# *"Stabilität und Kompatibilität applikationsfertiger Zytostatikazubereitungen ausgewählter neuer Zytostatika"*

**Dissertation**

**zur Erlangung des Grades**

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**im Promotionsfach Pharmazie**

**am Fachbereich Chemie, Pharmazie und Geowissenschaften der Johannes Gutenberg-Universität in Mainz**

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## *Erklärung*

Hiermit erkläre ich an Eides statt, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Ort, Datum

Iman Sarakbi

## *Executive Summary*

Ready-to-use parenteral preparations of anticancer medicinal products are typically prepared in pharmacy-based centralized aseptic preparation units. Microbiological stability of the preparations remains a primary issue and stability determining factor when assigning shelf lives to the preparations. For this reason, experimental studies were performed to evaluate the ability of four different pathogens (S. aureus, E. coli, P. aeruginosa, C. albicans) related to hospital infections to grow in ready-to-use, cytotoxic and non-cytotoxic parenteral products aseptically prepared in hospital pharmacies. Viability studies were performed under conditions that simulate optimal ones for the growth of micro-organisms (low drug concentrations, room temperature). In total 14 new anticancer small molecules and anticancer monoclonal antibodies were tested. Most of the drugs tested showed neither growth-retarding nor growth-supporting properties of the test organisms. An exception was trabectedin, which generated rapidly antibacterial activity.

Additional experiments with non-cytoxic drug preparations revealed that the selected strains lost viability in parenteral solutions containing vancomycin, phenylephrine or midazolam after a period of a few hours or days. Tranexamic acid solution and 50% w/v glucose solution generated species-specific antimicrobial activity. In the lipid containing emulsions, colony forming units increased rapidly. Low pH values and high osmolality are probably the reason for growth inhibition in midazolam and 50% glucose solutions, respectively. The antimicrobial activity of phenylephrine solutions is caused by the excipient sodium metabisulfite. According to the results, solutions for parenteral administration should be prepared under strict aseptic conditions and kept refrigerated whenever possible to inhibit the growth of any contaminating organism.

There are a number of originator publications and databases available dealing with the physicochemical stability of reconstituted and diluted cytotoxic preparations. A comprehensive information source in table format (STABIL-LISTE©) is compiled by the Pharmacy Department of University Medical Center in Mainz. In the updated version of STABIL- LISTE© 7th ed., 2015 information about the stability/compatibility of drug loaded beads as well as 13 recently approved anticancer drugs (alemtuzumab 10 mg/mL, amsacrine 1.5 mg/mL, belinostat, blinatumomab, cyclophosphamide Lyophilisat, nivolumab, obinutuzmab, pembroli-

zumab, plerixafor,ramucirumab, rituximab subcutaneous injection, trastuzumab subcutaneous injection trastuzmab emtansine) was added. Methods of data compilation are described, conflicting results and open questions were commented.

In order to reduce the adverse reactions of systemic chemotherapy, targeted transarterial administration (TACE) was introduced to treat patients suffering from HCC or liver metastases of CRC. Simultaneous administration of the embolic and chemotherapeutic agents is achieved by drug eluting beads. DC Bead™ are marketed in different diameters ranging from 70-900 µm and can be loaded with cationic anticancer drugs. Prior to administration of the drug loaded beads, they are mixed with nonionic contrast medium to guide the embolic agent to the selected site of tumor. In order to guarantee the complete delivery of cytotoxic agents to the tumor site, compatibility and stability of the admixtures are prerogative. Therefore, the compatibility of epirubicin-loaded and irinotecan-loaded DC Beads™ (bead size 70-150 µm, 100-300 µm) with nonionic contrast media (Accupaque™ 300 (Iohexol), Imeron® 300 (Iomeprol), Ultravist® 300 (Iopromid), Visipaque™ 320 (Iodixanol)) commonly used by the interventional radiologists was experimentally tested. Results showed that it is feasible to prepare admixtures of epirubicin-loaded DC Bead (bead size 70-150 µm, 100-300 µm) with selected non-ionic contrast media in centralized cytotoxic preparation units in advance because of stability of admixtures over a period of 7 days. Admixtures of irinotecan-loaded DC Beads (bead size 70-150 µm) with different types and volumes of non-ionic contrast media are incompatible and should only be mixed immediately prior to delivery procedure by the radiologists.

## *Kurzzusammenfassung*

Die parenterale Arzneimitteltherapie spielt immer noch eine wichtige Rolle in der Behandlung von Krebs. Applikationsfertige parenterale Zubereitungen von Anti-Krebs-Arzneimitteln werden in der Regel in den zentralen aseptischen Herstellungseinheiten der Krankenhausapotheken zubereitet. Die mikrobiologische Stabilität dieser Zubereitungen bleibt ein Hauptproblem in Bezug auf die Ermittlung ihrer Haltbarkeit. Aus diesem Grund sind zuverlässige Informationen Viabilität von Krankheitserregern in diesen Zubereitungen von hohem Interesse. Dementsprechend wurden experimentelle Untersuchungen mit ausgewählten fakultativ pathogenen Mikroorganismen (S. aureus, E. coli, P. aeruginosa, C. albicans) in Zubereitungen von insgesamt 14 neuartigen chemischen und biopharmazeutischen Antineoplastika durchgeführt. Die gewählten Wachstumsbedingungen sollten die schlechtesten Bedingungen für Patienten und optimale Bedingungen für das Keimwachstum simulieren. Die meisten getesteten neuen Antitumor-Arzneimittel zeigten weder wachstumshemmende noch wachstumsfördernde Eigenschaften. Eine Ausnahme stellte Trabectedin dar, das rasch bakterizid wirkte.

Zusätzliche Experimente mit aseptischen Zubereitungen ohne CMR-Eigenschaften zeigten, dass die ausgewählten Testkeime rasch die Vermehrungsfähigkeit in Zubereitungen von Vancomycin, Phenylephrin und Midazolam verloren. Glucose 50% w/v-Lösung und Tranexamsäurelösung wirkten spezies-spezifisch antimikrobiell. Die antimikrobielle Wirkung von Midazolam ist durch den sauren pH-Wert, von Glucose 50% durch die hohe Osmolarität und von Phenylephrin Lösung durch den Hilfsstoff Natriummetabisulfit verursacht. In den lipidhaltigen Emulsionen stieg die Zahl der Kolonie bildenden Einheiten rasch an. Komplexe Parenteraliazubereitungen sollten wann immer möglich unter aseptischen Bedingungen in der Apotheke zubereitet und gekühlt aufbewahrt werden.

Zusammen mit der mikrobiologischen Stabilität ist die physikalisch-chemische Stabilität der applikationsfertigen Infusions- oder Injektionslösungen bei der Festlegung der Haltbarkeit entscheidend. Ebenso ist die Stabilität der konzentrierten Stammlösungen eine Voraussetzung für die Weiterverwendung nach dem Erstanbruch. Es gibt zahlreiche Publikationen und auch Datenbanken, die die physikalisch-chemische Stabilität von rekonstituierten und verdünnnten Antineoplastika-Zubereitungen zum Gegenstand haben. In sehr komprimierter

Form werden diese Informationen in der STABIL-LISTE© dargestellt, die von der Apotheke der Universitätsmedizin Mainz herausgegeben wird. Die aktualisierte Version der STABIL-LISTE© 7. Aufl., 2015 bietet einen raschen Überblick über die Stabilität/Kompatibilität von Zytostatika beladenden Mikrosphären (drug-loaded beads) sowie 13 neuartigen Antitumor Arzneimitteln (Alemtuzumab 10 mg/ml, Amsacrin 1,5 mg/ml, Belinostat, Blinatumomab, Cyclophosphamid Lyophilisat Nivolumab, Obinutuzmab, Pembrolizumab, Plerixafor, Ramucirumab, Rituximab subkutan, Trastuzumab subkutan und Trastuzumab Emtansine). In dieser Arbeit werden die Methoden der Datenevaluierung und Ergebnisse beschrieben sowie offene Fragen kommentiert.

Um die Nebenwirkungen der systemischen Chemotherapie zu reduzieren, werden inoperable hepatozelluläre Karzinome (HCC) oder Lebermetastasen von Kolonkarzinomen heutzutage häufig simultan embolisiert und eine lokale Chemotherapie mit Zytostatika-beladenen Mikrosphären (DEB) durchgeführt. Die dafür indizierten DC Bead™ werden in verschiedenen Durchmessern von 70 bis 900 µm vermarktet und können mit kationischen Antineoplastika beladen werden. Vor der Applikation werden die DEB mit nicht-ionischen Kontrastmitteln gemischt, um die korrekte Platzierung in den Tumor versorgenden Arterien zu gewährleisten. Voraussetzung für eine effektive und sichere Therapie ist die Stabilität der beladenen Mikrosphären sowie die Kompatibilität mit dem Kontrastmittel. Daher wurde die Kompatibilität von Epirubicin-beladenden und Irinotecan-beladenden DC Beads™ des Durchmesser 70 bis 150 µm und 100 bis 300 µm mit häufig verwendeten, nicht-ionischen Kontrastmitteln (Accupaque™ 300, Imeron® 300, Ultravist® 300, Vispaque™ 320) untersucht. Im Ergebnis kann die Mischung von Epirubicin-beladenden DC Bead-Mikrosphären (70-150 µm, 100-300 µm) mit den ausgewählten nicht-ionischen Kontrastmitteln empfohlen werden, da Stabilität über 7 Tage nachgewiesen wurde. Mischungen aus Irinotecan beladenden DC Bead-Mikrosphären 70-150 µm mit verschiedenen Arten und Mengen von nicht-ionischen Kontrastmitteln erwiesen sich als inkompatibel, indem zu große Mengen Irinotecan unmittelbar freigesetzt wurden. Die Mischung sollte daher nur unmittelbar vor der Anwendung durch die applizierenden Radiologen selbst erfolgen.

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## *List of Abbreviations*

| Abbreviation | Connotation |
|---|---|
| ADKA | The German Society of Hospital Pharmacists (Bundesverband Deutscher Krankenhausapotheker e.V.) |
| AET | The antimicrobial effectiveness test |
| AMPS | 2-acrylamido-. 2-methylpropanesulphonate |
| API | active pharmaceutical ingredient as per USP-NF definition |
| ATP | adenosine triphosphate |
| BAK | The Federal chamber of German Pharmacists (Bundesapothekerkammer) |
| BCLC | Barcelona Clinic Liver Cancer |
| C.albicans | Candida. Albicans |
| CFU | Colony forming units |
| CHMP | Committee for medicinal products for human use |
| CPT-11 | Irinotecan |
| CRC | Colorectal carcinoma |
| cTACE | Conventional trans-arterial chemoembolization |
| DEB | Drug eluting beads |
| DEBDox | Doxorubicin eluting beads |
| DEBEPI | Epirubicin eluting beads |
| DEBIRI | Irinotecan eluting beads |
| Dox | Doxorubicin |
| DSM | Degradable starch microspheres |
| EASL | European Association for the Study of the Liver |

| | |
|---|---|
| E.faecium | Enterococcus faecium |
| EJHP | European journal of hospital pharmacy |
| EMA | European Medicines Agency |
| EMEA | European agency for the evaluation of medicinal products |
| EPI | Epirubicin |
| FDA | Food and Drug Administration |
| FPP | Final pharmaceutical preparation |
| FTM | fluid thioglycollate medium |
| G5 | 5%ig- Glucose solution |
| GMP | Good Manufacturing Practice |
| HCC | Hepatocellular Carcinoma |
| HEPA | high-efficiency particulate absorption |
| HPLC | High-performance liquid chromatography |
| HVAC | heating, ventilating, and air conditioning |
| ICH | International Conference of Harmonization |
| IPA | International pharmaceutical abstracts |
| JP | Japanese Pharmacopeia |
| LAF | Laminar air flow |
| mCRC | metastatic colorectal cancer |
| NECC | New England compounding center |
| P. aeruginosa | Pseudomonas aeruginosa |
| PCR | polymerase chain reaction |
| PDA | Photodiodenarray |
| Ph. Eur. | European Pharmacopeia |

| | |
|---|---|
| Pp | polypropylene |
| PVA | polyvinylalcohol |
| RFA | radiofrequency ablation |
| RH | relative humidity |
| RP-HPLC | Reversed Phase- High performance liquid chromatography |
| RSD | relative standard deviation |
| RT | room temperature |
| SAP-TACE | superabsorent polymer microspheres |
| S. aureus | Staphylococcus aureus |
| SCDM | soybean casein digest medium |
| SD | Standard deviation |
| SIRT | selective internal arterial radiotherapy |
| STACE | Supraselective trans-arterial chemoembolization |
| TACE | Trans-arterial chemoembolization |
| TAE | Trans-arterial embolization |
| TARE | Trans-arterial radioembolization |
| TKI | Tyrosine kinase inhibitors |
| TOCE | Trans-catheter oily chemoembolization |
| USP | United States Pharmacopeia |
| UV | Ultraviolet |
| WFI | Water for injection |
| WHO | World Health Organization |

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# 1 Introduction

In Germany, cancer is the second most often cause of death regardless the gender. Prostate cancer is the most frequent cancer disease in men and lung cancer the most common cause of death related to cancer. Among women, breast cancer is associated with highest incidence and mortality (1, 2). On one hand, the incidence of cancer increases in Germany, on the other hand 5 year survival rates of cancer patients significantly increased during the last three decades (> 61% for men, 67% for women) depending on the cancer entity. The average age of death for cancer patients has increased about two years over the same period and amounts to 73 years (3, 4) .

Early diagnosis and successful cancer therapies are the major objectives in cancer research, but also prevention of tumor diseases is of interest. The treatment of cancer is based on three major methods, i.e. surgery (removing the tumor and surrounding healthy tissue), radiotherapy and chemotherapy, which are utilized as mono- or combination therapy.

## 1.1 Anticancer drug therapy

Antineoplastic drug therapy, also named chemotherapy (CTx), encompasses various antineoplastic drug substances that have the ability to destroy the cancer cells by either stopping the growth of these cells (curative therapy) or controlling tumor growth. In patients with advanced stage of cancer disease, chemotherapy is administrated also as a palliative therapy to shrink and/or stop the tumor spreading in order to improve the quality of life and reduce pain in cancer patients.

Antineoplastic drugs are administered orally, topically, by instillation, subcutaneously, and mainly intravascularly. The latter way of administration is the preferred one in order to ensure rapid delivery of maximal tolerated dose of antitumor drugs and bioavailability. In Germany, since more than 25 years, chemotherapy products are prepared in the centralized pharmacy based cytotoxic preparation areas as ready-to-administer products in patient individual doses. The individual doses are calculated according to patients' body surface area ($m^2$) or the body weight (kg), and if necessary adopted to renal and hepatic function (5).

Because of the mutagenic, teratogenic and/or carcinogenic character of antineoplastic products, the centralized preparation is operated under protective measures, which decrease the contamination risk and enhance the safety of health care workers.

In pharmacy departments the preparation of ready-to-use intravenous anticancer drug solutions is performed according to different guidelines:

- PICS/PE 010-3 (6)
- Ph Eur 7.7 monograph Pharmaceutical preparations (7),
- United States Pharmacopoeia, monograph <797> (8)
- Council of Europe Resolution CM/Resap 2011 (9)
- Quality Assurance Guideline of the Federal chamber of German Pharmacists (BAK) Aseptic preparation and quality control of ready-to-use parenteral preparations with CMR properties category 1A or 1B (see Fig 1) (10)
- Guideline of the German Society of Hospital Pharmacists (ADKA): Aseptic preparation and quality control of ready-to-use parenteral preparations (11), which corresponds to the PICS/PE 010-3 (6)(12)

The preparation of antineoplastic drug solutions is usually performed separately from other parenteral preparations in dedicated clean rooms. During the preparation process essential goals are to be achieved simultaneously:

- protection of the products from microbial contamination
- protection of the staff and the preparation area from hazardous substances

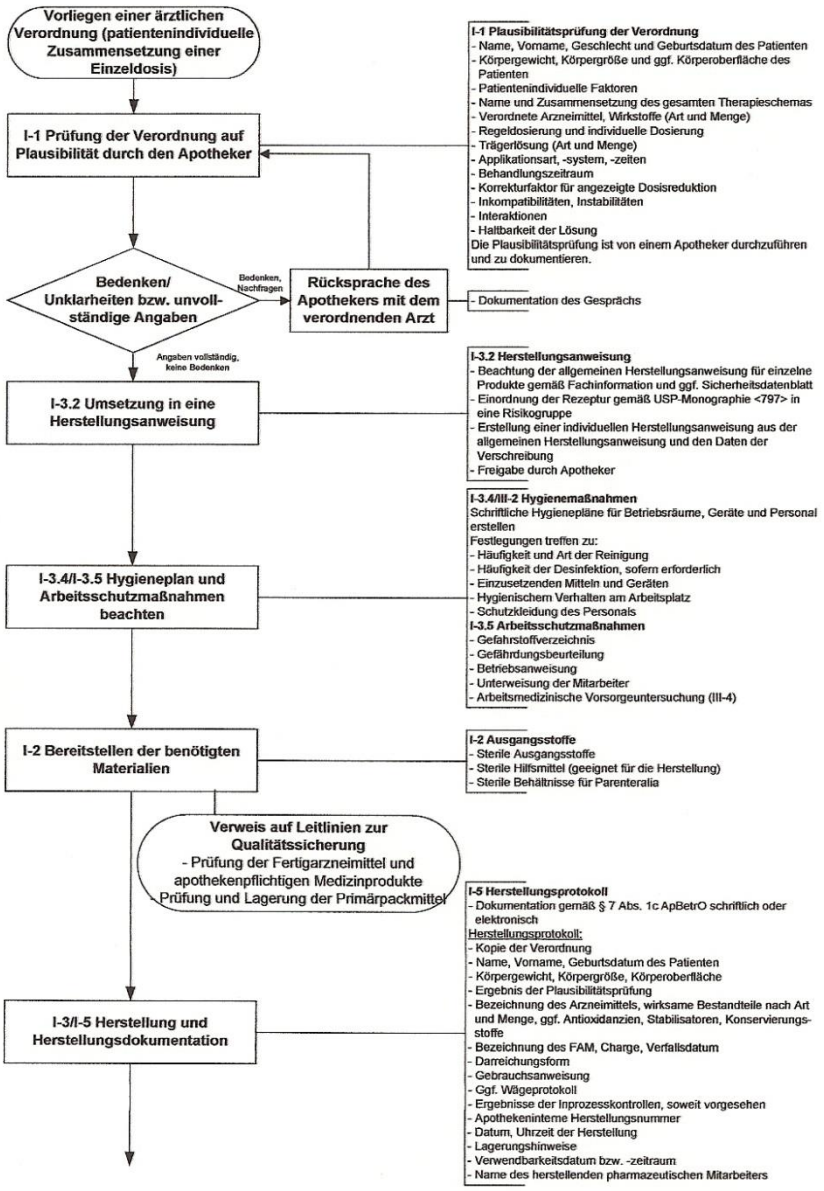
Ready-to-use anticancer drug preparations should be prepared in clean room areas equipped with safety cabinets or isolators with negative pressure, i.e. cytotoxic workbenches providing cleanroom class A in a grade B or C background rooms with negative pressure (6,8,10,11) Thereby the products are protected from airborne particulates and microbial contamination. Coincidently the environment and staff involved in this process are protected. As a matter of choice the exhausts of the cabinets and isolators should be connected to the open air (10,11). The working surfaces should also be designed to minimize contamination and facilitate easy cleaning.

An important concern is the safety of workers involved in the preparation process. But at the same time, the staff is considered to be the main source of microbial contamination during the aseptic preparation. Therefore suitable clean room clothing and hygiene measures are to be regarded. Operators should wear proper sterile clothing such as gloves, hair covers, masks, shoe covers and overalls and wash and disinfect hands by alcohol based gel. The cleaning and disinfection procedures should be monitored by microbial environmental monitoring. Last not least thorough education and continuous training programs for operators are to be implemented.

Very recently robotic systems were introduced to improve the aseptic preparation process of antineoplastic drugs. Fully automated preparation increases the safety of staff, saves time and costs, eases complete documentation of the process, and reduces preparation errors(12, 13).The fully automatic system APOTECAchemo is used in the pharmacy department of the University Medical Center Mainz. The operator loads the articles and drugs needed for preparation and unloads the finished preparations in the loading area of the robot. The aseptic preparation takes place in the production area. The process is controlled by various technologies like imaging, barcode reading, and weight control.

The preparation of anticancer drugs is not only designed to minimize the contact between the drugs and the operators involved in the preparation procedure, but also to limit the contamination of the patient areas and other health care staff and visitors. For these reasons it is recommended to seal the primary containers of ready-to-use antineoplastic parenterals and pack them into liquid-tight outer packaging in order to prevent any contamination by spillage.

## IV Aseptische Herstellung und Prüfung applikationsfertiger Parenteralia mit CMR-Eigenschaften der Kategorie 1A oder 1B



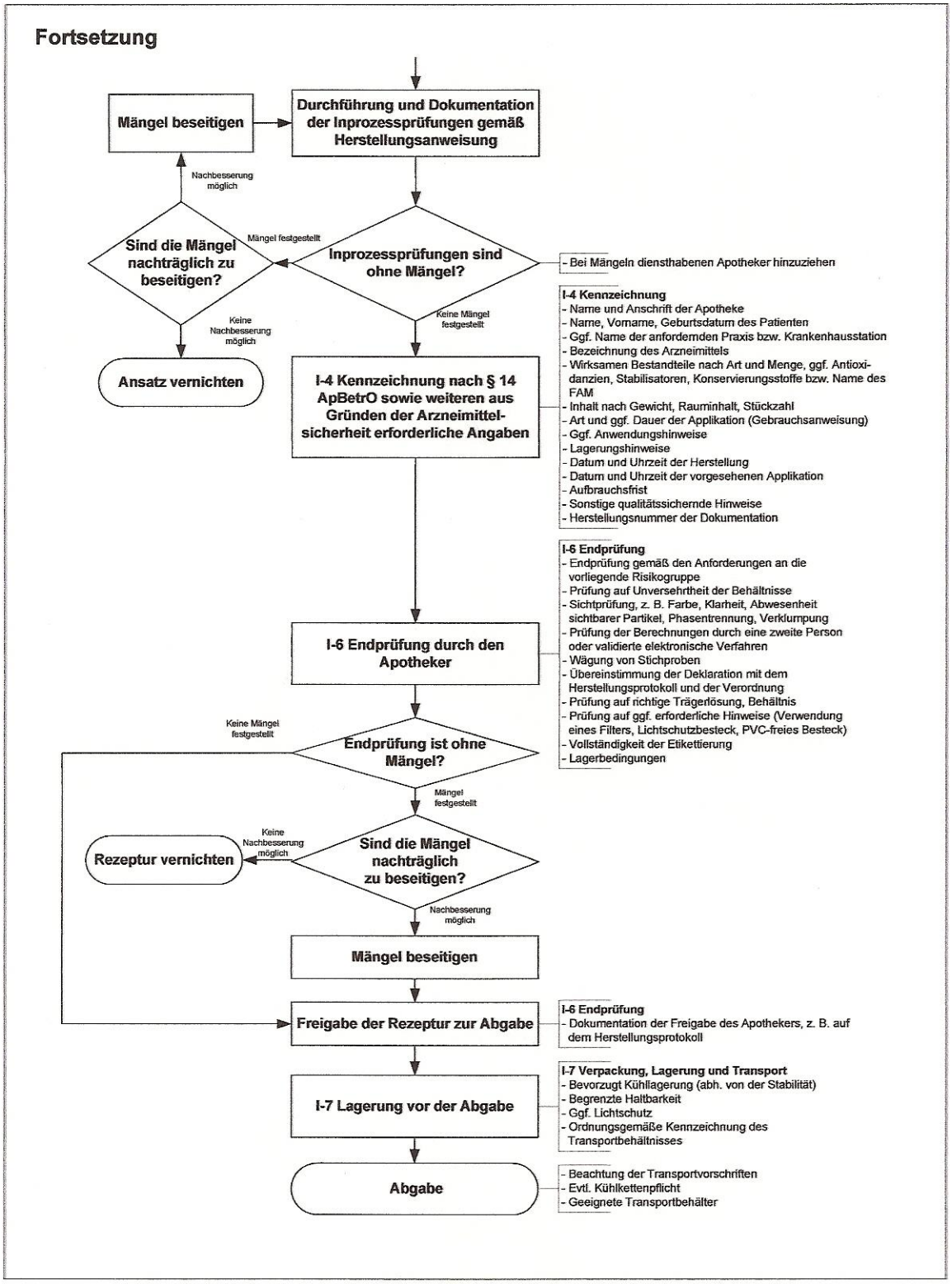



Figure 1 Flowchart of the preparation and quality control of ready-to-use preparations of antineoplastic drugs, taken from reference (6): Quality Assurance Guideline of the German Federal Chamber of Pharmacists: Aseptic preparation and quality control of ready-to-use parenteral preparations with CMR properties category 1A or 1B

## 1.2 Shelf life and stability of antineoplastic drug preparations

According to the German guidelines for aseptic preparation (10, 11), the expiry date of the ready-to-use preparations must be defined and indicated together with the storage conditions on the product label. In addition to physical and chemical stability, microbial and toxicological stability under designated conditions of storage and use are to be taken into account, when assigning expiry dates to the products.

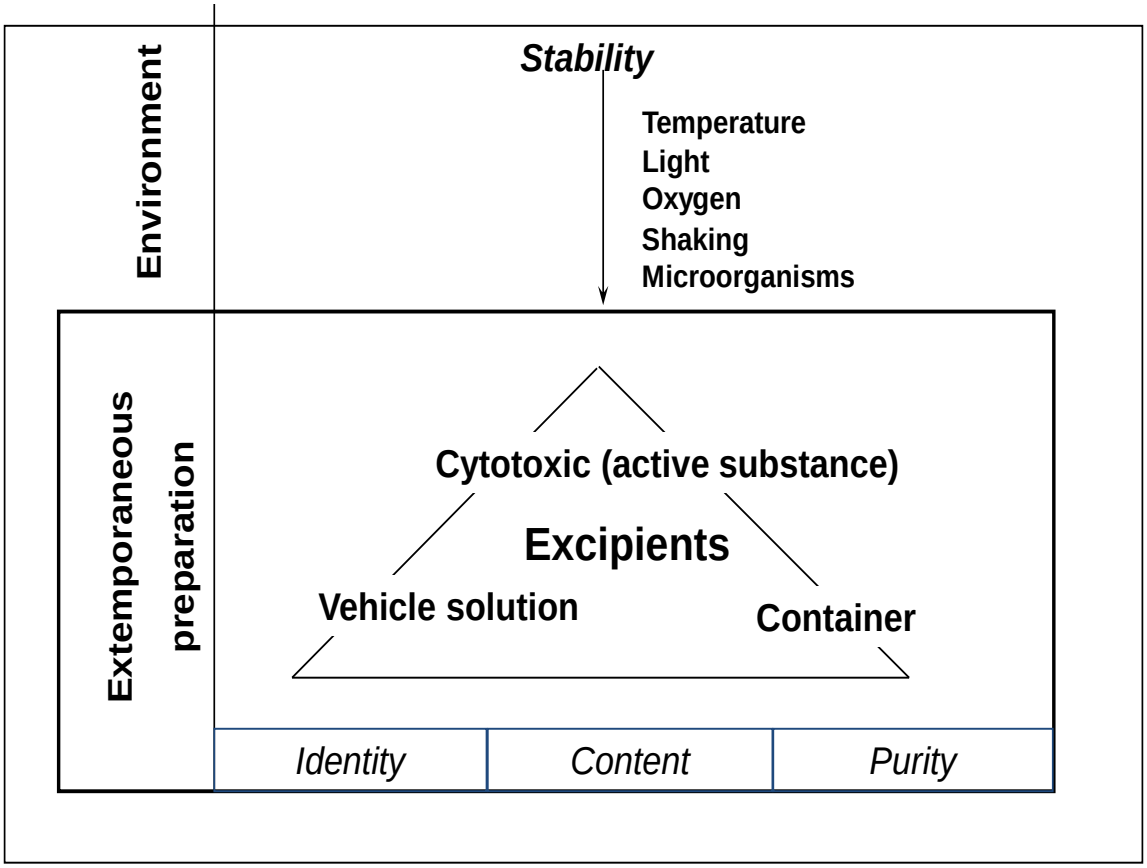



Figure 2: Scheme of factors impacting the stability of cytotoxic drug preparations including the internal and external (= environmental) conditions.

Stability of the ready-to-use preparations and also the stock solutions depends on internal and external parameters (compare Fig.2). Internal factors comprise the chemical structure and concentration of the active ingredient, structure and concentration of excipients, type of primary container (e.g. infusion bags, syringes), and type of the vehicle solution used for dilution. The most important stability determining external factors are temperature and light.

Today targeted anticancer therapy is performed with drug substances capable to interfere with structures in the malignant cells or tissues leading to targeted block of tumor growth, with fewer side effects (18). Targeted anticancer drugs are categorized according to their chemical structure as monoclonal antibody (mAb) drugs or small molecule drugs (19). The proteinaceous mAbs are sensitive to different environmental conditions like temperature, pH value and physico-chemical degradation. Physical instability of the protein may cause aggregation, precipitation, surface adsorption, and conformational changes, while the chemical degradation can be elucidated by oxidation, deamidation, isomerization, disulfide exchange, and peptide fragmentation. As a consequence, the preparation, administration, transportation and storage of these products have to be done under conditions which minimize the instability of the protein structure and thereby decrease the potential immunogenicity of degraded protein structures (20).

In assigning the expiration date or shelf life to pharmacy-prepared products it is common practice to accept a maximum decline of the content of the active substance of 10%, i.e. 90% of the nominal content. Purity may also vary only within defined limits. If toxic degradation products are formed, the content of these may account for a shorter shelf-life.

The stability of reconstituted and diluted drug solutions which are unpreserved is limited by the Note for Guidance of the CPM/QWP/159/96 to a period of 24 hours (21). The maximum storage period of 24 hours is determined by microbiological instability regardless of the extent of physical and chemical (in)stability (14). The storage period does not reflect experimentally determined physico-chemical and microbial stability of the antineoplastic solu-

tions, which may be longer than 24 h. Furthermore, the high cost of some anticancer drugs is in particular limiting factor of experimental stability studies (14, 15).

It is also useful and efficient to prepare ready-to-use antineoplastic drug solutions in advance with consequence to facilitate administering these products for outpatients over several days and reduce the time needed for patients in the hospital (16). More detailed and accurate knowledge of the physical, chemical and microbial stability of the preparations can be used to save time and money, and decrease the waste of antineoplastic preparations by re-using the sealed preparations.

However, when assigning in-use stability data to parenterals microbiologic stability cannot be neglected. Therefore, a particular objective of this thesis was to investigate the viability of micro-organisms in ready-to-use aseptically prepared parenteral products.

Extended in-use stability data can only be applied if there is scientifically based knowledge about the physico-chemical stability of the compounded products. There are a number of publications and various databases available dealing with physico-chemical stability of concentrated and diluted antineoplastic drug solutions under various conditions, such as drug concentration, vehicle (usually 0.9% sodium chloride or 5% glucose solution), container, and storage conditions (see Fig. 2). In German pharmacy based cytotoxic preparation units usually the stability information given in Stabil Liste® (see Figure 3) is utilized (17). The preparation of an updated version of Stabil Liste® in 2015 was another objective of the thesis.

| Arzneimittel | Fertigarzneimittel | | Stammlösung | | | Physikalisch-chemische Stabilität | | | | | Aufbewahrung der applikationsfertigen Lösung | Besonderheiten | Ausgewählte Kompatibilitäten | Ausgewählte Inkompatibilitäten |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rekonstituens | | | Stammlösung | | Infusionslösung | | | | | | |
| INN-Name (Abkürzungen) | Beispiele (Hersteller) | Gehalt [mg] | Art | Menge [ml] | Konzentration [mg/ml] | RT | 2-8 °C | Träger-lösung / Behältnis-material | RT | 2-8 °C | | | | |
| **Aciclovir** | Aciclovir-ratiopharm® 500 mg p.i. (ratiopharm GmbH) | 500 | Aqua ad inject. | 20 | 25 | 12 h | Gefahr der Auskristal-lisation! | NaCl 0,9% oder G 5 / PVC PE (PP) Polyisopren EVA | 28 d | 28 d Chemische Stabilität gegeben, aber auf Auskristal-lisationen achten! | Raumtemperatur | Auf Auskristallisation achten! | Amikacin, Cefalexin, Cefuroxim, Dexamethason, Dimenhydrinat, [Diphenhydramin], Doxorubicin, Doxycyclin, Enoxaparin, Filgrastim, Fluconazol, [Granisetron], [Granisetron], Heparin-Na, Hydromorphon, Ibuprofen, Lorazepam, [Metoclopramid], [Morphin], $NaHCO_3$, Penicillin G, [Piperacillin], Ranitidin, Rituximab, Tazobactam, Tobramycin, Trastuzumab, Vancomycin | Amifostin, Amsacrin, Bromglukonat, Caspofungin, Ceftazidim, Ciclosporin, [Diphenhydramin], Dobutamin, Dopamin, Fludarabin, Fluorouracil, Gemcitabin, [Granisetron], [Granisetron], Idarubicin, Levofloxacin, [Metoclopramid], [Morphin], Ondansetron, Paracetamol, [Piperacillin-Tazobactam], saure Lösungen, Vinorelbin |
| **Aclarubicin-HCl** (=Aclacinomycin) (ACM) | Aclaplastin® (medac) | 20 | NaCl 0,9% | 10 | 2 | 72 h | 14 d | NaCl 0,9% oder G 5 / (PVC) (PE) (PP) | 72 h | 7 d | Lichtgeschützt Kühl | | | Alkalische Lösungen, Heparin-Na, Paracetamol |
| **Aflibercept** | Zaltrap® 25 mg/ml (Sanofi) | 200 | _ | (8) | 25 | 8 h | 28 d | NaCl 0,9% oder G 5 / PVC PE PP | 8 h | 24 h | Lichtgeschützt Kühl | Konzentration der Infusionslösung auf 0,6 - 8 mg/ml einstellen. Applikation über 0,2 µm Inline-Filter aus Polyethersulfon. | | Alkalische Lösungen, Heparin-Na, Paracetamol |
| **Aldesleukin (=Interleukin 2)** | Proleukin® S (Novartis Pharma) | 18 Mio IE (= 1,1 mg) | Aqua ad inject. | 1,2 | 18 Mio IE ! (= 1,1 mg) | 48 h | 14 d | G 5 ! / PVC (PE) PP | 14 d | 14 d | Kühl | Nicht heftig schütteln! Für Interleukin-Konzentrationen < 0,1 mg/ml als Trägerlösung G 5 mit 0,1% Humanalbumin einsetzen (sonst Gefahr der Adsorption an Infusionszubehör)! Tiefgefrieren 1 Monat stabil! | Amikacin, Amphotericin B, Calciumgluconat, Diphenhydramin, Dopamin, Fluconazol, Fosaprepitant, Granisetron, Heparin-Na, KCl, Metoclopramid, Morphin, Ondansetron, Piperacillin, Ranitidin, Rituximab, Tobramycin | Bromglukonat, Gemcitabin, Lorazepam, NaCl, Paracetamol, Trastuzumab |
| **Alemtuzumab** | MabCampath® (Sanofi) | 30 | _ | (1) | 30 | 24 h | 28 d | NaCl 0,9% oder G 5 / PVC PE PP | 14 d 24 h | 28 d 24 h | Lichtgeschützt Kühl | Stabilitätsangaben gelten für Konzentrationen >/= 0,05 mg/ml. | | basische Lösungen, oberflächenaktive Lösungen |
| | Lemtrada® 12 mg (Genzyme) | 12 | _ | (1,2) | 10 | | | | | | | | | |
| **Amsacrin** (AMSA) | Amsidyl® (Nord Medica) | 75 (1,5 ml) | Verdünnungs-lösung = Milchsäure 0,035 m | 13,5 | 5 | 96 h | Nicht bekannt | G 5 ! / PVC PE PP | 7 d | 7 d Chemische Stabilität gegeben, aber auf Auskristal-lisationen achten! | Raumtemperatur | Unverdünnte Amsidyl®-Lösung kann mit B Braun Injekt Spritzen entnommen werden. Inkompatibel mit chloridhaltigen Lösungen (z.B. NaCl 0,9%) (Ausfällung von Amsacrin-HCl). Adsorption an Filtermaterial möglich! | Amikacin, Chlorpromazin, Cefuroxim, Dexamethasonphosphat-Na, Diphenhydramin, Famotidin, Fludarabin, Granisetron, [Granisetron], Haloperidol, Hydromorphon, Hydroxyethylstärke oral-Na, Hydroxyzin, Lorazepam, Morphin, $NaHCO_3$, Promethazin, Ranitidin, Tobramycin, Vancomycin | Amsacrin, Amphotericin B, Aztreonam, Ceftazidim, Ceftriaxon, Ciprofloxacin, Furosemid, Gemcitabin, [Granisetron], Heparin-Na, Methylprednisolon-21-hydrogensuccinat-Na, Metoclopramid, NaCl-Lösungen und neutral chloridhaltige Lösungen, Ondansetron, salzfreie Lösungen |
| | Amsalyo® (Prostrakan Pharma, Nord Medica) | 75 | Aqua ad inject. | 50 | 1,5 | 5 d | Nicht bekannt | | | | | | | |





**Figure 3 Excerpt from Stabil Liste® 2015**

Besides new targeted anticancer therapies, also new targeted procedures and techniques are developed. Transarterial chemoembolization (TACE) is a modern and targeted technique introduced in the last decade to treat patients suffering from HCC or liver metastases of CRC. During the TACE procedure embolizing particles and chemotherapeutic agents are delivered simultaneously into the blood vessels feeding the tumor. As a consequence, oxygen and nutrient supply of the tumor decreases. The antitumor agent is gradually released from the embolic device and distributed to the tumor tissue resulting in a longer contact time and carrying less systemic exposure and fewer side effects. Simultaneous administration of the embolizing particles and the chemotherapeutic agent is facilitated by injecting the chemotherapeutic agent loaded beads through a catheter into the targeted artery. Prior to administration of the drug eluting beads, they are mixed with non-ionic contrast medium to guide the embolic agent to the selected site of the tumor. The specific nonionic contrast medium should pledge to successful delivery of DC beads up to the tumor bed.

