should be in the range of 0.2% to 1% w/w. Prior to the preparation of KBr pellets the KBr was kept in the hot air oven for 1h maintained at 100°C. The KBr was removed from the oven and about 1g of the powder was finely ground using a mortar and pestle. To this 1000 mg of KBr, about 10 mg of the sample was added and mixed using mortar and pestle until a homogenous mixture was obtained. The finely powdered sample mixture was transferred to the pellet forming die and compressed into a pellet using 5-8 tons for 5 min. The clear transparent pellets were carefully removed from the die before the measurement. A blank spectrum was also recorded using the KBr alone prior to the sample measurements. Care was taken to produce clear pellets. The measurement was done using the Perkin Elmer system and the spectrum obtained was later processed using the Essential FTIR software.

Different components of the DRC were incorporated into the KBr pellet and the FTIR spectra were obtained. Additionally, simple physical mixture of DRC and Eudragit RS 100 was prepared in 1:1 ratio by weighing 0.5 g of DRC and 0.5 g of Eudragit RS 100 together and mixed mechanically using a pestle and mortar until a homogenous mixture was obtained.

4.15 Manufacturing of nicotine chewing gums by direct compression

The primary objective of this study was to develop a nicotine based gum formulation which may exhibit its usefulness in the qualification of the existing *in vitro* drug release testing apparatus and is not intended to demonstrate the superiority to the marketed formulations. For the manufacturing of chewing gums, commercially available DC gum bases were used. Each of the gum formulation contains either nicotine hydrogen tartrate salt or DRC or one of the coated DRC with the amount equivalent to 2 mg of nicotine. The rest of the components used for the manufacturing and optimization are listed in the corresponding result section. Unlike conventional methods employed to manufacture gum formulations involving melting and extrusion, an attempt was made to compress gums into tablets. The techniques involved in the manufacturing are discussed below.

4.15.1 Sieving

The tailored compressible gum base powders contain large aggregate of particles which may not be suitable for tabletting and affect the content uniformity and flowability of the final mixture. Therefore, the gumbase powders were dried at 40 °C for 12 h and sieved using 16 mesh (1.5 mm) to remove any large aggregates. The gumbase powders were stored then in an airtight container.

4.15.2 Mixing and blending

Accurately weighed quantities of all the excipients and the respective gumbases mentioned in the respective results section, excluding magnesium stearate and talc, were mixed and blended together for 10 min using a cone or double cone blenders. Prior to tabletting, the pre-blended components were mixed with the accurately weighed quantities of magnesium stearate and talc and blended further for 5 min and the mixture was directly transferred to the feed hopper of the tabletting machine.

4.15.3 Direct compression technique

The mixtures of different formulation described above were directly compressed using the Korsch EK 0 tabletting machine mounted with a custom made round flat surface punch and the die with beveled edges. The weight of the chewing tablets was adjusted to 1.5 grams/tablet, so that it contains approximately 2.0 g (8.62 g of Polacrilex resin) of bound nicotine. The chewing tablets were collected once constant weight was achieved.

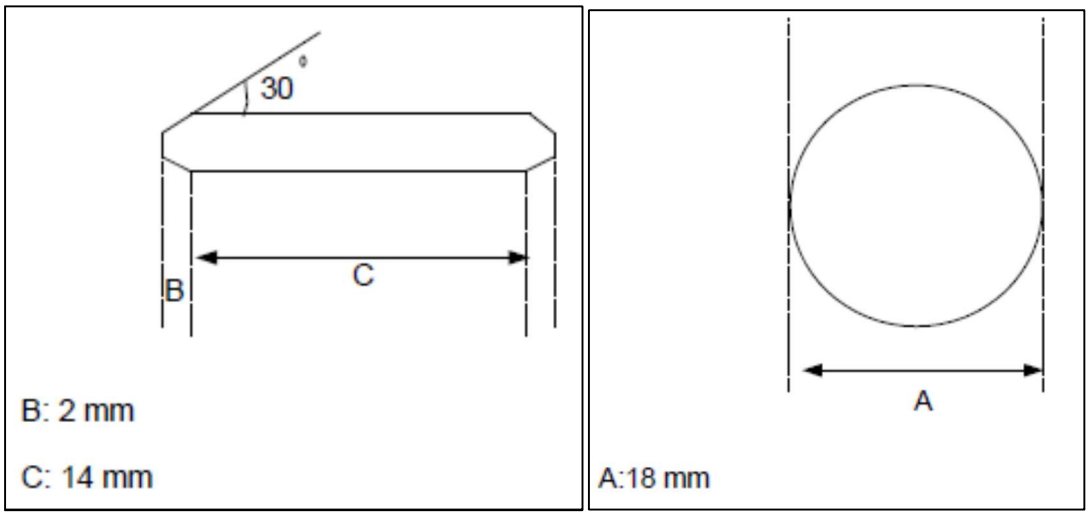


Figure 4-12. Schematic custom made tablet punch and dye for tabletting chewing gums

4.15.4 Evaluation of physical properties of gum formulations

The pharmaceutical quality of the gum formulations were tested using the techniques described in the pharmacopeia (USP 2015) to ensure that the end product meets all the necessary quality criteria and the reproducibility of the data is assured.

4.15.4.1 Evaluation of powder flow properties

The angle of repose is to characterize the flow properties of solids. Angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles. The results are reported to be dependent upon the method used. The values and the interpretation of angle of repose are given in the Table 4-14. Generally for pharmaceutical powders, the angle of repose should be ≤ 40°.

Table 4-14. Interpretation of angle of repose values

Flow property	Angle of repose (°)
Excellent	25–30
Good	31–35
Fair—aid not needed	36–40
Very poor	56–65
Very, very poor	>66

Taken from USP 2015.

4.15.4.1.1 Principle and methodology

The funnel with a retaining lip to retain a layer of powder on the base is fixed at a specific height. The height of the funnel is carefully adjusted to build up a symmetrical cone of powder. Care was taken to prevent vibration as the funnel is moved. The funnel height was

maintained approximately 2–4 cm from the top of the powder pile as it is being formed in order to minimize the impact of falling powder on the tip of the cone. The angle of repose was determined by measuring the height of the cone of powder (Nair, *et al.* 1997) and the base of the cone from the following equation,

Tan (α) = Height/0.5 x Base

The measurement of the powder flow was performed using the Pharmatest flow tester. The angle of repose (Tan α) and the time of flow were directly recorded and reported.

4.15.4.2 Compressibility

The compressibility index is an indirect measure of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials. The compressibility index and the Hausner ratio were determined by measuring both the bulk volume and the tapped volume of a powder.

Generally, the compressibility index of ≤15 and the Hausner ratio of 1.0-1.18 is considered suitable for the powder compaction and possess a good flowability.

The Carr index "C" is an indication of the compressibility of a powder. It is calculated by the formula,

$$C = 100 \times \frac{V_B - V_T}{V_B}$$

where,

V_B = freely settled volume of a given mass of powder

V_T = tapped volume of the same mass of powder

It can also be expressed as,

$$C = 100 \times \left[1 - \frac{\rho_B}{\rho_T} \right]$$

where,

ρB = the freely settled bulk density of the powder, and

ρT = tapped bulk density of the powder.

4.15.4.2.1 Methodology

Accurately weighed 100 g of the sample was transferred to the graduated 250 mL volumetric cylinder and the volume (ρB) was noted. The cylinder was tapped for 750 times using the Erweka tapped density tester and the volume (ρT) was noted. The procedure was repeated for additional 750 times until no further change in the volume was observed. The measurements were performed in triplicate and the final volume was noted. The compressibility of the gum formulation mixtures was evaluated.

4.15.4.3 Weight variation, friability and hardness

For the purpose of testing the uniformity of the weight, 20 individual units of chewing gums from respective formulation were individually weighed. Average weight of the gum and the relative standard deviation was calculated.

The friability of the gums (directly compressed chewing gum tablets) was determined by Roche Friabilator. It consists of a plastic chamber that revolves at 25 rpm, dropping the tablets through a distance of six inches in the friabilator. The tablets were reweighed. A weight difference of less than 0.5% was considered acceptable.

The hardness of solid dosage forms was tested to ensure the ability of the tablets to withstand the mechanical stress during handling, manufacturing, packaging and transport. Hardness generally measures the tablet crushing strength. 10 individual units of the gums were tested using the Erweka hardness tester and results are given in newton (N).

4.16 Method of data evaluation and interpretation

The evaluation and interpretation of the *in vitro* data is crucial in understanding the process involved in the release of the API during testing. The *in vitro* drug release data generated were tested wherever required using the statistical techniques described in the following sections.

4.16.1 Similarity test statistics (f_2)

Method of *in vitro* dissolution profile analysis and comparison has been widely discussed (Anderson, *et al.* 1998, Moore, *et al.* 1996, Podczeck 1993, Sathe, *et al.* 1996) since the inception of *in vitro* dissolution testing as a QC tool for routine analysis, particularly for the waiver of *in vivo* bioequivalence studies (BE). Different techniques have been proposed by various authors to investigate the difference or similarity between the profiles. The most important aspect of such mathematical treatment is to link the chemistry, manufacturing and control variables (CMC) to the dissolution behavior which in turn is used to determine the acceptable end product quality. In a biorelevant environment, the difference observed *in vitro* could be linked to the variability observed between the clinical batches and side batches that might have a greater impact on the bioavailability of the product.

The similarity test analysis provides an effective method of quantifying the difference in the release characteristics of the drug product. Currently, the test statistic is one of the primary requirements for testing and evaluating acceptance of inter-lot homogeneity and intra-lot variability within the product. In case of biowaiver applications for marketing authorization (Wang, *et al.*), direct comparison of the innovator product to the generic versions in terms of performance at various buffer solutions covering the entire physiological range of relevance is justified by the use of similarity test statistics. The requirement of such an evaluation is usually applicable for dosage forms where expected drug release is slower or sustained. Demonstration of similarity of the dissolution profiles is not necessary for IR dosage forms where Q > 85% in 15 min or less.

Currently marketed chewing gums are intended to be chewed for a period of 30 min or less and are expected to release its contents completely or at predetermined level to impart therapeutic efficacy. Since the release of active(s) from chewing gum formulations is dependent on a number of factors like chewing frequency, chewing distance etc., gum formulations cannot be characterized simply as an immediate or sustained release formulation. This holds true for at least all the investigated formulations containing nicotine, dimenhydrinate and caffeine as an active pharmaceutical ingredient.

The similarity test (f_2) proves to be a useful technique to quantify the difference observed between the profiles and to identify the apparatus setups that generate similar profiles for the same product.

The determination of f_2 values were first proposed by Moore, *et al.* 1996. Basically, it is a logarithmic transformation of the sum of the squared error. It considers the average sum of squares of the difference between the test and the reference product. The usefulness of the f_2 calculations were already demonstrated for many dosage forms where the expected drug release is modified or controlled and doesn't fall under the category of IR dosage forms with Q> 85% in less than 15min. The f_2 test statistics was applied to the drug release data from the *in vitro* release experiments to evaluate the apparatus and to study the influence of apparatus parameters on release characteristics. The information also provides the basis for recommending a comparative apparatus setup for *in vitro* drug release testing.

The formula proposed by Moore and Flanner to calculate the similarity factor (f_2) is given as;

$$f_2 = 50x\log\left\{\left[1+\left(\frac{1}{n}\right)\sum_{t=1}^{n} w_t\left(R_t - T_t\right)^2\right]^{-0.5} x100\right\}$$

Where n is number of time points, R_t and T_t are dissolution of reference and test products at time t and W_t is the optional weight factor. A value of f_2 greater than 50 represent the performance of the test and reference product is similar. Usually, the lower limit value of 50 is reached; when the observed difference in dissolution is 10%. With an increase in difference > 10%, the f_2 value falls below 50 and subsequently fails the f_2 criterion.

4.16.2 Mean dissolution time (MDT)

The measured amount of drug substance in a cumulative drug release profile can be considered as a probability that describes the time of residence of the drug substance in the dosage form (Podczeck 1993). In a normal dissolution/drug release testing, the dissolution profile can be considered as a distribution function of residence times of each molecule in the formulation. By this, the mean dissolution time (MDT) may be defined as the arithmetic mean value of any dissolution profile. If the amount of drug remaining in the product or the amount yet to be released is a plotted as a function of time, then the mean residence time (MRT) can be obtained from the profile.

The MDT of a given product from an *in vitro* dissolution/release data can be calculated using the formula;

$$MDT\,[\text{in vitro}] = \frac{\sum_{i=1}^{n} t_{mid}\,\Delta M}{\sum_{i=1}^{n} \Delta M}$$

MDT reflects the time for the drug to dissolve and is the first statistical moment of the cumulative dissolution process which provides an accurate drug release rate. Higher MDT values indicate greater drug retarding ability of the gum components with respect to the settings of the instrument.

4.16.3 Kinetic models for drug release

During the development of any dosage form, it is necessary to ensure that drug dissolution/release occurs in an expected manner to ensure adequate concentrations of the drug substance will available at the site of absorption. The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage form characteristics. In some cases, these mathematic models are derived from the theoretical analysis of the occurring process. In most of the cases the theoretical concept does not exist and some empirical equations have proved to be more appropriate. Drug dissolution from solid dosage forms can be described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time, t.

To describe the release kinetics, various kinetics models such as zero-order (Koester, *et al.* 2004), first-order (Koester, *et al.* 2004), Higuchi equation (Higuchi 1963), Korsmeyer-Peppas equation (Korsmeyer, *et al.* 1983, Korsmeyer, *et al.* 1984), Hixson-Crowell Equation (Hixson, *et al.* 1931), Weibull function (Langenbucher 1972) given in the equations 1 to 6, were fitted to the *in vitro* release data. Some diffusion models like Korsmeyer-Peppas are predicted to be valuable upto 60% release and the regression analysis for such models were restricted to that range. The underlying release mechanism is determined by the diffusional release exponent (n) of the Korsmeyer-Peppas model (Korsmeyer, *et al.* 1983). The coefficient of determination (R^2), slope and the residual sum of squares (SSR) are used to assess the fitness of the proposed model.

$Q_t = Q_0 + k_0 t$ (1)

$\text{Ln } Q_t = \ln Q_0 - k_1 t$ (2)

$Q_t = k_H t^{1/2}$ (3)

$M_t/M_\infty = K\, t^n$ (4)

$Q_0^{1/3} - Q_t^{1/3} = K_{HC}\, t$ (5)

$\text{Log}[-\ln(1-m)] = \beta \log(t-T_i) - \log a$ (6)

In all the kinetic models given, Q_0 and Q_t is the initial and the amount of drug released in time t, K_0, K_1, K_{HC} are release rate constants, M_t/M_∞ is the fractional solute release, n is the release exponent which characterizes the mechanism of drug release. The value of n = 0.45 and less than 1, indicates fickian and non-fickian (anomalous) release, values equal or greater than 1 for case II and super case II release mechanism (Korsmeyer, *et al.* 1983).

4.16.3.1 Release kinetics from Bhaskar and Boyd model

IERs are well known for their properties to deliver actives at a controlled rate (Bhaskar, *et al.* 1986, Boyd, *et al.* 1947). One interesting property "pore diffusion resistance" or "particle diffusion control" of the IER is well recognized to deliver drugs in a controlled manner. In order to produce a good formulation, it is essential to determine the controlling mechanism of release. While diffusivity is the pertinent parameter in case of particle diffusion control, film thickness (or the mass transfer coefficient) is relevant for film diffusion control. Normally, particle diffusion control is expected for drug release from resinate and hence the data is tested for particle diffusion control.

In many instances, the drug release from the DRC is controlled by one of the two mechanisms, diffusion of free drug in the resin matrix and the diffusion of drug across the thin liquid film at the surroundings of the resin particle (Jeong, *et al.* 2007, Reichenberg 1953). The model assumes that the DRC's are uniform spheres with radius "r" and the diffusion of the API within the matrix is the rate limiting step for the further release into the medium. In such a case, the fraction of the drug released F, is given by the equation,

$$F = \frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{e^{-n^2 Bt}}{n^2} \qquad (7)$$

Where, M_t and M_∞ are the amounts of drug released after time "t" and after infinite time, respectively. B is the rate constant, Di represents the effective diffusion coefficient of the exchanging ions inside the resin particle, and n is the summation variable. Generally, it is not possible to estimate the B-values from measured *F* using Eq. (7), because infinite terms are involved. Therefore, Reichenberg 1953 reduced the eq. (7) to estimate the *Bt* values based on F values as following.

For F >0.85, a first term approximation can be used and the equation can be reduced to

$$F = 1 - \frac{6}{\pi^2} e^{-Bt} \quad (8)$$

or

$$Bt = -log_e \frac{\pi^2}{6}(1 - F) = -2.303 log_{10}(1 - F) - 0.498 \quad (9)$$

If the value of F< 0.85, then the equation would be

$$Bt = 2\pi - \frac{\pi^2 F}{3} - 2\pi(1 - \frac{\pi F}{3})^{1/2} \quad (10)$$

$$=6.283\text{-}3.290F\text{-}6.283(1\text{-}1.047F)^{1/2} \quad (11)$$

If the plot *Bt* corresponding to the F value against time (t) gives a straight line, it can be assumed that drug diffusion within the resin matrix is the rate-limiting step (Atyabi, *et al.* 1996, Ichikawaa, *et al.* 2001, Motycka, *et al.* 1985 Jeong, *et al.* 2007).

Furthermore, Bhaskar, *et al.* 1986, derived from an reduced Reichenberg model, an equation to determine the value of B_t in a simple way and to estimate the diffusivity by the equation,

$$-\ln(1 - F) = \ln(Q_0/Q) = 1.59(\frac{6}{d_p}) D^{0.65} t^{0.65} \quad (12)$$

Particle diffusion control can be simply tested from this equation by estimating the linearity between the $\ln(Q_0/Q_t)$ and $F_{0.65}$. The slope of the resulting line is related to the diffusivity of the active(s) within the particle.

$$D = \frac{d_p^2}{36}(slope/1.59)^{1/0.65} \quad (13)$$

The constants 1.59 and the 0.65 proposed by Bhaskar, *et al.* 1986, is applicable to all the drug resin complexes. The *in vitro* drug release kinetics from the DRC was evaluated using the method described by Boyd, *et al.* 1947 and Reichenberg 1953. In our experiments, the *in vitro* nicotine release kinetics was estimated using the Boyd and Reichenberg model and the proposed Bhaskar's model.

4.17 Methods of *in vivo* data evaluation

In order to verify the *in vitro* drug release methodology developed, clinical data obtained from the sponsor (Zenara India Pvt. Ltd) and literatures (Choi, *et al.* 2003, Roteliste 2013, Russell, *et al.* 1985, FDA 1995) were used. The clinical data required suitable mathematical treatment prior to fitting the *in vitro* and *in vivo* data. Both model dependent and independent techniques have been used by authors to develop an *in vitro in vivo* correlation (Bastian, *et al.* 2004). For developing an IVIVC, nicotine based chewing gum formulations were chosen since data pertaining to the *in vivo* release have been reported in literatures and is publicly available. The description of methodologies used for the treatment of data is described in the following sections.

4.17.1 Wagner Nelson back calculation method

Wagner and Nelson developed an equation (Wagner, *et al.* 1963) for calculating absorption rate constant (K_a) and fraction of dose absorbed from plasma drug concentration time profile for an open-compartment model. The Wagner-Nelson method does not require a model assumption concerning the absorption process. The *in vitro in vivo* correlation is generated using pooled mean fraction of dose dissolved (FRD) and pooled mean fraction of dose absorbed (FRA) from two or more formulations.

The objective of the present study is to present a rearranged form of the Wagner-Nelson equation for evaluating IVIVC.

4.17.1.1 Validation of the clinical data to develop IVIVC

Gohel, *et al.* 2005 proposed a technique to validate a model using back calculation of Wagner Nelson method and to test the suitability of the *in vivo* data.

According to the Wagner - Nelson equation,

$$\frac{At}{A\infty} = \frac{Ct + Ke * \int_{t=0}^{t=t} Cdt}{Ke * \int_{t=0}^{t=\infty} Cdt} \qquad (14)$$

where,

A_t = Amount of drug absorbed at time‘t’

A_∞ = Amount of drug absorbed at time ‘infinite'

K_e = Elimination rate constant of the drug

$\int_{t=0}^{t=t} Cdt$ = Area under the curve of the plasma concentration versus time profile of drug, for time period between t = 0 to t = t

$\int_{t=0}^{t=\propto} Cdt$ = Area under the curve of the plasma concentration versus time profile of drug, for time period between t = 0 to t = ∞.

$Ft = \frac{At}{A\propto}$ = fraction of drug absorbed at time't',

$$Ft = \frac{Ct + Ke * \int_{t=0}^{t=t} Cdt}{Ke * \int_{t=0}^{t=\propto} Cdt} \quad (15)$$

However,

$$Ke * \int_{t=0}^{t=t} Cdt = \frac{F\infty * D}{Vd} \quad (16)$$

where,

D = Dose of drug administered
V_d = Apparent volume of distribution

Assuming that at infinite time, the administered dose is completely absorbed, i.e. F∞=1 in equation (16),

$$Ke * \int_{t=0}^{t=\infty} Cdt = \frac{D}{Vd} \quad (17)$$

The rearranged forms of Wagner - Nelson for time t and t+1 are given below:

$$F_t = \frac{Ct + Ke * \int_{t=0}^{t=t} Cdt}{D/Vd} \quad (18)$$

$$F_{t+1} = \frac{C_{t+1} + Ke * \int_{t=0}^{t=t+1} Cdt}{[D/Vd]} \quad (19)$$

Subtracting equation (18) from equation (19),

$$Ct + 1 = \frac{\frac{[2 * \Delta F * D]}{Vd} + Ct(2 - Ke * \Delta t)}{(2 + Ke * \Delta t)} \quad (20)$$

The *in vivo* plasma concentration time data is fit according to the equation (20) and the model is validated for its suitability.

The *in vivo* plasma concentration data obtained from the literature and the sponsor were fit to the above equation to test the validity of the data. This method can also be used to predict the *in vivo* plasma concentration from a biorelevant *in vitro* dissolution data used as an input

variable. In the current study, the back calculation method proposed by Gohel, *et al.* 2005, was used only to test the validity of the data and Wagner Nelson equation was used to determine the fraction of the dose (FRA) absorbed. The elimination rate constant K_{el} was obtained from the slope of the natural log (ln) transformed terminal data points of plasma concentration time data.

The FRA obtained was directly correlated to the *in vitro* data obtained for the nicotine chewing gums.

5 RESULTS

5.1 HPLC data analyses for model drug substances

5.1.1 Linearity of nicotine

The HPLC UV-detector response was linear in the concentration range of 1.93 µg/mL and 144.39 µg/mL. The maximum dosage strength currently available for nicotine containing chewing gums is 4 mg. The maximum expected concentration with a 100 % drug release would be 100 µg /mL considering the volume of drug release medium in the apparatus is 40 mL. The linear regression curve for nicotine is shown below in the graph.

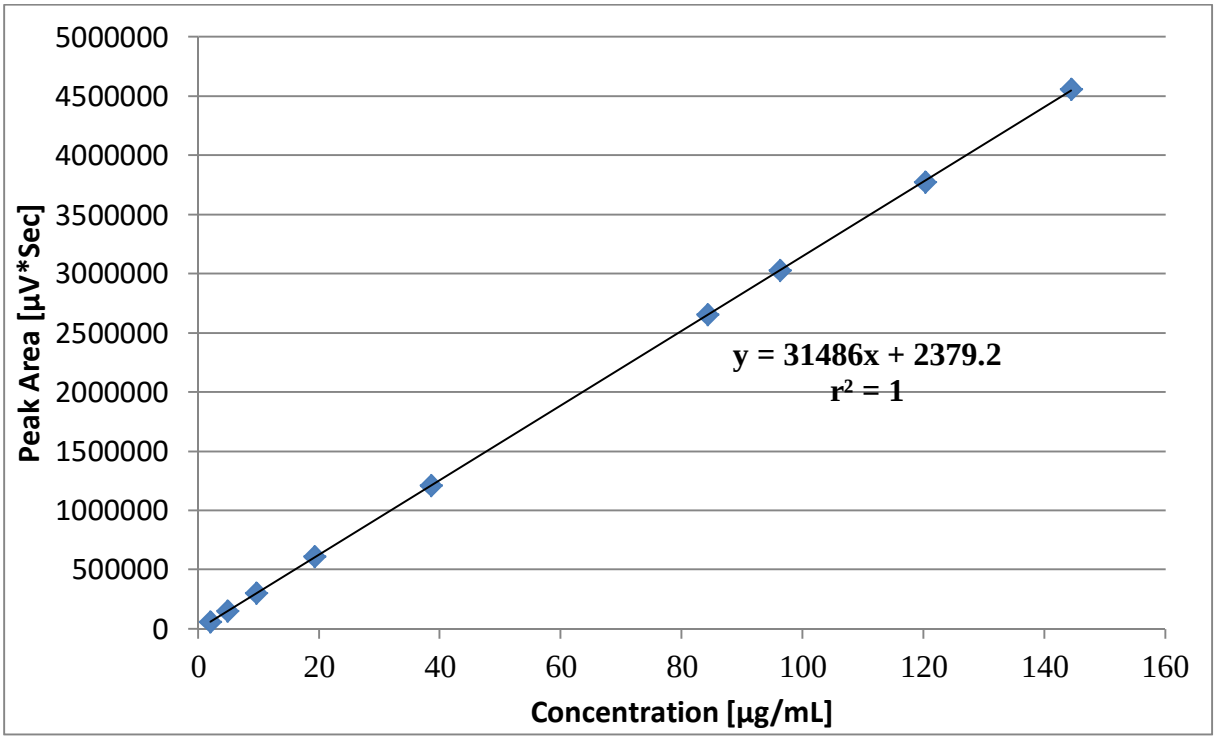


Figure 5-1. Nicotine external standard calibration curve

Table 5-1. Statistical parameters of linear regression analysis of nicotine

Index	Slope [m]	Y-Intercept [C]	Correlation coefficient [r^2]	RSD. resp. factor [%]
Value	31486	2379.2	0.99998	0.58

5.1.2 Linearity of dimenhydrinate

As far as the dimenhydrinate is concerned, it is available as a salt of diphenhydramine and 8-chlorotheophylline. Both of these compounds are eluted in the chromatogram with different retention times. The quantification of the compounds was individually calculated. The results of the analyses are shown in the Figure 5-2 - Figure 5-3.

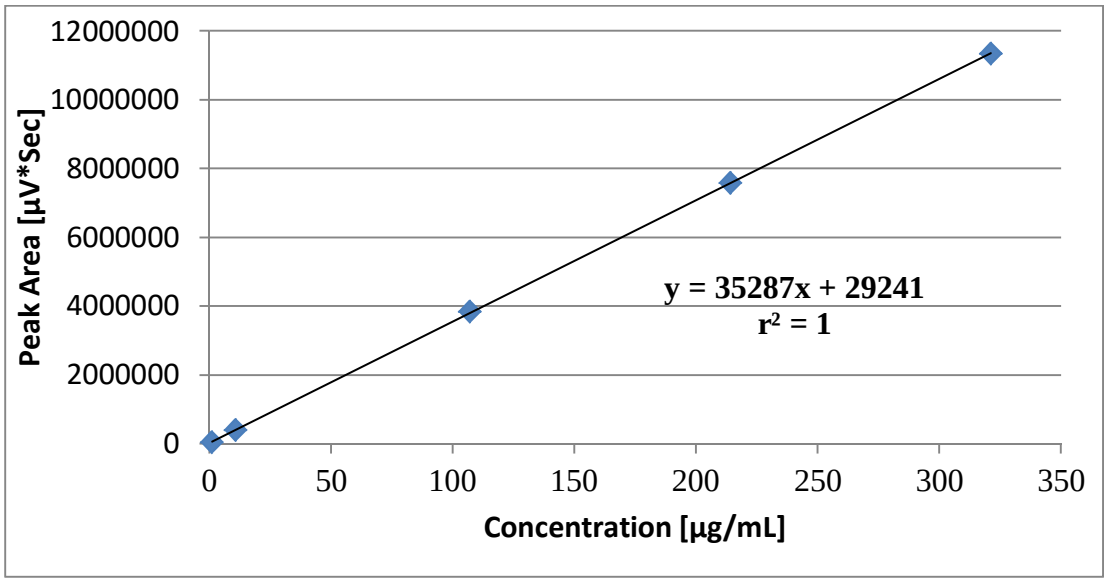


Figure 5-2. Diphenhydramine external standard calibration curve

Graph of calibration function of solutions containing diphenhydramine in the concentration range of 10 µg/mL and 320µg/mL.

Table 5-2. Statistical parameters of linear regression analysis for diphenhydramine

Index	Slope [m]	Y-Intercept [C]	Correlation coefficient [r2]	RSD. response. factor [%]
Value	35287	29241	1.00000	2.76

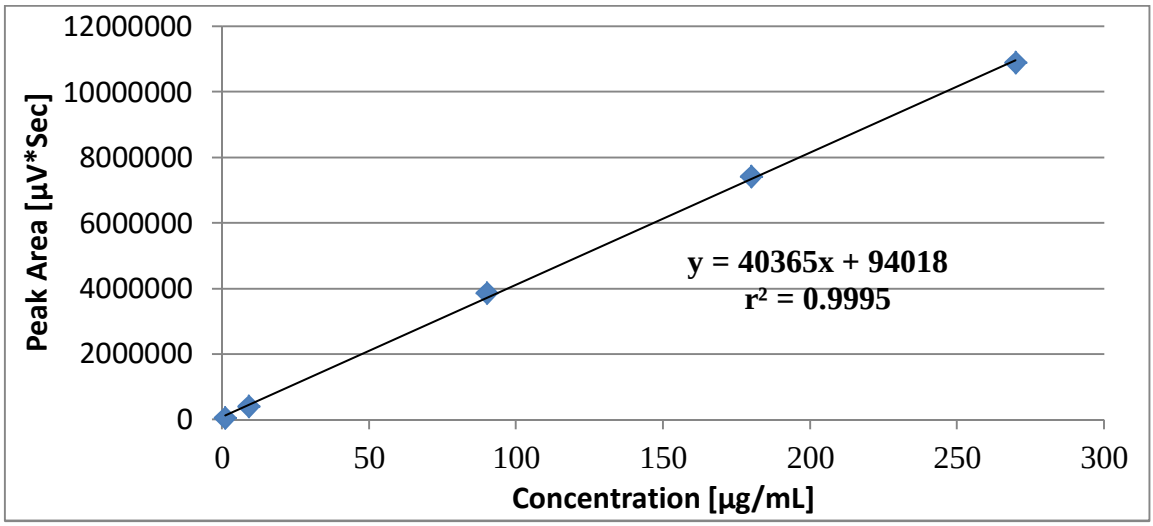


Figure 5-3. 8-Chlorotheophylline external standard calibration curve

Graph of calibration function of solutions containing 8-Chlorotheophylline in the concentration range of 0.9 µg/mL and 270 µg/mL

Table 5-3. Statistical parameters of linear regression analysis for 8-chlorotheophylline

Index	Slope [m]	Y-Intercept [c]	Correlation Coefficient [r^2]	RSD. Resp. factor [%]
Value	40364.85	94018	0.9995	6.04

5.1.3 Linearity of caffeine

The caffeine containing chewing gums are dosed at 40 mg per piece. The maximum theoretical concentration expected with complete drug release would be about 1000 µg/mL. For this purpose, the standard solutions containing caffeine were prepared in the range of 20.0 µg/mL and 1397.0 µg/mL.

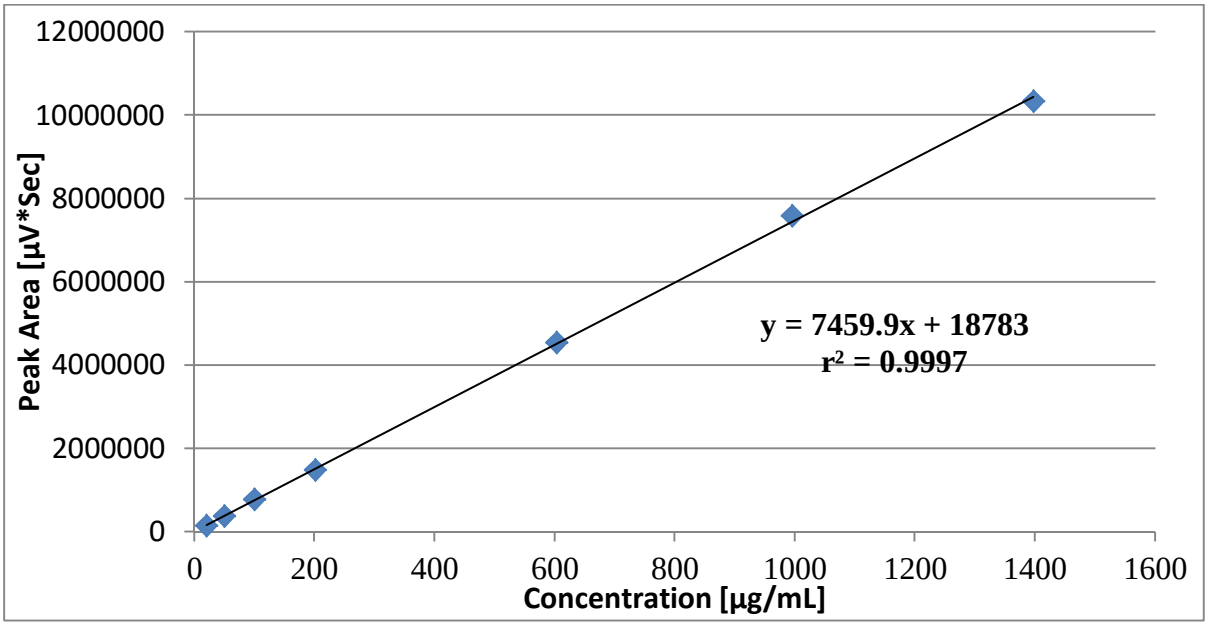


Figure 5-4. Caffeine external standard calibration curve

Graph of calibration function of solutions containing caffeine in the concentration range of 20 µg/mL and 1397 µg/mL

Table 5-4. Statistical parameters of linear regression analysis for caffeine

Index	Slope [m]	Y-Intercept [C]	Correlation coefficient [r^2]	RSD. resp. factor [%]
Value	7459.9	18783	0.9997	1.53

5.1.4 Solubility of dimenhydrinate

The solubility of dimenhydrinate in different buffer solutions was investigated to ensure that sink conditions are present and the dissolution is not limited by the solubility of the compounds. This point is particularly crucial since very low volume (40 mL) of the buffer solution is employed during the drug release testing of dimenhydrinate containing chewing gums. The results of the analysis are summarized in Table 5-5 and shown graphically in Figure 5-5 - Figure 5-6.

Table 5-5. Results of the dimenhydrinate solubility investigations

No	Media	Solubility (mg/mL)		
		Dimenhydrinate	8-Chloro-Theophylline	Diphenhydramine HCl
1	SGF pH 1.2	1.37	0.37	1.45
2	Phosphate buffer pH 4.5	1.57	0.4	1.53
3	Phosphate buffer pH 5.8	1.55	0.85	1.52
4	Phosphate buffer pH 6.8	1.6	1.5	1.54
5	Phosphate buffer pH 7.4	1.59	1.49	1.51

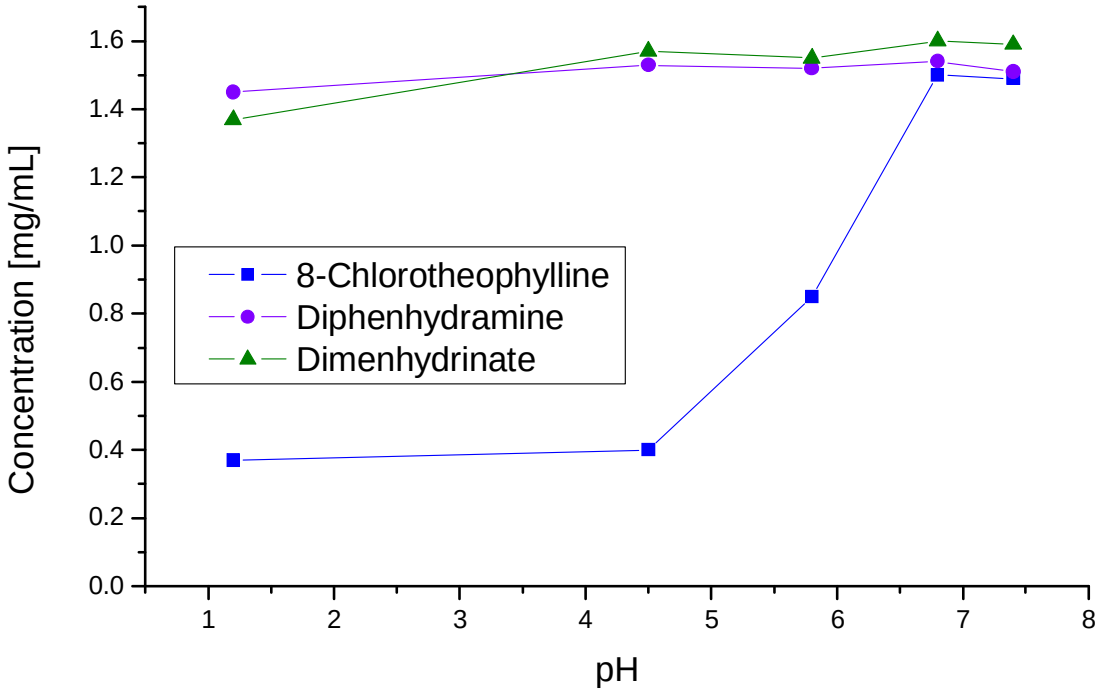


Figure 5-5. Solubility of dimenhydrinate, 8-chlorotheophylline and diphenhydramine hydrochloride

Graph showing the pH solubility profiles of different components of dimenhydrinate in different pH values at room temperature.

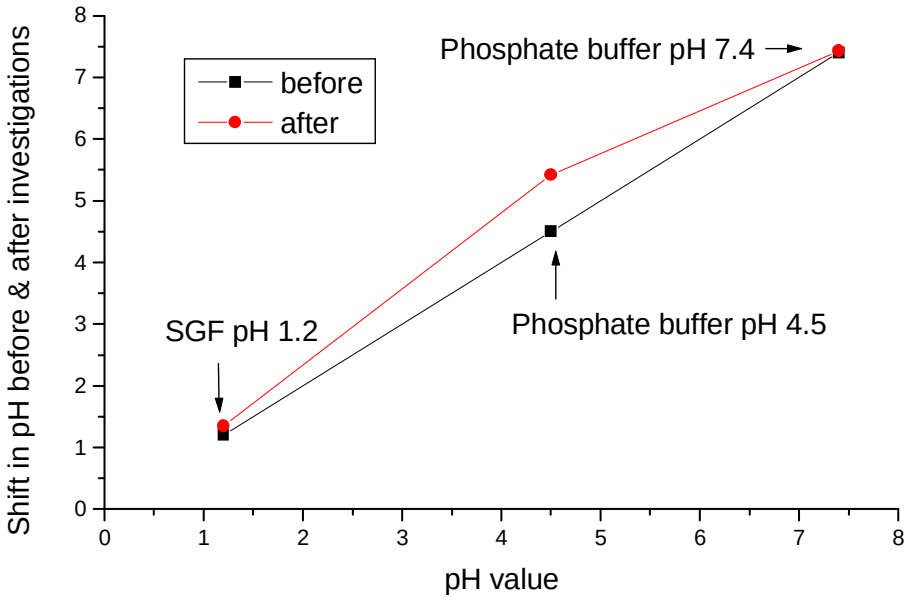


Figure 5-6. Influence of shift in pH value of buffer solutions during the solubility testing for dimenhydrinate

Findings and discussion

Based on the solubility results presented in the Table 5-5, it can be concluded that the dimenhydrinate and diphenhydramine HCl are freely soluble in aqueous media at concentrations > 1.5 mg/mL except at lower pH values (SGF pH 1.2). The solubility remains independent of pH values between pH 4.5 and pH 7.4. The 8-chlorotheophylline showed a clear pH-dependent solubility. From the above results, it is possible to conclude that the solubility will not be a rate limiting step during the drug release testing of chewing gums containing dimenhydrinate. During the drug release testing, a volume of 40 mL of the dissolution medium is sufficient to dissolve 100% of the drug theoretically provided that the other factors, for e.g., formulation, method of manufacturing, experimental setup has negligible influence on the solubility behavior.

For biorelevant drug release testing, the drug release medium should mimic the pH conditions of the saliva in the oral cavity. The pH values ranging from 6 to 7 are suitable to predict the bio-performance of the drug product.

5.2 Feasibility study results

The results of the feasibility study are summarized in the following sections.

5.2.1 Evaluation of content uniformity testing

The content uniformity for the nicotine containing chewing gums was tested on 10 individual pieces of gums from the two different products. The results of the analyses are presented in the Figure 5-7 and the data in the corresponding Table 5-6.

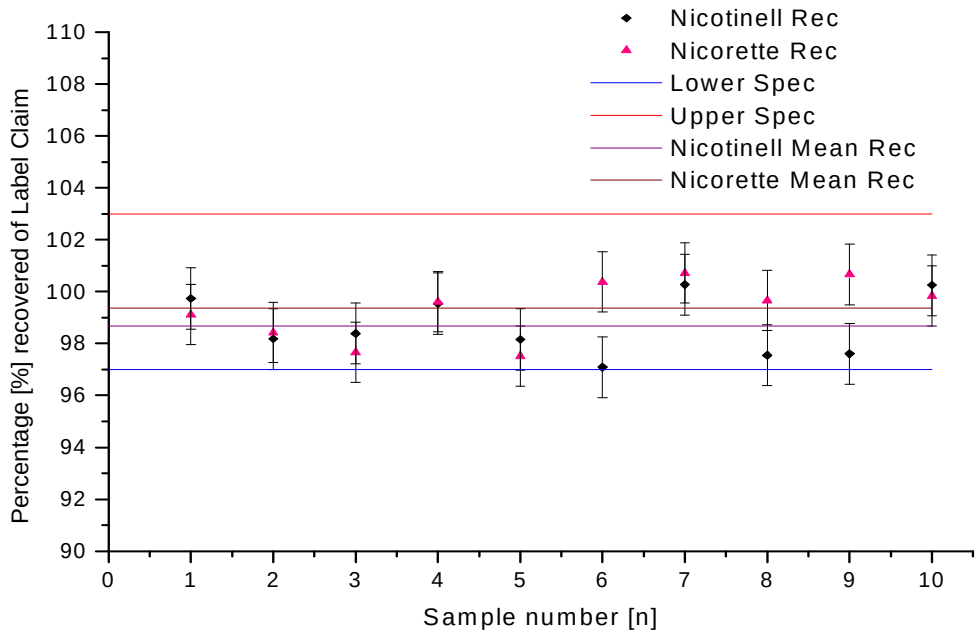


Figure 5-7. Individual and mean recovery of nicotine from Nicotinell 2 mg and Nicorette 2mg
*) Rec.: recovery, Spec.: specification

Table 5-6. Summary of content uniformity testing

Product	Mean recovery [mg]/ RSD [%]	Mean recovery [%]	Mean average wt. of gums [G]/ RSD [%]
Nicotinell 2 mg	1.97/1.19	98.68	1.1817/1.71
Nicorette 2 mg	1.99/1.15	99.48	1.2191/5.78

5.2.2 Results of “home-depot” method

The surface of nicotine based multisource gum formulations were suitably transformed using the “home-depot” apparatus at an applied force of 5 KN to obtain a uniform surface area. The *in vitro* release profiles are shown in Figure 5-8.

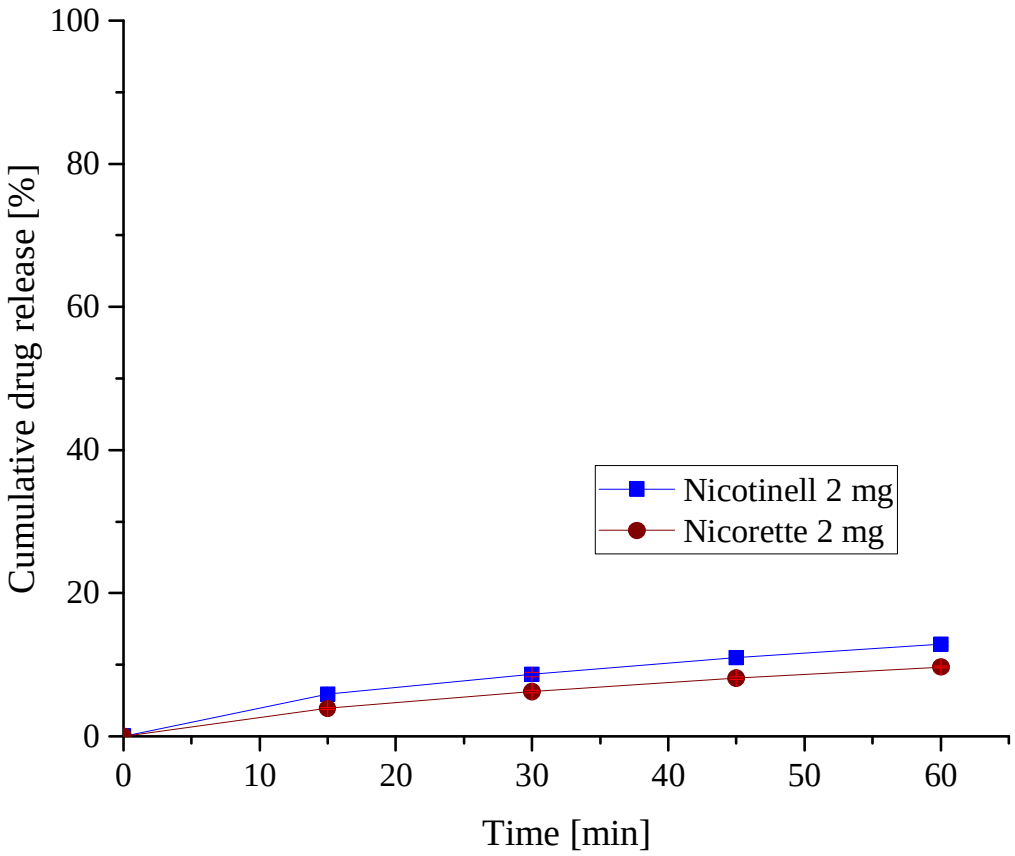


Figure 5-8. ***In vitro*** **release profiles of nicotine from multisource nicotine products using USP <2> paddle apparatus**

Results and discussion

A maximum amount corresponding to 13% of label claim was released from the formulations. The tests indicate the requirement of masticatory action for drug release from gum formulations and demonstrated the safety aspect of the MCG during accidental swallowing, where no significant release would be expected.

On the other hand it necessitates the need to employ a specialized apparatus for the performance testing of MCGs. Following the demonstration of inadequacy of the compendial instrument to effect drug release, feasibility study using chewing gum apparatus was initiated. Further studies were performed using the chewing apparatus described in the earlier section.

5.2.3 Nicotine based chewing gum formulations

The *in vitro* performance of multisource nicotine gum products was tested using both the apparatus; Ph. Eur apparatus A and B. The apparatus parameters and the proposed feasibility study design were already described. The drug release profiles of two nicotine gum products generated from each of the apparatus are reported individually and the influence of various apparatus parameters on release are shown in the sections thereafter.

5.2.3.1 *In vitro* release of nicotine from apparatus A

The *in vitro* drug release profiles generated are presented in Figure 5-9 to Figure 5-12 with respect to the product and corresponding apparatus. Figures having scales (x-axis) up to 2400 and 3600 cumulative strokes (chews) correspond to chewing frequencies of 40 chews/min and 60 chews/min.

The variables of the apparatus A include;

Vertical chewing distance: distance (mm) between the vertical chewing piston and the surface of the chewing chamber at its closest position during mastication (2 distances have been selected, 3 mm and 6 mm)

Horizontal chewing distance: distance (mm) between the two horizontal pistons within the chewing chamber at their closest position during mastication (3 distances have been selected, 0.3mm, 0.5 mm and 0.7 mm)

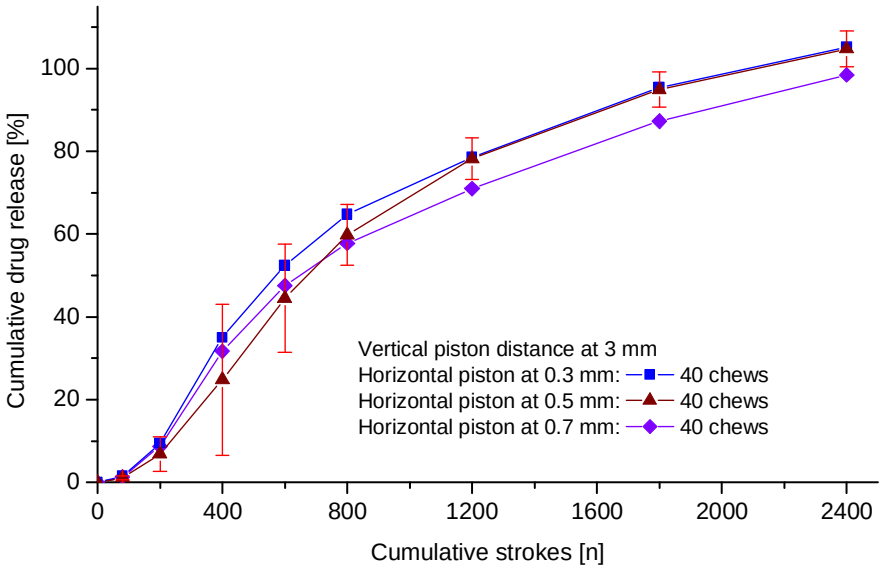


Figure 5-9. *In vitro* release of nicotine from Nicorette 2 mg freshmint gums using Ph.Eur. apparatus A

Drug release profiles represent data (n=3 ± SD) generated using 40mL of artificial saliva pH 6.2 at 37°C at a constant vertical distance (3 mm), chewing frequency (40 strokes/min) and variable horizontal chewing distance (0.3 mm vs. 0.5 mm vs. 0.7 mm)

Findings and discussion

It is shown in the Figure 5-9 that there is no significant difference observed between the drug release profiles generated using three different horizontal chewing distances (0.3mm vs. 0.5mm vs. 0.7mm). One possible explanation for this behavior is that the difference in chewing distance between all the setups is 0.2 mm which may not be adequate to increase the surface area of the gum matrix during mastication. However, a small difference in the drug release profiles can be observed between 0.3 mm and 0.7 mm due to an increase in the chewing distance.

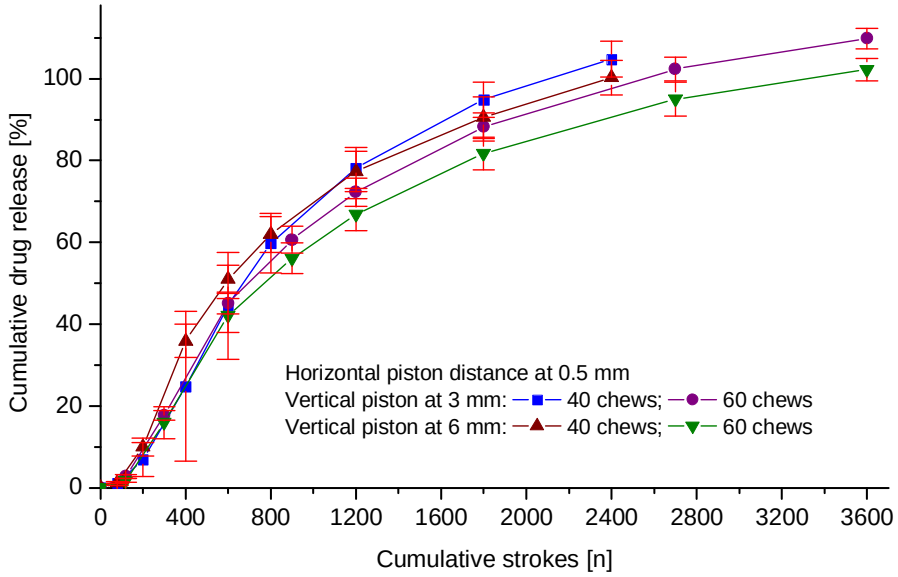


Figure 5-10. ***In vitro*** **release of nicotine from Nicorette 2 mg freshmint gums using Ph.Eur. apparatus A**

Drug release profiles represent data (n=3 ± SD) generated using 40mL of artificial saliva pH 6.2 at 37°C at a constant horizontal distance (0.5 mm), chewing frequency (40 strokes/min) and variable vertical chewing distance (3 mm vs. 6 mm)

Findings and discussion

The Figure 5-10 shows the drug release profiles generated using two different vertical piston distances (3 mm vs. 6 mm). It was observed that the difference in release between the apparatus setups is very small. But a trend in release behavior was observed for profiles at later time points with different chewing frequencies (40 chews vs. 60 chews). This is due to the fact that at a higher chewing frequency (60 chews), the contact time of the vertical piston with the gum matrix is significantly reduced. Therefore, the contact time necessary for the gum matrix to achieve the surface area of the vertical piston is reduced. This results in a reduction of drug release from the gum formulation.

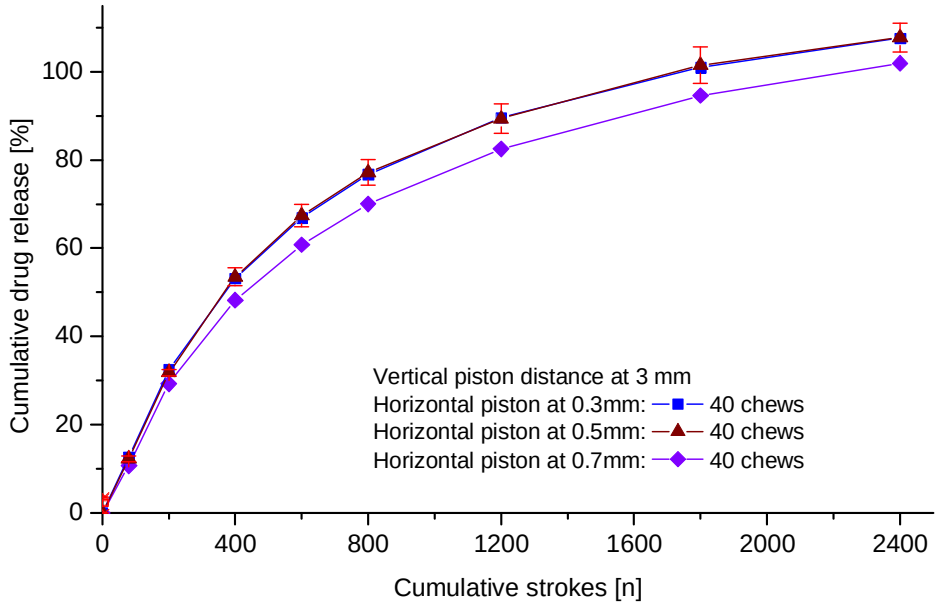


Figure 5-11. ***In vitro*** **release of nicotine from Nicotinell 2 mg mint gums using Ph.Eur. apparatus A**

Drug release profiles represent data (n=3 ± SD) generated using 40mL of artificial saliva pH 6.2 at 37°C at a constant vertical distance (3 mm), chewing frequency (40 strokes/min) and variable horizontal chewing distance (0.3 mm vs. 0.5 mm vs. 0.7 mm)

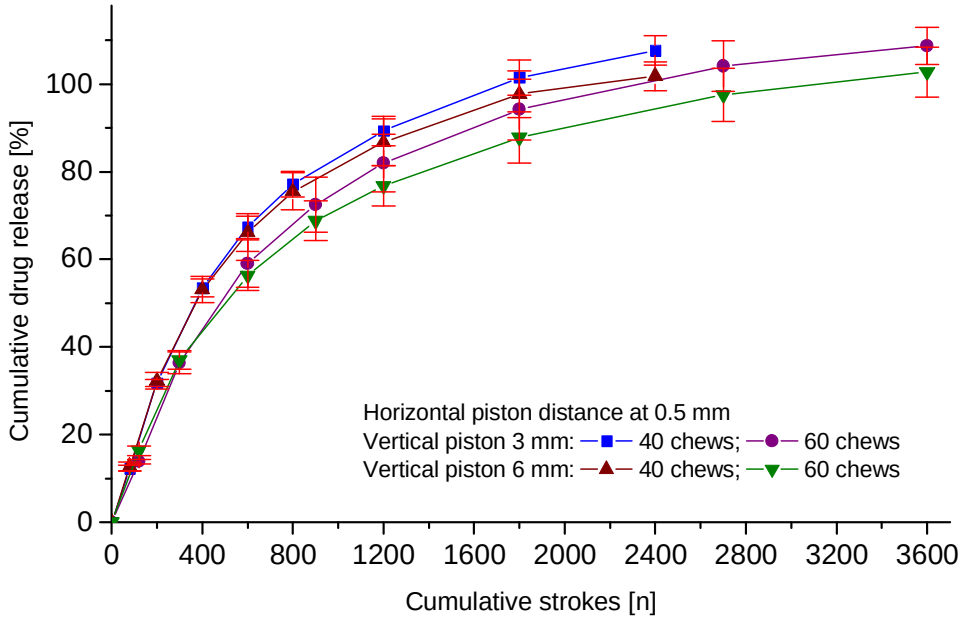


Figure 5-12. ***In vitro*** **release of nicotine from Nicotinell 2 mg Mint gums using Ph.Eur. apparatus A**

Drug release profiles represent data (n=3 ± SD) generated using 40mL of artificial saliva pH 6.2 at 37°C at a constant horizontal distance (0.5 mm), chewing frequency (40 strokes/min) and variable vertical chewing distance (3 mm vs. 6 mm)

Findings and discussion

The *in vitro* drug release profiles of nicotinell gum shown in Figure 5-11 and Figure 5-12 indicate a similar influence observed for the Nicorette gum using apparatus A.

