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Research Article

Theme: Advancements in Dissolution Testing of Oral and Non-Oral Formulations
Guest Editor: Sandra Klein

Influence of Dissolution Vessel Geometry and Dissolution Medium on *In Vitro* Dissolution Behaviour of Triamterene-Coated Model Stents in Different Test Setups

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Abstract. The aim of this study was to investigate if the geometry of the dissolution vessel, the dissolution medium volume and composition might contribute to the variation in drug release from drug-eluting stents (DES) in different test setups, which has been observed in previous *in vitro* studies. Therefore, DES containing triamterene as model substance were produced *via* fluidised-bed technology. Dissolution testing was carried out using different incubation setups, the reciprocating holder (USP Apparatus 7) and two flow-through methods, a method similar to the USP Apparatus 4 (FTC) and the vessel-simulating flow-through cell (vFTC) equipped with a hydrogel as a second compartment simulating the blood vessel wall. The results indicate that dissolution vessel geometry and medium volume had no influence on the release behaviour and only the flow-through cell methods yielded a lower dissolution rate than the incubation setups (80.6 ± 2.0% released in the FTC after 14 days compared to > 90% for all incubation setups). The composition of the hydrogel used in the vFTC also affected the dissolution rate (53.9 ± 4.5% within 14 days with a hydrogel based on phosphate-buffered saline compared to 78.2 ± 1.2% obtained with a hydrogel based on water) possibly due to different solubility of triamterene in the release media as well as interactions between the coating polymer and the release medium. Hence, the introduction of a hydrogel as a second compartment might lead to a more biorelevant test setup.

KEY WORDS: drug-eluting stent; *in vitro* dissolution testing; vessel-simulating flow-through cell; fluidised-bed technology; release medium.

INTRODUCTION

In-stent restenosis which is defined as re-narrowing of the treated artery with a stenosis of > 50% of the stented area is one of the major complications of bare metal stents (BMS) (1). Since this loss of lumen is caused by neointimal hyperplasia due to smooth muscle cell proliferation and migration, drug-eluting stents (DES) releasing anti-proliferative agents have been developed. Currently or previously marketed DES elute paclitaxel (2), sirolimus (also called rapamycin) (3,4) and its derivates (5–7). However, other active agents such as actinomycin D (8) and dexamethasone (9) and antibodies like glycoprotein IIb/IIIa receptor antibody (10) or anti-CD34 antibody (11) have been studied as well. Furthermore, dual drug-eluting stents either with anti-proliferative coatings on the abluminal and anti-thrombotic substances on the luminal site (12,13) or in the same or different layers (14) seem promising in order to treat in-stent restenosis and thrombosis simultaneously. For coating materials, durable polymers such as polyethylene-co-vinyl acetate (PEVA), poly-n-butyl methacrylate (PBMA), the tri-block co-polymer poly(styrene-b-isobutylene-b-styrene), phosphorylcholine (PC) and the co-polymer poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) (15) as well as biodegradable polymers (polylactic acid, polylactide-co-glycolide) have been used. In addition, polymer-free DES manufactured by various techniques have been employed (16).

Although the first DES received regulatory approval in 2002, release behaviour *in vivo* and *in vitro* has been investigated to a limited extent. Furthermore, *in vivo* studies generally focus on drug levels in the blood rather than the drug concentration in the blood vessel wall, which is the target site of the anti-proliferative agents. However, tissue concentration of the drug can only be determined in animal models after stent removal. Therefore, *in vitro* studies might be extremely helpful in estimating the release behaviour of DES. Mainly non-compendial methods such

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as incubation test setups with small dissolution medium volumes have been used to evaluate drug release from DES (17–19). Besides, compendial methods comprising the reciprocating cylinder (USP Apparatus 7) (20,21) or the flow-through cell (USP Apparatus 4) (22) have been considered for estimating drug release from DES. Nevertheless, none of these *in vitro* test setups take the distribution of the drug into the tissue into account. Thus, the vessel-simulating flow-through cell (vFTC) (23) was previously developed by our group introducing a hydrogel as a second compartment mimicking the tissue in which the stent is implanted. In a previous work, the release of sirolimus from the CYPHER™ stent was evaluated using different test setups (20). The results of this study showed a dependency of the release behaviour on experimental conditions such as dissolution medium volume and test setup (Fig. 1) despite maintenance of sink conditions, which could not be clarified completely. For example, using 2 mL medium in a glass vial with flat bottom yielded faster release than in 10 mL within a polypropylene test tube with a conical bottom. Adsorption could be excluded as the cause of this difference. Different hydrodynamic conditions due to varying dissolution vessel geometry might be an explanation for the observed high variability of release behaviour.