The preparation of loaded beads is done in the centralized pharmacy based preparation unit. Most often doxorubicin and irinotecan are loaded with different types and sizes of beads. Stability of the loaded beads has been established for 14 and 28 days under defined conditions of storage (22, 23). Epirubicin has also been used in Germany in TACE treatment and shown adequate efficacy in clinical trials (24). However, there is a lack of stability and compatibility data of drug-loaded DC Bead™. Thus a further goal of this thesis was to investigate the compatibility of epirubicin-loaded or irinotecan-loaded beads of different sizes (70-150 µm (M1), 100-300 µm) with various non-ionic contrast media commonly used by the interventional radiologists.

# 2 Microbiological instability of pharmaceutical preparations

In 2012 the New England Compounding Center NECC was indicted for an outbreak of fungal meningitis causing the death of 64 patients. The outbreak was traced to fungal contamination of compounded methylprednisolone suspension for epidural steroid injections (25). This deterrent example shows that Good Preparation Practice (GPP) of ready-to-use parenteral products is inconceivable without intensive precautions to keep the products sterile. Microbial contamination of these products during the preparation process, storage or administration to the patients must be strictly avoided. Otherwise viable microorgansims and/or endotoxins are parenterally administered to the patients and lead to infections, SIRS and sepsis.

**Microbial contaminants relevant for pharmacy-based aseptic preparation**

- Bacteria (bacteria, mollicutes, and bacterial endospores)

In general, bacterial contamination plays a key role among various contaminants relevant for pharmacy based aseptic compounding. Major causes for this phenomenon are the ability of bacteria to change their characteristics and adapt various environmental conditions, and their potential to multiply quickly if the amount of nutrients is sufficient in the surrounding conditions.. Mollicutes, also known as mycoplasmas are the smallest free-living prokaryotic pathogens. They are able to pass through typically used bacterial filters. In addition, the detection of mollicutes is an ongoing problem because they grow slowly in liquid or on solid media. PCR (polymerase chain reaction) based methods are less time-consuming and shown higher sensitivity (26). Bacterial endospores are non-reproductive forms produced by specific types of gram positive bacterial species like *Bacillus* and *Clostridium* under stressed environmental conditions i.e. heating, radiation and starvation to ensure their bacterial survival (27). Elimination of endospores is used as an essential indicator to validate and monitor the sterilization process (28).

- Fungi (yeasts, molds)

Fungal contamination is the second most relevant microbial contamination limiting the safety of pharmaceutical preparations (29). On one hand, several important APIs originate from fungi and on the other hand, fungi cause severe infections and generate toxic substances (mycotoxins). Depending on the morphology, fungi are commonly classified into two major groups, i.e. yeasts and molds.

Yeasts refer to unicellular organisms that grow usually by asexual reproduction (budding); hence a new cell is produced by one mother cell. Molds are multicellular fungi, which multiply by sexual or/and asexual reproduction pattern. The hyphal fungi may grow until forming a visible mass called mold (mycelium). Molds reproduce by formation of spores which are extremely robust. *Histoplasma* and *C. albicans* are dimorphic fungi capable to exist either in mold/hyphal/filamentous form or in the yeast form depending on the actual environmental conditions (30). Molds can enter pharmaceutical preparations from the air (e.g. insufficient retained heating, ventilating, and air conditioning systems and high-efficiency particulate absorption (HEPA) filters, loss of pressure difference between controlled preparation areas), material (e.g. wipes used to clean the cleanrooms, raw materials, packaging) and personnel (31). Of note, molds grow under refrigerated conditions, where the raw materials and products are usually stored. Thus, suitable disinfection solutions and processes of aseptic preparation areas must be capable to eliminate all microbial organisms including molds.

Other risky contaminants of pharmaceutical preparations are viruses, prions, endotoxins/pyrogens, and biofilms.

### 5.1. Factors influencing growth and survival of microorganisms in pharmaceutical preparations

The common factors supporting survival of microbial organisms are classified in:

- extrinsic factors like temperature
- intrinsic factors like water content, nutrients, pH, moisture, and oxygen.

#### Temperature

Depending on the optimum temperature for growth, microorganisms are divided into three groups (32):

- Psychrophiles: grow at 0-5 °C, not above 30 °C.
- Mesophiles: optimum growth temperature varies from 25-40 °C, no growth either above 45 °C or under 5 °C. This group includes the human pathogenic organisms that grow at 37 °C.
- Thermophiles: grow at temperatures above 55 °C.

Because of significant growth and multiplication of pathogens at 25-40 °C, it is recommendable to store the pharmaceutical preparations under refrigerated conditions (2-8 °C) and to use preservatives whenever possible to minimize improper microbial growth. However water for injection (WFI) as bulkware should be kept above 80 °C after distillation and prior to filling and sterilization in order to prevent microbial growth (28).

**Water activity:**

Water activity $a_w$ describes the ratio of water vapor pressure of a pharmaceutical product (p) to the vapor pressure of pure water at the same temperature ($p_o$): $a_w = p/p_o$.
Microbial growth in pharmaceutical preparations is proportional to water activity; it is inhibited at low values of $a_w$ and increases with increased $a_w$ values. Parenterally administered ready-to-use preparations generally have a water activity > 0.6 which enable microorganisms to grow.

**Nutrient content**:

Microorganisms require particular source of energy, , minerals and trace elements to enable their growth and keeping on metabolic functions. The amount and type of nutrients required is dependent upon the type of microorganism. A few species like pseudomonads can even grow in distilled water and reach concentrations up to $10^6$ CFU/mL (28).

**pH:**

The optimum pH range for the growth of microorganisms varies. A pH range of 6-7 supports the growth of most bacteria. Increasing acidity reduces bacterial growth. Pathogenic bacteria will not grow below pH 4.5 but they may survive long times in weakly acidic solutions. Pseudomonads grow at a pH range of 6-9-. Optimum pH for the growth of most fungi is pH 5 (28). Furthermore, the change of the pH value can influence other intrinsic factors like water activity and redox potential and thereby microbial growth.

**Redox potential:**

Microorganisms are categorized into 3 groups based on the oxygen concentration necessary for growth: aerobes (grow in presence of oxygen), anaerobes (grow in absence of oxygen), and facultative anaerobes (grow either in presence or absence of oxygen). This is now defined in terms of oxidation-reduction potential $E_h$ (28). Nutrient media used for microbiological testing of aerobes or anaerobes require specified redox potentials to ensure growth of the bacteria. The redox potential of aerobic medium is +300 mV and of anaerobic medium (e.g. thioglycolate) is -200 mV (28).

**Growth model of microorganisms in pharmaceutical preparations**

Microbial growth follows a sigmoid curve including a lag phase, log phase and stationary phase (compare curve A: growth in Figure 1). After entry of microorganisms into the medium, the microorganisms adapt to the environment and their number remains nearly unchanged (lag phase). After adaption to the medium, they grow rapidly in what is known log phase (exponential phase). The growth rate is arrested after a defined period because essential nutrients are limited; the number of organisms remains constant (stationary phase). Finally the organisms lose their viability (the end of survival or death phase). Fungi behave in a similar way (32).

In some cases, the number of colony forming units decreases initially and increases thereafter (compare curve D: regrowth in Figure 1). This may be observed preparations are inadequately preserved (28).

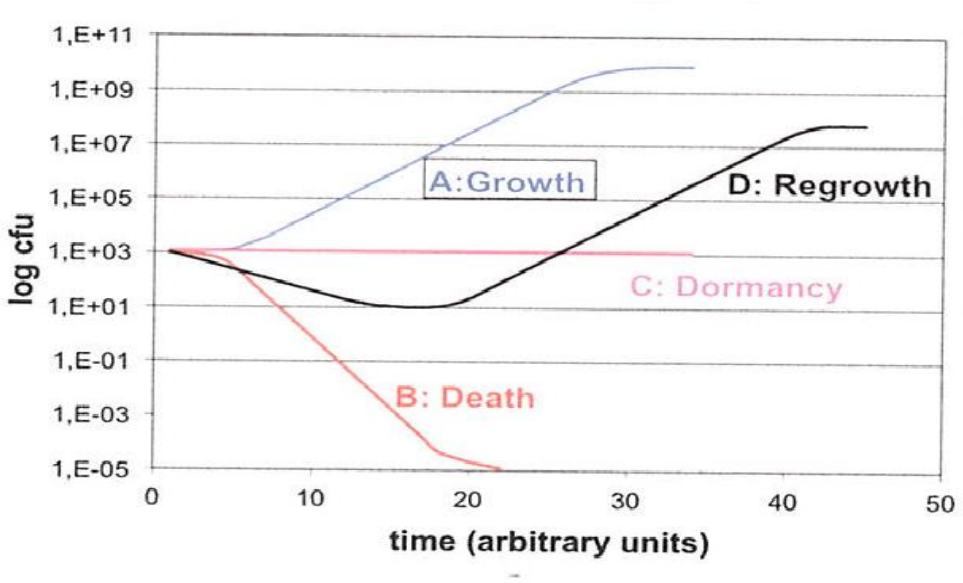



Figure 4: Number of colony forming units over time in different scenarios: growth, regrowth, dormancy and death (cited from ref.(28))

## 2.2. Origin of contamination of pharmaceutical preparations

Knowledge about sources and ways of contamination is necessary in order to avoid microbial contamination during aseptic preparation.

When the contamination occurs during or prior to the preparation it is called primary contamination. Secondary contamination occurs within the period of storage, shipping and usage of products.

- Personnel working in aseptic manufacturing areas are considered the main source of contamination. On the surface of the skin different types of microbes are located in a high number. Thus the main source of contamination is loss of skin scurf by shedding. Most of the bacterial species of the skin flora are non-pathogenic, while some are pathogens like *Staphylococcus aureus*. Shedding also results from improper gowning or unhygienic gloves which facilitate the direct contact between the operators and workplace. In addition, insufficient staff training and education are responsible for microbial contamination of clean room areas. From this perspective, it is important to provide personnel with adequate skills and to monitor the preparation process closely (28).
- Raw materials: Raw material from synthetic origin may be highly contaminated with aerobic spores. Gram positive spores and molds often originate from natural sources (32).
- Water: Water is an essential component in the majority of drug products and it used as vehicle to deliver the products or to clean the work place. However, it encourages the growth and survival of contaminants. From a microbial point of view, it is recommendable to use always freshly distilled und presterilized water in order to diminish the probability of organisms to grow (34).
- Environmental air: Airborn microbial contamination is reduced by filtration and pressure differentials between controlled zones (10). However, airborne contaminants (gram-positive bacilli, skin cocci, molds, bacterial spores) can enter preparation areas via dust, soil particles, or shedding of skin scurfs, and by means of raw materials (28, 32, 33).
- Equipment and Facilities: The equipment is rarely considered as source of microbial contamination, due to regularly cleaning and disinfection. Even though contaminants can enter products from facility equipment, because of inadequate cleaning. In the working area dry surfaces can be contaminated by gram positive rods, cocci and fungal spores, whilst wet surfaces like sinks, drains or any cleaning materials can be contaminated by waterborne species. It should be kept in mind, that the cleaning process should be performed carefully following strict regulations to avoid any opportunity of

contamination or/and cross contamination (32,33,34). Disinfectant solutions and sporicidal desinfectants should be selected according to the efficacy of disinfection and safety of personnel.

- Primary packaging and closures: Primary packaging is the first barrier between the environment and the pharmaceutical preparation. Contamination via primary packaging material is especially problematic for sterile preparations. Vials, infusion bottles, or ampoules should be sterile and pyrogen-free before filling (28). The root cause for secondary contamination is mainly insufficient closure integrity (28).

## 2.3. Sterility tests

Sterility of pharmaceutical preparations refers to the absence of all viable microorganisms including living microorganisms, pyrogens, and any particles that have the potential to reproduce, in order to limit the contamination rate of products and minimize the risks on the health care (35). Sterile drug products can be achieved by terminal sterilization or aseptic processing of sterilized components. The objective of sterility tests is to demonstrate that a batch is sterile. As the test method is a destructive one it is not applicable to patient individually prepared parenteral products. Moreover, the shelf life of the products is too short to complete a sterility test over 14 days. The applicability of rapid microbiological methods (RMMs) relying on various methods of microbial growth detection, including adenosine triphosphate (ATP), bioluminescence, $CO_2$ monitoring is under discussion. The recently harmonized Pharmacopoeia tests regulate sampling, sample preparation, inoculation, incubation and interpretation.

### Microbiological test methods

Sterility testing by microbiological test methods demonstrates that a product does not contain any viable microorganism. Suitability test are necessary to show that the growth of microorganisms is not inhibited by the antimicrobial activity of the product. The antimicrobial activity can be caused by the API itself or excipients in drug dosage form (36, 37). Moreover, the physiochemical properties of the drug product like pH, osmolality can impact the growth of microorganisms and lead to antimicrobial activity during the storage and usage period of the preparation. Microbiological testing with alternative methods like rapid microbiological methods, have to be validated and suitability for the intended purpose has to be demonstrated in comparison to the Pharmacopoeia method.

- **Ph. Eur. Membrane Filtration**

The Membrane Filtration test method is applied to filterable pharmaceutical products. After standardized filtration through a 0.45 micron pore filter, the filter is

added to growth media and incubated. The used media are thioglycolate medium (FTM) that promotes the growth of aerobic and anaerobic microorganisms and soybean casein digest medium (SCDM) that promotes the proliferation of aerobic bacteria, yeasts and molds (28, 38, 39).

- **Ph. Eur. Direct transfer**

This is the optimum test method for non-filterable medical products. The prepared sample is directly transferred into the appropriate media. During the incubation period of 14 days, the samples should be checked for turbidity every day (39).

- **ATP Bioluminescence**

The traditional methods used in sterility tests are limited by long incubation periods and the fact that only viable microorganisms can be recovered. The ATP bioluminescence method belongs to the rapid microbiological methods. Adenosine triphosphate (ATP) is a metabolite present in all microorganisms. It can be measured semiquantitatively with the luciferin/luciferase test system as relative light units (RTU) using a luminometer. Despite the widespread use of the ATP bioluminescence method in cleaning and disinfection procedures, it is not possible to use the method in order to evaluate the activity of different types of disinfectants or to validate the disinfection procedures in cleanroom areas (40).

- **Colorimetric growth detection ($CO_2$ monitoring technology)**

The colorimetric growth detection method is based on changes in color of media bottles, due to the production of carbon dioxide ($CO_{2)}$ by microbial metabolism over the growth period (41).

- **Non-growth based: solid state laser scanning cytometry**

This technology can be used to detect a single living cell, without any need to grow. A sample is filtered over am membrane filter. The membrane is mixed with a fluorogenic agent which living cells actively take up. The resulting fluorescence is measured by laser scanning cytometry to detect the source of each fluorescent event. Sample processing and test takes about 2 hours (28).

- **Antimicrobial effectiveness test (AET)**

The antimicrobial effectiveness test is used to measure the effectiveness of a preservative which is added to a multi-dose medicinal product to inhibit the proliferation of microorganisms during use (multiple usage of the product). The test is carried out during development and stability testing of multi-dose parenteral preparations (37). The AET is regulated in the United States Pharmacopeia (USP) <51>, the European Pharmacopeia (Ph. Eur. 8.0), and the Japanese Pharmacopeia (JP) 19 (36,37,42). The microorganisms used are *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* (not Ph. Eur.)*, Aspergillus brasiliensis* and *Candida albicans*. The test organisms are chosen because of their relationship to nosocomial infections (36,37,42). During the test for microbial kill a controlled inoculum of the challenge organism(s) is placed in suspension with the sample to be tested, and then the number of survivors determined at different time points.

According to the standardized AET test method, the viability and growth of test organisms in aseptically prepared ready-to-use preparations was investigated. Most of the tested anticancer and antiviral drug solutions did not exhibit antimicrobial activity. Exceptions were busulfan, carboplatin, cisplatin, dacarbazine, 5-flourouracil, mitomycin and streptozocin (43,44,46). Treosulfan and oxaliplatin solutions suppressed significantly the growth of *P. aeruginosa* (45). Biopharmaceutics (monoclonal antibodies) approved for anticancer therapy did neither support nor retard the microbial growth in the same test scenario. It is important to remember the microbial instability during the preparation and while determining the shelf life of parenterals used in anticancer therapy (46).

The aim of the following studies was to evaluate the growth of four different microorganisms in 14 chemical and biopharmaceutical medicinal products, which were approved for anticancer therapy after the year 2007 and in 17 non-cytotoxic ready-to-use parenteral products prepared in the hospital pharmacy of the University Medical Center Mainz. The microorganisms chosen are commonly associated with nosocomial infections and represent potential contaminants. The test conditions simulate the worst possible conditions for patients and optimal circumstances for the growth of microorganisms.

# *3 Viability of microorganisms in novel chemical and bio-pharmaceutical anticancer drug solutions*

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# Viability of microorganisms in novel chemical and biopharmaceutical anticancer drug solutions

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**Most anticancer drug products used in clinical practice lack antimicrobial properties. Therefore, materials and methods utilised to prepare the parenterally administered preparations must ensure sterility and avoid the introduction of contaminants and the growth of microorganisms. The aim of the study was to evaluate the growth potential of four different microorganisms in diluted ready-to-use novel chemical and biopharmaceutical anticancer drug preparations.**

**In three consecutive series, 14 different antineoplastic drugs were diluted to the lowest customary concentrations in polyolefin containers prefilled with 0.9 % sodium chloride or 5% dextrose solution. Aliquots (9 mL) of each anticancer drug solution were inoculated with 1 mL suspension of bacteria or fungi (*Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa* and *Candida albicans*) to achieve approximately $10^4$ microorganisms per mL. Pure vehicle solutions were used as positive controls in each series. The inoculated preparations were stored at room temperature (22°C) and protected from light. Samples (1 mL) were taken immediately and 1, 3, 5, 24, 48 and 144 hours after inoculation, processed and transferred to tryptic soy agar plates. The plates were incubated at 37°C and the colony-forming units counted after 24 hours.**

**The tested microorganisms remained viable in most of the anticancer drug solutions over a period of 144 hours after inoculation. Trabectedin was the only product generating distinct and rapid antibacterial activity. Viability of *C.albicans* was not affected by trabectedin, but growth of the fungus was retarded in temsirolimus-containing samples. Nab-paclitaxel suspension supported the growth of the selected bacteria and fungus.**

**Most of the novel anticancer drug products showed neither growth-retarding nor growth-supporting properties. Therefore, in pharmacy departments the anticancer drug products for parenteral administration should be prepared under strict aseptic conditions and refrigerated. Lack of antibacterial and antifungal properties should be considered when assigning extended expiry dates. Attention should be paid to the vulnerability of albumin-containing nab-paclitaxel suspensions to microorganism proliferation.**

**Key words:** Biopharmaceutical anticancer drug solution, microorganisms, antimicrobial activity.

## Introduction

The armamentarium of anticancer drugs is still constantly growing. Recently approved anticancer medicinal products comprise of the innovative signal transduction inhibitors, which can be administered orally, as well as novel small molecules and monoclonal antibodies, which are administered parenterally. The latter are mostly supplied as ready-to-use preparations individually prepared for specific patients by pharmacy-based centralised cytotoxic preparation units. The preparation process must take place in accordance with the regulatory framework of good preparation practice, such as the Pharmaceutical Inspection Cooperation Scheme (PIC/S) PE 010-3[1], the European Pharmacopoeia 7.7 Pharmaceutical Preparations[2], the United States Pharmacopeia Monograph <797>[3], and various national guidelines. Materials and methods used to prepare parenterally administered products must ensure sterility and avoid introduction of contaminants and the growth of microorganisms[2].

Quality tests of individual anticancer drug preparations are limited by singularity of each preparation and time restraints. In most cases, preparations are administered earlier than the results of sterility tests are available. However, the results of sterility tests, media fills and environmental monitoring programs are useful for process validation.

This is especially true because most of the anticancer drug preparations lack antimicrobial properties[4-7]. Of note, growth of microorganisms was not stimulated in previously tested monoclonal antibody-containing preparations[4]. There was no obvious direct link between chemical structures or

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pharmacological principles and antimicrobial activity in our previous studies. Moreover, there was no correlation between antifungal and antibacterial activity, and antimicrobial activity proved to be species specific.

During the validation of the sterility tests, the manufacturers of medicinal products have to determine the extent to which bacteria survive in the products. The aim of the present study was to evaluate the growth of four different microorganisms in diluted ready-to-use preparations of 14 chemical and biopharmaceutical medicinal products approved for anticancer therapy after the year 2007. The microorganisms chosen are commonly associated with nosocomial infections and represent potential contaminants. The test conditions simulate the worst possible conditions for patients and optimal circumstances for the growth of microorganisms.

## Materials and methods

The study was performed in three consecutive series, investigating the antimicrobial activity of four drug products in the first series (aflibercept, brentuximab-vedotin, nelarabine, ofatumumab), four drug products in the second series (arsenic trioxide, temsirolimus, ipilimumab, cabazitaxel), and six drug products in the third series (trabectedin, eribulin, fotemustine, nab-paclitaxel, panitumumab, clofarabine). During each of the three series, the vehicle solutions (0.9% sodium chloride (NaCl) solution, 5% dextrose solution) were tested in parallel and acknowledged as a positive control measure.

### *Preparation of inocula*

The preparation of inocula was carried out at the Institute of Medical Microbiology and Hygiene, University Medical Center Mainz, Germany. The four microorganisms selected for the study were *Staphylococcus aureus* ATCC strain 6538, *Pseudomonas aeruginosa* ATCC strain 15542, *Enterococcus faecium* ATCC strain 6057, and *Candida albicans* ATCC strain 10231. The microorganisms were cultured on agar plates (CASO-Agar, Oxoid Deutschland GmbH; LOT 1406767) for 24 hours (*C. albicans* 72 h) at 37°C.

Subsequently, the cultures were harvested and suspended in 0.9% NaCl solution. The concentration of microorganisms was adjusted by matching the turbidity of the suspensions with McFarland standards. The McFarland standards used to achieve a microorganism concentration of $10^8$ colony forming units (CFU)/mL were 0.5 for *S.aureus* and *E.faecium*, 0.2 for *P.aeruginosa*, and 2.5 for *C.albicans*. The inocula were further diluted with 0.9% NaCl solution to achieve viable counts of approximately $10^5$/mL.

### *Sample preparation*

The 14 antineoplastic drug products tested, solutions used for reconstitution and dilution, the final concentrations, and the in-house data regarding physicochemical stability of the preparations are shown in **Table 1**.

The drug products were aseptically reconstituted according to the manufacturers' recommendations in the centralised cytotoxic drug preparation unit of the Pharmacy Department of the University Medical Center Mainz, Germany. Vehicle solutions and container material were selected in order to guarantee maximal chemical and physical stability. Samples were prepared by injecting the calculated amount of each concentrated solution into a polyolefin bag containing a nominal volume of 50 mL 0.9% NaCl infusion solution (Freeflex Isotonische Kochsalzlösung Infusionslösung, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany; LOT 13GBS271, LOT 13GGS152) or a polyolefin bag containing a nominal volume of 250 mL 5% dextrose/glucose solution (Freeflex Glucosteril® 5% Infusionslösung, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany; LOT 14GD7107) in order to achieve the predetermined concentrations. These final concentrations were the lowest ones prescribed by the physicians in daily clinical practice.

Nelarabine injection solution (Atriance®) was transferred to an empty infusion bag (MIB300 - EVA mixing and infusion bag, Hegewald Medizinprodukte GmbH, 09638 Lichtenberg, Germany; LOT 3GH71302), and used without any further dilution. Nab-paclitaxel (Abraxane®, Celgene, München, Germany) suspension was prepared by adding 20 mL of 0.9% NaCl infusion solution (Freeflex Isotonische Kochsalzlösung Infusionslösung, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany; LOT 14GG7346) to 3 vials each and 7 mL aliquots were withdrawn directly from the vials.

Aliquots amounting to 9 mL were aseptically withdrawn in quadruplicate from each sample preparation (except nab-paclitaxel) and transferred to sterile empty containers (15 mL centrifuge tubes with screw caps, VWR International, Radnor PA, USA; LOT 317CE).

### *Inoculation*

Prior to inoculation, each tube containing bacterial or fungal suspension was carefully homogenised with an automatic shaker for 2 minutes in order to suspend CFU adhering to surfaces and to ensure the uniformity of the suspensions. Subsequently, 1 mL of the suspension was added to 9 mL of the freshly prepared drug product to achieve concentrations of approximately $10^4$ microorganisms per mL. As an exception, 7.0 mL of nab-paclitaxel suspension was inoculated with 0.7 mL of bacteria or fungal suspension to achieve the target concentration of microorganisms.

NaCl solution (0.9%) and 5% dextrose solution (same brand and batch number as the vehicle solution) inoculated with microorganisms in the same manner, served as positive controls. Dextrose solution (5%) served as a positive control for the fotemustine (Muphoran®, Servier) drug preparation and was only used in the third series. The inoculated preparations were stored at room temperature (22°C) and protected from light.

### *Sampling, incubation and analysis*

Samples (1 mL) were taken immediately and 1, 3, 5, 24, 48 and 144 hours after inoculation. Samples were diluted 1:10 consecutively three times by using tubes prefilled with 0.9% NaCl solution (NaCl (phys.) 9 mL 2084r-100p, Heipha Dr. Müller GmbH; LOT 125309). Finally 0.1 mL

**Table 1.** Characteristics of the anticancer drug preparations inoculated with microorganisms.

| Active drug substance | Brand name | Manufacturer | Lot No | Solution used for reconstitution | Vehicle solution | Test concentration [mg/mL] | Physico-chemical stability at room temperature |
|---|---|---|---|---|---|---|---|
| Aflibercept | Zaltrap® | Sanofi | 2F003A | – | 0.9% NaCl | 1.2 | 8 h |
| Arsenic trioxide | Trisenox® | Teva | PL4037301 | – | 0.9% NaCl | 0.04 | 24 h |
| Brentuximab-vedotin | Adcetris® | Takeda Pharma | 228427 | Water for injection | 0.9% NaCl | 0.4 | 24 h* |
| Cabazitaxel | Jevtana® | Sanofi | 3F854A | Ethanol 13% | 0.9% NaCl | 0.17 | 28 d |
| Clofarabine | Evoltra® | Genzyme | K2003H07 | – | 0.9% NaCl | 0.07 | 28 d |
| Eribulin mesilate | Halaven® | Eisai | 107568 | – | 0.9% NaCl | 0.07 | 28 d |
| Fotemustine | Muphoran® | Servier | 900113 | Ethanol 95% + water | 5% dextrose | 0.2 | 8 h |
| Ipilimumab§ | | Bristol-Myers Squibb | OC62104 | – | 0.9% NaCl | 1.5 | 24 h |
| Nelarabine | Atriance® | GlaxoSmithKline | C643808 | – | – | 800 mg† | 28 d |
| Ofatumumab | Arzerra® | GlaxoSmithKline | C623687 | – | 0.9% NaCl | 3.0 | 48 h |
| Nab-paclitaxel | Abraxane® | Celgene | 6200014A | 0.9% NaCl | – | 5 mg‡ | 8 h |
| Panitumumab | Vectibix® | Amgen | 1042168 | – | 0.9% NaCl | 3.07 | 24 h |
| Temsirolimus | Torisel® | Pfizer | AHRE/18 | Ethanol | 0.9% NaCl | 0.1 | 72 h |
| Trabectedin | Yondelis® | PharmaMar | 13065 | Water for injection | 0.9% NaCl | 0.005 | 30 h |

§ Clinical trial material; † undiluted test solution; ‡ undiluted test suspension; * refrigerated.

aliquots of the maximum diluted samples were transferred to tryptic soy agar plates (CASO-Agar, Oxoid Deutschland GmbH; LOT 1406767). The plates were incubated for 24 hours at 37°C and the CFU counted. As an exception, the plates used for culturing mixtures of temsirolimus test solution with *C.albicans* were incubated for an additional 72 hours; the CFU were counted after both periods. All microorganisms recovered were checked to confirm their identity with the organisms initially inoculated.

## Results

Detailed results of the viability of the four different strains in 14 different anticancer drug solutions and control solutions are given in **Tables 2–5**. Viability of the four test organisms in five different drug solutions (cabazitaxel, clofarabine, nab-paclitaxel, ofatumumab, trabectidin) are illustrated in **Figure 1**.

In most of the anticancer drug solutions, the selected strains remained viable over a period of 144 hours after inoculation. With the exception of nab-paclitaxel and trabectedin, the anticancer drug products did not have an impact on the viability of the microorganisms tested. The number of viable counts were species specific and reflected the growth in nutrient-poor vehicle solutions. The number of viable strains decreased in solutions inoculated with *S.aureus* and *E.faecium*, remained unchanged in solutions inoculated with *C.albicans* and increased in solutions inoculated with *P.aeruginosa*.

Trabectedin was the only product generating distinct and rapid antibacterial activity. Already 3 hours after inoculation, *S.aureus* and *E.faecium* had lost viability and 48 hours after inoculation *P.aeruginosa* had lost viability. However, viability of *C.albicans* was not affected by trabectedin (see **Figure 2**).

Growth of *C.albicans* was retarded when temsirolimus-containing samples were inoculated. No growth of *C.albicans* was detected after 24 hours of incubation. Growth was obvious after a further 3 days of incubation. The results given in **Table 5** were obtained after 4 days of incubation. The number of CFU remained unchanged over the test period. According to these findings, temsirolimus affects the growth of *C.albicans*, but does not affect the viability of *C.albicans*.

Growth promotion of the selected strains was not significant in monoclonal antibody drug solutions. However, nab-paclitaxel suspension supported the growth of the selected bacteria and fungus (see **Figure 3**).