5.2.3.2 *In vitro* release of nicotine from apparatus B

As far as the apparatus B is concerned, there were a total of 12 different combinations of apparatus parameters. The results are presented in Figure 5-13 - Figure 5-14.

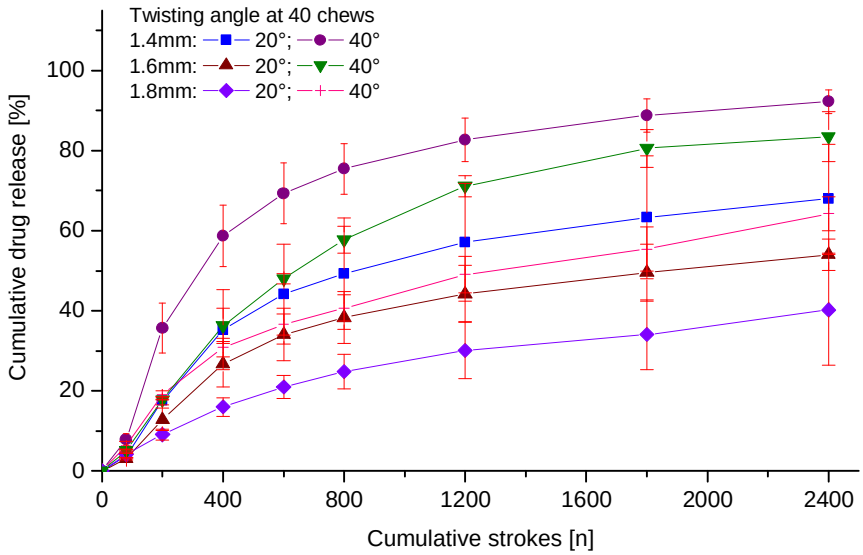


Figure 5-13. *In vitro* release of nicotine from Nicorette 2mg freshmint gums using Ph.Eur. apparatus B

Drug release profiles represent data (n=3 ± SD) generated using 40mL of artificial saliva pH 6.2 at 37°C at a constant chewing frequency (40 strokes/min) and variable chewing distance (1.4 mm vs. 1.6 mm vs. 1.8 mm) and twisting angle (20 ° vs. 40 °)

Findings and discussion

The results presented for Nicorette chewing gums in Figure 5-13 clearly indicate that the apparatus parameters have a strong influence on the release characteristics of nicotine. The order of release can be given as; 1.4mm/40° > 1.6mm/40° > 1.4mm/20° > 1.8mm/40° > 1.6mm/20° > 1.8mm/20°. It is clearly evident that the chewing distances 1.4 mm and 1.6mm in combination with larger twisting angle significantly affected the drug release. With the exception of 1.4 mm/20°, a clear relationship of the release profiles with the apparatus setup can be given. In general, the nicotine release at 40° > 20 ° twisting angle with the respective chewing distances (1.4 mm, 1.6 mm, 1.8 mm). This indicates that at a larger twisting angle (40°), the chewing surface of the gum matrix was more renewed than at 20°.

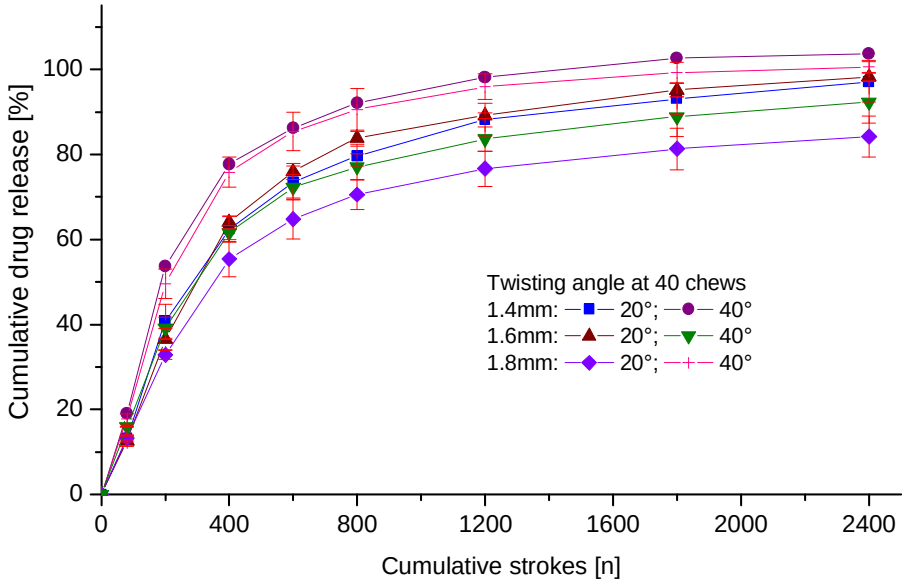


Figure 5-14. ***In vitro*** **release of nicotine from Nicotinell 2 mg mint gums using Ph.Eur. apparatus B**

Drug release profiles represent data (n=3 ± SD) generated using 40mL of artificial saliva pH 6.2 at 37°C at a constant chewing frequency (40 strokes/min) and variable chewing distance (1.4 mm vs. 1.6 mm vs. 1.8 mm) and twisting angle (20 ° vs. 40 °).

Findings and discussion

The Figure 5-14 shows the nicotine release profiles from nicotinell chewing gum formulation. The drug release profiles follow no particular order to establish a relationship between the apparatus setups and release characteristics. Quantitatively, a very small difference in release was observed for profiles between 1.4mm/20°, 1.6mm/20° and 1.6mm/40°. Since the standard deviation (controlled variability) of the profiles is relatively small compared to the Nicorette product, an apparatus error can be ruled out. The results were inconclusive and can only be verified using a spit-out study data.

The comparative product performance obtained from the gum products with different settings of the apparatus are shown in Figure 5-15Figure 5-28 in the appendix.

5.2.3.3 Comparative product performance from Apparatus A

The apparatus A individual parameters used for the release testing of nicotine from both the formulations have been investigated. Various parameters of the apparatus A have been tested and their influence on the release characteristics was investigated. The results of the analysis are displayed graphically in the Figure 5-15 - Figure 5-18.

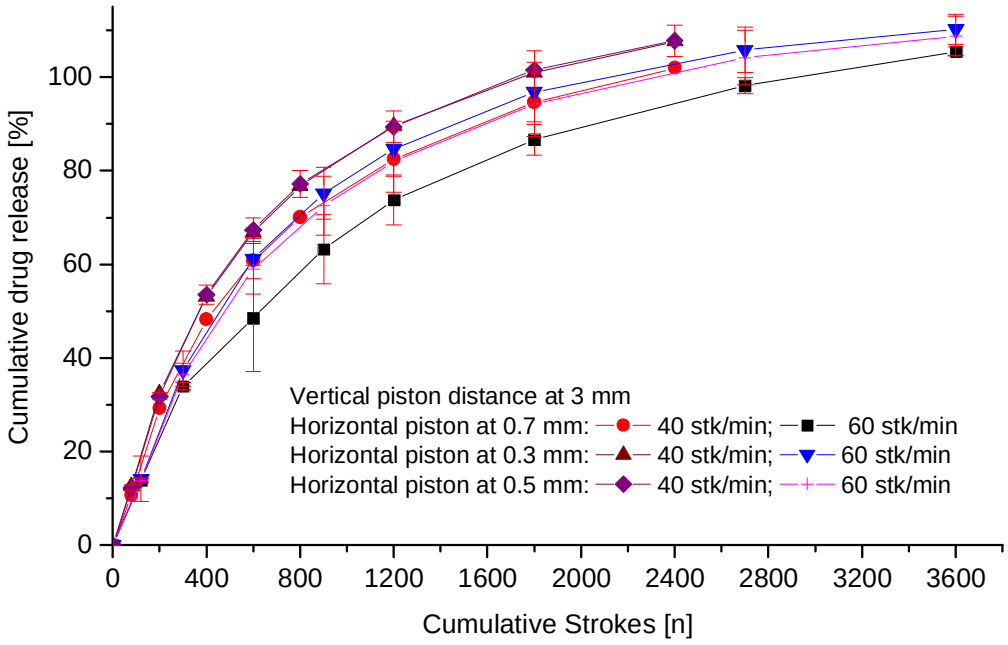


Figure 5-15. Influence of horizontal chewing distance at constant vertical piston distance (apparatus A, Nicotinell 2 mg mint)

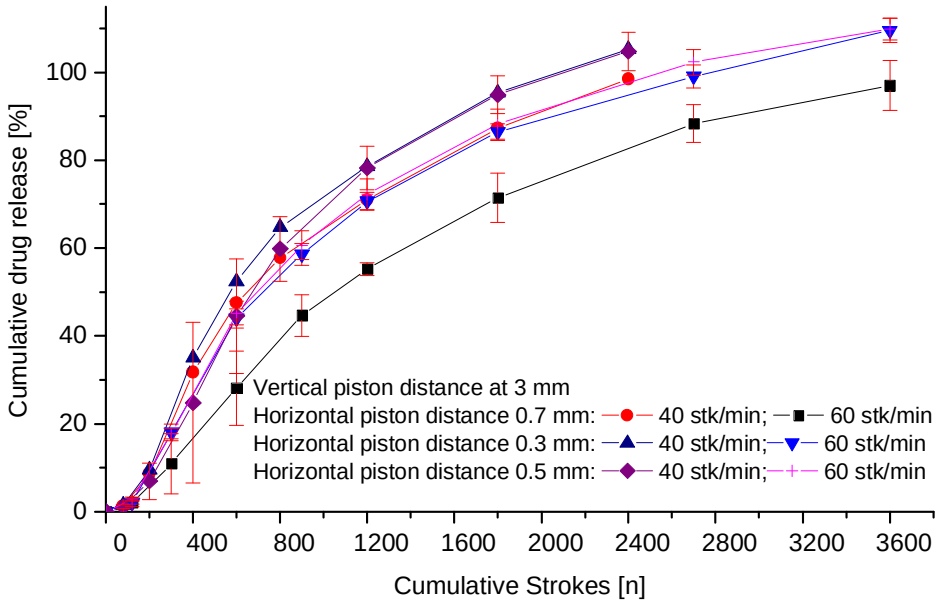


Figure 5-16. Influence of horizontal chewing distance at constant vertical piston distance (apparatus A, Nicorette 2 mg freshmint)

Both the drug release profiles represent data (n=3 ± SD) generated using 40 mL of artificial saliva pH 6.2 at 37°C at a constant vertical distance (3 mm), variable chewing frequency (40 strokes/min vs. 60 strokes/min) and horizontal chewing distance (0.3 mm vs. 0.5 mm vs. 0.7 mm)

For the purpose of comparison, the drug release profiles generated from apparatus A for the products Nicotinell and Nicorette under the same conditions were shown in the Figure 5-15 - Figure 5-18. The release using 60 strokes/min is slower than 40 strokes/min. This is due to the reduction in the time of contact between gum matrix and chewing surface.

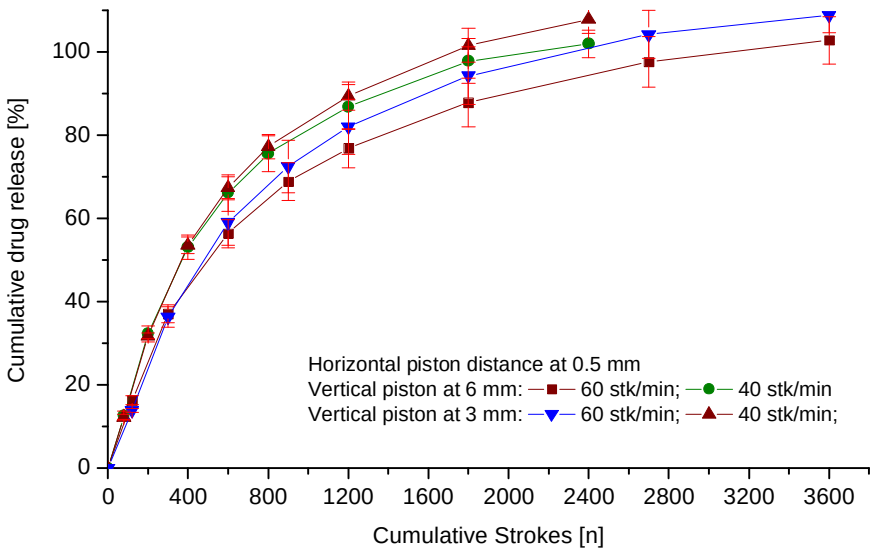


Figure 5-17. Influence of vertical chewing distance at constant horizontal piston distance (apparatus A, Nicotinell 2 mg mint)

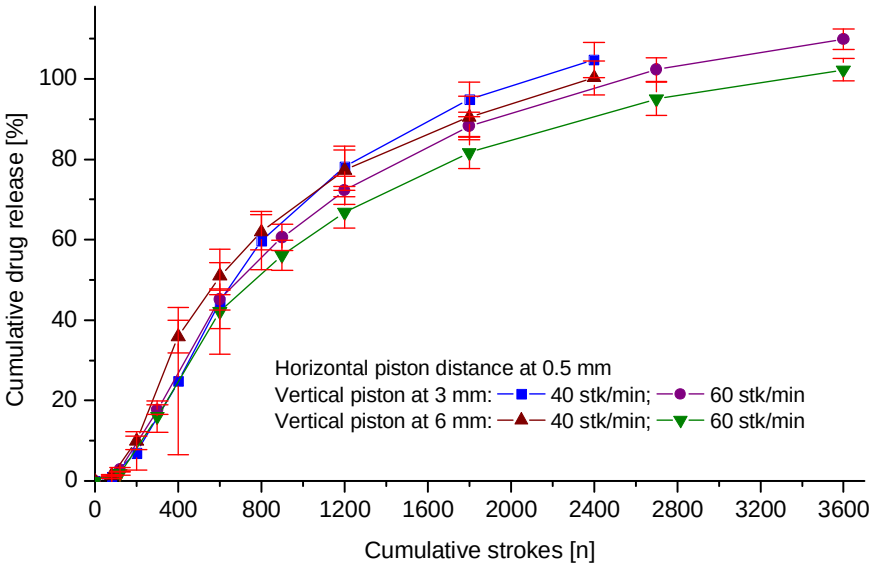


Figure 5-18. Influence of vertical chewing distance at constant horizontal piston distance (apparatus A, Nicorette 2 mg freshmint)

Both the drug release profiles represent data (n=3 ± SD) generated using 40 mL of artificial saliva pH 6.2 at 37°C at a constant horizontal distance (0.5 mm), variable chewing frequency (40 strokes/min vs. 60 strokes/min) and vertical chewing distance (3 mm vs. 6 mm).

Results and discussion

A small difference (Figure 5-15 - Figure 5-18) in release behavior was observed with respect to the apparatus setups. Comparing all the tested parameters, drug release at 40 strokes/min > 60 strokes/min. The release profiles of both the products confirms the assumption that the time of contact between chewing surface and gum matrix is essential to achieve a large surface area, thereby a better interaction between the drug release medium and gum components is established.

5.2.3.4 Comparative product performance from apparatus B

The apparatus B has gained the compendial status in the year 2012. The monograph describing the functionality of the apparatus has been elaborated. However, there are no recommendations or the guidelines related to the development of the *in vitro* drug release method. Different parameters of the apparatus were investigated to determine their influence on the release characteristics of the gum components. The results are shown graphically in the Figure 5-19 - Figure 5-28.

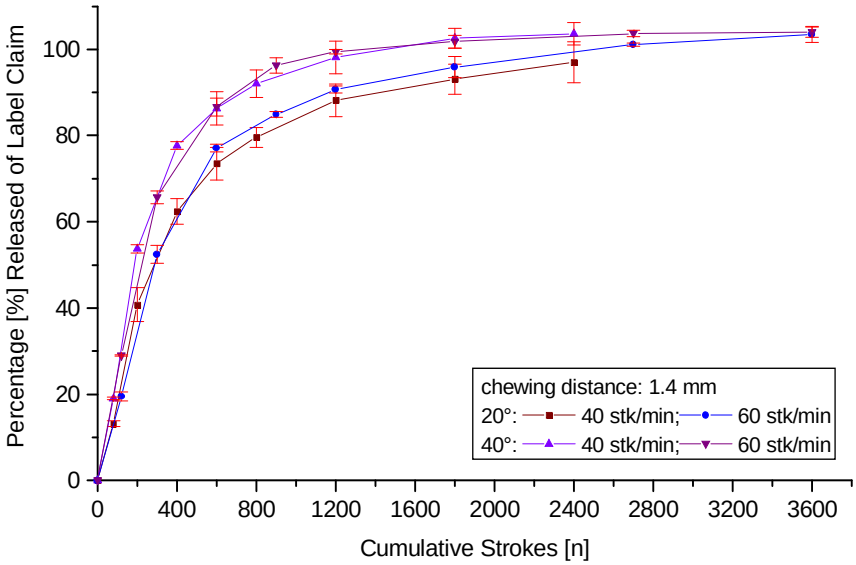


Figure 5-19. Influence of twisting angle at constant chewing distance (apparatus B, Nicotinell 2 mg mint)

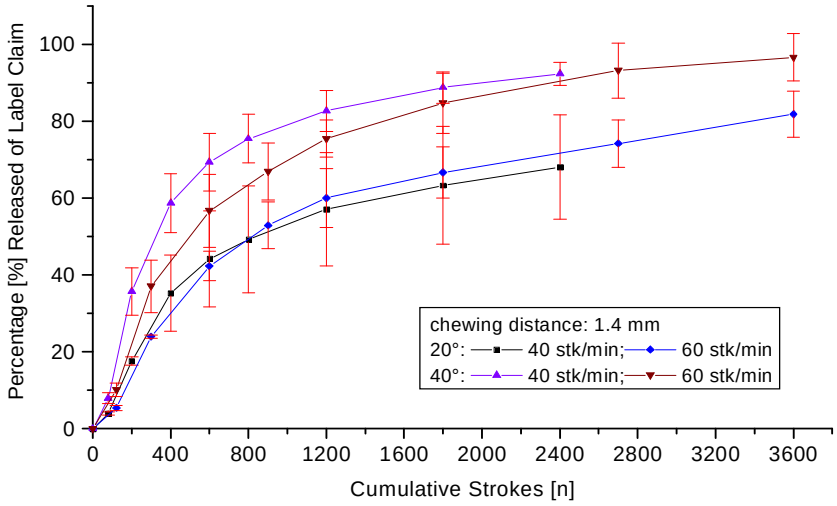


Figure 5-20. Influence of twisting angle at constant chewing distance (apparatus B, Nicorette 2 mg freshmint)

Both the drug release profiles represent data (n=3 ± SD) generated using 40 mL of artificial saliva pH 6.2 at 37°C at a constant chewing distance (1.4 mm), variable chewing frequency (40 strokes/min vs. 60 strokes/min) and twisting angle (20° vs. 40°).

Results and discussion

As observed in Figure 5-19 - Figure 5-20, significant difference in the release characteristics between products were found with respect to the apparatus setups. Unlike apparatus A, the difference in release between 40 strokes/min and 60 strokes/min is negligible. This is due to the fact that in case of apparatus B, the mastication process consists of compression of the gum to a predefined chewing distance followed by twisting action of 20° or 40°. In this way, a reduction in contact time is compensated by the twisting action, which provides an adequate time for expansion and renewal of gum surface.

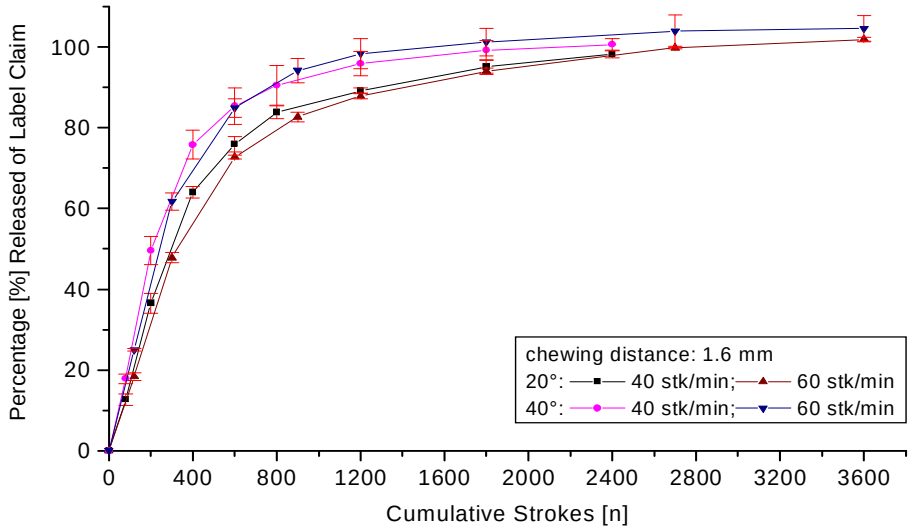


Figure 5-21. Influence of twisting angle at constant chewing distance (apparatus B, Nicotinell 2 mg mint)

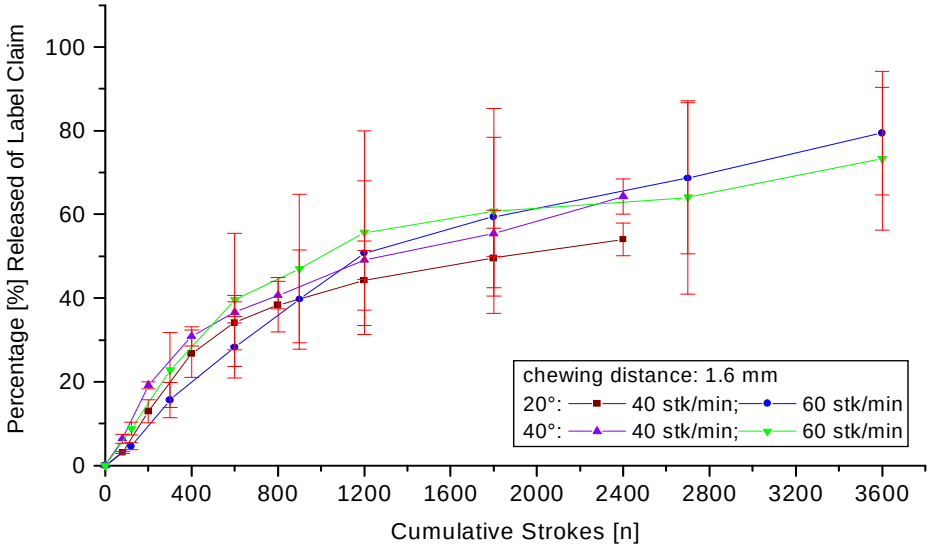


Figure 5-22. Influence of twisting angle at constant chewing distance (apparatus B, Nicorette 2 mg freshmint)

Both the drug release profiles represent data (n=3 ± SD) generated using 40 mL of artificial saliva pH 6.2 at 37°C at a constant chewing distance (1.6 mm), variable chewing frequency (40 strokes/min vs. 60 strokes/min) and twisting angle (20° vs. 40°).

Results and discussion

As seen in Figure 5-21 and Figure 5-22, the shape of nicotine release profiles from nicotinell is significantly different from Nicorette chewing gum under the same experimental conditions.

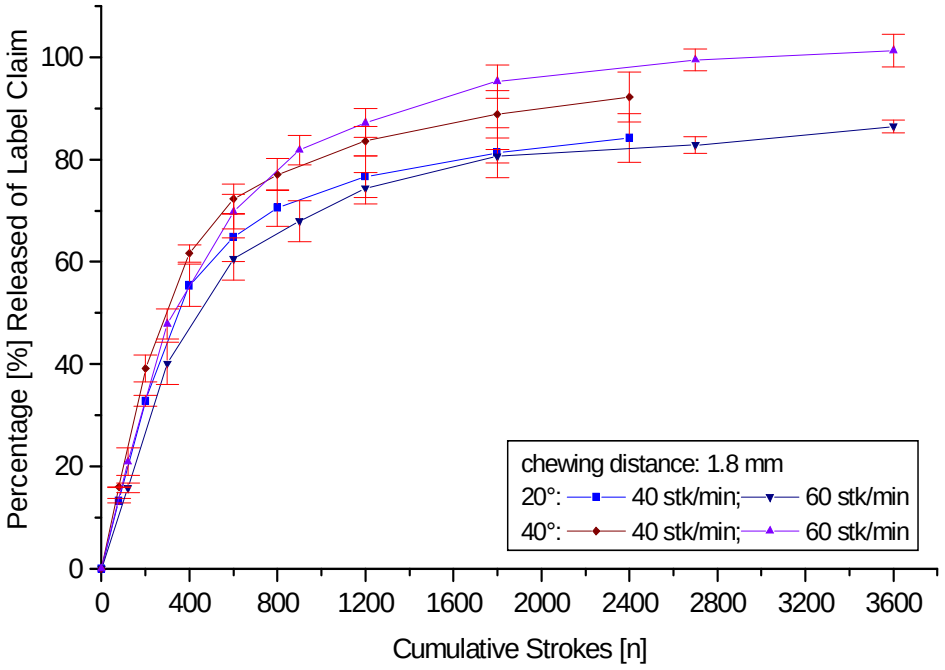


Figure 5-23. Influence of twisting angle at constant chewing distance (apparatus B, Nicotinell 2 mg mint)

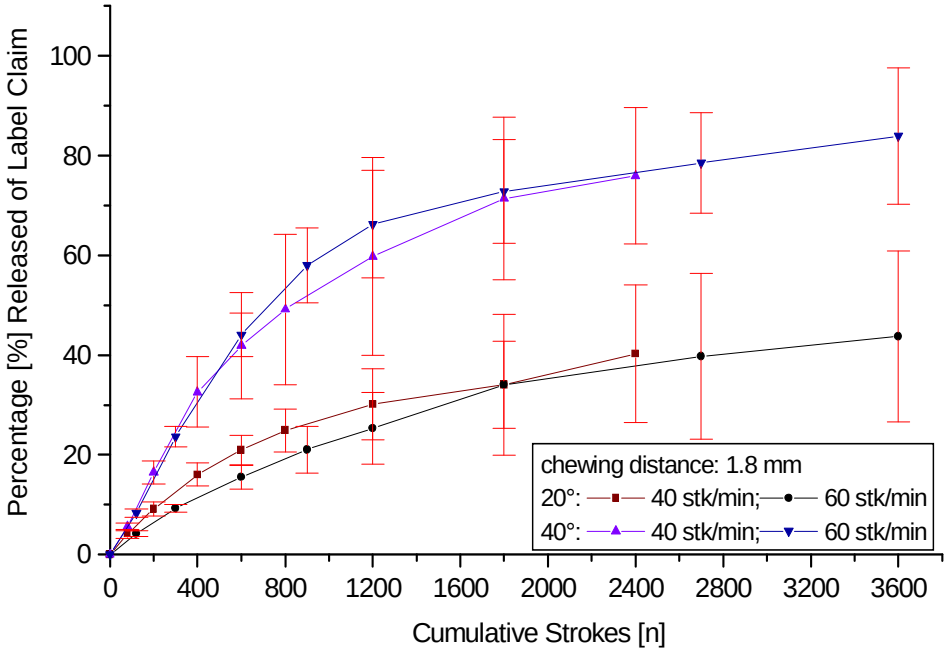


Figure 5-24. Influence of twisting angle at constant chewing distance (apparatus B, Nicorette 2 mg freshmint)

Both the drug release profiles represent data (n=3 ± SD) generated using 40 mL of artificial saliva pH 6.2 at 37°C at a constant chewing distance (1.8 mm), variable chewing frequency (40 strokes/min vs. 60 strokes/min) and twisting angle (20° vs. 40°).

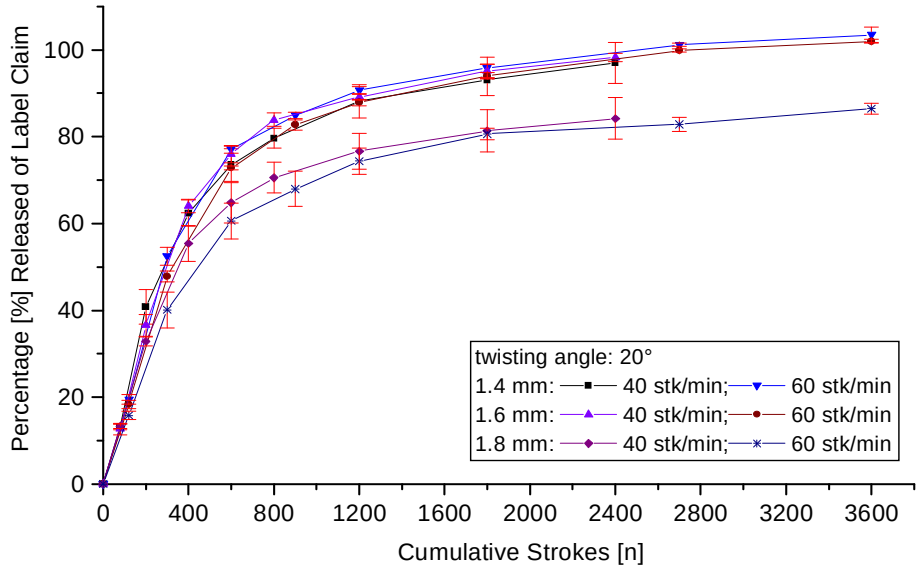


Figure 5-25. Influence of chewing distance at constant twisting angle (apparatus B, Nicotinell 2 mg mint)

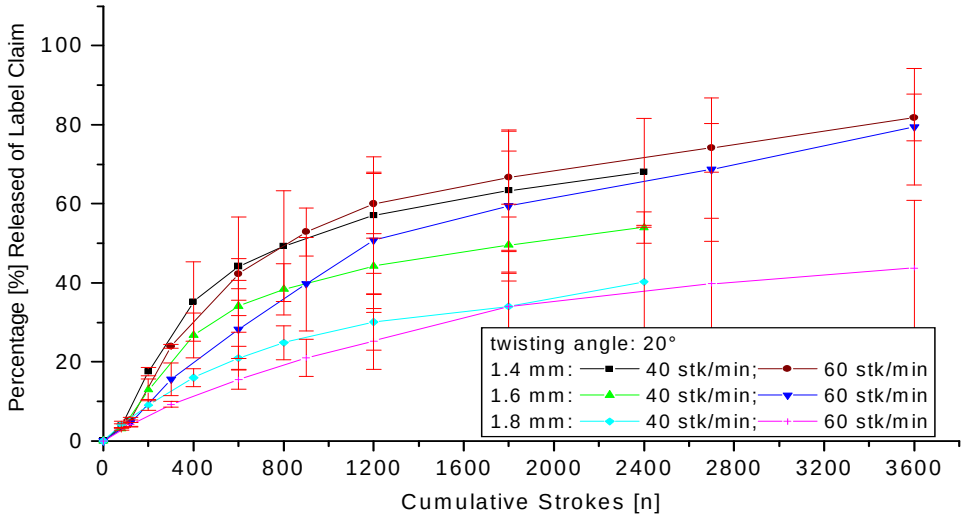


Figure 5-26. Influence of chewing distance at constant twisting angle (apparatus B, Nicorette 2 mg freshmint)

Both the drug release profiles represent data (n=3 ± SD) generated using 40 mL of artificial saliva pH 6.2 at 37°C at a constant twisting distance (20°), variable chewing frequency (40 strokes/min vs. 60 strokes/min) and chewing distance (1.4 mm vs. 1.6 mm vs. 1.8 mm).

Results and discussion

The *in vitro* drug release profiles in the Figure 5-25 and Figure 5-26 indicate that the two nicotine containing chewing gum products of same dosage strength (2 mg) release nicotine differently with the same apparatus setups. This clearly shows that the products are not comparable and cannot be substituted for one another. The gum products differ in their geometry and in their texture. The nicotinell gum product is rectangular in shape and a larger surface area and softer in texture compared to Nicorette which is square shaped and has a smaller surface area. Due to these properties, the release of nicotine from the Nicorette gum is

significantly slower than the Nicotinell gum. This phenomenon is also seen in the results shown in Figure 5-27 and Figure 5-28.

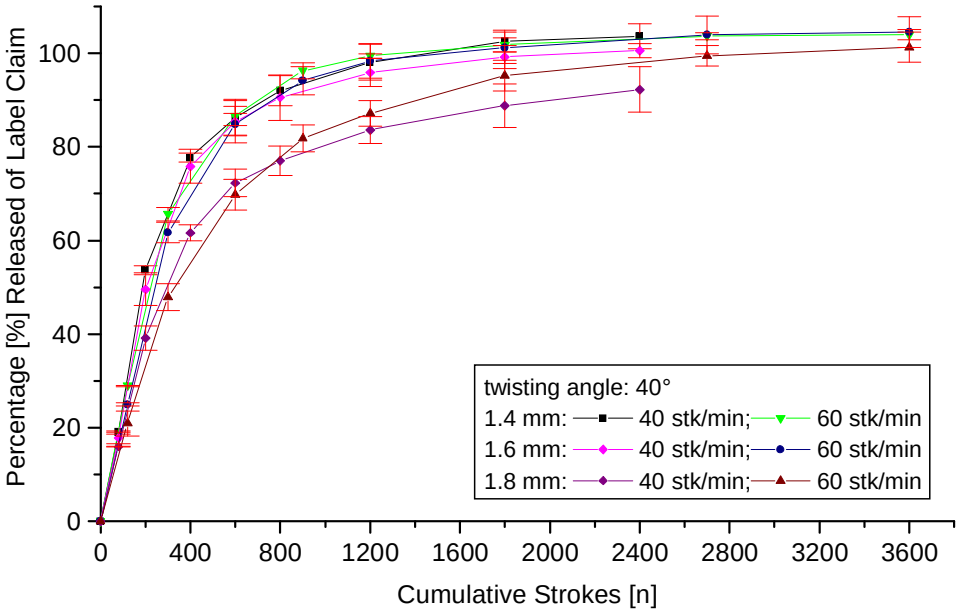


Figure 5-27. Influence of chewing distance at constant twisting angle (apparatus B, Nicotinell 2 mg mint)

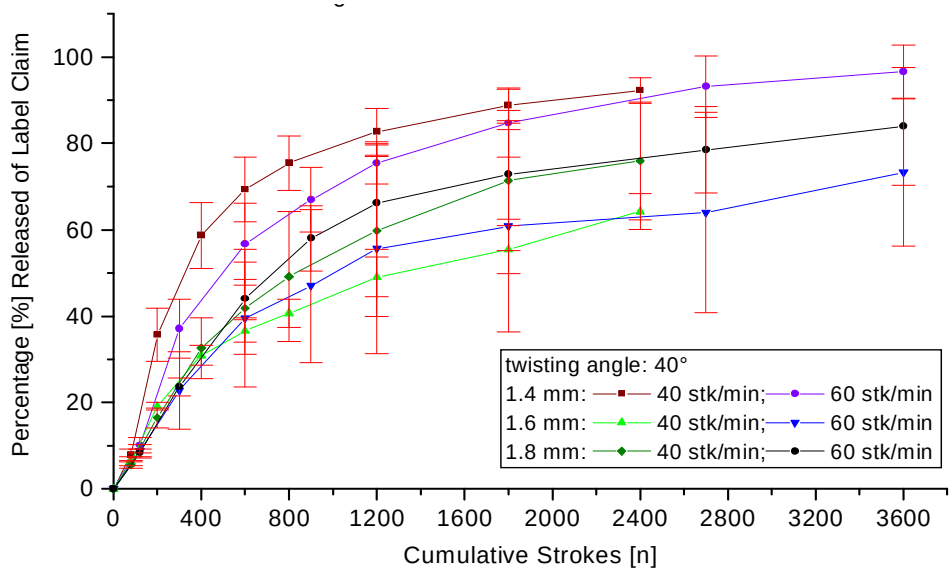


Figure 5-28. Influence of chewing distance at constant twisting angle (apparatus B, Nicorette 2 mg freshmint)

Both the drug release profiles represent data (n=3 ± SD) generated using 40 mL of artificial saliva pH 6.2 at 37°C at a constant twisting distance (40°), variable chewing frequency (40 strokes/min vs. 60 strokes/min) and chewing distance (1.4 mm vs. 1.6 mm vs. 1.8 mm).

Results and discussion

An increase in the twisting angle of 20° resulted in a small increase in the extent and rate of nicotine release from the gum products. This clearly indicates the release of any actives from chewing gum formulations can be directly linked to the rate of change of surface area available for release. With a 40° twisting angle, the extent of renewed surface available for interaction with the dissolution medium is increased.

Further *in vitro* release analyses were performed only using apparatus B due to its availability. The apparatus A is not commercially available. Nicotine based chewing gum of different dosage strengths and flavor was tested to determine the suitability of the developed *in vitro* drug release methodology.

5.2.3.5 Performance testing of commercially available nicotine gums

The *in vitro* drug release from 2 mg and 4 mg dosage strength Nicorette classic gum is represented graphically in Figure 5-29 and Figure 5-30. The data were generated from the apparatus B using varying chewing distance and twisting angle.

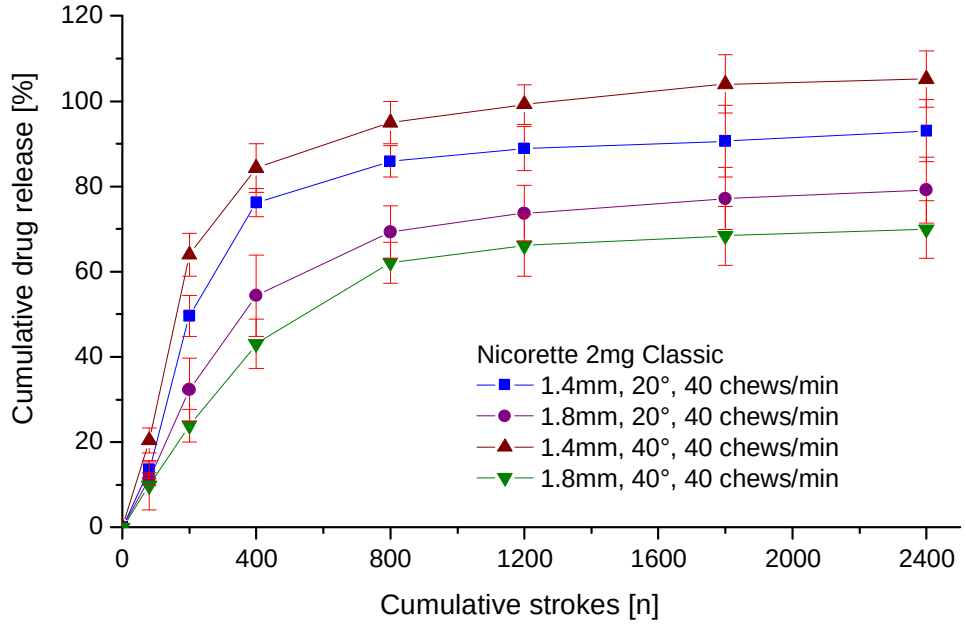


Figure 5-29. ***In vitro*** **release of nicotine from Nicorette 2 mg classic gums using apparatus B**

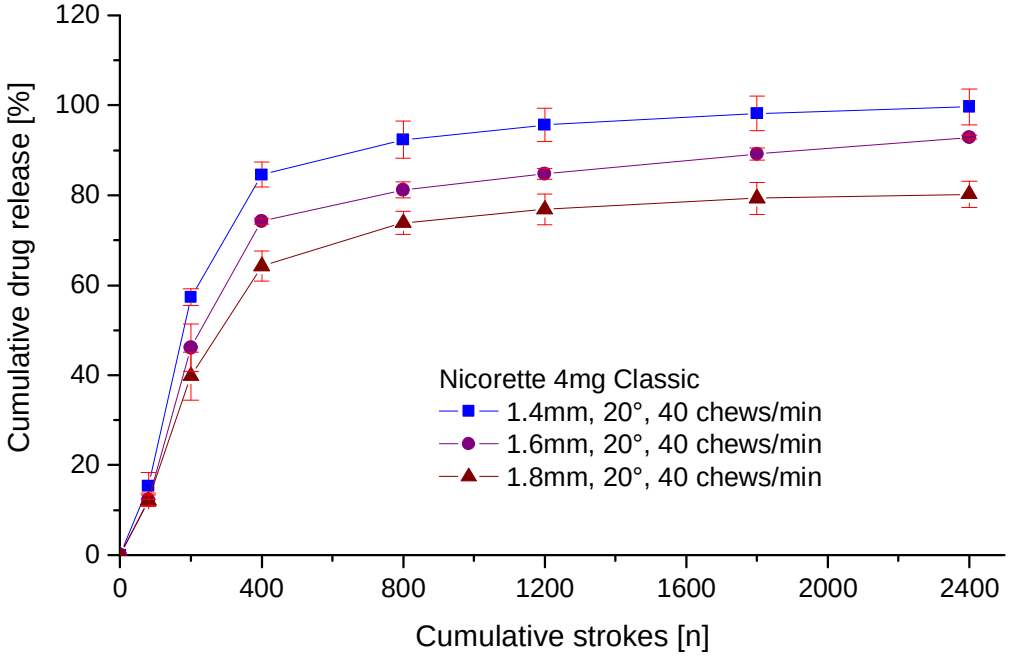


Figure 5-30. ***In vitro*** **release of nicotine from Nicorette 4 mg classic gums using apparatus B**

Both the drug release profiles represent data (n=3 ± SD) generated using 40 mL of artificial saliva pH 6.2 at 37°C at a constant twisting distance (20°), chewing frequency (40 strokes/min) and variable chewing distance (1.4 mm vs. 1.6 mm vs. 1.8 mm).

Results and discussion

The Nicorette classic gum product contains no flavoring agents. The release of nicotine from Nicorette classic gum indicates that the nicotine release has the following order; 1.4 mm/40°/40 strokes/min > 1.4 mm/20°/40 strokes/min > 1.8mm/20°/40 strokes/min > 1.8 mm/40°/40 strokes/min. As far as the chewing distance of 1.4 mm is concerned, the release at 40° twisting angle is more than at 20°. This was not observed in case of 1.8 mm chewing distance. This is due to the fact that the force exerted with the chewing distance of 1.8 mm may not be sufficient to compress the chewing gum of same surface area obtained in 1.4 mm. With an increase in the chewing distance, frictional force on the gum matrix is considerably reduced and resulting in unexpected release characteristics. This concludes that the data obtained for Nicorette chewing gums at 1.8mm chewing distance are inconclusive.

5.2.3.5.1 Summary

The findings from the presented results indicate that the *in vitro* drug release from the chewing gum formulations vary significantly with respect to the apparatus and the products as well. The drug release from Nicotinell formulation was rapid and complete with all the apparatus A setups and almost complete with the apparatus B setups.

In case of Nicorette formulations, the drug release was complete with the apparatus A and but not with the apparatus B setups. Larger deviations in the drug release profile (SD) were observed within an experimental setup and the gum product was found to be very sensitive, discriminating between the different instrument setups. This effect was more pronounced with tighter experimental parameters, for e.g., smaller distance between chewing jaws, twisting angle etc.

No significant difference in drug release profiles was found for the Nicotinell chewing gum formulations using the Apparatus A, whereas, small but distinct differences during the early phase of drug release was observed using the Apparatus B.

Generally speaking, differences in drug release observed are pertinent to the first 5 min of testing and it continued to exist until the end of investigation. Such a trend could be explained by the crumbling of gums at the initial mastication followed by the burst release of the API. Adjusting the chewing distance affected the release and when coupled with twisting motion, a clear rank order in release was observed. This effect is more pronounced for the Nicorette 2 mg than the Nicotinell 2 mg gums. In all the cases investigated, the drug release rate was in the following order; apparatus A (chewing distance): 0.3 mm > 0.5 mm > 0.7 mm, apparatus B (chewing distance): 1.4 mm > 1.6 mm > 1.8 mm; Twisting angle: 40° > 20°. This holds true for the other commercially available nicotine based gums as well.

Based on the results presented, it is possible to conclude that both the apparatus are capable of generating the drug release data which could be potentially used in the product development and routine quality control purposes.

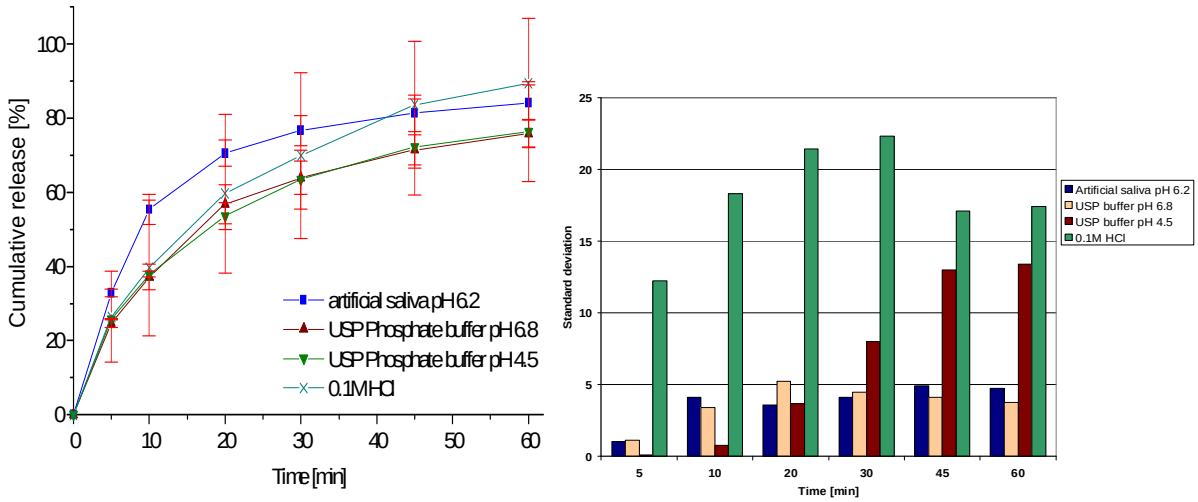
However in order to verify the suitability of the *in vitro* dissolution method for gum products, knowledge of their *in vivo* performance characteristics are necessary. Partial correlation of *in vitro* release data to the data obtained from *in vivo* studies on Catechins containing chewing gums using apparatus A was reported (Yang, *et al.* 2004). In case of nicotine gums, a correlation between an *in vitro* release and an *in vivo* chew-out study was demonstrated for Nicorette chewing gums of 2 mg dosage strength using apparatus B (Kvist, *et al.* 2000). The testing conditions were not biorelevant and such information is incomplete to arrive to any conclusion about the instruments.

5.2.3.6 Influence of apparatus B parameters on *in vitro* release

The apparatus B is seen as a potential design for release testing, since it is widely and commercially available and simplicity of its construction. This part of the analysis is focused with a perspective to evaluate this apparatus suitability in generating reproducible and reliable data. The developed *in vitro* drug release method was employed with the changes described in the individual following sections.

5.2.3.6.1 Evaluation of buffer influence

The influence of different buffer solutions over drug release was evaluated. The product performance in the compendial buffers of physiological relevance listed in the USP (USP 2015) was compared to that of the artificial saliva pH 6.2 (Hughes, *et al.* 2002). The drug release testing was performed using a single setup of the apparatus.



(a) (b)

Figure 5-31. Evaluation of different buffer solutions for *in vitro* drug release testing

a) In vitro drug release testing of nicotinell chewing gums using the bio-relevant setup (1.8mm; 20°; 40 strokes/min) b) plot of SD for drug release at all time points from different buffer solutions

Results and discussion

As shown in Figure 5-21, the *in vitro* drug release profiles of Nicotinell 2 mg mint gum in buffer solutions of physiological relevance indicate that the buffer pH has no influence over release with respect to nicotine based gums. However, analysis of the inter-individual variability demonstrate that the release profiles at pH 6.2 and pH 6.8 have a less variability (SD) compared to other buffer solutions. It was also found that the buffer solutions in the pH range of 6.0 to 6.8 are suitable for the bio-relevant *in vitro* release testing of nicotine based gum formulations.

Large inter-individual difference between the profiles was found in the presence of 0.1M HCl. This is due to the fact that the nicotine present in the gum is bound to a weak cation exchange

resin (nicotine polacrilex) which behaves like free acid below pH 4.0 resulting in variable degree of ion exchange interactions between free nicotine and hydrogen ions.

5.2.3.6.2 Evaluation of durability of chewing jaws

The durability of the chewing jaws after a single and multiple uses is shown in Figure 5-32. Drug release testing (n=3) was performed in apparatus B using a single pair of jaw for 1h using the standard setup of 1.4 mm chewing distance, 40 strokes/min and 20° twisting angle.

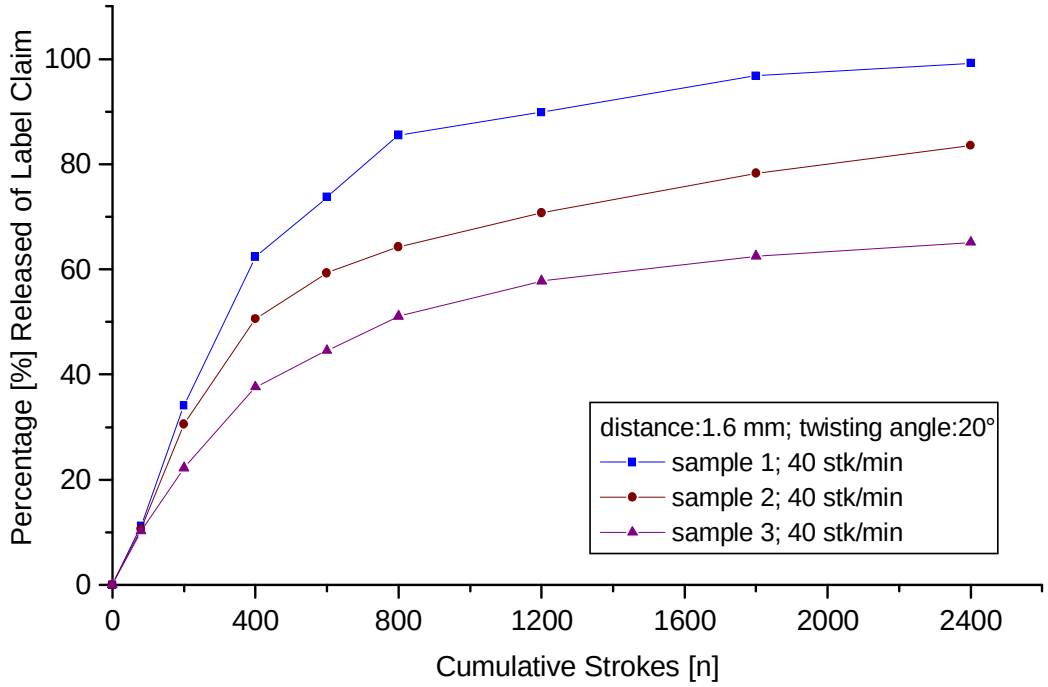


Figure 5-32. Influence of repeated usage of jaws over drug release tested using Nicotinell 2 mg mint gums

Results and discussion

The result of the drug release profiles generated by consecutive use of chewing jaws at a specific apparatus revealed that the jaws tend to undergo continuous change in the surface roughness which need to be verified on a product to product basis and when required, should be qualified after every single use. Since the chewing jaws used in apparatus B are sand blasted to provide frictional force during mastication, it is generally expected that the sand blasted surfaces wear-off after a specific period of use. This part of the study was to demonstrate the importance of surface roughness on drug release. The general recommendation is to employ always a new pair of jaws for drug release testing to eliminate undesirable effects of the chewing surface on release.

5.2.3.6.3 Evaluation of surface roughness of chewing jaws

The principle of operation of both apparatus is mastication. Unlike apparatus A, the apparatus B, consists of detachable chewing jaws. It is recommended that the chewing jaws have to be routinely qualified after single use. Surface roughness plays a key role in the drug release behavior. The surface roughness was measured on the upper jaw using surface roughness tester Mitutoyo SJ201. The jaws used for a single (n=1) and multiple investigations (n=3) were measured. A total of 8 jaws from each setup were taken and the individual surface roughness values (Ra) are shown graphically in Figure 5-23.

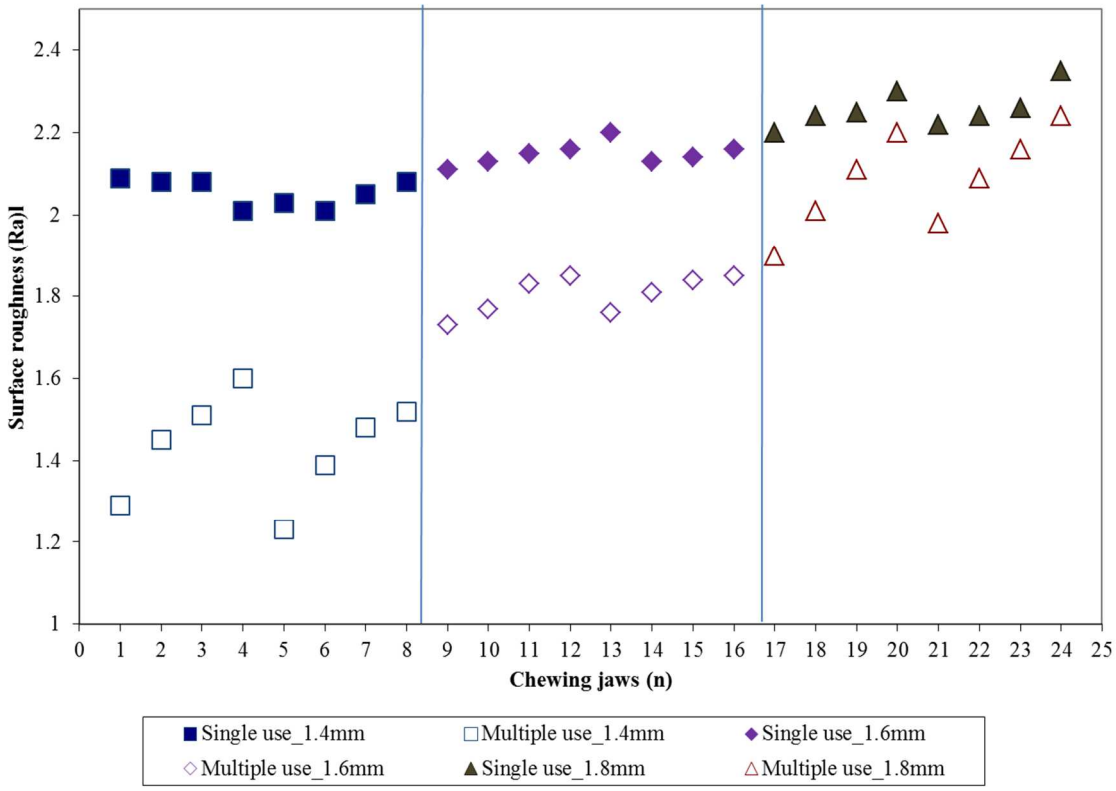


Figure 5-33. Surface roughness (Ra) of upper jaws measured after single and multiple uses

Results and discussion

The results of the consecutive or the repeated use of the chewing jaw indicated the diminishing surface roughness. This influence was seen directly from the drug release profiles generated from the same jaws. Later, it was quantitatively verified by the measurement of the surface roughness (Ra) resulting from a single and multiple use of jaws. The results (R_a) measured after a single-and-multiple uses are shown in Figure 5-33. The R_a values remain almost constant at higher chewing distance after a single use and a considerable reduction in the surface roughness after multiple uses particularly at a reduced chewing distance.

5.2.3.6.4 Evaluation of pre-tempering of gums

During mastication of the gumbase, the drug release is induced by the mechanical kneading force. The transition of the solid dosage form to a rubber glass elastic state at the human body temperature aid the kneading procedure smooth by the flow of gum components and to maintain the integrity of the dosage form itself. In order to understand the influence of pre-warming of the gums prior to drug release testing, the chewing gums were subjected to uniform heat treatment in a pre-tempered oven maintained at 37 °C ± 0.5 °C for an hour and then tested directly for drug release. The results shown in Figure 5-34 and Figure 5-35 indicate that the pre-warming of the gums alone has no significant influence over drug release.