Hence, it was the aim this study to evaluate the influence of the dissolution vessel geometry as well as different test setups on the release behaviour of DES. Moreover, the impact of different release media on the release behaviour was examined as well. Since marketing of the CYPHER™ stent was ceased at the end of 2011 and sirolimus is known for its high instability in aqueous media (24), model substance-coated DES were used for this study. Triamterene was used as a model drug for this purpose due to its fairly low solubility in water, which is typical for drugs applied *via* stents, and ease of detection.

MATERIALS AND METHODS

Materials

Triamterene and agarose were obtained from Sigma Aldrich Chemie GmbH (Steinheim, Germany). BMS (Pro Kinetic T6S15) were kindly donated by Biotronik SE & Co. KG (Berlin, Germany). Eudragit® RS 30 D (30% aqueous dispersion of poly(ethyl acrylate, methyl methacrylate) trimethylammonioethyl methacrylate chloride) was generously provided by Evonik Nutrition and Care GmbH (Darmstadt, Germany). Formic acid was obtained from AppliChem GmbH (Darmstadt, Germany) and triethyl citrate was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). All other chemicals were of analytical grade.

Phosphate-buffered saline according to Ph. Eur. (25) with a pH of 7.4 (PBS) was prepared by dissolving 2.38 g disodium hydrogen phosphate dodecahydrate, 0.19 g potassium dihydrogen phosphate and 8.00 g sodium chloride in deionised water and diluting to 1000.0 mL with deionised water resulting in a molar concentration of 145 mmol/L. pH was adjusted to 7.4 with orthophosphoric acid. Phosphate-buffered saline without sodium chloride (PB) and with a molar concentration of 8 mmol/L was prepared similarly by simply omitting sodium chloride. If not stated otherwise, media were used without prior filtration. All media were freshly prepared before the experiments and showed no signs of microbial contamination over 14 days.

Model Substance and Quantification

Triamterene was chosen as a model drug due to its low solubility in water. Physico-chemical properties of sirolimus (also known as rapamycin) and paclitaxel, which are currently used as active therapeutic agents in DES, as well as the model substance triamterene are given in Table I.

Furthermore, triamterene is easily detectable *via* fluorescence spectroscopy. Therefore, a microplate reader (Varioskan Flash, Thermo Scientific, Waltham, USA) was used for fluorimetric quantification with an excitation wavelength of 370 nm and an emission wavelength of 434 nm. For aqueous samples derived from dissolution test setups and solubility studies, calibration was performed using a dilution series of model substance-loaded medium in the range from 12 to 3000 µg/mL. Where necessary, aqueous samples were diluted with the corresponding medium. Calibration for methanolic samples derived from the determination of the residual amount and drug content was carried out dissolving