**Table 2.** Viability of *S.aureus* in anticancer drug solutions and control solutions.

| Drug/control solution | *S.aureus* (CFU log/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 3 h | 5 h | 24 h | 48 h | 144 h |
| 0.9% NaCl | 3.96 | 4.09 | 3.85 | 3.80 | 3.26 | 2.30 | n.a. |
| Aflibercept | 4.07 | 4.20 | 4.06 | 4.21 | 4.05 | 3.97 | 3.96 |
| Brentuximab-vedotin | 4.01 | 3.98 | 4.02 | 4.13 | 4.02 | 4.03 | 3.76 |
| Nelarabine | 4.00 | 3.77 | 3.75 | 3.72 | 3.20 | 3.00 | n.a. |
| Ofatumumab | 4.03 | 3.98 | 3.96 | 4.09 | 3.99 | 3.90 | 3.61 |
| 0.9% NaCl | 3.86 | 4.07 | 4.05 | 3.91 | 3.60 | 3.40 | 2.18 |
| Arsenic trioxide | 4.16 | 4.08 | 3.96 | 3.91 | 3.66 | 3.48 | n.a. |
| Cabazitaxel | 4.13 | 4.05 | 3.93 | 3.95 | 3.90 | 3.85 | n.a. |
| Ipilimumab | 4.04 | 4.06 | 4.14 | 4.13 | 4.13 | 3.99 | 4.04 |
| Temsirolimus | 4.08 | 4.12 | 4.11 | 4.09 | 4.04 | 3.97 | 2.74 |
| 0.9% NaCl | 3.78 | 3.84 | 3.62 | 3.65 | 3.28 | 2.60 | n.a. |
| 5% Dextrose | 4.09 | 4.05 | 4.04 | 4.04 | 3.59 | 3.26 | 2.40 |
| Clofarabine | 3.96 | 4.04 | 3.79 | 3.94 | 3.33 | 3.16 | 2.18 |
| Eribulin | 3.87 | 3.93 | 3.85 | 3.71 | 3.46 | 3.10 | 2.40 |
| Fotemustine | 4.06 | 4.06 | 3.98 | 3.99 | 3.56 | 3.18 | 2.13 |
| Nab-paclitaxel | 4.01 | 4.04 | 3.99 | 4.05 | 5.02 | 5.78 | 6.02 |
| Panitumumab | 4.18 | 4.05 | 4.14 | 4.17 | 3.97 | 3.92 | 3.48 |
| Trabectedin | 3.26 | 2.74 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

**Table 3.** Viability of *P.aeruginosa* in anticancer drug solutions and control solutions.

| Drug/control solution | *P.aeruginosa* (CFU log/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 3 h | 5 h | 24 h | 48 h | 144 h |
| 0.9% NaCl | 3.65 | 3.10 | 3.37 | 3.22 | 2.95 | 4.66 | 6.15 |
| Aflibercept | 3.71 | 3.63 | 3.37 | 3.49 | 5.22 | 5.29 | 6.28 |
| Brentuximab-vedotin | 3.72 | 3.37 | 3.62 | 3.33 | 5.34 | 6.18 | 6.41 |
| Nelarabine | 3.48 | 3.47 | 3.06 | 3.02 | 3.04 | 4.31 | 4.62 |
| Ofatumumab | 3.68 | 3.56 | 3.56 | 3.30 | 3.18 | 3.41 | 3.56 |
| 0.9% NaCl | 3.67 | 3.38 | 3.38 | 3.35 | 3.64 | 4.24 | 6.04 |
| Arsenic trioxide | 3.50 | 3.44 | 3.40 | 3.23 | 3.34 | 4.49 | 6.25 |
| Cabazitaxel | 3.69 | 3.78 | 3.85 | 3.82 | 4.95 | 5.68 | 6.18 |
| Ipilimumab | 3.63 | 3.55 | 3.68 | 3.70 | 3.94 | 3.77 | 5.00 |
| Temsirolimus | 3.71 | 3.66 | 3.64 | 3.51 | 3.94 | 5.17 | 5.61 |
| 0.9% NaCl | 3.95 | 3.78 | 3.87 | 3.72 | 4.20 | 5.25 | 6.12 |
| 5% Dextrose | 3.68 | 3.59 | 3.51 | 3.54 | 3.16 | 5.39 | 5.50 |
| Clofarabine | 3.66 | 3.46 | 3.30 | 3.13 | 3.88 | 5.54 | 6.19 |
| Eribulin | 4.07 | 3.95 | 3.84 | 3.82 | 4.41 | 5.16 | 5.63 |
| Fotemustine | 3.77 | 3.61 | 3.60 | 3.55 | 3.00 | 2.85 | 3.02 |
| Nab-paclitaxel | 3.69 | 3.65 | 3.87 | 3.86 | 6.24 | 6.90 | 7.22 |
| Panitumumab | 3.64 | 3.72 | 3.42 | 3.36 | 3.22 | 4.29 | 6.36 |
| Trabectedin | 3.64 | 3.60 | 3.44 | 2.93 | 2.40 | 0.00 | 0.00 |

**Table 4.** Viability of *E.faecium* in anticancer drug solutions and control solutions.

| Drug/control solution | *E.faecium* (CFU log/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 3 h | 5 h | 24 h | 48 h | 144 h |
| 0.9% NaCl | 3.92 | 3.88 | 3.95 | 3.94 | 3.85 | 3.80 | 2.94 |
| Aflibercept | 3.93 | 3.95 | 3.91 | 3.93 | 4.31 | 3.86 | 4.03 |
| Brentuximab-vedotin | 3.89 | 4.06 | 3.94 | 4.04 | 3.95 | 3.79 | 3.88 |
| Nelarabine | 3.92 | 3.95 | 3.95 | 4.05 | 3.94 | 4.02 | 3.93 |
| Ofatumumab | 3.98 | 4.11 | 3.69 | 3.84 | 3.08 | 3.11 | 2.60 |
| 0.9% NaCl | 4.09 | 4.12 | 4.04 | 4.03 | 3.92 | 3.87 | 3.76 |
| Arsenic trioxide | 4.13 | 4.07 | 4.02 | 4.00 | 4.05 | 3.97 | 3.86 |
| Cabazitaxel | 3.95 | 4.00 | 4.03 | 4.01 | 3.47 | 3.45 | 2.95 |
| Ipilimumab | 4.02 | 4.08 | 4.08 | 4.07 | 4.07 | 3.89 | 3.87 |
| Temsirolimus | 4.09 | 4.01 | 4.07 | 4.09 | 3.97 | 3.92 | 3.91 |
| 0.9% NaCl | 4.00 | 4.07 | 3.97 | 3.97 | 3.99 | 4.01 | 3.76 |
| 5% Dextrose | 4.10 | 4.15 | 4.05 | 4.08 | 3.80 | 3.45 | 2.95 |
| Clofarabine | 4.13 | 4.10 | 4.02 | 4.11 | 4.07 | 3.97 | 3.89 |
| Eribulin | 4.15 | 4.10 | 4.14 | 4.00 | 4.01 | 3.98 | 3.27 |
| Fotemustine | 4.06 | 4.05 | 4.02 | 4.03 | 4.01 | 3.81 | 3.45 |
| Nab-paclitaxel | 4.10 | 4.10 | 4.12 | 4.17 | 5.35 | 6.02 | 4.93 |
| Panitumumab | 4.07 | 4.06 | 4.04 | 4.06 | 4.09 | 4.02 | 3.93 |
| Trabectedin | 3.78 | 3.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

**Table 5.** Viability of *C.albicans* in anticancer drug solutions and control solutions.

| Drug/control solution | *C.albicans* (CFU log/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 3 h | 5 h | 24 h | 48 h | 144 h |
| 0.9% NaCl | 4.16 | 4.04 | 4.00 | 4.10 | 4.15 | 4.74 | 4.92 |
| Aflibercept | 3.99 | 4.10 | 3.98 | 4.04 | 4.55 | 4.89 | 4.84 |
| Brentuximab-vedotin | 4.01 | 4.01 | 3.99 | 4.12 | 4.05 | 3.96 | 4.39 |
| Nelarabine | 3.97 | 4.03 | 4.05 | 4.14 | 4.78 | 4.81 | 4.99 |
| Ofatumumab | 4.03 | 4.04 | 3.97 | 4.06 | 4.36 | 4.66 | 4.54 |
| 0.9% NaCl | 4.19 | 4.19 | 4.17 | 4.12 | 4.23 | 4.33 | 4.71 |
| Arsenic trioxide | 4.10 | 4.10 | 4.14 | 4.13 | 4.20 | 4.22 | 4.84 |
| Cabazitaxel | 4.09 | 4.17 | 4.00 | 4.06 | 4.15 | 4.72 | 4.85 |
| Ipilimumab | 4.19 | 4.14 | 4.17 | 4.16 | 4.33 | 4.64 | 4.28 |
| Temsirolimus | 4.07 | 4.10 | 4.08 | 4.05 | 4.11 | 4.11 | 4.04 |
| 0.9% NaCl | 4.11 | 4.05 | 4.08 | 4.16 | 4.23 | 4.34 | 4.26 |
| 5% Dextrose | 4.15 | 4.25 | 4.23 | 4.27 | 4.77 | 4.90 | 4.94 |
| Clofarabine | 4.18 | 4.10 | 4.09 | 4.12 | 4.53 | 4.63 | 4.70 |
| Eribulin | 4.10 | 4.04 | 4.14 | 4.11 | 3.31 | 3.54 | n.a. |
| Fotemustine | 4.13 | 4.09 | 4.14 | 4.24 | 4.99 | 5.06 | 5.18 |
| Nab-paclitaxel | 4.09 | 4.24 | 4.17 | 4.11 | 4.82 | 5.17 | 6.36 |
| Panitumumab | 4.05 | 4.12 | 4.08 | 4.23 | 4.80 | 5.13 | 5.03 |
| Trabectedin | 4.12 | 4.01 | 4.04 | 4.06 | 4.66 | 4.90 | 5.11 |

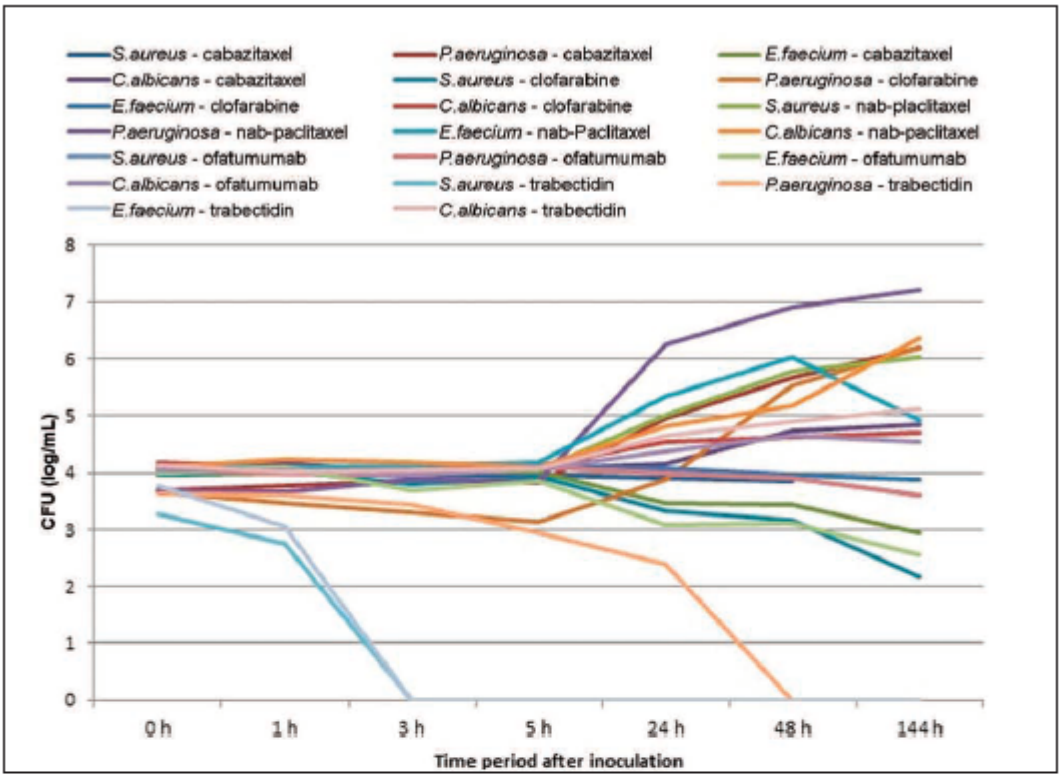


**Figure 1.** Viability of the four test organisms in diluted solutions of cabazitaxel, clofarabine, nab-paclitaxel, ofatumumab and trabectidin used as examples.

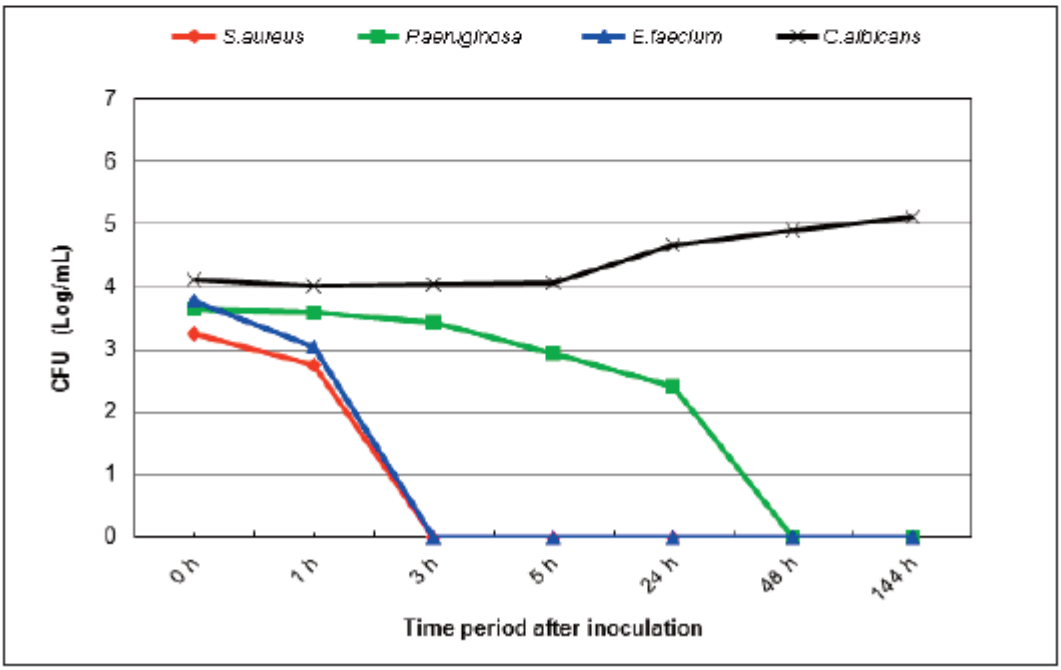


**Figure 2.** Viability of test microorganisms in trabectedin preparations 0.005 mg/mL and 0.9% NaCl vehicle solution.

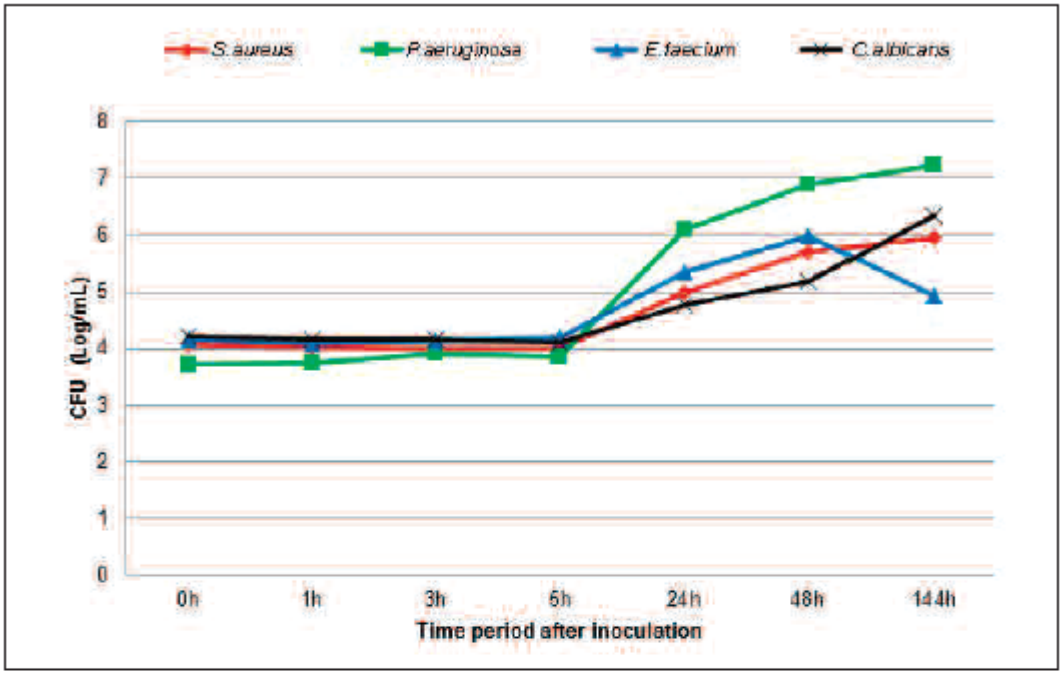


**Figure 3.** Viability of test microorganisms in nab-paclitaxel suspension (5 mg/mL).

## Discussion

Consistent with the results of the previous studies[4–7], microorganisms retained viability in anticancer drug preparations and proliferated when transferred to proper growth media. Viability patterns of the microorganisms in the control solutions reflected the known viability of the selected species in nutrient-poor solutions over the test period. However, the experiments were designed to provide optimal conditions for the viability of microorganisms and worst conditions for the antimicrobial activity of the drug preparations.

The lowest drug concentrations used in clinical practice were chosen and inoculated test solutions were stored at 22°C. The lack of antimicrobial activity may, therefore, be caused by concentrations lower than the minimal inhibitory concentrations. Because dilution is not recommended by the manufacturer, nelarabine and nab-paclitaxel were inoculated without further dilution. Despite the high concentrations, nelarabine did not restrict the viability of microorganisms and nab-paclitaxel even promoted the viability of bacteria.

Regardless of the physicochemical stability of the drug solutions, samples were withdrawn up to 5 days after inoculation. According to the summaries of product characteristics and known literature, physicochemical stability of aflibercept[8], fotemustine[9] and nab-paclitaxel[10] is limited to 8 hours at room temperature. Arsenic trioxide[11], brentuximab-vedotin[12], ipilimumab[13] and panitumumab[14] are reported to be physicochemically stable over 24 hours and trabectidin[15] over 30 hours at room temperature. Shelf-lives of ofatumumab[16] and temsirolimus[17] are also shorter than 5 days when stored at room temperature (see **Table 1**). Therefore, viability of the microorganisms can be related to the parent drug substance and/or degradation products.

Trabectedin binds to the minor groove of DNA thereby affecting several transcription factors, DNA binding proteins and DNA repair pathways. The resulting cell cycle perturbation causes *in vivo* antiproliferative activity against a range of human tumour cell lines[18]. To the best of our knowledge, this is the first report about the bactericidal activity of trabectedin. This finding should be kept in mind when sterility tests are evaluated, because of the chance of false-negative results. The spectrum of antibacterial activity was not in the scope of our tests and it remains to be determined whether the antibacterial activity is general.

In accordance with previously reported results[4], the antibody drug solutions exhibited neither antibacterial nor antifungal activity and did not encourage the reproduction of the microorganisms. So far, it is obvious that the microorganisms selected for inoculation are not able to use antibody-containing preparations as a nutrient source. The microbial growth potential of antibody-containing preparations is similar to that of chemical substance-containing preparations.

The growth-supporting activity of nab-paclitaxel suspension must be related to the human serum albumin-containing nanoparticles. The medicinal product contains 100 mg paclitaxel and 800 mg albumin as lyophilised powder which is reconstituted with 20 mL of 0.9% NaCl solution[19]. The resulting suspension contains 5 mg paclitaxel and 4% albumin. The growth-promoting activity

of human albumin (final concentration 1.25%) admixed to 0.9% NaCl solution or total parenteral nutrition solutions is well-known[20]. The test procedure, inoculated strains and results are similar to those reported here. Moreover, there is an article describing *Enterobacter cloacae* bloodstream infections that had been traced to contaminated human albumin harbouring in cracks in the vials[21].

It is the responsibility of the pharmacist to assign expiry dates to the prepared ready-to-use parenteral products. Physicochemical as well microbiological stability should be taken into account when the expiry dates are determined. According to our written policies, expiry dates are limited to 72 hours (storage at 2–8°C or room temperature) as long as physicochemical stability of the preparations is proven over this period. If physicochemical stability is not proven within this interval, the expiry dates are assigned according to the known physicochemical stability. Whenever possible, the preparations should be kept refrigerated in order to diminish microbial instability.

## Conclusions

Most of the novel anticancer drug products tested showed neither growth-retarding nor growth-supporting properties. Therefore, in pharmacy departments the solutions for parenteral administration should be prepared under strict aseptic conditions, and appropriate quality assurance programs should be established in order to guarantee the sterility of drug preparations. Except for trabectedin, end-product sterility testing may be performed without further dilution or inactivation. The lack of antibacterial and antifungal properties should be considered when assigning extended expiry dates to ready-to-use solutions of most antineoplastic drugs. Attention should be paid to the vulnerability of albumin-containing nab-paclitaxel suspensions to microorganism proliferation. All ready-to-use preparations should be kept refrigerated whenever possible to inhibit the growth of any contaminating organism.

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# *4 Viability of selected microorganisms in non-cytotoxic aseptic preparations*

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# Viability of Selected Microorganisms in Non-Cytotoxic Aseptic Preparations

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**Abstract**

**Background:** Numerous ready-to-use parenteral solutions are aseptically prepared in pharmacy-based aseptic preparation units. Microbiological stability of the preparations is influenced by the cleanroom environment, the complexity of the aseptic process, conditions during administration and the microbiological vulnerability of the products.

**Object:** The aim of the study was to evaluate the ability of four different pathogens related to hospital infections to grow in ready-to-use, non-cytotoxic parenteral products aseptically prepared in hospital pharmacies.

**Method:** In four consecutive series the antimicrobial activity of the following products was tested: caspofungin 35 mg or 70 mg in 250 mL 0.9% NaCl solution (NS), micafungin 0.5 mg/mL in NS, vancomycin 5 mg/mL in G5/G10, heparin-sodium 1 IE/mL in NS, epinephrine 0.02 mg/mL in G5, norepinephrine 0.01 mg/mL in G5, phenylephrine 0.1 mg/mL, KCl solution 0.8 mmol/mL, trace elements 1:1 in G5/G10, midazolam 1 mg/mL injection solution, tranexamic acid 100 mg/mL injection solution, 50% glucose solution, SMOFlipid 20% lipid emulsion, 1% propofol injection.

Nine milliliter aliquots of each test solution were inoculated with 1 mL suspension of selected strains, i.e. *S. aureus*, *P. aeruginosa*, *E. faecium* or *C. albicans*. Samples of the inoculated solutions were taken in predefined intervals up to 144 h and transferred to tryptic soy agar plates. The plates were incubated at 37°C and colony forming units (CFUs) counted after 24 h for bacteria and after 72 h in the case of *C. albicans*.

**Results:** Most of the tested preparations induced no growth inhibition of the tested organisms. The selected strains lost viability in preparations containing vancomycin, phenylephrine or midazolam after a period of a few hours or days. Glucose 50% w/v solution generated antimicrobial activity against *P. aeruginosa* and *C. albicans* immediately after inoculation. In tranexamic acid solutions only *P. aeruginosa* lost viability after 48 h of inoculation. In the lipid containing emulsions, CFUs increased rapidly. Low pH values and high osmolality are probably the reason for growth inhibition in midazolam and 50% glucose solutions, respectively. The antimicrobial activity of phenylephrine solutions is caused by the excipient sodium metabisulfite.

**Conclusion:** The lack of antimicrobial properties of ready-to-use, non-cytotoxic solutions should be considered while determining the shelf-life of the products. Ready-to-use preparations should be kept refrigerated whenever possible to inhibit the multiplication of any contaminating organism.

**Keywords:** growth, microorganism, non-cytotoxic preparations, viability

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## Introduction

Today in most German hospitals various ready-to-use or ready-to-administer parenteral products are prepared in the hospital pharmacy departments. The portfolio of products encompasses cytotoxic as well as non-cytotoxic preparations. Typical non-cytotoxic product types are parenteral nutrition solutions, electrolytes, emergency drugs, analgesics, antibiotics, and antifungals. But the pharmacy based preparation service is never all-encompassing and a lot of preparations are still to be reconstituted in clinical areas. The quality-assured aseptic processing within pharmacies is performed following national (e. g. Apothekenbetriebsordnung [1], ADKA Guideline [2]) and international regulations and guidelines (PIC/S PE 010-3 [3], Ph.Eur.7.7 Monograph Pharmaceutical Preparations [4], Council of Europe Resolution CM/ResAP (2011)1 [5], USP Monograph <797> Pharmaceutical Compounding – Sterile Preparations [6]). By pharmacy-based aseptic processing in well-controlled environments and quality assurance driven procedures the risk of preparation errors and microbial contamination is considerably reduced. Sterility tests of extemporaneously and batchwise prepared products are limited by

singularity of each preparation and time restraints. However the results of sterility tests, media fills, and environmental monitoring programs are useful for process validation. Preparation in uncontrolled environments such as clinical areas is associated with a higher potential for microbiological contamination and an increased risk of systemic infection after administration. For the individual product types different factors influencing the risk of microbial contamination and infection are discussed. The specific factors are complexity of the preparation, time between preparation and administration, extended periods of administration, elevated temperatures during administration by infusers and other ambulatory devices and last not least the growth potential of the preparation. Multiple additions of multiple components, mixing of large volumes and growth promoting qualities of the mixed components increase the risk of microbial contamination of the preparations and the risk of infection for the patients. If preparations are contaminated with microorganisms, viability and growth of the specific microorganisms in the specific preparations determine the infection risk for the patient. Previously we investigated the growth of microorganisms in antineoplastic drug preparations and found out that most preparations lack antimicrobial properties and monoclonal antibody preparations do not stimulate the growth of microorganisms [7–11]. From the literature it is known, that lipid emulsions support bacterial and fungal growth and bear an increased risk for iatrogenic infections [12–20]. Species-specific growth inhibition is reported for heparin 100 U/mL [21], midazolam [22] and local anaesthetics [23]. Recently the lack of growth inhibition of some vasopressor infusion solutions was published [24]. For a number of parenteral products prepared in our central intravenous additive service information about growth promotion or inhibition is missing. Moreover tests are done under different experimental conditions and extrapolation of the results is hardly possible. Therefore the aim of the study was to evaluate the ability of four different pathogens related to hospital infections (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Candida albicans*) to grow in 17 ready-to-use solutions typically prepared in the aseptic preparation unit of the Pharmacy Department of the University Medical Center Mainz. The chosen test conditions promoted the growth of germs and simulated daily practice.

## Material and methods

The viability of the selected microbes was tested by inoculation of 17 non-cytotoxic ready-to-use aseptic preparations belonging to the portfolio of the pharmacy based aseptic preparation service. The experiments were carried out in four consecutive series. In the first series the antimicrobial activity of caspofungin 35 mg or 70 mg in 250 mL 0.9% NaCl solution (NS), micafungin 50 mg in 100 mL 0.9% NS, heparin sodium 1 IE/mL in NS and 50% w/v glucose solution was tested. In the second series adrenaline (epinephrine) 0.02 mg/mL in G5, noradrenaline (norepinephrine) 0.01 mg/mL in G5, trace elements in G5 1:1, and vancomycin 5 mg/mL in G5 were examined. In the third series trace elements 1:1 in G10, midazolam1 mg/mL, lipid emulsion 200 mg/mL and vancomycin 5 mg/mL in G10 were inspected. In the fourth series phenylephrine 0.1 mg/mL, 0.8 mmol/mL potassium chloride solution, 1% propofol injection emulsion, and tranexamic acid injection solution 100 mg/mL was tested. Details about the material used and characteristics of the test solutions are given in Table 1.

Pure vehicle solutions (0.9% NaCl solution infusion solution 100 mL (Free Flex Isotonische KochsalzLösung NaCl 0.9, Fresenius Kabi, lot 13HLS251, expiration date 10/2016), glucose 5% infusion solution and glucose 10% infusion solution prepared from glucose 70% (Glucose 70%, 500 mL, B Braun, lot 144238062), water for injection (Ampuwa® 1,000 mL, Fresenius Kabi, lot 14H129, expiration date09/2017)) were used as control solutions.

## Preparation of inocula

The microorganisms used in this study were *Staphylococcus aureus* ATCC strain 6538, *Pseudomonas aeruginosa* ATCC strain 15442, *Enterococcus faecium* ATCC strain 6057, and *Candida albicans* ATCC strain 10231. The strains were cultivated at the Institute of Medical Microbiology and Hygiene of the University Medical Center Mainz, Germany and cultured on agar plates (BD Trypticase™ Soy Agar, BD Medical, Heidelberg, Germany, Lot 5019173, expiration date 12/04/2015) at 37 °C for 24 h in the case of bacteria. *C. albicans* was cultured for 72 h at 37 °C. The cultures were collected and suspended in 0.9% NaCl solution. After that the density of bacterial suspensions was adjusted by using McFarland standards (0.5 for *S. aureus* and *E. faecium*, 0.2 for *P. aeruginosa* and 2.5 for *C. albicans*) to obtain suspensions of approximately $10^8$ CFUs per milliliter. To achieve suspensions with a density of $10^5$ CFU/mL, the suspensions were diluted with 0.9% sodium chloride solution.

**Table 1:** Characteristics of the test solutions and the medicinal products used for preparation.

| Active drug substance | Medicinal product | Lot No | Solution used for reconstitution | Vehicle solution | Test concentration [mg/mL] | Physico-chemical stability at room temperature(25 ± 2 °C) |
|---|---|---|---|---|---|---|
| Adrenaline (Epinephrine hydrochloride) | Suprarenin® 1 mg/mL, 25 mL Sanofi –Aventis | 20150128 | – | G5 | 0.02 | 14 d[35] |
| Caspofungin35 mg in 250 mL 0.9 % NaCl | Cancidas® 70 mg Powder for reconstitution | 20150216Cas35 | WFI | 0.9% NaCl | 0.14 | 60 h[36] |
| Caspofungin 70 mg in 250 mL 0.9 % NaCl | Cancidas® 70 mg Powder for reconstitution | 20150216Cas70 | WFI | 0.9% NaCl | 0.28 | 60 h[36] |
| Glucose infusion solution 50% w/v | Glucose 70 % B Braun (100 mL) | 20150204G50 | | Aqua ad inject. | 500 | 20 months[37] |
| Heparin Sodium | Heparin-Sodium- 5.000-ratiopharm® | 20150109H50 | G5 | 0.9% NaCl | 1 IE/mL | 365 d[38] |
| Trace elements | InzolenInfantibus S® | 20150224Inz5 | – | G5 | 1:1 | 54 d[35] |
| Trace elements | InzolenInfantibus S® | 20150225Inz10 | – | G10 | 1:1 | 54 d[35] |
| KCl | Potassium chloride 40 mmol/50 mL Pharmacy compounded bulk solution | 20150106KCl40 | – | | 0.8 mmol/mL | 36 months[39] |
| Micafungin | Mycamine® | 02284012 | 0.9 % NaCl | 0.9% NaCl | 0.5 | 15 d[40] |
| Midazolam | Midazolam-Hameln 1 mg/mL | 442070 | – | – | 1 | 36 d[41] |
| Noradrenaline | (Norepinephrinehydrochloride) | Arterenol® 1 mg/mL, 25 mL | 4F079A | – | G5 | 0.01 |
| 28 d[35] | | | | | | |
| Phenylephrine hydrochloride | Pharmacy compounded bulk solution* | 20150216 | 0.9 % NaCl | | 0,1 | 180 d[35] |
| Propofol | Propofol®lipuro 10 mg/mL | 14471035 | – | – | 10 | – |
| Lipidemulsion | SMOFlipid 200 mg/mL | 16HGO152 | – | – | 200 | – |
| Tranexamic acid | Pharmacy compounded bulk solution | 20150116 | WFI | – | 100 | 180 d[35] |
| Vancomycin | Vancomycin CP 1000HikmaPharma GmbH | 20150226 | WFI | G5 | 5 | 17 d[42] |
| Vancomycin | Vancomycin CP 1000HikmaPharma GmbH | 20150226 | WFI | G10 | 5 | 17 d[42] |

Notes: d = days, h = hours, WFI = Water for injection. *The excipients contained are sodium-metabisulfite, trisodium citratedihydrate, citric acid monohydrate, water for injection.

## Preparation of samples and analysis

The tested product solutions were aseptically prepared in the pharmacy based centralized aseptic preparation unit at the University Medical Center Mainz, Germany in a cleanroom environment following Good Preparation Practice Guidelines. Physical and chemical stability of the preparations is proven for at least 5 days (see Table 1). Nine milliliters of each freshly prepared test solution were aseptically transferred in quadruplicate to a sterile empty 15 mL centrifuge tube with screw cap (VWR International Randor PA, USA, Lot No-827CB-27C) and inoculated with 1 mL suspension of bacteria or fungus (*S. aureus, P. aeruginosa, E. faecium, C. albicans*) to achieve a concentration of $10^4$ CFU/mL. The inoculated test solutions were stored at 22°C. An aliquot of one mL was withdrawn immediately and at predetermined time intervals (1, 3, 5, 24, 48, and 144 h) and diluted 1:10 three times by using tubes prefilled with 0.9 % NaCl solution (NaCl (phys.) 9 mL 2084r-100p, Heipha Dr. Müller GmbH, lot 125309, expiration date 16/10/2014 or lot 129336, expiration date 02/08/2015). From each degree of dilution, 0.1 mL aliquots were withdrawn and transferred to tryptic soy agar plates (BD Trypticase™ Soy Agar, BD Medical, Heidelberg, Germany, lot 5019173, expiration date 12/04/2015, and BD BBL™ Stacker ™ Plates, lot 5027214, expiration date19/04/2015) in duplicate ($n = 6$). The plates were incubated at 37 °C and the colony forming units counted after 24 h of incubation for bacteria and 72 h for *C. albicans*. The 6 results counted for each species and time interval were checked for plausibility. A representative value was selected and given as CFU log/mL in table format. For each combination of the 17 aseptically prepared products and the 4 different microorganisms the growth curve was constructed by plotting the number of CFUs per milliliter (expressed as logarithm) against the time interval post inoculation.

## Results

Most of the tested aseptic preparations affected the growth of the test organisms in the same manner as the control solutions (water for injection, NS, G5, G10). The number of CFUs remained unchanged (*E. faecium, C. albicans*), decreased (*S. aureus*) or increased (*P. aeruginosa*) over a period of five days (see Tables 2–5).

**Table 2:** Viability of *S. aureus* in non-cytotoxic drug preparations and control solutions.

| Drug/control solution | *S. aureus* (CFU log/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 3 h | 5 h | 24 h | 48 h | 144 h |
| Caspofungin 35 mg in 250 mL 0.9 %NaCl | 3.84 | 3.04 | 2.30 | 0 | 0 | 0 | 0 |
| Caspofungin 70 mg in 250 mL 0.9 %NaCl | 3.67 | 2.00 | 0 | 0 | 0 | 0 | 0 |
| Glucose infusion solution 50% w/v | 3.68 | 3.43 | 3.61 | 3.67 | 3.69 | 3.56 | 0 |
| Heparin Sodium | 3.64 | 3.60 | 3.48 | 3.55 | 3.40 | 2.85 | 0 |
| Micafungin | 3.62 | 3.66 | 3.70 | 3.68 | 3.76 | 3.65 | 3.24 |
| 0.9% NaCl infusion solution | 3.59 | 3.59 | 2.74 | 3.52 | 3.54 | 2.18 | 0 |
| Adrenaline | 3.62 | 3.42 | 3.41 | 3.13 | 2.60 | 2.40 | 0 |
| Noradrenaline | 3.51 | 3.47 | 3.37 | 3.06 | 2.65 | 2.30 | 0 |
| Vancomycin in G5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trace elements:G5 1:1 | 3.74 | 3.64 | 3.54 | 3.41 | 3.34 | 2.88 | 0 |
| Glucose infusion solution 5% w/v | 3.43 | 3.39 | 3.35 | 3.22 | 2.81 | 2.60 | 0 |
| Trace elements:G10 1:1 | 3.74 | 3.68 | 3.68 | 3.58 | 3.33 | 3.11 | 0 |
| Vancomycin in G10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SMOFlipidemulsion | 3.80 | 3.81 | 3.85 | 4.02 | 6.87 | n. c. | n. c. |
| Midazolam | 3.81 | 3.78 | 3.51 | 3.37 | 2.60 | 0 | 0 |
| Glucose infusion solution 10% w/v | 3.61 | 3.60 | 3.54 | 3.43 | 3.30 | 2.88 | 0 |
| KCl 40 mmol/50 mL | 3.80 | 3.69 | 3.68 | 3.64 | 3.53 | 3.31 | 2.54 |
| Phenylephrine | 3.59 | 3.36 | 0 | 0 | 0 | 0 | 0 |
| Propofol | 3.86 | 3.86 | 3.85 | 4.04 | 5.80 | 6.87 | n. c. |
| Tranexamic acid | 3.63 | 3.65 | 3.54 | 3.54 | 3.48 | 3.48 | 3.50 |
| Water for injection | 3.85 | 3.78 | 3.76 | 3.75 | 3.60 | 3.54 | 3.00 |

Note: n. c. = more than countable.

**Table 3:** Viability of *P. aeruginosa* in non-cytotoxic drug preparations and control solutions.

| Drug/control solution | *P. aeruginosa* (CFU log/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 3 h | 5 h | 24 h | 48 h | 144 h |
| Caspofungin 35 mg in 250 mL 0.9 %NaCl | 3.48 | 3.29 | 3.00 | 2.88 | 3.38 | 3.52 | 5.67 |
| Caspofungin 70 mg in 250 mL 0.9 %NaCl | 3.51 | 3.30 | 2.48 | 2.18 | 2.70 | 2.80 | 4.60 |
| Glucose infusion solution 50% w/v | 3.45 | 3.26 | 2.70 | 2.85 | 0 | 0 | 0 |
| Heparin Sodium | 3.48 | 3.40 | 3.30 | 3.42 | 4.54 | 6.75 | 7.18 |
| Micafungin | 3.37 | 3.11 | 3.00 | 3.19 | 4.37 | 5.78 | 6.27 |
| 0.9% NaCl infusion solution | 3.40 | 3.06 | 3.08 | 3.10 | 3.65 | 5.27 | 6.07 |
| Adrenaline | 3.41 | 3.00 | 2.98 | 2.93 | 4.32 | 5.53 | 6.05 |
| Noradrenaline | 3.11 | 3.11 | 2.95 | 2.78 | 4.25 | 4.62 | 6.75 |
| Vancomycin in G5 | 3.05 | 2.68 | 0 | 0 | 0 | 0 | 0 |
| Trace elements:G5 1:1 | 3.45 | 3.44 | 3.40 | 3.76 | 4.59 | n. c. | n. c. |
| Glucose infusion solution 5 % w/v | 3.18 | 3.04 | 2.85 | 2.54 | 4.19 | 5.42 | 5.75 |
| Trace elements:G10 1:1 | 3.70 | 3.38 | 3.32 | 3.19 | 4.67 | n. c. | n. c. |
| Vancomycin in G10 | 3.29 | 3.00 | 2.54 | 2.50 | 0 | 0 | 0 |
| SMOFlipidemulsion | 3.58 | 3.35 | 3.48 | 3.54 | 6.68 | n. c. | n. c. |
| Midazolam | 3.54 | 0 | 0 | 0 | 0 | 0 | 0 |
| Glucose infusion solution 10% w/v | 3.42 | 3.30 | 3.10 | 2.85 | 2.70 | 3.48 | 5.45 |
| KCl 40 mmol/50 mL | 3.41 | 3.40 | 3.39 | 3.39 | 3.23 | 3.02 | 2.98 |
| Phenylephrine | 3.36 | 2.98 | 0 | 0 | 0 | 0 | 0 |
| Propofol | 3.78 | 3.54 | 3.26 | 3.29 | 3.73 | 6.71 | n. c. |
| Tranexamic acid | 3.30 | 3.30 | 3.08 | 2.81 | 2.78 | 0 | 0 |
| Water for injection | 3.30 | 3.24 | 3.27 | 3.27 | 4.10 | 4.18 | 4.72 |

Note: n. c. = more than countable.

**Table 4:** Viability of *E. faecium* in non-cytotoxic drug preparations and control solutions.

| Drug/control solution | *E. faecium* (CFU log/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 3 h | 5 h | 24 h | 48 h | 144 h |
| Caspofungin 35 mg in 250 mL 0.9 %NaCl | 3.00 | 0 | 0 | 0 | 0 | 0 | 0 |
| Caspofungin 70 mg in 250 mL 0.9 %NaCl | 3.19 | 0 | 0 | 0 | 0 | 0 | 0 |
| Glucose infusion solution 50% w/v | 3.404 | 3.30 | 3.59 | 3.57 | 3.38 | 3.02 | 2.93 |
| Heparin Sodium | 3.44 | 3.99 | 3.99 | 3.65 | 3.59 | 3.61 | 3.27 |
| Micafungin | 3.72 | 3.61 | 3.72 | 3.65 | 3.72 | 3.41 | 3.31 |
| 0.9% NaCl infusion solution | 3.60 | 3.60 | 3.53 | 3.54 | 3.65 | 3.62 | 3.65 |
| Adrenaline | 3.78 | 3.65 | 3.67 | 3.60 | 3.67 | 3.60 | 3.43 |
| Noradrenaline | 3.59 | 3.60 | 3.60 | 3.70 | 3.65 | 3.53 | 3.57 |
| Vancomycin in G5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trace elements:G5 1:1 | 3.60 | 3.65 | 3.73 | 3.81 | 3.57 | 3.57 | 3.51 |
| Glucose infusion solution 5 % w/v | 3.79 | 3.76 | 3.70 | 3.66 | 3.62 | 3.94 | 3.93 |
| Trace elements:G10 1:1 | 3.37 | 3.65 | 3.68 | 3.66 | 3.67 | 3.54 | 3.54 |
| Vancomycin in G10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SMOFlipidemulsion | 3.76 | 3.84 | 3.98 | 4.06 | 6.70 | n. c. | n. c. |
| Midazolam | 3.65 | 3.60 | 3.40 | 3.33 | 0 | 0 | 0 |
| Glucose infusion solution 10% w/v | 3.64 | 3.59 | 3.69 | 3.56 | 3.68 | 3.69 | 3.02 |
| KCl 40 mmol/50 mL | 3.50 | 3.38 | 3.44 | 3.54 | 3.41 | 3.48 | 3.46 |
| Phenylephrine | 3.41 | 3.45 | 3.30 | 3.28 | 0 | 0 | 0 |
| Propofol | 3.60 | 3.70 | 3.64 | 3.81 | 6.16 | 6.02 | 6.18 |
| Tranexamic acid | 3.67 | 3.66 | 3.53 | 3.51 | 3.64 | 3.72 | 3.74 |
| Water for injection | 3.51 | 3.54 | 3.59 | 3.60 | 3.87 | 3.56 | 3.51 |

Note: n. c. = more than countable.