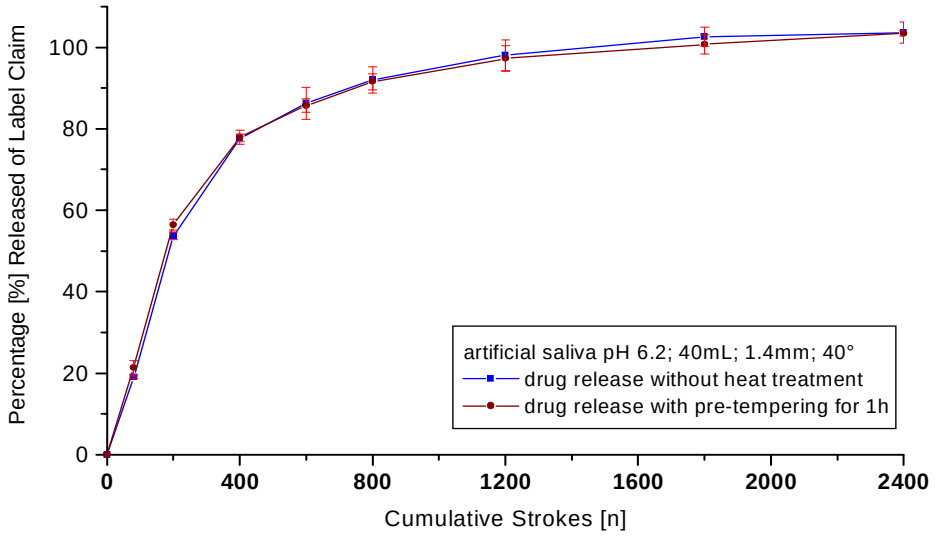


Figure 5-34. Influence of pre-warming of gums on release of nicotine from Nicotinell 2 mg mint (apparatus B)

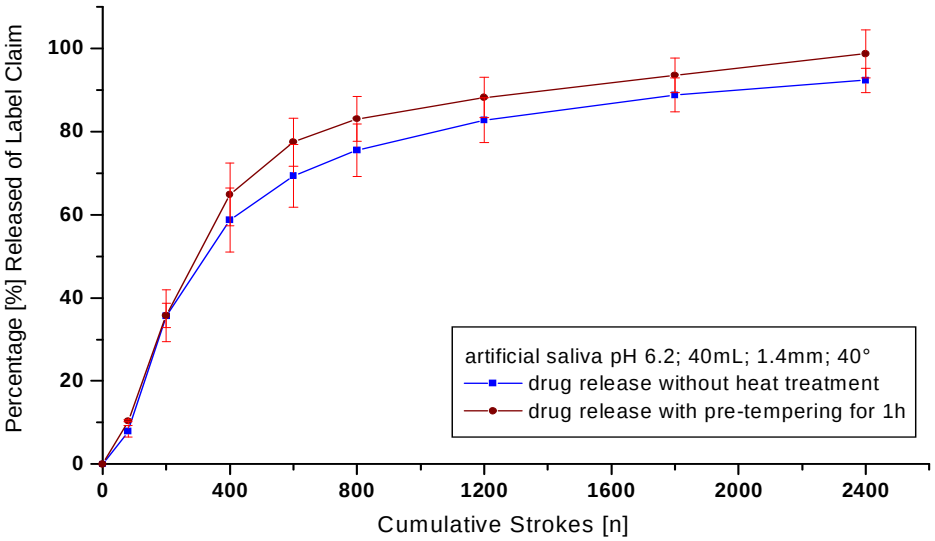


Figure 5-35. Influence of pre-warming of gums on release of nicotine from Nicorette 2mg freshmint (apparatus B)

Results and discussion

The effect of pre-tempering of the gums indicated no significant difference in the release behavior between the pre-tempered and the normal chewing gum. The activation of the inactive solid gum depend on number of factors such as a mastication, transition to a rubber elastic state and the diffusion of the medium to facilitate the above said processes. Pre-tempering under physiological temperature alone was not sufficient to activate the gum. This concludes that the pre-tempering is not necessary for the investigated nicotine containing chewing gum formulations.

5.2.3.6.5 Evaluation of the influence of nylon nets

The recommendation from the manufacturer of the apparatus B is to use a pair of standardized nylon mesh for the release testing. During the release testing, the gum piece was placed between the nylon nets which prevent the gum particles leaving the chewing surface and keep them intact during chewing. This is necessary for gum matrix that crumbles into pieces during the initial phase of mastication. The Nicorette and Nicotinell gums are manufactured using the traditional/conventional methods by employing melting and extruding the gumbase mixture. The purpose of this study was to evaluate whether it is mandatory to use nylon nets for release testing. The drug release testing was performed using Nicorette sample which was found to be very sensitive and the average drug release values (n=3) with the standard deviation are shown in Figure 5-36.

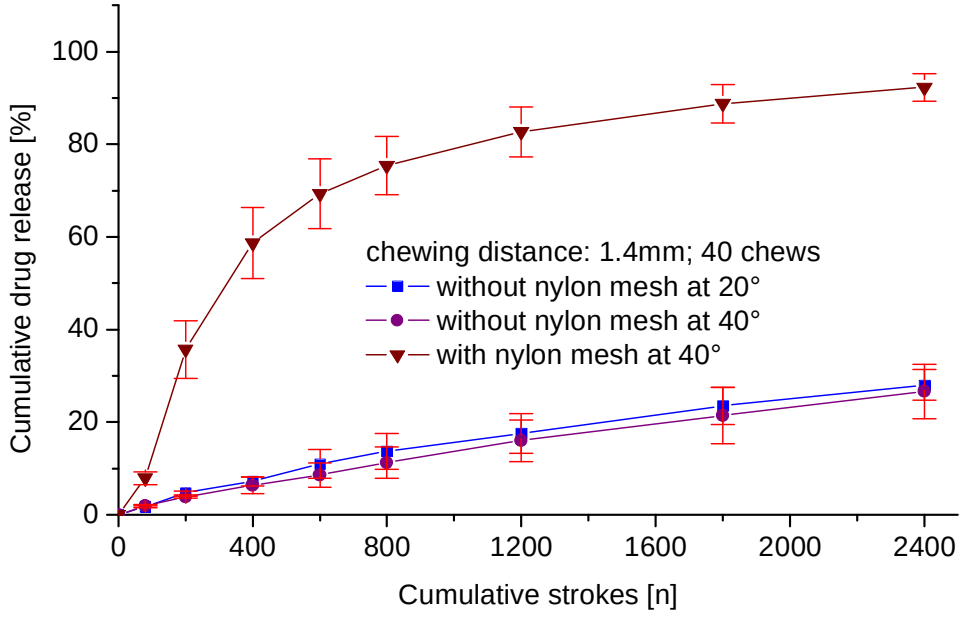


Figure 5-36. ***In vitro*** **drug release profiles of Nicorette 2 mg freshmint with and without nylon gum (apparatus B)**

Results and discussion

The use of nylon meshes for the release testing clearly indicated that the release testing cannot be performed without the nylon mesh at least for the gum products investigated. One of the primary reasons could be that the forces necessary for the renewal of chewing surface on the gum is considerably reduced in the absence of nylon nets. Even when the twisting angle is increased from 20° to 40°, no impact on the release was observed. This necessitates the use of nylon nets for all the performance testing on chewing gums. Since the inclusion of apparatus B in the Ph. Eur., the manufacturer has standardized the design specifications for all the components. Nylon nets geometry and the mesh size are also standardized by the manufacturer.

5.2.3.6.6 Flavor influence on *in vitro* release

Different flavors of the nicotine based gums were tested using the developed *in vitro* release method. The results are shown graphically in Figure 5-37.

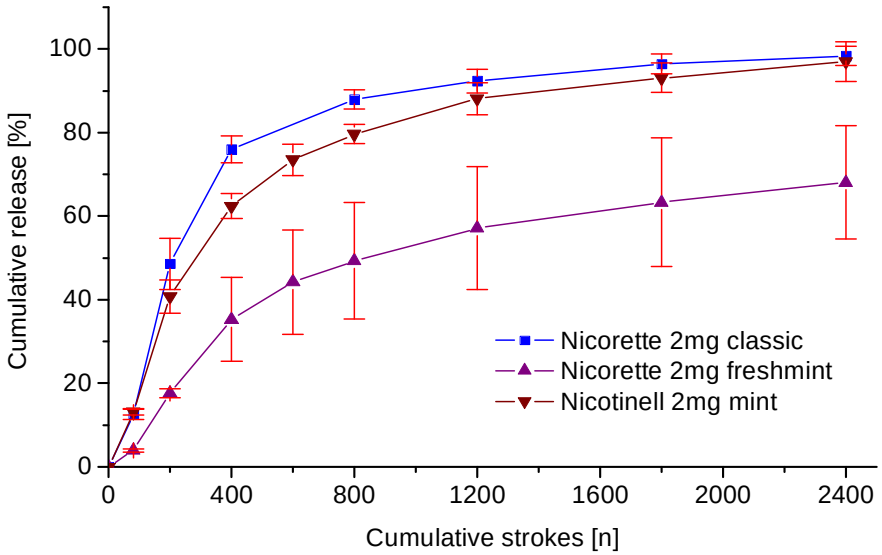


Figure 5-37. *In vitro* release of nicotine from different chewing gum formulations

Drug release profiles generated using the apparatus B at the following setup: 1.4mm/20°/40 strokes, 40mL of artificial saliva pH 6.2 at 37°C.

Results and discussion

The *in vitro* drug release profiles shown in Figure 5-37 clearly indicate that the release of nicotine from gum matrix is affected by the type of flavor. As seen, the classic gum which does not contain any flavor has a faster release of nicotine compared to the freshmint flavor from the same manufacturer. This is due to the fact that the mint flavor which is a volatile oil renders the gum matrix softer at the early phase of release testing. In case of classic gum, it was observed that the gum matrix crumbles during initial mastication resulting in a faster release of nicotine. However, it was not possible to compare the release of nicotine from gum

products of different manufacturers (Nicorette freshmint vs Nicotinell) as they differ in texture, composition and possibly method of manufacturing.

As an overall conclusion, it can be stated that the methodology and the apparatus parameters proposed were found to be suitable for the *in vitro* performance testing of the chewing gums containing API, particularly gums manufactured by conventional techniques like hot melt extrusion.

5.2.3.7 Evaluation of f_2 test statistics

The f_2 test statistics was performed for the nicotine based gum formulations. Since the *in vitro* data were generated using both the apparatus, it was assumed that the f_2 analysis would provide critical information about the apparatus parameters. For the feasibility study, 8 different setups for apparatus A and 12 for apparatus B were considered. The data generated were directly compared with each one of the apparatus setup to the other based on the two products. The results of the analysis have been summarized and represented graphically. The results were also published (Gajendran, *et al.* 2012).

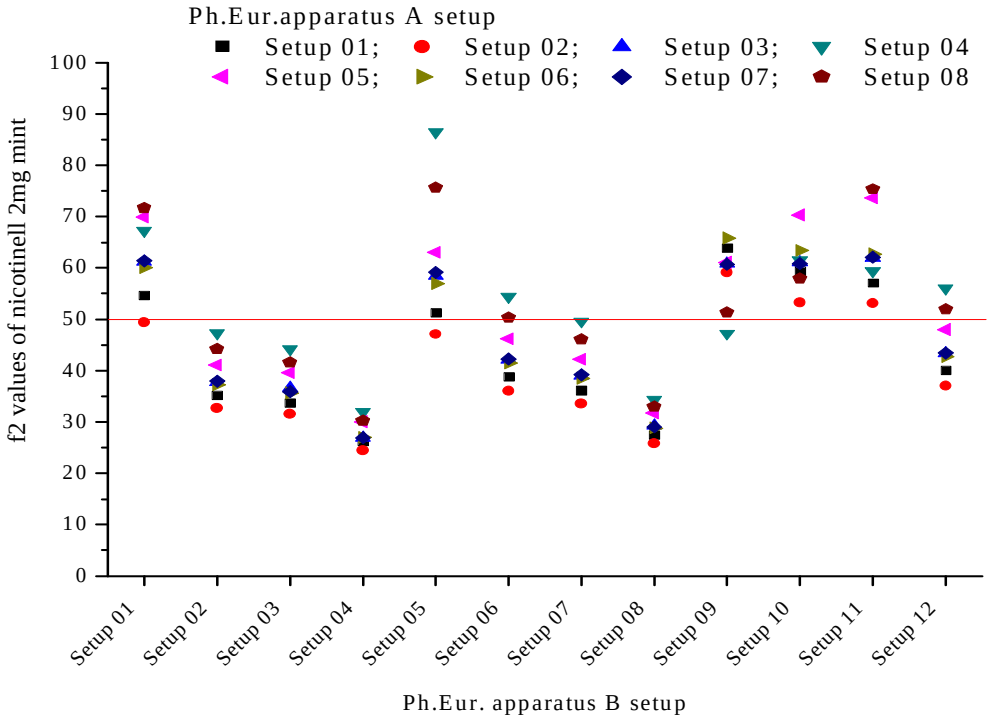


Figure 5-38. Comparison of similarity factor (f_2) values for Nicotinell 2mg mint gums

The apparatus A and B parameters for the results shown in Figure 5-38 and Figure 5-39 are given in Table 4-7 and Table 4-8. The objective was to assess the interchangeability of the apparatus for a gum product thereby a similar drug release profiles are generated under specific apparatus parameters (setups). The measure of evaluation of profile similarity is achieved by f_2 analysis. Value of $f_2 > 50$ for profiles under comparison signifies their

similarity. Since both of the apparatus A and B are described in the Ph. Eur., it is necessary to link the setups from apparatus for the purpose of interchangeability.

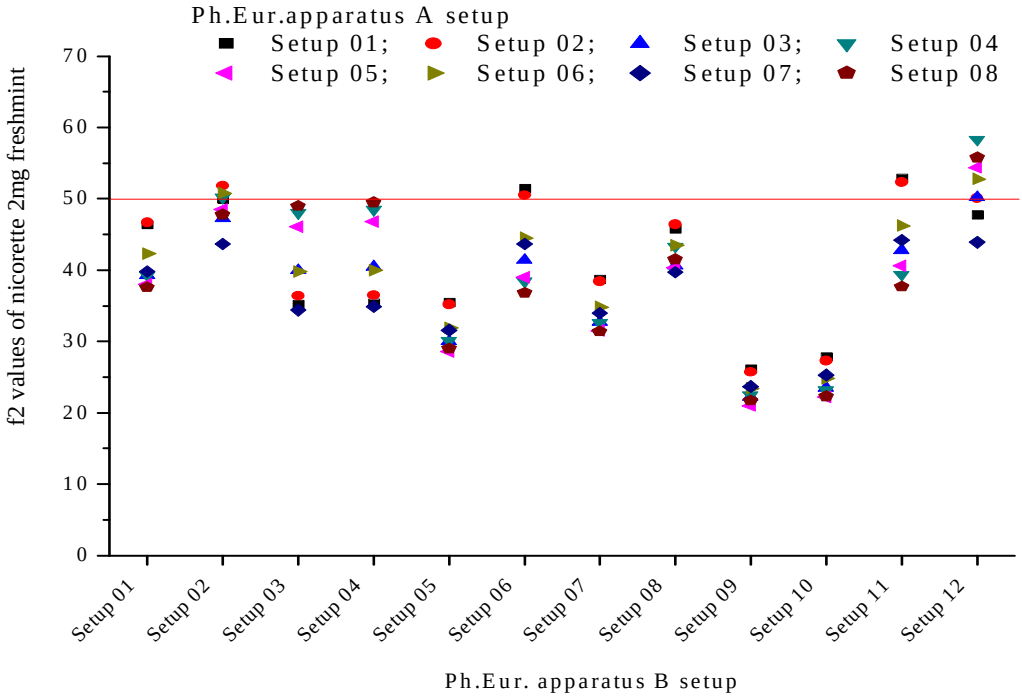


Figure 5-39. Comparison of similarity factor (f_2) values for Nicorette 2 mg freshmint gums

Plot of similarity factor values (f_2) determined between the in vitro dissolution data generated from the Ph. Eur. apparatus A and apparatus B for the Nicorette 2mg freshmint drug product. Data from 8 different setups of apparatus A is compared to the 12 different setups of the apparatus B. The mean drug release data was derived from n=3 dissolution runs at each apparatus setup.

Results and discussion

The results of the $f_2$test statistic are presented graphically in Figure 5-38 and Figure 5-39 for the gum products Nicotinell and Nicorette. As described in the objective, the purpose was to demonstrate the suitability of the instruments in the routine quality control and development purposes. Due to the differences in the construction and operation of the instruments under study, it would not be possible to compare the *in vitro* drug release results from the apparatus directly even with the same product. The differences in drug release profiles could be attributed by several factors, beginning from instrument design to the formulation of the product. In order to understand the optimal instrument parameters which make the direct comparison plausible, similarity factor (f_2) calculation was used to identify the similar drug release profiles generated by both the apparatus and their corresponding operational parameter.

The purpose is to test the interchangeability of the apparatus for a given product. As seen in Figure 5-38 and Figure 5-39, the red lines mark minimum requirements (f_2 = 50) to accept the profiles are similar. Values below this indicate the dissimilarity of the profiles compared.

From the results, it is seen that more similar profiles were generated by the apparatus for Nicotinell 2 mg mint gums. This is due to the soft texture of the nicotinell gum product which has more elasticity during mastication. This provides better kneading and renewal of chewing surfaces even with a minimal force exerted by the apparatus B.

On the other hand, very few similar profiles ($f_2 < 50$) were generated between the apparatus A and B for Nicorette 2 mg freshmint gums. However, none of the apparatus parameters compared resulted in f_2 values close to unity ($f_2 = 100$). This indicates that the apparatus interchangeability for the gum products is not generally recommended or requires prior knowledge on the performance of the product *in vivo* to verify the suitability.

5.2.3.8 Evaluation of mean dissolution times

The mean drug release / dissolution time (MDT) calculated from the *in vitro* drug release data from both the apparatus with respect to the product is shown in Figure 5-40. The lines passing through the data points indicate the average MDT obtained from all the apparatus setup used.

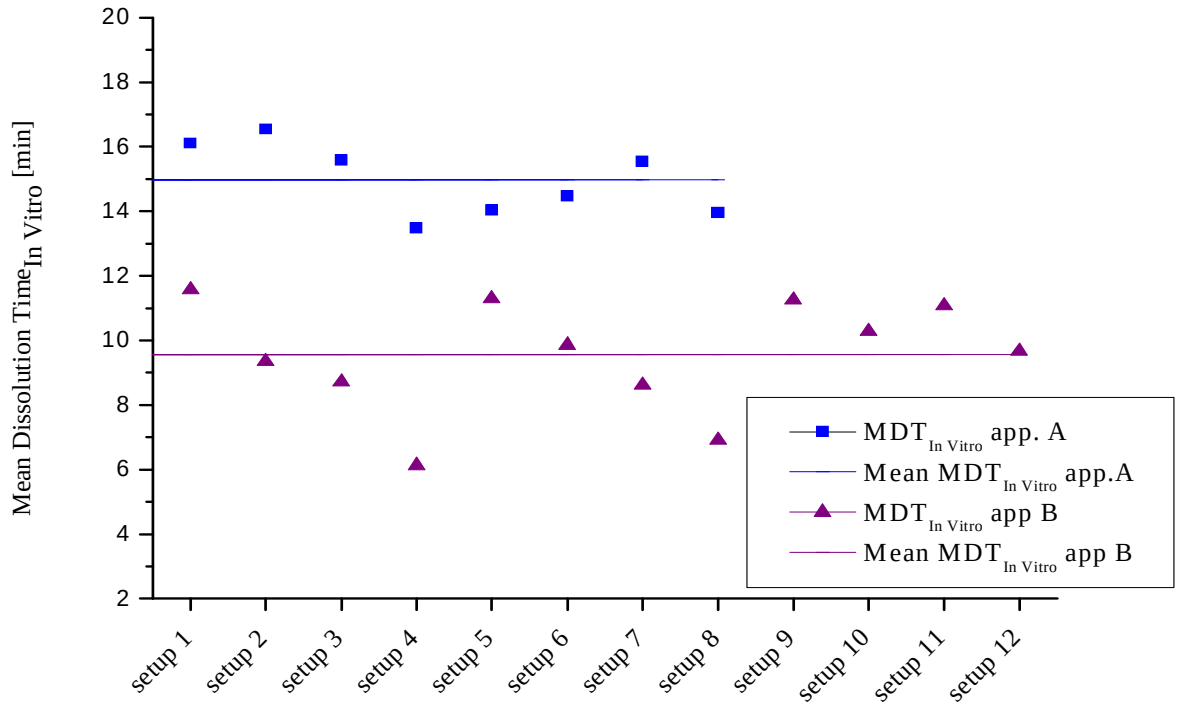


Figure 5-40. Mean dissolution times of Nicotinell 2 mg mint gums (apparatus A and B)

The apparatus setup parameters for Figure 5-40 and Figure 5-41are given in Table 4-7 and Table 4-8. Plot shows the $MDT_{(in\ vitro)}$ obtained for Nicotinell 2 mg mint gums. There are 8 different setups for apparatus A (MDT values shown in square) and 12 different setups for apparatus B (MDT values shown in triangle). The straight lines indicate average MDT values for the gum products.

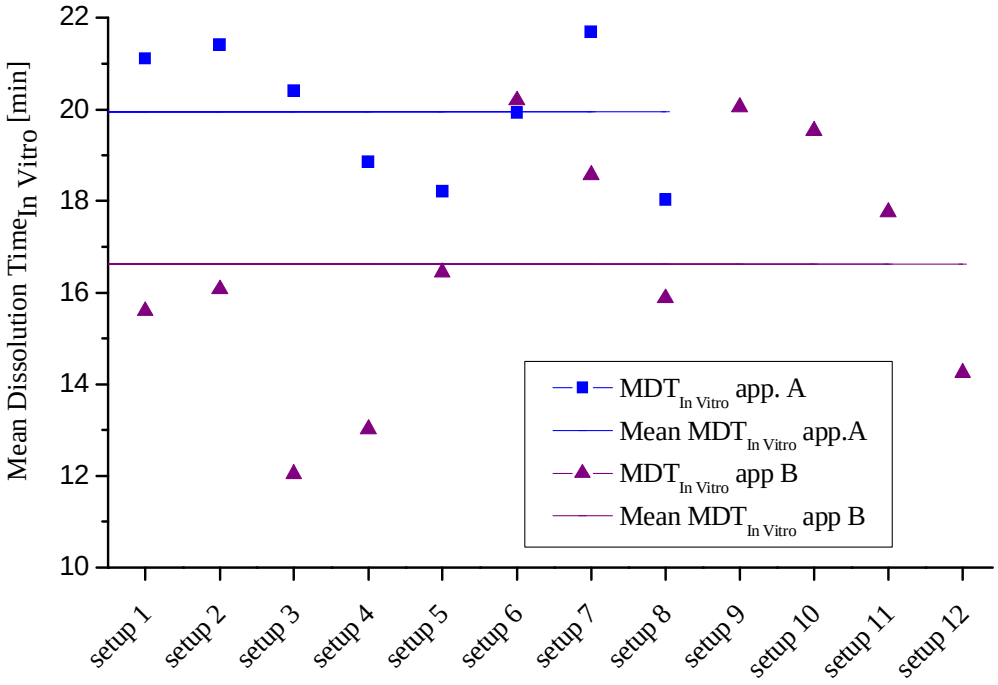


Figure 5-41. Mean dissolution times of Nicorette 2mg freshmint gums (apparatus A and B)

Plot shows the $MDT_{(in\ vitro)}$ obtained for Nicorette 2 mg freshmint gums. There are 8 different setups for apparatus A (MDT values shown in square) and 12 different setups for apparatus B (MDT values shown in triangle).

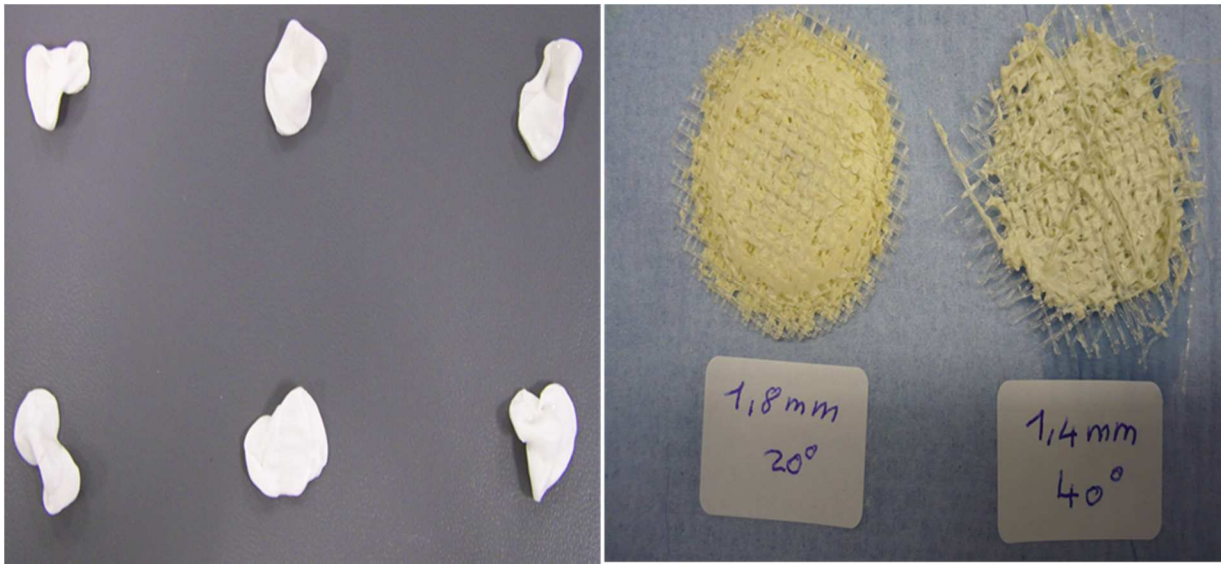


Figure 5-42. Chewing gums after mastication by apparatus A and B

Appearance of chewing gums after mastication for 60 min. Figure on the left for apparatus A and the figure on the right for apparatus B

Results and discussion

As observed from the Figure 5-40 and Figure 5-41, the MDT values for Nicorette and Nicotinell chewing gums using apparatus A are less scattered than the MDT values obtained from apparatus B. This phenomenon is usually expected with such a system since strong masticatory chewing force is exerted on the gum products in apparatus A than B and the

sensitivities of drug products to the *in vitro* release methodology is suppressed. A clear difference in the chewing pattern can be seen in the Figure 5-42.

The analysis of the drug release data from both the apparatus using a model independent approach revealed shorter mean dissolution times [MDT] in apparatus B than apparatus A for both nicotine gum formulations, even at conditions when complete drug release was not observed. This explains that the drug release rate was higher during the initial masticatory period and the later phase of drug release was influenced by the product characteristics. During this period, the water soluble excipients are released, rendering the gum hard enough to resist mastication which eventually led to the incomplete release profiles. This was not the case with apparatus A where the robust construction and greater mechanical forces acting on the gum product overwhelms the sensitiveness of the products, so that no appreciable difference in drug release profiles could be observed.

5.2.3.9 Release kinetics from nicotine gum formulations

The results of best fit model for all the apparatus setups are summarized in Table 5-7 - Table 5-10. The purpose was to describe the release kinetics from the gum formulations and to evaluate the influence of the variables which may change the mechanism of release. The results are also shown graphically Figure 5-43 - Figure 5-44 for an overview. The value of r^2 close to 1 demonstrates the goodness of fit for the particular model.

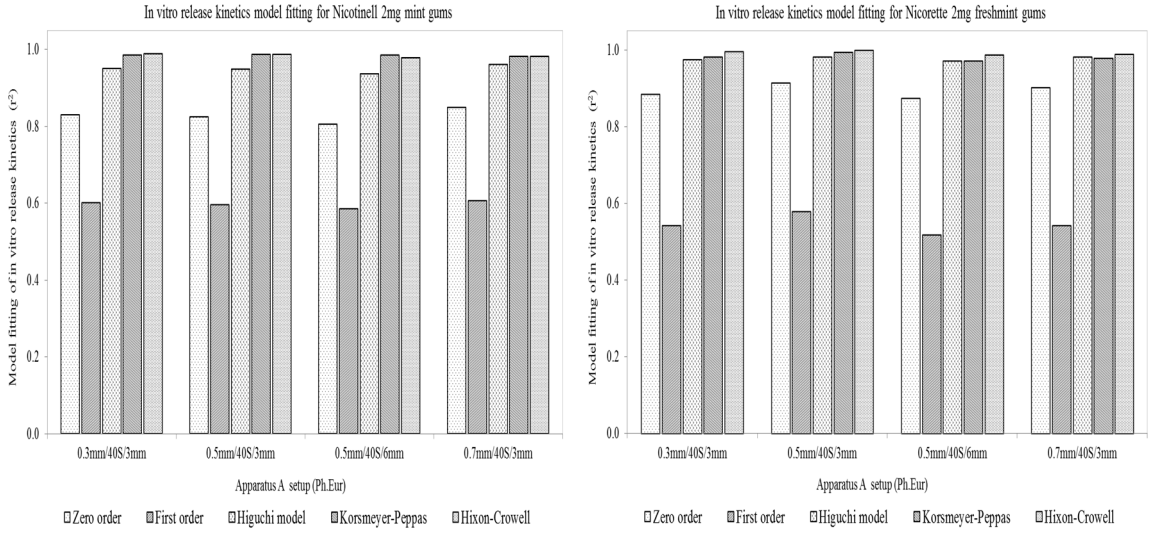
Table 5-7. Summary of *in vitro* release kinetics evaluation from Nicotinell 2 mg mint gums using apparatus A

Apparatus setup*)	Zero Order		First Order		Higuchi Model		Korsmeyer-Peppas			Hixson-Crowell	
	r^2	k_0 (h^{-1})	r^2	k1 (h^{-1})	r^2	k_H ($h^{-1/2}$)	r^2	n value	K_{kp} (h^{-n})	r^2	k_{HC} ($h^{-1/3}$)
0.3mm/3mm	0.8293	1.4867	0.6010	0.0274	0.9506	14.9206	0.9849	0.8315	2.1172	0.9898	-0.0792
0.5mm/3mm	0.8255	1.4945	0.5962	0.0278	0.9484	15.0150	0.9867	0.8503	2.1901	0.9868	-0.0793
0.5mm/6mm	0.8050	1.3878	0.5856	0.0265	0.9361	14.0285	0.9851	0.8235	2.0705	0.9787	-0.0731
0.7mm/3mm	0.8494	1.4335	0.6063	0.0288	0.9618	14.2992	0.9815	0.8662	2.0200	0.9812	-0.0657

Table 5-8. Summary of *in vitro* release kinetics evaluation from Nicorette 2 mg freshmint gums using apparatus A

Apparatus setup*)	Zero Order		First Order		Higuchi Model		Korsmeyer-Peppas			Hixson-Crowell	
	r^2	k_0 (h^{-1})	r^2	k_1 (h^{-1})	r^2	k_H ($h^{-1/2}$)	r^2	n value	K_{kp} (h^{-n})	r^2	k_{HC} ($h^{-1/3}$)
0.3mm/3mm	0.8837	1.7553	0.5408	0.0524	0.9737	17.2715	0.9807	1.6592	4.0062	0.9947	-0.0683
0.5mm/3mm	0.9122	1.8396	0.5777	0.0590	0.9796	17.8702	0.9921	1.7636	4.1495	0.9978	-0.0690
0.5mm/6mm	0.8732	1.6561	0.5165	0.0525	0.9698	16.3606	0.9692	1.7128	4.0649	0.9863	-0.0589
0.7mm/3mm	0.9000	1.6369	0.5414	0.0535	0.9812	16.0209	0.9773	1.6904	3.6975	0.9874	-0.0532

*) the apparatus setup represents the different combination of the horizontal and vertical pistons in the chewing chamber of the apparatus



(a) (b)

Figure 5-43. Evaluation of *in vitro* release kinetics of nicotine from a) Nicotinell 2 mg mint b) Nicorette 2 mg freshmint (apparatus A)

Results and discussion

Among the five kinetic models used to assess the release kinetics, it was found that the r2 values were predominantly close to 1 for Higuchi, Korsmeyer and Hixon-Crowell models. This suggests that the nicotine release from chewing gum formulation is controlled by more than one process. This involves the diffusion of drug molecules within the gum matrix (Higuchi model) in combination with polymer relaxation (Korsmeyer-Peppas model) and constant change in the particle size and surface area (Hixson-Crowell model). The value of the release exponent (n) in Korsmeyer-Peppas equation ranging from 0.82 to 1.76 suggests that the release mechanism is a combination of non-fickian and super case II transport which translates the mechanism of drug transport as a function of polymeric matrix relaxation. The r^2 value of zero order release > 0.8 indicates that the drug release is independent of the amount of nicotine present in the gum matrix.

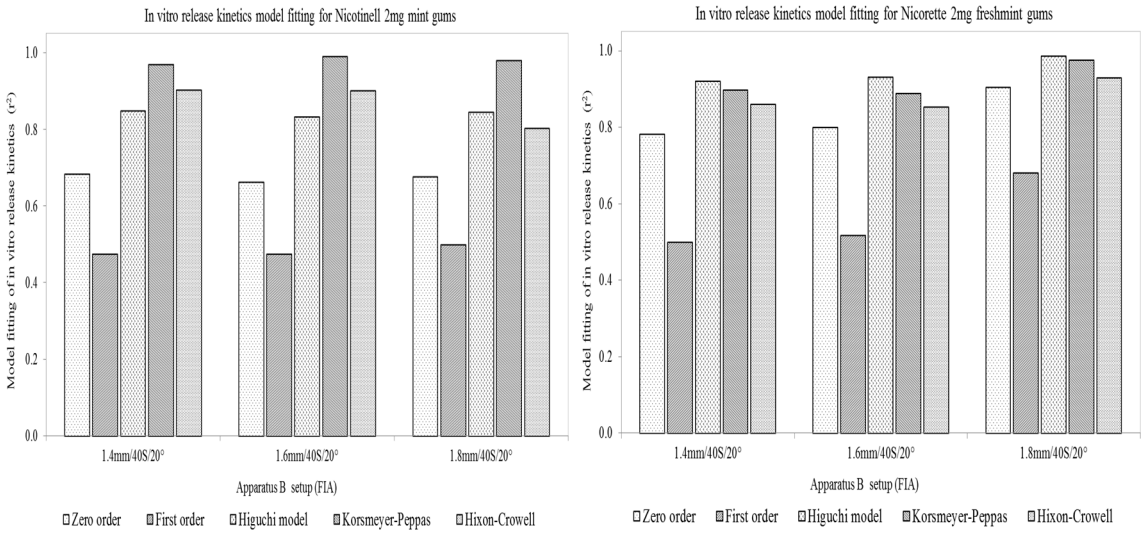
Table 5-9. Summary of *in vitro* release kinetics evaluation from Nicotinell 2 mg mint gums using apparatus B

Apparatus setup*)	Zero Order		First Order		Higuchi Model		Korsmeyer-Peppas			Hixson-Crowell	
	r^2	k_0 (h^{-1})	r^2	k_1 (h^{-1})	r^2	k_H ($h^{-1/2}$)	r^2	n value	K_{kp} (h^{-n})	r^2	k_{HC} ($h^{-1/3}$)
1.4mm/20°	0.6833	1.1699	0.4760	0.0227	0.8484	12.2198	0.9692	0.9825	3.6282	0.9027	-0.0469
1.6mm/20°	0.6622	1.2130	0.4749	0.0237	0.8321	12.7458	0.9907	1.0151	3.9477	0.9010	-0.0514
1.8mm/20°	0.6774	1.0113	0.4999	0.0219	0.8443	10.5832	0.9806	0.8013	1.9669	0.8027	-0.0297

Table 5-10. Summary of *in vitro* release kinetics evaluation from Nicorette 2 mg freshmint gums using apparatus B

Apparatus setup*)	Zero Order		First Order		Higuchi Model		Korsmeyer-Peppas			Hixson-Crowell	
	r^2	k_0 (h^{-1})	r^2	k_1 (h^{-1})	r^2	k_H ($h^{-1/2}$)	r^2	n value	K_{kp} (h^{-n})	r^2	k_{HC} ($h^{-1/3}$)
1.4mm/20°	0.7817	0.9717	0.4985	0.0333	0.9200	9.8816	0.8965	0.8716	0.8138	0.8599	-0.0223
1.6mm/20°	0.8002	0.7798	0.5165	0.0340	0.9315	7.8865	0.8891	0.7953	0.5403	0.8529	-0.0159
1.8mm/20°	0.9050	0.5775	0.6801	0.0308	0.9853	5.6486	0.9749	0.6588	0.4025	0.9296	-0.0108

*) the apparatus setup represents the different combinations of the chewing distance with a constant twisting angle and chewing frequency.



(a) (b)

Figure 5-44. Evaluation of *in vitro* release kinetics of nicotine from a) Nicotinell 2 mg mint b) Nicorette 2 mg freshmint (apparatus B)

Results and discussion

The results presented in Table 5-9 and Table 5-10 indicate that the mathematical relationship fitted the best with the Higuchi, Korsmeyer-Peppas and Hixson-Crowell model with r^2 values close to 1. The results presented were generated for the nicotine gum products using the apparatus B. The release exponent value (n) ranging from 0.65 to 1 indicates the release mechanism from the gum formulations is a mixed phenomenon commonly called as anomalous transport (non-fickian and super case II transport) involving drug diffusion in hydrated matrix followed by polymer relaxation.

The r^2 values for the mathematical models tested differ between the apparatus A and apparatus B. There were many instances where $r^2 > 0.9$ was observed for drug release data generated from apparatus A than B. This is due to the fact that force exerted by the apparatus A is considerably more than apparatus B which results in better kneading effect and a constant masticatory force throughout the chewing process. Due to this effect, the nicotine release from the gum matrix resembles zero-order release and is similar and almost complete in most of the apparatus setup parameters. However, it can inferred from the Figure 5-43 and Figure 5-44 that the highest r^2 values were observed for Higuchi, Korsmeyer-Peppas and Hixson-Crowell models indicating the nicotine release kinetics from gum products using apparatus A and B were same and was not influenced by the apparatus setups. Applying these mathematical models can be empirical and no definitive conclusion can be drawn to explain the dominating mass transport mechanism.

5.2.4 Dimenhydrinate based chewing gum formulations

Following the demonstration of the suitability of the developed drug release methodology with nicotine based gums; performance testing was extended to the commercially available dimenhydrinate chewing gums using the apparatus B. Since no monographs related to product quality for this gum in the Ph. Eur. exist, assay testing was performed using the method developed for the nicotine gums. The results are presented in the following section.

5.2.4.1 Dimenhydrinate content in chewing gums

The assay samples were prepared from 3 different samples using the method described under content uniformity testing. The samples were measured in duplicate and the individual results are reported.

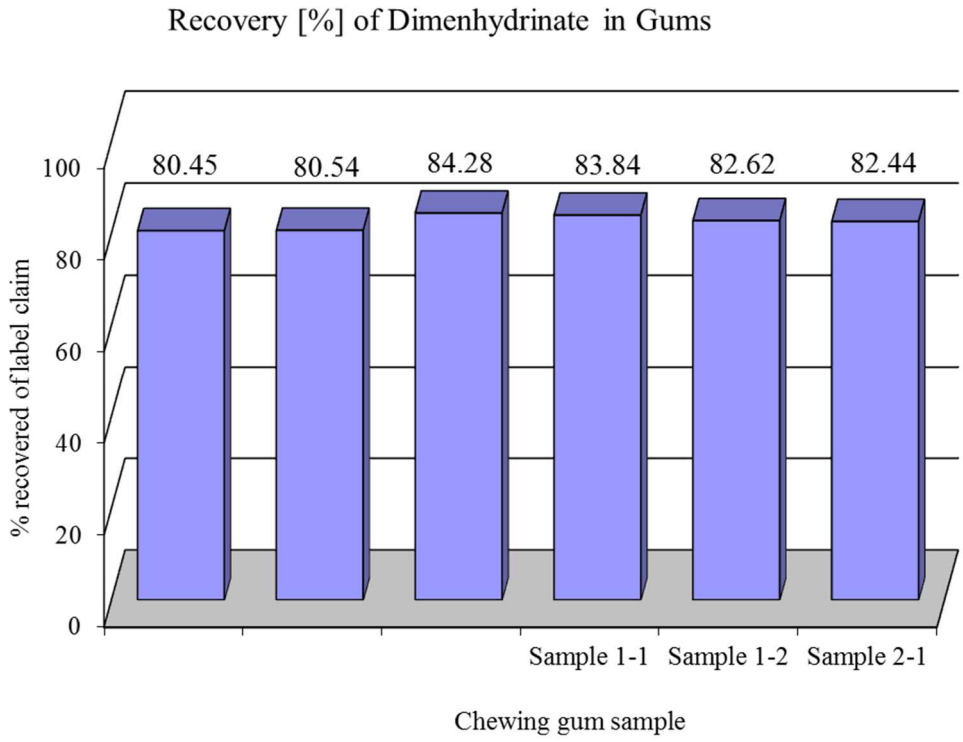


Figure 5-45. Recovery of dimenhydrinate from gum formulations

Bars represent % recovered from individual gums. Assay/extraction method developed for nicotine chewing gums was used to extract the dimenhydrinate from gums. Average amount extracted was about 82% of the labelled amount (dosage strength of dimenhydrinate is 20 mg/ gum)

Results and discussion

The maximum amount of dimenhydrinate recovered from the chewing gum is between 80% and 85%. It was not possible to extract the drug substance completely using the developed method. Other alternative methods involving solvents like chloroform and cyclohexane was also attempted and the recoveries were found to be less than the assay method described for nicotine. The incomplete release during the *in vitro* dissolution testing could be explained

using this phenomenon of binding between the drug substance and the gum components. However the results were consistent within the test with a maximum variability of 5 %.

5.2.4.2 *In vitro* drug release testing of dimenhydrinate containing chewing gums

The intention of release testing of dimenhydrinate containing chewing gums was due to the fact that unlike the nicotine based gums, dimenhydrinate gums are manufactured using a direct compression technique. The gum is having MA in Germany and is available as OTC product. A central fracture of the gum reveals the dry blends of the excipients indicating the method of manufacturing which is a simple tabletting (direct compression technique). Upon crushing, the gum usually disintegrates and allows the medium or the saliva (*in vivo*) to wet the components, which subsequently turns the wet gumbase to attain the elasticity to behave like a normal chewing gum mass. This technique allows the manufacturer to employ normal tabletting machines to produce gums that combines the advantage of using thermo-labile drug substances to be incorporated into the gum safely which otherwise is complicated with a hot melt extrusion technology.

The pharmacological activity of both the diphenhydramine and 8-chlorotheophylline has already been described and well investigated (Shahrzad, *et al.* 2005,Kvist, *et al.* 1999). During the drug release testing, it was expected that the dimenhydrinate salt will dissociate in the dissolution medium into diphenhydramine and 8-chlorotheophylline. The quantification of any one of the compound will correspond to the release behavior of the other provided the ratio or proportion of the salt is taken into consideration. In order to avoid any errors during analysis by the HPLC, the peak areas obtained for diphenhydramine and 8-chlorotheophylline were added and quantified using an external standard calibration method using Dimenhydrinate as a reference standard. The result of the *in vitro* drug release testing is presented in Figure 5-46.

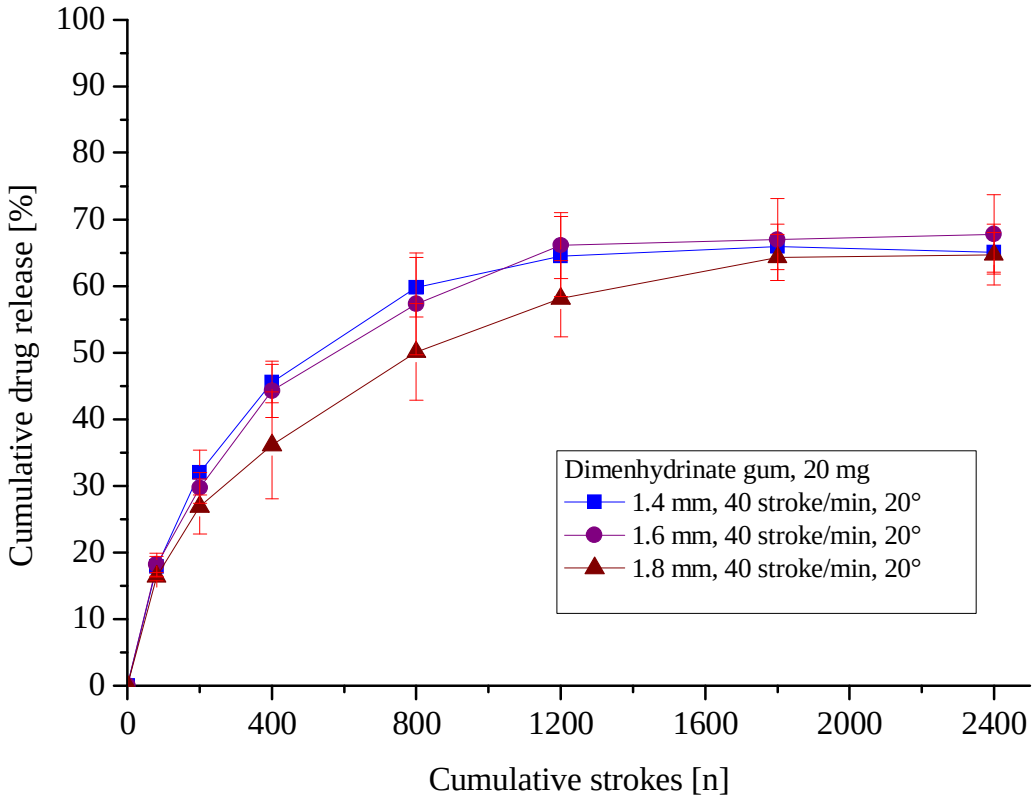


Figure 5-46. ***In vitro*** **release of dimenhydrinate from the gum product**

The data were generated using variable chewing distance of the apparatus B maintained at constant chewing frequency and twisting angle. Drug release profiles represent data (n=3 ± SD) generated using 40mL of artificial saliva pH 6.2 at 37°C.

Results and discussion

A visual observation of the drug release profiles shown in Figure 5-46 indicates absence or a very small influence of the chewing distance on drug release. The maximum amount of drug released at the end of testing is about 70 % of the label claim. The solubility investigations of dimenhydrinate indicated that the solubility (1.6 mg/mL) is not a rate limiting factor for dissolution/drug release. One explanation might be that drug substance remained/trapped within the gum matrix during the dissolution process. It would be difficult to determine the residual content in the cud gum after drug release studies since the gum components adhere to the nylon mesh. The *in vitro* drug release profile for chewing distances 1.4 mm and 1.6 mm are nearly identical. The profiles of 1.4 mm and 1.6 mm reached the asymptote at about 30 min (1200 cumulative strokes) of testing and at 45 min (1800 cumulative strokes) for 1.8 mm chewing distance. The presence of asymptote generally indicates the completion of *in vitro* drug release process. There is still a difference of about 10 % to 12 % between the assay and drug release value. In case of dimenhydrinate chewing gums, the label claim states 20 mg of dimenhydrinate in the gum product. This leads to the conclusion that the rest of dimenhydrinate is still bound to the gum matrix and was not released during the chewing process. The reason might be the force necessary to transform the gum product to a thickness corresponding to the pre-adjusted chewing distance (1.4 mm/ 1.6 mm/1.8 mm) is inadequate

to knead the gum for a renewable surface. Chewing distance < 1.4 mm is not suitable since the chewing jaws tend to compress the gum matrix much longer that affects the operation of the chewing apparatus. The decision to choose smallest chewing distance is always product dependent (gum product thickness, texture and softness) and can be achieved only using trial and error methods. In order to evaluate the influence of another buffer solution, further drug release testing was carried out in a compendial buffer adjusted to pH 6.8.

5.2.4.3 Evaluation of buffer influence on dimenhydrinate release

The buffer pH 6.8 was chosen to keep the drug release testing within the physiological range. Besides, the solubility data of dimenhydrinate indicated that the solubility of diphenhydramine and 8-chlorotheophylline are similar in the pH range of 6 to 7. The results of the analysis are shown in Figure 5-47.

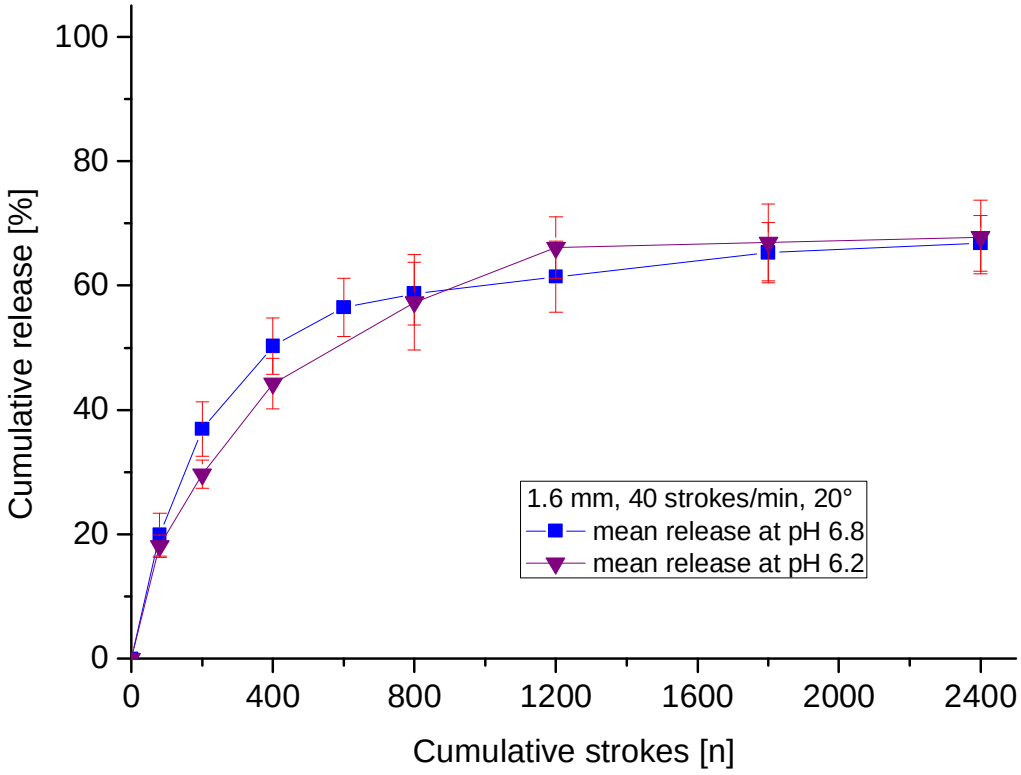


Figure 5-47. Influence of buffer solutions on dimenhydrinate release
Data represent in vitro drug release at constant apparatus B setup (1.6mm, 20°, 40 strokes/min)

Results and discussion

The results shown in Figure 5-46 indicate that the release of dimenhydrinate from the chewing gum is not influenced by the change in the apparatus parameters. The difference in release observed between the drug release profiles was negligible. This is confirmed quantitatively by the f_2 test statistic summarized in Table 5-11. It can also be inferred that the profiles reach asymptote after 1200 strokes for chewing distances adjusted to 1.4 mm and

1.6 mm. In case of 1.8 mm chewing distance, an asymptote was observed at 1800 strokes. Moreover a change in the dissolution medium pH with one of the apparatus setup yielded the same result which is shown in Figure 5-47. This indicates, under normal conditions of testing, a complete release from the formulation is not expected. It is also expected that the *in vivo* release would be same for the drug product. However, clinical data was not found in the public domains to verify the *in vitro* release methodology.

5.2.4.4 Evaluation of f_2 test statistics

The *in vitro* drug release data from the different apparatus B setup was treated using the f_2 statistic proposed by (Moore, *et al.* 1996). The method of evaluation is described in the earlier section. The results of the test statistic are summarized in Table 5-11.

Table 5-11. f_2 test statistic for dimenhydrinate containing chewing gums using variable chewing distance of the apparatus B

Test parameter	**Apparatus setup (chewing distance as a primary variable)**		
	1.4mm vs. 1.6mm	**1.4mm vs. 1.8mm**	**1.6mm vs. 1.8mm**
f_2 statistic	85	62	64

Results and discussion

As shown in Table 5-11, the drug release profiles of apparatus setup 1.4 mm and 1.6 mm are very similar compared to that of other setups. However, a value of $f_2>50$ is always acceptable. With regard to the dimenhydrinate gums, all the profiles are considered to be similar. There is no significant influence of the chewing distance on drug release.

5.2.4.5 Evaluation of mean dissolution times (MDT)

The results of the *in vitro* mean dissolution times (MDT) are summarized below in Table 5-12.

Table 5-12. Evaluation of MDT of dimenhydrinate containing chewing gums

Time [min]	**1.4mm/40strokes/20°**	**1.6mm/40strokes/20°**	**1.8mm/40strokes/20°**
AUMC 0	0.00	0.00	0.00
AUMC 2	17.86	18.17	16.40
AUMC 5	49.39	40.25	36.75
AUMC 10	102.08	109.35	69.00
AUMC 20	213.30	196.05	210.00
AUMC 30	116.00	219.50	200.00
AUMC 45	55.88	32.63	232.50
AUMC 60	-44.63	42.00	21.00
AUMC 0-60	509.87	657.95	785.65
$MDT_{in\ vitro}$ [min]	**7.83**	**9.71**	**12.14**

Results and discussion

The MDT of dimenhydrinate containing chewing gum shows a constant difference in the MDT with respect to the chewing distance. The difference in MDT between the two consecutive apparatus setup is < 3 min. In comparison to the nicotine based chewing gums, the release behavior was found to be similar and the value lies almost in this range for the apparatus B.

5.2.4.6 Release kinetics from dimenhydrinate gum formulations

The *in vitro* drug release kinetics was evaluated using the method described earlier in the section.4.16.3 The results of the analysis are shown in Figure 5-48 and summarized in Table 5-13.

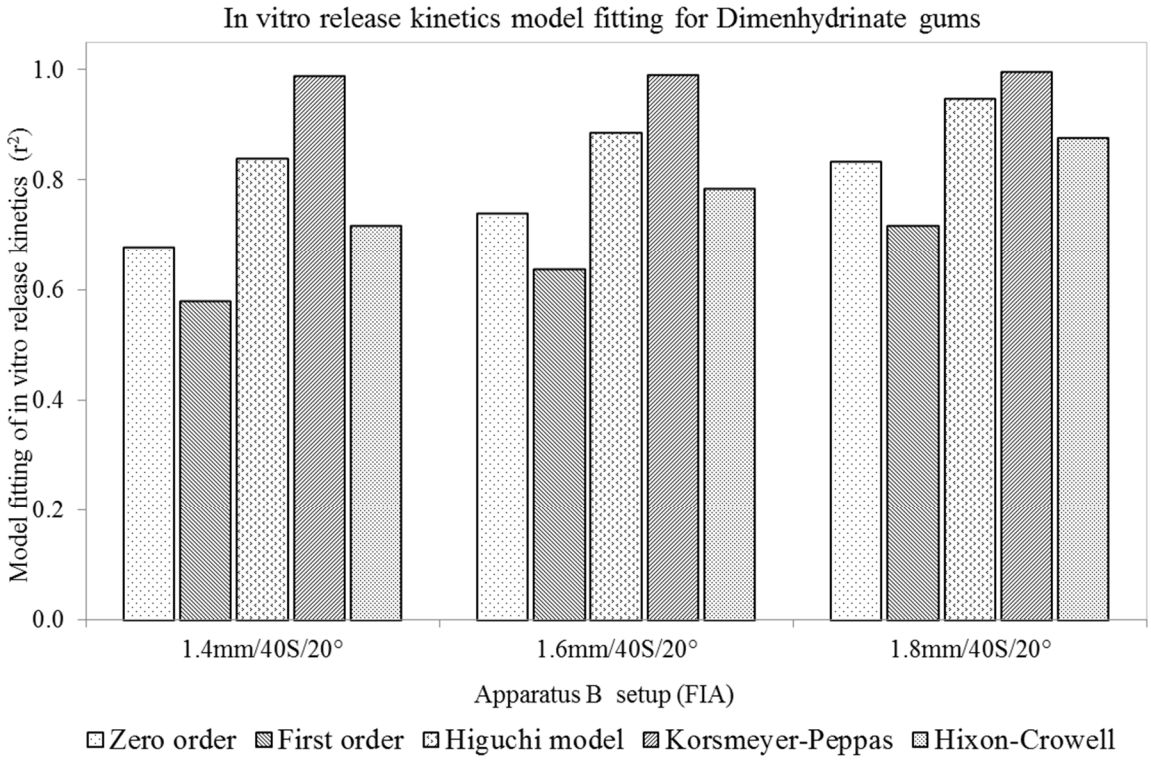


Figure 5-48. Evaluation of in-vitro release kinetics of dimenhydrinate gum formulation

The data were generated using the Ph. Eur. apparatus B at different chewing distance shown in the diagram.