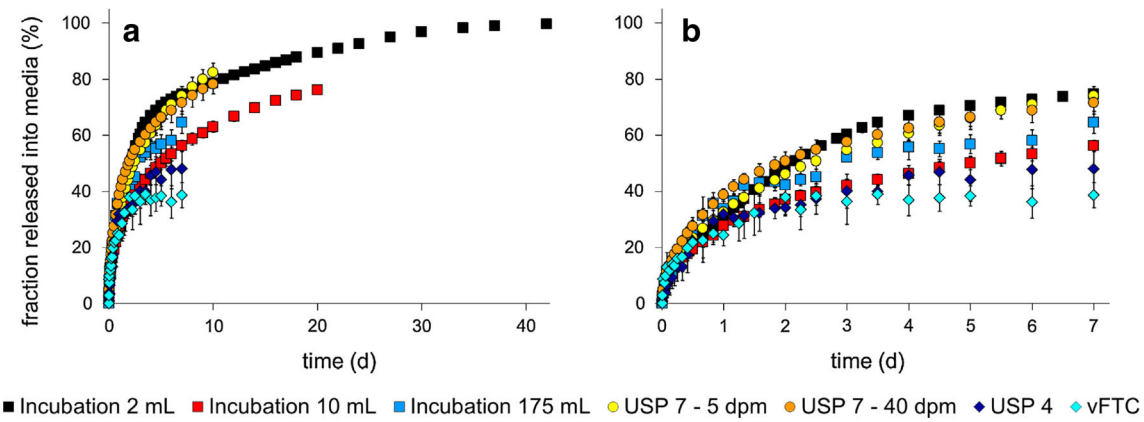

Fig. 1. Reprinted from (20) with permission from Elsevier. Comparison of the normalised release profiles of the CYPHER™ stent obtained using the different methods including three incubation methods (media volumes of 2, 10 and 175 mL), two reciprocating holder apparatus methods (USP Apparatus 7 at dip rates of 5 and 40 dpm) and two flow-through methods (conventional USP Apparatus 4 and vessel-simulating flow-through cell (vFTC) with hydrogel compartment) depicted for the entire individual testing period (**a**) and for the first 7 days (**b**); $n = 3$, means ± SD (20)

Table I. Physico-chemical Properties of Triamterene and Active Agents Currently Used in Drug-Eluting Stents

	Triamterene	Sirolimus	Paclitaxel
Solubility in water	27.7 μg/mL (25) 45 μg/mL (26)	2.6 μg/mL (27)	1 μg/mL (28)
Octanol–water partition coefficient	1.250 (29)	5.77 (30)	4.40 (31)

triamterene in a mixture of methanol and PBS in concentrations ranging from 6 to 1500 μg/mL, and methanolic samples were diluted with PBS accordingly. The amount of triamterene released into hydrogels was determined with a set of dilutions of triamterene in PBS mixed with 2% agarose in PBS resulting in a calibration range from 1.2 to 300 μg/mL. Hydrogel samples were further diluted with PBS.

Model Substance Coating of Drug-Eluting Stents

BMS were coated with Eudragit® RS 30 D and triamterene according to a modified procedure described elsewhere (26). Briefly, triamterene was dissolved in formic acid and mixed with deionised water and the plasticiser triethyl citrate leading to a fine precipitation of triamterene. Subsequently, the polymer dispersion was added under continuous stirring. The composition of the coating liquid is given in Table II.

The coating was performed *via* a bottom-spray fluidised-bed process using a Mini-Glatt fluidised-bed apparatus. Parameters of the procedure are shown in Table III. Two hundred fifty stents were coated under addition of 377 steel springs of similar measures (length 14.6 mm, diameter 1.76 mm, Gutekunst & Co. KG, Germany). These springs were added to assure sufficient filling of the product container and adequate fluidisation, which could not be achieved using 250 stents.

In order to evaluate the homogeneity of the coating, stents were studied by fluorescence microscopy (BZ-8000, Keyence Corporation, Japan, excitation wavelength 360 nm, emission wavelength 460 nm) and scanning electron microscopy (SEM, excitation voltage 5 kV, PHENOM® desktop SEM, FEI Company, USA) after sputter-coating with gold–palladium under argon for 90 s using the Mini Sputter Coater SC7620 (Quorum Technologies Ltd., UK).

Uniformity of Mass and Triamterene Content

Ten coated stents were randomly chosen and evaluated for coating mass and drug content, respectively. For determination of coating mass, stents were weighed before and after incubation in 10 mL acetone for 24 h at 37°C in a shaking incubator (IKA KS 3000 i control, IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 250 rpm. The coating mass was calculated from the difference of the stent masses before and after removal of the coating. For analytical reasons, content uniformity was evaluated by incubation of each stent in 10 mL methanol at 37°C in a shaking incubator at 250 rpm followed by additional incubation steps in 1 mL methanol under the same conditions. Since the solubility of the drug and the polymer was slightly lower in methanol compared to acetone, the first elution was performed for 72 h, whereas further incubations steps were carried out for 24 h. Complete dissolution of the drug was assumed when fluorescence was below threefold the value obtained without triamterene. Methanolic eluates were analysed for triamterene content as described above.