Table 5: Viability of *C. albicans* in non-cytotoxic drug preparations and control solutions.

| Drug/control solution | *C. albicans* (CFU log/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 3 h | 5 h | 24 h | 48 h | 144 h |
| Caspofungin 35 mg in 250 mL 0.9 %NaCl | 2.30 | 0 | 0 | 0 | 0 | 0 | 0 |
| Caspofungin 70 mg in 250 mL 0.9 %NaCl | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Glucose infusion solution 50% w/v | 2.90 | 0 | 0 | 0 | 0 | 0 | 0 |
| Heparin Sodium | 2.73 | 2.50 | 2.35 | 2.30 | 2.60 | 2.60 | 3.62 |
| Micafungin | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.9% NaCl infusion solution | 3.08 | 2.48 | 2.60 | 2.60 | 2.85 | 3.08 | 3.72 |
| Adrenaline | 2.93 | 3.00 | 3.18 | 3.40 | 4.13 | 4.44 | 4.49 |
| Noradrenaline | 2.80 | 3.20 | 3.31 | 3.45 | 4.01 | 4.27 | 4.65 |
| Vancomycin in G5 | 3.11 | 3.02 | 2.65 | 2.00 | 0 | 0 | 0 |
| Trace elements:G5 1:1 | 3.06 | 3.30 | 3.28 | 3.40 | 4.45 | 4.81 | 5.06 |
| Glucose infusion solution 5% w/v | 3.10 | 3.10 | 3.12 | 3.39 | 3.90 | 3.88 | 3.92 |
| Trace elements:G10 1:1 | 3.11 | 3.14 | 3.15 | 3.37 | 4.44 | 4.72 | 4.97 |
| Vancomycin in G10 | 3.15 | 3.10 | 3.00 | 2.98 | 2.70 | 2.52 | 0 |
| SMOFlipidemulsion | 3.04 | 3.20 | 3.40 | 5.39 | 5.43 | n. c. | n. c. |
| Midazolam | 3.04 | 3.02 | 0 | 0 | 0 | 0 | 0 |
| Glucose infusion solution 10% w/v | 3.41 | 3.36 | 3.29 | 3.37 | 3.71 | 3.80 | 3.98 |
| KCl 40 mmol/50 mL | 3.20 | 3.16 | 3.12 | 3.08 | 3.08 | 3.04 | 4.43 |
| Phenylephrine | 3.20 | 3.00 | 0 | 0 | 0 | 0 | 0 |
| Propofol | 3.06 | 3.30 | 3.30 | 3.65 | 4.88 | 5.54 | 5.95 |
| Tranexamic acid | 3.33 | 3.29 | 3.31 | 3.30 | 3.80 | 4.30 | 5.16 |
| Water for injection | 3.19 | 3.15 | 3.16 | 3.18 | 3.77 | 3.79 | 3.70 |

Note: n. c. = more than countable.

These results reflect the species-specific capability of the microorganisms to survive and grow in nutrient-deficient solutions. However, the tested microorganisms lost viability in preparations containing vancomycin, phenylephrine, and midazolam after a period of a few hours or few days (see Figures 1 and 2).

*C. albicans* rapidly lost viability in caspofungin and micafungin containing test solutions (see Figure 3). Thereby the proven antimicrobial activity of vancomycin and the echinocandins, which are used as an antibiotic and antifungals, respectively, confirmed the validity of the experimental design and the results.

Species-specific antibacterial activity was observed in tranexamic acid solutions. Only *P. aeruginosa* lost viability 48 h post inoculation, while the other strains tested remained viable. Glucose 50 % w/v solution also generated species-specific antibacterial activity against *P. aeruginosa*. Antifungal activity of glucose 50 % against *C. albicans* got already obvious 1 hour post inoculation. Caspofungin and micafungin exhibited strong antifungal activity as expected. Noteworthy, caspofungin containing test solutions in both concentrations exhibited antibacterial activity against *S. aureus* and *E. faecium*. Viability of *P. aeruginosa* was not affected (see Figure 3). Moreover, micafungin did not inhibit bacterial growth. In some of the test solutions *C. albicans* tends to grow (compare Table 5).

The lipid containing formulation of 1% propofol emulsion and the 20 % SMOFlipid emulsion served as nutritive media for all selected microorganisms and the number of CFUs increased rapidly (see Figure 4).

Trace elements mixed with G5 or G10 stimulated the growth of *P. aeruginosa* and *C. albicans*, but had no influence on the growth of the tested Grampositive bacteria (see Tables 2–5).

## Discussion

Viability and growth of microorganisms in pharmaceutical preparations is directed by extrinsic (e. g. temperature, oxygen) and intrinsic factors (e. g. type and concentration of ingredients). It is well known that refrigeration retards the growth of microorganisms. The experiments were performed to increase our knowledge about the intrinsic factors of ready-to-use parenteral preparations compounded in the pharmacy department except the drugs used in anticancer therapy. The portfolio of products encompasses different indications, e. g.

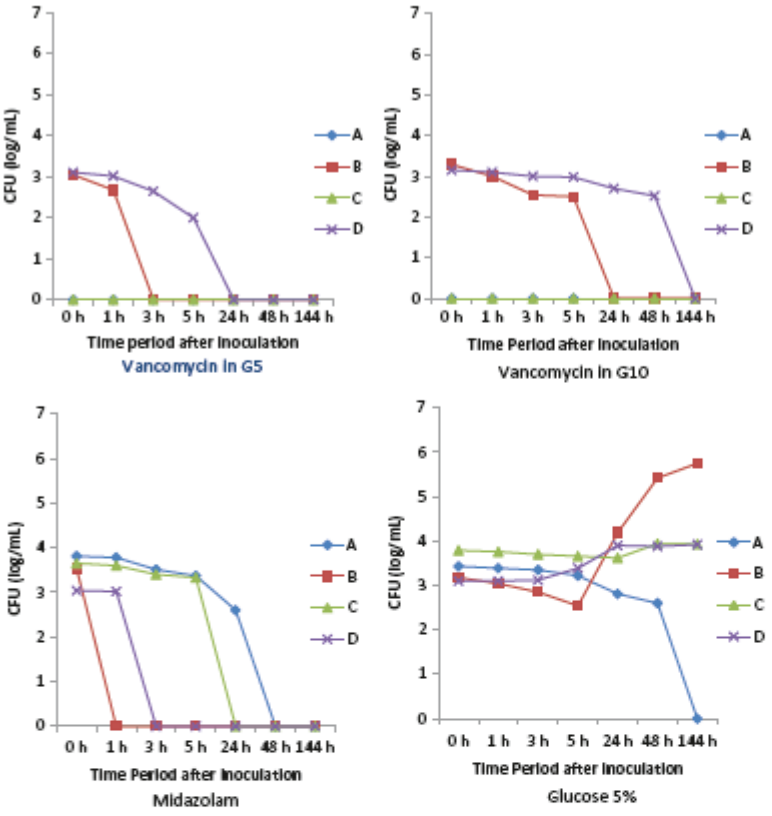


**Figure 1:** Viability of selected microorganisms (A: *S. aureus*; B: *P. aeruginosa*; C: *E. faecium*; D: *C. albicans*) in vancomycin or midazolam containing test solutions and Glucose 5% as control solution. CFU = colony forming units.

parenteral nutrition, catecholamines, antibiotics, antifungals, anaesthesia and the use in different patient groups (paediatric patients, intensive care patients, operating theatre patients). These preparations contain different active substances and excipients and have different physicochemical characteristics. The experimental conditions of the intrinsic factors were chosen according to clinical practice. All preparations contained liquid water and some of them contained nutrient sources, minerals or trace elements. The extrinsic factors were the same throughout the study. As a reasonable compromise of typical temperature conditions in clinical practice and optimum growth temperature for pathogenic bacteria (37 °C) the experiments were carried out at room temperature (22 °C). The inoculum size was kept unchanged and simulated low level contamination. The experiments were performed in duplicate for each ready-to-use preparation at each time interval. Each sample was tested in three degrees of dilution (in total 6 experiments). This allows the detection of the influence of the CFU concentration and of process errors. Because these are biological experiments, not the average but a representative value was chosen to be presented as result.

The growth inhibition detected in some preparations is not related to the active substance, but to the physicochemical parameters such as pH and osmolality. Bacterial growth is inhibited by low pH, while the optimum pH for the growth of most fungi is pH 5. Therefore, low pH values are most probably the reason for growth inhibition in midazolam injection solutions [20, 22]. The pH values of diluted vancomycin infusion solutions amount to pH 3–5. That might be the reason for the growth inhibition of *P. aeruginosa* and *C. albicans* recognized in our experiments.

The phenylephrine injection preparation (pH 5) contains citric acid and sodium metabisulfite. The comparatively high concentration of sodium metabisulfite (2 mg/mL) reduces the redox potential of the phenylephrine preparations, what may explain the observed antimicrobial activity. This assumption is confirmed by the fact that inhibition of microbial growth did not occur in the adrenaline and noradrenaline containing preparations

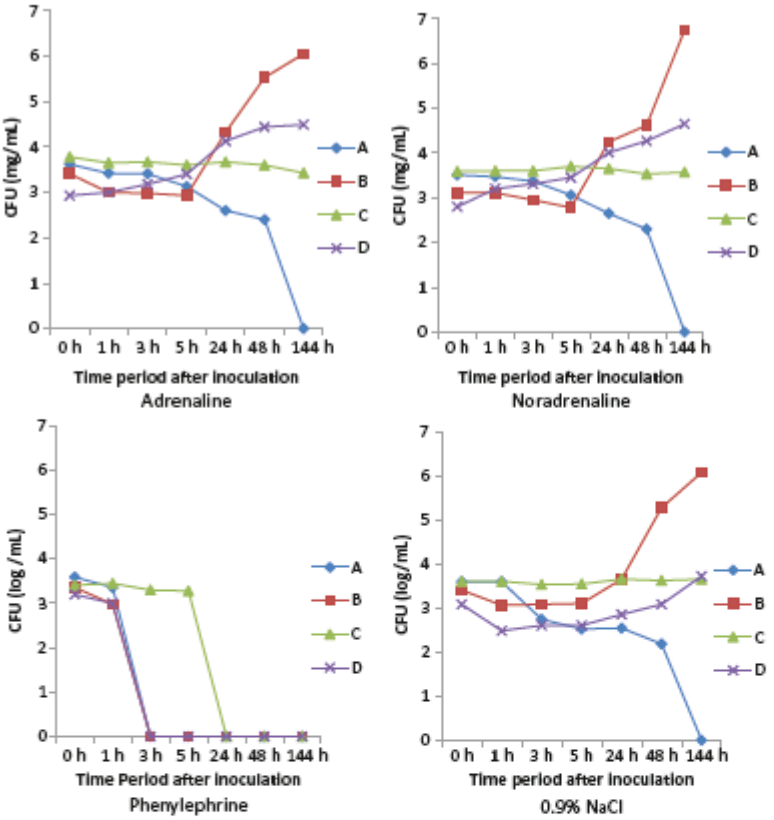


Figure 2: Viability of the four test organisms (A: *S. aureus*; B: *P. aeruginosa*; C: *E. faecium*; D: *C. albicans*) in diluted solutions of adrenaline, noradrenaline, and phenylephrine and 0.9% NaCl solution as control solution. CFU = colony forming units.

containing small amounts of sodium metabisulfite (0.072 mg/mL). Similar findings were reported by Bostan et al. [24]. Among the tested catecholamine preparations only dobutamine preparations showed antimicrobial activity because of high sodium metabisulfite concentrations compared to adrenaline and noradrenaline preparations [24]. Notably, the stimulation of bacterial growth by catecholamines [25, 26] is eliminated by the antioxidative excipients in the medicinal products. High osmolality is also known to inhibit microbial growth what explains the antimicrobial of glucose 50% preparations [13, 27]. Antifungal activity of glucose 50% against *C. albicans* got already obvious 1 hour post inoculation and is commonly known.

To our knowledge there is no information available about the antimicrobial activity of tranexamic acid containing preparations. The fact that growth inhibition was only given for *P. aeruginosa* suggests that the activity is substance specific.

According to the studies of Rosett et al. the antimicrobial activity of heparin is a result of the reduction of divalent cations from the growth media [21]. The lack of antimicrobial activity of the heparin 1 IE/mL containing preparations is to be explained by the low heparin concentration and the experimental conditions used. Obviously the low amount of heparin in our preparations was insufficient to bind the cations essential for bacterial growth. During *in vivo* experiments also high concentrations of heparin sodium lacked antibacterial activity against *S. aureus* [28]. The same explanation is applicable for the missing antimicrobial activity of the 0.8 molar potassium chloride preparations. Different concentrations and experimental designs lead to inconsistent results [29, 30].

Fat emulsions like 1% propofol injection and 20% lipid emulsion are known to serve as growth medium for a number of microorganisms and as origin of bloodstream infections [31, 32]. The lack of antimicrobial activity is related to the high value of pH (7–8.5) and the high lipid content serving as nutrient source. Fat emulsion serves as an excellent non-nitrogen energy source for a number of microorganisms, including bacteria and fungi.

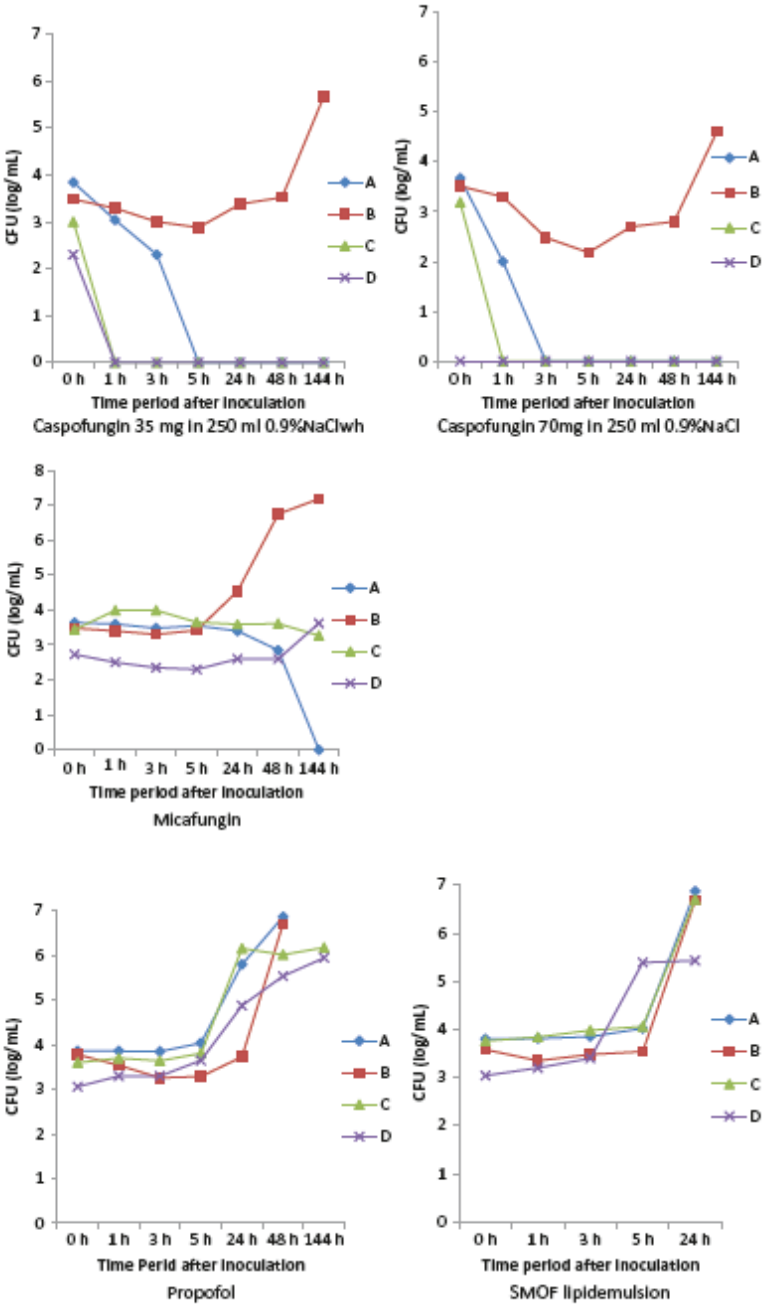


Figure 3: Viability of the four test organisms (A: *S. aureus*; B: *P. aeruginosa*; C: *E. faecium*; D: *C. albicans*) in diluted solutions of caspofungin and micafungin. CFU = colony forming units.

Figure 4: Viability of the four test organisms (A: *S. aureus*; B: *P. aeruginosa*; C: *E. faecium*; D: *C. albicans*) in lipid containing solutions, i. e. propofol 1% and SMOFlipid 20 % emulsion. CFU = colony forming units.

When microbial growth in five different commercially available lipid emulsions was tested no difference in growth patterns due to the nature of the oil or its concentration was observed [33]. SMOFlipid 20 % consists of fish oil, soybean oil, medium chain triglycerides, and olive oil and supported the growth of the selected bacteria and yeast in a similar manner. In both settings the number of CFUs rapidly increased to $\geq 10^6$ over a period of less than 24 h after inoculation. Pure fat emulsions and lipid containing total parenteral nutrition solutions

are the most vulnerable preparations and should always be prepared under strict aseptic conditions and should not be stored or infused more than 12 h after preparation [33, 27].

The species-specific growth promoting activity of the trace elements mixed with glucose solutions is not yet reported in the literature. But plausibility is given as *P. aeruginosa* and *C. albicans* grow in nutrient deficient solutions and microorganisms require also trace elements for their growth [34].

## Conclusion

As most of the tested parenteral preparations did not generate antimicrobial activity, preparation should be done under strict aseptic conditions in order to avoid any microbial contamination. Furthermore the insufficient antimicrobial properties of ready-to-use solutions should be considered while determining the shelf-life of the products. Lipid containing preparations should be kept refrigerated whenever possible to inhibit the multiplication of any contaminating organism.

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## Bionotes

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Irene Krämer is currently Director of the Pharmacy Department, University Medical Center, Johannes Gutenberg-University Hospital, Mainz and is also a Professor for clinical pharmacy at the Pharmacy School of Johannes Gutenberg-University. She completed her postdoctoral thesis in Pharmaceutical Technology entitled: Development, quality assurance, and optimization of ready-to-use parenteral solutions in the integrated cancer care concept. Her special interests include oncology pharmacy, infectious diseases, and aseptic drug preparation. She is doing research projects in the field of physicochemical and microbiological stability of cytotoxic drugs, compatibility of admixtures of nebulizer solutions, and monitoring of medication compliance.

# 5. *Physical and chemical instability of ready-to-use drug preparations*

Since more than 25 years, ready-to-use preparations of antineoplastic drugs are prepared in patient individual doses in pharmacy based cytotoxic preparation units. The aim is to protect health care workers from occupational hazard and the preparations from environmental contamination and cross contamination. Adequate information about the integrity, stability, environment and process in combination with education, training, and experiences are relevant factors regarding the quality of the preparations(8). Valid knowledge about the stability of ready-to-use anticancer drug preparations also offers new prospects for cost savings, prolonged or continuous administration and home chemotherapy. Generally, the stability of ready-to-use antineoplastic drug preparations is labeled by the manufacturer, according to the microbiological instability, and not due to physicochemical instability (14). Stability of drug preparations depends on the stability of the active ingredient as well as the excipients and dosage form developed by the manufacturers, the stability and compatibility of containers and vehicle solutions used for diluted products and external factors like temperature.

***Definition of stability***

The term stability refers to ability of drug products to remain the same potency of active ingredient (s) as it had at the time of compounding under defined conditions and during the labeled storage and use period possess (47).

Instability includes undesired changes in physical appearance of the preparation, or/and the degradation of active pharmaceutical ingredient (API), leading to decreased activity ingredients and/or increased side effects and toxicity.

Incompatibility refers to alteration of drug preparations by reactions between the API and the solution for reconstitution, vehicle solution, primary container, another medicinal product, or the devices and catheters used for administration (47).

Physical, chemical, and microbiological stability are typically the most important terms used while determining the shelf life of a medicinal product. Most studies focus on the chemical stability while few studies reported data about physical and microbiological stability.

Microbiological stability implies the freedom of the parenterally administered medicinal products from any viable microorganisms in order to ensure the sterility during the preparation, storage and use. Of note, determining the microbial stability plays a key role in determining the shelf life of final pharmaceutical preparation (FFP) because of the lack of data about microbial stability.

## *5.1. Chemical instability*

Chemical instability describes the decrease in the amount or concentration of API or/and excipients in the final drug product as a consequence of various chemical reactions like hydrolysis, oxidation, photolysis and epimerization leading to reduced efficacy and safety.

- **Hydrolysis**

Hydrolysis phenomenon plays the major role in chemical instability, and occurs due to functional groups such as ester, amide, ß-lactam bonds in the chemical structure of most drugs that are liable to undergo hydrolytic reaction (48). Often the hydrolysis rate depends on the pH value and temperature(48, 49).

- **Oxidation**

Oxidation reactions may affect the API or/and excipients by exposure to ambient air or other oxidizing agents (49). The rate of oxidation depends on the chemical structure of the products and also pH and temperature.

Therefore, optimum pH value, reduced contact with the atmosphere, and addition of antioxidants as excipients can be the key to diminish the rate of drug degradation (47-49).

- **Photo degradation (photolysis)**

Some medicinal products are affected by various light conditions (sun light, daylight or artificial light) and encompassing wavelengths in the range from 290-800 nm. Light may catalyze the oxidation-reduction or hydrolysis degradation pathways. In some cases, photolysis gets obvious discoloration of drug and excipients. In order to limit the light induced degradation of sensitive products, it is recommendable to use proper container material or secondary packing (47, 48).

- **Isomerization-Racemization**

Isomerization refers to a process, in which the drug is converted into its isomer that has lower or no therapeutic activity and in some cases seems to be the reason of adverse effects. Racemization means the conversion of optically active API into its enantiomers which present different chemical reactions and undesirable therapeutic activity (50).

- **Drug-excipient interaction**

Excipients are usually used in pharmaceutical formulation to ensure the stability or release of the active ingredient. However antioxidants or surfactants can also be associated with physiochemical instability as well as drug degradation (48, 51).

Other pathways of chemical instability include dehydration, decarboxylation, polymerization and catalysis.

## *5.2. Physical instability*

Physical instability implies changes of physical parameters of drug products. Physical instability is expressed as alteration in the elegance of drug, uniformity in the appearance of drug content or drug release rate (47). Moreover, the state of drug impacts the physical changes of the drug. In solid state formulations, polymorph changes and amorphorization are considered the important phenomena leading to physical instability of drug, while precipitation, lack of solubility, and contents/container interaction with continent or packing material are more commonly phenomenon in liquid drug dosage forms (50).

- **Precipitation**

Precipitation is one of the most common causes of physical instability, especially in the parenteral drugs. It occurs by settling of solid particles of API or destabilized excipient out of the drug solution as a result of changing in their solubility.

Monitoring the pH value of final drug product and adding co-solvents to the nonpolar drug formulations may improve the solubility of API and drug formulation. Furthermore, using surfactants and adding complexation agents can enhance the solubility of various hydrophobic drugs in the hospital pharmaceutical preparations. In this case, these preparations maintain their bioavailability, safety, and identity (48)

- **Amorphorization/ Polymorphs**

Amorphorization process describes the status of drug substance, at which drug molecules possess the highest energy and indiscernible crystalline phases under specific environmental conditions. While polymorphs refer to state of drug at which it can be able to identified as two or more than crystalline forms (52, 53).

Despite the advantage of amorphous drug form in improving the solubility and consequently the bioavailability of poorly-soluble drugs, amorphorization phenomenon affects in some cases the release of active substance and minimizes the stability and quality of drug products (52-54).

Under physical changes term, set of mechanisms may impact the quality of drug dosage forms like loss of water, water absorbance, loss of volatile compounds, changes in particle sizes, viscosity, taste (flavor) and color changes; mostly as a response to chemical changes(pH, light exposure), etc.

## *5.3. Factors affecting the stability and the rate of degradation*

Physicochemical stability of medicinal products (stock solution, diluted infusion solution) is influenced by different factors:

- **pH**

Each drug has its optimum specific pH value or pH range, at which it maintains its physiochemical properties stable and guarantees optimum solubility of the API and its physiological availability in the target tissue (47). Moreover, pH has an influence on the rate of degra-

dation by increasing the hydrolysis and/or oxidation rate, when the pH values fall below or go beyond the specification limits.

Utilization of buffer-independent pH rate in formulation of pharmaceutical product attribute retaining the active substance and drug dosage form stable (48).

- **Temperature**

The increase of temperature mostly raises the degradation rate of aqueous drug preparations. Alteration of the storage temperature of solid dosage forms stimulates melting, change of polymorphic states of API or excipient, or loss of the water. Often the increase of temperature is associated with changes in humidity (51). Aggregation is a common phenomenon of denatured monoclonal antibody molecules (mAB) and occurs as a result of freezing-thawing and elevated temperature (48, 50).

- **Light**

Chemical instability of a few drugs is induced by daylight, fluorescent light or UV light by accelerated redox reactions or polymerization. (47-49). For instance, the aqueous solutions of doxorubicin, epirubicin, and daunurubicin hydrochloride, at low concentrations, should be stored light protected in polypropylene container in order to prevent the photo degradation (56).

- **Moisture**

Moisture content of API (bulk drug) and excipients in solid dosage forms is a critical issue regarding physicochemical and microbial stability. Not just API, excipients and packing materials, but also the manufacturing process can be considered as a source of moisture. Moisture content has an influence on the hydrolysis and dissolution rate and attributes to changes of physical and chemical parameters.

In addition, the growth of microorganisms is stimulated by moisture and attention must be paid to the impact of water on the microbial stability of pharmaceutical products, especially with parenteral preparations because most of parenterals are normally prepared by using an aqueous vehicle.

## *5.4. Stability Testing*

The main purpose of the stability testing is to identify the quality and integrity of API as well as the final drug product during the shelf life and storage conditions claimed, in order to guarantee patient safety.

Stability testing is applied at different stages of drug development providing data about stability of API, pharmaceutical formulation, excipients, and packaged drug product. Of note, the stability study profile takes care for the variation of environmental factors (temperature, humidity, light) and packaging materials. Stability test must follow ICH or WHO guidelines (57). EMA Guidelines (European agency for the evaluation of medicinal products) are provided by the committee for medicinal products for human use (CHMP) as additional guidelines in the European Union in order to esure the quality and safety of pharmaceutical products (58).

**ICH Guidelines**

ICH Guidelines are international guidelines implemented with the aim to enhance the safety and quality of new pharmaceutical products within specific global zones (i.e. European Union, Japan, and USA) (57). According to the ICH guidelines, the main purpose of stability testing is to supply evidence that there are no changes in regarding the specifications of API and FPP.

According to the ICH and WHO guidelines, the world is categorized into four climatic zones (I-IV) in order to harmonize the conditions used for stability testing and facilitate licensing applications in all climatic zones. The climatic zones are based on two main storage conditions, i.e. temperature and relative humidity. The following table presents the climatic zones under predetermined specific storage conditions (57).

Table 1: Characterization of climatic zones

| Climatic zone | Definition of climatic zone | Storage conditions |
|---|---|---|
| I | Temperate climate | 21 °C, 45% RH |
| II | Subtropical and Mediterranean | 25 °C, 60% RH |
| III | Hot, dry climate | 30 °C, 35% RH |
| IV a | Hot, humid climate | 30 °C, 65% RH |
| IV b | Hot, very humid climate | 75 °C, 75% RH |

The ICH guidelines are vaiid in the climatic zone I and II (i.e. US, Japan, EU).

Moreover, ICH guidelines focus on different issues of stability of FPP (compare Table 2).

Table 2: Categories of stability tests

| Codification | Type of stability tests |
|---|---|
| Q1A(R2) | Stability testing of new drug substances and products (59) |
| Q1B | Photostability testing of new drug substances and products (60) |
| Q1C | Stability testing for new dosage forms (61) |
| Q1D | Bracketing and matrixing designs for stability testing of new drug substances and products (62) |
| Q1E | Evaluation of stability data (63) |
| Q1F | Stability data package for registration (64) |

**Stability study methods and storage conditions (59)**

Based on the phase of drug product development, climatic zone, and drug dosage form, stability studies are classified as follows:

- **Long term stability (real stability)**

The aim of this test is to reveal the decomposition and physicochemical stability of API and the pharmaceutical product under recommendable storage conditions over a particular time period. According to the ICH guidelines, the long term stability testing is performed in early stages of drug product development and done every three months in the first year, every six months in the second year, and annually afterwards.

- **Accelerated stability testing**

During accelerated stability studies, the API is exposed to forced degradation conditions at predetermined intervals, i.e. 0, 3, 6 months at elevated temperatures (recommendable to

be set at 40 °C), humidity, agitation, and light. Accelerated test conditions are considered to be the worst case conditions for API and drug product.

- **Intermediate stability testing**

Intermediate stability testing is performed if any specific change in the drug properties is observed throughout the accelerated stability testing. In this case, the test is applied at the temperature 30 °C±2 °C and relative humidity 65% during a period of 12 months and at four time intervals (0, 3, 6, 12 months).

In general, the stability testing criteria used are related to the proposed storage conditions of the medicinal products. Recommended test methods and conditions are given in Table 3.

Table 3: Recommended test conditions and methods according to ICH Guidance

| Recommended storage conditions | Stability testing methods | | |
|---|---|---|---|
| | Long term | Accelerated | Intermediate |
| Ambient temperature | 25 °C ± 2°C/ 60% RH±5%<br>or<br>30 °C ± 2°C/ 65% RH±5% | 40 °C ± 2°C/ 75% RH±5% | 30 °C ± 2°C/ 65% RH±5%<br>or<br>No Intermediate stability testing |
| Refrigerated | 5 °C ± 3°C | 25 °C ± 2°C/ 60% RH±5% | No chance for Intermediate stability testing |
| Frozen | -20 °C± 5 °C | No chance for accelerated stability testing | No chance for Intermediate stability testing |

Stability assessment is challenging, as it requires evaluation and characterization of of breakdown products as part of the analysis of the parent product.

# *6 Die STABIL- LISTE© 7. Auflage 2015*

## *Physikalisch-chemische Stabilität, Kompatibilität und Inkompatibilität parenteral applizierbarer Zytostatika, Virustatika und Supportivtherapeutika*

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Physikalisch-chemische Stabilität, Kompatibilität und Inkompatibilität parenteral applizierbarer Zytostatika, Virustatika und Supportivtherapeutika

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## Einleitung

Die zentrale Herstellung applikationsfertiger Zytostatikalösungen in der Apotheke erfolgt oftmals nicht unmittelbar vor der Anwendung, sondern im Voraus, z.B. für die Versorgung am Wochenende. Zur Sicherstellung der erforderlichen Qualität der zubereiteten Infusions- oder Injektionslösungen benötigt der verantwortliche Apotheker daher Daten zu deren physikalisch-chemischen Stabilität. Ebenso kann das Weiterverwenden von Anbrüchen der zur Zubereitung eingesetzten konzentrierten Stammlösungen nur bei Vorliegen entsprechender Stabilitätsdaten erfolgen. Die in den Packungsbeilagen oder Fachinformationen der Zytostatika-Fertigarzneimittel angegebenen Aufbrauchsfristen von maximal 24 Stunden nach Anbruch bzw. Zubereitung werden meistens unter mikrobiologischen Aspekten festgelegt [51]. Erfolgt die Zubereitung jedoch unter validierten und kontrollierten aseptischen Bedingungen in der Apotheke, kann die Aufbrauchsfrist basierend auf der physikalisch-chemischen Stabilität der jeweiligen Lösung festgesetzt werden.

Eine evidenz-basierte Übersicht zur physikalisch-chemischen Stabilität von Zytostatikalösungen wird seit Jahren von der Apotheke der Universitätsmedizin Mainz in Tabellenform publiziert (Stabil-Liste©). In dieser Liste sind tabellarisch für jedes Zytostatikum die physikalisch-chemischen Stabilitätszeiträume der Stamm- bzw. Infusionslösungen aufgeführt, die nach sorgfältiger Bewertung aller bekannten Stabilitätsdaten zum Zeitpunkt der Erstellung von den Autoren empfohlen werden können. Die Liste wird in der Regel alle 2 Jahre aktualisiert, zuletzt im März 2015 [93]. Im Folgenden werden das Vorgehen bei der Aktualisierung der Stabil-Liste© 2015 sowie Änderungen und Ergänzungen in dieser Auflage vorgestellt. Darüber hinaus werden im Diskussionsteil die wichtigsten Fragen (FAQs) beantwortet, die die Autoren in den letzten Monaten zur aktualisierten Stabil-Liste© erreichten.

**Material und Methode**

Die Stabilitätsliste wurde zunächst um die Daten aller neuzugelassenen bzw. kurz vor der Zulassung befindlichen onkologischen Wirkstoffe ergänzt.

Im zweiten Schritt erfolgte die Aktualisierung der Stabilitäts- bzw. In/Kompatibilitätsdaten für alle aufgenommenen Arzneimittel.

***Datenquellen***

Alle Ergebnisse der in der Apotheke der Universitätsmedizin Mainz durchgeführten experimentellen Untersuchungen wurden unabhängig von deren Publikationsstatus eingefügt. Anschließend wurden die Publikationen oder Informationen externer Autoren zusammengestellt. Hierfür wurden mehrere Quellen genutzt. Die Apotheke der Universitätsmedizin Mainz sammelt kontinuierlich alle Informationen zur Stabilität von onkologischen Arzneimitteln wie Originalpublikationen, mündliche Präsentationen oder Posterbeiträge auf Kongressen sowie Informationen von pharmazeutischen Herstellern oder von Kollegen, z.B. aus der ADKA Mailing-Liste. Zur Vervollständigung dieser Sammlung wurde im März 2015 unmittelbar vor der Neuauflage der Stabil-Liste© in der Datenbank International Pharmaceutical Abstracts (IPA) [53] auf Vorhandensein neuer Publikation zur physikalisch-chemischen Stabilität von Zytostatika gesucht. Die Suche erfolgte für jeden Wirkstoff anhand der kombinierten Stichwörter „Stabilität“ und „Wirkstoff“ sowie „Kompatibilität“ und „Wirkstoff“. Der Suchzeitraum wurde auf die Veröffentlichungsjahre 2013 bis 2015 begrenzt, da die letzte Auflage der Stabil-Liste© von April 2013 stammte. Alle in der Datenbank gefundenen Abstracts wurden hinsichtlich der Relevanz für die Erstellung der Stabil-Liste© bewertet. Für als verwertbar eingestufte Abstracts wurde der Volltext der Originalpublikation beschafft. Zudem wurden die Ausgaben Januar 2014 bis März 2015 der von der Apotheke der Universitätsmedizin Mainz abonnierten wissenschaftlichen Zeitschriften systematisch nach Publikationen zur Stabilität und/oder (In)-Kompatibilität onkologischer Wirkstoffe durchsucht. Dabei handelte es sich um die Annals of Pharmacotherapy, American Journal of Health-System Pharmacy, International Journal Pharmaceutical Compounding, European Journal of Hospital Pharmacy und das Journal of Oncology Pharmacy Practice. Abschließend wurden die entsprechenden Wirkstoffmonographien der Stabilis-Datenbank, Version 4.0 [54] durchgesehen. In der Stabilis-Datenbank sind oftmals anderweitig nicht zugängliche Vortragsfolien oder Abstracts der französischen Arbeitsgruppen zu finden.