It can be inferred from the Figure 5-48 the data were best fitted to the Higuchi and Korsmeyer-Peppas equations with the correlation coefficient (r^2) of 0.84 and 0.95. Summary of the release kinetic data is presented in the Table 5-13.

Table 5-13. Summary of *in vitro* release kinetics evaluation from dimenhydrinate 20 mg gums using apparatus B

Apparatus setup*	Zero Order		First Order		Higuchi Model		Korsmeyer-Peppas			Hixson-Crowell	
	r^2	k_0 ($h^{-1)}$	r^2	k_1 (h^{-1})	r^2	k_H ($h^{-1/2}$)	r^2	n value	K_{kp} (h^{-n})	r^2	k_{HC} ($h^{-1/3}$)
1.4mm/20°	0.6777	0.7200	0.5789	0.0173	0.8385	7.4374	0.9885	0.5275	1.0675	0.7178	-0.0173
1.6mm/20°	0.7403	0.7887	0.6377	0.0186	0.8852	8.0086	0.9908	0.4814	0.9228	0.7848	-0.0193
1.8mm/20°	0.8340	0.8028	0.7175	0.0202	0.9472	7.9446	0.9965	0.4674	0.8032	0.8755	-0.0188

*) the apparatus setup represents the different combinations of the chewing distance with a constant twisting angle and chewing frequency.

Results and discussion

The analysis of *in vitro* dissolution data clearly shows that the release from the gum matrix is diffusion controlled (anomalous transport). For dosage forms, an ideal condition for the zero order drug release would be with “n” approaching unity. In this situation, the release follows neither zero order nor the first order. It is usually expected with such kind of system where the API is dispersed in an insoluble gum matrix. The diffusion of the API within the matrix is controlled by a number of factors. In other words, the diffusion or the permeability of the drug in the gum matrix is determined by the state of the polymer gum matrix. Temperature and the wettability of the gum play an important role and aids in the transition of non-porous gum matrix to a glassy rubber elastic state.

5.2.5 Caffeine based chewing gum formulations

Caffeine containing chewing gums are multi-source products available in the market. One gram of caffeine dissolves in 46 mL water (Merck Index), which corresponds to a solubility of 21.7 mg/mL. Both the media (water and artificial saliva) are supposed to be comparable, as the pH value in this range should not influence the solubility of caffeine. The caffeine content per serving is 80 mg (one serving consists of two pieces of chewing gum); therefore, the maximum concentration in 40 mL dissolution medium is 2 mg/mL. Solubility is sufficient and the sink condition is assumed in the given volume of 40 mL drug release medium.

5.2.5.1 *In vitro* drug release testing of caffeine containing chewing gums

The *in vitro* release of caffeine from chewing gum formulation was tested using different apparatus setups. The influence of various parameters on drug release was investigated. The results of the *in vitro* release testing are shown in Figure 5-49 - Figure 5-50.

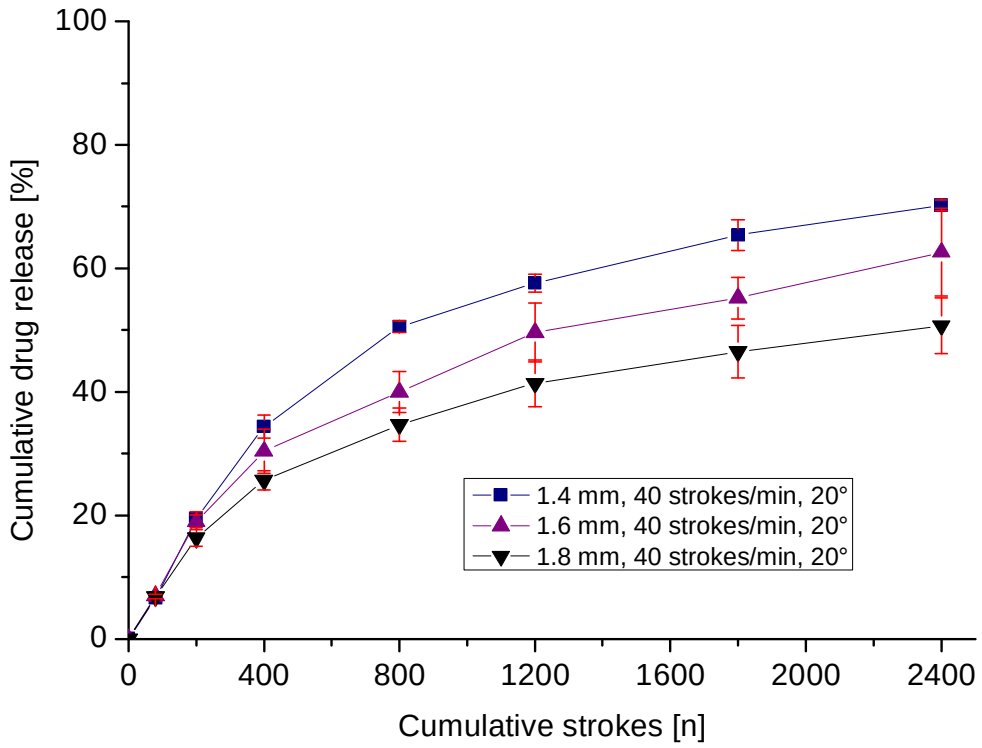


Figure 5-49. *In vitro* release of caffeine from the gum product at constant twisting angle

The data were generated using variable chewing distance of the apparatus B maintained at constant chewing frequency. The twisting angle is set to 20°. Drug release profiles represent data (n=3 ± SD) generated using 40mL of artificial saliva pH 6.2 at 37°C.

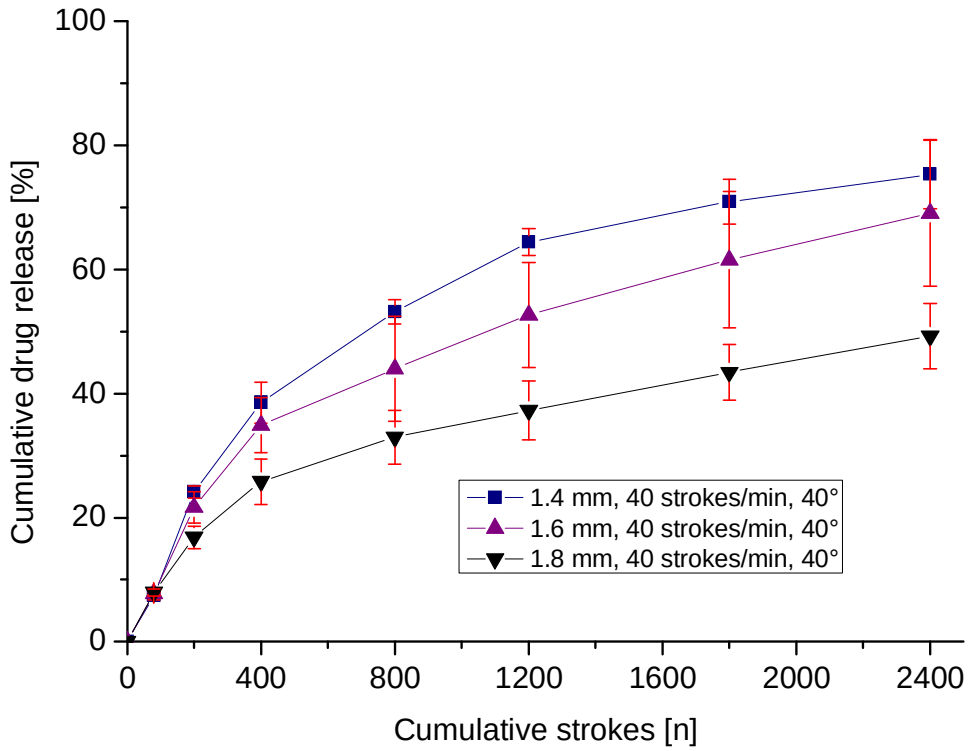


Figure 5-50. In-vitro release of caffeine from the gum product at constant twisting angle

The data were generated using variable chewing distance of the apparatus B maintained at constant chewing frequency. The twisting angle is set to 40°. Drug release profiles represent data (n=3 ± SD) generated using 40mL of artificial saliva pH 6.2 at 37°C.

Results and discussion

As far as the caffeine containing chewing gums are concerned, the *in vitro* drug release profiles shown in Figure 5-49 and Figure 5-50 indicates that the release is not complete within the tested time period of 60 min. Absence of asymptote in the profiles indicates that the release was incomplete. It might not be relevant to extend the drug release testing time further to completion, since the condition may not reflect the physiological process. Chewing gum products are generally designed to deliver active substances within 30 to 45 min of chewing. Extended mastication results in a loss of texture and softness of the gum matrix as the excipients dissolve over time leaving a hard gum residue. Besides, the *in vitro* release of caffeine from gums shows that it follows a clear rank order. The release is faster in case of 1.4 mm as compared to 1.8 mm chewing distance. Profiles from both the figures indicate the apparatus setup at two different chewing angles, 20° and 40°. When compared and evaluated for their influence on drug release, the effect is less pronounced at 1.8 mm even with an increased twisting angle. This is due to the fact that the kneading process was not effective during the release testing.

5.2.5.2 Evaluation of f_2 test statistics

The f_2 values between the profiles generated with 20° and 40° twisting angle were compared. The results of the analysis are shown in Table 5-14 and Figure 5-51.

Table 5-14. Evaluation of f_2 values between apparatus setups at 20° and 40° twisting angle

Apparatus setups	1.4mm/40strokes/40°	1.6mm/40strokes/40°	1.8mm/40strokes/40°
1.4mm/40strokes/20°	68	73	42
1.6mm/40strokes/20°	49	69	54
1.8mm/40strokes/20°	38	82	82

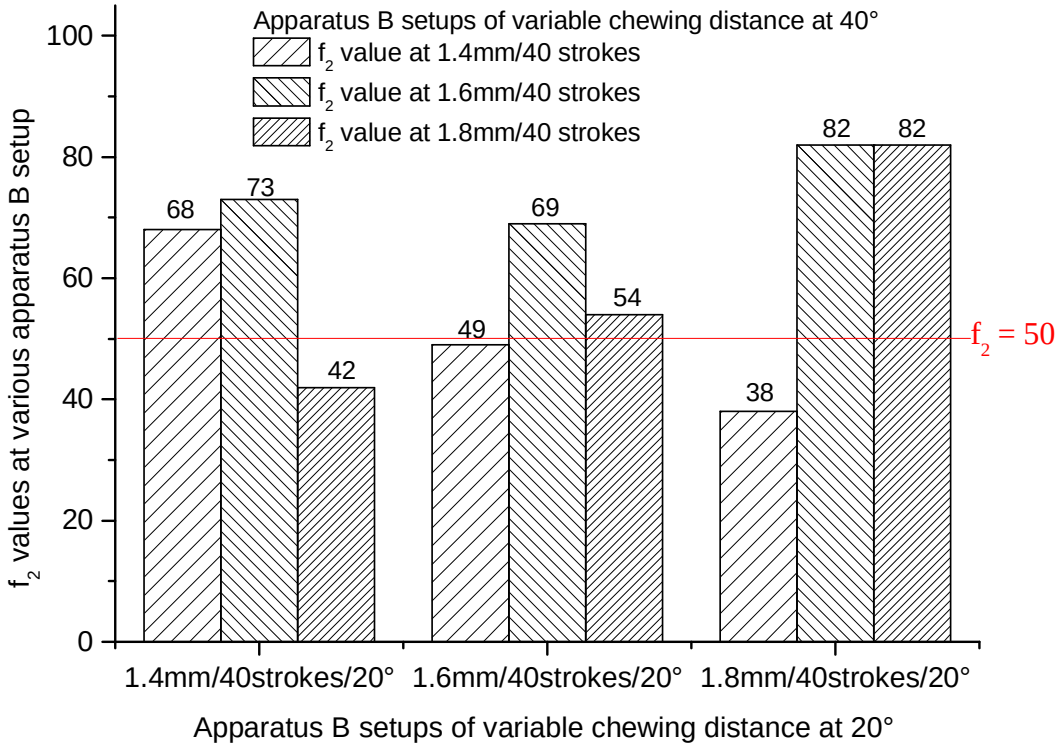


Figure 5-51. Comparison of drug release data (f_2) of caffeine containing chewing gums using various apparatus setup

Results and discussion

The bars in the Figure 5-51 represent the f_2 values obtained by comparing the *in vitro* data from various apparatus setups at 20° and 40° twisting angle. The line on the y axis at 50 indicates the minimum value required to accept the similarity between the profiles. A value of $f_2 > 50$ indicates that the profiles under comparison are similar and the difference observed in release at each time point is less than 10% of label claim. From the Figure 5-51, it can be seen that the similarity is always observed for profiles with similar chewing distance independent of the twisting angle at all the parameters tested. Using the apparatus setup of 1.8 mm/40 strokes/20°, the test yielded a similar f_2 values, and shows that the twisting angle has no

influence on the drug release. This is expected since the frictional forces are reduced at a higher chewing distance which in turn reduces the surface modification of the gum during the masticatory process resulting in less renewable surface for further drug release.

5.2.5.3 Release kinetics of caffeine gum formulations

The *in vitro* drug release data were fitted to the various models described earlier. The best fit is determined from the correlation coefficient. The results of the analysis is shown in the Figure 5-52 and summarized in the Table 5-15.

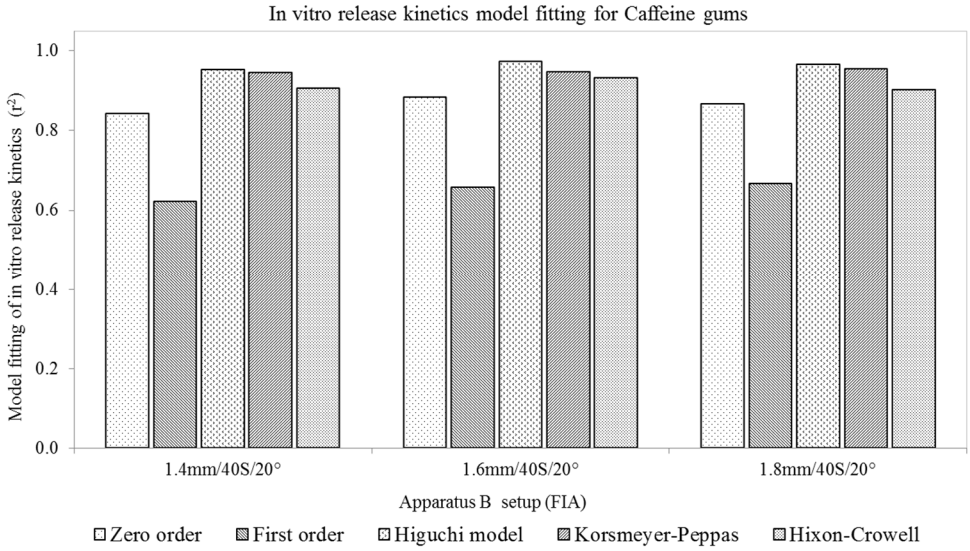


Figure 5-52. Evaluation of in-vitro release kinetics of caffeine from the gum formulation

The data were generated using the Ph. Eur. apparatus B at different chewing distance shown in the diagram.

Table 5-15. Summary of *in vitro* release kinetics evaluation from Caffeine 40 mg containing gums using apparatus B

Apparatus setup*	Zero Order		First Order		Higuchi Model		Korsmeyer-Peppas			Hixson-Crowell	
	r^2	k_0 ($h^{-1)}$	r^2	k_1 (h^{-1})	r^2	k_H ($h^{-1/2}$)	r^2	n value	K_{kp}(h^{-n})	r^2	k_{HC} ($h^{-1/3}$)
1.4mm/20°	0.8432	1.0166	0.6215	0.0311	0.9534	10.0383	0.9450	0.7250	0.8058	0.9066	-0.0238
1.6mm/20°	0.8842	0.8704	0.6581	0.0289	0.9734	8.4805	0.9465	0.6099	0.6263	0.9320	-0.0190
1.8mm/20°	0.8673	0.6947	0.6669	0.0269	0.9667	6.8102	0.9550	0.5672	0.5071	0.9033	-0.0140

*) the apparatus setup represents the different combinations of the chewing distance with a constant twisting angle and chewing frequency.

Results and discussion

Like dimenhydrinate chewing gums, the best fit is observed for the Higuchi model and the Korsmeyer-Peppas model which explains the mechanism of release is predominantly non-fickian transport (anomalous) with the value of 0.85 < n> 0.5.

5.2.5.4 Evaluation of mean dissolution times (MDT)

The results of the *in vitro* mean dissolution times (MDT) are summarized below in the Table 5-16 and Table 5-17.

Table 5-16. Evaluation of MDT of caffeine containing chewing gums at 20° twisting angle

Time [min]	**1.4 mm/40 Strokes/ 20°**	**1.6 mm/40 Strokes/ 20°**	**1.8 mm/40 Strokes/ 20°**
AUMC 0	0.00	0.00	0.00
AUMC 2	6.57	6.96	6.85
AUMC 5	45.22	42.14	33.29
AUMC 10	111.98	85.43	69.68
AUMC 20	241.95	144.00	135.90
AUMC 30	176.50	240.50	167.00
AUMC 45	292.50	209.63	192.38
AUMC 60	253.05	390.08	219.98
AUMC 0-60	1127.77	1118.73	825.06
$MDT_{in\ vitro}$ [min]	16.06	17.86	16.27

Table 5-17. Evaluation of MDT of caffeine containing chewing gums at 40° twisting angle

Time [min]	**1.4 mm/ 40 Strokes/ 40°**	**1.6 mm/40 Strokes/ 40°**	**1.8 mm/40 Strokes/ 40°**
AUMC 0	0.00	0.00	0.00
AUMC 2	7.44	7.79	7.95
AUMC 5	58.56	48.51	31.08
AUMC 10	107.93	99.45	67.35
AUMC 20	219.15	136.50	107.55
AUMC 30	280.50	217.00	107.25
AUMC 45	245.25	333.00	229.88
AUMC 60	231.00	394.28	307.13
AUMC 0-60	1149.82	1236.53	858.18
$MDT_{in\ vitro}$ [min]	15.26	17.90	17.42

Results and discussion

The purpose of calculating the MDT is to describe the *in vitro* drug release profiles and to differentiate curves of different shape and extent. As far as the caffeine containing chewing gums are concerned, the MDTs are found to be similar which was quantitatively confirmed by the f_2 values. The observed differences in MDTs are in agreement with the apparatus setups. The area under the first moment curve (AUMC) upto 60 min indicates a difference in the extent of release of caffeine from chewing gums. The area ($AUMC_{0-60}$) is slightly higher for apparatus setups with 40 ° twisting angle than with 20 °C. The $AUMC_{0-60}$ for 1.8 mm

chewing distance at 20° and 40° is considerably less than the AUMC observed for 1.6 mm and 1.8 mm chewing distance. Even then, the MDT $_{[in\ vitro]}$ has indicated no significant difference exists between the apparatus setups. One possible explanation could be that, in all the tested apparatus parameters, caffeine release were incomplete based on the stated label claim. The difference in AUMC among the release profiles confirms the difference in the extent of release exists. As the MDT calculation considers the release upto the time point tested, the MDT values are based on the individual incomplete profiles. In these situations, it would be more reliable to compare the AUMC values to understand the extent of drug release rather than to rely on the MDT values alone.

5.3 *In vitro in vivo* correlation

The method of developing *in vitro in vivo* correlation (IVIVC) has already been described in the section 1.6. The availability of clinical/*in vivo* data is of at most importance during the development of *in vitro* drug release methodology. It provides valuable data about the performance of the product *in vivo*, which in turn would be helpful in developing a meaningful and biorelevant methodology which could later be adapted to the routine quality control testing. The nicotine gums are widely marketed and abundance of literatures is available for nicotine gums which were primarily used for the product approval. But the product approvals for MCGs are based on the BE studies and until now are not waived based on the *in vitro* data. This is because of the fact that there is a lack of data to support the relationship between an *in vivo* and *in vitro* data for MCGs. Garg, *et al.* 2016 demonstrated the BE of nicotine based gums products of two different dosage strengths (2 mg and 4 mg). This concludes still the acceptance of generic gum products is based on BE approach. Therefore it became necessary to investigate the possibilities to establish the relationship between an *in vitro* and *in vivo* data and assess the whether an *in vitro* method can be a potential method to predict *in vivo* performance.

In this section, different types of clinical data obtained from literatures, sponsors and from preliminary chew-out studies were evaluated to test whether the developed *in vitro* drug release methodology to some extent simulate the *in vivo* release.

5.3.1 Evaluation of literature data

Publically accessible *in vivo* data were taken to evaluate the suitability of the developed *in vitro* drug release method. During the literature search, only nicotine based *in vivo*/clinical data were found (Hukkanen, *et al.* 2005, Russell, *et al.* 1985, Choi, *et al.* 2003, FDA 1995, 1999).

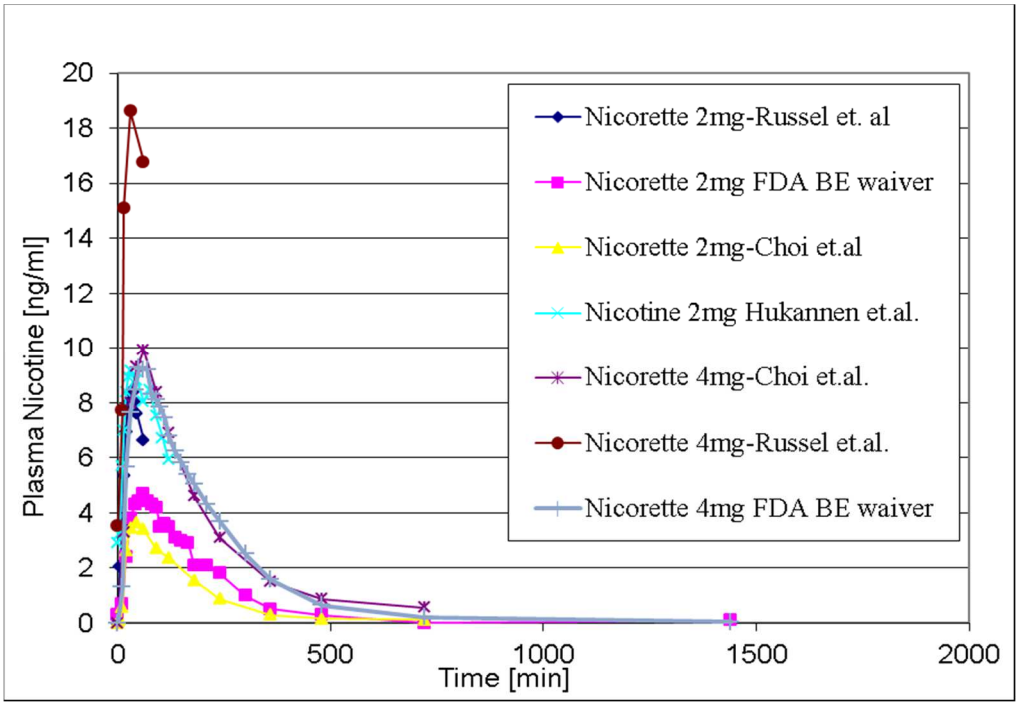


Figure 5-53. Plasma concentration time profiles reported in literatures for Nicorette 2 mg and 4 mg based chewing gums

The *in vivo* data reported in the literatures (Russell, *et al.* 1985) was treated using the Wagner Nelson method (Wagner, *et al.* 1963) to derive the fraction of dose (FRA) absorbed. Steps involved in the treatment to the data are shown in the Figure 5-54.

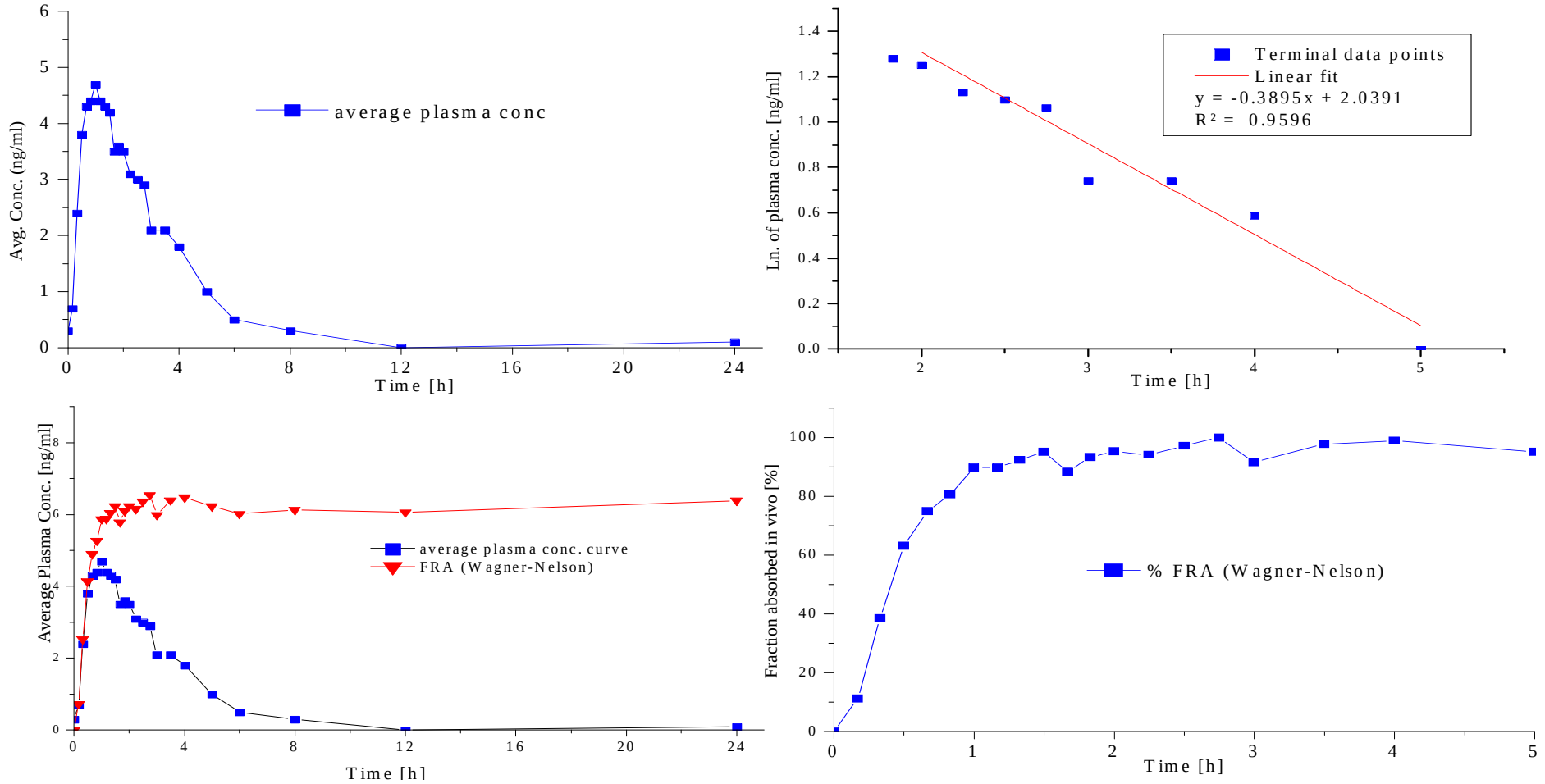


Figure 5-54. Evaluation of nicotine gum 2mg clinical data obtained from literature

The data was taken from the literature (Russell, et al. 1985) and the average plasma concentration time graph was reconstructed (upper left). Linear plot of Ln transformed terminal data points (upper right) to determine the elimination rate constant. Deconvoluted data (FRA) using Wagner-Nelson method is shown in lower left and % absorbed in vivo in lower right.

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5.3.1.1 Superimposability of the *in vitro in vivo* profiles

The fraction of the dose absorbed (FRA) calculated earlier using Wagner-Nelson method from the literature reported data was compared with the *in vitro* nicotine release profiles of nicotine.

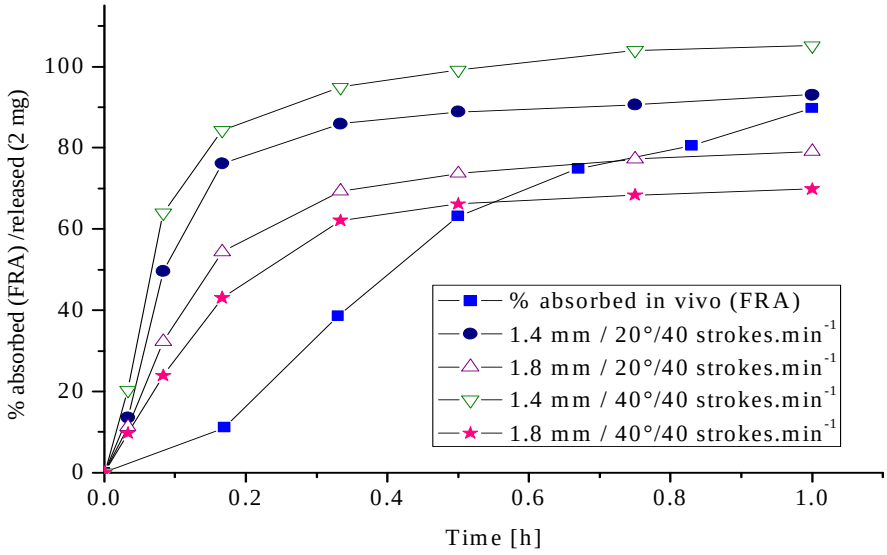

Figure 5-55. ***In vitro* and *in vivo* release profiles of nicotine gums dosed at 2 mg**
The in vivo absorption profiles were obtained using Wagner-Nelson method.

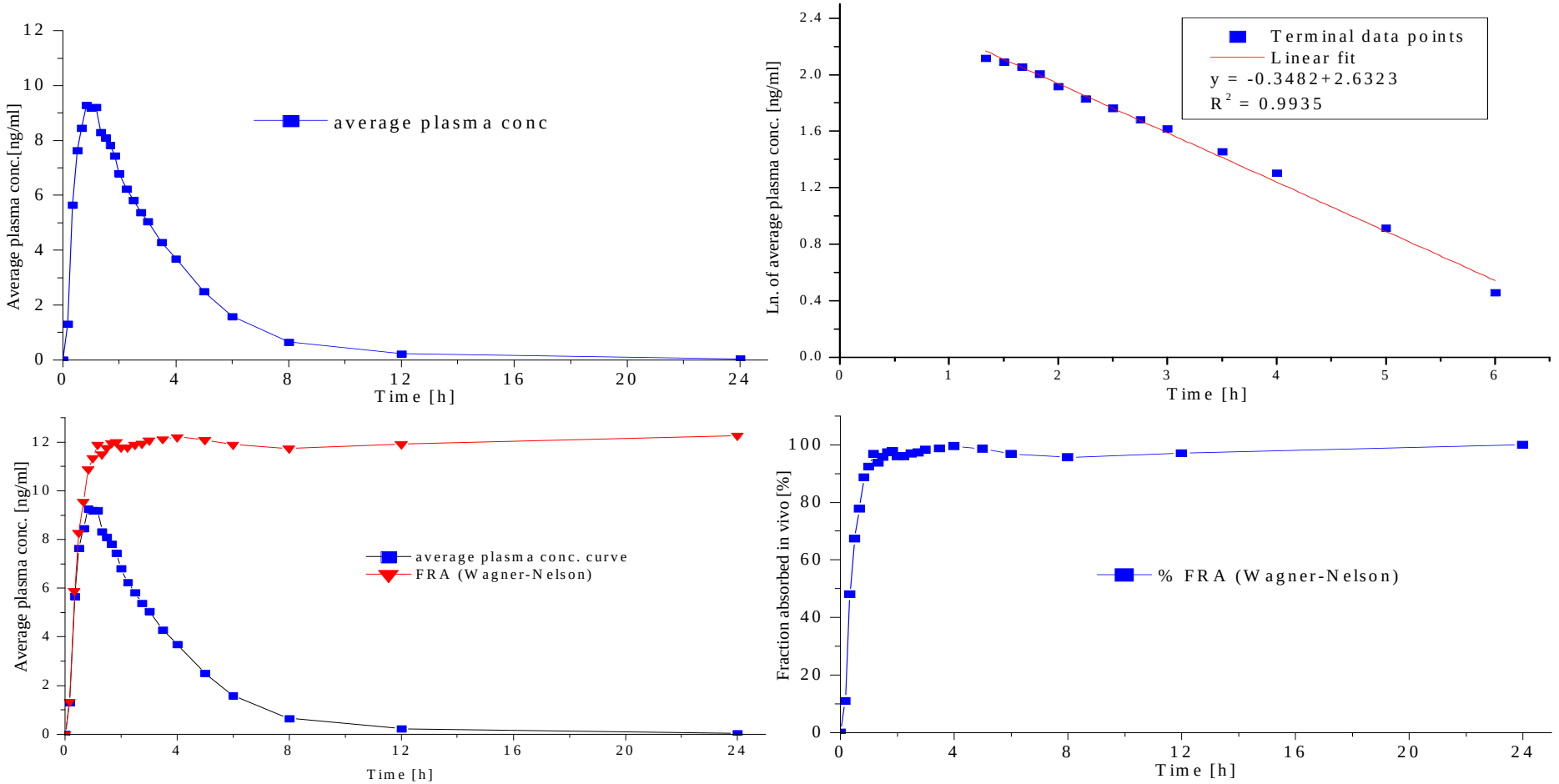

Figure 5-56. Evaluation of nicotine gum 4 mg clinical data obtained from the literature

The data from the original publication was taken from the literature (Choi, et al. 2003) and the average plasma concentration time graph was reconstructed (upper left). Linear plot of Ln transformed terminal data points (upper right) to determine the elimination rate constant. De-convoluted data (FRA) using Wagner-Nelson method is shown in lower left and % absorbed in vivo in lower right.

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5.3.1.2 Superimposability of the *in vitro in vivo* profiles

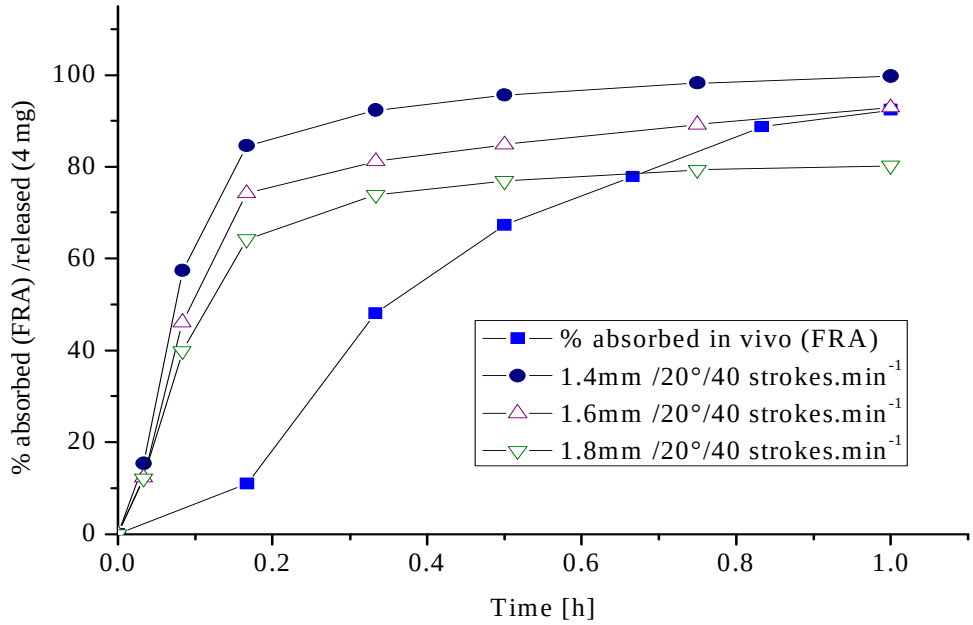


Figure 5-57. ***In vitro* and *in vivo* release profiles of nicotine gum dosed at 4 mg**
The in vivo absorption profiles were obtained by Wagner-Nelson method.

The overlay of the *in vitro* and *in vivo* drug dissolution/release curves for both the dosage strengths indicate that the drug release *in vivo* is much slower than that of the *in vitro* release. This is particularly more evident at the initial and at the end time points. The fraction of the drug absorbed *in vivo* reached to one of the *in vitro* release amounts (1.8 mm chewing distance) only at time points > 30 min (0.5h). This is obviously an initial approach to develop a relationship between the *in vitro* and *in vivo* parameters. Any conclusion based on the above results could not be arrived since there are a number of factors that affect the release of an API both *in vitro* and *in vivo*. The purpose of this approach is to develop a meaningful relationship between various parameters of release so that the developed *in vitro* method could be a potential indicator of product performance. Based on the results, it was concluded that more reliable *in vivo* data performed in a very standardized setup were necessary to estimate the *in vitro in vivo* relationships.

5.3.2 Evaluation of nicotine 4 mg clinical data

The pharmacokinetic data (PK) of nicotine chewing gum 4 mg dosage strength was obtained from Zenara Pharma India Pvt. Ltd. The study was conducted in the year 2010- 2011 as part to demonstrate the bioequivalence two gum products (reference and test formulation) of 4 mg dosage strength for market authorization. The summary of the pharmacokinetic data is provided in the Table 5-18. The test was conducted with 12 subjects.

Table 5-18. Summary of pharmacokinetic data for 4 mg nicotine gum

Index	C_{max} (ng/mL)	T_{max}	$AUC_{(0-t)}$ (ng·h/mL)	$AUC_{(0-\infty)}$ (ng·h/mL)	λ_z (1/h)	$t_{½}$Nair, *et al.*
Average [n=12]	8.307	1.23	33.271	40.065	0.2309	3.56
Std. Dev.	3.570	0.65	19.671	21.110	0.0999	1.47
RSD [%]	42.98	52.68	59.12	52.69	43.27	41.36

Estimated elimination rate constant from terminal slope, K_{el} = -0.2748 ng/mL.h^{-1}.

The average C_{max} obtained is consistent with the results reported in the literature. Additionally, the data reported in the Rote-Liste from Germany is shown in Figure 5-58.

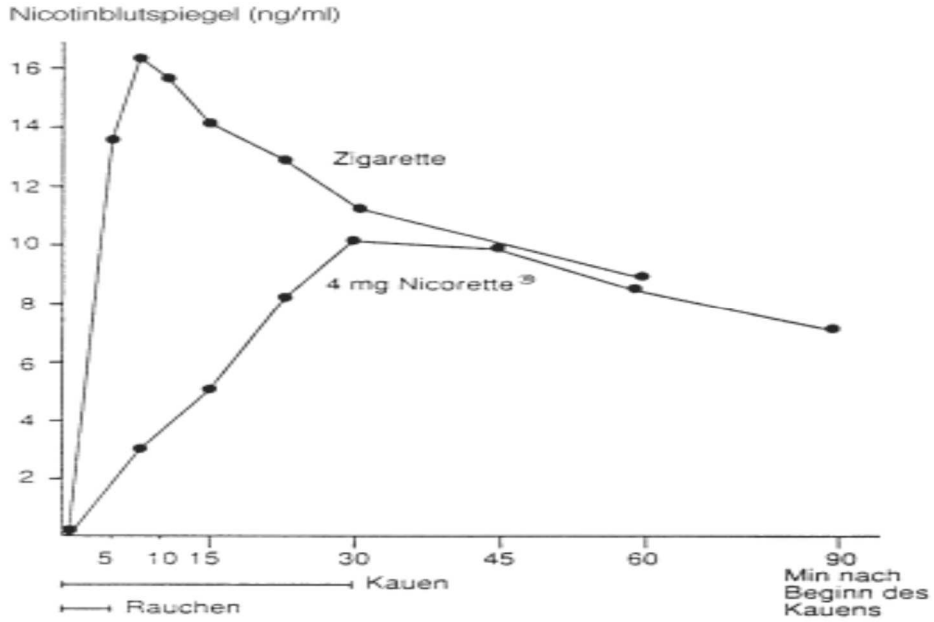


Figure 5-58. Plasma-concentration time profile achieved after intake of 4 mg nicotine chewing gum marketed in Germany

Plasma nicotine concentration observed after cigarette smoking shown for comparative purpose. Figure was taken from Roteliste 2013, Germany.

The Figure 5-58 was taken from the Rote-Liste (2013), Germany and shows absorption of nicotine from Nicorette chewing gums and cigarette smoking. Since this data has been published in the Rote-Liste, it is assumed that the precision and correctness of the data should be acceptable. It is therefore used for the comparative evaluation.

Additionally, the clinical data was also obtained from Zenara Pharma India Pvt. Ltd (Table 5-19). Therefore validity of the Wagner Nelson back calculation was tested by the method proposed by Gohel, *et al.* 2005. For this purpose, the elimination rate constant (K_{el}) is calculated from the intravenous (IV) infusion data of nicotine solution obtained from the Rote-Liste.

The reported pK parameters are as follows;

Volume of distribution (V_d) = 3 L/Kg
Plasma half-life ($T_{1/2}$) = ~ 2 h
Plasma Clearance (Cl) = 70 L/h

Average weight was considered about 75 kg. Using the given data, the elimination rate constant was calculated using the formula;

Elimination rate constant (K_{el}) = $\frac{Cl}{Vd\ (total)}$

$V_{d\ (total)}$ = V_d (L/kg) x Weight (kg)

Using the PK parameters given above, the plasma concentration was calculated using the equation described earlier in the section methods of evaluation,

$$Ct + 1 = \frac{\frac{[2 * \Delta F * D]}{Vd} + Ct(2 - Ke * \Delta t)}{(2 + Ke * \Delta t)}$$

The results of the analysis are summarized in the Table 5-19 and graphically shown in the Figure 5-59.

Table 5-19. Summary of the pharmacokinetic data obtained from Zenara Pharma for nicotine 4 mg reference gum product

Time [h]	Obs. plasma conc. [ng/ml]	At/Vd	Fraction abs. [FRA]	Calc. plasma conc. (ng/ml)	Pred. error (PE %)
0	0.00	0.00	0.00	0.00	0
0.17	3.06	3.13	0.25	4.38	43.05
0.33	5.49	5.76	0.46	6.58	19.99
0.50	7.11	7.69	0.62	7.91	11.19
0.67	8.09	9.04	0.73	8.64	6.77
0.83	7.76	9.10	0.73	7.76	0.00
1.00	7.14	8.84	0.71	7.01	-1.82
1.17	7.04	9.09	0.73	7.12	1.27
1.33	6.77	9.16	0.74	6.79	0.29
1.50	6.38	9.11	0.73	6.35	-0.46
1.67	6.03	9.07	0.73	6.01	-0.41
1.83	5.63	8.96	0.72	5.57	-1.06
2.0	5.80	9.41	0.76	5.97	3.01
2.5	5.05	9.46	0.76	5.03	-0.34
3.0	4.27	9.38	0.76	4.20	-1.52
4.0	3.17	9.39	0.76	3.14	-0.89
6.0	1.86	9.65	0.78	1.94	4.68
8.0	1.48	10.30	0.83	1.69	14.88
10.0	1.41	11.11	0.90	1.66	17.72
12.0	1.14	11.66	0.94	1.34	17.07
14.0	1.18	12.39	1.00	1.40	19.55

The goodness of fit is demonstrated using the correlation of the observed vs. predicted plasma concentration. The value of $R^2 = 0.9720$ indicates the proposed back calculation of Wagner-Nelson method is valid for the current evaluation.

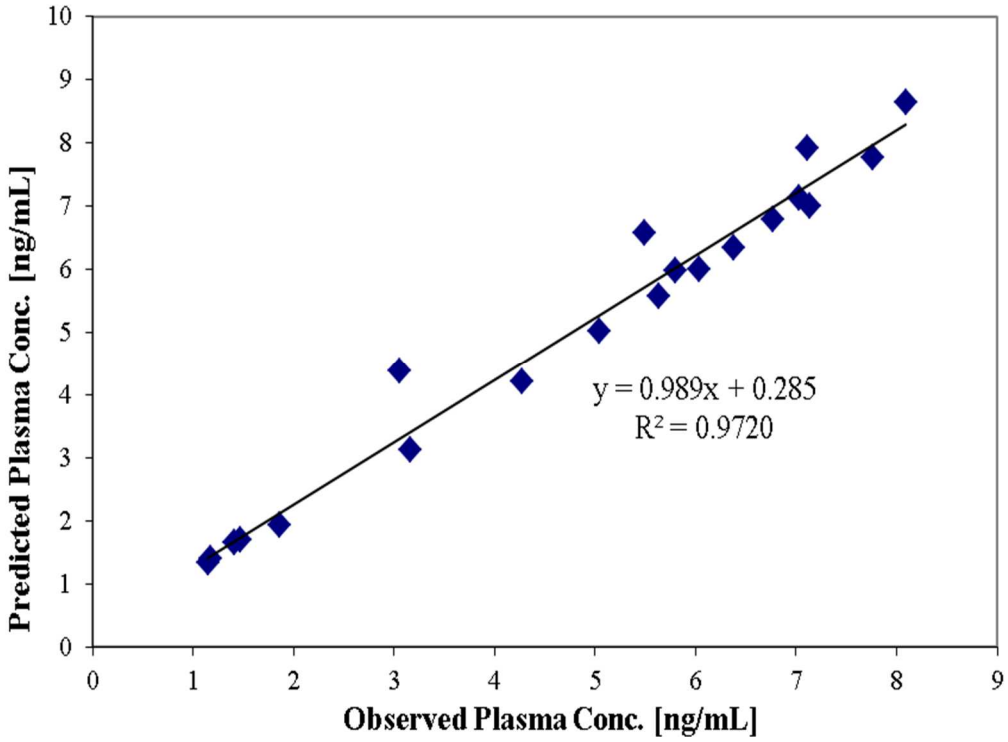


Figure 5-59. Plot of predicted vs. observed plasma concentration time profile for nicotine 4 mg dosage form

From the Figure 5-59, it can be seen that there is a good agreement between the predicted and observed plasma concentration. The predicted values indicate that the Wagner-Nelson back calculation is useful in determining the validity of the *in vivo* data. It was therefore decided to verify the developed *in vitro* methodology for nicotine based gums using this *in vivo* data.

The individual and the average *in vivo* plasma concentration profiles are shown in the Figure 5-60. Additionally, the fraction of the dose (FRA) absorbed calculated using the Wagner-Nelson method is also shown. Linear portion of terminal data points from the average plasma concentration time profile showing the elimination phase was taken to estimate the elimination rate constant (K_{el}). A plot of natural log transformed (Ln) plasma concentration vs. time is also shown and the corresponding slope gives the K_{el}.

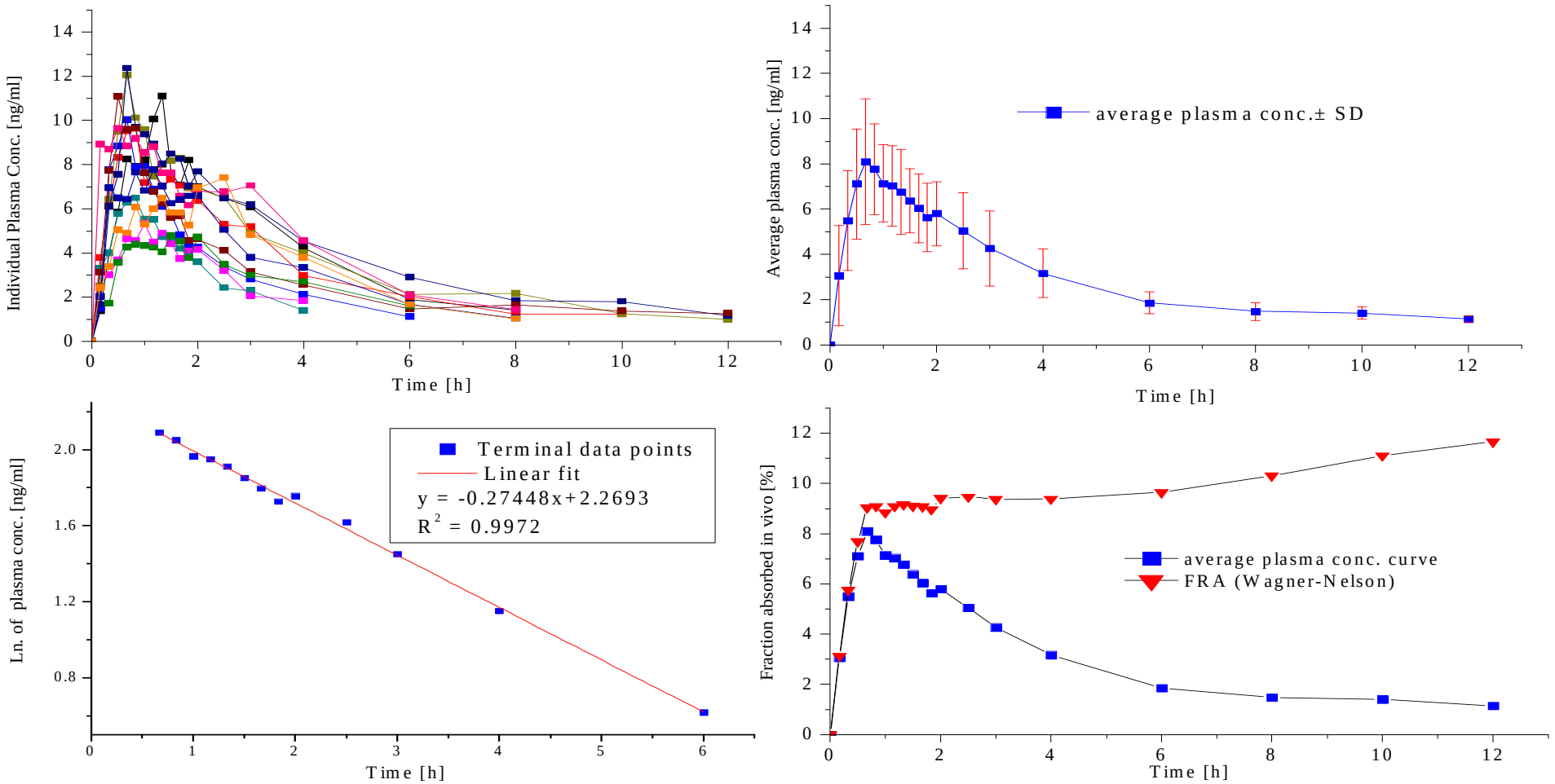


Figure 5-60. Transformation of *in vivo* data of nicotine (4 mg) based gum using a non-compartmental model dependent approach (Wagner-Nelson method) to derive FRA

Individual plasma concentration profiles shown in upper left, average plasma concentration profile with SD in upper right, linear fit for the terminal data points to determine the elimination rate constant (k_{el}) and the goodness of fit shown by the R^2 value in the lower left and lower right shows an average plasma concentration-time profile with the FRA obtained using Wagner-Nelson method

From the plasma concentration time plot, it was observed that there is a large inter-individual variability with respect to the subjects. The *in vivo* fraction absorbed data is correlated to the *in vitro* data obtained from a different setup of apparatus B. The goodness of fit is given by the value of correlation coefficient (R^2).

5.3.2.1 Evaluation of the *in vitro-in vivo* profiles

The FRA estimated from the PK data of 4 mg nicotine gum was correlated with the *in vitro* data from the Nicorette 4 mg classic flavor. The *in vitro* data was generated using the apparatus B at various setups. The result of the estimation is show in the Figure 5-61.

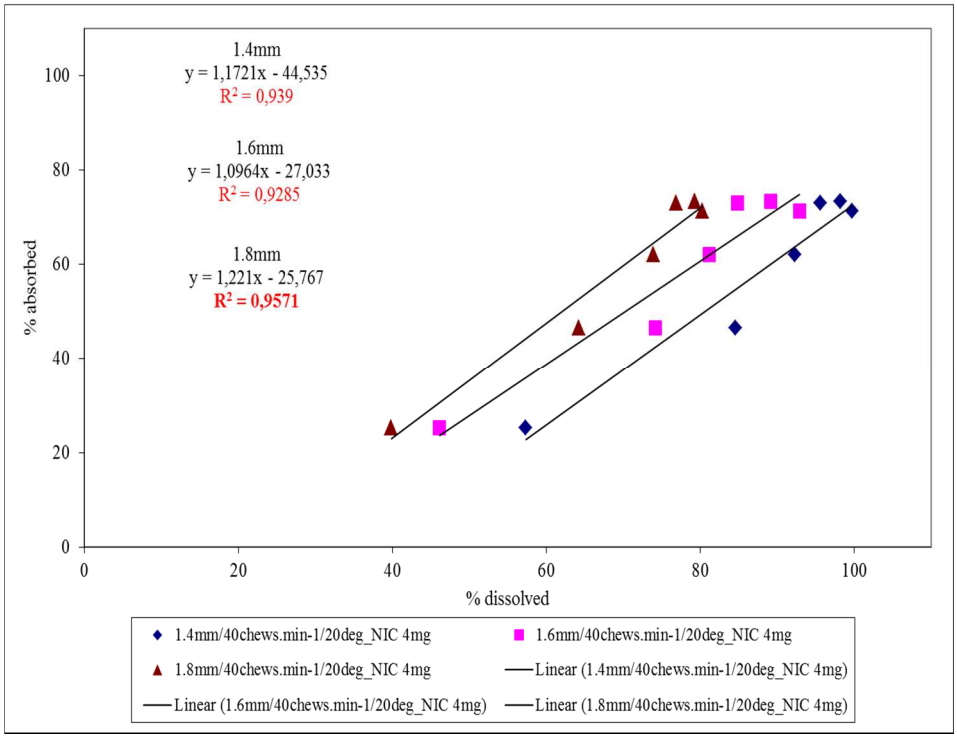


Figure 5-61. ***In vitro in vivo* correlation of 4 mg nicotine chewing gums**

The lines indicate the goodness of fit between the in vitro drug release data at selected time points and the in vivo release data obtained after deconvolution using Wagner-Nelson method.

Results and discussion

Though the correlation between the *in vitro* and *in vivo* data is acceptable (based on r^2), there is no superimposability of the *in vitro* and *in vivo* profiles observed. The results of the *in vitro* drug release testing have shown that the nicotine release profiles differ with respect to the apparatus setups. However, a point to point relationship (level A correlation) could not be established between the *in vitro* dissolution profiles and the *in vivo* clinical data. In other words, the *in vitro* release testing did not reflect the *in vivo* release behavior. The possible explanations could be as follows;

Medicated chewing gums differ from the other conventional solid oral dosage forms with respect to the mechanism of release. Since the site of application is the oral cavity and the prerequisite for release is the masticatory action, variability in blood plasma concentration is a common phenomenon. The force and the frequency of chewing vary considerably and are dependent on individual characteristics. Additionally, complete absorption of the API through the buccal mucosa or within the oral cavity is not expected, as part of the API is swallowed which in turn is partially metabolized by the liver enzymes and along the gut wall. This will result in variable plasma concentration time profiles.

The goal of *in vitro* drug release testing with respect to IVIVC is to predict the performance of product *in vivo* thereby it can serve as a surrogate for bioequivalence studies. The plasma concentration time data do not reflect the actual amount of drug available for absorption and also the site of release is not accessible to evaluate the *in vivo* release characteristics. As discussed, many mathematical models were developed to interpret the plasma /urinary excretion data to determine the concentration at the site of absorption.

Moreover it is nearly impossible to mimic or to simulate the complete *in vivo* situation. Here the purpose of *in vitro* release test is to mimic the *in vivo* situation as close as possible if not complete. In case of medicated chewing gums, the drug release kinetic is completely different. The dosage form is directly accessible at any point of time during its application. Since the goal is to predict the *in vivo* drug release characteristics, an alternative method like a chew-out study approach might be more appropriate for the medicated chewing gum products.

5.3.3 Development of alternative BA approach for the proposed IVIVC model

Understanding the performance of the drug product *in vivo* plays a key role in the development of meaningful *in vitro* drug release methodology. For most of the oral transmucosal dosage forms where the oral cavity is the site of application, the release is expected to be immediate and comparable to conventional immediate release (IR) solid oral dosage forms. This is due to the fact that the residence time of dosage forms within the oral cavity is limited (exceptions are the buccal patches). With most of dosage forms intended to deliver drugs within the oral cavity, their release is often immediate (sublingual tablets, orally disintegrating tablet etc.) and do not require *in vitro* dissolution and bioequivalence studies to demonstrate sameness of the products.

In case of functional chewing gums, the mode and the mechanism of release and the site of application differ significantly from other dosage forms. As the release of actives within the oral cavity is voluntarily controlled (chewing frequency), the therapeutic concentrations can be meaningfully interpreted when instructions of chewing/mastication were followed. Therefore a standardized approach is necessary to extract meaningful information from the clinical studies. Gajendran, *et al.* 2012, demonstrated the *in vivo* predictive power of the model to verify the *in vitro* drug release methodology.