Table II. Composition of Coating Liquid

Substance	Fraction percentage (*w*/*w*)
Eudragit® RS 30 D	3.51
Triethyl citrate	0.19
Triamterene	0.24
Formic acid	8.22
Deionised water	87.83

Solubility

Solubility of triamterene in three different release media (PBS, PB and deionised water) was determined using a conventional saturation shake-flask method as well as the facilitated dissolution method (FDM).

For the shake-flask method, excess amounts of solid triamterene were placed in 75 mL of the respective release medium and maintained in a water bath for 120 h at 37°C under constant stirring (300 rpm). Samples were taken at predetermined time points and filtered through a 10-μm poroplast filter (ERWEKA, Heusenstamm, Germany) and a 0.45-μm PVDF filter (Whatman™, GE Healthcare, Little Chalfont, UK). Due to the use of unbuffered media, pH was measured at the beginning and the end of the experiment (Hamilton Minitrode, Bonaduz, Switzerland).

In order to overcome the low equilibration rates and the poor wetting of triamterene by aqueous systems, a facilitated dissolution method as described by Higuchi *et al.* (27) was performed to estimate the solubility as well. Therefore, a limited amount of a water-immiscible solvent, in which

Table III. Coating Parameters

Parameters	Chosen equipment/set value
Equipment	Micro-Kit product container Wurster bottom plate
Nozzle diameter	0.5 mm
Inlet air temperature	40°C
Process pressure	0.15 bar
Spray pressure	0.5 bar
Spray rate	1.48 g/min

triamterene is significantly more soluble, was added to the system leading to an increased interfacial area. n-Octanol was chosen for the immiscible solvent since triamterene has a considerably higher solubility in n-octanol than in water (28). An excess amount of triamterene was mixed with 10 mL of the respective release medium and 400 μL n-octanol, vortexed and kept for 120 h in a shaking incubator at 37°C and 100 rpm. At predetermined time points, samples were centrifuged (4400 rpm, 10 min, Eppendorf Centrifuge 5702R, Wesseling-Berzdorf, Germany); n-octanol was removed; and the aqueous phase was filtered through a 0.45-μm PVDF filter, diluted with the correspondent medium and analysed for triamterene content and pH.

In order to quantify the difference in solubility between the three media, the empirical Setschenow constant K_{salt} was calculated by the Setschenow equation (Eq. 1) (29).

$$\log \frac{S}{S_0} = -K_{salt} C_{salt} \quad (1)$$

where S and S_0 are the solubilities of triamterene determined *via* the shake-flask method after 120 h in the aqueous salt solution and in deionised water, respectively, and C_{salt} is the molar concentration of the salts.

Dissolution Test Setups

Release of triamterene from DES was evaluated in four different test setups. In each setup, three to six triamterene-coated stents were examined. If not stated otherwise, samples were withdrawn after 5, 15 and 30 min and 1, 2, 4, 8, 12, 16, 24, 48, 72, 96, 168, 192, 216, 240, 264 and 336 h. The duration of the experiments of 14 days was selected in order to ensure either complete dissolution of the drug or that at least a plateau phase has been reached. PBS pH 7.4 was used as dissolution medium and sink conditions (defined as the saturation solubility in the dissolution medium being at least three times higher than the drug concentration) were maintained throughout all the experiments. An overview of all dissolution test setups is given in Table IV. Prior to dissolution testing, stents were expanded using a balloon catheter (Pantera 3.5/25, Biotronik, Switzerland) and a catheter pump (20/30 Indeflator, Abbott, USA). An inflation pressure of 10 atm for 60 s resulted in an anticipated total stent diameter of 3.76 mm.

Incubation Setups

Three different volumes of release medium were evaluated in an incubation setup. In addition to a small volume of 2 mL as it is often described in the literature (17–19), 10 and 75 mL were used as well in order to provide comparability between the incubation setups and the reciprocating holder or the flow-through cells, respectively. Release medium was changed completely at each sampling time point for incubation in 2 and 10 mL to ensure sink conditions. Here, stents were removed with a wire, carefully blotted with a lint-free paper and transferred into a new container with fresh preheated release medium of 37°C. For incubation in 75 mL, two samples of 750 μL were withdrawn at each time point and replaced with 1.5 mL fresh medium of 37°C.

In order to determine the influence of the dissolution vessel geometry, different vessels with either flat, conical or round base were used as well. Descriptions of the investigated vessels can be found in Table IV.