***Bewertung***

Alle gesammelten Veröffentlichungen und Informationen wurden vollständig gelesen und hinsichtlich der Validität der enthaltenen Daten bewertet. Die Bewertung orientierte sich an den ICH Quality Richtlinien Q1 und Q2 [50] und vor allem an der 2011 veröffentlichten europäischen Konsensusempfehlung zur Durchführung von Stabilitätsuntersuchungen onkologischer Wirkstoffe [10]. Nur als valide eingestufte Untersuchungen fanden bei der Aktualisierung der Stabilitäts-und Kompatibilitätsdaten Berücksichtigung.

## Ergebnisse

***Neue onkologische Wirkstoffe***

Insgesamt wurden 8 neue onkologische Wirkstoffe in die Stabil-Liste© aufgenommen (s. Tabelle 1).

**Tabelle 1**: Neu in die Stabil-Liste© 2015 aufgenommene Wirkstoffe mit Datenquellen und Hinweisen zur Festlegung der Aufbrauchsfrist

| **Wirkstoff** | **Fertig-arzneimittel** | **Datenquellen** | **Hinweis** |
|---|---|---|---|
| Belinostat | Beleodaq™ | pi[1] Beleodaq® [91] | _ |
| Blinatumomab | Blincyto™ | pi[1] Blincyto™ [6] | _ |
| Nivolumab | Opdivo™ | pi[1] Opdivo™ [19] | Stabilität der Stammlösung mikrobiologisch determiniert |
| Obinutuzumab | Gazyvaro™ | FI[2] Gazyvaro™ [84] | Stabilität der Stammlösung mikrobiologisch determiniert |
| Pembrolizumab | Keytruda™ | pi[1] Keytruda™ [69] | Stabilität der Stammlösung mikrobiologisch determiniert |
| Plerixafor | Mozobil® 20 mg/ml | Kim 2015 [63] | _ |
| | | FI[2] Mozobil® [88] | _ |
| | | Yuan 2013 [99] | _ |
| Ramucirumab | Cyramza™ | pi[1] Cyramza™ [31] | Stabilität der Stammlösung mikrobiologisch determiniert |
| Trastuzumab Emtansine | Kadcyla® | FI[2] Kadcyla® [85] | _ |

[1] pi = prescribing information
[2] FI = Fachinformation

Zudem waren zum Recherchezeitpunkt für 5 Wirkstoffe neue Fertigarzneimittel erhältlich, die sich hinsichtlich der Formulierung deutlich von den bereits zugelassenen Fertigarzneimitteln dieser Wirkstoffe unterschieden. Diese Tatsache erforderte das Einfügen von zusätzlichen Datensätzen zu den jeweiligen Wirkstoffen: bei Alemtuzumab Lemtrada® 12 mg neben MabCampath®, bei Amsacrin Amsalyo® neben Amsidyl®, bei Cyclophosphamid Endoxan® Lyophilisat neben Endoxan® und bei Rituximab und Trastuzumab die Formulierungen zur subkutanen Anwendung Mabthera® SC bzw. Herceptin® SC neben den i.v. zu applizierende Lösungen (s. Tabelle 2).

**Tabelle 2**: Neu in die Stabil-Liste© 2015 aufgenommene Datensätze zu neuen Formulierungen bekannter Wirkstoffe mit Datenquellen und Hinweisen zur Festlegung der Aufbrauchsfrist

| **Wirkstoff** | **Fertigarzneimittel** | **Datenquellen** | **Hinweis** |
|---|---|---|---|
| Alemtuzumab | Lemtrada® 12 mg | _ | Daten von Mabcampath® Übertragen |
| Amsacrin | Amsalyo® | Packungsbeilage Amsalyo [80] | _ |
| Cyclophosphamid | Endoxan® Lyophilisat | _ | Daten von Endoxan® Trockensubstanz übertragen |
| Rituximab | MabThera® SC | FI[1] MabThera® SC [86] | _ |
| | | Müller 2015 [71] | _ |
| Trastuzumab | Herceptin® SC | FI[1] Herceptin® SC [87] | _ |
| | | Nalenz 2014 [72] | |

[1] FI = Fachinformation

Für neu zugelassene Wirkstoffe bzw. für neue Formulierungen sind nur selten Stabilitätsdaten zu finden, die über die Angaben der entsprechenden Fachinformationen und Packungsbeilagen hinausgehen. Die Aufbrauchsfristen sind in der Regel aus mikrobiologischen Gründen auf maximal 24 h bei Kühllagerung begrenzt [51]. Sofern das Fertigarzneimittel als flüssiges Konzentrat formuliert ist und keine Hinweise auf eine Sauerstoffempfindlichkeit des Wirkstoffs zu finden sind, kann davon ausgegangen werden, dass durch eine Entnahme mittels Kanüle oder Spike die physikalisch-chemische Stabilität der verbleibenden Restlösung nicht relevant beeinträchtigt wird. Für solche Konzentrate wird in der Stabil-Liste© die physikalisch-chemische Stabilität nach Anbruch mit 28 Tagen bei Kühllagerung angegeben. Dies gilt auch für Pembrolizumab (Keytruda™), das in den USA zusätzlich als flüssiges Injektionskonzentrat in identischer Formulierung (ausgenommen Lösungsmittel Aqua ad inject.) zugelassen ist [69].

***Mit Zytostatika beladene DC Bead™***

Für vier Zytostatika wurden erstmals eigenständige Datensätze für deren Ladung in DC Bead™ Mikrosphären aufgenommen (s. Tabelle 3). Die Zytostatika beladenen DC-Bead™ werden zur arteriellen Chemoembolisation bei Lebertumoren oder Lebermetastasen eingesetzt. Die besondere Anwendungsform erforderte die zusätzliche Darstellung der zu empfehlenden Beladungszeiten und -bedingungen und den daraus resultierenden Beladungsraten. Für alle Wirkstoffe ist gezeigt, dass die Beladung von DC Bead™ mit Zytostatika umso rascher erfolgte, je kleiner der Durchmesser und damit je größer die Oberfläche der DC Bead™ ist und je mehr Bewegung der Mischung während des Beladungsprozesses zugeführt wird. Für die beiden Anthracyclin-Derivate Doxo- und Epirubicin verlief die Beladung effektiver, wenn nicht das Fertigkonzentrat (2 mg/ml), sondern die mit Aqua ad inject. zu 25 mg/ml rekonstituierte Trockenformulierung verwendet wurde [41, 49, 92]. Mit der 25 mg/ml Lösung wurden für Doxorubicin in Abhängigkeit von der DC Bead™ Größe (70-700 µm) nach 1-2 Stunden unter gelegentlichem Schwenken der Vials Beladungsraten von >98% erreicht [41]. Entsprechende Beladungsraten (>99%) wurden mit Epirubicin für DC Bead™ der Größe 70-300 µm bereits nach 30 Minuten unter gelegentlichem Schwenken erzielt [92].

Die physikalisch-chemische Stabilität der mit Zytostatika beladenen DC Bead™ sowie die Integrität der Zytostatika konnte ausreichend belegt werden. Für Epirubicin

und Irinotecan konnte die Stabilität für 28 Tage bei Raumtemperatur gezeigt werden [60, 92]. Mit Doxorubicin beladene DC Beads erwiesen sich über 14 Tage bei 2-8 °C stabil [41], für Topotecan wurde die Stabilität über 7 Tage bei Raumtemperatur untersucht und belegt [58].

Für die zur Beladung zu empfehlenden hochkonzentriert rekonstituierten Anthracyclin-Stammlösungen sind keine experimentell bestimmten Daten zur Stabilität bekannt. Für Doxorubicin wurden Untersuchungen mit maximal 7,5 mg/ml [40] publiziert, welche die physikalisch-chemische Stabilität über 30 Tage bei Kühllagerung belegen. Anthracyclinlösungen sind bekanntlich insbesondere in höheren Konzentrationen sehr stabil. Zudem lässt die nachgewiesene Integrität von Doxo- und Epirubicin über 14 bzw. 28 Tage Lagerung nach Beladung von DC Beads mit den hochkonzentrierten Lösungen den Rückschluss zu, dass die hochkonzentrierten Stammlösungen über 28 Tage stabil sind.

Des Weiteren wurden verschiedene Untersuchungen zur Kompatibilität der beladenen DC Beads mit unterschiedlichen nichtionischen Kontrastmitteln durchgeführt. Für die mit Doxo- oder Epirubicin beladenen Beads konnte die Stabilität bzw. Kompatibilität nach Mischung mit ausgewählten nicht-ionischen Kontrastmitteln für 7 Tage bei 2-8 °C gezeigt werden [41, 90]. Im Gegensatz dazu erwiesen sich mit Irinotecan beladene DC Beads als inkompatibel mit nicht-ionischen Kontrastmitteln. Die Mischungen führten zu einer rapiden Abnahme der Irinotecan-Beladungsrate innerhalb weniger Stunden [60, 89]. Es kann daher nicht empfohlen werden die mit Irinotecan beladenen DC Beads zentral in der Apotheke mit Kontrastmitteln zu mischen, statt dessen sollte dies durch den Radiologen unmittelbar vor der Anwendung erfolgen.

**Tabelle 3**: Datensätze zu mit Zytostatika beladenen DC Bead™

| Arzneimittel | Fertigarzneimittel | | Stammlösung | | | Beladung DC Bead™ | | | Physikalisch-chemische Stabilität beladene Beads | | Aufbewahrung der applikationsfertigen Lösung | Besonderheiten | Ausgewählte Kompatibilitäten | Zeitraum der Kompatibilität und Aufbewahrung der Mischung | Ausgewählte Inkompatibilitäten |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rekonstituens | | | | | | | | | | | | |
| INN-Name | Name (Hersteller) | Gehalt [mg] | Art | Menge [ml] | Konzentration [mg/ml] | Menge [mg / 2 ml Bead] | Durchmesser [µm] | Zeit (Bel.rate) / Bedingung | RT | 2-8 °C | | | | | |
| **DC Bead™ mit Doxorubicin-HCl** | Doxorubicin NC 2 mg/ml (NeoCorp AG) | 100 | – | (50) | 2 | 50 | 70 - 150 | 4 h (98%) / gelegentl. Schwenken | – | 14 d | Lichtgeschützt Kühl | – | Omnipaque™ 350 (Iohexol) (nur: 100-700 µm) | 7 d bei 2-8 °C | – |
| | | | | | | | 500 - 700 | 12 h (>99%) / kont. Schwenken | | | | | | | |
| | | | | | | 75 | 70 - 150 | 5 h (98%) / gelegentl. Schwenken | | | | | | | |
| | Doxorubicin Actavis® (Actavis Deutschland GmbH) | 50 | Aqua ad inject. | 2 | 25 | 50 - 75 | 70 - 150<br>100 - 300 | 1 h (98%) / gelegentl. Schwenken | – | 14 d | Lichtgeschützt Kühl | | | | |
| | | | | | | | 300 - 500 | 1,5 h (98%) / gelegentl. Schwenken | | | | | | | |
| | | | | | | | 500 - 700 | 2 h (98%) / gelegentl. Schwenken | | | | | | | |
| | | | | | | 50 | 100 - 500 | 10 min. (>99%) / kont. Schwenken | | | | | | | |
| | | | | | | 50 | 500 - 900 | 1 h (>99%) / kont. Schwenken | | | | | | | |
| | | | | | | 50 | 900 - 1200 | 4 h (>99%) / kont. Schwenken | | | | | | | |

| Arzneimittel | Fertigarzneimittel | | Stammlösung | | | Beladung DC Bead[TM] | | | Physikalisch-chemische Stabilität beladene Beads | | Aufbewahrung der applikationsfertigen Lösung | Besonderheiten | Ausgewählte Kompatibilitäten | Zeitraum der Kompatibilität und Aufbewahrung der Mischung | Ausgewählte Inkompatibilitäten |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rekonstituens | | | | | | | | | | | | |
| INN-Name | Name (Hersteller) | Gehalt [mg] | Art | Menge [ml] | Konzentration [mg/ml] | Menge [mg / 2 ml Bead] | Durchmesser [µm] | Zeit (Bel.rate) Bedingung | RT | 2-8 °C | | | | | |
| DC Bead[TM] mit Epirubicin-HCl | Epimedac[B] (medac) | 200 | – | (100) | 2 | 76 | 70 - 150<br>100 - 300 | 6 h (95%)<br>statisch<br>6 h (90%)<br>12 h (96%)<br>initiales Schwenken | 28 d | – | Lichtgeschützt Raumtemperatur | – | Accupaque™ 300 (Iohexol)<br>Imeron® 300 (Iomeprol)<br>Ultravist® 300 (Iopromid)<br>Visipaque ™ 350 (Iodixanol) | 7 d bei 2-8 °C | – |
| | Farmorubicin[B] (Pfizer) | 50 | Aqua ad inject. | 2 | 25 | 75 | 70 - 300 | 0,5 h (99%)<br>initial u. nach 15 min. Schwenken | 28 d | – | Lichtgeschützt Raumtemperatur | | | | |

| Arzneimittel | Fertigarzneimittel | | Stammlösung | | | Beladung DC Bead™ | | | Physikalisch-chemische Stabilität beladene Beads | | Aufbewahrung der applikationsfertigen Lösung | Besonderheiten | Ausgewählte Kompatibilitäten | Zeitraum der Kompatibilität und Aufbewahrung der Mischung | Ausgewählte Inkompatibilitäten |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rekonstituens | | | | | | | | | | | | |
| INN-Name | Name (Hersteller) | Gehalt [mg] | Art | Menge [ml] | Konzentration [mg/ml] | Menge [mg / 2 ml Bead] | Durchmesser [µm] | Zeit (Bel.rate) Bedingung | RT | 2-8 °C | | | | | |
| **DC Bead™ mit Irinotecan** | Campto® (Pfizer Pharma GmbH) | 100 | – | (5) | 20 | 100 | 100 - 500 | 2 h (96%)<br>gelegentl. Schwenken<br>2 h (98%)<br>kont. Schwenken | 28 d | 14 d | Lichtgeschützt Raumtemperatur | Nur unmittelbar vor Applikation mit Kontrastmitteln (auch den unter Inkompatibilität genannten Kontrastmitteln) mischen. | – | – | Accupaque™ 300 (Iohexol) (1:1)<br>Accupaque™ 350 (Iohexol) (1:6)<br>Imeron® 300 (Iomeprol) (1:1)<br>Imeron® 400 MCT (Iopromid) (1:4, 1:6)<br>Optiray™ 300 (Ioversol) (1:1, 1:2, 1:4, 1:6)<br>Optiray™ 350 (Ioversol) (1:1, 1:2, 1:4, 1:6)<br>Solutrast® 370 (Iopamidol) (1:4, 1:6)<br>Ultravist® 300 (Iopromid) (1:1)<br>Ultravist® 370 (Iopromid) (1:4, 1:6)<br>Xenetix® 350 (Iobitrodol) (1:4, 1:6) |
| | | | | | | | 500 - 700 | 2 h (95%)<br>kont. Schwenken | | | | | | | |
| | | | | | | | 700 - 900 | 2 h (93%)<br>kont. Schwenken | | | | | | | |
| | Irinotecan Accord® (Accord Healthcare Limited) | 500 | – | (25) | 20 | 100 | 70 - 150 | 2 h (99,5%)<br>initiales Schwenken<br>5 h (100%)<br>statisch | 28 d | – | Lichtgeschützt Raumtemperatur | | | | |
| **DC Bead™ mit Topotecan** | Hycamtin® 4 mg (Glaxo SmithKline) | 4 | Aqua ad inject. | 4 | 1 | 4 | 300 - 500 | 6 h (>92%)<br>statisch<br>1 h (>90%)<br>2 h (>92%)<br>kont. Schwenken | 7 d | – | Lichtgeschützt Raumtemperatur | – | – | – | – |

***Neue Daten zu bekannten Wirkstoffen***

Insgesamt wurden 43 neue Veröffentlichungen und Informationen zu bereits in der vorherigen Auflage der Liste enthaltenen Wirkstoffen gefunden. Davon flossen 26 in die Neuauflage ein (s. Tabelle 4). Die restlichen Daten wurden entweder als nicht valide bewertet oder lieferten keine neuen Erkenntnisse bzw. blieben mit ihren Untersuchungszeiträumen hinter den bereits bekannten Stabilitätszeiträumen zurück.

**Tabelle 4**: Neue Informationsquellen zu bestehenden Datensätzen

| Wirkstoff | Informationsquelle | Berücksichtigt bei der Aktualisierung der Stabil-Liste© 2015 |
|---|---|---|
| Alemtuzumab | Goldspiel 2013 [343] | Ja |
| Amsacrine (AMSA) | Packungsbeilage Amsalyo [80] | Ja |
| Asparaginase | pi[1] Elspar® [67] | Ja |
| Azacitidin | Walker 2012 [97] | Teilweise, da Daten nicht plausibel |
| | Legeron 2013 [65] | Nein, längere Stabilität bekannt und Daten nicht plausibel |
| Azathioprin | FI[2] Imurek® [9] | Ja |
| Bevacizumab | Morand [68] | Nein, entsprechende Stabilität bereits bekannt |
| | Gonzales 2015 [35] | Nein, Daten nicht valide |
| | Khalili 2015 [62] | Nein, entsprechende Stabilität bereits bekannt |
| | Palmer 2013 [74] | Nein, entsprechende Stabilität bereits bekannt |
| | Pereboom 2015 [79] | Nein, entsprechende Stabilität bereits bekannt |
| Bleomycin | Hexal 2013 [44] | Ja |
| | Hexal 2013 [42] | Nein, längere Stabilität bekannt |
| Bortezomib | Walker 2014 [98] | Ja |
| | Berruezo Garcia 2013 [17] | Nein, Daten nicht plausibel |
| | Lipp 2013 [66] | Nein, längere Stabilität bekannt |
| Busulfan | Houot 2013 [48] | Nein, entsprechende Stabilität bereits bekannt |
| Calciumfolinat | Karbownik 2013 [61] | Ja |
| | Hexal 2013 [44] | Nein, längere Stabilität bekannt |
| | Hexal 2013 [42] | Nein, längere Stabilität bekannt |
| Carboplatin | Hexal 2013 [44] | Nein, entsprechende Stabilität bereits bekannt |

| | | |
|---|---|---|
| Carmustin | Fresenius 2013 [52] | ja |
| Cetuximab | Gonzales 2015 [36] | Nein, Daten nicht valide |
| | Gonzales 2015 [38] | Nein, längere Stabilität bekannt |
| Cisplatin | Hexal 2013 [44] | Nein, längere Stabilität bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| Clofarabin | FI$^2$ Evoltra® [33] | Ja |
| Cyclophosphamid | Baxter 2014 [15] | Ja |
| | Gebrauchsinfo 2011 [12] | Ja |
| Cytarabin | FI$^2$ Cytarabin Accord [1] | Ja |
| Decitabin | Janssen 2015 [56] | Nein, valide Daten bekannt |
| Dexrazoxan | Zhang 2014 [101] | Ja |
| Docetaxel | Hexal 2013 [44] | Nein, längere Stabilität bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| | Physicochemical Data Docetaxel Accord 20 [2] | Nein, entsprechende Stabilität bereits bekannt |
| Doxorubicin | Escudero-Ortiz 2014 [32] | Ja |
| | Hexal 2013 [44] | Nein, längere Stabilität bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| Epirubicin | Amandine [3] | Ja |
| | Hexal 2013 [44] | Nein, längere Stabilität bekannt |
| Etoposid | Hexal 2013 [44] | Nein, längere Stabilität bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| Fludarabin | Trittler 2010 [94] | Ja |
| | Hexal 2013 [44] | Nein, entsprechende Stabilität bereits bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| 5-Fluorouracil | Hexal 2013 [44] | Nein, längere Stabilität |

| | | |
|---|---|---|
| | | bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| Gemcitabin | Hexal 2013 [44] | Nein, entsprechende Stabilität bereits bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| Ifosfamid | Zhang 2014 [100] | Nein, entsprechende Stabilität bereits bekannt |
| Ipiliumumab | Bardo 2013 [11] | Ja |
| Irinotecan | Hexal 2013 [44] | Nein, entsprechende Stabilität bereits bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| Lenograstim | FI$^2$ Granocyte® [28] | Ja |
| Methotrexat | Hexal 2013 [44] | Nein, längere Stabilität bekannt |
| | Hexal 2013 [42] | Nein, längere Stabilität bekannt |
| Mitoxantron | Hexal 2013 [44] | Nein, längere Stabilität bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| Ofatumumab | FI$^2$ Arzerra® [73] | Ja |
| Oxaliplatin | Escudero-Ortiz 2014 [32] | Ja |
| | Hexal 2013 [44] | Nein, längere Stabilität bekannt |
| | Hexal 2013 [42] | Nein, entsprechende Stabilität bereits bekannt |
| Paclitaxel | Hexal 2013 [45] | Nein, entsprechende Stabilität bereits bekannt |
| | Hexal 2013 [43] | Nein, entsprechende Stabilität bereits bekannt |
| | Hexal 2014 [46] | Nein, entsprechende Stabilität bereits bekannt |
| Paclitaxel Albumin-gebunden | Celgene 2014 [25] | Ja |
| Panitumumab | Apotheke Mainz 2014 [7] | Ja |
| Pentostatin | FI$^2$ Nipent [47] | Ja |
| Pixantron | cti Life Sciences 2013 [29] | Ja |
| Rituximab | Zhang 2013 [102] | Nein, entsprechende Stabilität bereits bekannt |

| | | |
|---|---|---|
| Temozolomid | FI[2] Temodal® [70] | Ja |
| Thiotepa | Riemser 2013 [82] | Ja |
| | SMPC Tepadina® [83] | Nein, valide Daten bekannt |
| Trastuzumab | Paul 2013 [77] | Nein, entsprechende Stabilität bereits bekannt |
| | Gonzales 2015 [39] | Nein, Daten nicht valide |
| Vincristinsulfat | Hexal 2013 [44] | Nein, längere Stabilität bekannt |

[1] pi = prescribing information
[2] FI = Fachinformation

**Diskussion**

Die in der Stabil-Liste© aufgeführten Daten zur physikalisch-chemischen Stabilität wurden durch sorgfältige Analyse der Originalliteratur bzw. durch eigene experimentelle Untersuchungen gewonnen und unterscheiden sich von den Angaben der Zulassungsinhaber in den Gebrauchs- und Fachinformationen. Sofern das Produkt nicht unverzüglich angewendet wird, liegen die Aufbewahrungszeiten und Bedingungen in der Verantwortung des Anwenders. Unter Berücksichtigung der mikrobiologischen Instabilität ist die Verwendbarkeit bei Zubereitung in patientennahen Bereichen auf maximal 24 Stunden zu begrenzen. Bei Zubereitung unter Reinraumbedingungen in der Apotheke ist die mikrobiologische Instabilität zu berücksichtigen und die Verwendbarkeit entsprechend der mikrobiologischen Validierung zu begrenzen.

Eine immer wieder geführte Diskussion ist die Frage, ob die Stabilität bis 95% oder 90% des Nenngehalts als gegeben anzusehen ist. Dies kann nicht pauschal beantwortet werden und sollte auch unter klinischen Aspekten, wie therapeutischer Index eines Wirkstoffs, maximal tolerable Menge von toxischen Zersetzungsprodukten und speziellen Applikationswegen (Aggregate, Partikel bei intrathekaler, intraokularer Applikation) im Einzelfall entschieden werden [10]. Wenn toxische Zersetzungsprodukte entstehen und die Arzneibuchmonographien Grenzwerte für diese enthalten (z.B. bei Cisplatin), müssen diese selbstverständlich berücksichtigt werden. Für die neu aufgenommenen Wirkstoffe sind uns derartige Grenzwerte nicht bekannt. Bei

allen Biopharmazeutika müssen Aggregate als immunogen und daher als stabilitätsbegrenzend untersucht und beachtet werden. Für den Anwender könnte es bei sehr instabilen Wirkstoffen hilfreich sein, wenn in der Stabil-Liste© die Angabe der Haltbarkeit um die gewählte Grenze bzw. den Hinweis auf toxische Zersetzungsprodukte ergänzt wird.

Folgende Fragen werden häufig zur Stabil-Liste© gestellt und diskutiert.

***Welches Lösungsmittel sollte zur Rekonstitution des Cyclophosphamid Lyophilisats verwendet werden und wie stabil ist die resultierende Lösung?***

Cyclophosphamid Lyophilisat unterscheidet sich von der Pulverform durch zusätzlich enthaltenes Mannit. Dieses hat nach unserem Dafürhalten keinen Einfluss auf die Stabilität nach Rekonstitution. Der entscheidende stabilitätsdeterminierende Faktor für Cyclophosphamid ist die Temperatur [20]. Die Untersuchungen zur Stabilität der rekonstituierten Cyclophosphamid Stammlösung aus der Pulverform über 28 Tage bei 2-8 °C stammen aus 1973 [20] und 1984 [64]. Sie wurden mit dem damals vom Hersteller empfohlenen Rekonstitutionsmittel Aqua ad injectabilia durchgeführt. Erst viele Jahre später wurde vom Hersteller zur Rekonstitution die NaCl 0,9% Injektionslösung empfohlen [14], um die Osmolarität der rekonstituierten Lösung der Osmolarität des Blutes anzunähern. Analog wurde für das Lyophilisat zunächst Aqua ad inject. [12] und später NaCl 0,9% [13] als Rekonstitutionsmittel empfohlen. Gemäß Informationen der Herstellerfirma Baxter Oncology [15] reduziert sich der Cyclophosphamidgehalt des mit NaCl 0,9% Lösung rekonstituierten Lyophilisats bei einer Temperatur <8 °C um 0,3% pro Tag. Gleichzeitig bestätigt der Hersteller, dass die beiden Fertigarzneimittel Cyclophosphamid Pulver und Cyclophosphamid Lyophilisat sowohl mit NaCl 0,9% Lösung als auch mit Aqua ad injectabilia rekonstituiert werden können [15]. Daher können die Stabilitätsangaben über 28 Tage bei 2-8 °C sowohl auf die Stammlösung rekonstituiert mit Wasser oder NaCl 0,9% Lösung als auch auf die Stammlösung aus Lyophilisat oder Pulver angewendet werden.

***Kann ein Anbruch von Panitumumab (Vectibix®) aufbewahrt werden?***

Gemäß einer Stabilitätsuntersuchung mittels ELISA von Ikesue et al. [55] sind die Panitumumab Stammlösung nach Anbruch (20 mg/ml) und daraus zubereitete Infusionslösungen (2,5 mg/ml) über mindestens 14 Tage bei 2-8 °C stabil. Nach 14 Ta-

gen betrug der Gehalt an Panitumumab noch 102,7% bzw. 100,5% des Ausgangsgehalts. Die Konzentration der Stammlösung zum Zeitpunkt t0 betrug im Mittel 20,00 mg/ml, die der Infusionslösung 2,55 mg/ml ± 0,18 (Soll: 2,5 mg/ml). Die Publikation enthält den Hinweis, dass die Panitumumab-Stammlösung zur Zubereitung der Infusionslösung ohne nennenswerten Wirkstoffverlust durch einen 0,22 µm Filter gefiltert wurde. In der Praxis wird bei Aufbewahrung des Anbruchs innerhalb weniger Stunden eine mehr oder weniger starke Trübung durch proteinöse Strukturen beobachtet. Gemäß Produktinformation [4] handelt es sich bei Vectibix® um ein farbloses Konzentrat, das durchscheinende bis weiße, sichtbare amorphe, proteinöse Panitumumab-Partikel enthalten kann. Aufgrund zahlreicher Reklamationen hat die Herstellerfirma Amgen eine Untersuchung in Auftrag gegeben. Diese kam zu dem Ergebnis, dass der Wirkstoffgehalt nach Verdünnung in NaCl 0,9% Lösung und anschließender Filtration bei 92% und damit innerhalb der Endproduktspezifikation (80 – 125%) lag [5]. Der Wirkstoffgehalt des unverdünnten Konzentrats nach Anbruch wurde nicht untersucht. Aus den beiden Informationen lässt sich ableiten, dass die Ausflockung von Panitumumab bekannt ist und toleriert wird. Offensichtlich unterliegt der Panitumumab-Gehalt des Konzentrats nach Anbruch durch die Ausflockung einer hohen Variabilität, so dass die Aufbewahrung des Anbruchs nicht empfohlen werden kann. Die Applikation der Infusionslösung über einen Inline-Filter der Porengröße 0,2 µm mit niedrigem protein-bindenden Potential ist zwingend erforderlich. Dieser Hinweis wird in die nächste Auflage der Stabil-Liste© aufgenommen.

***Ist die Haltbarkeit der Decitabin Infusionslösung auf 3 Stunden zu begrenzen?***

Gemäß Rundschreiben der Herstellerfirma Janssen beträgt die Aufbrauchsfrist für Decitabin Infusionslösungen nach Verdünnung mit vorgekühlten Infusionslösungen nicht wie bisher empfohlen 9 Stunden sondern nur 3 Stunden [56]. Grundlage dieser Änderung waren keine neuen Untersuchungen, sondern eine Neubewertung der vorhandenen Daten unter Berücksichtigung engerer Grenzwerte. Unabhängig von der Herstellerfirma untersuchten Patel et al. [75] die Stabilität von Decitabin nach Verdünnung mit vorgekühlten Trägerlösungen (NaCl 0,9% oder Glucose 5% Lösung). Der Decitabingehalt der Infusionslösungen betrug nach einem Zeitraum von 7 Stunden bei 2-8 °C und anschließender 3-stündiger Lagerung bei Raumtemperatur 92% des Ausgangsgehalts. Nach Zubereitung mit ungekühlten Trägerlösungen und Aufbewahrung bei Raumtemperatur lag der Wirkstoffgehalt nach 3 Stunden bei

93% (1,0 mg/ml) bzw. 90% (0,1 mg/ml). Zur Reduktion der Aufbrauchsfrist von Decitabin Infusionslösungen auf 3 h besteht daher aus unserer Sicht keine Veranlassung.

***Sind die Angaben zur Stabilität der mit vorgekühltem Aqua ad inject. rekonstituierten Azacitidin Suspension valide?***

Die Stabilität von Azacitidin in Lösung ist stark temperaturabhängig [8]. Das Stabilitätsoptimum liegt bei pH 6-8 [23]. Azacitidin hydrolysiert in neutralem, wässrigen Milieu reversibel, aber rasch zu der ringoffenen Form N-formylguanylribosylharnstoff (RGU-CHO), welches langsamer irreversibel zu RGU (Guanylribosylharnstoff) deformyliert wird [16, 26]. Vieillard et al. untersuchten die Stabilität von Azacitidin (100 mg) nach Rekonstitution mit gekühltem Aqua ad inject. (4 °C, 4 ml) zu einer Suspension (25 mg/ml) über einen Zeitraum von 10 Tagen [96]. Die Gehaltsbestimmung erfolgte mittels HPLC mit UV-Detektion. Die Peaks der beiden Zersetzungsprodukte RGU-CHO und RGU waren deutlich vom Azacitidin-Hauptpeak getrennt. Vieillard et al. stellten analog zu den Untersuchungen mit Azacitidin Lösung von Hartigh et al. [30] und Cheung et al. [27] fest, dass auch bei der Azacitidin Suspension die Zersetzung in zwei Phasen verläuft, einer ersten, schnellen Phase (vermutlich die Gleichgewichtsreaktion zur ringoffenen Form CHO-RGU) und einer zweiten, langsameren Phase (vermutlich die Deformylierung zu RGU). Unmittelbar nach Rekonstitution betrug der Azacitidingehalt bereits nur 96,85% ± 1,29%. Innerhalb von 5 Tagen reduzierte sich der Gehalt auf absolut 95%, nach 10 Tagen auf absolut ca. 88%. Der gefundene Azacitidingehalt wurde nicht (wie bei Stabilitätsuntersuchungen üblich) als Prozentsatz der zum Zeitpunkt t0 gefundenen Konzentration ausgedrückt, sondern als Prozentsatz der Nominalkonzentration 25 mg/ml. Mit diesem Vorgehen wird vermieden, dass bei Wirkstoffen mit hoher initialer Zersetzungsrate die gefundenen Konzentrationen an nicht zersetztem Wirkstoff falsch zu hoch gewertet werden.

Vergleichbare Ergebnisse fand Tutino für die Azacitidin Suspension [95]. Nach Rekonstitution mit kaltem Aqua ad inject. und Lagerung bei 2-8 °C für 22 Stunden, gefolgt von einer 30 minütigen Erwärmung auf 25 °C reduzierte sich der Azacitidingehalt nur um absolut 2,7%.

Die Herstellerfirma Celgene selbst gibt die Zersetzungsrate einer Azacitidin Suspension zubereitet mit zimmerwarmem Aqua ad inject. und Lagerung bei 2-8 °C mit

0,1% pro Stunde an. Damit läge der Azacitidingehalt einer Suspension nach 50 Stunden bei 95% und nach 100 Stunden bei 90%. Allerdings wurde die Zersetzungsrate nicht anhand des Azacitidingehalts, sondern indirekt anhand des Gehalts der Zersetzungsprodukte ermittelt. Entsprechend der oben beschriebenen Zersetzungskinetik stieg der Gehalt der Zersetzungsprodukte innerhalb der ersten beiden Stunden um ca. 1,2%, in den folgenden Stunden nur um 0,083%/h. Nach Suspension mit kaltem Aqua ad inject. (4 °C) und Lagerung über 72 Stunden bei 2-8 °C gefolgt von 30 Minuten Lagerung bei Raumtemperatur wurde eine Zersetzungsrate von 0,12%/h ermittelt. Der Azacitidingehalt reduzierte sich innerhalb von 72,5 Stunden um 8,7%. Die Zersetzungsrate von Azacitidin Suspension bei Raumtemperatur beträgt 2,5%/h [23].

Legeron et al. [65] ermittelten die Stabilität von Azacitidin nach Suspension mit 4 °C kaltem Aqua ad inject. mit nur 48 Stunden. Allerdings zeigten die Werte zu den verschiedenen Untersuchungszeitpunkten starke Schwankungen. Der Azacitidin Gehalt reduzierte sich nach 12 Stunden (t12h) nur um 0,72%, nach weiteren 12 Stunden (t24h) plötzlich um weitere 3,1%, nach weiteren 24 Stunden (t48h) wieder nur um weitere 0,41% und nach weiteren 24 Stunden (t72h) wieder um weitere 2,89%. Die gefundenen Werte lassen sich nicht mit der für Azacitidin bekannten Zersetzungskinetik plausibilisieren.

Ebenso können die HPLC-Untersuchungen von Walker et al. [97] als nicht valide bewertet werden. Für alle Untersuchungszubereitungen wurde zum Zeitpunkt 0 ein Azacitidin Gehalt von deutlich über 25 mg/ml gefunden. Diese Werte müssen als falsch zu hoch angenommen werden, zumal bei der Methodenvalidierung für die Wiederfindungsrate eine mittlere Abweichung von 4,25% angegeben wurde. Da alle Werte der späteren Untersuchungszeitpunkte auf den Wert zum Zeitpunkt t0 bezogen wurden, müssen die Ergebnisse dieser Untersuchung insgesamt in Frage gestellt werden.

***Warum ist Abraxane® in der Stabil-Liste© als unverdünnte Infusionslösung länger stabil als im Vial? Muss zur Applikation ein Filter verwendet werden?***

Laut Stabil-Liste© ist Abraxane® als Stammlösung im Originalbehältnis für 8 Stunden bei 2-8 °C stabil und als Infusionszubereitung im Infusionsbeutel für 48 Stunden bei 2-8 °C stabil. Diese Angabe erscheint nicht plausibel, da Abraxane® unverdünnt appliziert wird. Die Angaben stammen von der Herstellerfirma Celgene [24]. Die Stabi-

lität der Stammlösung wurde entsprechend dieser Informationen in einem Infusionsbeutel aus PVC über einen Zeitraum von 48 Stunden, im Originalvial jedoch nur über einen Zeitraum von 8 Stunden untersucht. Unserer Auffassung nach kann man die Daten über 48 h im PVC-Beutel auch auf die Lagerung im Originalbehältnis aus Glas übertragen. Daher wurde in die Stabil-Liste© unter Besonderheiten der Hinweis aufgeführt, dass die Stabilität im Originalvial nicht länger als 8 h untersucht wurde.
Kürzlich erreichte uns auch der Hinweis, dass die Aufbrauchsfrist von Abraxane® nach Erstanbruch in der US-amerikanischen prescribing information im Originalvial mit 24 h bei 2-8 °C angegeben wird [21]. Diese Angabe wird bei der nächsten Auflage der Stabil-Liste© übernommen.
Gemäß Fachinformation [22] kann Abraxane®-Suspension in Infusionsbeutel aus PVC oder non-PVC-Materialien injiziert werden. Aus theoretischen Überlegungen heraus kann Kompatibilität und Stabilität von mindestens 48 h in allen Primärpackmitteln, einschließlich EVA-Infusionsbeuteln, angenommen werden.
Im Januar 2014 wurde ein Rote-Hand-Brief bzgl. sichtbarer Fäden in der rekonstituierten Abraxane®-Suspension herausgegeben, mit dem Hinweis, bei Vorhandensein sichtbarer Fäden sei Abraxane® über einen Filter der Porengröße 15 µm zu infundieren. Ein Filter mit kleinerer Porengröße darf nicht verwendet werden [25]. Diese Hinweise wurden in die aktuelle Auflage der Stabil-Liste© aufgenommen. Inzwischen wird in der Fachinformation generell die Applikation über einen 15 µm-Filter empfohlen, da die Verwendung von Einmalspritzen, welche Silikonöl als Gleitmittel enthalten bei Kontakt mit Abraxane® zur Bildung proteinöser Fäden führen kann. Dieses Phänomen ist auch von anderen proteinösen Arzneistoffen bekannt [18, 57].