In a classical bioavailability approach (BA), the fraction of the dose absorbed is obtained after suitable mathematical treatment of the plasma-concentration time curve which may not be completely reflective of *in vivo* release behavior owing to the inter-individual differences. In case of medicated gums, the insoluble gum base which acts a vehicle/carrier is always readily accessible during the complete course of administration. As long as the chewing gum is masticated in the oral cavity, it can be removed at any point of time and can be analyzed using a validated analytical approach to determine the amount of API remaining in the masticated gum matrix (cud gum). Based on the label claim, the amount of API released from the gum matrix can be calculated by;

API released/dissolved from gum = Label Claim (L.C) – API remained in gum matrix

This principle forms the basis of the spit-out approach to derive the *in vivo* release data which is more reliable to demonstrate the suitability of the *in vitro* methodology.

Using this methodology, the authors (Kvist, *et al.* 2000) have demonstrated the *in vitro in vivo* relationships for a multisource nicotine based gum product. The *in vitro* and *in vivo* profiles are shown in the Figure 5-62.

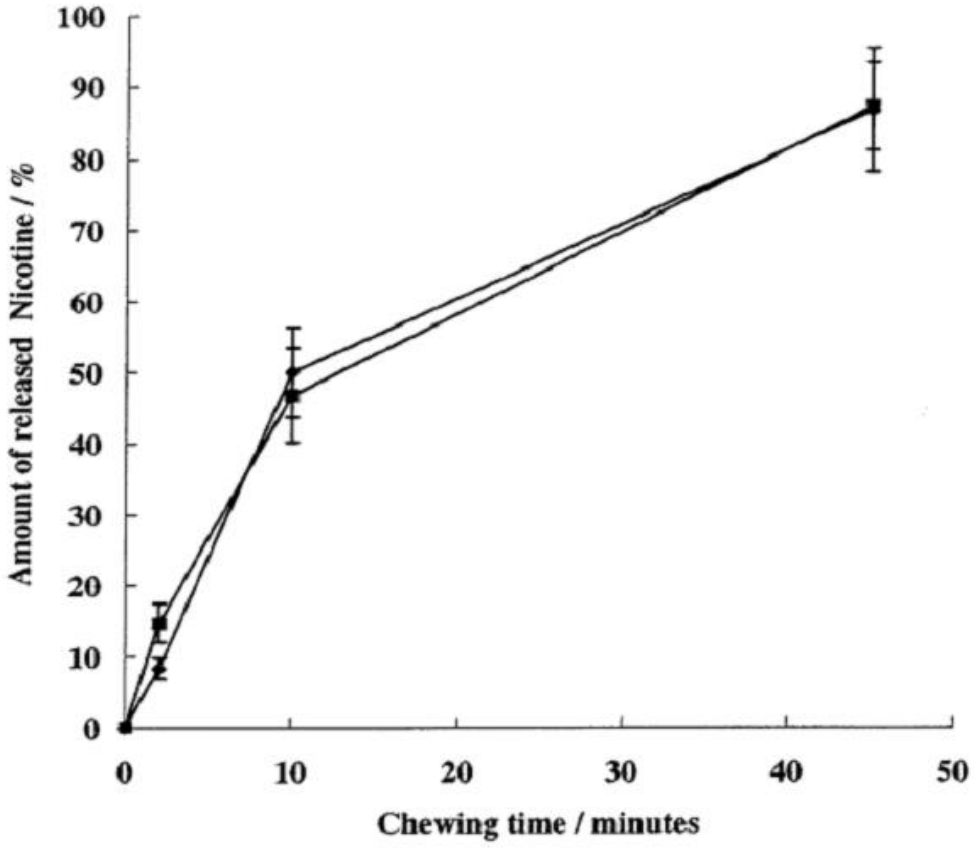


Figure 5-62. ***In vitro*** **and** ***in vivo*** **(spit out) release profiles of Nicorette 2 mg chewing gum**
Data taken from Kvist, et al. 1999, Kvist, et al. 2000. Key: in vivo release profile (square), in vitro release profile (diamond). Settings: stroke frequency- 30 strokes/min, distance between the jaws-1.8 mm.

In vitro drug release data for Nicorette 2 mg chewing gums generated from apparatus B (Kvist, *et al.* 2000) is shown in Figure 5-62. The *in vivo* data was generated using the chew-out study. The vitro and *in vivo* chew out test conditions were optimized and well controlled to eliminate any variable for e.g., chewing frequency, which may influence the release rate of the actives. The superimposability of the results indicates that under standardized test conditions, the *in vivo* release is comparable to that of the developed *in vitro* method. This way, the *in vitro* methodology could be verified using the *in vivo* chew out data. Such a method could be used successfully in developing the specifications for commercial products in QC environments.

5.3.3.1 Verification of *in vitro* methodology by *in vivo* chew out data

Based on the results from the literature data, an attempt was made to determine the suitability of the *in vitro* drug release methodology developed for the Nicotinell 2 mg product. For this purpose, a pilot study was organized. Nicotinell 2 mg mint chewing gum, batch # 000013747 was bought from a pharmacy in Germany and was used to conduct the chew-out study. The test panel consisted of 3 volunteers habitual to smoking tobacco and chewing nicotine gums. The volunteers chewed a piece of gum in resonance to a metronome which was preset to 40

acoustic tones/min. Pieces of cud (rest) gum was removed from the oral cavity after a specific period of time and were frozen at -80°C before being used for the analysis.

For the purpose of analysis, the frozen cud gum samples were ground to a coarse powder and dissolved in n-heptane and the test analysis was continued as described in the assay/content uniformity testing method for nicotine based gums. The samples were analyzed using a HPLC-UV method previously developed and validated for assay. The experimental design of the chew out study is summarized in Table 5-20.

Table 5-20. Study parameters for chew out study

Test panel (n)	3
Test products	Nicotinell 2 mg
Batch	# 000013747
Chew out time points [min]	2, 5, 15, 30, 45, 60
Gums per chew out time	1
Approx. chew frequency	40 chews/min
Time synchronization	Metronome & calibrated Stop watch

The amount of nicotine present in the cud gum at each specific time was used to construct the release profile. Since the label claim was given for the gum product, the nicotine remaining can be easily interpreted as the nicotine released *in vivo*. The results of the analysis are presented in the following section.

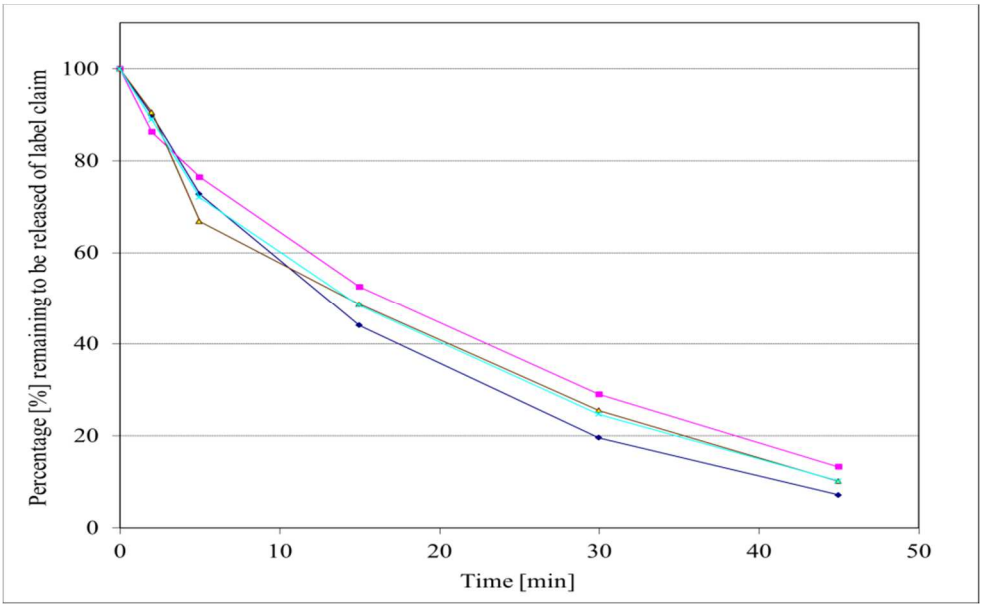


Figure 5-63. Individual (n=3) chew out profiles of nicotine from Nicotinell 2 mg gums

The chew-out release profiles shows the amount of nicotine available for release over time. Data points in the profile indicate the drug release from individual piece of chewing gum tested for the corresponding time point. The profiles represent the data obtained from 3 individual volunteers who chewed a new piece of gum for every time interval (a total of 5 individual pieces of gum /person, 1gum piece/time point).

From the given data, it is possible to calculate the release of nicotine from the gum product, which is given by;

Amount [%] released = (100 - Amount [%] remaining to be released)

By this way, *in vivo* release of the active from the drug product can be realistically evaluated and could be directly compared to the *in vitro* drug release data. Relevance of the *in vitro* data to the chew out data was tested using correlation analysis and the goodness of fit is given by the correlation coefficient (R^2) value. Data generated from different setup of apparatus A and B are correlated to the chew out data. The results of the analysis are presented in Figure 5-64 and Figure 5-65.

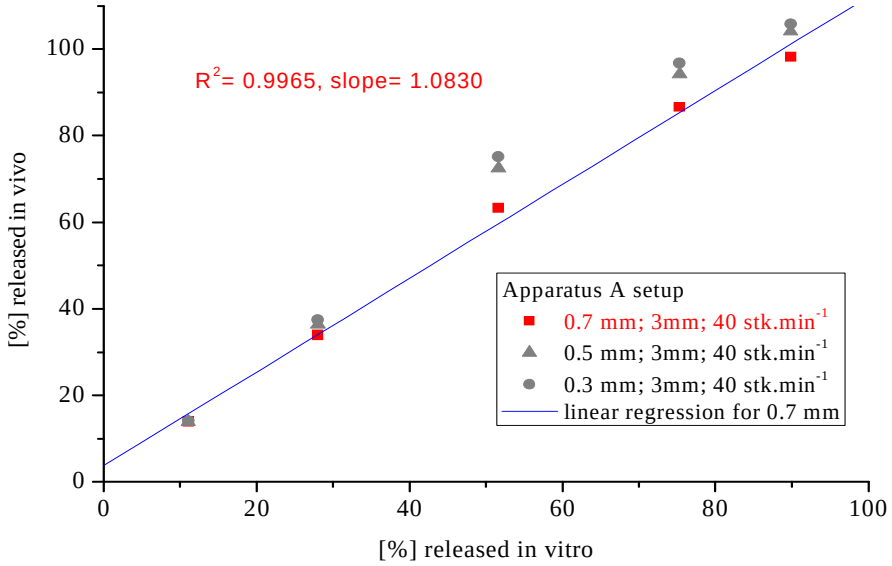


Figure 5-64. ***In vitro in vivo*** **correlation of Nicotinell 2 mg freshmint gums (apparatus A)**

The legend in square indicates the specific setup of the apparatus which correlated well with the chew out data.

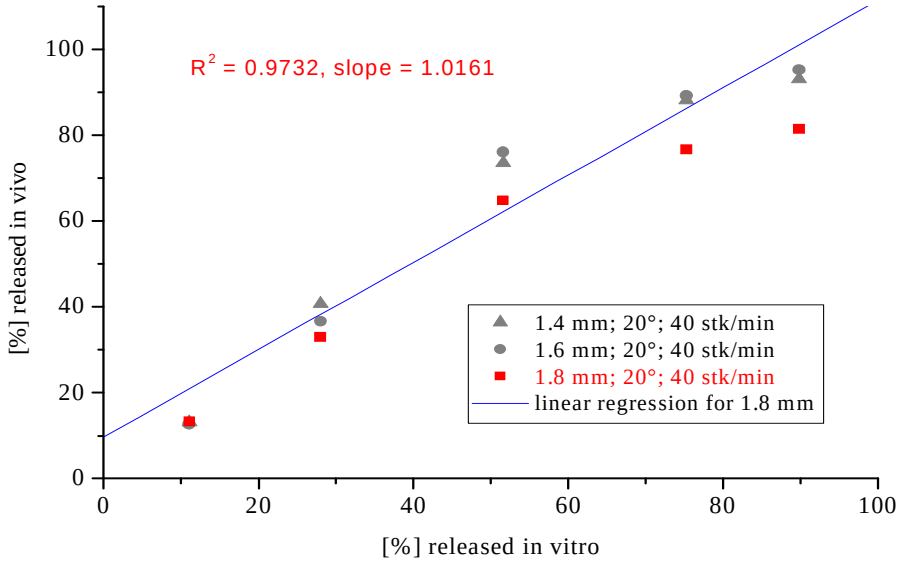


Figure 5-65. ***In vitro in vivo*** **correlation of Nicotinell 2 mg mint gums (apparatus B)**

The legend in square indicates the specific setup of the apparatus which correlated well with the chew out data.

An overlay of the *in vitro* profiles with the apparatus setups that match closely to the chew-out profile is shown in Figure 5-66.

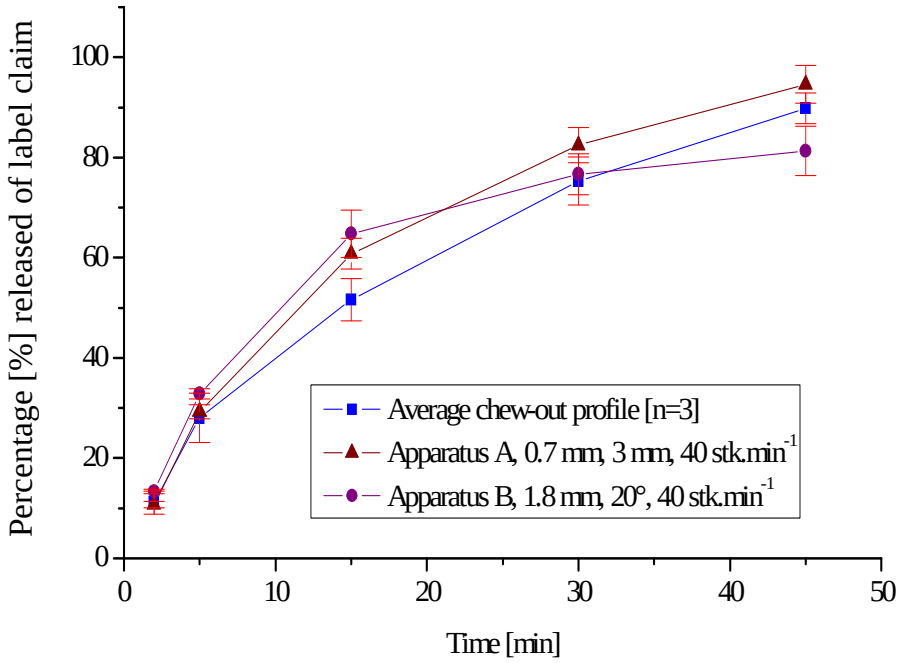


Figure 5-66. Average *in vitro* and chew-out release profile of nicotine from Nicotinell 2 mg mint (batch # 000013747)

The chew- out release profile represents n=3 ± SD calculated by the amount of nicotine remaining in the gum after a specific chewing time period. In vitro release data (n=3 ± SD) is obtained from corresponding apparatus setup shown in the legend.

Results and discussion

From the Figure 5-64 and Figure 5-65, it can be observed that both the apparatuses are capable of generating data comparable to that of the *in vivo* mastication under controlled conditions.

The coefficient of determination (R^2) values indicates the appropriate apparatus setup that best describes the *in vivo* release behavior. However, a better correlation (R^2) with the chew-out data was observed for apparatus A.

This is due to the fact that the masticatory force of apparatus B on gums is highly variable within the dissolution run and strongly dependent on the chewing distance. When the smallest possible chewing distances between the apparatuses were compared, the 0.3 mm chewing distance from apparatus A imparts more compression force than the 1.4 mm chewing distance from apparatus B. At 1.8 mm chewing distance (apparatus B), the force is considerably less than at 1.4 mm.

It can be seen from the Figure 5-66 that the *in vitro* drug release behavior is similar to that of the chew out data. As shown in Figure 5-66, this effect is more pronounced at time points > 30 min where the nicotine release rate was further reduced. Another reason for such

behavior is related to the gum matrix relaxation rate (Jójárt, *et al.* 2013). After 30 min of mastication, the relaxation of the gum matrix is diminished due to the solubilization of most of the excipients and rendering the gum less elastic. This subsequently leads to a less mechanical kneading and a decrease in the release rate of nicotine from the gum matrix. Therefore, the validity of the results for an IVIVC could be given upto 30 min. The 30 min time period is considered more realistic and accounts for a normal chewing time period.

The apparatus setup exhibiting highest correlation (R^2) to that of the chew out data can be regarded generally as a biorelevant setup which can be further used for the QC purposes to test the performance of the products. The results also indicate the suitability of the developed *in vitro* drug release methodology for routine QC testing purposes. The chewing apparatus are interchangeable for the tested product at the given test conditions.

The chew-out method to assess the *in vivo* performance of the gum product is found to be more reliable than the conventional methods used for demonstrating the IVIVC of the dosage form. Using this approach the best estimates could be made about the drug release methodology and verify its usefulness in the development stages of the formulation.

The method could also be useful as a supporting tool for the biowaiver applications provided the pharmaceutical equivalency between the test and the reference product is established. The chew out method could also be effectively used to predict the API release of the different products and will be a true indicator of *in vivo* performance.

5.4 Formulation development of nicotine gum by direct compression (DC)

Nicotine is a naturally occurring alkaloid and is extracted mostly from tobacco plants. Nicotine as an alkaloid is extremely volatile and is unsuitable to be used as an active ingredient in the solid dosage forms. Alternatively, nicotine salts available as nicotine bitartrate or nicotine hydrogen tartrate (USP 2015) is readily water soluble and stable, suitable for oral administration.

Since the aqueous solubility of nicotine is rapid, incorporation of nicotine salt in chewing gums results in rapid release within minutes of chewing. We have shown in our experiments that the release of nicotine salt was rapid and independent of the formulation variables.

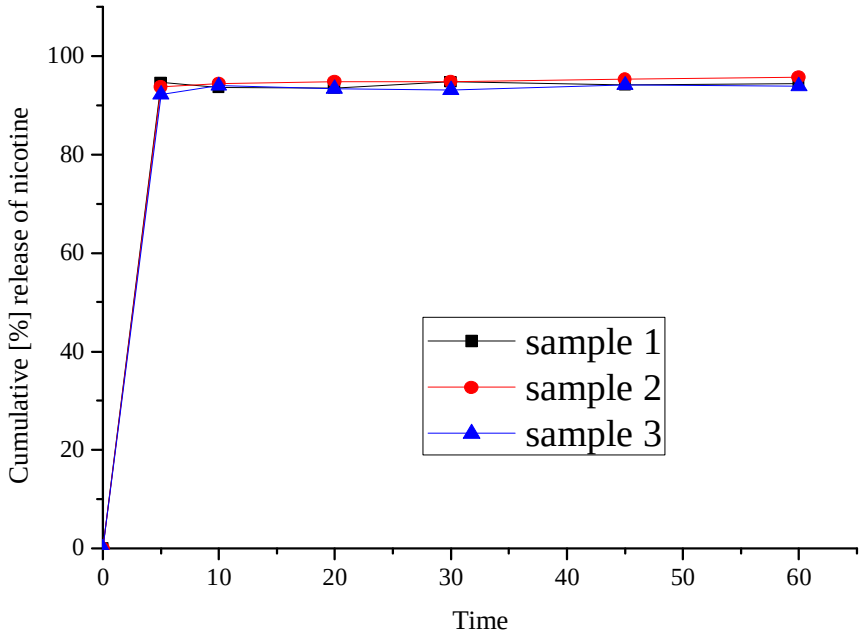


Figure 5-67. Release of nicotine from directly compressed chewing gum formulation

In vitro drug release data generated from Ph.Eur. apparatus B, using 40 mL of artificial saliva pH 6.2 maintained at 37 °C with the following apparatus setup; chewing distance 1.4 mm; twisting angle 20° and chewing frequency 40 strokes/min.

5.4.1 Ion exchange resins

5.4.1.1 Influence of nicotine resin ratio on loading

Nicotine was loaded onto the resin according to the method described in section 4.11.4. The results of the analyses are discussed in the following sections.

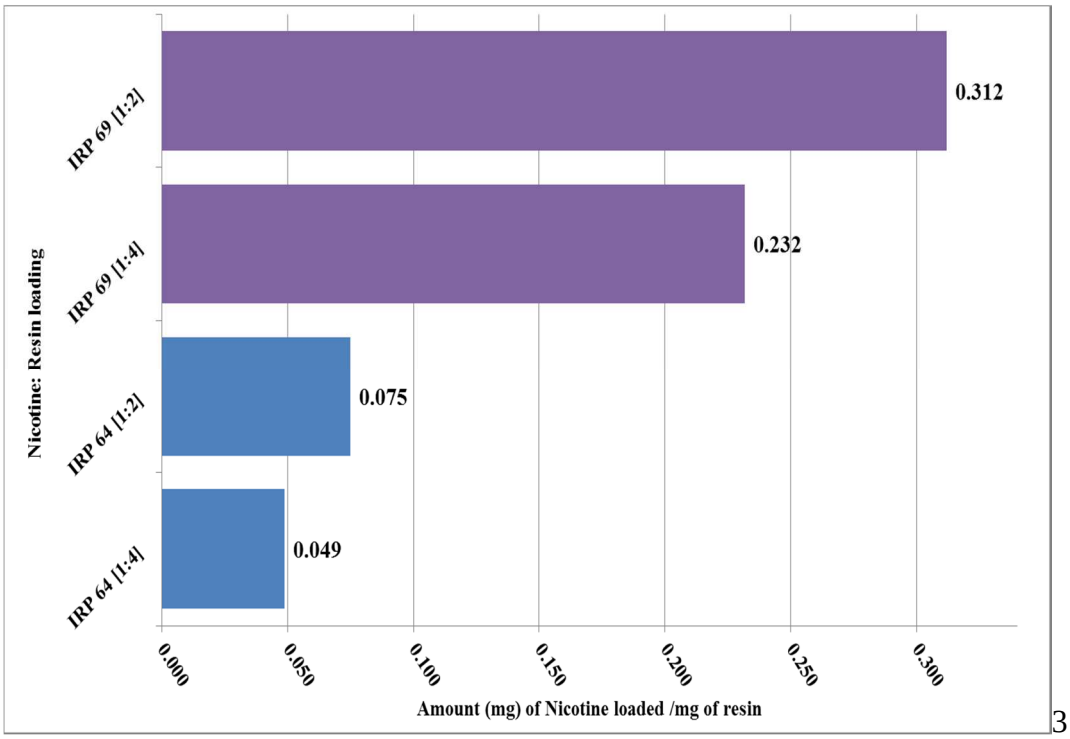


Figure 5-68. Loading of nicotine onto Amberlite IRP resin at different ratios

As observed from Figure 5-68, the Amberlite IRP 69 resin has a higher loading compared to that of the Amberlite IRP 64 resin. Since the loading of nicotine onto Amberlite IRP 64 is not effective in the tested medium (0.01M HCl) it was decided to proceed with the optimization of loading with the Amberlite IRP 69 resin. As far as the IRP 69 is concerned, increasing the amount of available nicotine twice the initial amount does not increase the effective ion exchange capacity. The optimal loading of nicotine to resin ratio [1:4] was considered suitable for further studies.

Furthermore, a rapid release of nicotine would be normally expected from the IRP 64 resinate due to the weak ion exchange group namely the carboxylic acid [-COOH]. Commercially available nicotine gum products contain nicotine loaded IRP 64 resins, resulting in faster release of nicotine. The Figure 5-69 shows the release characteristics of nicotine loaded on to the Amberlite IRP 64 resin at different concentrations. The release was complete within 5 min independent of the amount of loading.

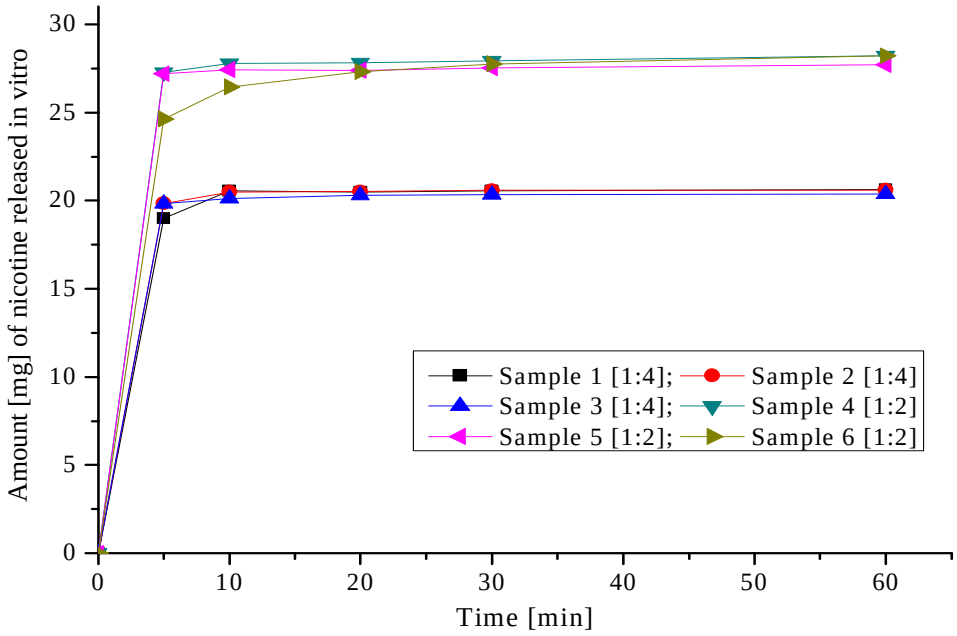


Figure 5-69. Individual *in vitro* release of nicotine from Amberlite IRP 64 resinate loaded at two different ratios

Test conditions: USP apparatus 2, 50 rpm, 500 mL artificial saliva pH 6.2 at 37 °C. Samples represent in vitro release profiles of DRCs loaded at 1:2 and 1:4 ratios

5.4.1.2 Evaluation of loading of nicotine onto Amberlite IRP 69 resins

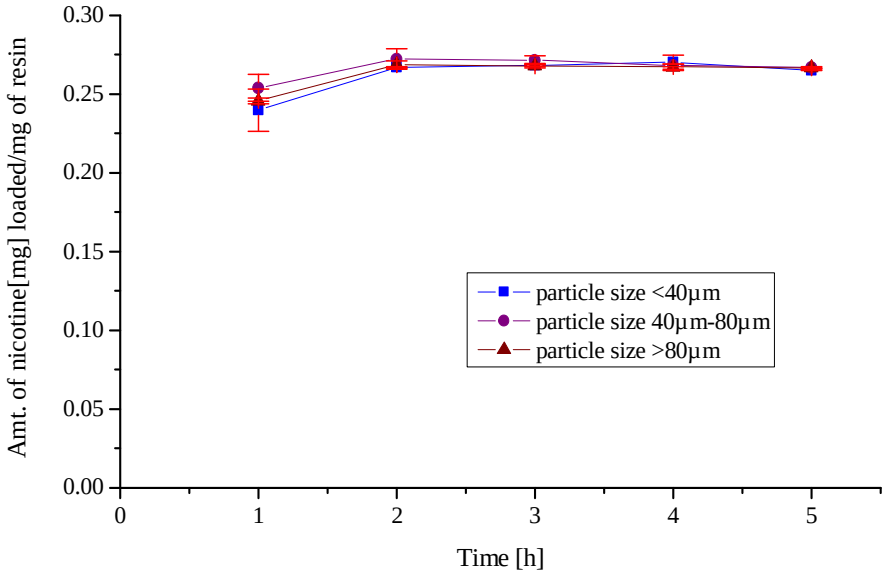


Figure 5-70. Evaluation of particle size on loading of nicotine onto Amberlite IRP 69

Graph represents data replicates n=3±SD for each of the resin particle size

It can be seen from the results (Figure 5-70) that almost 95% of the dissolved nicotine is loaded within 1 h and was almost complete within 2 h. The method is found to be reproducible and can be successfully employed for the batch loading of nicotine onto the resin. Additionally, no difference in the absorption of nicotine onto resin based on the particle

size was found. Usually, commercially available Amberlite resins contain particles of different sizes ranging from few microns to 150 microns and are non-homogenous. The bigger and coarse particles in the final product may have an unpleasant feel and can act as a disintegrant in the formulation. Hence particles size ranging from 40 µm to 80 µm was chosen for further studies.

Following the demonstration of suitability of the method to load nicotine, the process was scaled-up to produce drug resin complex (DRC).

Table 5-21. Loading efficiency of nicotine onto Amberlite IRP 69 resin

Sample Name	Amt. [g] in supernatant found	Loading nic (mg)/resin(mg)	Loading efficiency [%]
IRP 69 1/1	0.2917	0.231	92.22
IRP 69 1/2	0.2919	0.231	92.22
IRP 69 2/1	0.3080	0.229	91.79
IRP 69 2/2	0.3085	0.229	91.77

nic: nicotine

It can be seen from the results, that about 92% of total nicotine is loaded onto the resin and the method is found to be very effective, robust and reproducible for batch production of nicotine resinates [DRC].

5.4.1.3 Results of particle size analysis by laser diffraction

The results of the particle size analyses are shown in Figure 5-71 to Figure 5-73. It indicates that the average particle size had changed after impregnation using PEG 6000 solution. The purpose was to retard the rapid ion exchange/ drug release and to control the swelling of resinates which are primarily responsible for release. The summary of the results are also shown in the Table 5-22.

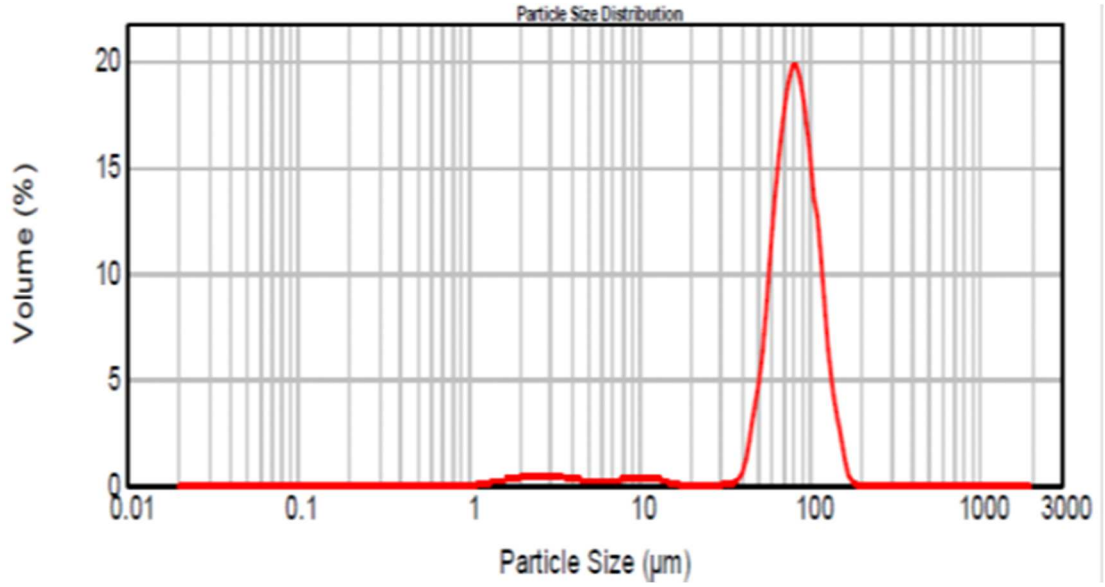


Figure 5-71. Particle size distribution of initial DRC

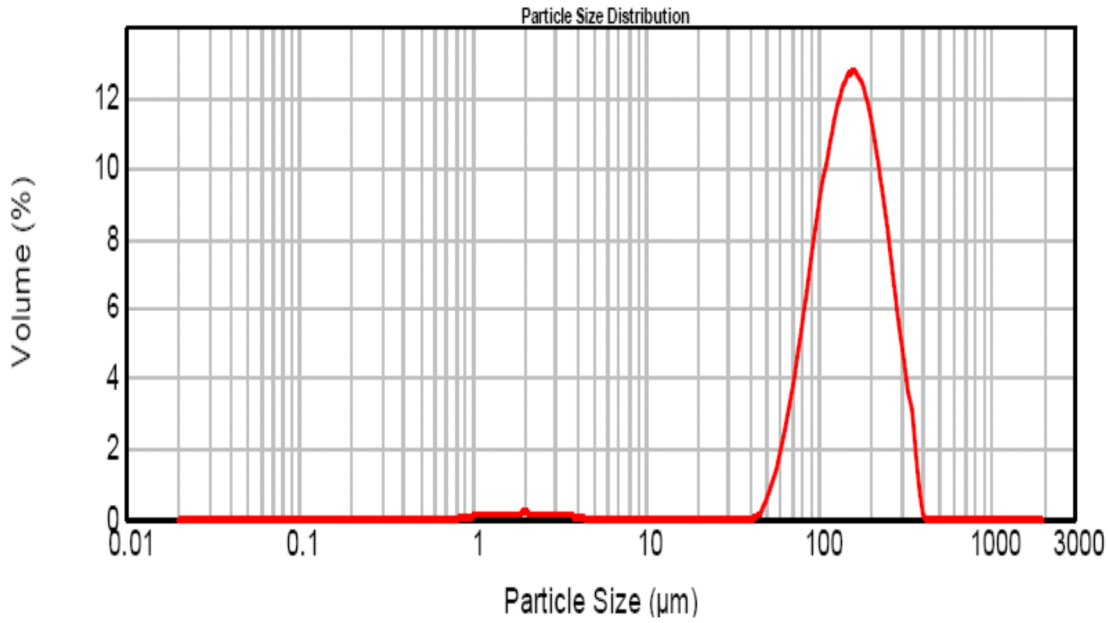


Figure 5-72. Particle size distribution of DRC impregnated with solution of PEG 6000 2 % w/v and dried for 24 h

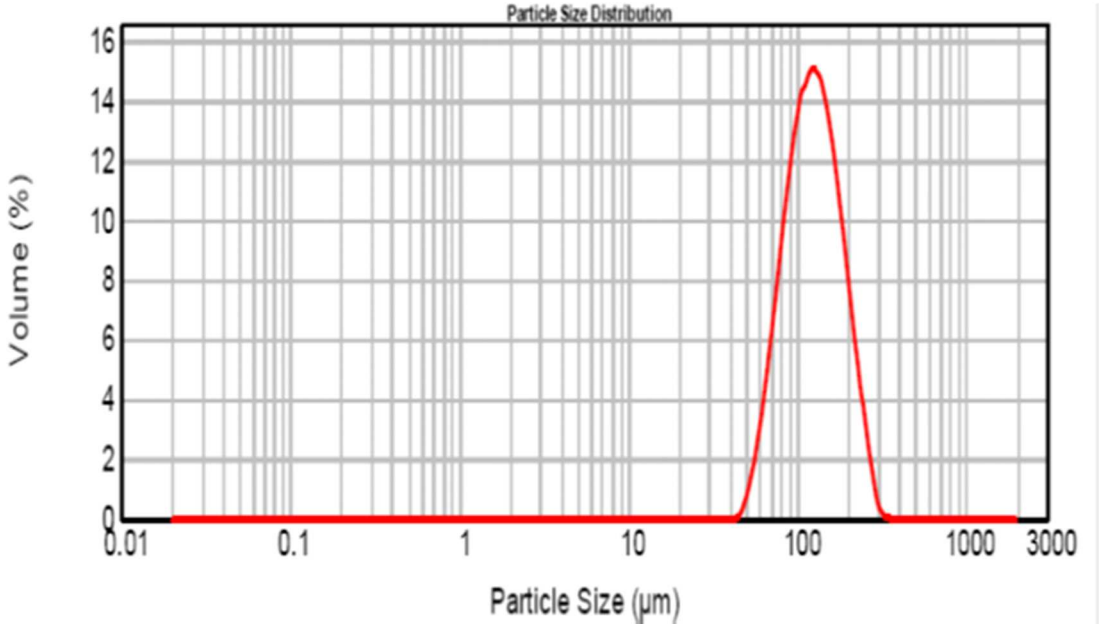


Figure 5-73. Particle size distribution of DRC impregnated with solution of PEG 6000 2 % w/v and dried for 48 h

Table 5-22. Summary of particle size determination by laser diffraction

Parameter	**Drug Resin Complex [DRC] particles**		
	DRC	**DRC -PEG 6000 (24 h drying at 60 °C)**	**DRC-PEG 6000 (48h drying at 60 °C)**
d10 [µm]	52.192	81.546	75.106
d50 [µm]	80.529	151.526	123.387
d90 [µm]	116.912	264.824	202.075

The average particle size represented by d50 has changed from 81 µm to 123 µm after impregnation with PEG 6000 solution and dried at 60° for 48 h in a hot air oven. The measurement of particle size was also performed on PEG impregnated DRC after 48 h of drying. The results are in agreement with the results published by the authors (Pisal, *et al.* 2004, Conaghey, *et al.* 1998, Pisal, *et al.* 2004, Pisal, *et al.* 2004). The influence of PEG treated on the release of nicotine from the DRC's were further investigated.

5.4.1.4 *In vitro* drug release methodology

During the initial phase of the study, influence of the amount of loading of nicotine onto resin and its release performance were tested. It was generally assumed that the release of nicotine by the ion exchange process will be accelerated with higher loading ratios. The release is a first order process and further can be controlled by the presence of unloaded IER available for ion exchange during the release process. *In vitro* release testing of DRC was performed using USP paddle apparatus to ensure whether the release of nicotine follows our assumption. The results of the analysis are presented in Figure 5-74.

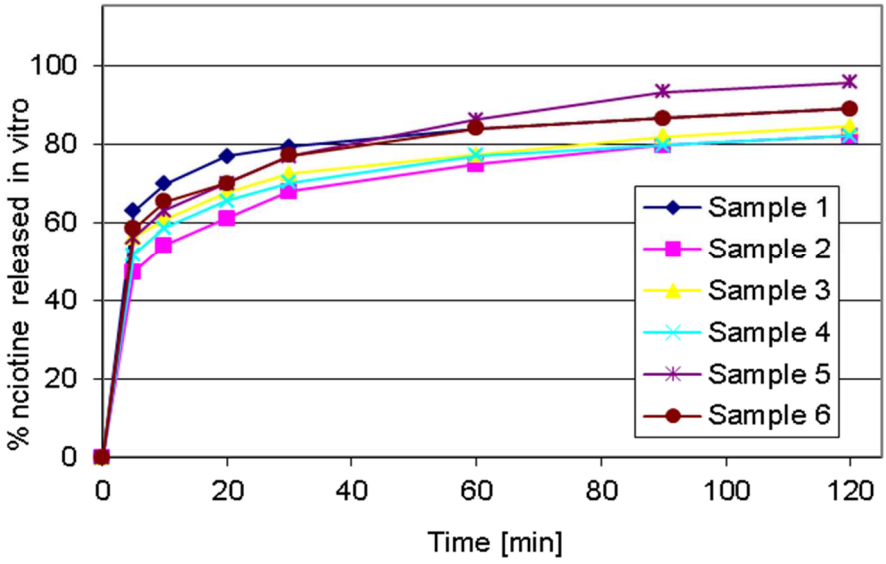


Figure 5-74. Individual *in vitro* release profiles of nicotine from Amberlite IRP 69-DRC
Test conditions: paddle apparatus at 50 rpm using 500 mL of artificial saliva pH 6.2 at 37 °C.

The composition of the Amberlite IRP69-DRC used for the release testing is shown in Figure 5-74 are given in Table 4-10.

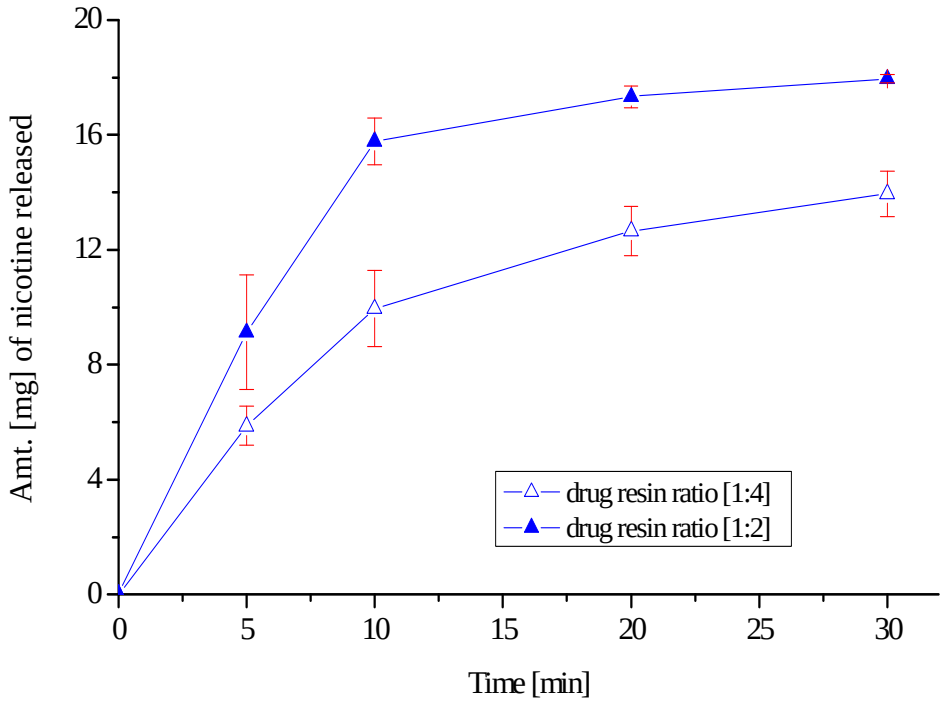


Figure 5-75. Average *in vitro* release of nicotine from DRC loaded in different ratios using USP <2> paddle apparatus

Drug release profiles represent average n=6± SD of the two differently loaded DRCs. The profiles are generated using the following test setup. USP apparatus <2>, 500 mL artificial saliva pH 6.2 maintained at 37 °C, paddle rotation 50 rpm.

The results clearly indicate that the release of nicotine from the two different loaded DRC is dependent on loading ratios. The release was rapid at 1:2 loading and completed within 30 min which is observed from the asymptote of the profile. Compared to the 1:2 loading ratios, 1:4 loaded ratios were found to be optimal for further studies.

Additionally, the influence of paddle speed on release was also tested to evaluate the rate of release which is directly proportional to the ion exchange rate. This may be crucial information for the development of dosage form. Since dissolution itself depends on the rate at which the drug boundary layer is agitated and renewed, so that a concentration gradient created would enhance the dissolution/drug release. In case of chewing machines, this agitation rate may be slower and unlike stirring apparatus, the active moiety is embedded in a gum matrix, which further hinders the release rate of the substance. However, the paddle apparatus has provided information necessary to understand the key factors involved to control the release mechanism. These results cannot be directly interpreted to final formulation design, but a step closer to understand, tailor and optimize the product.

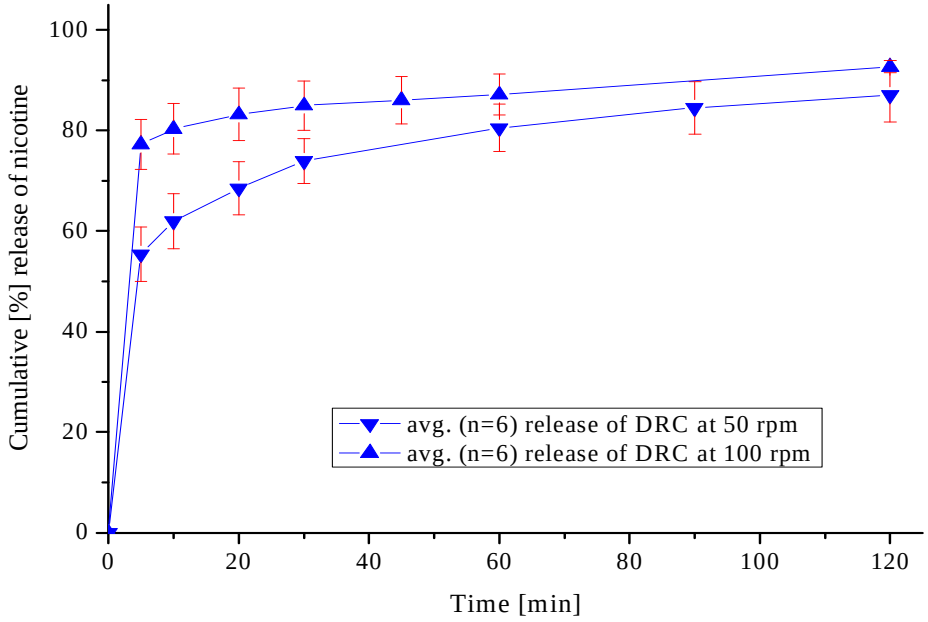


Figure 5-76. Average *in vitro* release of nicotine from DRC using USP <2> paddle apparatus

Drug release profiles represent average n=6± SD of the DRC. The profiles are generated using the following test setup. USP apparatus <2>, 500 mL artificial saliva pH 6.2 maintained at 37 °C, paddle rotation 50 rpm /100 rpm

The drug release testing was performed until 48 h. The profiles show a burst release at the beginning of testing and are about 52% and 79% respectively at 5 min for both paddle rotation speeds. The amount released at 48 h was considered complete as the curve approached asymptote after 24 h (data not shown) and the corresponding amount was taken to calculate the % dissolved over time.

5.4.1.5 Influence of buffer solution on release

The influence of different buffer solutions on the release of nicotine from DRC processed differently was evaluated. Prior to the loading of nicotine, resins were washed as described earlier to eliminate the presence of fine particles (<40 µm) that may hinder the loading process and the release characteristics. The performance of DRCs was tested using the paddle apparatus at 50 rpm.

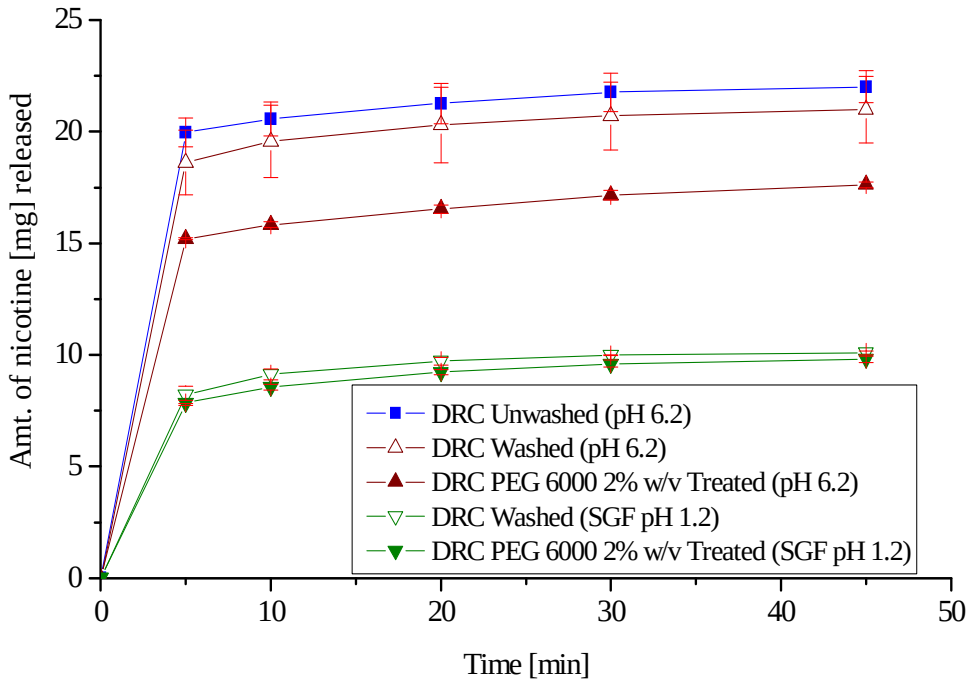


Figure 5-77. Average *in vitro* release of nicotine from DRC prepared and treated using different techniques in different buffer solutions

Drug release profiles represent average n=6± SD of the DRCs. The profiles are generated using the following test setup. USP apparatus <2>, 500 mL of different media indicated in the legend, maintained at 37 °C, paddle rotation 50 rpm.

No significant difference in release behavior was observed between the washed and unwashed resins in SGF pH 1.2 and artificial saliva pH 6.2. However, the nicotine release in acidic pH 1.2 is significantly reduced compared to that of pH 6.2 independent of prior treatment to the DRCs. Burst release observed in pH 6.2 was significantly reduced after treatment with the PEG 6000 2 % w/v solution. The results are in agreement with the literature (Pisal, *et al.* 2004). The assumption is that the PEG treatment reduced the swelling of DRCs and retards the ion exchange process. The particle size measurement by laser diffraction technique confirmed the permanent change in the particle size with PEG treatment. For further studies, the DRCs treated with the PEG solution were chosen.

5.4.1.6 Evaluation of release kinetics from DRC

The goodness of fit for the Boyd and Bhaskar model is shown in the Figure 5-78.

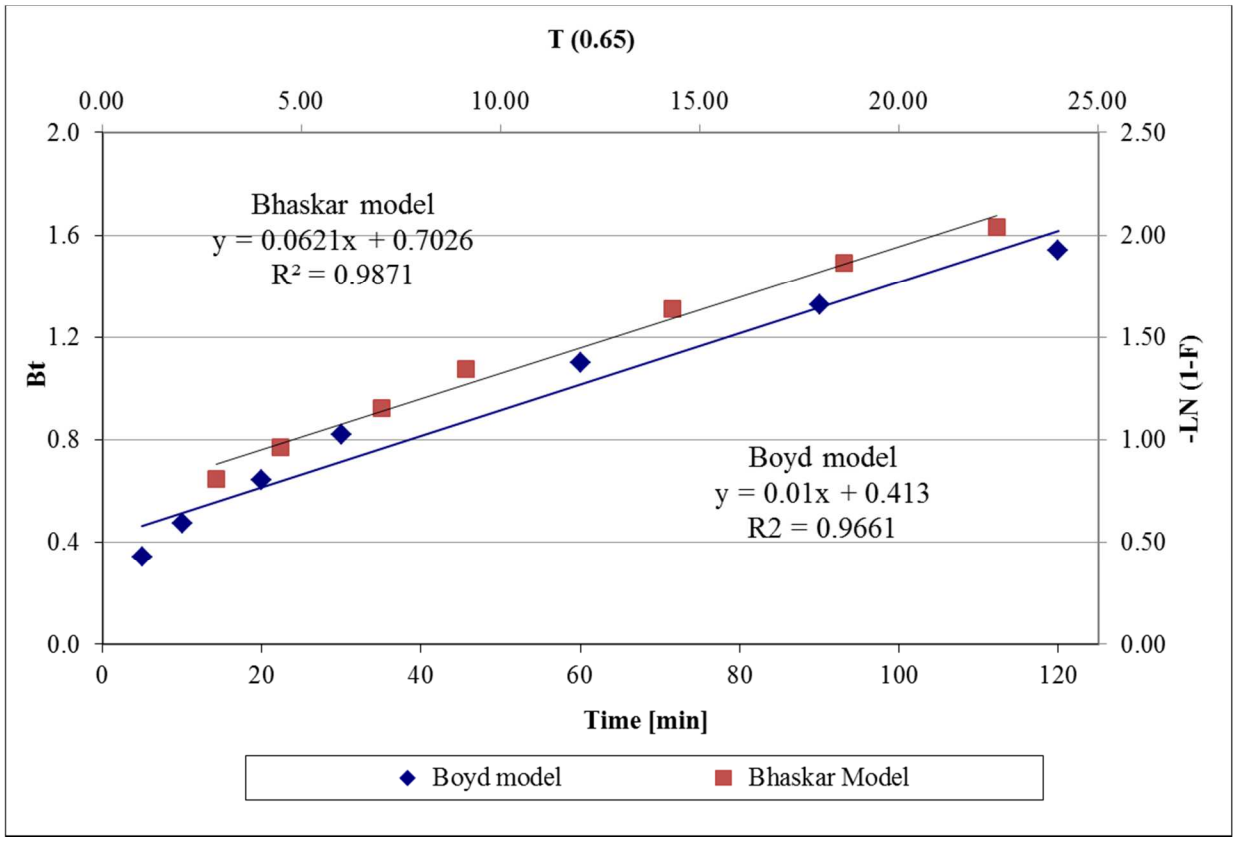


Figure 5-78. *In vitro* release kinetics of nicotine from DRC

Graph represents the data derived from in vitro nicotine release from 6 replicates. Linear fit for Boyd /Reichenberg and Bhaskar's model. The Bt values on the y-axis and time on the x-axis is for the Boyd/Reichenberg model and the second y-axis –LN (1-F) and the second x-axis with $T_{(0.65)}$ values indicate the Bhaskar model. The legend below refers to corresponding model.

As seen from the correlation values, the model fits better for the data. This confirms the drug release from the IER is diffusion controlled.

5.4.1.7 Coating and micro-encapsulation of DRC

In vitro drug release on the coated DRC samples were performed using the USP paddle method described earlier. Since the Eudragit L100 dissolved rapidly at above pH 6.0, acetate buffer pH 4.5 was used to evaluate the release of nicotine from the coated DRCs.

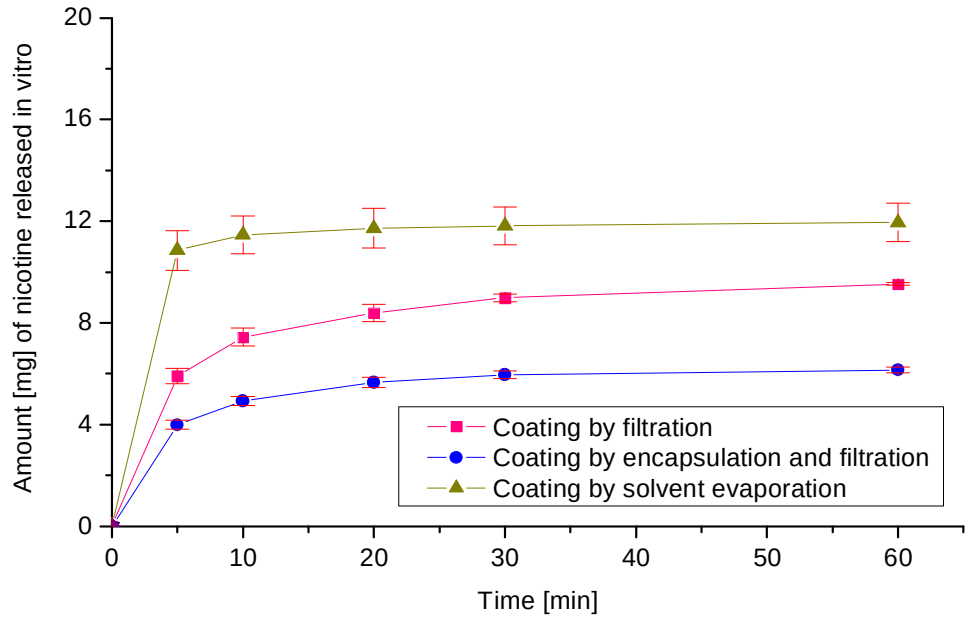


Figure 5-79. Evaluation of suitability of the methods for coating DRCs

Test conditions: USP paddle apparatus at 75 rpm, 500 mL of acetate buffer pH 4.5 maintained at 37°C, n=3

As indicated in the result (Figure 5-79), the method was able discriminate different encapsulation techniques employed to coat the DRCs. Burst release was observed for DRCs encapsulated using the filtration method. Coating by encapsulation and filtration was found to be more effective in controlling the release than the other methods. The drug release rate was well controlled by the formation of Eudragit L100 film layer around the DRC inhibiting the rapid diffusion of medium into the resin particle. The encapsulation method was found to be more suitable than other methods and was chosen for further optimization.

The coating by encapsulation and filtration was already investigated by many authors to encapsulate different resinates. Following the preliminary results, the coating method was further investigated to evaluate the influence of various excipients used for coating and their effect on release of nicotine from the DRCs. Five different compositions containing various amounts of excipients given in the Table 5-23 were prepared and tested for the nicotine content and release.

Table 5-23. Composition for coating the DRC

Composition	Amounts in each formulation				
	F1	F2	F3	F4	F5
DRC [mg]	1000	1000	1000	1000	1000
Eudragit L100 [mg]	1000	1000	1000	1000	1000
PEG 4000 [mg]	70	150	150	150	150
Tween 20 [mg]	40	40	80	40	80
Dichloromethane [mL]	10	10	10	20	20
Acetone [mL]	1	1	1	1	1
Polyvinyl alcohol [mg]	375	375	375	375	375
Deionized water [mL]	150	150	150	150	150

The formulations were tested for the nicotine content. Accurately weighed 100 mg of the coated DRC was transferred to a beaker containing 100 mL of artificial saliva pH 6.2 maintained at 37 °C. The medium was stirred for about 1h at 600 rpm using a magnetic stirrer. Then the samples were withdrawn, filtered and suitably diluted before the HPLC-UV determination. The experiment was performed in duplicate and the results are presented in the Figure 5-80.