Additional short-term experiments were conducted with both different media and different hydrogels. Stents were incubated either in deionised water, PB or PBS in 100 mL screw cap glass flasks as described in the incubation setup with 75 mL. Samples of 1 mL were withdrawn after 5, 15, 30, 60, 120 and 240 min, and the media reduction was compensated by the addition of 1 mL fresh medium preheated to 37°C. In addition, 2% agarose hydrogels were prepared by dissolving agarose in either hot deionised water or hot PBS (90°C in each case to guarantee complete dissolution of the agarose) under stirring. Stents were mounted on a wire and fixed inside of the screw cap glass flasks, and 75 mL of the agarose sols of 50°C were poured over the stents. After 30, 60, 120 and 240 min, stents were resected from the hydrogels. The hydrogels were diluted with boiling medium to dissolve the agarose and the amount of triamterene in the sol was determined.

All vessels were placed in a shaking incubator at 37°C and 100 rpm with the exception of incubations in 75 mL screw cap glass flasks, which were conducted in a water bath of 37°C under constant stirring using a magnetic stir bar (250 rpm).

Reciprocating Holder (USP Apparatus 7)

The compendial reciprocating holder (USP Apparatus 7, 400-DS, Agilent Technologies Inc., USA), originally designed for transdermal delivery systems, was chosen for another test setup because of its high degree of standardisation and the possibility to use small media volumes as low as 5 mL. Equipped with a non-compendial stent holder, the suitability of the reciprocating holder for testing drug-eluting stents has already been demonstrated (20,21). Two different dip rates (5 and 40 dips per minute) with amplitudes of 20 mm were applied to subject the stents to varying mechanical stress. Stents were mounted on a stent holder (Fig. 2a) and dipped into 10 mL release medium in the dissolution cells at 37°C. At predetermined time points, cells were emptied automatically *via* a port at the cell bottom and filled with fresh medium equilibrated to 37°C. Samples of 750 μL were withdrawn automatically as well, transferred to 2 mL HPLC vials and analysed for triamterene content.

Flow-Through Methods

Two flow-through methods were used for dissolution testing of the triamterene-coated stents. First, a method similar to the compendial flow-through cell as described in the pharmacopoeias (USP Apparatus 4, (30)) was utilised (FTC). For the experiments conducted here, a slightly modified cell was used as described below for the vFTC. Second, stents were tested in a vFTC (Fig. 2b, c) previously described (23). For this purpose, the compendial flow-through cell for large tablets was modified as follows: the cylindrical part was elongated by 10 mm and the conical part was shortened accordingly and filled with glass beads (one large glass bead of 5 mm at the inlet of the cell and several 1 mm glass beads on top) to achieve laminar flow inside the flow-through cell. The cylindrical part of the vFTC was filled with a 2% agarose hydrogel

Table IV. Overview of Experimental Conditions and Apparatuses/Vessels for Different Release Test Setups

Method	Media volume	Apparatus/Vessels	Sampling
Incubation	2 mL	Screw cap glass vial, volumetric capacity 4 mL, size 45 mm × 14.7 mm, flat base	Complete media change
Incubation	10 mL	Screw cap polypropylene test tube, volumetric capacity 15 mL, size 120 mm × 17 mm, conical base	Complete media change
Incubation	10 mL	Screw cap polypropylene test tube, volumetric capacity 13 mL, size 100 mm × 16 mm, round base	Complete media change
Incubation	10 mL	Screw cap glass vial, volumetric capacity 12 mL, size 66 mm × 18.5 mm, flat base	Complete media change
Incubation	75 mL	Screw cap glass flask, volumetric capacity 100 mL, size 105 mm × 56 mm, flat base	Withdrawal of 2 × 750 μL samples and replenishment with 1.5 mL of fresh media of 37°C
USP 7	10 mL	Small volume reciprocating holder apparatus with 10 mL glass cells and stent holder, amplitude of 2 cm at a rate of 5 dips per minute (dpm) or 40 dpm, respectively	Automated withdrawal of 750 μL samples and subsequent complete automated media change
Flow-through cell (FTC)	75 mL	Polymethylmethacrylate flow-through cell, closed loop, flow rate of 35 mL/min	Withdrawal of 2 × 750 μL samples and replenishment with 1.5 mL of fresh media of 37°C
Vessel-simulating flow-through cell (vFTC)	75 mL	Polymethylmethacrylate flow-through cell, closed loop, flow rate of 35 mL/min, agarose hydrogel as a second compartment	Withdrawal of 2 × 750 μL samples and replenishment with 1.5 mL of fresh media of 37°C