***Können die Daten der Stabil-Liste von einem Fertigarzneimittel auf ein anderes Fertigarzneimittel übertragen werden?***

In der Stabil-Liste© werden nur beispielhafte Fertigarzneimittel genannt. Die Stabilitätsuntersuchungen wurden nicht zwingend mit diesen Fertigarzneimitteln durchgeführt. Unserer Auffassung nach können bei identischer Formulierung Stabilitätsdaten in der Regel problemlos von einem auf ein anderes Fertigarzneimittel übertragen werden. Die Bewertung der Vergleichbarkeit von Formulierungen fällt bei Konzentraten oftmals leichter als bei Trockensubstanzen, deren Löslichkeit durch Unterschiede in der quantitativen Zusammensetzung von Hilfsstoffen variieren kann. Vorsicht ist bei der Übertragung von Daten geboten wenn die Fertigarzneimittel mit Lösungs-

vermittlern formuliert sind. Die physikalische Stabilität solcher Wirkstoffe lässt sich nur schwer vorhersagen und kann bereits durch Veränderungen in der Herstellung von den lösungsvermittelnden Hilfsstoffen stark beeinträchtigt sein. Bei Hinweisen auf eine ggf. unterschiedliche physikalisch-chemische Stabilität von ähnlich formulierten Fertigarzneimitteln sind in der Stabil-Liste© zu dem betreffenden Wirkstoff zwei Datensätze mit unterschiedlichen Stabilitätsangaben durch eine durchgezogenen Linie konsequent getrennt. Ein Hinweis auf die unterschiedlichen Formulierungen ist in diesen Fällen unter Besonderheiten aufgeführt, z.B. bei Fludarabin und Paclitaxel.

***Welcher Filter ist zur Applikation von Wirkstoff xy geeignet?***

Wenn in der Packungsbeilage oder Fachinformation Filter zur Applikation empfohlen bzw. vorgeschrieben sind, finden sich die entsprechenden Hinweise in der Stabil-Liste® in der Spalte Besonderheiten zusammen mit der Angabe der Porengröße und der Materialeigenschaften. Im Sinne der Patientensicherheit und zur Arbeitserleichterung auf Station empfiehlt es sich, in der Apotheke den entsprechenden Filter zu der jeweiligen Zubereitung beizupacken. Bei einigen Fertigarzneimitteln werden passende Filter bereits vom Hersteller kostenlos zur Verfügung gestellt. Der Markt der Filter ist sehr groß und unübersichtlich, so dass von der Apotheke eine wirkstoffbezogene Filter-Liste erstellt werden sollte. Zur Reduktion der Verwechslungsgefahr sollte dabei die Auswahl auf 2-3 Filter reduziert werden. Eine Publikation hierzu befindet sich in Vorbereitung [81].

***Wie sind die Ergebnisse der Immunoassay Untersuchungen zu diversen Antikörpern zu bewerten?***

Anlässlich des EAHP Kongresses 2015 wurden von der gleichen Autorengruppe mehrere Poster zur Stabilität von Antikörperzubereitungen (Bevacizumab [35], Cetuximab [36, 38], Trastuzumab [39], Infliximab [37]) präsentiert. Die Untersuchung der Stabilität der Antikörper fand ausschließlich mit einem Immunoassay für Bevacizumab und Trastuzumab oder mit einem Immunoassay ergänzt durch andere Methoden für Cetuximab und Infliximab statt. Nach den Regeln der ‚Guten Stabilitätsuntersuchungen' sind zur Bewertung der Stabilität von monoklonalen Antikörpern die Ergebnisse beispielsweise folgender Methoden in der Zusammenschau maßgeblich: sichtbare und nicht-sichtbare Partikel, Trübung, pH, Osmolarität, Größen-

ausschlusschromatographie, Ionenaustausch-HPLC, RP-HPLC mit UV Detektion, biologische Assays (am besten zellbasiert und Effektmessung). Ausschließlich auf einem Immunoassay basierend, können keine validen Aussagen zur Stabilität von Antikörperzubereitungen getroffen werden. Bei den präsentierten ELISA Untersuchungen für Bevacizumab und Trastuzumab fällt auf, dass jeweils am Tag 3 nach Erstanbruch die ‚biologische Aktivität' stark abnimmt (auf 80% bzw. 50% der Ausgangsaktivität) und dann bis Tag 57 bzw. Tag 15 weitgehend unverändert bleibt. Die Aktivitätsabnahme ist unabhängig von der Lagertemperatur (4 °C, -20 °C, -80 °C). In den Fachinformationen beider Fertigarzneimittel ist die Lagerung ausschließlich bei 4 °C empfohlen und wird explizit darauf hingewiesen, dass das Konzentrat bzw. die rekonstituierte Lösung nicht eingefroren werden sollen. Zusammen mit den schlechten Wiederfindungsraten und der hohen Variabilität der Wiederholpräzision und Vergleichspräzision sind die Untersuchungsergebnisse für die Stabilitätsbewertung als nicht valide einzustufen. Sie wurden in der Stabil-Liste© nicht berücksichtigt, zumal sie im Widerspruch zu anderen von uns als valide eingestuften Untersuchungsergebnissen stehen. Sehr umfangreiche Untersuchungen wurden beispielsweise zu Bevacizumab Zubereitungen durchgeführt [62, 68, 76, 78] und die Stabilität der Anbrüche und Zubereitungen über mindestens 28 Tage gezeigt. Das gleiche gilt für Trastuzumab Zubereitungen für die mit verschiedenen physikalisch-chemischen Methoden gezeigt wurde, dass Stabilität über 28 d gegeben ist [59, 77]. Zudem weisen die Autoren bei den Präsentationen zu Cetuximab und Infliximab auf die nicht konsistenten Ergebnisse der biologischen Aktivität und verschiedener HPLC-Methoden hin, die sie nicht erklären können.

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# 7 Compatibility of drug eluting DC Bead™ with different types of non-ionic contrast media

## *Treatment of patients with Hepatocellular carcinoma*

Hepatocellular carcinoma (HCC, also hepatic carcinoma) is the second most important cause of cancer-related death in the world(1). Nowadays the Barcelona Clinic Liver Cancer (BCLC) - Staging System is used to determine the diagnosis, prognosis and the best treatment strategy for the non-resectable HCC patients. The optimum strategy depends on cancer characteristics (number and size of invaded nodules, macrovascular invasion, extrahepatic metastasis) and cirrhosis related variables (liver function and portal hypertension) (65-67). According to the BCLC classification (see Fig 1), different stages are distinguished which are guiding the therapy strategy.

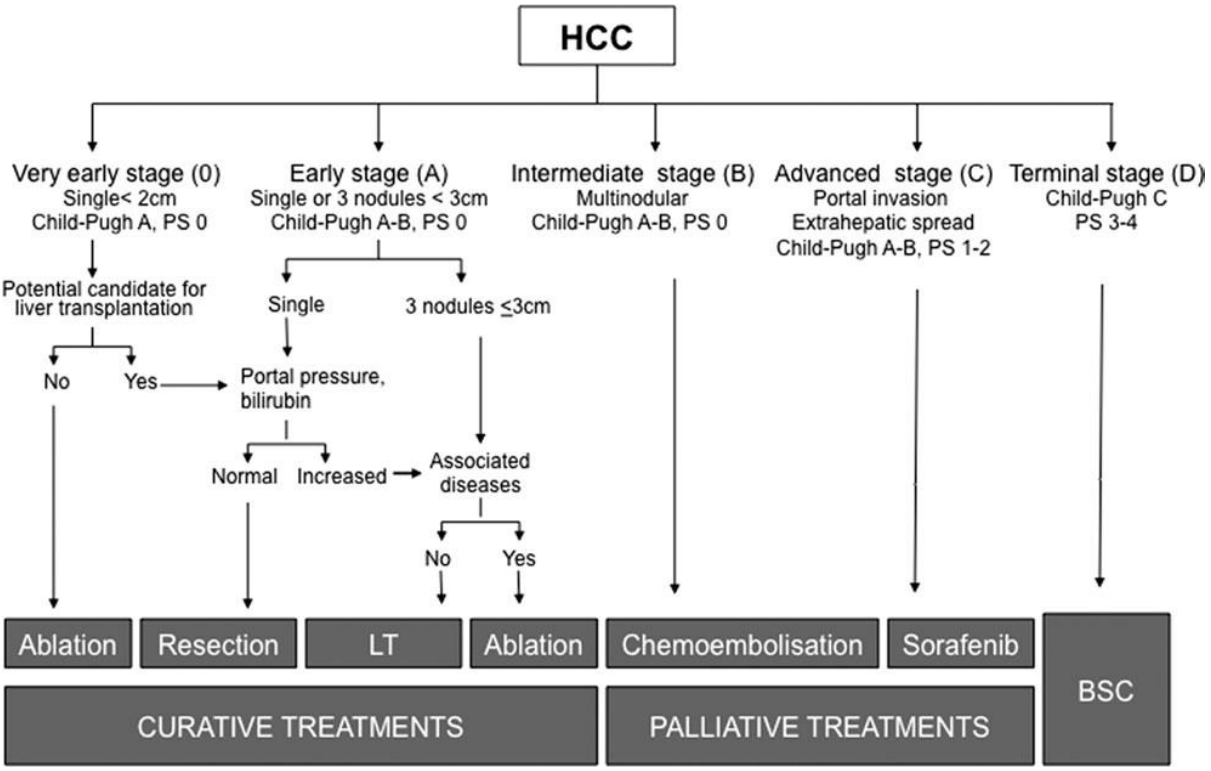


Figure 5. Liver cancer staging system defined by the BCLC group in order to determine the treatment of HCC patient. (PS = performance status 0-4, LT = liver transplantation (cited from ref. (68, 696)).

Curative therapy includes surgical treatment (hepatic resection as primary therapy for patient with HCC and a non-cirrhotic liver, liver transplantation for patients with cirrhotic liver and single nodule smaller than 5 cm or with maximal three lesions of 3 cm or less), percutaneous ethanol injection (PEI), destruction of the tumor tissue by various methods of ablation as cryotherapy, laser therapy, focused ultrasound, microwave ablation as a local ablation therapy and in particular radiofrequency ablation (RFA). RFA is utilized via electrodes with high radio-frequency waves which destroy the tumor tissue by heating (70, 71).

The multi-kinase inhibitor sorafenib is commonly used to treat HCC patients in order to increase life-expectancy and to retard the progress of HCC in patients with advanced hepatocellular carcinoma (70, 72, 73).

Trans-arterial embolization (TAE or bland embolization) or a combination of TAE and other therapies is used as a standard therapy in the palliative setting (74). The blood feeding of the tumor is maintained by the hepatic artery and thus by embolization of the hepatic artery oxygen and nutrient supply can be stopped and attribute to shrink the tumor. Depending on the hepatic reserve, the transarterial embolization can be performed as

- bland embolization (TAE),e.g. with superabsorbent polymer microspheres (SAP-TACE) (75)
- radioembolization (TARE), i.e. local radiation therapy or selective internal arterial radiotherapy (SIRT) with $^{90}$Yttrium loaded on micro-spheres. The spheres consist of glass (*TheraSpheres,* MDS Nordion)(14) or resin (*SIR-Spheres,* Sirtex Medical) (76).
- combination of cytotoxic drugs with iodized oily fluid that takes a role in delivering the chemotherapeutic agents (TOCE)
- combination of embolizing agents with chemotherapy = chemoembolization (TACE) where the cytotoxic drug is delivered prior to or simultaneously with the embolization agent. The reduced-blood flow keeps the cytotoxic agent in tumor bed with more selectively impact on the tumor cells.

## *7. 1 Trans-arterial Chemoembolization (TACE) therapy*

Nowadays the TACE treatment is the preferred treatment in patients with advanced stage HCC in German interventional radiology centers despite the absence of a consistent TACE protocol (77, 78). Moreover, the TACE procedure includes either transient occlusion by employment gelatin sponge segments (marketed as Gelfoam®), degradable starch microspheres (DSM) as an embolic agent, or permanent occlusion by using stainless steel coils, liquid embolics or polyvinylalcohol (PVA) particles.

DSM consists of hydrolyzed cross-linked starch particles. Because of the biodegradation by amylase within circa 35 min only temporary embolization is achieved and the chemoembolization can be repeated several times (79). Embocept® S is a widespread DSM product manufactured by the company PharmaCept. It causes less side effects and higher drug accumulation levels into tumor tissue than other devices, possibly because of portal washout (80). However there is no evidence for the impact of DSM on the survival or response rate compared to other TACE devices in patients suffering from advanced unresectable HCC (81).

Non-spherical PVA-embolizing particles lead to incomplete occlusion of targeted arteries because of the irregular shape and variable sizes of the microspheres. The granulometric distribution of the microspheres results in distal or untargeted occlusion and perivascular inflammatory changes. From this point of view, it is important to choose a proper embolic device in the right shape, homogenous size distribution, low elastic properties, and colored particles to ease the following of the injection. The marketed spherical embolic products do not have the same properties and are characterized by different sizes and embolization endpoints. Therefore, the interventional radiologists have to take into account the differences and to follow the manufacturers' recommendations in order to obtain the optimal results

According to the European Association for the Study of the Liver (EASL), conventional transarterial chemoembolization (cTACE) is approved as the first-line therapy for patients who are not able to undergo to surgery, transplantation or ablation (68). cTACE is performed by administration of an adequate amount of emulsified oily radiopaque fluid (usually Lipidiol®) mixed with chemotherapeutic agents like doxorubicin, irinotecan, mitomycin, and cisplatin. Irinotecan (CPT-11) revealed to be more advantageous than other anticancer

drugs (82). After delivery of the emulsion, the hepatic artery is embolized with Gelfoam® segments or microspheres (71, 77, 83, 84). Supraselective transarterial chemoembolization (sTACE) refers to injection of the embolic agent particular into selectively branches of hepatic artery that feed the tumor. The aim is to achieve better targeting of antineoplastic drug delivery as well as to minimize both the dosage of injected drug and the damage of surrounding healthy tissues (85). It is applied to patients with defined limited preserved liver function.

Drug eluting beads- facilitate a highly effective simultaneous embolization and local chemotherapy, also known as DEB-TACE. In this procedure, microspheres loaded with an anticancer agent embolize the feeding artery of tumor and ensure the controlled release of the cytotoxic drug, leading to localized tumor necrosis.

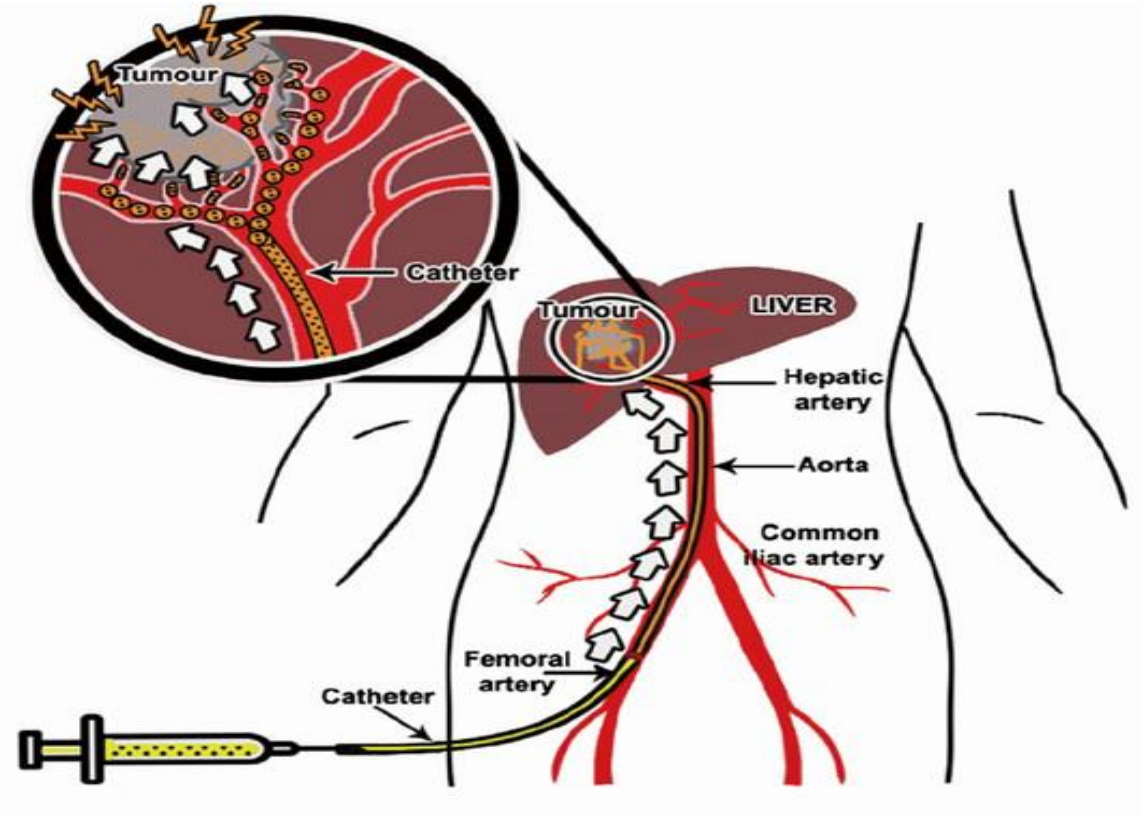


Figure 6. Illustration of transarterial chemoembolization treatment (cited by ref. (86))

The supplementary safety and efficacy of DEB-TACE among the TACE procedures has been proved in clinical trials by increased tumor response (87-89) and lower rates of side effects caused by the decreased systemic exposure (90). DEB-TACE treatment was associated with higher survival rates than cTACE and enhanced quality of life in patients with advanced

stages of HCC (91, 92). On the other hand there are study reports showing no significant difference in the safety and efficacy of DEB-TACE in comparison to cTACE. There are no unambiguous data supporting the preferred use e of a specific chemotherapeutic agent or a specific combination. Doxorubicin and irinotecan are considered to be the best choice for patients with HCC and metastatic colorectal cancer (mCRC) (93, 94). Doxorubicin, epirubicin, irinotecan and topotecan are cationic drugs which can be loaded to negatively charged microspheres. However, other efficacious antineoplastic agents like cisplatin and mitomycin cannot be loaded to the beads because of lack or limited cationic properties (95). In addition to the type of the chemotherapeutic agents, the choice of the bead size is also crucial for the success of the treatment. Smaller beads can settle deeper in the tumor and thus cause more tumor necrosis (96). The greater surface area of the smaller beads resultsin more rapid drug loading into the beads and is in turn associated with faster release of loaded drug (97, 98). Currently, the preferred size of microspheres is 100-300 microns diameter (83)

## *Loadable, commercially available Microsphere Products*

There are different types of embolizing agents currently marketed that have the ability to deliver the antitumor therapeutic agent to the targeted tumor. Medical products liecensed to treat HCC patients in Europe and the USA are: HepaSphere™ (known in the USA as Quadrasphere™), DC Bead® (LC bead™ in the USA) and Embozene Tandem®. All these products are constructed with negatively charged substructures, in order to be capable to uptake cationic drugs. By addition of positively-charged chemotherapeutic agent to the mixture, cation exchange is induced.

- **HepaSphere™ microspheres**

HepaSphere microspheres are produced by the company Merit Medical System. The two monomers vinyl acetate and methyl acrylate are used to form sodium acrylate alcohol copolymer. This design allows the negatively charged acrylate to interact with the cationic cytotoxic drugs. The microspheres are packaged in dry status without a solvent, to facilitate loading of aqueous solutions (99). The bead sizes range from 30-200 μm. The loading and elution rates of loaded drugs are based on both particle size and loading method (100).

- **Embozene TANDEM ® microspheres**

Embozone Tandem microspheres are licensed in Europe since 2012 and are aimed to prolonged release of the anticancer drug through utilizing smaller calibrated microsphere sizes (101). They consist of a non-organic perfluorinated polymer (Polyzene®-F) with a sodium carboxylate group which allows the ion exchange with cationic drugs. Embozene TANDEM ® microspheres are coming to the market as a suspension in prefilled syringes as primary packaging material. They are available in three tightly calibrated sizes 40 ± 10 µm, 75 ± 15 µm and 100 ± 25 µm, which guarantee the stability of the microsphere sizes during the loading process and under storage conditions (the size changes are less than 5%) (102). These microspheres have been designed to ensure lower eluting rates of the loaded drugs with higher penetration into the peripheral vessels of the tumor bed (101, 102).

- **DC Bead®**

DC Bead® is a CE marked hydrogel microsphere product developed by Biocompatibles International, meanwhile BTG International Ltd. The PVA hydrogel microspheres are cross linked with negatively charged sulfonate groups, with the purpose to release a loaded drug locally in a controlled behavior. The DC Bead® sizes available vary in diameter from the largest size (900-1200) µm to the smallest size of 70-150 µm (= M1) (103). DC Bead® is marketed in vials as 2 ml microspheres suspended in 6 ml physiological buffered saline, colored blue to facilitate the injection.

The loading process, loading period, and loading capacity depends on the type of DEB products and chemotherapeutic agent used. The loading features of doxorubicin into different microsphere products are given as an example in Table 5.

Table 4: Characters of doxorubicin-loaded currently available types of microsphere products

| Doxorubicin | DC Bead® | Hepasphere™ | EmbozeneTandem® |
|---|---|---|---|
| Loading amount | 37.5 mg/mL (100-300 µm or M1) | 25 mg/mL (400-600µm) | 50 mg/mL (40 /75/100µm) |
| Loading period | 60 min | 60 min | 60 min |
| Loading efficacy | 98±3 % | 82- 100% | 98±2 % |
| Drug release | 27±2 % | 18±7 % | - |
| Size changes after loading | ≤ 20 % | - | <5 % |

### *Loading process of DC Bead®*

The loading process of the beads can be carried out in the original DC Bead® vial when the drug volume to be used is smaller than the vial size. The loaded beads are transferred into a syringe for the application. Otherwise the unloaded beads are shaken, transferred via an 18-gauge needle into a syringe and loaded there. At first the excess buffered saline is expelled as far as possible via a 5 µm filter needle, in order to limit the competition with cationic drugs to bind to sulfonate binding sites. Attention should be paid during removing the buffered saline to avoid aspiration any beads. The bead slurry is then admixed with the calculated amount of the drug solution. The period of loading depends on various factors, such as total drug dosage, drug concentration, microsphere size, and agitation conditions (compare Fig. 7.).

Prior to the application, the excess supernatant is to be removed, as well as the drug loaded bead suspension is mixed with non-ionic contrast medium to guide the delivery into the targeted vessel. After the administration controlled and extended release of the chemotherapeutic agent is most relevant.

The compatibility of Dox-loaded beads (DEBDox) with selected non-ionic contrast media is proven for up to 7 days. No degradation products of Dox were observed the remaining loading rate amounted to > 99 % when the admixtures were stored under refrigerated conditions (22). In contrast, the admixture of irinotecan loaded beads (DEBIRI) (bead size 100-300 µm, 300-500 µm) and different non-ionic contrast media resulted in 5-10% immediate release of irinotecan from the beads when the samples were stored at room temperature and light protected (23).

Nowadays the smaller bead diameter is recommended to use in TACE procedures and DC Bead M1 (bead size 70-150 µm) is widely introduced into the market. Therefore it is important to evaluate the compatibility of the novel bead size 70-150 µm loaded with epirubicin or irinotecan and mixed with different nonionic contrast media which are commonly used by radiologists to guide the injection to the targeted tumor site. For epirubicin loaded beads, the study was planned to be performed with four different non-ionic contrast media over a period of 7 days while the samples are stored light protected under refrigerated conditions. In the same way, the compatibility of mixtures of DEBIRI and four different volumes of seven non-ionic contrast media was planned to be studied up to a period of 24 hours while stored at room temperature.

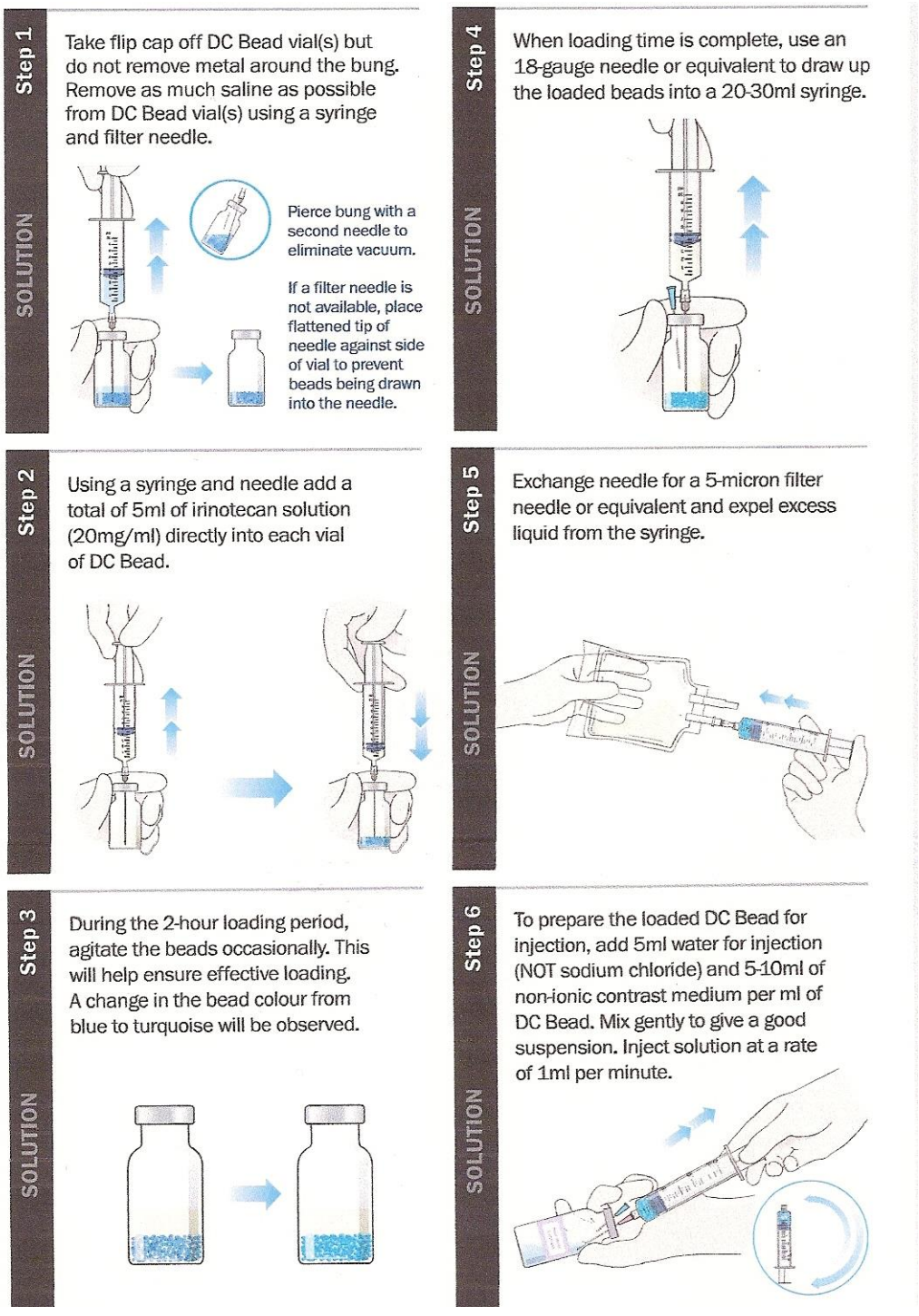


Figure 7. Steps of loading irinotecan solution into DC Bead ® according to the manufacturer's instruction leaflet (cited from ref. (104))

## *7.2 Compatibility of Epirubicin-loaded DC Bead™ with different contrast media*

Iman Sarakbi and Irene Krämer

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## *Epirubicin*

Epirubicin (or 4-epidoxorubicin) is the 4-epimer of doxorubicin and differs from Dox in the orientation of hydroxyl group at 4-carbon of sugar molecule.Like doxorubicin it belongs to the anthracycline cytotoxic category. Clinical trials started in 1980 (105), and epirubicin was approved for human use in Europe in 1982 (106). Epirubicin is used in patients with various types of tumors like breast cancer, gastric carcinoma, ovarian cancer, soft tissue sarcoma, and pancreatic cancer.

Epirubicin has the ability to intercalate into DNA and form complexes thus inhibiting the nucleic acid synthesis. Intercalation of DNA also prompts the DNA cleavage by topoisomerase II, leading to cytocidal activity (apoptosis) which is supported by the generation of free radicals in the biotransformation. Furthermore, epirubicin has shown the ability to bind to cell membranes and plasma proteins, resulting in increasing the membrane fluidity and permeability. Pronounced cytostatic activity takes place in the synthesis phase of the cell cycle (107). The effectivity of anthracyclines depends on the dose administered and the commonly observed toxicities are cardiotoxicity and myelosuppression. It is important to note, that the cardiotoxicity of anthracyclines is irreversible and the use limited to maximum cumulative doses. The cardiotoxicity is maybe due to the free radicals (107) and lack of the inactivating enzyme in the heart tissue.

**Epirubicin-loaded DC Bead™**

Several clinical studies were performed with epirubicin loaded beads (DEBEPI) (108-111). A validated loading procedure, compatibility and stability of the loaded beads is a prerogative for the safe and effective use of the beads. The loading of 75 mg epirubicin per 2 ml DC Bead™ (bead size 70-150 µm=M1, 100-300 µm) was analyzed in our working group. Physico-chemical stability of the epirubicin loaded beads was given over at least 28 days regardless of the formulation of epirubicin used, when the samples were stored light protected at room temperature. (112)

Loading of the epirubicin injection concentrate 2 mg/mL required 6 hours while the loading period of epirubicin powder for reconstitution 50 mg (reconstituted with 2 mL water for injection to achieve the concentration 25 mg/mL) was 2 hours. This longer loading time of

epirubicin injection concentrate is due to the lower concentration gradient. Moreover, the loading rate of epirubicin into DC Bead™ is dependent on the concentration of loading solution, size of the beads and agitation conditions applied during the loading process. Independent from the epirubicin formulation used during the loading process, the elution rate of epirubicin relies on the bead size as well as both the volume and ionic strength of elution medium (112).

Because there is a lack of data about the compatibility of epirubicin loaded DC Bead™ of the bead size 70-150 µm and 100-300 µm with different types of non-ionic contrast media , the aim of this study was to evaluate the amount of epirubicin eluted after mixing with four different non-ionic contrast media and storage over a period of 7 days under refrigerated conditions and light protection. In addition the purity of epirubicin eluted should be investigated.

Original Article

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# Compatibility of epirubicin-loaded DC bead™ with different non-ionic contrast media

**Iman Sarakbi and Irene Krämer**

**Abstract**

**Purpose:** The aim of this study was to determine the compatibility of epirubicin-loaded DC bead™ with different non-ionic contrast media over a period of seven days when stored light protected under refrigerated conditions.

**Methods:** DC bead™ (2 ml) (Biocompatibles UK Ltd) of the bead size 70–150 µm (= DC bead M1) or bead size 100–300 µm were loaded with 75 mg epirubicin powder formulation (Farmorubicin® dissolved in 3 ml water for injection to a concentration of 25 mg/ml) or 76 mg epirubicin injection solution (Epimedac® 2 mg/ml) within 2 h or 6 h, respectively. After removal of the excess solution, the epirubicin-loaded beads were mixed in polypropylene syringes with an equal volume (~1.5 ml) of contrast media, i.e. Accupaque™ 300 (Nycomed Inc.), Imeron® 300 (Bracco S.p.A), Ultravist® 300 (Bayer Pharma AG), Visipaque™ 320 (GE Healthcare) and agitated in a controlled manner to get a homogenous suspension. Syringes with loaded beads in contrast media were stored protected from light under refrigeration (2–8°C). Compatibility was determined by measuring epirubicin concentrations in the suspensions in triplicate on day 0, 1, and 7. A reversed phase high-performance liquid chromatography assay with ultraviolet detection was utilized to analyze the concentration and purity of epirubicin.

**Results:** Mixing of epirubicin-loaded beads with different non-ionic contrast media released 0.1–0.5% of epirubicin over a period of 24 h, irrespectively, of the DC bead™ size or type of contrast media. No further elution or degradation was observed after seven days when the admixtures were stored protected from light under refrigeration.

**Conclusion:** Compatibility of epirubicin-loaded DC bead™ with an equal volume of different contrast media in polypropylene syringes is given over a period of seven days. Due to a maximum elution of 0.1–0.5% of epirubicin from loaded DC bead™, admixtures with contrast media can be prepared in advance in centralized cytotoxic preparation units. Microbiological aspects have to be considered when determining the expiration date of the product.

**Keywords**

Drug eluting beads, epirubicin, contrast media, compatibility, high-performance liquid chromatography assay

## Introduction

Hepatocellular carcinoma (HCC) is a widespread primary liver tumor that ranks worldwide second in cancer-related death.[1,2] Depending on the stage of HCC, different treatment options are used which comprise surgical resection, transarterial chemo embolization (TACE), systemic chemotherapy, radio frequency ablation (RFA), selective internal radiation therapy (SIRT) and others.[3] TACE is also employed in liver metastases of colorectal carcinoma. The combination of the cytotoxic drug substance and the embolic agent is locally administered to the tumor site via a microcatheter. The embolic agent blocks the artery which feeds the tumor, leading to oxygen and nutrient deficiency in the tumor bed. Simultaneous administration is feasible with drug-loaded microspheres, such as DC bead™. DC bead™ consist of non-degradable

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**Table 1.** Physiochemical characteristics of the non-ionic contrast media.

| Contrast media (brand name) | Active substance | pH value | Viscosity at 20°C (mPas) | Viscosity at 37°C (mPas) | Osmolality at 37°C (mOsm) |
|---|---|---|---|---|---|
| Accupaque™ 300 | Iohexol | 6.8–7.6 | 11.6 | 6.1 | 640 |
| Imeron® 300 | Iomeprol | 6.5–7.2 | 8.1 | 4.5 | 510 |
| Ultravist® 300 | Iopromid | 6.5–8.0 | 8.7 | 4.6 | 610 |
| Visipaque™ 320 | Iodixanol | 6.8–7.7 | 26.6 | 11.8 | 290 |

hydrogel microspheres, which are copolymerized from polyvinyl alcohol (PVA) and unsaturated monomer 2-acrylamido-2-methylpropane sulfonate (AMPS).[4] The negatively charged sulfonate moieties of cross-linked PVA-AMPS permit to load and release positively charged drugs, for instance anthracyclines like doxorubicin and epirubicin (acting by DNA intercalation and topoisomerase II inhibition) or camptothecin derivatives like irinotecan or topotecan (acting by topoisomerase I inhibition).[5] The ion exchange capacity of DC bead™ loaded with cationic cytotoxic drugs represents the mechanism of controlled release in the tumor. The loading level and release of cationic drugs are limited by the number of sulfonate moieties in the microspheres. Stability and compatibility of drug-loaded beads are essential for safe administration. Epirubicin represents the epimer of doxorubicin differing in the configuration of the hydroxyl group at C4 in the sugar moiety. It exhibits the same antitumor activity like doxorubicin but less cardiotoxicity.[6,7] The physicochemical properties of epirubicin and doxorubin are highly similar, and stability of epirubicin in aqueous solutions depends mainly on the pH. Acid induces hydrolysis of the glycosidic bond and in alkaline solutions degradation of the aglycone structure takes place.[8–10] Prior to injection the drug-loaded DC bead™ suspension is mixed with non-ionic contrast medium to guide the injection to the targeted tumor. The aim of the study was to determine the compatibility of epirubicin-loaded DC bead™ with different non-ionic contrast media over a period of seven days when stored light protected under refrigeration (2–8°C). The contrast media selected are those commonly used during the TACE procedures by interventional radiologists.