The amount of resin present in the weighed amount of the coated DRC is determined by the following formula;

$$\text{Amount of DRC [mg]} = \frac{\text{Amount of nicotine found [mg]}}{Loading\ factor\left[\frac{mg\ nicotine}{mg\ resin}\right]}$$

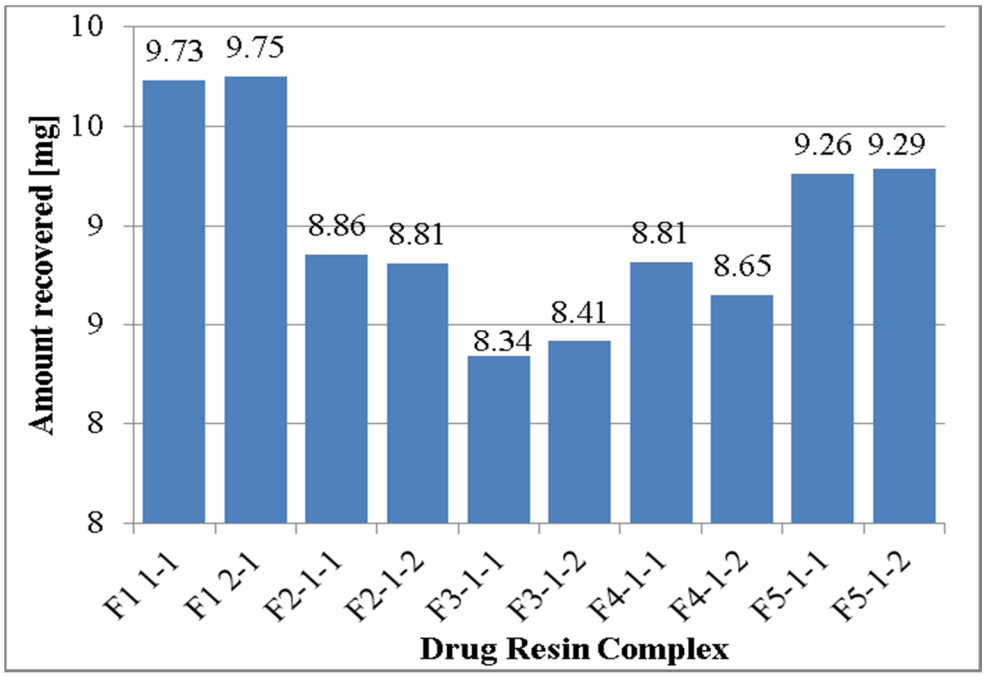


Figure 5-80. Assay of nicotine content in the Eudragit L100 coated IRP 69-DRC

100 mg of Eudragit coated DRC (theoretically equivalent to 50 mg DRC containing 11.5 mg of available nicotine) was taken for the assay determination. Experiment was performed in duplicate.

The *in vitro* drug release testing was performed for all the coated DRCs using the paddle method described in section 4.11.9.1.

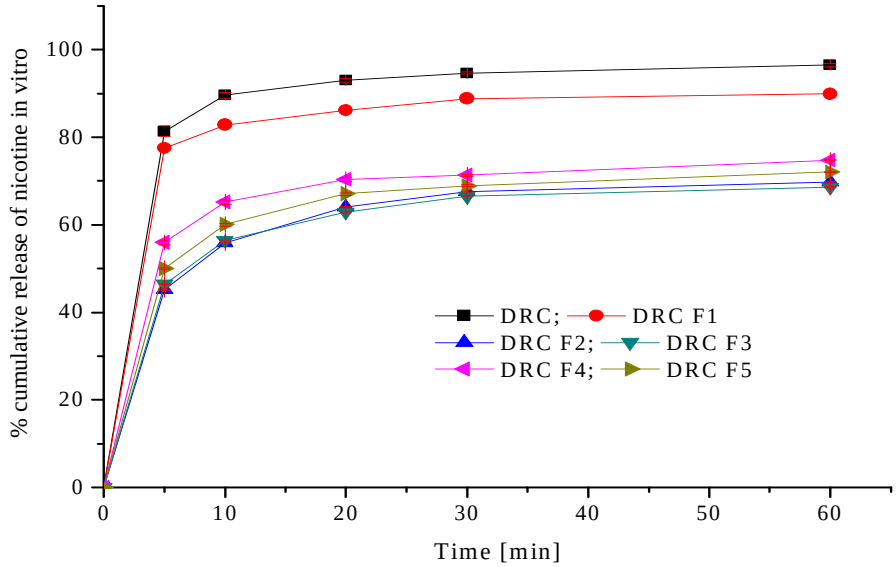


Figure 5-81. Average *in vitro* release of nicotine from Eudragit L100 coated IRP 69-DRC

Test conditions: USP paddle apparatus at 75 rpm, 500 mL of acetate buffer pH 4.5 maintained at 37°C.

The content determination shows that about 85% of the DRC is contained in the weighed amount (100 mg coated DRC). The end point of nicotine release from coated DRC is indicated by the asymptote of the drug release curve. For the tested DRC's, the drug release

was in the following order; DRC (uncoated)> DRC F1> DRC F4> DRC F5> DRC F2> DRC F3.

It was found from the release experiments that the presence of PEG 4000 affected the coating and release properties. The PEG acts as a wetting agent and also as a plasticizer for the polymer film layer around the DRC. The formulations F2 – F5 contained same amount of PEG and the release profiles of them showed a small difference. On the other hand, the F1 contained less amount of PEG in comparison to the rest of the formulations indicated a faster release profile. Additionally, the release of nicotine from an uncoated DRC is also presented in Figure 5-81, which shows a fastest release among the formulations (F1 –F5) compared. This confirms the effect of PEG in controlling the release of nicotine from the Eudragit L coated DRCs (Pisal, *et al.* 2004).

Earlier it was found that the release of nicotine from the DRC is pH dependent. In drug release media of pH > 4.0, the nicotine release was found to be complete. There is no effective control of nicotine release from DRC's in artificial saliva pH 6.2.

From the coated formulations F1-F5, the lowest nicotine release was observed for F2 and F3. The profiles of F2 and F3 were found to be similar. Among the two, F2 was chosen since the only difference in composition between the formulations is the amount of Tween 20 present (F2: 40 mg, F3: 80 mg) which was found to have no influence on the release characteristics. Moreover, for F2 formulation, the variability observed (SD) was found to be less than other formulations. The *in vitro* release testing was performed additionally in two other buffer solutions and compared for release behavior.

5.4.1.8 Influence of pH on the release of nicotine from coated DRCs

The effectiveness of coating was further tested by the influence of differently buffered solutions to dissolve the Eudragit L100. As shown in Figure 5-82, the drug release is completely dependent on the pH following the coating. The Eudragit L 100 is soluble in aqueous media above pH 6.0.

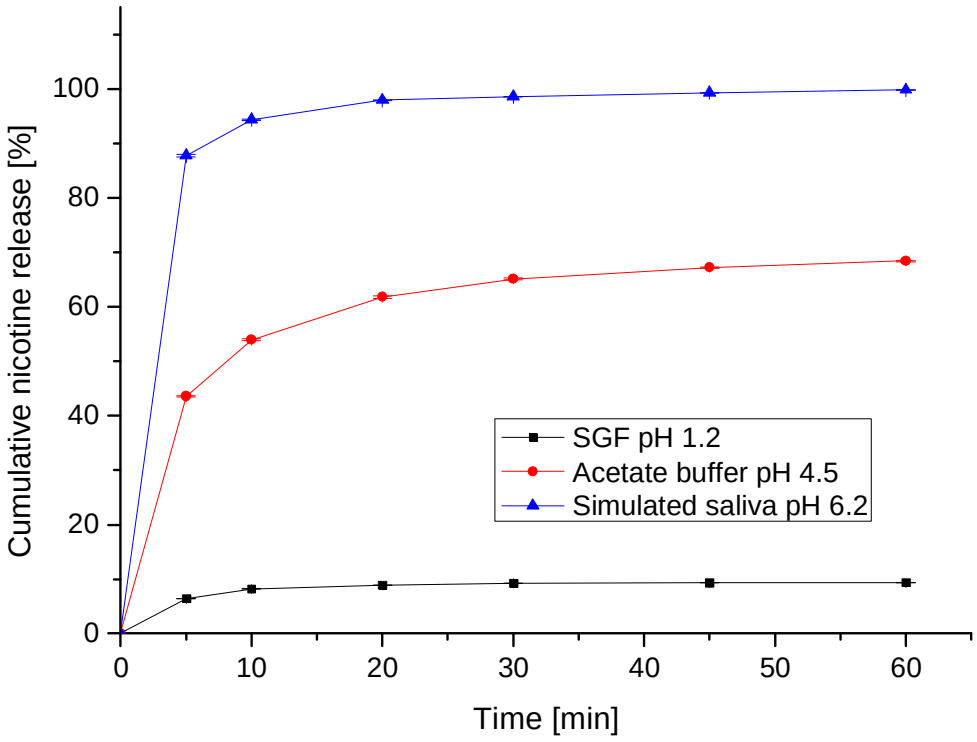


Figure 5-82. Influence of pH on the release of nicotine from the pH sensitive polymer Eudragit L 100 coated DRC–F2

Test conditions: USP paddle apparatus 2 at 75 rpm, 500 mL of buffered solution at 37 °C, data represent n=3 ±SD.

The results indicate the suitability of the Eudragit L100 for coating. The method can be effectively implemented for actives where the gastric resistance is required. In case of chewing gums, the resin particles containing nicotine are aqueous insoluble and during the chewing process, part of the disintegrated gum particles along with the DRC are swallowed and exposed to the acidic environment. Coating using Eudragit L100 may be useful in such situations. However, the Eudragit L100 indicated a high solubility characteristic in salivary pH 6.2. Therefore a burst release of nicotine and partial absorption in the oral cavity is expected. The rest of the nicotine release is exposed to the acidic environment in the stomach. The suitability of artificial saliva pH 6.2 for the biorelevant *in vitro* release testing of chewing gums was already demonstrated (Gajendran, *et al.* 2012). Buffer solutions between pH 6-7 are recommended in the Ph. Eur. for the performance testing of gum formulations.

Since DRCs coated using Eudragit L100 do not lead to an effective release control at the pH of saliva, an attempt was made to encapsulate the DRCs using pH independent polymers. Such a method would be better for substances that require a sustained and controlled release of active(s) independent of the pH value at the site of release.

5.4.1.9 *In vitro* nicotine release from Eudragit RS 100 coated DRC

The result of nicotine release from the Eudragit RS 100 coated DRC's is shown in the Figure 5-83. The method of microencapsulation and the amount of excipients used are described earlier in the methodology section. The performance of the micro-encapsulated DRC was tested by the *in vitro* dissolution testing by the methods established and optimized previously. The results were compared with the uncoated DRC in Figure 5-83.

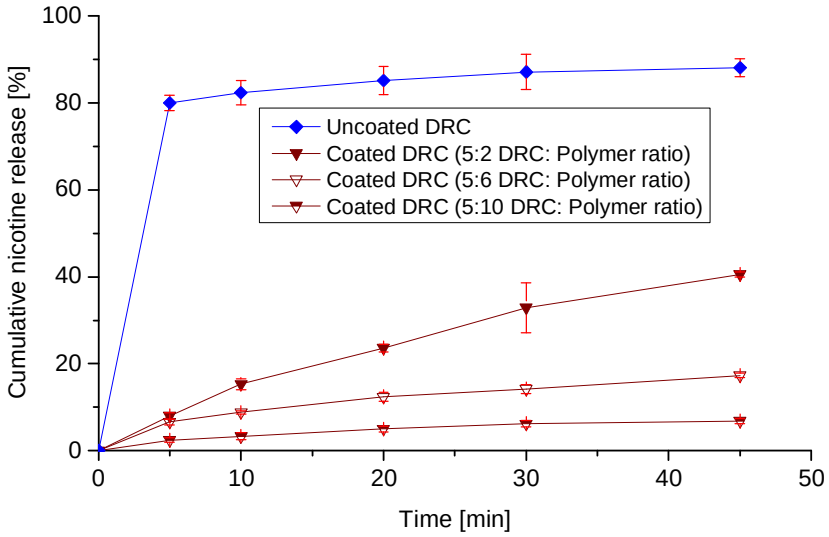


Figure 5-83. ***In vitro*** **nicotine release from coated and uncoated DRC's using different concentrations of Eudragit RS100.**

The tests were performed using USP 2 apparatus at 75 rpm in 500 mL of artificial saliva pH 6.2 maintained at 37 °C, n=3 units ±SD

The *in vitro* drug release clearly indicates that the coat to core ratio (polymer to DRC ratio) has a large influence in controlling the release of nicotine from the DRC. Since Eudragit RS 100 is practically insoluble in water, the drug release is diffusion controlled. In order to optimize the coating method, design of experiments (DoE) was adopted to study the influence of various factors influencing the release and to obtain optimal coating parameters for desired release characteristics.

5.4.1.10 Coating optimization by DoE

The efficiency of the process of coating by microencapsulation was further optimized by implementing DoE. The simulation of combination of different factors assumed to have influence on the release characteristics are given in the Table 4-13. Additionally, the suitability of the coating process was assessed by the leaching and release testing experiments.

5.4.1.10.1 Evaluation of drug leaching during microencapsulation

During the microencapsulation process, the amount of nicotine released into the external aqueous phase was investigated. This procedure would provide evidence to justify the suitability of the method for microencapsulation. For this purpose of determining the amount of nicotine released in to the external phase, about 3 mL of the PVA solution was removed from the solution containing the coated resin samples and filtered using a 0.45µm filter before filling into the HPLC vials. The nicotine present in the samples was quantified using a previously established and validated HPLC-UV method. The result of the analysis is shown in the Figure 5-84.

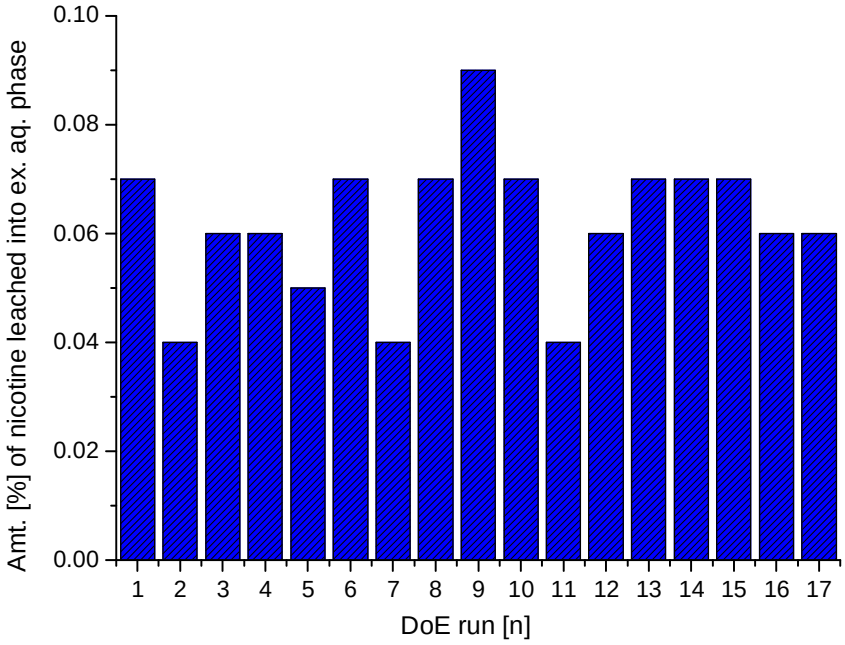


Figure 5-84. Evaluation of nicotine leaching into external phase

The data represents the samples from the 17 DoE runs. The results indicate only a small fraction (about 0.09% of theoretical nicotine content) of nicotine from the encapsulated DRCs leached into the external aqueous phase. This proves that the method is suitable for coating the DRCs.

5.4.1.10.2 *In vitro* drug release testing

The microencapsulated DRCs were tested for their nicotine release using the USP paddle method used for general testing of the DRCs. The testing was performed in triplicate and the average *in vitro* drug release is shown in the Figure 5-85 and data are summarized in Table 5-24

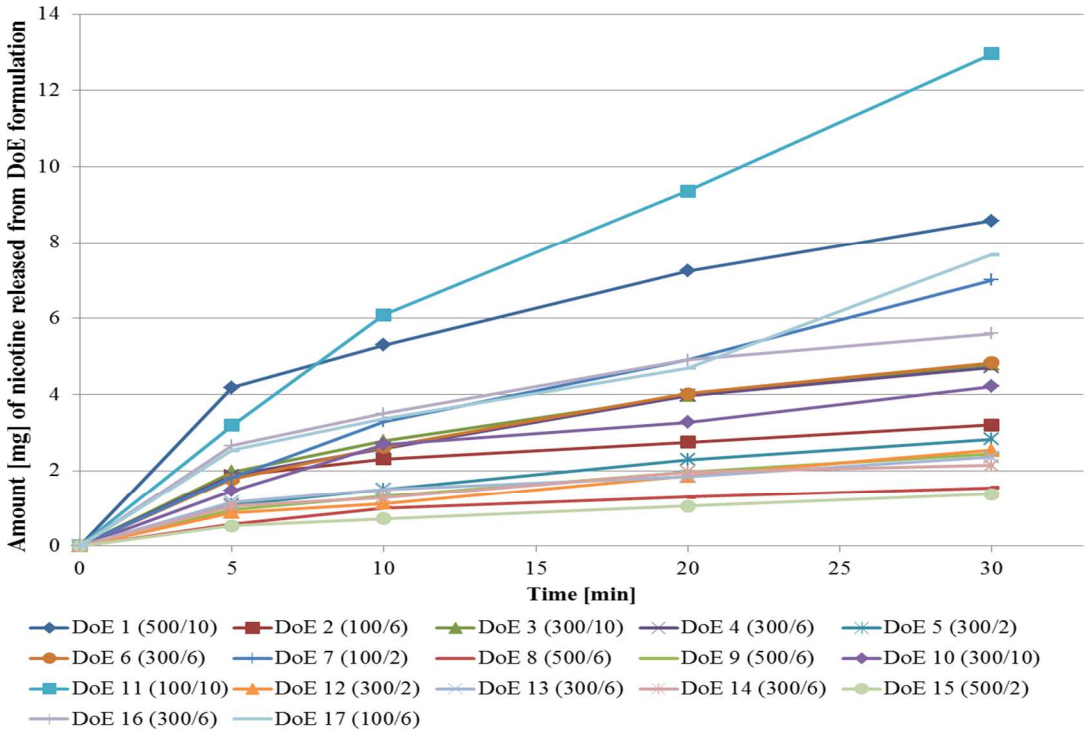


Figure 5-85. *In vitro* drug release from coated nicotine resinates obtained by DoE

Numbers in the brackets represent amount of Eudragit RS 100 in mg and volume of dichloromethane (mL) used in the design.

Test conditions: USP 2, 75 rpm, 500 mL artificial saliva pH 6.2 at 37 °C, 10µm cannula filters for pre-filtration and 0.45µm PVDF filters for HPLC analysis. The composition and amounts corresponding to each DoE run is given in Table 4-13.

Table 5-24. Statistical summary of nicotine release from DoE formulations

DoE run	Amount of Eudragit RS 100 [mg]	Amount of Phosphatidyl choline [mg]	Vol. of DCM [mL]	Amount [mg] of nicotine released			
				5 min	10 min	20 min	30 min
1	500	12.5	10	4.19	5.30	7.25	8.58
2	100	5	6	1.86	2.32	2.76	3.20
3	300	5	10	1.97	2.79	3.98	4.80
4	300	12.5	6	1.88	2.59	3.98	4.71
5	300	5	2	1.08	1.51	2.29	2.83
6	300	12.5	6	1.78	2.62	4.03	4.84
7	100	12.5	2	1.82	3.29	4.91	7.01
8	500	5	6	0.59	1.01	1.30	1.55
9	500	20	6	0.96	1.33	1.98	2.45
10	300	20	10	1.47	2.69	3.27	4.22
11	100	12.5	10	3.20	6.09	9.36	12.97
12	300	20	2	0.89	1.13	1.86	2.54
13	300	12.5	6	1.17	1.50	1.85	2.36
14	300	12.5	6	1.06	1.29	1.97	2.14
15	500	12.5	2	0.53	0.73	1.06	1.38
16	300	12.5	6	2.66	3.51	4.91	5.60
17	100	20	6	2.54	3.38	4.70	7.67

DCM: dichloromethane

The coating method was able to provide a sustained release of nicotine from resinates. The burst release of nicotine from resinates could be well controlled with the solvent evaporation coating method.

The *in vitro* release of nicotine from the resin complex was investigated. The summary of all *in vitro* drug release experiments are presented in the Figure 5-85. From the results, it can be observed that the nicotine release from all the formulations is well controlled in the artificial saliva pH 6.2. General assumption is that with the higher polymer concentration, the drug release is controlled by the thickness of polymer film formation around the DRC. The volume of the internal phase also affects the coating process and hence the nicotine release. From the results presented, it can be observed that a combination of high polymer amount and lower internal solvent volume can be used to achieve higher coat to core ratio. Higher concentrations of phosphatidyl choline in the internal phase significantly affected the release characteristics. Usually in formulation studies, phosphatidyl choline is used as a wetting agent. In the current study, in order to reduce the interfacial tension at the drug resinate - polymer solution interface, phosphatidyl choline has been used. One drawback of employing Phosphatidyl choline is the agglomeration of the particles which was observed at high concentration levels which might also affect the flow properties. The primary objective is to optimize the nicotine release from chewing gum formulations. The current coating study using the DoE could be successfully used as a tool to optimize the release characteristics from the resin complex (DRC).

5.4.1.11 Analysis of DoE data

The *in vitro* drug release data from the 5 min and 10 min time points were considered for the evaluation. The data at the selected time points are assumed to be more reflective of the coating characteristics. Different models were initially fit to the data and found that the response surface linear model was acceptable. The data were natural log transformed before the analysis. The ANOVA statistics was used to test the significance of the model.

Table 5-25. ANOVA test to evaluate the goodness of fit for the model and the factors at 5 min drug release

Source	Sum of squares	df	Mean square	F value	p-value prob > F
Mean vs Total	1.25	1	1.25		
Linear vs Mean	3.22	3	1.07	24.79	< 0.0001*
2FI vs Linear	0.06	3	0.02	0.36	0.7828
Quadratic vs 2FI	0.08	3	0.03	0.39	0.7701
Cubic vs Quadratic	0.04	1	0.04	0.43	0.5588
Residual	0.25	3	0.08		
Total	4.89	14	0.35		

*) suggest that the model effect is significant

Table 5-26. ANOVA test to evaluate the goodness of fit for the model and the factors at 10 min drug release

Source	Sum of squares	df	Mean square	F value	p-value prob > F
Mean vs Total	6.87	1	6.87		
Linear vs Mean	3.66	3	1.22	17.01	0.0002*
2FI vs Linear	0.03	3	0.01	0.09	0.9621
Quadratic vs 2FI	0.17	3	0.06	0.49	0.7046
Cubic vs Quadratic	0.18	2	0.09	0.67	0.5734
Residual	0.41	3	0.14		
Total	11.32	15	0.75		

*) suggest that the model effect is significant

Table 5-27. ANOVA table of individual factors for 5 min drug release

Source	Sum of squares	df	Mean square	F value	p-value prob > F
A-Eudragit RS100	2.0377	1	2.0377	47.1322	< 0.0001*
B-Phosphatidyl Choline	0.0470	1	0.0470	1.0869	0.3217
C-Dichloromethane	0.6346	1	0.6346	14.6792	0.0033*

*) suggest that the model effect is significant

Table 5-28. ANOVA table of individual factors for 10 min drug release

Source	Sum of squares	df	Mean square	F value	p-value prob > F
A-Eudragit RS100	2.2722	1	2.2722	31.6639	0.0002*
B-Phosphatidyl Choline	0.0133	1	0.0133	0.18555	0.6750
C-Dichloromethane	0.8599	1	0.8599	11.9827	0.0053*

*) suggest that the model effect is significant

The Model F-value of 24.79 and 17.01 implies that the effect of linear model is significant. There is only a 0.01% and 0.02% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case,

the factors Eudragit RS 100 and dichloromethane (A & C) are significant model terms. Values > 0.1 indicate that the model terms are not significant.

From the table it was also observed that phosphatidyl choline has no significant effect on microencapsulation procedure. For further statistical analyses, the influence of phosphatidyl choline was not considered.

Table 5-29. Coefficient of determination for predicted and actual values of drug release

Coefficient of determination	**Drug release**	
	5 min	**10 min**
R-Squared	0.8815	0.8227
Adj R-Squared	0.8459	0.7743
Pred R-Squared	0.7986	0.7011
Adeq Precision	16.1414	13.5293

The "Pred. R-Square" of 0.7986 and 0.7011 is in reasonable agreement with the "Adjusted R-Squared" of 0.8459 and 0.7743 for both the response variables 5 min and 10 min drug release. The “Adeq. Precision” measures the signal to noise ratio. A ratio greater than 4 is usually desirable. The ratio of 16.1414 and 13.5293 indicates an adequate signal for the model and could be used to navigate the design space.

The final linear equation in terms of coded and actual factors is given by;

Ln (5 min release) = +0.41-0.39 x A+0.039 x B+0.47 x C
Ln (10 min release) = +0.77-0.43 x A+0.041 x B+0.51 x C

Evaluation of other factors to estimate the goodness of fit for the *in vitro* drug release data was also tested. The results of the analysis are shown in the Figure 5-86 to Figure 5-97.

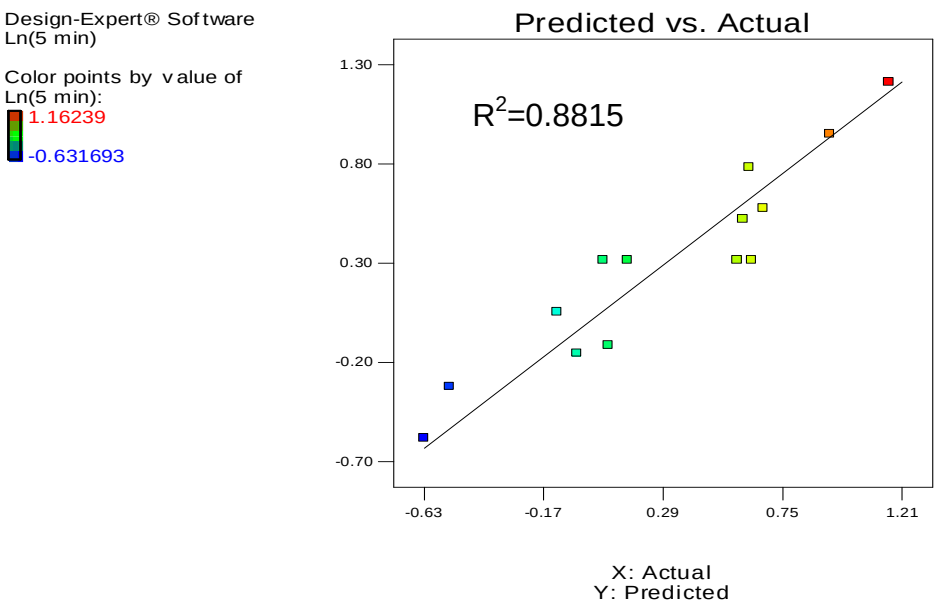


Figure 5-86. Correlation of predicted vs. actual values for drug release at 5 min

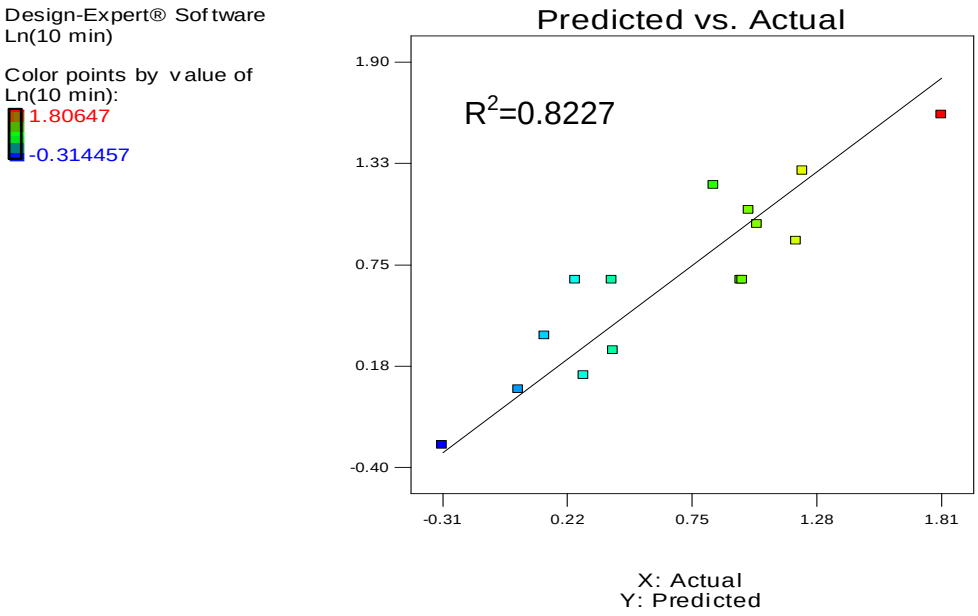


Figure 5-87. Correlation of predicted vs. actual values for drug release at 10 min

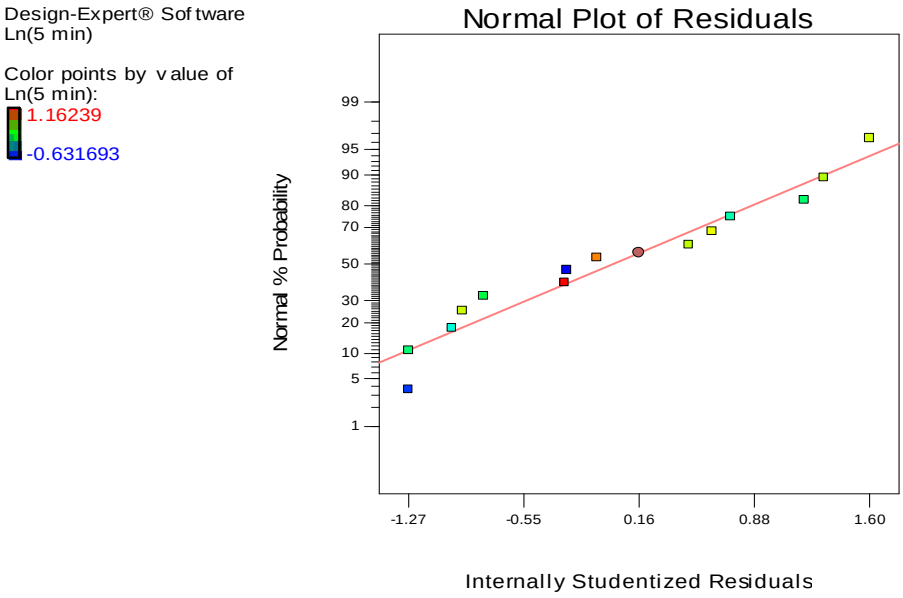


Figure 5-88. Normal plot of residuals for the model terms described in DoE Table 4-13 at 5 min release

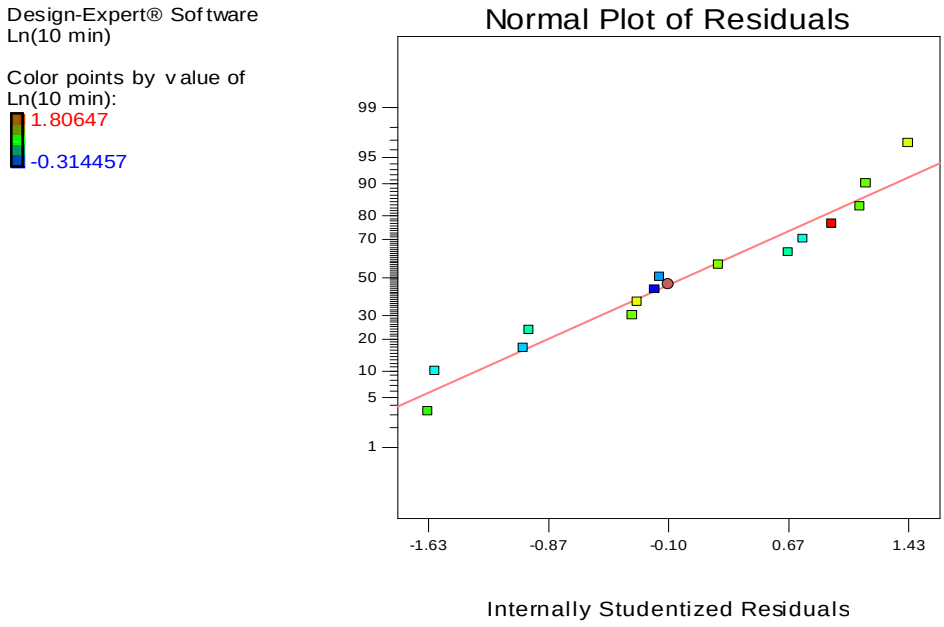


Figure 5-89. Normal plot of residuals for the model terms described in DoE Table 4-13 at 10 min release

The normal probability plot for the internally studentized residuals is given in the Figure 5-89. The purpose of the plot is to evaluate whether the residuals from the design follow a normal distribution. The residuals plotted are distributed around the regression line with no definite patters or trend. In cases where trends demonstrate the presence of definite patters like “S-shaped” curve. Transformation of data may be required to provide a better estimate.

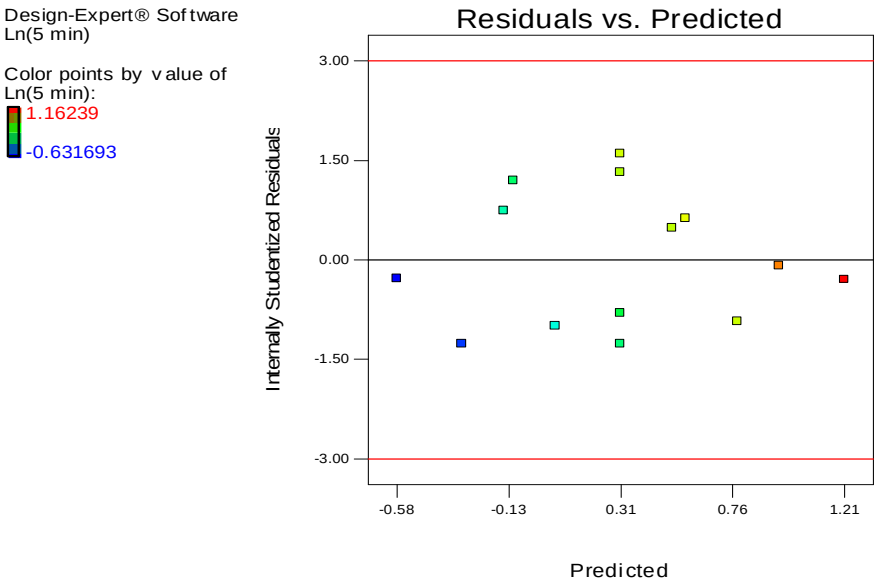


Figure 5-90. Internally studentized residuals for drug release at 5 min vs. the predicted values from the DoE

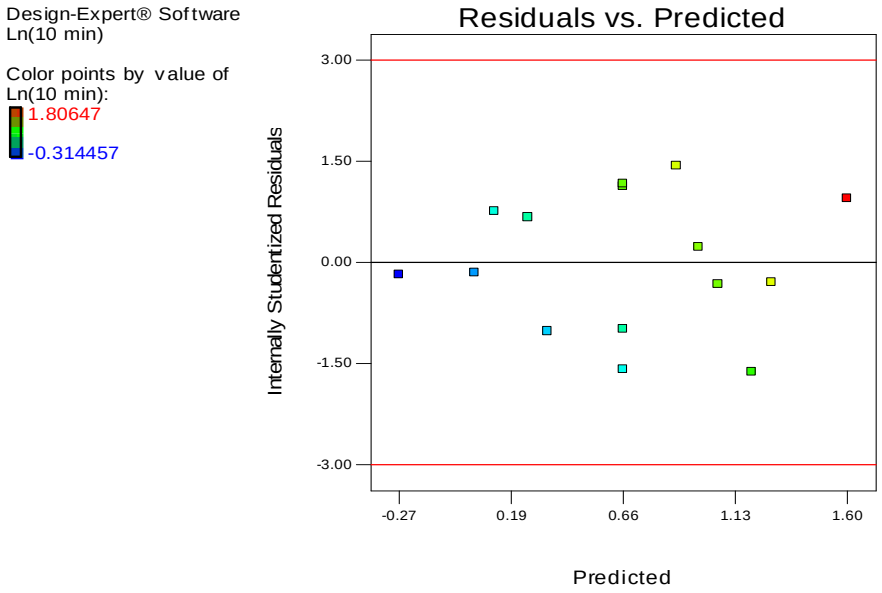


Figure 5-91. Internally studentized residuals for drug release at 10 min vs. the predicted values from the DoE

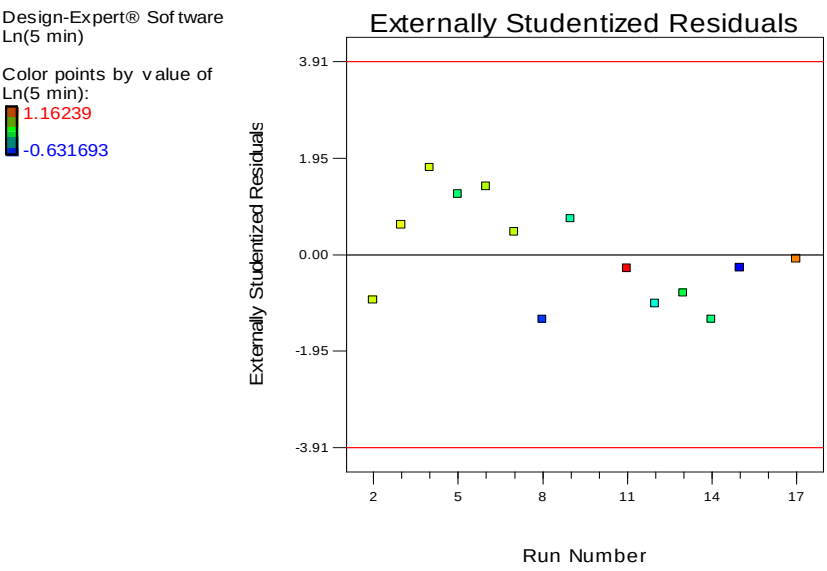


Figure 5-92. Externally studentized residual plot for drug release at 5 min for all experimental runs

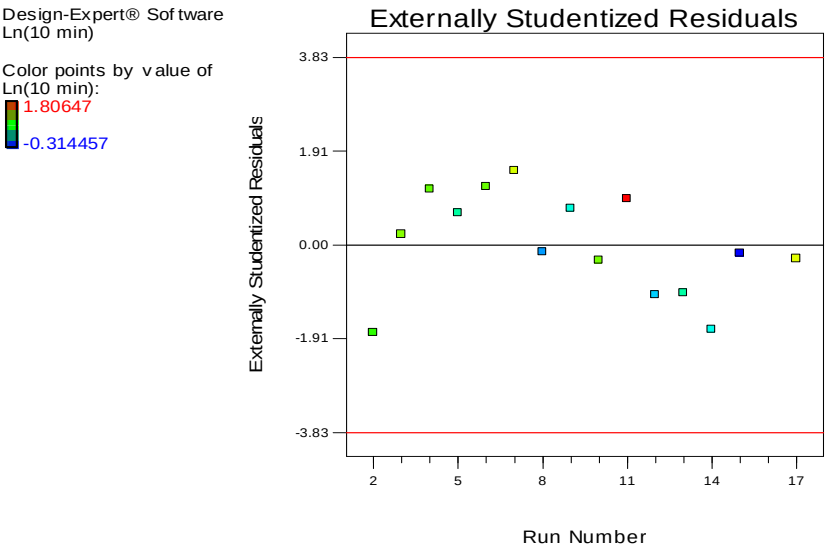


Figure 5-93. Externally studentized residual plot for drug release at 10 min for all experimental runs

An externally studentized residual plot was used to determine the outliers in the model taking into consideration all the runs in the experimental design. This takes into account that number of standard deviations the actual value deviates from the predicted values. As indicated in the graph that none of the points are outside the 95% confidence limits represented by the red lines.

The fitness of the selected experimental design was further evaluated using the box-cox plots to determine the outliers in the model.

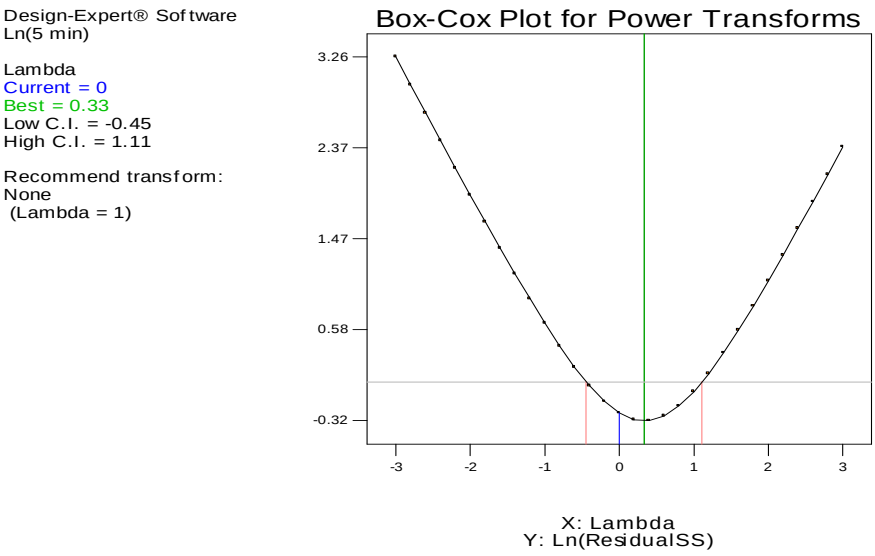


Figure 5-94. Box –Cox plot for drug release at 5 min

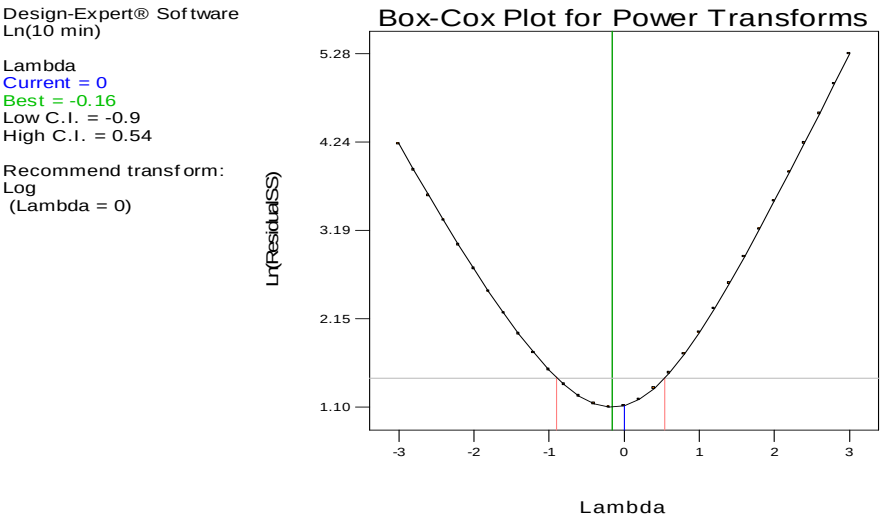
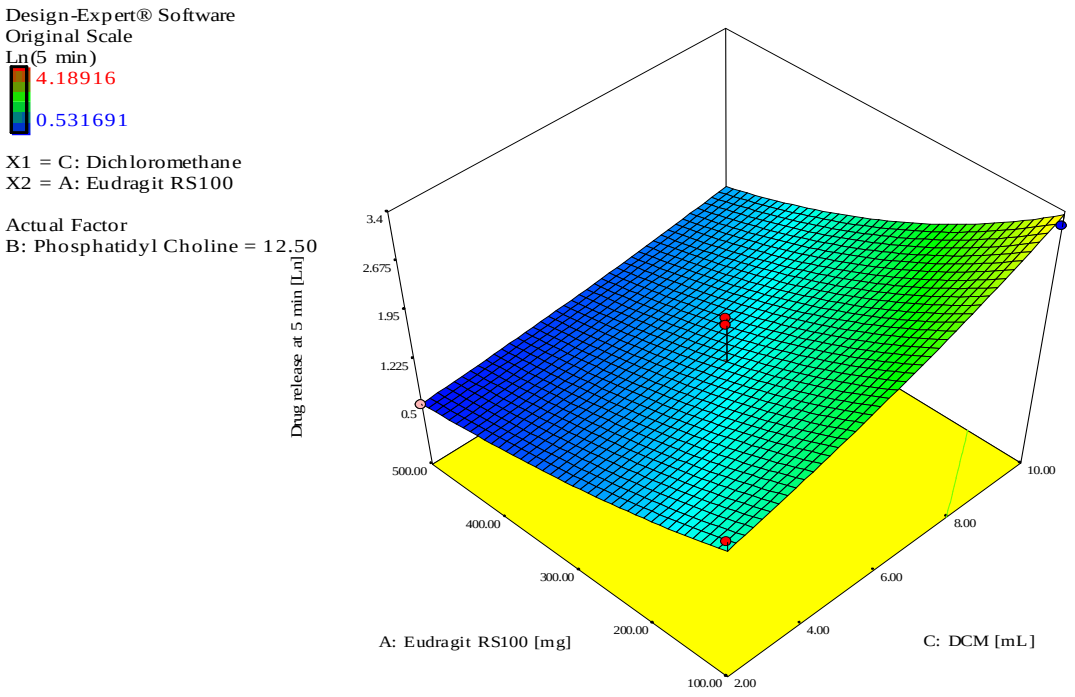
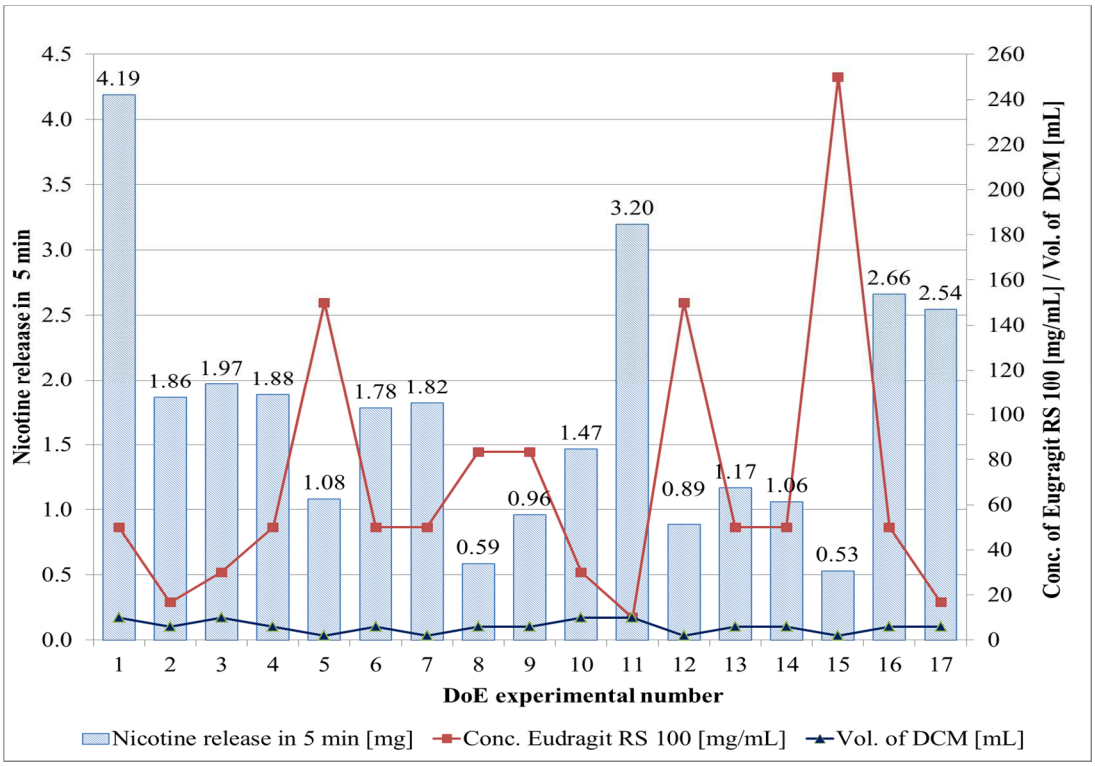


Figure 5-95. Box –Cox plot for drug release at 10 min

The box-cox plot was used to determine if a power law transformation is required for data treatment. The minimum sum of squared residuals (residual SS) is represented by the lambda value. The figure above shows the minimum lambda value of 1 with the calculated 95% confidence interval. Since the estimated lambda value is within the interval. Transformation of the data for this analysis is not recommended. The proposed model could be successfully used to navigate the design space in the model.



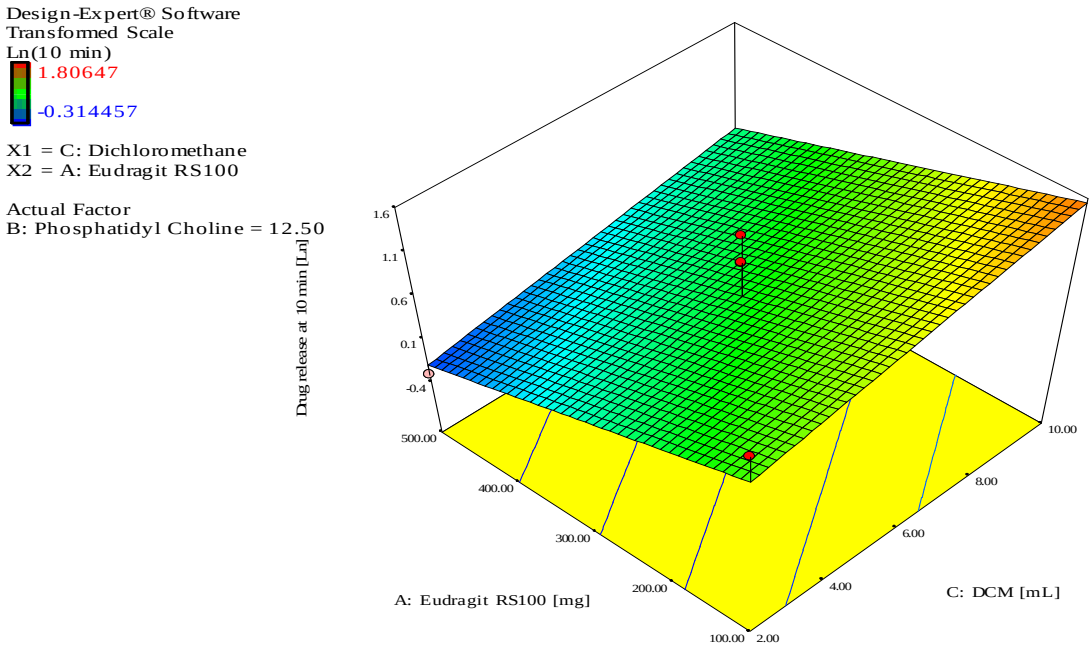
(a)



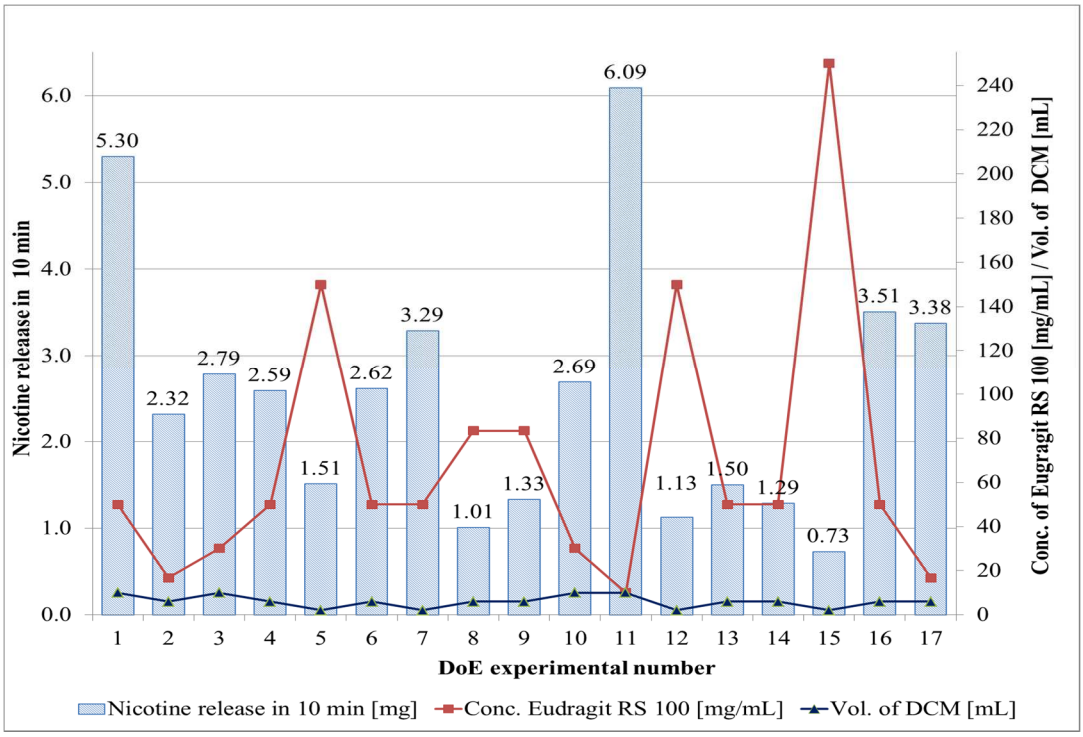
(b)

Figure 5-96. Evaluation of nicotine release from DoE formulations at 5 min time point

a) Surface response plot for 5 min nicotine release from micro-encapsulated DRCs, b) perturbation plot showing the influence of different factors on nicotine release, c) graphical overview of nicotine release and the corresponding amount of factors used in DoE formulations.



(a)



(b)

Figure 5-97. Evaluation of nicotine release from DoE formulations at 10 min time point

a) Surface response plot for 10 min nicotine release from micro-encapsulated DRCs, b) graphical overview of nicotine release and the corresponding amount of factors used in DoE formulations.

The plot was constructed from the natural log transformed 5 min and 10 min drug release data collected from the 17 DoE runs. The surface response plot shows the influence and the interaction of various factors, amount of Eudragit RS100 and the volume of DCM affecting the drug release.

Results and discussion

The results presented in the Figure 5-96 - Figure 5-97 shows the nicotine release from all the DoE formulations at 5 min and 10 min time point. The bars in the graph indicate the amount of nicotine released. The red and green lines indicate the corresponding concentrations of Eudragit RS100 and volume of dichloromethane used. In the DoE design, Eudragit RS 100 concentrations ranging from 10 mg/mL to 250 mg/mL were used. As far as the Eudragit RS 100 concentrations are concerned, it was generally assumed that at higher concentrations, the release rate of nicotine will be controlled. However, this effect was very less when the difference in concentrations is very less. As seen in these figures, the slowest release of nicotine was observed for DoE 15 (Eudragit RS 100 conc.: 250 mg/mL) and the highest release for DoE 11 (Eudragit RS 100 conc.: 10 mg/mL) with an exception of DoE 1 where the nicotine release was unexpected (Eudragit RS 100 conc.: 50 mg/mL). One possible explanation could be that the polymer coating might have ruptured during the drying or drug release testing. Moreover increasing the concentration of Eudragit RS 100 in a DCM shows no linear response in the *in vitro* drug release characteristics. This concludes that a varying combination of polymer to solvent system is necessary to achieve a desired coating property. Similarly, increasing the volume of DCM reduces the thickness of the film formed which results in a faster release rate of nicotine.

Further optimization of the model was found not necessary since the *in vitro* drug release as a response factor may not be a suitable indicator to optimize the coating efficiency. This is due to the fact that the *in vitro* drug release testing itself is influenced by a number of factors like conditions of testing (apparatus parameters, media, temperature etc.). The surface response methodology plot indicates the effect of combination of the factors, Eudragit RS 100 and volume of dichloromethane on the microencapsulation of the DRCs. DoE aids in determining the precise combination of various factors necessary to obtain the desired end product with a minimum number of trials. The prediction of interactions of the various factors within the design space is a primary advantage of DoE. In the present situation, the DoE model is only able to predict the effect of combination of these factors than the influence of individual factors for the optimization of coating method.

5.4.1.12 Results of fourier-transform infra-red analysis

The results of all the FTIR measurements are shown in the Figure 5-98.