simulating the blood vessel wall and the surrounding tissue and introducing an additional compartment. The hydrogel was prepared as described above. Initially, only a 2% agarose gel based on deionised water was used. Because of the test results, the influence of different hydrogel compositions was further evaluated using PBS as medium for the agarose hydrogel. An acrylic disc was placed on top of the glass beads in the vFTC, and the warm agarose sol was casted in the cylindrical part. A placeholder was introduced in the centre of the sol creating a lumen of 3 mm diameter in the solidified hydrogel when removed, and the DES were expanded inside the lumen prior to dissolution testing. After 14 days, the hydrogel was removed from the cell and the stent was resected. After dilution with boiling medium, the amount of triamterene in the hydrogel was evaluated.

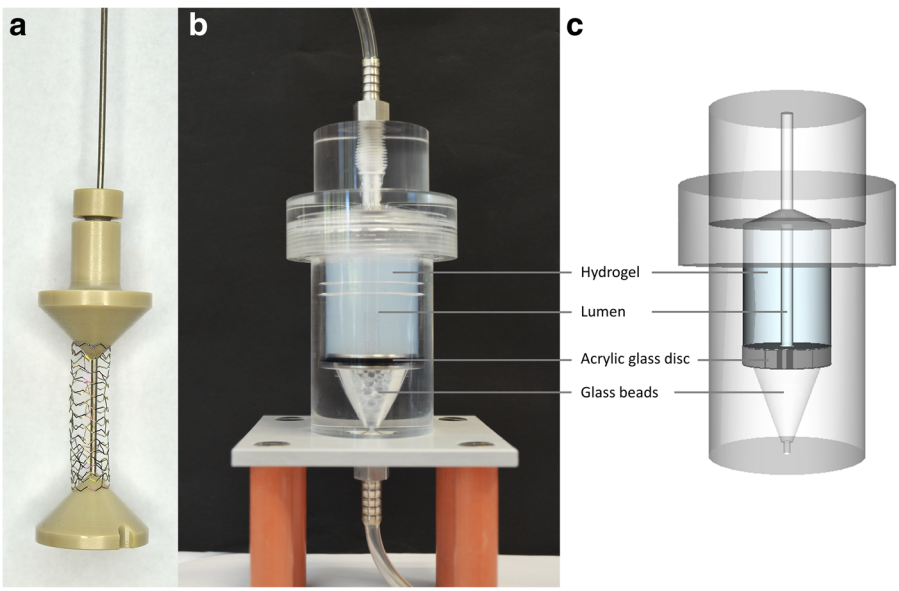

Fig. 2. Non-compendial stent holder for the reciprocating holder apparatus (**a**) and photograph (**b**) and schematic (**c**) of the vessel-simulating flow-through cell equipped with a hydrogel

For comparability, the cell constructed for the vFTC was used for either of the flow-through methods, but without hydrogel for the compendial method. For both methods, cells were operated in a closed loop with 75 mL release medium. Medium was circulated with a peristaltic pump (Ismatec, IDEX Health & Science GmbH, Wertheim, Germany) at a flow rate of 35 mL/min simulating the blood flow rate in coronary arteries (31). Release medium container (screw cap glass flask as used in the incubation setup) and flow-through cells were placed in a water bath at 37°C and the medium was continuously stirred at 250 rpm. Two 750 μL samples were collected at the time points mentioned above and replaced with 1.5 mL fresh medium of 37°C.

Determination of Residual Triamterene Content

Residual triamterene content of the stents after dissolution testing was determined by incubation of the stents in 5 mL methanol for 72 h at 37°C and 100 rpm in a shaking incubator followed by a second extraction in 1 mL methanol for 24 h. A third extraction step was carried out if the second step yielded fluorescence that was three times higher than fluorescence of the blank.