## Material and methods

Two commercially available formulations of epirubicin hydrochloride, i.e. Epimedac 2 mg/ml, 100 ml, injection solution (medac, Hamburg, Germany, lot E130347B(05/2015) and Farmorubicin® 50 mg HL, rapid dissolution powder (Pfizer Pharmacia GmbH, Berlin, Germany, lot 3S6014A) to be reconstituted with 2 ml of water for injection, were used for loading. DC bead™ were received from Biocompatibles UK Ltd, BTG International Group Company, Camberley, UK in the two smallest size ranges available: size 70–150 µm (=M1), lot V10413, size 100–300 µm, lot V10505. Each size is marketed in 10 ml glass vials with 2 ml beads suspended in 6 ml physiological buffered saline. The non-ionic contrast media utilized in the compatibility tests were Accupaque™ 300, 50 ml; Imeron® 300, 500 ml; Ultravist® 300 10 ml; and Visipaque™ 320, 50 ml (Table 1).

## High-performance liquid chromatography method

Each sample was assayed three times by a validated stability indicating high-performance liquid chromatography (HPLC) assay with photodiode array detection (PDA) to analyze the concentration and purity of epirubicin. The HPLC system consisted of a Waters 717 plus Autosampler, a Waters 510 HPLC-pump, and a Waters 996 photodiode array detector. Waters Empower pro, Empower 2 software, version 6.10.01.00 was used to acquire and analyze the data. The concentrations of epirubicin were determined by using a Symmetry® column C18 (250 × 4.6 mm) with a particle size of 5 µm (lot 022338079, MZ-Analysentechnik, Mainz, Germany). The mobile phase consisted of 27.5% acetonitrile (ACN) HPLC Gradient Grade (lot 134 622, 2.5 L, Promochem, Wesel, Germany) and 72.5% 0.05 M potassium dihydrogen phosphate buffer solution (PBS) (pH = 4.6). The PBS pH = 4.6 was prepared by solving 6.8 g potassium dihydrogen phosphate (Merck, Darmstadt, Germany, lot A0185177031) in 1000 ml water HPLC Gradient Grade (Applichem, Darmstadt, Germany, 2.5 L, lot 4Q009403). The pH was adjusted by using 85% ortho phosphoric acid (AppliChem GmbH, Darmstadt, Germany, lot 7A005123). The washing solution consisted of 95% water HPLC grade and 5% ACN HPLC grade. The flow rate was set at 1.5 ml/min, and the injection volume was 10 µL. PDA wavelength was 190–600 nm with the detection wavelength of 479 nm. Under these conditions, the retention time of the epirubicin peak was about 5–7 min (see Figures 1 to 3). The identity of the epirubicin peak

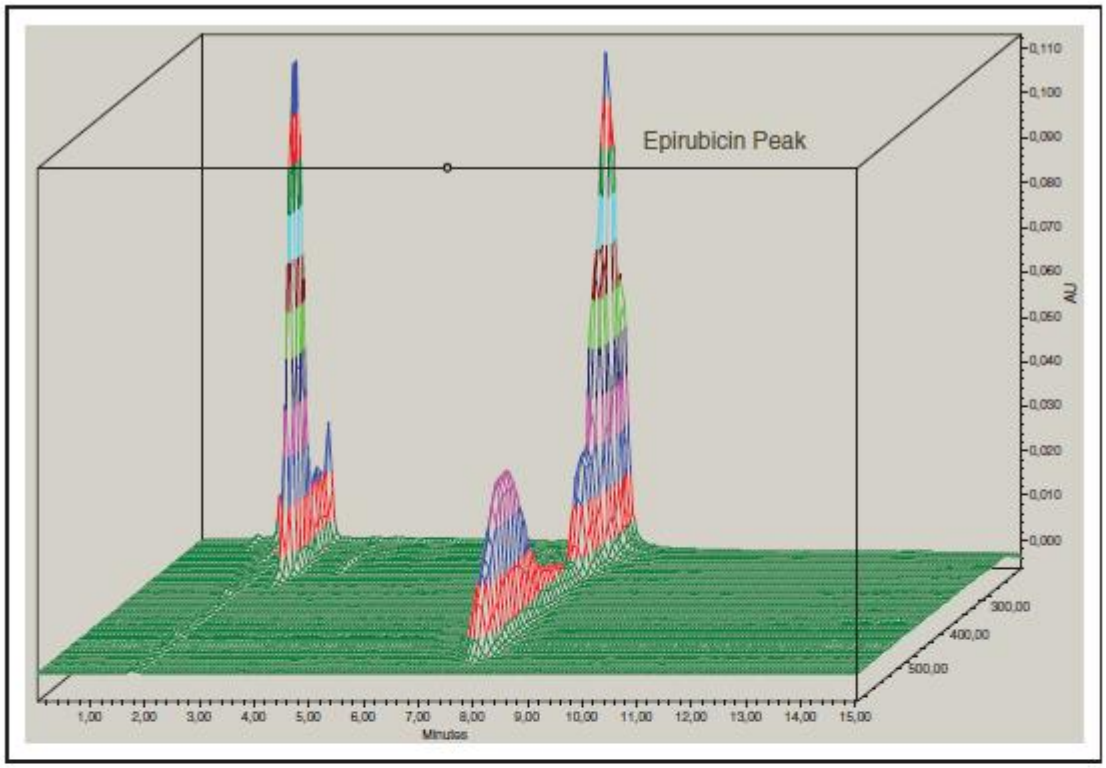


**Figure 1.** HPLC PDA chromatogram of epirubicin.

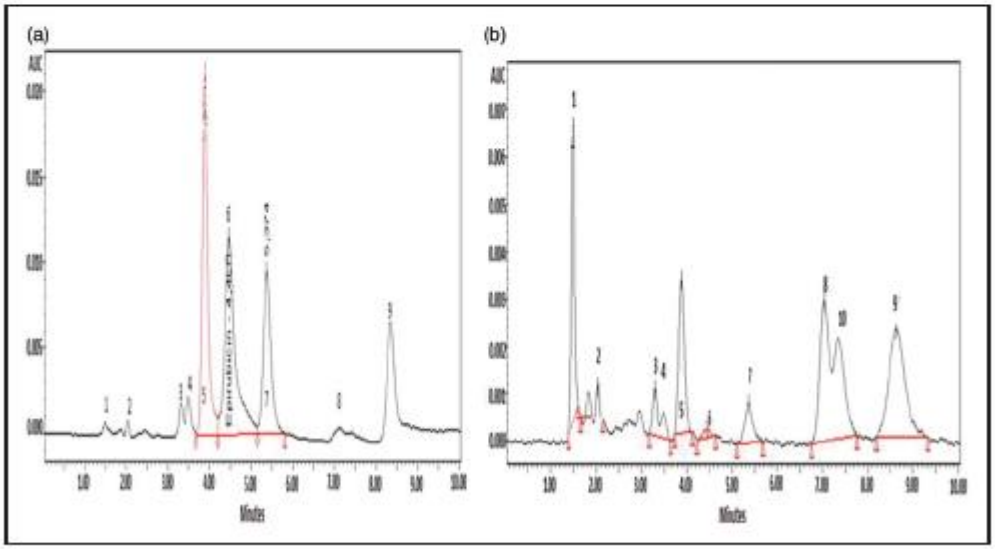


**Figure 2.** Chromatograms of alkaline (1 mol NaOH, pH 13) degradaded epirubicin solution (2 mg/ml); (a) without heating, (b) after heating.
Peak no 6: undegraded epirubicin; peak no 2: glucose; other peaks: degradation products.

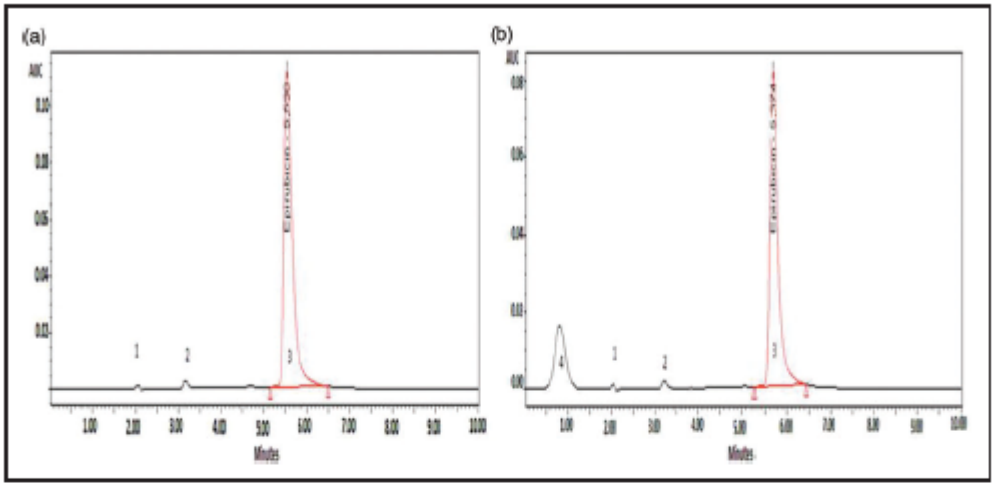


**Figure 3.** Chromatograms of acid (HCl, pH 0.2) degradaded epirubicin solution (2 mg/ml); (a) without heating, (b) after heating. Peak no 3: undegraded epirubicin; peak no 1 = glucose; peaks no 2, 4: degradation products.

was confirmed by concentration-dependent changes of the peak area and PDA chromatograms. Run time was set at 10 min.

### *Suitability of the HPLC assay*

Suitability of the HPLC method was proven by analyzing forced degraded samples of epirubicin injection concentrate. The injection solution (2 mg/ml epirubicin) was acidified with hydrochloride acid in order to achieve pH values of 0.2, 1.0, 2.0, and 3.6 and was heated in a water bath at 60°C for 2 h. The injection solution (2 mg/ml epirubicin) was alkalinized with 1 mol sodium hydroxide solution to pH = 13 and heated in the same manner. Solutions were diluted with phosphate buffer 1:5 and assayed. Under alkaline pH conditions, the color of the epirubicin solution changed from red to deep purple. The peaks of the degradation products did not interfere with epirubicin parent peak. After heating, intact epirubicin was not detectable any longer (see Figure 2). Acidic degradation resulted in the hydrolysis of the glycosidic bond and formation of the deep red-colored aglycone doxorubicinon. The peak of the aglycone degradation product did not interfere with the epirubicin parent peak (see Figure 3). These results confirm the suitability of the HPLC method implemented.

## Validation of the HPLC assay

The method was validated following the ICH Harmonised Tripartite Guideline for Validation of analytical procedures: text and methodology Q2 (R1).

### *Linearity and calibration curve*

In order to study the linearity of the calibration curve, epirubicin solutions of different concentrations (n = 15) were prepared by diluting a 1:10 dilution Epimedac® injection concentrate with calculated amounts of PBS in order to achieve the nominal epirubicin concentrations 3, 5, 10, 20, 30, 50, 80, 90, 100, 110, 120, 150, 170, 180, and 200 µg/ml. Aliquots of the calibration standards were injected in triplicate. The calibration curve was constructed by analyzing plots of the peak area versus epirubicin concentrations. The correlation coefficient of $R^2 = 0.998375$ proved linearity over the concentration range. The equation of calibration curve was $y = 6.9464\,x + 40.283$.

### *Accuracy*

Accuracy was evaluated with four different quality control solutions (20 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml) and five-fold injection. The mean recovery was 100.05% ± 0.27% (n = 20). The accuracy was 100.57 ± 0.24% for 20 µg/ml, 99.84 ± 0.71% for 50 µg/ml, 99.64 ± 1.72% for 100 µg/ml, and 100.19 ± 0.57% for 150 µg/ml.

### *Intra-day precision*

Intra-day precision was determined by five-fold injection of four quality control solutions nominally containing 20 µg/ml, 50 µg/ml, 100 µg/ml, and 150 µg/ml epirubicin. Intra-day precision expressed as relative standard deviation was 0.99% for 20 µg/ml, 1.54%

for 50 µg/ml, 1.53% for 100 µg/ml, and 1.94% for 150 µg/ml epirubicin.

### Inter-day precision

Inter-day precision was determined by four-fold injection of four quality control solutions (20 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml epirubicin) on five different days. Inter-day precision expressed as relative standard deviation was 3.25% for 20 µg/ml, 0.95% for 50 µg/ml, 0.75% for 100 µg/ml, and 0.89% for 150 µg/ml.

### Limit of detection (LOD)

The limit of detection was calculated by the equation LOD = 3.3 SD/s, where s represents the slope of the calibration curve and SD the standard deviation of the peak area. The LOD amounted to 0.025 µg/ml of epirubicin.

### Limit of quantification (LOQ)

The limit of quantification was calculated by the equation LOQ = 10 SD/s, where s represents the slope of the calibration curve and SD the standard deviation of the peak area. The LOQ amounted to 0.075 µg/ml of epirubicin.

## Experiments

Bead-slurry (8 ml) either DC bead™ 100–300 µm or DC beadM1™ was transferred via an 18-gauge needle into an empty 50 ml or 10 ml syringe made of polypropylene (PP). After sedimentation of the beads, the excess solution was expelled via a 5 µm filter needle; 2 ml beads remained in the syringe.

### Loading of DC bead™ with 2 mg/ml epirubicin hydrochloride solution

38 ml epirubicin injection solution 2 mg/ml was transferred into a 50 ml syringe. The syringe was connected by a female/female connector to the 50 ml syringe containing the bead slurry and the epirubicin solution was pushed into the syringe. These experiments had to be performed with 76 mg epirubicin referring to clinical practice and volumetric measurement of the drug solution with a 50 ml syringe calibrated in milliliters. Loading was performed under static conditions over a period of 6 h. Each test suspension was prepared in triplicate.

### Loading of DC bead™ with 25 mg/ml epirubicin hydrochloride solution

Each vial of epirubicin powder for reconstitution 50 mg was reconstituted with 2 ml water for injection. The resulting epirubicin concentration amounted to 25 mg/ml; 3 ml of reconstituted epirubicin injection solution 25 mg/ml was drawn up into the bead containing 10 ml syringe to load the beads with 75 mg epirubicin under static conditions over a period of 2 h. Each test suspension was prepared in triplicate.

At the end of the loading period, the color of the beads had changed from blue to red and the color of excess solution had become lighter. The excess solution was removed from syringes by pushing it into empty bags. Samples were withdrawn from the excess solutions and the concentration of epirubicin measured by HPLC. Prior to the HPLC assay, samples were diluted with PBS (pH = 4.6) 1:3 when Epimedac® was used for loading or 1:40 when Farmorubicin® was used for loading in order to obtain concentrations in the range of calibration curve. The loading rate was calculated by using equation (1).

$$\text{Loading rate (\%)} = \frac{\left\{\begin{array}{l}\text{initial drug concentration in excess solution} \\ -\text{residual drug concentration in excess solution}\end{array}\right\}}{\text{initial drug concentration in excess solution}} \times 100 \quad (1)$$

### Compatibility of epirubicin-loaded beads with different non-ionic contrast media

After removal of the excess solutions, the epirubicin-loaded beads were mixed with different contrast media (Accupaque™ 300, Imeron® 300, Ultravist® 300, Visipaque™ 320) in a 1:1 ratio and agitated in a controlled manner to obtain a homogeneous suspension. Samples (0.3 ml) were withdrawn via a 5 µm filter needle and diluted 1:5 with PBS pH = 4.6 on day 0, 1, and 7. Aliquots were injected three times and assayed by HPLC in order to determine the remaining loading level of epirubicin in the beads.

Calculation of the percentage rate eluted was performed by the following equation:

$$\text{Percentage rate eluted (\%)} = \frac{\text{Epirubicin concentration in contrast medium}}{\text{Epirubicin concentration in loaded beads}} \times 100 \quad (2)$$

## Results

The loading level of epirubicin in DC bead™ 100–300 µm amounted to 93% and 98% when 2 ml DC beads™ were loaded with 76 mg of epirubicin (injection formulation, 2 mg/ml) over 2 h and 75 mg

**Table 2.** Epirubicin loading levels of epirubicin-loaded DC Bead™ (bead size 70–150 µm, 100–300 µm) mixed with different contrast media and stored protected from light under refrigeration.

| | | Percentage rate epirubicin loaded (%) ± RSD (n = 3) | | | |
|---|---|---|---|---|---|
| Contrast medium (CM) | DC bead™ size | Day 0 before mixing with CM | Day 0 mixed with CM | Day 1 mixed with CM | Day 7 mixed with CM |
| Accupaque™ 300 | 70–150 µm | 98.81 ± 0.44 | 98.81 ± 0.13 | 98.81 ± 0.36 | 98.70 ± 0.35 |
| | | 98.03 ± 0.44 | 98.03 ± 0.29 | 98.03 ± 0.45 | 97.80 ± 0.36 |
| | | 98.36 ± 0.27 | 98.36 ± 0.50 | 98.16 ± 0.03 | 98.20 ± 0.54 |
| | 100–300 µm | 98.73 ± 0.21 | 98.73 ± 0.27 | 98.63 ± 0.35 | 98.56 ± 0.11 |
| | | 98.63 ± 0.21 | 98.43 ± 0.72 | 98.43 ± 0.05 | 98.43 ± 0.29 |
| | | 99.46 ± 0.27 | 99.25 ± 0.31 | 99.35 ± 0.87 | 99.35 ± 0.06 |
| Imeron® 300 | 70–150 µm | 99.36 ± 0.01 | 99.16 ± 1.66 | 99.06 ± 0.53 | 99.16 ± 0.35 |
| | | 99.36 ± 0.00 | 99.16 ± 0.53 | 99.16 ± 0.59 | 99.26 ± 0.65 |
| | | 99.35 ± 0.00 | 99.13 ± 0.54 | 99.13 ± 0.21 | 99.13 ± 0.42 |
| | 100–300 µm | 97.43 ± 0.84 | 97.23 ± 0.10 | 97.32 ± 0.20 | 97.32 ± 0.29 |
| | | 97.93 ± 1.24 | 97.87 ± 0.11 | 97.87 ± 0.28 | 97.83 ± 0.14 |
| | | 98.29 ± 0.57 | 98.20 ± 0.01 | 98.16 ± 0.05 | 98.16 ± 0.21 |
| Ultravist® 300 | 70–150 µm | 99.17 ± 0.33 | 98.70 ± 0.03 | 98.70 ± 0.68 | 98.70 ± 0.42 |
| | | 98.99 ± 0.40 | 98.66 ± 0.47 | 98.63 ± 1.22 | 98.63 ± 0.27 |
| | | 98.79 ± 0.29 | 98.49 ± 1.01 | 98.49 ± 1.70 | 97.19 ± 0.45 |
| | 100–300 µm | 98.86 ± 1.95 | 98.66 ± 0.12 | 98.66 ± 0.25 | 98.56 ± 0.15 |
| | | 98.88 ± 0.60 | 98.60 ± 0.62 | 98.60 ± 1.18 | 98.60 ± 1.28 |
| | | 98.83 ± 0.54 | 98.63 ± 0.92 | 98.02 ± 1.38 | 98.02 ± 0.39 |
| Visipaque™ 320 | 70–150 µm | 99.42 ± 0.35 | 99.25 ± 0.83 | 99.25 ± 0.25 | 99.13 ± 0.45 |
| | | 99.16 ± 0.48 | 98.96 ± 0.15 | 98.86 ± 0.17 | 98.86 ± 0.16 |
| | | 99.15 ± 0.52 | 98.94 ± 0.34 | 98.94 ± 0.36 | 98.94 ± 0.48 |
| | 100–300 µm | 96.52 ± 0.27 | 96.42 ± 0.40 | 96.42 ± 0.04 | 96.42 ± 0.69 |
| | | 97.97 ± 0.65 | 97.88 ± 0.28 | 97.86 ± 0.75 | 97.86 ± 0.62 |
| | | 98.44 ± 0.38 | 98.30 ± 0.06 | 98.20 ± 0.08 | 97.00 ± 0.32 |

Note: Percentage rate expressed as mean ± relative standard deviation (RSD) of triplicate assays of each test solution. Beads were loaded with 25 mg/ml epirubicin loading solution.

epirubicin (powder formulation, 25 mg/ml) over 6 h, respectively. The loading levels were about 1% higher when DC beadM1™ 70–150 µm were loaded. The resulting loading levels amounted to 94% and 99% for epirubicin solution 2 mg/ml and 25 mg/ml, respectively. These results are in accordance with those reported for doxorubicin loading, showing that higher concentrations of the loading solution and smaller bead sizes (due to surface area effects) result in faster loading.[11,12] Mixing of various non-ionic contrast media with the epirubicin-loaded DC bead™ (70–150 µm, 100–300 µm) resulted in a total release of only 0.1–0.5% epirubicin over a period of 24 h. Differences depending on the DC bead™ diameter size and the concentration of the epirubicin loading solutions thereby did not get obvious. Moreover, the release rate was independent from the type of contrast medium used even though there are differences in the physiochemical properties of the contrast media (see Table 1). No epirubicin degradation products were detected in the HPLC chromatograms. Detailed results are given in Tables 2 and 3. No further elution or degradation was observed after seven days when the admixtures with contrast media were stored protected from light under refrigeration. With regard to the minimal release of epirubicin after mixing of loaded DC bead™ with different contrast media, the admixtures can be classified as compatible and stable over a minimum of seven days when stored under refrigeration.

## Discussion

In order to determine the concentration and purity of epirubicin, at first the HPLC method previously reported by Sobczak et al. was implemented.[14] But the resulting epirubicin peak was not symmetric and feasible for accurate peak area calculation. Therefore, an alternative HPLC assay was developed by testing

**Table 3.** Epirubicin loading level of DC Bead™ (bead size 70–150 µm, 100–300 µm) mixed with different contrast media and stored protected from light under refrigeration.

| | | Percentage rate epirubicin loaded (%) ± RSD (n = 3) | | | |
|---|---|---|---|---|---|
| Contrast medium (CM) | DC bead™ size | Day 0 before mixing with CM | Day 0 mixed with CM | Day 1 mixed with CM | Day 7 mixed with CM |
| Accupaque™ 300 | 70–150 µm | 92.42 ± 0.21 | 92.36 ± 0.27 | 92.36 ± 0.33 | 92.02 ± 0.31 |
| | | 93.82 ± 0.50 | 93.78 ± 0.03 | 93.07 ± 0.23 | 92.84 ± 0.31 |
| | | 94.06 ± 0.44 | 93.88 ± 0.12 | 94.06 ± 0.18 | 93.87 ± 0.12 |
| | 100–300 µm | 92.39 ± 0.18 | 92.36 ± 0.01 | 92.38 ± 0.32 | 92.39 ± 0.23 |
| | | 92.71 ± 0.15 | 92.63 ± 0.02 | 92.71 ± 0.59 | 92.71 ± 0.37 |
| | | 92.55 ± 0.02 | 92.50 ± 0.08 | 92.37 ± 0.85 | 92.46 ± 0.30 |
| Imeron® 300 | 70–150 µm | 94.77 ± 0.73 | n.a. | 92.36 ± 0.88 | 92.36 ± 0.01 |
| | | 84.14 ± 0.31 | n.a. | 83.04 ± 0.10 | 84.03 ± 0.57 |
| | | 94.49 ± 1.64 | n.a. | 94.09 ± 1.07 | 94.09 ± 0.10 |
| | 100–300 µm | 93.03 ± 0.60 | n.a. | 92.93 ± 0.06 | 92.93 ± 0.23 |
| | | 93.12 ± 1.04 | n.a. | 92.84 ± 0.39 | 92.93 ± 0.08 |
| | | 93.00 ± 1.81 | n.a. | 92.81 ± 0.11 | 92.91 ± 0.50 |
| Ultravist® 300 | 70–150 µm | 94.22 ± 0.74 | 94.04 ± 1.90 | 93.92 ± 0.25 | 93.92 ± 0.15 |
| | | 95.67 ± 2.12 | 95.37 ± 1.71 | 95.37 ± 1.18 | 95.37 ± 1.28 |
| | | 94.84 ± 0.75 | 94.54 ± 1.57 | 94.54 ± 1.38 | 94.54 ± 0.39 |
| | 100–300 µm | 93.14 ± 0.25 | 93.14 ± 0.31 | 93.13 ± 0.56 | 93.13 ± 0.08 |
| | | 93.04 ± 0.51 | 92.86 ± 0.99 | 92.86 ± 0.15 | 92.86 ± 0.11 |
| | | 93.23 ± 0.09 | 93.03 ± 0.69 | 93.03 ± 0.61 | 93.03 ± 0.29 |
| Visipaque™ 320 | 70–150 µm | 96.17 ± 4.08 | 95.98 ± 0.21 | 95.97 ± 0.56 | 95.97 ± 0.40 |
| | | 97.40 ± 0.71 | 97.21 ± 0.09 | 97.21 ± 0.02 | 97.21 ± 0.54 |
| | | 96.96 ± 1.08 | 96.76 ± 0.37 | 96.76 ± 0.29 | 96.76 ± 0.84 |
| | 100–300 µm | 94.07 ± 3.64 | 93.97 ± 0.22 | 93.97 ± 0.16 | 93.97 ± 0.40 |
| | | 94.09 ± 1.36 | 93.89 ± 0.47 | 93.98 ± 0.21 | 93.98 ± 0.19 |
| | | 94.18 ± 0.55 | 94.07 ± 0.66 | 94.07 ± 0.91 | 94.07 ± 0.21 |

Note: Percentage rate expressed as mean ± relative standard deviation (RSD) of triplicate assays of each test solution. Beads were prepared with 2 mg/ml epirubicin loading solution.
n.a.: data not available because of technical reasons.

different types of columns and mobile phases (ACN: Water and ACN: PBS in different ratios and different pH values) as well as different flow rates in the range from 0.7 ml/min to 1.5 ml/min. Finally, a Symmetry® column C18, a mobile phase consisting of 72.5% PBS (pH 4.6) and 27.5% ACN, and a flow rate of 1.5 ml/min revealed to be suitable for the separation and quantification of epirubicin. This HPLC assay was shown to be stability indicating and valid. Of note, minimal variations of the liquid phase caused shifts in the retention time (see Figures 1 to 3). The small differences between the epirubicin loading levels of the different test solutions when the same bead sizes and the same concentration of the loading solution were used are to be explained by the loss of a few beads during the loading procedure and preparation of samples. Therefore, the results are given for each test combination on its own and not for the average of three test combinations. However, it is obvious that the loading level of epirubicin increases about 1% when DC bead™ M1 is loaded and otherwise unchanged parameters. This phenomenon results from the larger surface area of beads with a lower diameter and more efficient loading during the same period. Using a 2 mg/ml concentrated epirubicin solution for loading the same loading efficiency is only reached after a longer loading period. After 6 h, loading was not completed, but sufficient in order to test the admixture compatibility with contrast media.

Like other cytotoxic preparations, the loading of DC bead™ is in general performed in a centralized pharmacy-based cytotoxic preparation unit. As the drug-loaded beads are administered admixed to contrast media, the question arises whether compatibility of the admixtures is given and whether admixtures with

contrast media can be prepared in advance by the pharmacy staff. To give a valid answer to these questions, the compatibility and stability of epirubicin-loaded beads with four different non-ionic contrast media which are conventionally used by the interventional radiologists, were studied over a maximum period of seven days.

The results confirm compatibility and stability of the admixtures with different non-ionic contrast media. The minimal percentage rates (0.1–0.5%) of epirubicin eluted did not allow the identification of differences caused by the bead sizes or the loading levels. Of note, any influence of the viscosity or osmolality of the various contrast media on the elution rate was not verifiable for the same reason. Potential degradation products of epirubicin are not to be expected and most probably existing only below the LOD. Adsorption of epirubicin to the PP syringes or HPLC glass vials can also be excluded. Hecq et al. also reported stability of doxorubicin after admixture of doxorubicin-loaded DC bead™ with Omnipaque 350 and storage over seven days under refrigeration.[12] In their experiments, higher concentrations of doxorubicin and impurities were measured in the samples utilized after seven days. However, the experiments clearly show that there is no significant elution of epirubicin or doxorubicin from loaded DC bead™ after admixture and storage with non-ionic contrast media. The ionic binding of the positively charged anthracycline drugs to the sulfonate groups of DC bead™ is much stronger than this of irinotecan, which is eluted by contrast media in a percentage rate up to 10% after mixing.[13]

## Conclusion

Compatibility of epirubicin-loaded DC bead™ with an equal volume of different contrast media in PP syringes is given over a period of seven days. Due to a maximum elution of 0.1–0.5% of epirubicin from loaded DC bead™, admixtures with contrast media can be prepared in advance in centralized cytotoxic preparation units. Microbiological aspects have to be considered when determining the expiration date of the product.

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## *7.3 Compatibility of Irinotecan-loaded DC Bead™ with different volumes and types of non-ionic contrast media*

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## *Irinotecan*

Irinotecan (known as CPT-11) which is a semisynthetic analogue of camptothecin, has been approved by FDA (food and drug administration) in 1998 to treat patients with colorectal cancer after failure of treatment with 5-flourourcil (113). Today irinotecan is used in the first and the second line regimens to treat metastatic colorectal carcinoma. The first line regimens include combination of irinotecan with 5-fluorouracil and leucovorin (Folfiri) what attributes significantly to improve the response rate. The second line regimens include combinations of irinotecan with oxaliplatin (Folfoxiri) (114) or with monoclonal anti-EGFR (Epidermal Growth Factor Receptor) or anti-VEGF (Vascular Endothelial growth Factor) antibodies (115). Irinotecan acts by inhibition of topoisomerase I, resulting in single or double DNA strand breaks during the S phase of cell cycle. As there is no sufficient repair mechanism cell death occurs (116).

It is important to note that the intact lactone ring of irinotecan is in charge of cytotoxic activity, and an acidic environment (pH<3.5) guarantees impaired lactone structure (117, 118).

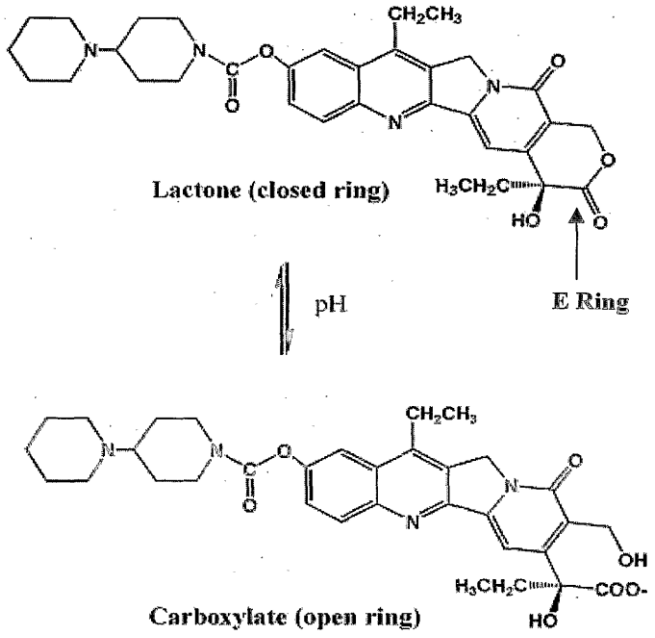


Figure 8: pH dependent equilibrium of lacton and carboxylate form of irinotecan

Irinotecan loaded beads can be utilized for loco-regional treatment of liver metastasis of colorectal cancer. Effectivity and safety are facilitated by extended contact time and controlled release into the targeted tumor site. Combination therapy of TACE with DEBIRI and oral

capecitabine in patients with mCRC and liver-dominant disease causes less toxicity than the systemic treatment with irinotecan (118). The efficacy of the treatment with irinotecan loaded beads depends on the amount of irinotecan penetrated into the tumor bed, i.e. the delivered amount of irinotecan (119). Increased response rates and optimum tolerance profiles are achieved in patients with liver metastasis by administration of DEBIRI with the bead size 70-150 µm=M1 compared to patients receiving DEBDox under the same conditions (120).

The ability of irinotecan to be loaded into DEB is related to its protonated amino function that binds strongly to the negatively charged sites in the microspheres. The loading rate is determined by the bead size, presence of ions in the surrounding medium, and the loaded amount of irinotecan (121). The physico-chemical properties of irinotecan, the loading stability and elution rate has been investigated in *in vitro* and pre-clinical studies with different sizes of DC Beads (average range 70-900 µm) (23, 120, 121). The maximum amount of irinotecan loaded per ml beads is 50 mg within the loading time of 2 hours. The resulting loading rate is 96-100% depending on the bead size (23, 122, 123). The study of Kaiser et al. revealed stability of DEBIRI over a period of 28 days when using beads of the size ranges 100-300 µm and 300-500 µm and storage refrigerated under light-protection (23). In addition the compatibility of DEBIRI (bead size 100-300 µm, 300-500 µm) with selected non-ionic contrast media was studied by Kaiser et al. and an immediate release of 5-10% of the loaded amount got obvious. The resulting recommendation was to refrain from mixing DEBIRI with non-ionic contrast medium in advance e.g. in centralized cytotoxic preparation units (23). More recently DC Bead™ M1 (bead size 70-150 µm) were introduced into the market and data about the effect of mixing DEBIRI (bead size M1) with non-ionic contrast media are not available. The aim of this study was to evaluate the compatibility of the novel bead size 70-150 µm loaded with irinotecan up to 50 mg per 1 ml bead and suspended homogenously with seven different non-ionic contrast media which are commonly used by radiologists to guide the administration of DEBIRI to the targeted tumor site. Up to four different volumes (5, 10, 20, 30 mL) of the non-ionic contrast media were admixed and the release of irinotecan investigated over a maximum period of 24 hours and storage at room temperature.

Original article

# Compatibility of irinotecan-loaded DC Bead with different volumes and types of non-ionic contrast media

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## ABSTRACT

**Objectives** Irinotecan-loaded microspheres are used for simultaneous embolisation and chemotherapy of liver metastases of colorectal carcinoma. The aim of the study was to evaluate the compatibility of recently introduced DC Bead*M1* (bead size 70–150 µm) loaded with irinotecan after admixture with different types and volumes of non-ionic contrast media over a maximum period of 24 h and storage at room temperature.

**Methods** Test suspensions were prepared by loading 2 mL DC Bead*M1* with 100 mg irinotecan within 2 h. The loading efficiency was determined by measuring the concentrations of irinotecan in the excess solutions via a reversed phase high pressure liquid chromatography (RP-HPLC) assay with ultraviolet detection. The compatibility of irinotecan-loaded DC Bead*M1* with different types and volumes of contrast media was studied by mixing 2 mL loaded bead slurry each with up to four different volumes (5, 10, 20, 30 mL) of seven different contrast media. Samples were withdrawn after 30 min, 1, 2, 4, 8 and 24 h. Admixtures were stored light protected at room temperature over the observation period. The concentrations of eluted irinotecan were measured in triplicate samples using the RP-HPLC assay.

**Results** Mixing of irinotecan loaded beads with non-ionic contrast media decreased the irinotecan loading efficiency between minimum 2.5% and maximum 17% over the observation period of 24 h. The rate and amount of irinotecan eluted from the beads varied relying on the type and volume of contrast medium admixed. However, no further elution or degradation was observed after the rapid release during the first 8 h.

**Conclusions** Because of the rapid and extensive release of irinotecan, it is not recommendable to prepare admixtures of irinotecan-loaded DC Bead*M1* with contrast media in centralised cytotoxic preparation units in advance. Admixture should be performed with the smallest possible amount by the radiologists immediately prior to the delivery procedure.

## INTRODUCTION

Colorectal carcinoma (CRC) is a widespread type of cancer, reported to be the second and third most often diagnosed cancer in female and male patients, respectively.[1] Although treatment options improved during the past years in the developed countries, mortality is still high.[2] Besides surgery of the localised carcinoma, multimodality therapy comprising chemotherapy regimens, radiation and palliative care are used to treat advanced CRC.[3]

Transarterial chemoembolisation (TACE) is an outstanding technique used to treat liver cancer and also liver metastases of CRC and other tumours. By local administration of drug-eluting microspheres, high concentrations of antineoplastic drugs and embolisation of the arteria supplying the tumour are accomplished. Simultaneous administration of the embolising and pharmacological principles in the form of a drug device combination, like DC Bead, represents the most advanced type of TACE.[4] DC Bead allows the load of cationic drugs and the controlled release of the cytotoxic agent within the tumour bed. The DC Bead microspheres consists of a polyvinyl alcohol backbone cross linked with unsaturated 2-acrylamido-2-methylpropane sulfonate sodium salt to obtain negatively charged sulfonate moieties, which allow the loading of cationic drugs. Among the typically loaded antitumour drugs are doxorubicin, epirubicin, topotecan and irinotecan, each in the hydrochloride salt form. The positively charged drugs are loaded and released in a controlled manner through an ionic charge–charge interaction. The drug-loaded beads are administered via a microcatheter after mixing with non-ionic contrast media (CM) to guide the injection and to facilitate the selective delivery of the drug eluting beads (DEB) to the targeted tumour.