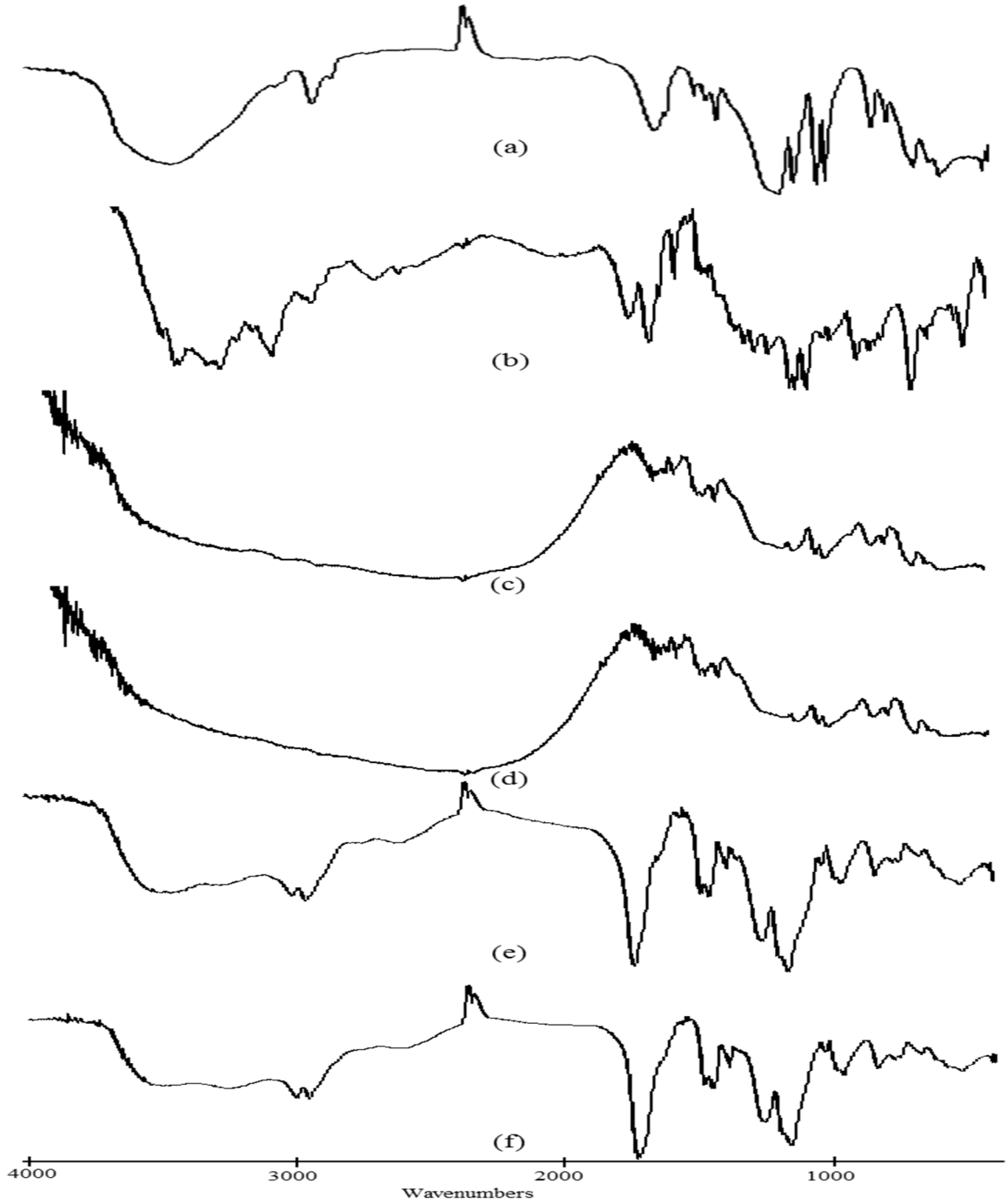


Figure 5-98. FTIR spectra of different components of the coated drug resin complex

a) Amberlite IRP 69 b) Nicotine hydrogen tartrate c) Nicotine- resin complex (DRC) d) DRC-Polyethylene Glycol (PEG) e) microencapsulated DRC-Eudragit RS100 f) physical mixture of DRC-Eudragit RS100 and PEG.

Results and discussion

The FT-IR spectra were obtained for the different components of the microencapsulation system. The primary objective was to determine any possible chemical interaction between the coating materials and the DRC. The results of the FTIR obtained are shown in the Figure 5-98.

For nicotine, a large peak of water at 3319 $cm^{-1}$was observed and the peak at 1658 cm^{-1} indicates aromatic C=N bond stretching and aromatic C=C stretching at 1739 cm^{-1}. The aliphatic part of the molecule is present in the very strong C-H valence vibrations from 3000 to 2800 cm^{-1} of the drug. Moreover, the drug spectrum shows a prominent absorption bands between 2100 and 2600 corresponding to NH+ stretching vibration in the tertiary amine group of the drug disappeared in the resinate due to the formation of new bonds between the resin and the drug. This is further confirmed by the absence of such bands in the physical mixture containing the DRC and polymer. At 681 cm^{-1} and 882 cm^{-1}, C-H plane bending of the mono-substituted pyridine ring is also observed. This band is also observed in the DRC and PEG treated resinates. In the figure shown, it is possible to observe the similarity between the spectra for microencapsulated DRC-Eudragit RS100 (e) and the physical mixture (f) containing DRC. Eudragit RS 100 and PEG in amounts corresponding to the formulation, indicating the absence of interaction between the excipients used. The prominent peaks in the Eudragit RS 100 spectra include the peak at 1155 cm^{-1} (C-CO-C stretching), 1393 cm^{-1} (asymmetric CH_3 bending), 1451 cm^{-1} (symmetric CH_3 bending) and one at 1736 cm^{-1} (C=O stretching). It is also evident from the FTIR spectra that the physical mixture of Eudragit RS100. FTIR spectra of nicotine hydrogen tartrate and Amberlite IRP 69 indicated that there was no appreciable interaction between the drug, resin and the polymer. The results were also consistent with the spectra of physical mixture containing Eudragit RS100. However the intensities of the bands differed considerably reflecting the amounts present in the potassium bromide (KBr) disc.

The results showed that no chemical interaction or changes took place during preparation of the formulations and the drug was found to be stable. The results presented for nicotine conforms to the data presented by Bobiak, *et al.* 2005 and also to data found in the database (http://www.chm.bris.ac.uk/motm/nicotine/E-propriete.html).

5.4.2 Directly compressed chewing gum formulations

Following the optimization of DRC coating using the DoE, the chewing gums were manufactured according to the method described in section 4.15.

5.4.2.1 Formulation of chewing gums with different gumbases

The results of various analyses obtained for various gum formulations are presented in the following sections.

Table 5-30. Composition of nicotine gum formulations using different gumbases

Components	Formulation				
	FG1	FG2	FG3	FG4	FG5
Weight of DRC [g]	0.58	0.58	0.58	0.58	0.58
Pharmagum M [g]	85.43	-	-	-	-
PG Nutra TA [g]	-	85.43	-	-	-
PG Nutra PEPP TA [g]	-	-	85.43	-	-
All in one gum SF Extra [g]	-	-	-	85.43	
All in one gum SF cool [g]	-	-	-	-	85.43
Sorbitol [g]	10.00	10.00	10.00	10.00	10.00
Magnesium stearate [g]	2.00	2.00	2.00	2.00	2.00
Talc [g]	2.00	2.00	2.00	2.00	2.00

5.4.2.2 Content uniformity testing

The content uniformity testing was performed using the single step liquid-liquid (LLE) extraction assay method described earlier in the section 4.8.1 for marketed products. The results of the analyses for the 5 different formulations manufactured using different gumbases are shown in the Figure 5-99.

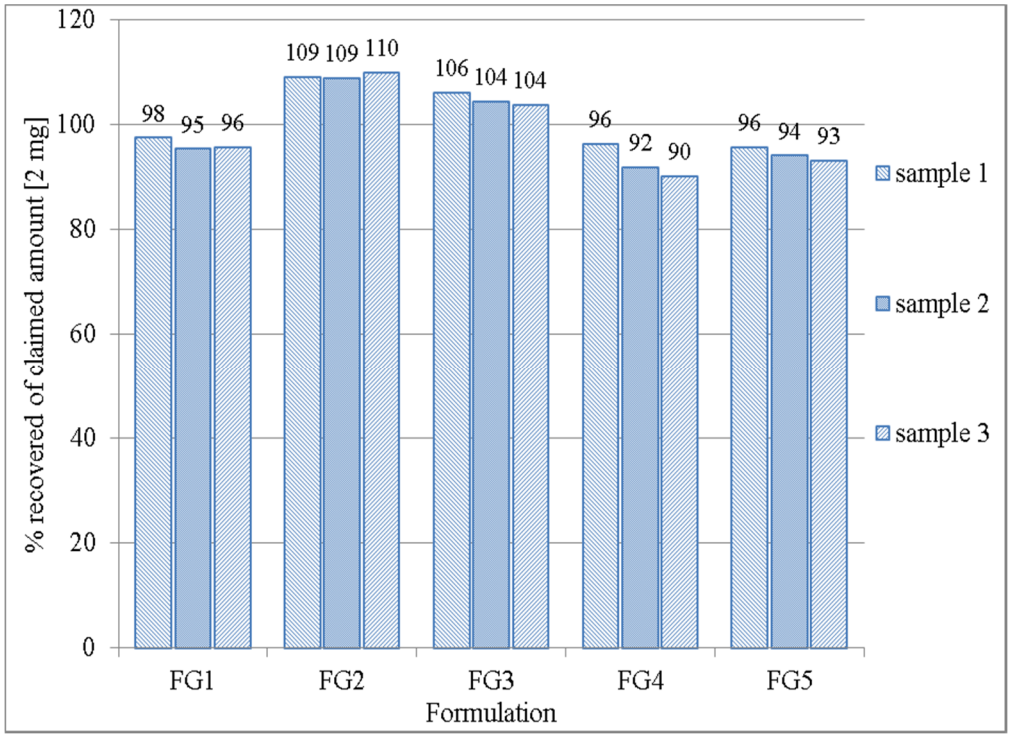


Figure 5-99. Content uniformity testing of different gumbase formulations

Data represents % nicotine recovered from individual pieces of gums. The amount of nicotine recovered from the gum formulations was found to be acceptable for the initial phase.

The results of the analyses are not conclusive to select a formulation for further optimization.

Inhomogeneity of the formulation samples can be directly inferred from the results of the content uniformity testing. Mixing and blending may have an impact on the homogeneity of the samples. Besides the organoleptic and physical characteristics of the gum products helps to choose an acceptable formulation. Additionally, the pre-formulated gum base samples other than the FG1 contained particles of wide range and mixing for a short time prior to compression results in a non-homogenous mixture. Therefore the blending and mixing time was increased from 5 min to 10 min. However the decision of selecting one formulation for further optimization was based on their *in vitro* release characteristics.

The physical properties of the gum components were further evaluated to determine their suitability. One of the primary tools in identifying the optimal product characteristics is the performance testing using the apparatus setup (apparatus B) that was found to be biorelevant.

5.4.2.3 Evaluation of *in vitro* drug release from different gumbase formulations

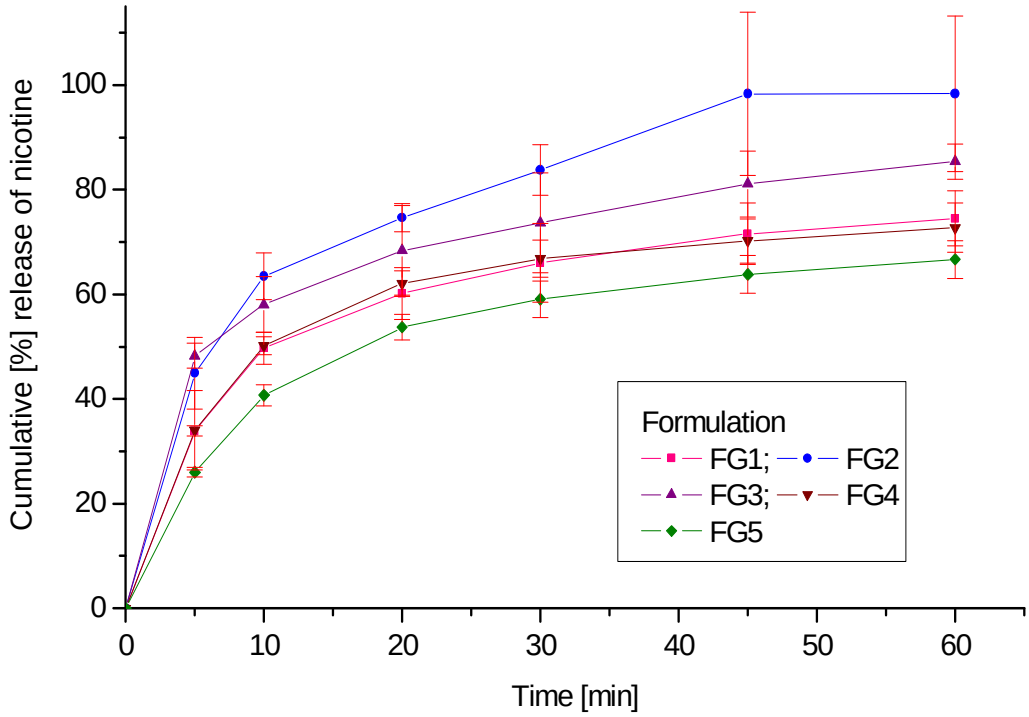


Figure 5-100. Cumulative average release profiles of nicotine formulations from different gumbases

Data represents n=3± SD for each formulation. Test conditions include apparatus B setup with a chewing distance of 1.8mm, 20° twisting angle, 40 chew/ min, 40 mL of artificial saliva pH 6.2 maintained at 37 °C.

From the results presented in Figure 5-100**,** it can be observed that the nicotine release of FG2 formulation was faster than rest of the formulations. The rate of release is in the following order; FG2 > FG3 >FG4 > FG1 > FG5. The variability observed (shown in red bars) within a drug release profile is highest for the formulation FG2. Incomplete release within the tested time was observed for all the formulations except FG2. This effect is related to the composition of the directly compressible (DC) gumbases itself, since the composition of formulations is identical except the DC gumbases. As these pre-formulated gumbases were obtained from different manufacturers for the purpose of testing, the ratio of gum matrix to the other excipients present in the DC gumbases may be different. This leads to a difference in the release of nicotine from the formulations. Among the tested formulations, FG5 was found to be acceptable. In other words, nicotine release from the FG5 is comparable to that of the marketed nicotine products under similar tested conditions. Therefore it was decided to select FG5 for further optimization.

5.4.2.4 Evaluation of release kinetics from different gumbases

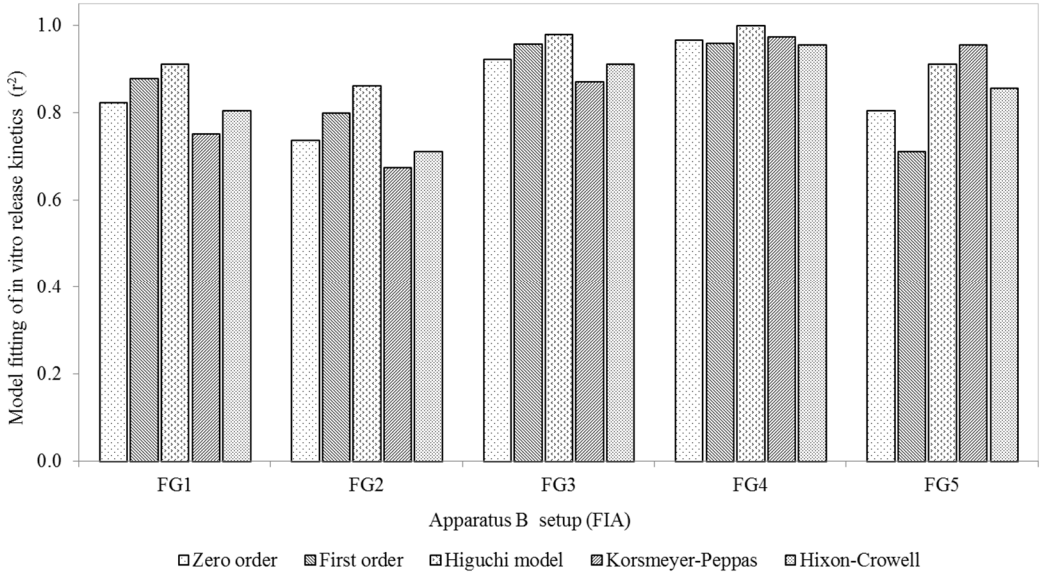


Figure 5-101. Evaluation of release kinetics of formulations based on different gumbases

Release kinetic data derived from the in vitro release data of 3 replicates. The best model fit was obtained for the Higuchi model and the Hixon Crowell model which explains the diffusion controlled release from the matrix and as well as the DRC's. The mechanism of release is further explained from the Korsmeyer model and the individual values are given in the table 14 of the Appendix

5.4.2.5 Optimization of formulation FG5

The basis of initial formulation design with various gumbases is to determine the suitability of the gumbases for tabletting. The formulation design was kept minimal (F1 to F6) to see the influence of gum bases over release. The tableting force was not monitored during the manufacturing of chewing gums. For the chewing gum formulations manufactured, the force required by the chewing apparatus to masticate the gum was found to be higher (about 7-8 bar of compressed air) than the conventional marketed chewing gum products. Problems were also encountered in the flow of pre-formulated mixture and the compaction resulted in partial chipping and capping of tablet gum formulations.

From the results presented, the formulation FG5 was found to be acceptable with the release behavior and was chosen for further optimization. The following FG5 formulation using a single commercially available gumbase (DC) was chosen to determine the influence of other excipients on the release behavior.

5.4.2.6 Formulation of DC gums using All in One (AIO) SF cool gumbase

The results of the analyses to determine the influence of excipients on release are presented in the following sections.

Table 5-31. Optimized and final composition of nicotine DC gums

Components	Formulation (FG5)					
	F1	F2	F3	F4	F5	F6
Weight of DRC [mg]	603.8	604.74	603.57	603.71	605.1	-
Theoret. amt. of Nicotine / 1.5 g of gum [mg]	2.002	2.005	2.001	2.001	2.006	2.003
Nicotine [g]	-	-	-	-	-	0.38
Gumbase [g] (AIO SF cool)	85.43	85.43	85.43	85.43	85.43	85.43
Magnesium Stearate [g]	2		2	2	2	2
Talc [g]	2		2	2	2	2
Vinylec K [g]	1.5	1	1	1.5	1.5	1.5
Microcrystalline Cellulose [g]	4	-	-	4	-	4
Polyethylene Glycol 6000 [g]	1.5	1.5	1	1	1.5	1
Sorbitol [g]	3	-	-	-	3	-
Prosolv [g]	-	12	8	4	4	4
Final weight [g]	100.0	100.5	100.0	100.5	100.0	100.3

After the initial testing with all the gumbases, formulations with the corresponding excipients listed in the table were manufactured on a small scale. The compression force on the tablet machine was adjusted manually until the tablets produced were homogenous without any defects. The weight of individual tablets was adjusted to 1.5 g/piece to contain 2 mg of active nicotine. The results of the physical evaluation of the formulations are provided in the following sections.

5.4.2.7 Physical evaluation of the gum formulations

The following physical evaluations were based on the methods described in the USP and the Ph.Eur.

The following section contains the summary of the evaluations undertaken to evaluate the suitability of the pre-formulation mixture for the tabletting purpose.

Table 5-32. Summary of compressibility of formulation mixture for formulation FG5

Formulation	Avg. [n=3]. bulk density [g/L]	Avg. [n=3]. tapped density [g/L]	Carr's Index	Hausner ratio
F1	0.71	0.79	10.29	1.11
F2	0.75	0.77	2.99	1.03
F3	0.68	0.77	11.27	1.13
F4	0.72	0.80	8.96	1.10
F5	0.72	0.78	7.46	1.08
F6	0.73	0.80	8.96	1.10

Table 5-33. Results on the evaluation of flow properties of gum formulation mixture

Index	Evaluation parameters to determine the angle of repose of different formulation mixtures									
	F1 [α]	Flow [s]	F2 [α]	Flow [s]	F3 [α]	Flow [s]	F4 [α]	Flow [s]	F5 [α]	Flow [s]
Run 1	30.6	12.3	30.6	11.7	31.1	11.4	30.3	12	29.3	11.41
Run 2	30.7	12.4	31.4	11.6	29.9	11.4	30.2	11.6	29.6	10.9
Run 3	30.1	11.33	30.1	12	30.3	11.4	31.8	12.5	30.1	11.2
Mean	30.47	12.01	30.70	11.77	30.43	11.40	30.77	12.03	29.67	11.17
SD	0.32	0.59	0.66	0.21	0.61	0.00	0.90	0.45	0.40	0.26
RSD [%]	1.06	4.92	2.14	1.77	2.01	0.00	2.91	3.75	1.36	2.29

Table 5-34. Summary of friability of the chewing gum formulations (n=5) at 25 rpm for 10 min

Formulation	Weight [g] before test	Weight [g] after test	Loss [%]
F1	7.417	7.391	0.35
F2	7.277	7.246	0.43
F3	7.472	7.431	0.55
F4	7.111	7.009	1.43
F5	7.285	7.242	0.59

Table 5-35. Summary of data representing uniformity of mass and crushing strength

Formulation	Avg. wt. [g] of gums (n=10 units)	Std. dev.	Crushing strength (N)	Std. dev.
F1	1.49	0.01	50.70	2.36
F2	1.47	0.01	40.20	3.29
F3	1.51	0.01	52.50	2.88
F4	1.47	0.01	55.50	4.06
F5	1.48	0.00	50.00	2.36
F6	1.49	0.01	48.10	3.48

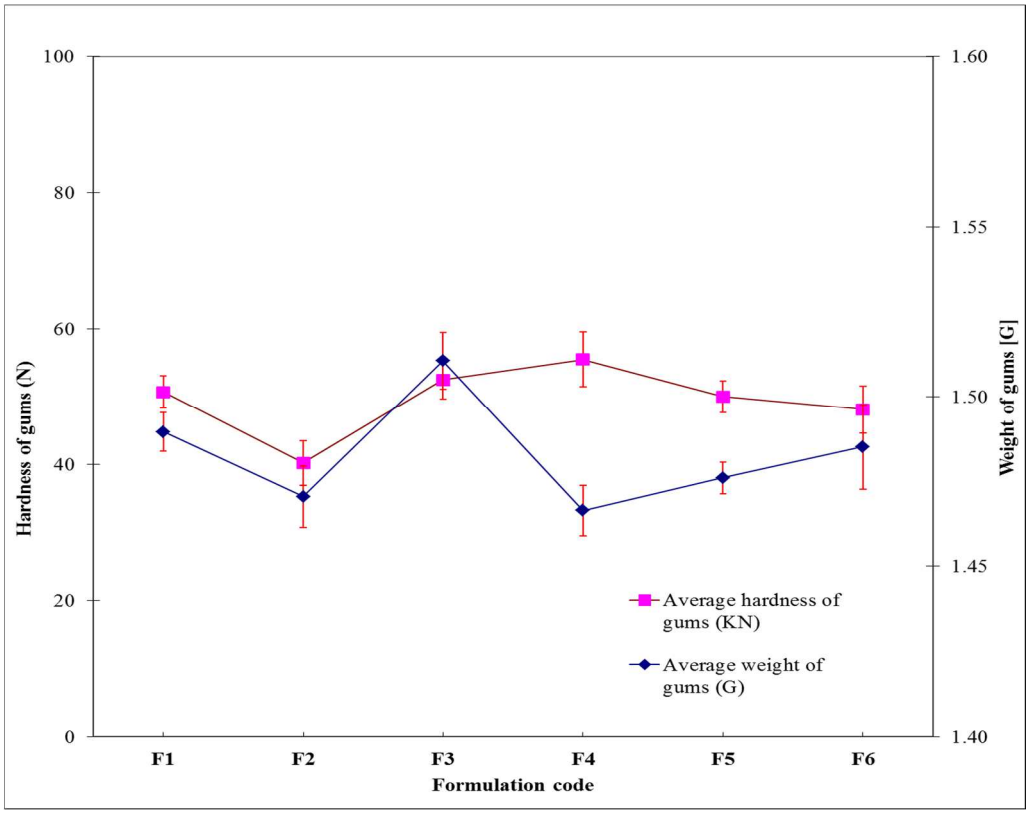


Figure 5-102. Evaluation of weight variation and crushing strength of gums

Plot shows the average (n=10) weight of different gum formulations manufactured using the direct compression technique.

Table 5-36. Summary of parameters for porosity determination

Parameter	Formulation					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Vol. of compact	2.89	3.18	2.94	2.79	2.90	2.89
Apparent density (p_a)	0.5151	0.4621	0.5133	0.5265	0.5095	0.5145
True density (p_T)	0.79	0.77	0.77	0.80	0.78	0.80
Porosity (ε)	35.19	40.03	33.32	33.78	34.72	35.44

Table 5-37. Summary of content uniformity testing results for different FG5 formulations

Parameter	Formulations				
	F1	**F2**	**F3**	**F4**	**F5**
Percent [%] recovered of claimed amount (n=3) ± SD	96.25 ± 1.16	109.42 ± 0.54	104.77 ± 1.22	92.88 ± 3.17	94.38 ± 1.36

5.4.2.7.1 Evaluation of drug release characteristics

The *in vitro* drug release testing was performed using the Ph. Eur. apparatus B using the test conditions described earlier for the marketed nicotine gum products. Cumulative drug release profiles are shown in the Figure 5-103.

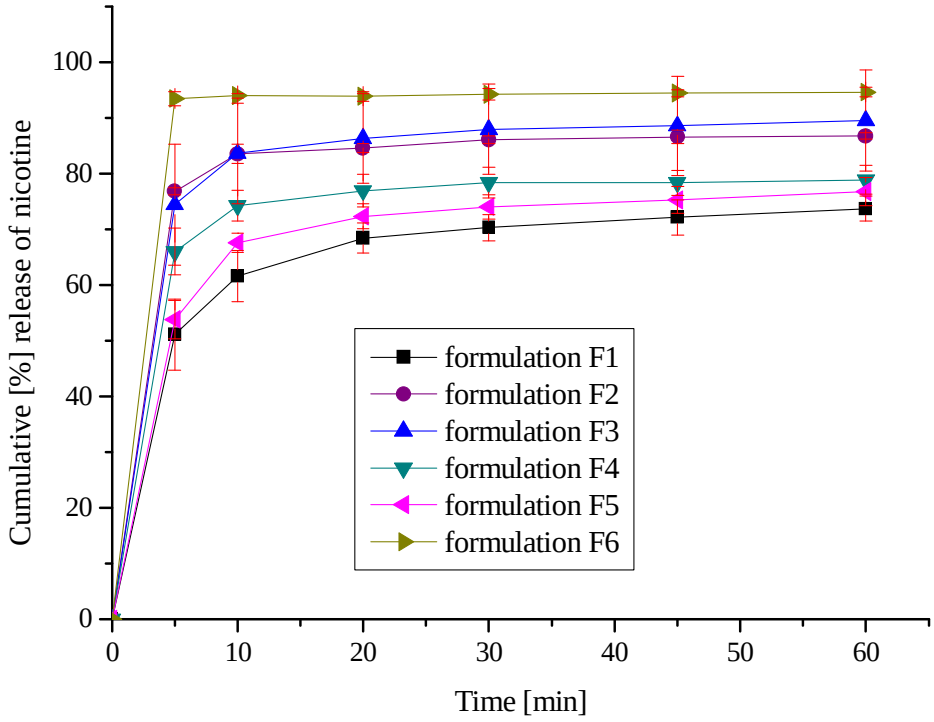


Figure 5-103. Average cumulative *in vitro* release profiles of nicotine from 6 different gum formulations

Data represent n=3 ± SD runs for each formulation. All the formulations as shown in the table contain same type and amount of gumbase. Formulation F6 contains Nicotine hydrogen tartrate instead of DRC. Apparatus setup include chewing distance 1.8 mm. twisting angle 20° and chewing frequency 40 chew/min.

The *in vitro* drug release data from all the formulations shows a difference in their release behavior particularly during the initial phase of mastication. The formulations F1 to F5 contain same amount of gumbase and differ quantitatively with the composition and presence of other excipients. The formulation F6 contains nicotine hydrogen tartrate as an API instead of DRC. A maximum difference in release between the profiles was observed only during the initial phase. The F5 formulation indicates comparatively faster release than F1 formulation. The composition of these formulations differ only the presence of Prosolv in F5. This concludes that the presence of Prosolv which acts as a super-disintegrant increases the disintegration of gum particles during the initial mastication and hence the release rate of nicotine. This effect is also evident among the formulations F2, F3 and F4, where the amount of Prosolv present in the composition is different. Formulations F2 and F3 exhibit a faster release profiles than F4. Additionally, the presence of sorbitol in the formulations F1 and F5 acts as a softening agent. After the initial mastication, the DRCs are well distributed within the gum matrix resulting in a less burst release at the start of mastication.

5.4.2.7.2 Evaluation of release kinetics

The kinetics of *in vitro* release from the gum formulations were evaluated using the models described in section 4.16.3. The *in vitro* dug release data of the formulations F1 to F5 were fit to different kinetic models and the results of the analysis are presented in the Figure 5-104 and the data are given in Table 17 of the appendix.

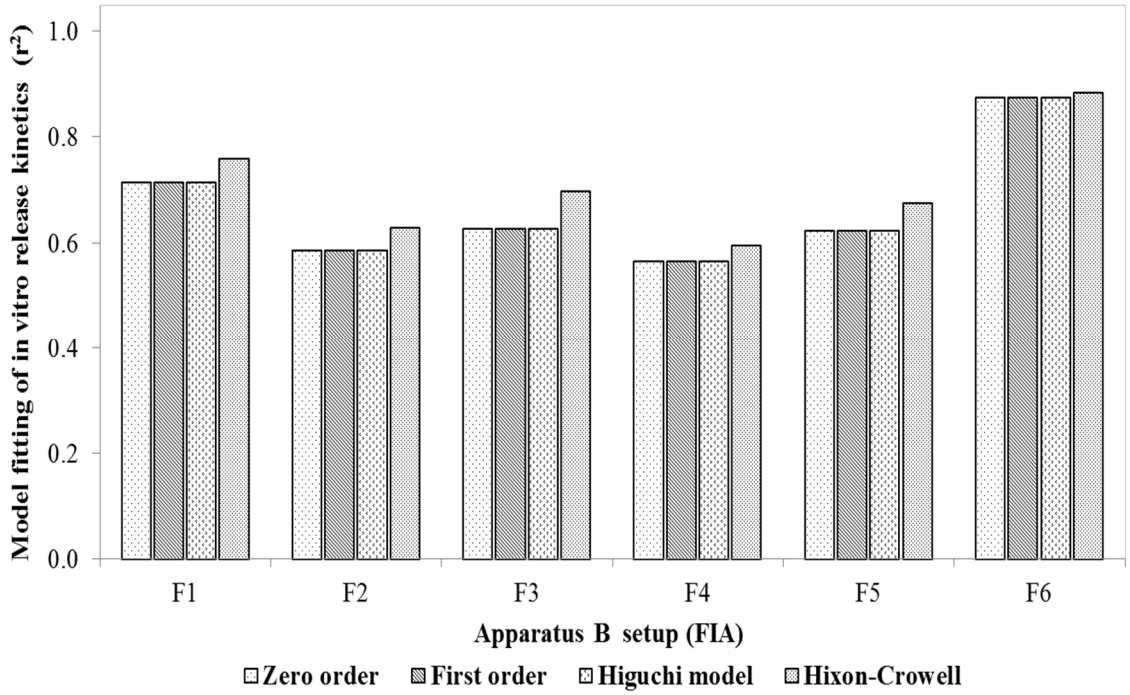


Figure 5-104. Evaluation of release kinetics of different formulations based on gumbase FG5

Release kinetic data derived from the in vitro release data of 3 replicates. The best model fit couldn't be obtained for any specific gum formulation since the rate of nicotine release after first 5min of mastication is similar for all the formulations. Korsmeyer-Peppas model failed to explain the release characteristics as the release at the second time point is more than 60% for the tested formulations.

Following the drug release results presented for the gum formulations F1-F5, it was concluded that the release of nicotine is additionally influenced by the excipients present in the formulation. Evaluations including the tablet compaction, porosity, weight variation, hardness and flow-properties were found to be acceptable. Among all the formulations investigated, formulation F5 was chosen since the Carr's index and Hausner ratio of 7.46 and 1.08 indicates a good flowability and compressibility of the formulation mixture. However during mixing and blending, content uniformity of this formulation close to 100 % could not be reached.

One possible explanation could be the mixing time of the excipients. In order to improve the content uniformity, the mixing time was increased from 5 min to 10 min before the addition of lubricants like magnesium stearate and talc. The content uniformity test was repeated for the formulation F5 to evaluate the influence of blending time.

Table 5-38. Summary of content uniformity data for formulation F5

Index	Average wt. 10 gums [g] ± SD	Average (n=10) recovery [%] ± SD
Formulation F5	1.502 ± 0.01	106 ± 3.67

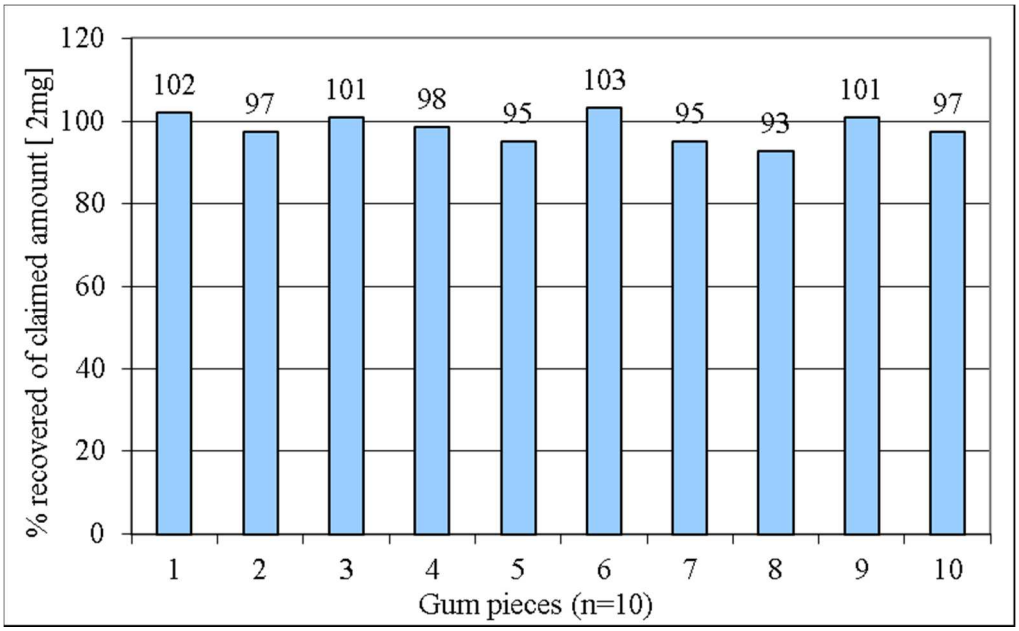


Figure 5-105. Content uniformity analysis of final formulation F5

Individual pieces of gums were weighed and assayed for the nicotine content to evaluate the suitability of the manufacturing process. The USP acceptance criterial for L1 is met for the content uniformity of dosage units.

5.4.2.7.3 Evaluation of water uptake

The amount of water absorbed and retained within the gum formulation F5 was evaluated by soaking the gum formulations in water contained in a glass petri-dish. The amount of water absorbed is determined by the weight gain of the formulation within the specified time period. The experiment was performed in triplicate and the individual results of the analysis are shown in the Figure 5-106.

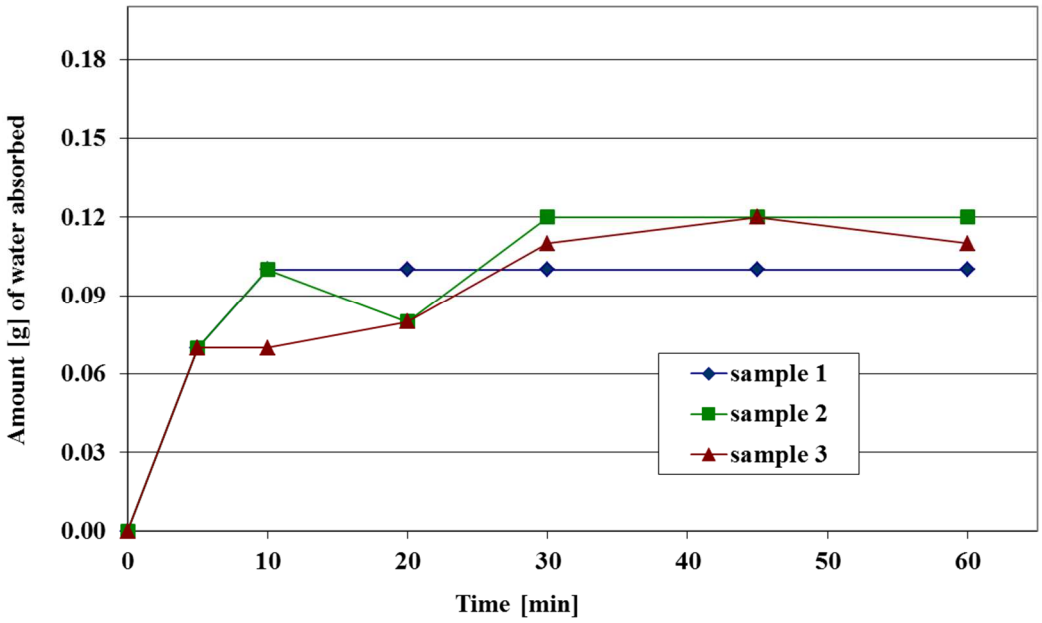


Figure 5-106. Evaluation of water uptake for formulation F5

Amount of water absorbed by the individual pieces of gum formulation F5 are shown in the graph. The rate of intake is fast and rapid for the entire sample at the beginning of testing and remains constant from 30 min to 60 min with a total amount of about 0.11 g. No further absorption occurs in this time. The values indicate the acceptable wetting properties of the dosage form which is essential for any dissolution/drug release to occur.

5.4.2.7.4 Evaluation of drug release characteristics

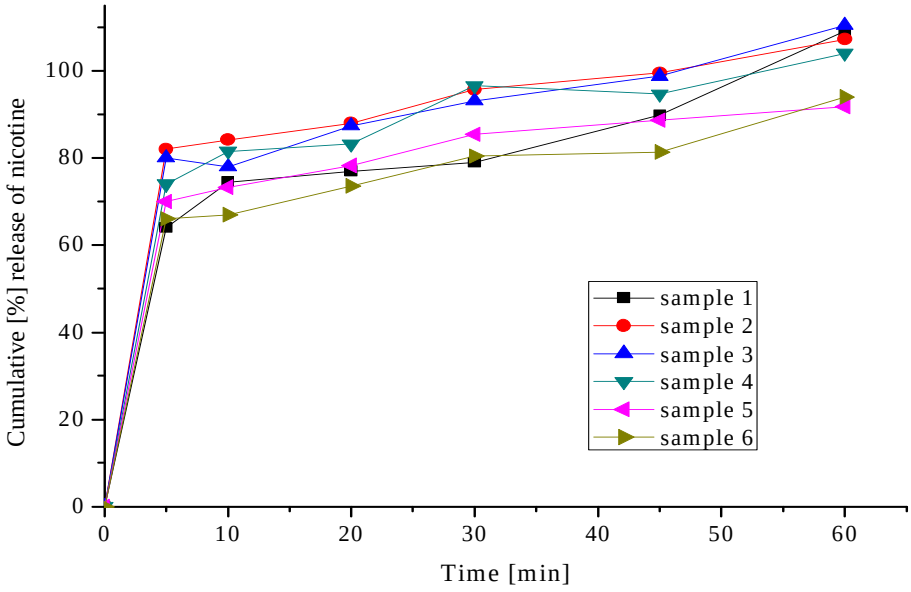


Figure 5-107. Individual release profiles of nicotine from the formulation F5

Drug release data is generated from Ph. Eur. apparatus B using following setup. Chewing distance 1.4 mm, twisting angle 20° and chewing frequency 40 strokes/min, 40 mL of artificial saliva pH 6.2 maintained at 37° C.

It can be inferred from the *in vitro* release results presented in Figure 5-107 that nicotine release was complete within the time period tested. The drug release rate was rapid at the start of the testing reaching above 60% within 5 min and controlled thereafter.

Since the IVIVC was established for the marketed nicotine products shown in the previous section "*in vitro in vivo* correlation", the biorelevant test conditions of the apparatus B for the marketed nicotine products were found to be at 1.8 mm chewing distance with a 20° twisting angle and 40 strokes/min chewing frequency. In order to evaluate the performance and influence of chewing distance on drug release, data from the two different apparatus setup were compared and shown in the Figure 5-108.

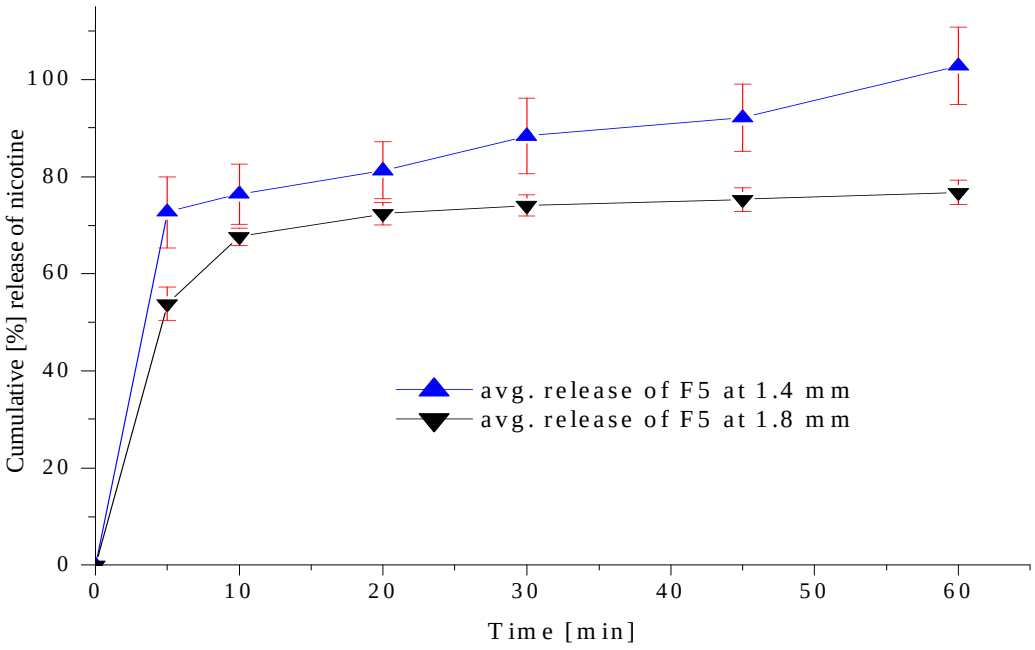


Figure 5-108. Average release profiles of nicotine release from formulation F5 with different apparatus setup

Data is generated from Ph. Eur. apparatus B using following setup; Chewing distance 1.4 mm and 1.8, twisting angle 20° and chewing frequency 40 chews/.min, 40 mL of artificial saliva pH 6.2 maintained at 37° C.

As shown in Figure 5-108, the difference is drug release was observed at the initial phase of testing and the nicotine release was complete within the time period tested (60 min) at 1.4 mm chewing distance. At 1.8 mm chewing distance, the profiles reached an asymptote at 20 min. No further release of nicotine from the formulation was observed. This is related to the absence of elasticity of the gum formulations after a certain period of mastication. As the excipients dissolve with time, the mass of gum matrix is significantly reduced. This results in a gum thickness smaller than the chewing distance resulting in minimal or no kneading effect on the gums. With this method, it was possible to demonstrate the sensitivity of the gum to discriminate the apparatus setup, namely chewing distance.

5.4.2.7.5 Evaluation of influence of binders and hardness on release

The suitable coating of the DRC complex identified using the design of experiments (DoE) was used as an active and incorporated into the gum base F5 formulation. With all the developed formulations, crumbling was always observed during the initial mastication of the gums. In order to keep the gum intact, poly-vinyl-pyrrolidine (PVP K30) at about 2.5% w/w and 5% w/w was used. The mass of the PVP was compensated from the mass of the gumbase powder used. The PVP was added to the gumbase mixture shortly before the addition of lubricants such as magnesium stearate and talc. The flow properties of the formulation were additionally tested to ensure free flow of the mixture in the hopper feed of the tabletting machine. The hardness of the gumbase formulation was adjusted around 20 N and 30 N for both the formulations respectively. The composition of the formulation is given in the Table 5-39.

Table 5-39. Composition of optimized formulation containing coated DRC

Components	**Formulation (F5) with coated DRC**		
	F5_coated DRC	**F5a _coated DRC**	**F5b_coated DRC**
Coated DRC [g]	0.8	0.8	0.8
Gumbase (AIO SF cool) [g]	85.2	82.7	80.2
Mg stearate [g]	2.0	2.0	2.0
Talc [g]	2.0	2.0	2.0
Vinylec K [g]	1.5	1.5	1.5
PEG 6000 (plasticity) [g]	1.5	1.5	1.5
Sorbitol [g]	3.0	3.0	3.0
Prosolv [g]	4.0	4.0	4.0
PVP K30 [g]	-	2.5	5.0
Final weight (% w/w)	100.0	100.0	100.0

Theoretical weight of nicotine in the coated DRC is adjusted to 2 mg/1.5 g of compressed gum piece.

5.4.2.8 Physical evaluation of the DoE optimized formulation

The flow properties of the formulations containing PVP K30 as a binding agent were evaluated using the method described earlier for other formulations. Since the physical composition of the formulation F5_coated DRC is same as that of the formulation F5 except that it contains coated DRC, the flow property was not evaluated.

Table 5-40. Summary of flow properties of optimized DoE formulations containing PVP K30

Formulation	**Avg. [n=3]. Bulk density [G/L]**	**Avg. [n=3]. Tapped density [G/L]**	**Carr's Index [C.I]**	**Hausner ratio**
F5a _coated DRC	0.71	0.78	9.68	1.11
F5b_coated DRC	0.74	0.78	5.00	1.05

Table 5-41. Summary of data representing uniformity of mass and crushing strength of optimized formulation

Formulation [adjusted crushing strength]	Avg. wt. of gums (n=10)	Std. dev.	Crushing strength (N)	Std. dev.
F5a coated DRC [20N]	1.507	0.0023	23.9	0.7379
F5a coated DRC [30N]	1.5058	0.0090	34.6	2.5906
F5b coated DRC [20N]	1.4838	0.0090	22.1	1.3703
F5b coated DRC [30N]	1.5027	0.0110	36.9	0.8756
F5 DoE optimized	1.5002	0.0052	40.7	2.6268

Table 5-42. Summary of friability of the chewing gum formulations

Formulation	Weight [g] before test	Weight [g] after test	Loss [%]
F5a _coated DRC [30N]	7.520	7.502	0.24
F5b_coated DRC [30N]	7.512	7.498	0.19

Data represents n=5 units tested at 25 rpm for 10 min

It was found that the chewing gum formulations manufactured with a crushing strength of 20 N failed the friability test. The gums were disintegrated during the test. Therefore, the chewing gum formulations manufactured with an average crushing strength of 30 N was only reported in Table 5-42.

Table 5-43. Summary of porosity determination for optimized formulation

Parameter	Formulation			
	F5a coated DRC [20N]	F5b coated DRC [20N]	F5a coated DRC [30N]	F5b coated DRC [30N]
Vol. of compact [mL]	3.30	3.25	3.12	3.12
Apparent density [$p\alpha$]	0.46	0.46	0.48	0.48
True density [pT]	0.78	0.78	0.78	0.78
Porosity [$\mathcal{E}$]	41.80	41.61	38.46	38.29

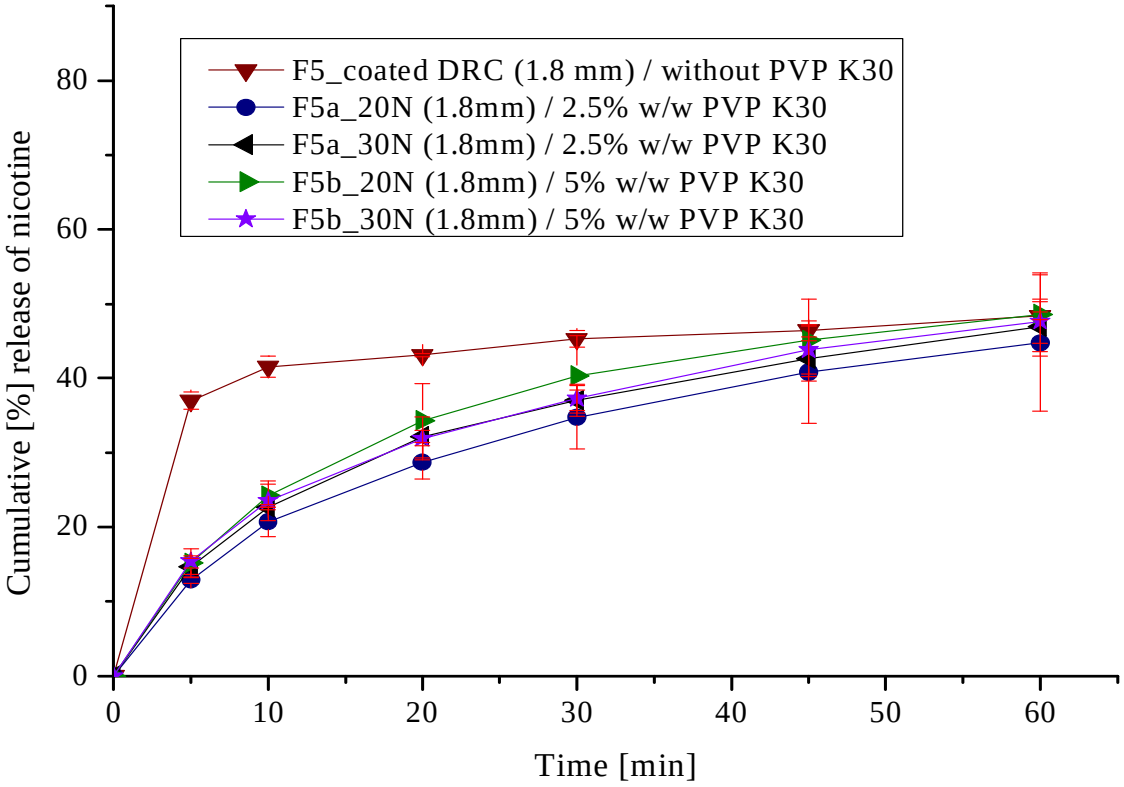


Figure 5-109. Average *in vitro* release profiles of nicotine from different gum formulations

Graph represents average (n=3 ± SD) in vitro nicotine release. Data is generated using Ph. Eur. apparatus B using following apparatus setup; chewing distance of 1.8 m, 40 mL of artificial saliva pH 6.2 maintained at 37 °C. The twisting angle of 20° and chewing frequency of 40 strokes/min is common to all the release testing experiments.

The *in vitro* nicotine release profiles were generated using the apparatus setup which was earlier identified as a biorelevant for the marketed nicotine gum products. As shown in Figure 5-109, the nicotine release from the formulation F5 coated DRC without PVP was rapid at the beginning of testing and the release tend to be slower for the formulations compressed with different amounts of PVP K30 and crushing strength. As a summary, it can be concluded that the amount of PVP K30 and crushing strength has no observable difference in release behavior. However, the overall influence of the presence of PVP K30 to the other formulations without the PVP K30 can be directly observed from the profile trend. The PVP as a binding agent limits the disintegration of the gum particles at the start of mastication, which results in controlled exposure of surface for further dissolution / drug release.

5.4.2.8.1 Evaluation of DoE optimized final formulation

In order to evaluate the suitability of the gum to discriminate the apparatus parameters, performance testing was carried out using the different chewing distances of apparatus B. The effect of chewing distance on release was evaluated. The results of the analysis are presented in the Figure 5-110.

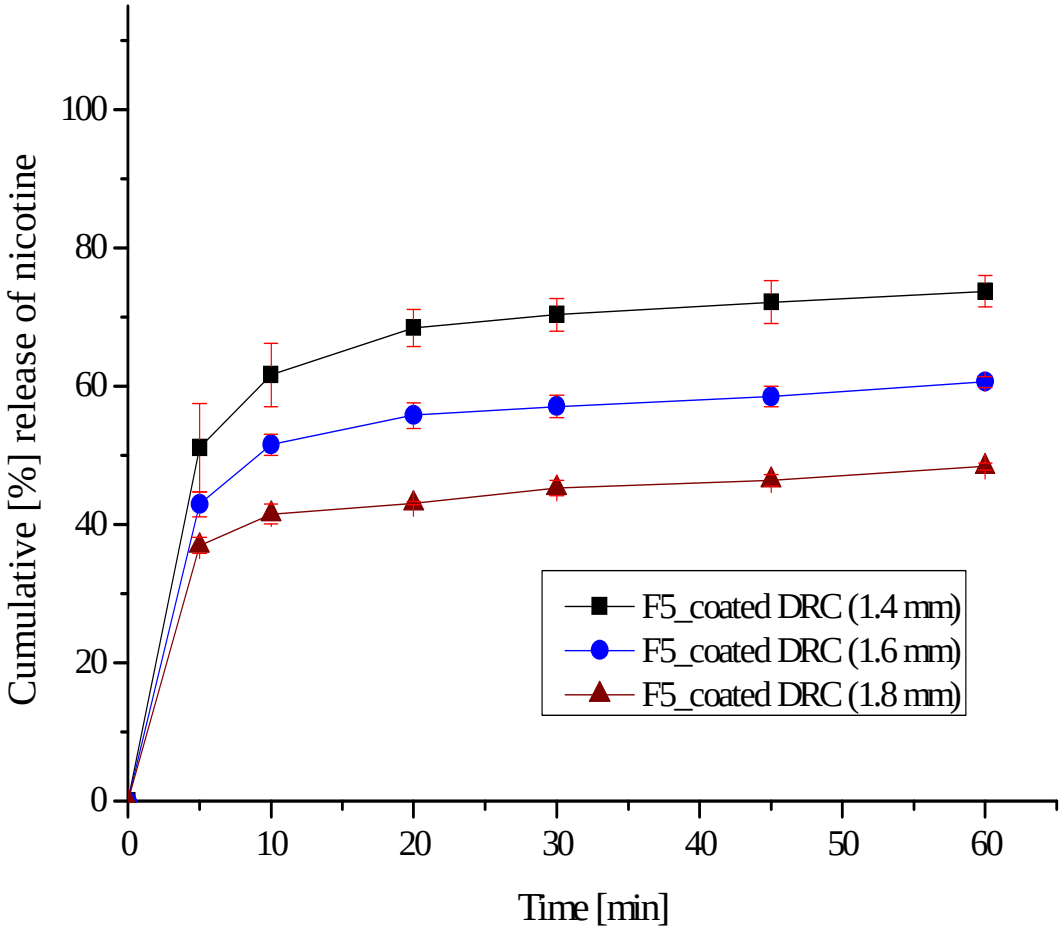


Figure 5-110. Average *in vitro* drug release profiles of nicotine at different apparatus setup

Graph represents average (n=3 ± SD) in vitro nicotine release. Data is generated using Ph. Eur. apparatus B at variable chewing distance. 1.4 mm vs. 1.6 mm vs. 1.8 mm using 40 mL of artificial saliva pH 6.2 maintained at 37 °C. The twisting angle of 20°and chewing frequency of 40 strokes/min is common to all the release testing experiments.

From the results shown in Figure 5-110, it can be clearly observed that the chewing distance affected the release of nicotine from chewing gum formulations. The drug release rate clearly follows the rank order which is in accordance with the previous results obtained with the marketed chewing gum products. The difference in release behavior observed during the first 20 min was carried until the end of the drug release period. The asymptote of the drug release profile can be related to the absence of mastication due to the thickness of the gum which in smaller than the distance between the chewing jaws. Additionally the loss of elasticity of the gum contributes to this release trend. In this way, it can be summarized that the change in chewing distance affected the nicotine release from the formulation..

5.4.2.8.2 Evaluation of release kinetics for DoE optimized formulation

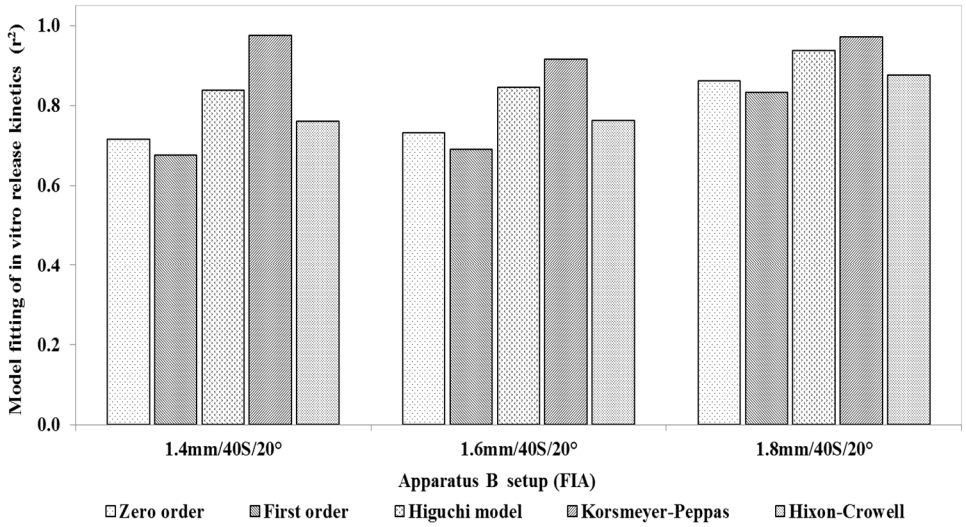


Figure 5-111. Evaluation of release kinetics of nicotine from DoE optimized formulation using variable chewing distance of Ph.Eur. apparatus B

Release kinetic data derived from the in vitro release data of 3 replicates. The best model fit was observed for Higuchi model and Hixon-Crowell cube root model. The mechanism of release is cannot be explained by the Korsmeyer-Peppas model as the in vitro release is rapid and reaches more than 60% within 10 min of testing.