Mechanism of Drug Release

Cumulative triamterene release was fitted according to the first-order equation, the Higuchi model and the Korsmeyer–Peppas model. The first-order equation

$$M_t = M_0 \cdot e^{-k_1 \cdot t} \tag{2}$$

describes the release from a system where the release rate is concentration dependent. M_t and M_0 are the amount of drug released at each time point and the initial amount in solution, respectively, and k_1 is the first-order release constant. Following the Higuchi model (32), drug release is described as a square root time-dependent diffusion process based on Fick's law as

$$\frac{M_t}{M_\infty} = k_\mathrm{H} \cdot t^{0.5} \tag{3}$$

$\frac{M_t}{M_\infty}$ is the percentage of drug released at each time point relative to the percentage of overall release and k_H is the Higuchi dissolution constant.

Korsmeyer and Peppas (33) established a relation described by

$$\frac{M_t}{M_\infty} = k_\mathrm{KP} \cdot t^n \tag{4}$$

with the exponent n used to characterise different release mechanisms. For thin films, an exponent of 0.5 determines the release mechanism as Fickian diffusion, whereas exponents of 0.45 and 0.43 were obtained for diffusion processes in cylindrical and spherical samples (34). Coefficients of correlation (r^2) were used to evaluate the accuracy of the fit and data was fitted up to a drug release of 60%.

RESULTS

Coating Process and Evaluation of the Coated Devices

The coating process was suitable to coat 250 stents (and 377 steel springs) simultaneously within 30 min. The mass gain of the coated batch amounted to 77% of the totally sprayed solids. Four stents were excluded from the batch due to deformation.

Fluorescence microscopic images before and after dilatation of the stent indicated uniform distribution of triamterene on the stent surface (Fig. 3a, b). No defects or bridges between struts were observed. SEM images confirmed a continuous layer on the surface with a slightly grainy structure (Fig. 3c). Single spots, which lacked sufficient coating, were observed at the ending of the stents where presumably the highest mechanical stress occurred during fluidisation. Even on expanded stents, no cracks in the coating and only small defects on the strut bends were visible indicating that dilatation had only little effect on the stent coating (Fig. 3b).

The average coating mass of ten observed stents was 375 ± 12 μg. Individual coating masses were within 95 and 106% of the average coating mass (Fig. 4a). Determination of triamterene content yielded similar results with individual contents of 86 to 109% of an average content of 76.3 ± 5.0 μg (Fig. 4b).

Solubility

A shake-flask method and the facilitated dissolution method were used to determine the solubility of triamterene in PBS, PB and deionised water over 120 h. Irrespective of the method used, the determined solubility was considerably lower in PBS (13.0 ± 0.5 μg/mL for the shake-flask method and 12.1 ± 0.2 μg/mL for the FDM) than in deionised water (45.0 ± 0.4 and 44.6 ± 0.8 μg/mL). Furthermore, a decrease in solubility from approximately 30 μg/mL after 4 h to about 10 μg/mL after 48 h was observed for PBS, which was faster using the shake-flask method (Fig. 5). Equilibrium was reached after 24 h in deionised water and PB, whereas equilibrium solubility in PBS was achieved after 48 h. Solubility of triamterene in PB was almost equal to that in deionised water; however, slightly higher values were obtained using FDM (49.7 ± 0.4 μg/mL) compared to the shake-flask method (41.8 ± 0.7 μg/mL).

Equation 1 yielded a Setschenow constant K_salt of 3.72 ± 0.08 for triamterene in PBS having a molar concentration of 145 mmol/L. For PB with a molar concentration of 8 mmol/L, a K_Salt of 4.06 ± 0.58 was calculated.

Dissolution Tests

In order to evaluate the influence of different dissolution medium volumes on the dissolution rate of triamterene-coated model stents, three different volumes were examined. Figure 6a depicts that the use of 75 mL resulted only in a slightly faster initial triamterene release within the first 4 days, whereas the use of 2 and 10 mL yielded similar dissolution profiles. Additionally, the effect of dissolution vessel geometry was negligible as well, as can be seen in Fig. 6b. Results obtained in the USP Apparatus 7 were comparable to those