Irinotecan is a semisynthetic analogue of camptothecin acting as topoisomerase-1-inhibitor after systemic and locoregional administration.[5 6] It is used in combination with fluorouracil and monoclonal antiepidermal growth factor receptor or antivascular endothelial growth factor antibodies in patients suffering from metastatic or unresectable CRC.[5] Irinotecan (CPT-11) is rapidly metabolised by hydrolysis of the carbamate ester moiety by liver carboxylesterase to SN-38. Irinotecan and its active metabolite SN-38 bind to the topoisomerase 1—DNA complex (specifically in the S-phase of the cell cycle) forming a triple complex, which prevents religation of DNA strands, and overlaps with the replication fork. Thereby, DNA replication is stopped, and the irreversible damage results in cell death.[7] The intact lactone ring of irinotecan and its derivatives is a prerequisite for cytotoxic activity. However, the lactone structure is sensitive to pH changes, and undergoes reversible hydrolysis. Acidic pH values (pH<3.5) favour the lactone ring form while alkaline conditions induce ring opening and lack of cytotoxic activity.[7–9] High loading efficiency and adequate stability of intact irinotecan to the DC Bead microspheres (bead size 100–300 µm) has been proven by in vitro studies some years ago.[8 10] The beads retain the lactone stability and prolong the half-life of the lactone form, which can provide an improved anticancer effect.[8] Mixing of 2 mL irinotecan-loaded beads (DEBIRI) (bead size

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100–300 µm, 300–500 µm) with 6.5 mL non-ionic contrast media rapidly decreased the loading efficiency of irinotecan by 5%–10%.[9] More recently, DC Bead*M1* (bead size 70–150 µm) was introduced in the market. The aim of these experiments was to evaluate the compatibility of the novel bead size 70–150 µm loaded with irinotecan (100 mg/2 mL) and mixed with seven different non-ionic contrast media, which are commonly used by radiologists to guide the administration of DEBIRI to the targeted tumour site. Compatibility was planned to be studied with up to four different volumes (5, 10, 20, 30 mL) of non-ionic contrast media over a maximum period of 24 h while stored at room temperature.

## MATERIAL AND METHODS

DC Bead*M1* (bead size 70–150 µm, lot: V10647-DEV-(02/2017) was received from Biocompatibles, BTG, Camberley, UK, as a grant. The DC Bead vials were labelled as R&D only, Not for Human Use. Each 10 mL DC Bead vial contains 2 mL beads and 6 mL physiological buffered saline.

Commercially available Irinotecan Accord 20 mg/mL, 25 mL, Accord Healthcare, Sage House, 319 Pinner Road, North Harrow, Middlesex, UK, (lot: P11760-(09/2015) contains nominally 500 mg irinotecan HCl 3 $H_2O$.

The samples were prepared in Omnifix polypropylene (PP) syringes Luer Lock Solo 20 mL (lot: 2B24048-(02/2019), B Braun Melsungen AG, Melsungen, Germany) or Original Perfusor syringes 50 mL (lot: 13L1982027, B Braun Melsungen AG, Melsungen, Germany). The syringes were used as primary packaging material.

The non-ionic contrast media used in this study were Accupaque 350 (100 mL, CH-B: 12322355-(12/2016), GE Healthcare Buchler, Braunschweig, Germany; iohexol-containing Accupaque is also marketed under the trade name Omnipaque), Imeron 400 MCT (200 mL, CH-B: 3110592-(09/2018), Bracco Imaging Deutschland, Konstanz, Germany), Optiray 300 (50 mL, CH-B: 13J2685-(09/2016), Mallinckrodt Deutschland, Hennef, Germany), Optiray 350 (50 mL, CH-B: 13G09214-(06/2016)), Solutrast 350 (50 mL, CH-B: 3G09214-(07/2018), Bracco Imaging Deutschland, Konstanz, Germany), Ultravist 370 (50 mL, CH-B: 41872C-(02/2017), Bayer Vital, Leverkusen, Germany) and Xenetix 350 (50 mL, CH-B: 13WF048F-(10/2016), Guerbet, Sulzbach, Germany).

### HPLC assay

The irinotecan concentrations were determined by using a previously published, validated reversed-phase HPLC method.[6] The HPLC system consisted of a Waters 510 HPLC pump, a Waters PDA-Detector 996 and a Waters Autosampler 717plus. Waters Empower pro, Empower² software, V6.10.01.00 was used to acquire and integrate the data. The separation was performed with the PerfectChrom 100CN column, particle size 5 µm, 250 mm×4.6 mm (MZ-Analysentechnik, Mainz, Germany).

The mobile phase used consisted of 75% 0.02 M potassium dihydrogen phosphate solution (2.72 g $KH_2PO_4$ (lot: AM0444477 402, Merck, Darmstadt, Germany) dissolved in 1 L water HPLC Gradient Grade (lot: 4Q009403, 2.5 L, Applichem, Darmstadt, Germany)) plus 25% of acetonitrile HPLC Gradient Grade (lot: 134622, 2.5 L, Promochem, Wesel, Germany). The injection volume was 20 µL and the flow rate 1.0 mL/min. The detector wavelength was set at 225 nm and the run time at 15 min. Under these conditions, the retention time of lactone-ring-closed irinotecan amounted to about 9 min.

Calibration was performed with eight concentrations of irinotecan solutions ranging from 15 to 200 µg/mL and threefold injection. The resulting calibration curve constructed from plots of peak area versus concentration showed linearity with a correlation coefficient of 0.9982.

Intraday precision of the HPLC assay was determined by fivefold injection of three standard concentrations of irinotecan solutions 50, 110 and 175 µg/mL on the same day. The relative SDs amounted to 0.63% for 50 µg/mL, 0.66% for 110 µg/mL and 2.7% for 175 µg/mL.

In order to determine interday precision, freshly prepared irinotecan solutions of the concentrations 30, 50, 100 and 150 µg/mL were injected four times on five different days. The interday precision given as relative SD (%) amounted to 1.6% for 30 µg/mL, 2.5% for 50 µg/mL, 2.4% for 100 µg/ mL and 0.2% for 150 µg/mL.

### Loading of DC Bead*M1* with 20 mg/mL irinotecan hydrochloride solution (100 mg nominal dose)

The content of a DC Bead*M1* vial was transferred to a 20 mL PP syringe (when 5 or 10 mL contrast media were added) or to a 50 mL syringe (when 20 or 30 mL of contrast media were added) via an 18-gauge needle. The packing solution was removed via a 5 µm filter needle until receiving a visually dry bead slurry mixture. Five millilitres of irinotecan infusion concentrate (ie, nominal dose 100 mg) were added to the DC Bead slurry in the PP syringe, and the admixture was agitated in a controlled manner (inverting the syringe 10 times). During the following loading process, admixtures were not agitated. Each test suspension was prepared in triplicate. In total, 51 DC Bead*M1* vials were loaded with irinotecan hydrochloride (50 mg/1 mL beads). According to the calculated value and experimental results, the sulfonate sites in 1 mL beads are saturated with 50 mg irinotecan through charge–charge interaction, and further addition does not increase the loading efficiency.[10]

After 2 h of loading, the beads had settled down in the syringes, and the excess solution was removed through a 5 µm filter needle. In order to maintain the hydration of the loaded DC Bead, 2 mL of water for injection (WFI) (Aqua ad iniectabilia, 50 mL, lot: S1311199 (31/08/2017), Department of Pharmacy, University Medical Center, Mainz, Germany) were added to each syringe, the syringe closed by a combi stopper and again inverted 10 times. DEBIRI were stored at room temperature under light protection (covered with aluminium foil) over a period of 24 h.

From the excess solutions removed after the loading procedure, 0.5 mL samples were withdrawn via a 5 µm filter needle into 1 mL BD Luer-Lok tip, and the concentration of irinotecan was measured by HPLC. Prior to the HPLC assay, samples were diluted 1:4 with 0.9% NaCl solution (Infusion Solution, 100 mL, freeflex infusion bag, lot: 13HBS051 (01/2016), Fresenius Kabi, Bad Homburg, Germany) in order to fit the calibration curve. Samples were acidified with 10 µL 1% $H_3PO_4$ (85% orthophosphoric acid, lot: 3R004802, AppliChem, Darmstadt, Germany, diluted to 1% phosphoric acid with water HPLC Gradient Grade) to pH 3.5, thereby favouring the lactone form of irinotecan. Samples were assayed in triplicate, and the percentage rate of irinotecan loaded calculated:

$$\text{Loading rate (\%)} = \frac{\text{initial drug concentration in solution} - \text{residual drug concentration in solution}}{\text{initial drug concentration in solution}} \times 100 \quad (1)$$

### Compatibility of irinotecan-loaded DC Bead*M1* with different non-ionic contrast media

To each syringe containing a suspension of DC Bead*M1* in 2 mL WFI, the predetermined volumes of the seven different contrast media were added, and the admixtures were agitated in a controlled manner by inverting the syringe 10 times at each predetermined interval in order to measure the amount of irinotecan eluted into the injection admixture (WFI and non-ionic CM). During the observation period, the test suspensions were stored light protected at room temperature. 0.5 mL aliquots of the supernatant solution were withdrawn via a 5 µm filter needle after 30 min, 1, 2, 4, 8 and 24 h of storage. Aliquots were diluted 1:25 with 0.9% NaCl solution, acidified with 10 µL 1% $H_3PO_4$, and assayed in triplicate by HPLC. The percentage rate of irinotecan remaining loaded in the beads was calculated by the equation:

$$\text{Percentage rate of irinotecan remaining loaded after admixture with CM (\%)} = \frac{\text{drug concentration in solution before admixture} - \text{drug concentration in solution after admixture}}{\text{drug in solution before admixture}} \times 100 \quad (2)$$

## RESULTS

The average loading level of irinotecan measured was 99.58% per 2 mL DC Bead*M1*, varying from 99.47% to 99.73%. The loss of individual beads during the loading procedure was minimal, and only minimal differences were observed between the measured concentrations and the nominal concentration of 100 mg irinotecan per 2 mL DC Bead*M1* loaded.

When up to four different volumes of seven different contrast media were admixed to the irinotecan-loaded DC Bead*M1* suspensions, significant amounts of irinotecan were eluted depending on the type and volume of contrast medium added. In addition, the type and volume of contrast medium added, determined the elution rate. When 5, 10, 20 and 30 mL of Optiray 300 or Optiray 350 were admixed to irinotecan-loaded DC Bead*M1*, after a period of 30 min, about 1%–2%, 3%, 4%, and 8%–9% of the irinotecan was eluted (see figures 1 and 2). The higher the volume of Optiray 300 or Optiray 350 admixed was, the more rapid irinotecan was eluted from the beads. Over the next 8 h, the percentage rate of irinotecan released went up to 2%, 5%, 7%–9% and 12%–16%. After 8 h, the equilibrium of the exchange was almost achieved. During the following 16 h, only small additional amounts of irinotecan were eluted.

When 20 or 30 mL of Imeron 400 MCT, Ultravist 370, Solutrast 370 and Xenetix 350 were admixed to irinotecan-loaded DC Bead*M1*, the eluted amount of irinotecan increased in each case when the bigger volumes of contrast media were used (compare figures 3 and 4). Irinotecan was released in large part after 8 h, and only small additional amounts were released until to the end of the observation period of 24 h.

For Accupaque 350, only one scenario was tested. Admixing 30 mL of Accupaque 350 to irinotecan-loaded DC Bead*M1* caused the release of 4% irinotecan after 30 min and 7% after 8 and 24 h.

In figure 4, the amounts of irinotecan eluted after admixing the same volume, that is, 30 mL, of each contrast medium to the loaded beads are given in order to illustrate the influence of the type of contrast media admixed. About 3%–5% of irinotecan were released after 30 min and 5%–7% after 24 h when Imeron 400 MCT, Accupaque 350 and Solutrast 370 were added. However, in the case of Xenetix 350, the amount of irinotecan released increased from 5% after 30 min to 12% after 24 h of admixture. When Optiray 350, Ultravist 370 and Optiray 300 were admixed, the initial release amounted to 7%–8%, and increased to 14%–17% after 24 h. The differences in rate and amount of irinotecan released are to be explained by different osmolalities and viscosities of the contrast media products (compare table 1). No degradation products of irinotecan were detected in HPLC chromatograms of the admixtures. However, because of the rapid release of irinotecan, compatibility of the admixtures of DEBIRI with contrast media is not given.

## DISCUSSION

Prior to the delivery via a microcatheter, drug-loaded microspheres have to be admixed with contrast media in order to guide the administration. There are different factors known to influence the compatibility of drug-loaded beads with contrast media. During the compatibility studies, the bead size (70–150 µm) and the type and amount of drug-loaded (irinotecan, 100 mg/2 mL beads) remained unchanged whereas the type and volume of contrast media varied. These parameters were chosen because small bead sizes are preferably used nowadays, and irinotecan is known to be more rapidly eluted after

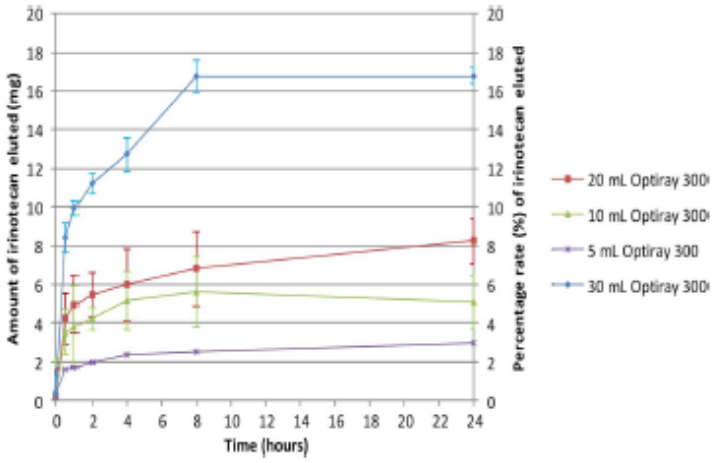


**Figure 1** Percentage rate (%) of irinotecan eluted from DEBIRI after mixing with different volumes of Optiray 300. Error bars indicate the relative SD.

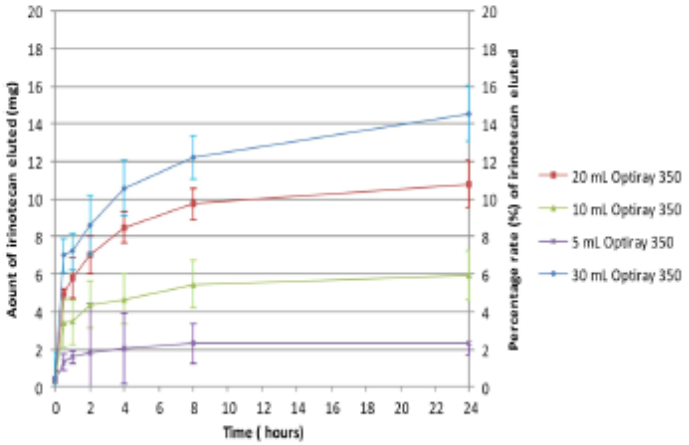


**Figure 2** Percentage rate (%) of irinotecan eluted from DEBIRI after mixing with different volumes of Optiray 350. Error bars indicate the relative SD.

admixture with contrast media than doxorubicin.[9 11] Various types and volumes of contrast media were tested with regard to clinical practice. The volume of contrast media used to administer 2 mL of beads depends on the clinical situation. With an eye to clinical practice, 5–30 mL of contrast media was chosen to be tested. Blank or reference solutions were not tested because it is well known that irinotecan is not released when the loaded irinotecan beads are placed in deionised water.[8 10] In order to determine the elution rate of irinotecan in the admixtures, samples were withdrawn at different predetermined time intervals up to a period of 24 h. Thereby, the influence of the type and volume of contrast media on the elution rate got obvious.

There are no studies published investigating the compatibility of DEBIRI with such a broad spectrum of contrast media. In our previous studies regarding DEBIRI, we used 6.5 mL of three different contrast media.[9] After 24 h, the amount eluted was about 5% in admixtures with Imeron 300, 10% with Ultravist 300 and 10% with Accupaque 300. The elution of the drug loaded in the beads is determined by the ion-exchange mechanism and the diffusive-conductive transport of the drug from beads.[8 10] Because irinotecan has no self-aggregation properties, the release rate depends on the ionic interactions with the sulfonate moieties of the beads only, and the elution is much faster than for doxorubicin and epirubicin. The differences in rate and amount of irinotecan released after admixture of DEBIRI with contrast media are to be explained by different formulations, osmolalities and viscosities of the formulated contrast media (compare table 1). The storage temperature influences the release by higher diffusion coefficients and via the impact on viscosity of the solutions. High osmolality and low viscosity favour the elution of irinotecan. This explains the low amount of irinotecan eluted from loaded beads after admixture with Imeron 400 MCT and Accupaque 350. On the other hand, for Optiray 300, which features low osmolality and low viscosity, a rapid initial release and the highest amount of irinotecan released was detected. In accordance with the Stokes equation, high viscosity reduces the diffusion coefficient and the elution rate of irinotecan, which might be an argument for preferential use of high-viscosity contrast media in the TACE procedures.

Physicochemical stability of irinotecan-loaded DC Bead*M1* was shown over a period of 28 days.[9] Due to acidification (pH <3) of the test samples before running the HPLC assay, ring-open and ring-closed irinotecan are determined as a sum, and the influence of the pH of the contrast media on lactone-carboxylate equilibrium remains unknown. Of note, the pH values of the pure contrast media are very similar. Because it is already known that loading of irinotecan into the beads shifts the equilibrium between the active lactone and carboxylate in favour of the

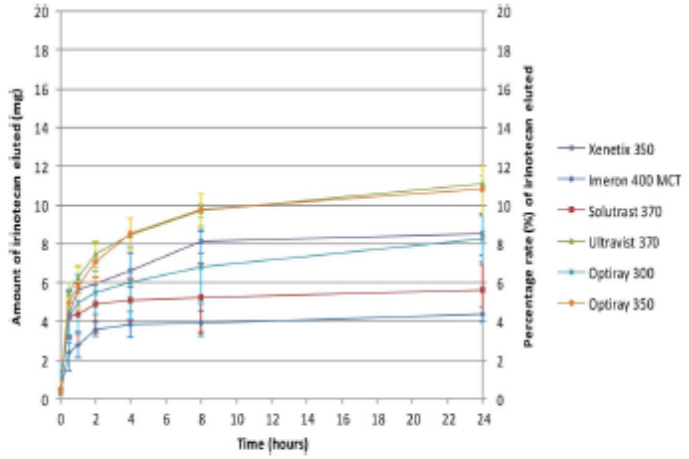


**Figure 3** Percentage rate (%) of irinotecan eluted from DEBIRI after mixing with 20 mL of six different non-ionic contrast media. Error bars indicate the relative SD.

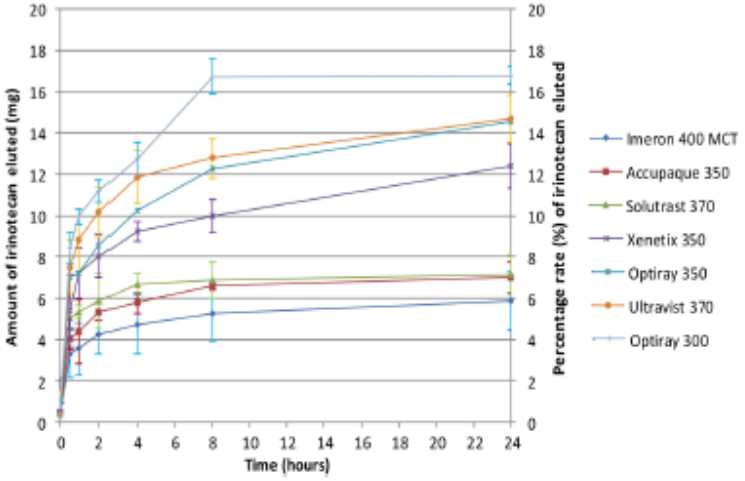


**Figure 4** Percentage rate (%) of irinotecan eluted from DEBIRI after mixing with 30 mL of seven different non-ionic contrast media. Error bars indicate the relative SD.

lactone form, the influence of the contrast medium on the pH-dependent equilibrium is most probably a subordinate one. Tang *et al*[8] showed that the major fraction eluted was the lactone form when the beads were placed in phosphate buffered solution (PBS) with neutral pH. The half-life of the lactone form eluted into the PBS depends on the size of the beads, and suggests that the higher lactone fraction is favoured by sustained release from larger bead sizes.[8] The high loading concentrations of the beads (50 mg/mL) also increase hydrophobicity of the loaded beads and retain the drug elution rate.[10]

No degradation products were detected, and the elution of irinotecan is testified as the only relevant incompatibility parameter. Moreover, during the assay validation tests, pure irinotecan drug solution was admixed with contrast medium, and the irinotecan content determined as a sum remained unchanged. However, the amount of irinotecan initially released was (except for Imeron 400 MCT) ≥5 mg, which corresponds to ≥5% loss of irinotecan loaded when 30 mL of contrast media were admixed. Therefore, the admixtures are categorised as incompatible.

## CONCLUSION

The amount of irinotecan released from loaded DC Bead*M1* after admixture with seven different contrast media added up to 2.5% and maximum 17% of the loaded amount over the observation period, depending on the type and volume of contrast media used. Because of the rapid release (3%–9%) during the first 8 h, it is not recommendable to prepare the admixture of irinotecan-loaded beads with the bead size 70–150 µm with contrast medium in centralised cytotoxic preparation units in advance. Admixtures with contrast media should be performed with the smallest possible amounts by the radiologists immediately prior to the delivery procedure.

**Table 1** Product characteristics of the non-ionic contrast media used (according to the summary of product characteristics)

| Contrast medium | Active substance | pH value | Viscosity at 20°C (mPas) | Viscosity at 37°C (mPas) | Osmolality at 37°C Osm/kg $H_2O$ |
|---|---|---|---|---|---|
| Accupaque 350 | Iohexol | 6.8–7.6 | 23.3 | 10.6 | 0.78 |
| Imeron 400 MCT | Iomeprol | 6.9–7.2 | – | 12.6±1.1 | 0.726 |
| Optiray 300 | Ioversol | 6.0–7.4 | 8.2 (25°C) | 5.5 | 0.645 |
| Optiray 350 | Ioversol | 6.0–7.4 | 14.3 (25°C) | 9.0 | 0.78 |
| Solutrast 370 | Iopamidol | 6.5–7.5 | – | 9.5 | 0.799 |
| Ultravist 370 | Iopromide | 6.5–8.0 | 20.1 | 9.5 | 0.77 |
| Xenetix 350 | Iobitridol | 7.3 | 21 | 10 | 0.915 |

### What this paper adds

**What is already known on this subject?**

- Mixtures of non-ionic contrast media and loaded microspheres are used for simultaneous chemoembolisation of liver tumours and metastases.
- Mixing of irinotecan-loaded beads (size >100 µm) with selected non-ionic contrast media rapidly decreases the loading efficiency of irinotecan by 5%–10%.

**What is not yet known?**

- Compatibility of the irinotecan-loaded DC Bead*M1* (size 70–150 µm) with non-ionic contrast media is unknown.

**What this study adds?**

- Scientifically proven data about the rapid decrease of the loading efficiency of irinotecan-loaded DC Bead*M1* (size 70–150 µm) after admixture with up to four volumes of seven different non-ionic contrast media.
- The recommendation not to prepare admixtures of irinotecan-loaded DC Bead*M1* with contrast media in centralised cytotoxic preparation units in advance.

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**Competing interests** None.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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# 8 Summary

Ready-to-use antineoplastic drug preparations are usually prepared in the patient individual dose in hospital pharmacies under strict aseptic conditions. Thereby medication errors during preparation and administration are reduced. However knowledge about the microbial and physicochemical stability of the ready to use anticancer drug preparations is necessary.

## Viability of micro-organisms in novel chemical and biopharmaceutical anticancer drug solutions

Preparation of ready-to-use cytotoxic solutions should be performed under strict aseptic conditions in order to guarantee the freedom from pathogenic microorganisms and pyrogens. In other words, the sterility of these products plays a key role in drug safety. However, sterility testing cannot be carried out in accordance with the pharmacopoeia because of the singularity of each preparation and administration of preparation before getting the results of sterility tests. Of note, the viability of microorganisms in anticancer drug preparations is unknown. Therefore viability of micro-organisms, i.e. S. aureus, P. aeruginosa, E. faecium, C. albicans which are commonly related to nosocomial infections and represent potential contaminants were tested in novel chemical and biopharmaceutical anticancer drug preparations. The test conditions simulate the worst possible conditions for patients and optimal circumstances for the growth of microorganisms. The tested antineoplastic drug products (aflibercept, brentuximab-vedotin, nelarabine, ofatumumab, arsenic trioxide, temsirolimus, ipilimumab, cabazitaxel, trabectedin, eribulin, fotemustine, nab-paclitaxel, panitumumab, clofarabine) were aseptically reconstituted according to the manufacturers' recommendations in the centralized cytotoxic drug preparation unit of our Pharmacy Department and 9 mL of each sample preparation were aseptically transferred in quadruplicate to sterile empty containers, and agitated with 1 mL of each microbial suspension to achieve concentrations of approximately $10^4$ micro-organisms per mL and get homogenized suspensions. 0.1 mL samples were transferred immediately and after 1, 3, 5, 24, 48, and 144 hours of inoculation to tryptic soy agar plates in duplicate. The plates were incubated for 24 hours at 37 °C and the CFU counted while the inoculated preparations were stored at room temperature (22 °C) and protected from light. Most of anticancer drug solutions showed neither growth-

retarding nor growth-supporting properties. An exception was trabectedin solution which has antibacterial activity.

Therefore in pharmacy departments the solutions for parenteral administration must be prepared under strict aseptic conditions and appropriate quality assurance programs should be established in order to guarantee the sterility of drug preparations. The lack of antimicrobial properties should be considered during determination of expiration dates of ready-to-use solutions of most antineoplastic drugs. Furthermore, it is recommendable to keep all ready-to-use preparations refrigerated whenever possible to inhibit the growth of any contaminating organism.

## Viability of selected microorganisms in non-cytotoxic aseptic preparations

Pharmacy-based preparation of ready-to-use parenteral solutions should be accompanied by quality tests including monitoring of environmental conditions, media fills and sterility tests of dummy solutions dedicated additional preparations.

Seventeen aseptically prepared ready-to-use parenteral preparations (caspofungin 35 mg in 250 mL 0.9% NaCl solution, 70 mg/250 mL 0.9 % NaCl solution, micafungin 0.5 mg/mL in 0.9% NaCl solution, vancomycin 5 mg/ml in G5 and G10, heparin-sodium 1 IE/mL, epinephrine 0.02 mg/mL in G5, norepinephrine 0.01 mg/mL in G5, phenylephrine 10 mg/mL, KCl solution 0.8 mol/mL, trace elements 1:1 in G5 and G10, midazolam 5 mg/mL injection, tranexamic acid injection solution 100 mg/mL, 50% glucose solution, SMOF Lipid 20% emulsion, 1% propofol injection emulsion) were inoculated with selected micro-organisms including i.e. S. aureus, P. aeruginosa, E. faecium, and C. albicans. Over a period of 7 days the growth retarding or growth promoting character of the preparations was studied Nine mL aliquots of each test solution were inoculated with 1 mL suspension of selected strains and agitated to achieve homogenous test suspensions. 0.1mL of the inoculated suspensions was withdrawn at predetermined time points (0, 1, 3, 5, 24, 48, 144 hours) and transferred to tryptic soy agar plates in duplicate. The plates were incubated at 37 °C and colony forming units counted after 24 hours. The selected microorganisms remained viable in most test preparations. Few tested preparations including vancomycin, phenylephrine or midazolam were able to inhibit the growth of selected strains after a period of a few hours or days. Glucose 50% w/v solution and tranexamic acid solution generated antibacterial activity against

P. aeruginosa respectively immediately and after 48 h of inoculation. In glucose 50% w/v solution, C. albicans lost viability directly after inoculation. The insufficient antimicrobial properties of ready-to-use, non-cytotoxic solutions should be considered while determining the shelf-life of the products. Ready-to-use preparations should be kept refrigerated whenever possible to inhibit the multiplication of any contaminating organism

## Updated data about physical and chemical stability of anticancer drug preparations

Stability data reported in the SmPC and other official documents are rather limited by microbiological instability than by physical and chemical instability. Known physicochemical stability data over prolonged periods facilitate the preparation of ready-to-use products in advance, especially for weekends and home chemotherapy treatment. There are a number of originator publications and databases available dealing with the physicochemical stability of reconstituted and diluted cytotoxic preparations. A comprehensive information source in table format (STABIL-LISTE©) is compiled by the Pharmacy Department of University Medical Center in Mainz. In the updated version of STABIL-LISTE© 7th ed., 2015 information about the stability/compatibility of drug loaded beads as well as 12 recently approved anticancer drugs (alemtuzumab 10 mg/mL, amsacrine 1.5 mg/mL, belinostat, blinatumomab, nivolumab, obinutuzmab, pembrolizumab, plerixafor, ramucirumab, rituximab subcutaneous injection, trastuzumab subcutaneous injection trastuzmab emtansine) was added. Methods of data compilation are described, conflicting results and open questions were commented. In the new edition of STABIL-LISTE© for the first time data about the stability and compatibility of drug eluting DC Bead™ were implemented. These data result from the following experimental studies.

## Compatibility of epirubicin-loaded DC Bead™ with different non-ionic contrast media

Transarterial chemoembolization (TACE) is the optimum treatment for patients who are not able to undergo to surgery, transplantation or ablation of HCC. During DEB-TACE treatment, the negatively charged substructures of microspheres can be loaded with cationic antineo-

plastic drugs in order to deliver the cytotoxic agent continuously over prolonged period by drug controlled release.

Loading of the beads with doxorubicin, epirubicin, irinotecan or topotecan is usually performed in a pharmacy-based aseptic preparation unit. The physio-chemical stability of epirubicin loaded beads over a period of 28 days was reported previously. However, there is a lack of data about the compatibility of admixtures of epirubicin-loaded DC Bead™ (bead size 70-150 µm (M1) and 100-300 µm) with commonly used non-ionic contrast media. Compatibility was studied by mixing2 mL DC Bead™ (Biocompatibles UK Ltd) with diameters of 70-150 µm (= M1) and 100-300 µm with epirubicin 75 mg of a reconstituted powder formulation 25 mg/mL epirubicin (Farmorubicin®) or epirubicin 76 mg of injection concentrate 2 mg/mL (Epimedac® 2 mg/ml) within 2 h or 6 h, respectively.

Compatibility experiments were performed over a period of 7 days after mixing epirubicin loaded beads (bead size M1, 100-300 µm) with an equal volume (~1.5 ml) of contrast media, i.e. Accupaque™ 300 (Nycomed Inc.), Imeron® 300 (Bracco S.p.A), Ultravist® 300 (Bayer Pharma AG), Visipaque™ 320 (GE Healthcare) to get a homogenous suspension. Concentrations of epirubicin in tested suspensions were tested on day 0, 1, and 7 by using RP-HPLC assay with UV. Mixing of epirubicin-loaded beads with an equal volume of different non-ionic contrast media released 0.1-0.5% of epirubicin over a period of 7 days irrespectively of the DC Bead™ size or type of contrast media and the purity of epirubicin into EPIBED in the presence of non-ionic contrast medium was more than 99.5%. No further elution or degradation was observed after 7 days when the admixtures were stored protected from light under refrigeration. Thereby admixtures of epirubicin-loaded DC Bead™ with non-ionic contrast media can be prepared in advance in centralized cytotoxic preparation units. Moreover, microbiological aspects should be considered while determining the expiration date of the product

## Compatibility of Irinotecan-loaded DC Bead™ with different volumes and types of nonionic contrast media

Irinotecan loaded beads were utilized for loco-regional treatment of liver mCRC and showed high efficacy and safety resulting from extended contact time with tumor site, controlled

release of irinotecan into the targeted tumor bed and less toxicity comparable with systemic treatment with irinotecan. More recently DC Bead™ M1 (bead size 70-150 µm) were introduced into the market, despite the lack of data about the compatibility of admixtures of DEBIRI (bead size M1) with different types and volumes of non-ionic contrast media commonly used commonly by radiologists. After removal of physiological buffered saline, 2 ml DC Bead™ M1 (Biocompatibles, BTG) were loaded with 100 mg irinotecan infusion concentrate within 2 hours. The loading efficiency was determined by measuring the concentrations of irinotecan in the excess solutions via a reversed phase HPLC assay with ultraviolet detection and amounted to 99.7%. The compatibility experiments of admixtures of DEBIRI with different types and volumes of contrast media were studied by mixing 2 ml DEBIRI bead slurry each with up to four different volumes (5, 10, 20, 30 mL) of seven different contrast media (Accupaque™ 350, Imeron®400 MCT, Optiray™, Optiray™350, Solutrast®350, Ultravist®370, and Xenetix®350 ). Each sample was tested in triplicate and withdrawn after 30, 60, 120, 240, 480, and 1440 min. Admixtures were stored light-protected at room temperature over the observation period. Mixing of DEBIRI with nonionic contrast media decreased the irinotecan loading efficiency between 2.5% and 18% over the observation period of 24 h depending on both the type and volume of contrast medium admixed. Therefore, the advanced preparation of admixtures of IRIDEB and non-ionic contrast media is unsuitable and inconvenient for preparation in a pharmacy-based cytotoxic preparation unit.

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# *10 Curriculum vitae*

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| **Schulausbildung:** | 2001-2002 | Gymnasium: Almothana bin Haretha in Homs. Abschluss: Abitur (Note: 233/240; 97,08) |
| **Praktische Arbeit:** | 2007 – 2011 | Assistentin an der Albaath Universität, Homs, Syrien |
| | 2007-2009 | Selbstständige Apothekerin, Iman Sarakbi Alrastan, Homs, Syrien |
| | 2009-2011 | Selbstständige Apothekerin, Iman Sarakbi, in |

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|---|---|---|
| | | Zentrum der Stadt Homs, Syrien |
| **Preise** | 2006 | Preis der Albaath Universität für ausgezeichnete Leistungen als drittbester Student des 2. und 4. Studienjahres |
| **Sprachkenntnisse** | Arabisch | Muttersprache |
| | Englisch | Vortragsqualität, gut in Wort und Schrift. Niveau B2 |
| | Deutsch | Gut in Wort und Schrift. Niveau B2 |
| **EDV-Kenntnisse:** | Betriebssysteme MS Windows<br>MS Office, Internet | |

**Präsentationen**

26-28 June 2014: The 2nd European congress of Oncology Pharmacy, Poster, "Compatibility of Epirubicin-loaded DC Bead™ with different contrast media", Poland, Krakow.

25-27 March 2015: 20th Congress of the European Association of Hospital Pharmacists, Poster, "Compatibility of Irinotecan-loaded DC Bead ™ with different volumes and types of nonionic contrast media", Germany, Hamburg

07-09 October 2015: 18th GERPAC conference, Oral presentation, "Viability of selected microorganisms in non-cytotoxic aseptic preparations", France, Hyeres.

28 November 2015: ADKA, PhD Student Conference, Oral presentation, "Compatibility of Irinotecan-loaded DC Bead ™ with different volumes and types of nonionic contrast media", Germany, Aachen.

**Publications**

Viability of micro-organisms in novel chemical and biopharmaceutical anticancer drug solutions
Iman Sarakbi, Matteo Federici, Irene Krämer
European Journal of Parenteral & Pharmaceutical Sciences 2015;20(1): 5-12

Compatibility of Epirubicin-loaded DC Bead™ with different contrast media
Iman Sarakbi, Irene Krämer
Journal of Oncology Pharmacy Practice 2016;22: 195-204

Compatibility of Irinotecan-loaded DC Bead ™ with different volumes and types of nonionic contrast media
Iman Sarakbi, Judith Thiesen, Irene Krämer
European Journal of Hospital Pharmacy 2016;**23**: 38-43

Viability of selected microorganisms in non-cytotoxic aseptic preparations
Iman Sarakbi, Rita Heeb, Judith Thiesen, Irene Krämer
Pharmacy Technology in Hospital Pharmacy 2016, 1:

Die STABIL- LISTE© 7. Auflage 2015: Physikalisch-chemische Stabilität, Kompatibilität und Inkompatibilität parenteral applizierbarer Zytostatika, Virustatika und Supportivtherapeutika
Judith Thiesen, Iman Sarakbi, Irene Krämer
Krankenhauspharmazie, 2016 (under review).