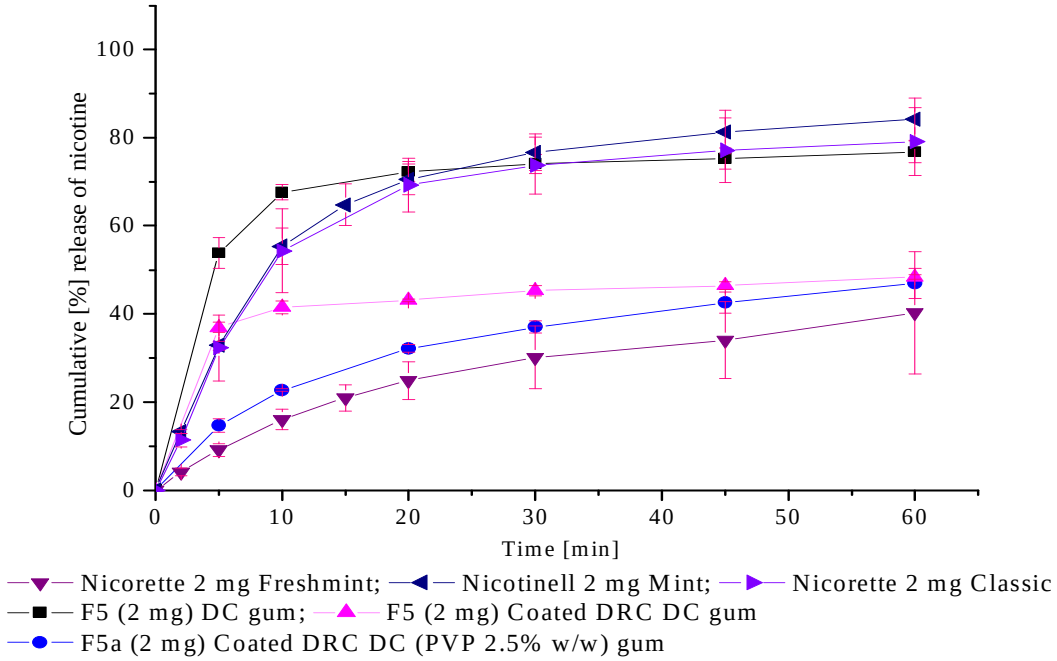


Figure 5-112. Average *in vitro* nicotine release profiles of marketed vs. directly compressed formulations

Graph represents average (n=3 ± SD) in vitro nicotine release. Data is generated using Ph. Eur. apparatus B using the following setup. Chewing distance: 1.8 mm. twisting angle: 20°, chewing frequency: 40 strokes/min. All the investigations were performed in 40 mL of artificial saliva pH 6.2 maintained at 37 °C.

Results and discussion

The *in vitro* drug release profiles of all the nicotine based chewing gum products are plotted in the Figure 5-112. For the purpose of comparison, data from similar apparatus setup were also shown. As discussed earlier the marketed chewing gum formulations are manufactured using the conventional technique which involves melting, blending and extruding into gum pieces. The manufacturing technique is cumbersome and requires special apparatus for the manufacture. On the other side, various formulations of the DC are manufactured using a pre-tailored gum base using a direct tabletting technique. The method of manufacturing is similar to those conventional tabletting methods. Like any conventional dosage forms the product quality test may be employed to test the biopharmaceutical quality. The drug release from the dosage form can be optimized as per the customer needs. From the results it is clearly shown the possibilities to alter/modify the drug release using various formulation parameters. The inter-individual variability within the release profiles is comparatively lower than that of the marketed product using the apparatus tested. The gums can be successfully employed as a platform or a carrier to deliver drugs within the oral cavity at a rate acceptable with respect to the therapeutic efficacy while maintaining higher patient compliance.

6 GENERAL DISCUSSION

6.1 Product performance testing

Medicated chewing gums provide a recent platform for the delivery of active pharmaceutical ingredients. Oral trans-mucosal drug delivery has gained a tremendous importance and has paved the way for the development of various dosage forms and provided room for extensive research for the pharmaceutical scientists. As a potential pharmaceutical dosage form, the key aspects of quality control were limited to the manufacturing sector and the importance of performance evaluation was not given utmost importance until the adoption of a suitable chewing gum apparatus by the Ph. Eur. This stipulated the need to develop techniques to determine the actual release of actives from the gum product which may prove its usefulness in the dosage form development and adopt a method for quality control purposes.

Earlier, several investigators have worked on the development of chewing apparatuses that are able to mimic the *in vivo* oral physiology. The primary objective is to simulate the masticatory action where a real-time chewing of gum product in the oral cavity could be established. Initially, the apparatus A was adopted by the Ph. Eur. in the year 2000 which is not commercially available worldwide and the data generated were restricted to the chewing gum manufacturers. One of the primary concerns was to get hands-on experience on the instrument to evaluate its suitability in the dosage form development.

Standardization and commercialization was another aspect that lacked in the instrument. The development of apparatus B and its commercial availability with the ease of operation even well before it was adopted in the Ph. Eur. revealed its acceptance worldwide. Following its acceptance in the Ph. Eur., the geometries of the apparatus were standardized. The description and functionality of both the apparatuses have been described in the earlier sections of this thesis.

This dissertation was focused on the quality control aspects (performance testing, assay, and partial validation of analytical methodology) of the medicated chewing gums. At the beginning of this project, not many commercially available chewing gums were available in the market. They were limited to the nicotine based chewing gums from different manufacturers, and dimenhydrinate containing chewing gum was used primarily to treat travel sickness. The published data both *in vitro* and *in vivo* were limited and or otherwise not accessible.

At this juncture, goals of the dissertation were set to develop suitable *in vitro* drug release testing methodology to evaluate the performance of gum products and to evaluate accessible and available chewing gum apparatuses. Prior to this, the possibilities of employing a

compendial dissolution testing apparatus described in the USP and Ph. Eur. to evaluate the performance of medicated chewing gums were considered. Tests conducted in the USP paddle apparatus with sinkers revealed that mastication is an important step for release of drug substances from the dosage form. The *in vitro* drug release method was then developed for nicotine based chewing gum from two different manufacturers. Nicorette is an originator product having marketing authorization in Germany and a generic product Nicotinell were chosen for the comparative study. Various parameters of the apparatus were tested using the two products. The results of nicotine based chewing gum data were later verified using the dimenhydrinate and caffeine based chewing gums. The results conform to the earlier findings with the nicotine chewing gums.

Both the apparatuses are capable of demonstrating sufficient masticatory action to affect the drug release. The apparatus parameters namely the chewing distance, chewing frequency and twisting angle (apparatus B) individually or in combination affected the release rate of the actives present in the dosage form. To what intensity and extent does this affect the release behavior was investigated. The interchangeability of the apparatus for a specific product was another question addressed.

Assessment of preliminary *in vitro* drug release study data obtained for the commercially available nicotine based chewing gum products using the apparatus A and B demonstrated the suitability of the apparatuses for *in vitro* drug release testing.

The different setups of the apparatus B resulted in various *in vitro* dissolution profiles for the nicotine based chewing gum products. For specific products like Nicorette freshmint gums, the difference observed in the drug release profiles could be directly linked to the parameters like chewing distance, twisting angle etc. A clear understanding on the influence of combination of these factors on release of nicotine could not be deduced. Generally it was observed that a smaller chewing distance (for e.g., 1.4 mm) in combination with a large twisting angle (40 degrees) resulted faster release of the nicotine contained in the chewing gum matrix. The apparatus B provided more discriminatory release profiles than apparatus A for the various parameters tested. This is due to the nature of construction of the apparatus. The apparatus B may find its usefulness in the development of chewing gum formulations. Since the chewing gumbase itself is a delivery device, the physicochemical properties of the excipient and the gum mass will influence the release of the active ingredients. The optimization of organoleptic properties like texture and feel contribute to the success of the final formulation. The apparatus B was able to pick any such differences in the formulation which can be seen in the *in vitro* drug release profiles from different flavors of nicotine gum products. This was also shown quantitatively using f_2 calculations. It is also generally recommended to use a pair of qualified new chewing jaws for every release testing. The

surface roughness of the jaws significantly affected the drug release behavior. Another aspect to consider is the use of pair nylon nets which prevent the disintegrated gum components leaving the chewing area during initial mastication. Conditioning of the gums prior to release testing at specific temperature was found to have negligible influence on release rate; however it prevented the disintegration process to some extent.

As far as the apparatus A is concerned, the strong mechanical forces during mastication conceal the product sensitivities, hence the release behavior does not discriminate between the tested gum products. The apparatus A will be useful in testing the assay or the content in the dosage form since there is always a complete release of the active at the end of testing period particularly when a narrow or tight apparatus setup is adopted.

As of 2012, both the apparatuses (A and B) are compendial in the Ph. Eur. However no recommendations with the respect to the selection of apparatus for release testing were made in the monographs. Selection of suitable apparatus for a specific product is based on the purpose of testing (product development, bio-relevant, content uniformity etc.), understanding of the products know-how's and obviously the prior experience. With the data readily available from both apparatus for a specific product, the interchangeability could be evaluated. From the feasibility study results, it was found that only a few combination of apparatus parameters yielded similar or comparable drug release profiles. The acceptance of similarity of profiles generated from the apparatuses is therefore based on the results of the f_2 test statistics. It is generally recommended that prior knowledge on the product performance is necessary to interchange the apparatus.

6.2 Verification of *in vitro* test methodology

In order to characterize the performance of the nicotine based chewing gum products and to investigate the influence of various apparatus parameters on release, a feasibility study was designed. The study included various parameters of both the apparatuses and two nicotine gum products commercially available in the market (Germany). From the feasibility study it was concluded that the profiles generated with apparatus B are more discriminating than the apparatus A. But how reliable these differences observed were could only be verified with the *in vivo* data. Unlike conventional dosage forms, the absorption of the released active begins in the oral cavity and continues along the GI tract resulting in highly variable blood plasma concentration. Moreover, the release is voluntarily controlled by the subject which is the rate of chewing, and the availability of saliva for dissolution adds further variability. The clinical data extracted from the literatures available in the publicly accessible sources were found to be highly variable and inconsistent between the studies. For many of these studies, the purposes were to compare the nicotine amounts in plasma following the cigarette smoking and chewing the gum. These data were analyzed by the Wagner-Nelson method.

Efforts to establish the *in vitro in vivo* correlation with the clinical data from the same batch to that of the *in vitro* dissolution failed to establish a one to one level A correlation between the *in vitro* and *in vivo* profiles. It is unclear from the reported literatures how the variables like chewing frequency were controlled. The approval of generic version of nicotine based chewing gum products was based on the BE studies. No literatures were found in an attempt to develop a bio-relevant *in vitro* drug release predictive of *in vivo* drug release behavior. Considering the advantages of the dosage form, a chew-out study model was proposed as an alternative to the bioavailability approach and was able to demonstrate the superimposability of the *in vitro in vivo* profiles. This eliminated the need to design complex clinical studies and mathematical treatment of the data to obtain the desired results. Using this methodology, the *in vivo* physiological variables like chewing frequency are standardized and the results obtained were consistent with the *in vitro* data. The corresponding apparatus setup that correlated with the data is considered as biorelevant setup. This methodology may prove itself to be useful in demonstrating the equivalence of medicinal gum products in terms of SUPAC based marketing authorization. On the other hand, data generated using the chew-out study methodology would be useful in verifying the suitability of the apparatus for the *in vitro* drug release testing. This will open a new perspective on the drug release testing of medicated chewing gums to measure and predict its performance. In this part of the thesis, a stepwise development of *in vitro* drug release testing methodology was explored and the importance of various aspects of performance testing to assess the product quality emphasized.

6.3 Formulation development

Development of an *in vitro* drug release method for various active(s) contained in the commercially available chewing products led to an understanding of the processes (compression of gum mass and simultaneous renewal of chewing surfaces) involved during mastication of gums and subsequent release of the actives. Without a standardized apparatus and an optimized *in vitro* drug release methodology, the data generated from the apparatus may not be reliable. Therefore, suitability of apparatuses used for the *in vitro* release testing of medicated chewing gums in a routine GMP laboratory has be considered. During the feasibility study, the apparatus parameters necessary for developing and optimizing the release testing method was investigated. However, verifying the performance of the apparatus with the aid of commercially available chewing gums formulations is not feasible due to the following reasons;

- Variability within the drug release test is large and demonstrates rapid release of the active(s) at the early phase of release testing. Due to this variability, the influence of apparatus parameters like chewing distance is not well differentiated.

- Commercially available multi-source chewing gums of same dosage strength of the active(s) tend to exhibit different release profiles under same experimental conditions.
- Performances of dose similar chewing gum products having MA cannot be compared directly and hence migrating between the products for performance testing will result in inconsistent results.
- Employing one particular gum product to verify the performance of the apparatuses (performance qualification) would increase the dependency to one manufacturer and the availability of that product.

Due to the above mentioned reasons, it was decided to explore the possibilities to manufacture chewing gums by a simple direct tabletting technique, which may prove to be reliable in verifying the performance of the apparatuses in a development and GMP environment. Dry preformulated gumbases are available from multiple sources of manufacturers for nutraceutical purpose. After screening various gumbases, suitable gumbases were identified based on the flow properties, stickiness, compressibility and particle size. Nicotine was chosen as a model drug, so that a direct comparison with a conventional nicotine chewing gum is possible. The conventional gums unlike the DC gums are manufactured using hot melt extrusion process. In this study, different aspects of chewing gum manufacturing have been investigated. Starting from the selection of ion exchange resins to loading of nicotine onto the resin using a batch process, coating of resin in a small scale using a solvent coacervation technique, modifying the release characteristics to attain the desired release profile were evaluated.

A single step batch process to load nicotine within a very short time span (<2 h) was found to be effective. The nicotine loading ratio (amount of nicotine loaded per unit weight of resin) was reflected in the dissolution testing. Rapid and burst release was observed with the higher loading ratios. Pre-treatment of the resins prior to loading includes elimination of unwanted small and large particles, both of which significantly affected the nicotine release behavior. Post treatment with PEG solution after loading with nicotine reduced the water uptake by resins and hence reduced the burst release. Lab scale coating using simple coacervation techniques using pH independent polymer (Eudragit RS 100) was found to be effective in modifying the release of nicotine from DRC.

Investigation of other components (type of organic solvents like dichloromethane and its volume, emulsifiers and plasticizers like lecithin) used for the coating process provided a good understanding of the variables and their associated impact on the release behavior. Design of experiments (DoE) helped in choosing an optimum amount of components to

obtain a desired coating level. With the application of DoE, it was possible to predict the response (release of nicotine) of the variables within the design space and optimum coating parameters. Limitations of this coating approach include lack of scaling up possibilities. Most of the coating process was performed on a lab scale with limited quantities. However the information obtained from the coating process can be successfully adapted to the fluid bed coating techniques. This study can be used as a platform to explore the possibilities of drug delivery systems using ion-exchange resins (IER).

Another aspect of the formulation study was to evaluate the possibilities to manufacture nicotine containing chewing gums using the direct compression technique. Formulation of different gumbases and excipients along with the DoE optimized Eudragit RS 100 coated DRC were tested for their performance using apparatus B. The *in vitro* drug release methodology was found to discriminatory with respect to the various apparatus setups. The release testing methodology described in this thesis can be used to qualify the chewing apparatuses A and B described in the European Pharmacopeia using any standardized gum product. Methods to manufacture chewing gums by direct compression technique are optimized and necessary quality attributes have been evaluated. The described method could be utilized to manufacture chewing gums containing various actives by a tabletting technique and extends room for the future research. This thesis might serve as an initiative platform to further research in order to incorporate more active drug substances in gum products for better and efficient drug delivery.

7 ABSTRACT

Evaluation of the performance of medicated chewing gum requires suitable chewing apparatus to simulate the masticatory action. In an *in vitro* environment, the release of any active pharmaceutical ingredient (API) present in chewing gum formulations is controlled by various apparatus parameters that include the distance between chewing jaws, rate of chewing (chewing frequency) and the twisting angle of chewing jaws. In this study, influence of all these factors in various combinations was evaluated using Apparatus A and B described in the chapter 2.9.25 of the European Pharmacopeia (Ph. Eur.) for commercially available nicotine based chewing gum products.

Nicotine containing chewing gums were chosen as a model drug product to develop the *in vitro* drug release methodology and to assess the suitability of the apparatus. The *in vitro* release testing methodology was further extended to Dimenhydrinate and Caffeine containing chewing gum products using Apparatus B due to its commercial availability. The *in vitro* release pattern from various chewing gum products indicated that the release of API(s) vary with respect to the product, apparatus type and setup parameter. Interchangeability of the apparatus for nicotine gum product was found only for a few apparatus parameters verified using the similarity test (f2) statistical approach. For most of the investigated gum products, highest release of the active was observed for the apparatus parameters with smallest chewing distance (apparatus A: 0.3 mm/3 mm, apparatus B: 1.4 mm), highest twisting angle 40° (apparatus B) and chewing frequency of 60 strokes/min (apparatus A and B). Verification of *in vitro* release methodology was performed using *in vivo* chew out approach and also from the *in vivo* clinical data. Correlation between the *in vitro* data and *in vivo* chew out data was observed. No *in vitro in vivo* correlation (IVIVC) could be established using the *in vitro* and *in vivo* clinical data.

Further experiments were performed with a goal of manufacturing nicotine containing chewing gums using a tableting technique. As a first step, a batch process to load nicotine onto a strong cation exchange resin was optimized. Nicotine to resin ratio was maintained 1:4. The nicotine loaded resins were coated using pH neutral polymer Eudragit RS 100. *In vitro* drug release experiment was used to assess the performance. The uncoated and Eudragit RS 100 coated resin complex were used as an API for manufacturing nicotine chewing gums. Various combinations of formulations containing different gumbases and excipients were used to formulate nicotine gum formulations. It was shown from the experiments that the release of nicotine salt > uncoated nicotine resinate > coated nicotine resinate present in gum formulations. Using this approach, possibilities to manufacture chewing gums by conventional tableting technique and to tailor the release of nicotine was explored. This work may provide a basis for further research to incorporate many more APIs of different physicochemical properties in chewing gum formulations.

8 ZUSAMMENFASSUNG

Die Bestimmung der Performance (Qualität) medizinischer Kaugummis erfordert geeignete Gerätschaften zur Simulation des Kauprozeses. In einer *in vitro* Umgebung wird die Freisetzung jeglicher vorhandener Wirkstoffe (API: Active Pharmaceutical Ingredient) aus den Kaugummiformulierungen durch Variation verschiedener Geräteparameter bestimmt, wie z.B. dem Abstand zwischen den Kaubacken, der Geschwindigkeit des Kauvorgangs (Kaufrequenz) und dem Torsionswinkel der Kaubacken. In der folgenden Studie wurde der Einfluss all dieser Faktoren in verschiedenen Kombinationen mittels Apparatur A und B gemäß dem Kapitel 2.9.25 der European Pharmacopeia (Ph. Eur.) für kommerziell erhältliche Nikotin enthaltende Kaugummiprodukte untersucht.

Nikotin enthaltende Kaugummis wurden als Modellarzneiform zur Entwicklung der *in vitro* Freisetzungsmethode und zur Feststellung der Eignung der Apparatur ausgewählt. Darüber hinaus wurde die *in vitro* Freisetzungsmethode auf Dimenhydrinat und Koffein enthaltende Kaugummiprodukte unter Verwendung der Apparatur B erweitert, da diese kommerziell erhältlich ist. Das Freisetzungsverhalten verschiedener Kaugummiprodukte wies darauf hin, dass die Freisetzung des Wirkstoffes in Abhängigkeit von Produkt, Gerätetyp und Geräteparameter variiert. Eine Austauschbarkeit der Geräte für Nikotin enthaltende Kaugummiprodukte konnte nur für wenige Geräteparameter unter Verwendung des statistischen Similarity Test (f2) verifiziert werden. Für die meisten untersuchten Kaugummiprodukte wurde die höchste Freisetzung des Wirkstoffs bei Geräteparametern mit dem kleinsten Kauabstand (Apparatur A: 0.3 mm/3 mm, Apparatur B: 1.4 mm), dem höchsten Torsionswinkel von 40° (Apparatur B) und einer Kaufrequenz von 60 Hüben pro Minute (Apparatur A und B) festgestellt. Verifiziert wurde die *in vitro* Freisetzungsmethode mittels der *in vivo* „Chew-Out“ Methode und mit klinischen *in vivo* Daten. Eine Korrelation zwischen *in vitro* Daten und *in vivo* „Chew-Out“ Daten konnte aufgezeigt werden, wohingegen keine *in vitro in vivo* Korrelation (IVIVC) zwischen den *in vitro* und klinischen *in vivo* Daten festgestellt werden konnte.

Mit dem Ziel, unter Nutzung der Tablettier Technik weiteres Nikotin enthaltende Kaugummis herzustellen, wurden weitere Versuche durchgeführt. Im ersten Schritt wurde ein Herstellungsprozess zur Beladung von Nikotin auf ein starkes Kationenaustauschharz optimiert. Das Verhältnis von Nikotin zu Harz wurde auf 1:4 gesetzt. Die mit Nikotin beladenen Harze wurden mit dem pH neutralem Polymer Eudragit RS 100 überzogen. Die *in vitro* Freisetzung des Wirkstoffes wurde zur Bestimmung der Performance (Qualität) herangezogen. Die nicht überzogenen und die mit Eudragit RS 100 überzogenen Harzkomplexe wurden als API zur Herstellung Nikotin enthaltender Kaugummis verwendet. Verschiedene Kombinationen von Formulierungen und eine Vielzahl verschiedener Kaumassen und Hilfsstoffe wurden verwendet um Nikotin enthaltende Formulierungen

herzustellen. Es wurde experimentell festgestellt, das sich die Freisetzungsgeschwindigkeit wie folgt verhält: reines Nikotinsalz enthaltende Formulierungen > nicht überzogenes Nikotinharz enthaltende Formulierungen > überzogenes Nikotinharz enthaltende Kaugummiformulierungen. Unter Verwendung dieser Herangehensweise wurden Möglichkeiten zur Herstellung von Kaugummis mittels konventioneller Tablettiertechnik und zur angepassten Freisetzung von Nikotin eruiert. Diese Arbeit steht die Grundlage für weitere Untersuchungen zur Einarbeitung vieler weiterer Wirkstoffe mit unterschiedlichen physiko-chemischen Eigenschaften in Kaugummi Formulierungen dar.

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Presentations, Posters & Papers

- Key presenter on the topic Preparation and evaluation of controlled release behavior of nicotine loaded ion exchange resins for manufacturing chewing gums by direct compression, Controlled Release Society (CRS), Washington DC, 2011.
- Invited speaker at the APV conference on IVIVC of Special Dosage Forms, Mainz, Germany, 2008.
- Invited speaker at the FIP/RPSGB (Royal Pharmaceutical Society of Great Britain): What's New with The In-Vitro Drug Release? Workshop for *In vitro* drug release testing of special dosage forms, London, 2008.
- Poster on Controlled release behavior of nicotine loaded ion exchange resins for manufacturing chewing gums by direct compression technique, PharmSciFair, Prague, 2011
- Poster on Suitability of *in vitro* drug release testing apparatus for medicated chewing gums through demonstration of *in vitro-in vivo* relationship (IVIVC), Los Angeles, 2009.
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10 APPENDIX

Table 1. Average *in vitro* drug release profiles of Nicorette 2mg freshmint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			1.4mm/ 40Strokes/20	SD [n=3]	1.4mm/ 60Strokes/20°	SD [n=3]	1.4mm/ 40Strokes/40°	SD [n=3]	1.4mm/ 60Strokes/40°	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	3.89	0.43	5.39	0.67	7.91	1.38	10.13	1.81
5	300	200	17.58	1.08	23.96	0.45	35.69	6.19	37.08	6.82
10	600	400	35.27	10.00	42.32	3.81	58.71	7.66	56.68	9.47
15	900	600	44.20	12.51	52.90	6.09	69.33	7.53	66.96	7.43
20	1200	800	49.28	13.95	60.01	7.64	75.45	6.30	75.46	4.86
30	1800	1200	57.10	14.74	66.67	6.73	82.68	5.40	84.68	7.81
45	2700	1800	63.33	15.36	74.16	6.15	88.78	4.11	93.20	7.12
60	3600	2400	68.05	13.57	81.83	5.94	92.29	2.96	96.61	6.11

Table 2. Average *in vitro* drug release profiles of Nicorette 2mg freshmint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			1.4mm/ 40Strokes/20	SD [n=3]	1.4mm/ 60Strokes/20°	SD [n=3]	1.4mm/ 40Strokes/40°	SD [n=3]	1.4mm/ 60Strokes/40°	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	3.08	0.26	4.90	0.89	6.36	1.04	8.36	1.93
5	300	200	12.93	2.78	18.02	0.40	19.15	0.85	26.65	8.34
10	600	400	26.73	5.68	32.38	2.47	30.89	2.30	48.29	6.72
15	900	600	34.11	6.50	46.48	2.05	36.60	2.56	56.90	6.38
20	1200	800	38.36	6.51	60.52	4.70	40.68	3.27	68.18	15.30
30	1800	1200	44.23	7.08	70.21	4.50	49.06	4.60	73.41	15.66
45	2700	1800	49.56	7.08	78.52	8.41	55.45	5.55	75.90	14.95
60	3600	2400	54.03	3.93	87.82	3.96	64.25	4.19	82.67	7.15

Table 3. Average *in vitro* drug release profiles of Nicorette 2mg Freshmint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			1.4mm/ 40Strokes/20	SD [n=3]	1.4mm/ 60Strokes/20°	SD [n=3]	1.4mm/ 40Strokes/40°	SD [n=3]	1.4mm/ 60Strokes/40°	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	4.17	0.89	3.86	0.08	5.11	0.35	7.88	0.32
5	300	200	9.12	1.44	9.42	1.01	17.81	0.05	24.30	2.35
10	600	400	16.04	2.29	16.55	2.26	36.28	4.35	46.24	3.20
15	900	600	20.97	2.93	23.28	3.61	48.02	1.22	61.92	4.62
20	1200	800	24.89	4.30	28.82	5.46	57.78	3.32	71.95	6.05
30	1800	1200	30.13	7.11	40.28	13.00	71.16	2.64	77.81	8.30
45	2700	1800	34.05	8.72	47.44	14.14	80.59	4.70	83.94	5.00
60	3600	2400	40.25	13.83	52.28	12.37	83.45	6.23	90.95	8.88

Table 4. Average *in vitro* drug release profiles of Nicotinell 2mg Mint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			1.4mm/ 40Strokes/20	SD [n=3]	1.4mm/ 60Strokes/20°	SD [n=3]	1.4mm/ 40Strokes/40°	SD [n=3]	1.4mm/ 60Strokes/40°	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	13.13	0.68	19.49	1.06	19.05	0.34	28.94	0.14
5	300	200	40.77	3.95	52.47	2.01	53.69	0.95	65.67	1.46
10	600	400	62.40	3.00	77.09	0.90	77.71	0.94	86.63	2.07
15	900	600	73.49	3.74	84.89	0.67	86.26	3.86	96.28	1.76
20	1200	800	79.61	2.31	90.68	0.84	92.06	3.20	99.45	0.48
30	1800	1200	88.16	3.84	95.88	2.40	98.09	3.79	101.83	1.49
45	2700	1800	93.09	3.55	101.13	0.34	102.60	2.31	103.67	0.74
60	3600	2400	97.02	4.75	103.45	1.83	103.63	2.64	104.00	1.15

Table 5. Average *in vitro* drug release profiles of Nicotinell 2mg Mint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			1.4mm/ 40Strokes/20	SD [n=3]	1.4mm/ 60Strokes/20°	SD [n=3]	1.4mm/ 40Strokes/40°	SD [n=3]	1.4mm/ 60Strokes/40°	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	12.66	1.37	18.33	0.97	17.83	1.23	24.97	0.34
5	300	200	36.57	2.48	47.84	1.27	49.58	3.51	61.72	2.16
10	600	400	64.04	1.50	72.80	0.46	75.83	3.61	84.87	2.29
15	900	600	75.97	1.92	82.72	1.20	85.41	4.51	94.20	2.99
20	1200	800	83.87	1.55	87.88	0.74	90.56	4.88	98.34	3.71
30	1800	1200	89.13	0.68	94.01	0.78	95.88	3.00	101.22	3.35
45	2700	1800	95.13	1.56	99.85	0.36	99.20	2.42	103.97	4.04
60	3600	2400	98.24	0.95	101.93	0.43	100.59	1.45	104.57	3.28

Table 6. Average *in vitro* drug release profiles of Nicotinell 2mg Mint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			1.4mm/ 40Strokes/20	SD [n=3]	1.4mm/ 60Strokes/20°	SD [n=3]	1.4mm/ 40Strokes/40°	SD [n=3]	1.4mm/ 60Strokes/40°	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	13.30	0.48	15.78	0.91	15.94	0.11	20.92	2.68
5	300	200	32.85	1.02	40.13	4.13	39.13	2.62	47.88	2.89
10	600	400	55.37	4.12	60.57	4.12	61.63	1.67	69.81	3.35
15	900	600	64.77	4.71	67.95	4.04	72.31	2.94	81.83	2.91
20	1200	800	70.54	3.57	74.38	3.02	77.03	3.13	87.18	2.79
30	1800	1200	76.66	4.12	80.63	1.29	83.63	2.87	95.28	3.26
45	2700	1800	81.32	4.91	82.83	1.67	88.86	4.67	99.48	2.17
60	3600	2400	84.19	4.75	86.47	1.28	92.25	4.86	101.30	3.20

Table 7. Average *in vitro* drug release profiles of Nicorette 2mg Freshmint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			0.7 mm/ 60strokes/3mm	SD [n=3]	0.7 mm/ 40strokes/3mm	SD [n=3]	0.3 mm/ 40strokes/3mm	SD [n=3]	0.3 mm/ 60strokes/3mm	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	2.08	0.97	1.29	0.16	1.56	0.49	2.08	0.37
5	300	200	10.90	6.91	8.66	1.78	9.48	6.22	18.06	1.88
10	600	400	28.09	8.52	31.71	3.13	35.04	8.55	43.99	2.20
15	900	600	44.66	4.70	47.53	4.84	52.42	6.69	58.58	2.50
20	1200	800	55.21	1.40	57.73	4.58	64.73	5.94	70.62	2.09
30	1800	1200	71.45	5.63	70.94	1.99	78.60	7.22	86.42	1.86
45	2700	1800	88.32	4.31	87.27	7.27	95.38	5.67	99.05	2.64
60	3600	2400	97.00	5.69	98.47	6.18	105.24	4.95	109.53	2.74

Table 8. Average *in vitro* drug release profiles of Nicorette 2mg Freshmint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			0.7mm/ 60strokes/3mm	SD [n=3]	0.7mm/ 40strokes/3mm	SD [n=3]	0.3mm/ 40strokes/3mm	SD [n=3]	0.3mm/ 60strokes/3mm	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	1.83	0.45	1.29	0.19	1.10	0.45	2.83	0.44
5	300	200	15.95	3.86	9.96	2.22	6.92	4.15	17.70	1.17
10	600	400	42.09	4.17	35.91	4.07	24.79	18.31	45.17	2.66
15	900	600	56.13	3.81	50.94	3.40	44.54	13.06	60.62	3.29
20	1200	800	66.81	3.93	61.92	4.38	59.78	7.31	72.27	3.47
30	1800	1200	81.67	4.02	77.31	4.97	78.21	5.03	88.23	3.43
45	2700	1800	95.02	4.14	90.53	5.07	94.90	4.28	102.33	2.92
60	3600	2400	102.27	2.76	100.27	4.26	104.74	4.39	109.86	2.50

Table 9. Average *in vitro* drug release profiles of Nicotinell 2mg Mint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			0.7mm/ 60strokes/3mm	SD [n=3]	0.7mm/ 40strokes/3mm	SD [n=3]	0.3mm/ 40strokes/3mm	SD [n=3]	0.3mm/ 60strokes/3mm	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	13.90	0.31	10.69	0.65	12.62	0.93	14.16	4.86
5	300	200	33.95	0.81	29.28	1.41	32.49	1.81	37.41	4.07
10	600	400	48.51	11.36	48.21	2.60	53.15	2.20	61.20	4.33
15	900	600	63.25	7.35	60.79	3.06	66.86	2.30	75.15	5.55
20	1200	800	73.73	5.29	70.01	3.37	76.73	2.66	84.71	5.83
30	1800	1200	86.63	3.26	82.50	3.50	89.60	3.40	96.80	6.39
45	2700	1800	98.18	1.70	94.59	3.77	101.00	3.43	105.82	4.89
60	3600	2400	105.47	0.97	101.93	3.76	107.63	2.63	110.18	3.26

Table 10. Average *in vitro* drug release profiles of Nicotinell 2mg Mint at variable chewing distance and chewing frequency

Time [min]	Cumulative strokes [n] 60/min	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
			0.7mm/ 60strokes/3mm	SD [n=3]	0.7mm/ 40strokes/3mm	SD [n=3]	0.3mm/ 40strokes/3mm	SD [n=3]	0.3mm/ 60strokes/3mm	SD [n=3]
0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	120	80	16.36	1.10	12.73	1.05	12.30	0.67	13.87	0.49
5	300	200	37.08	2.14	32.28	1.90	31.79	0.75	36.38	2.47
10	600	400	56.36	3.40	53.13	2.93	53.52	2.00	59.01	5.41
15	900	600	68.83	4.57	66.11	4.34	67.38	2.56	72.49	6.29
20	1200	800	76.83	4.66	75.53	4.26	77.20	2.92	82.03	6.61
30	1800	1200	87.86	5.88	86.77	5.33	89.39	3.37	94.23	6.91
45	2700	1800	97.57	6.11	97.75	5.37	101.52	4.08	104.15	5.81
60	3600	2400	102.79	5.69	101.88	3.28	107.74	3.30	108.79	4.19

Table 11. Average *in vitro* drug release profiles of Nicorette 2mg classic gum at variable chewing distance and chewing frequency of apparatus B

Time [min]	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
		1.4mm/ 40 strokes/20°	SD [n=3]	1.4mm/ 40 strokes/40°	SD [n=3]	1.8mm/ 40 strokes/20°	SD [n=3]	1.8mm/ 40 strokes/40°	SD [n=3]
0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	80	13.56	2.15	20.41	2.89	11.36	1.50	9.78	5.65
5	200	49.62	4.75	63.99	5.02	32.29	7.42	23.88	3.79
10	400	76.21	3.32	84.33	5.64	54.34	9.58	43.06	5.86
20	800	85.86	3.69	94.97	4.89	69.29	6.11	62.09	4.85
30	1200	88.90	5.22	99.22	4.64	73.71	6.48	66.12	7.17
45	1800	90.60	8.40	104.01	6.86	77.15	7.31	68.40	6.93
60	2400	93.05	7.27	105.19	6.54	79.12	7.74	69.88	6.78

Table 12. Average *in vitro* drug release profiles of Nicorette 4mg classic gum at variable chewing distance and chewing frequency of apparatus B

Time [min]	Cumulative strokes [n] 40/min	*In vitro* nicotine released [%] of label claim at different apparatus setup							
		1.4mm/ 40Strokes/20	SD [n=3]	1.6mm/ 40Strokes/20°	SD [n=3]	1.8mm/ 40Strokes/20°	SD [n=3]	1.4mm/ 40Strokes/2°	SD [n=3]
0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	80	15.38	2.96	12.31	1.53	12.17	1.18	15.38	2.96
5	200	57.36	1.84	46.13	5.29	39.84	5.35	57.36	1.84
10	400	84.58	2.82	74.22	0.66	64.25	3.33	84.58	2.82
20	800	92.33	4.14	81.21	1.77	73.90	2.57	92.33	4.14
30	1200	95.63	3.73	84.82	1.21	76.87	3.43	95.63	3.73
45	1800	98.20	3.80	89.18	1.31	79.32	3.58	98.20	3.80
60	2400	99.70	3.97	92.89	0.42	80.23	2.89	99.70	3.97

Table 13. Summary of *in vitro* nicotine release from DRC (Amberlite IRP 69)

Time [min]	5	10	20	30	45	60	90	120	1200	1440
Avg (%). Rel (50 RPM)	55.41	61.94	68.49	73.93	-	80.52	84.50	86.99	-	99.98
SD	5.44	5.47	5.28	4.46	-	4.71	5.26	5.27	-	0.99
Avg (%). Rel (100 RPM)	77.22	80.33	83.19	84.96	86.04	87.14	92.67	95.30	95.30	-
SD	4.94	4.99	5.23	4.94	4.67	4.05	1.25	1.53	1.53	-

Table 14. Summary of *in vitro* nicotine release from different gumbases

Time [min]	Formulations									
	FG1		FG2		FG3		FG4		FG5	
	Avg. rel.[%]	SD (n=3)	Avg. rel.[%]	SD (n=3)	Avg. rel.[%]	SD (n=3)	Avg. rel.[%]	SD (n=3)	Avg. rel.[%]	SD (n=3)
5	33.92	0.99	44.96	6.85	48.24	2.37	34.06	7.62	26.01	0.88
10	49.70	3.11	63.44	4.46	58.05	5.28	50.18	1.77	40.71	2.00
20	60.15	4.95	74.65	2.72	68.38	8.59	62.11	2.43	53.70	2.42
30	66.03	7.50	83.72	4.84	73.65	9.53	66.81	3.59	59.07	3.50
45	71.58	5.90	98.32	15.60	81.09	6.34	70.23	4.24	63.83	3.60
60	74.49	5.29	98.37	14.85	85.38	3.37	72.74	4.72	66.69	3.61

Table 15. Evaluation of release kinetics from different gumbase formulations

Formulation	Zero Order		First Order		Higuchi Model		Korsmeyer-Peppas			Hixson-Crowell	
	r2	k0 $^{(h-1)}$	r^2	k1 $^{(h-1)}$	r^2	kH $^{(h-1/2)}$	r^2	n value	Kkp $^{(h-n)}$	r^2	kHC ($h^{-1/3}$)
FG1	0.8218	0.6537	0.7366	0.0120	0.9213	6.9778	0.9665	0.4097	0.9411	0.8844	-0.0184
FG2	0.8774	0.9735	0.7996	0.0125	0.9556	10.2418	0.9580	0.3655	1.3186	0.9212	-0.1104
FG3	0.9115	0.6384	0.8602	0.0095	0.9778	6.6664	0.9986	0.2565	0.9184	0.9674	-0.0226
FG4	0.7515	0.6034	0.6746	0.0112	0.8696	6.5431	0.9738	0.4334	0.9981	0.8108	-0.0166
FG5	0.8042	0.6608	0.7103	0.0143	0.9106	7.0889	0.9544	0.4076	0.7826	0.8549	-0.0164

Table 16. Summary of *in vitro* nicotine release from different formulations of gumbase formulation FG5 at 1.8 mm chewing distance

Time [min]	Formulations of FG5											
	F1		F2		F3		F4		F5		F6	
	Avg. rel. [%]	SD (n=3)	Avg. rel. [%]	SD (n=3)	Avg. rel. [%]	SD (n=3)	Avg. rel. [%]	SD (n=3)	Avg. rel. [%]	SD (n=3)	Avg. rel. [%]	SD (n=3)
5	51.14	6.39	76.85	0.70	74.45	10.89	66.04	4.23	53.82	3.45	93.49	1.23
10	61.64	4.63	83.54	1.70	83.63	8.97	74.28	2.76	67.61	1.74	94.02	0.40
20	68.47	2.72	84.63	0.85	86.34	8.01	76.93	2.95	72.35	2.25	93.89	0.82
30	70.37	2.34	86.10	0.64	88.00	8.12	78.40	2.75	74.07	2.20	94.23	0.99
45	72.17	3.12	86.62	1.24	88.59	8.92	78.35	2.26	75.28	2.43	94.47	0.68
60	73.74	2.26	86.74	0.59	89.60	9.07	78.91	2.58	76.79	2.48	94.67	0.93

Table 17. Evaluation of release kinetics from different formulations of FG5

Formulation	Zero Order		First Order		Higuchi Model	Hixson-Crowell	
	r^2	$k0^{(h-1)}$	r^2	$k1^{(h-1)}$	r^2	r^2	kHC ($h^{-1/3}$)
F1	0.7157	0.3409	0.6766	0.0054	0.8379	0.7608	-0.0106
F2	0.5870	0.1361	0.5741	0.0016	0.7157	0.6291	-0.0069
F3	0.6278	0.2107	0.6071	0.0025	0.7548	0.6993	-0.0111
F4	0.5663	0.1753	0.5501	0.0024	0.7023	0.5970	-0.0066
F5	0.6236	0.3186	0.5852	0.0048	0.7513	0.6774	-0.0105
F6	0.8762	0.0188	0.8751	0.0002	0.8992	0.8866	-0.0019

Table 18. Summary of *in vitro* nicotine release from DoE optimized formulation at variable chewing distance

Time [min]	Optimized Formulation					
	1.4mm		1.6mm		1.8mm	
	Avg. rel. [%]	SD (n=3)	Avg. rel. [%]	SD (n=3)	Avg. rel.[%]	SD (n=3)
5	51.14	6.39	42.94	1.80	36.97	1.18
10	61.64	4.63	51.57	1.57	41.57	1.45
20	68.47	2.72	55.80	1.87	43.12	0.24
30	70.37	2.34	57.11	1.60	45.29	1.11
45	72.17	3.12	58.53	1.46	46.43	0.83
60	73.74	2.26	60.64	0.75	48.38	0.52

Table 19. Evaluation of release kinetics from DoE optimized formulation at variable chewing distance

Formulation	Zero Order		First Order		Higuchi Model	Hixson-Crowell	
	r^2	$k0^{(h-1)}$	r^2	$k1^{(h-1)}$	r^2	r^2	kHC ($h^{-1/3}$)
1.4 mm	0.7155	0.3409	0.6764	0.0054	0.8377	0.7606	-0.0106
1.6 mm	0.7324	0.2591	0.6911	0.0049	0.8446	0.7624	-0.0067
1.8 mm	0.8619	0.1779	0.8322	0.0041	0.9366	0.8757	-0.0040

Table 20. Summary of *in vitro* nicotine release from formulations compressed with different crushing strength at 1.8mm

Time[min]	Formulations							
	F5a20N		F5a30N		F5b20N		F5b30N	
	Avg. rel. [%]	SD (n=3)	Avg. rel. [%]	SD (n=3)	Avg. rel. [%]	SD (n=3)	Avg. rel. [%]	SD (n=3)
5	12.89	0.39	14.69	1.47	15.17	0.62	15.42	1.71
10	20.67	1.97	22.71	0.31	24.23	1.55	23.55	2.63
20	28.72	2.26	32.17	0.85	34.31	4.96	31.90	2.90
30	34.75	4.27	37.04	1.35	40.32	5.44	37.27	1.93
45	40.80	6.90	42.64	2.42	45.14	5.50	43.80	3.24
60	44.74	9.20	46.94	3.37	48.61	5.57	47.68	2.97

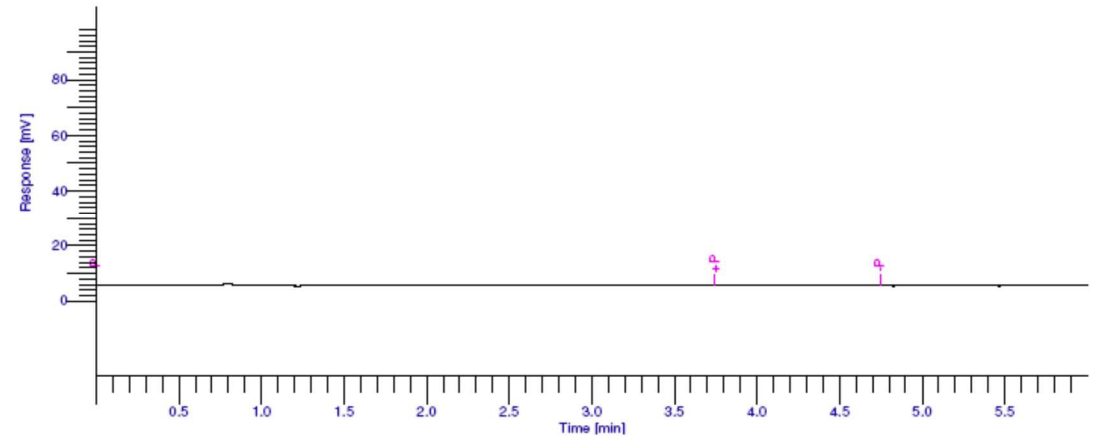

Figure 1. Representative chromatogram for the mobile phase (MeOH/buffer pH 6.5, 2/98 v/v)

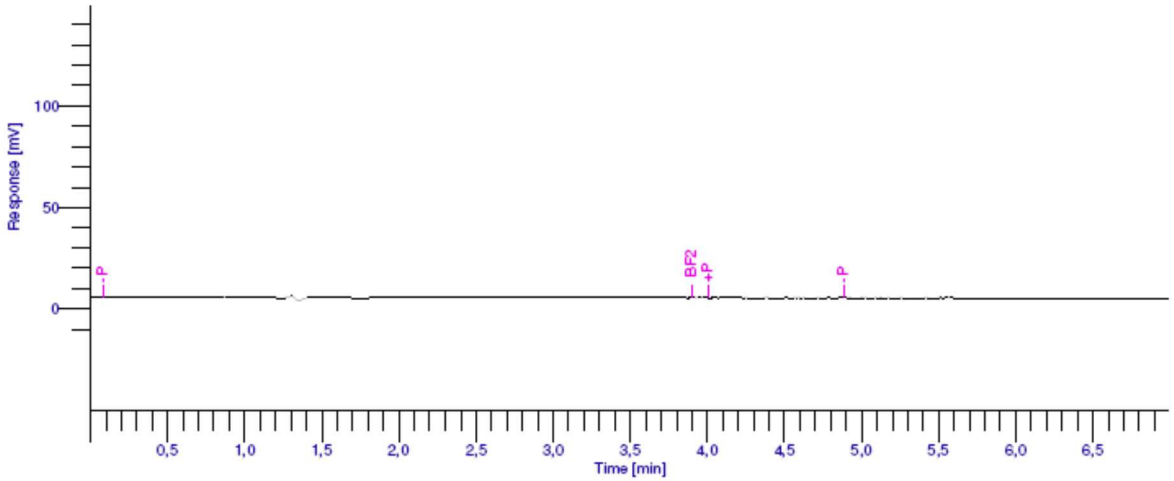

Figure 2. Representative chromatogram of artificial saliva pH 6.2

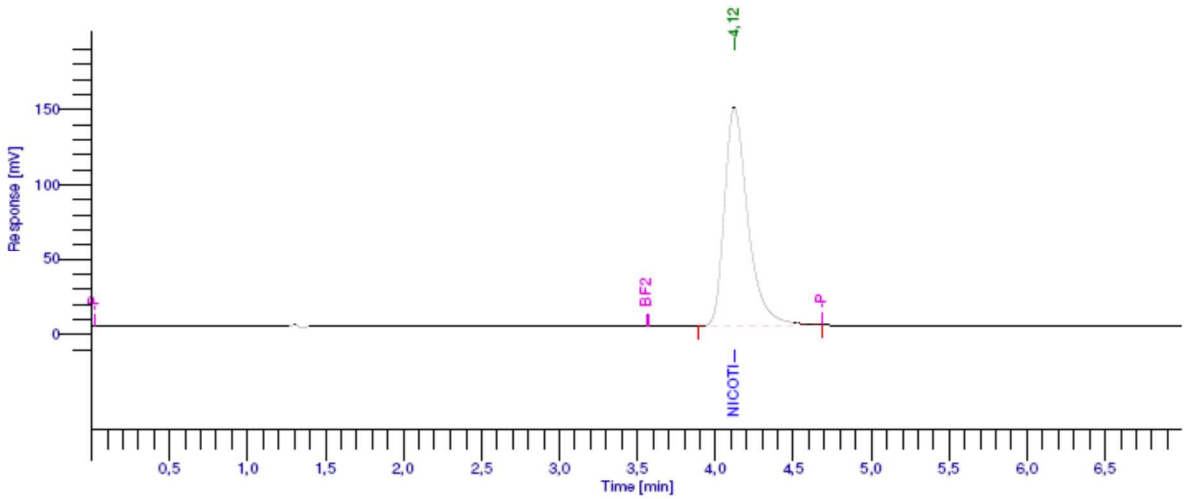

Figure 3. Representative chromatogram of Nicotine bitartrate (reference) in artificial saliva pH 6.2

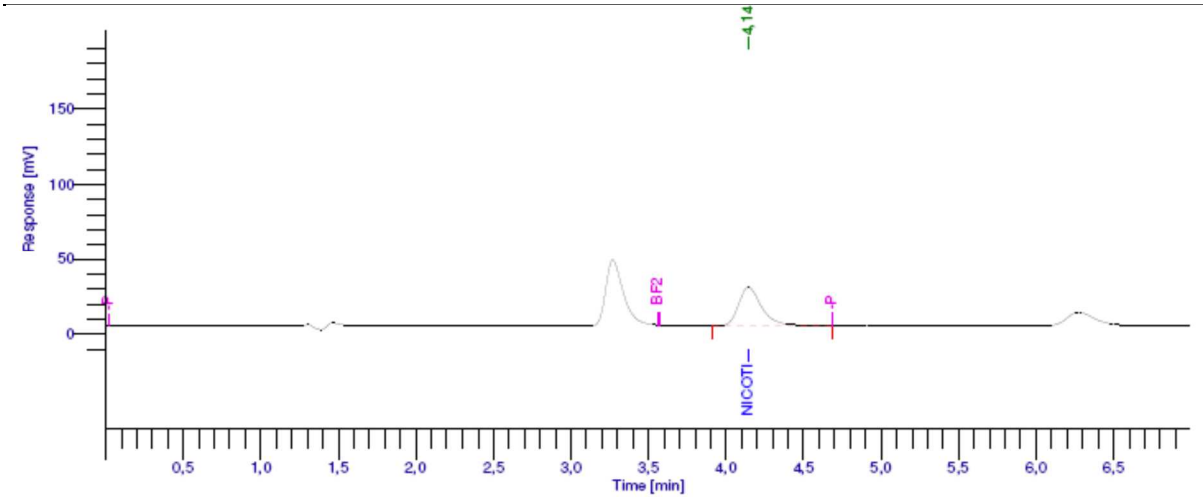

Figure 4. Representative chromatogram of Nicotinell 2 mg mint sample solution

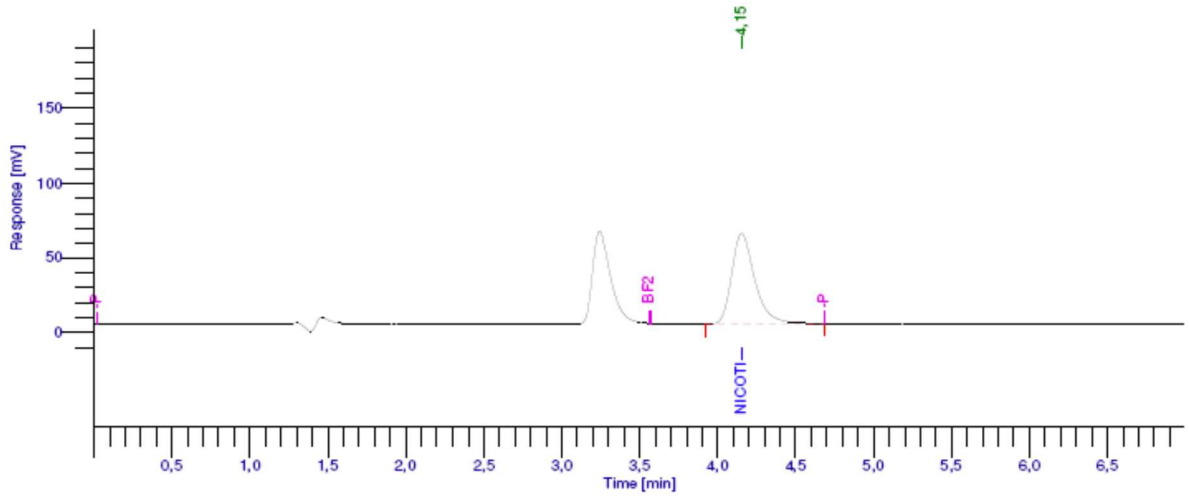

Figure 5. Representative chromatogram of Nicorette 2 mg freshmint sample solution

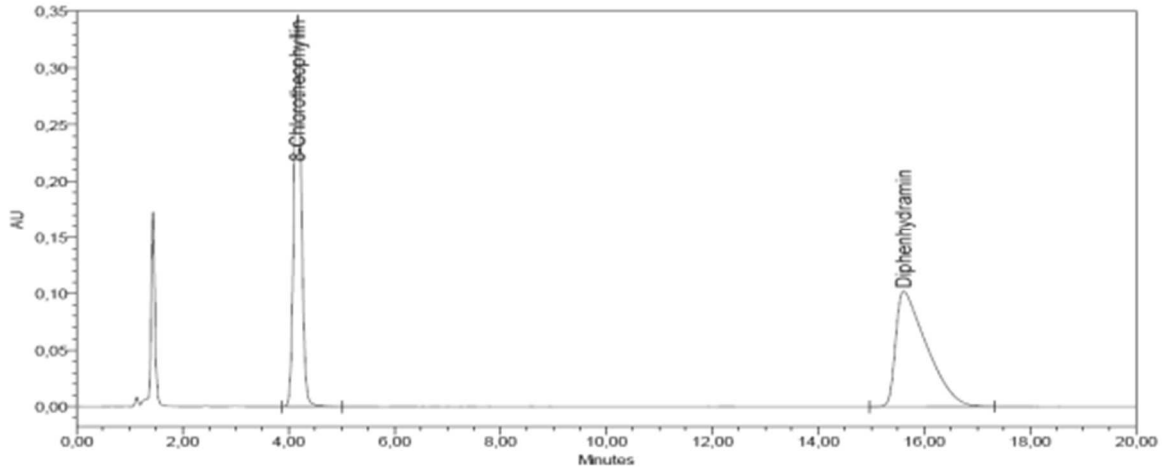

Figure 6. Representative chromatogram of dimenhydrinate (reference) in artificial saliva pH 6.2

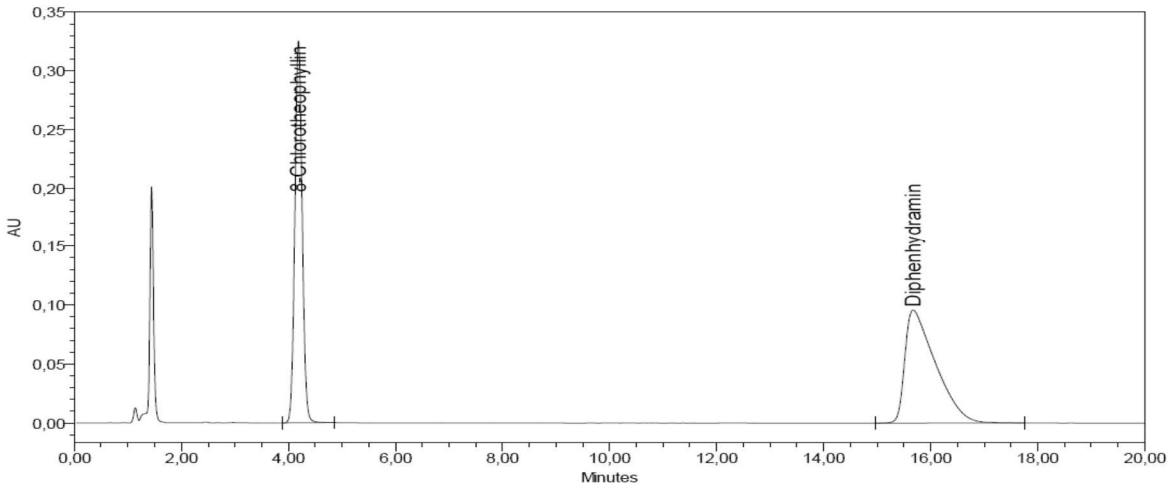

Figure 7. Representative chromatogram of dimenhydrinate sample solution in artificial saliva pH 6.2