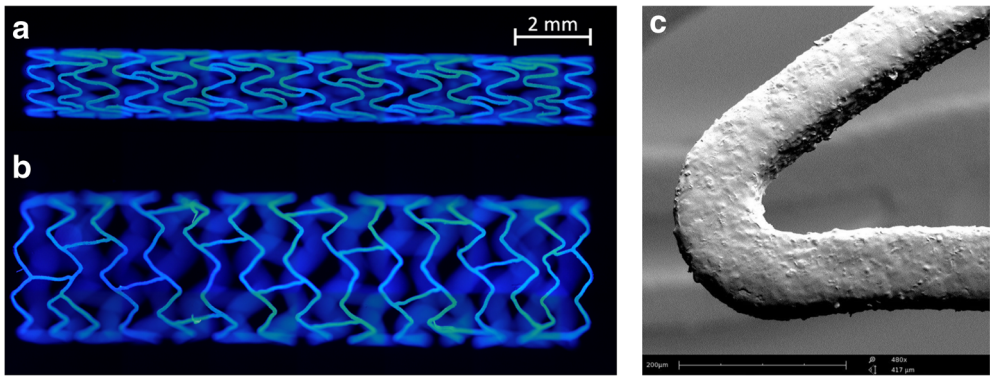

Fig. 3. Fluorescence microscopic image of stent before (**a**) and after dilatation (**b**) and scanning electron microscopic image of a stent before dilatation (**c**)

from a simple incubation method, and the dip rate had no influence on the release rate (Fig. 6c). For all incubation setups and the USP Apparatus 7, initial release was fast with more than 55% triamterene released after 1 day and around 70% (88.3 ± 5.5% for incubation in 75 mL) after 2 days. Subsequently, the release rate decreased over the next days with over 90% release after 7 days. After 14 days, release was almost completed with less than 3% of overall detected triamterene remaining in the coating.

In contrast, in test setups using the flow-through apparatus, release was incomplete after 14 days. Figure 6d depicts that, using the FTC as well as the vFTC with a hydrogel based on deionised water, approximately 80% of overall detected triamterene was released into the release medium until termination of the experiments. However, the introduction of a hydrogel as a second compartment had hardly any impact on the release into the medium. Actually, initial triamterene release even increased with 63.3 ± 2.0% for the vFTC compared to 51.8 ± 1.4% for the FTC after 24 h and total triamterene release (sum of triamterene recovered from the medium and hydrogel, if applicable) was slightly higher in the vFTC (93.0 ± 1.8%) than in the FTC (80.6 ± 2.0%) as well. In order to elucidate if the composition of the hydrogel might affect the release behaviour as well, the influence of the dissolution medium was further evaluated in short-term incubation experiments over 4 h (Fig. 7).

Obviously, release into hydrogel was lower after 4 h compared to stirred liquid media; 99.4 ± 0.9% of overall detected triamterene was released using deionised water as release medium, whereas the fraction released into the hydrogel based on this medium was only 83.1 ± 2.6%. Similar observations were made for PBS (42.6 ± 1.4%) and hydrogel based on PBS (27.6 ± 1.0%) although release was considerably lower when PBS was used either as medium or as hydrogel base compared to deionised water. The use of PB as release medium resulted in a faster triamterene release (75.8 ± 6.1% after 4 h) than the use of PBS.

As a result of the short-term experiments, release was investigated again using the vFTC, but this time equipped with a hydrogel based on PBS. Figure 8 shows that the use of a hydrogel based on PBS resulted in a considerable decrease in triamterene release with as little as 53.9 ± 4.5% after 14 days. Moreover, only 42.8 ± 5.3% of total triamterene was released in the initial 24 h.

These findings could be confirmed by investigating the amount of triamterene recovered from the hydrogel and the stent as can be seen in Fig. 9. The fraction of triamterene detected in the hydrogel after 14 days was considerably lower for the vFTC with the hydrogel based on PBS 8.2 ± 1.2% compared to 14.8 ± 4.4% in the vFTC with hydrogel based on deionised water. Thus, the least overall triamterene release in flow-through setups was observed in the vFTC based on PBS with a total release (sum of hydrogel and media release) of 62.2 ± 4.7% compared to 80.6 ± 2.0% for the FTC and 93.0 ± 1.8% for the vFTC with the gel based on deionised water.

Mechanism of Drug Release

Release data obtained in two different test setups, namely incubation in test tubes with conical base and the FTC, were fitted according to the first-order equation and the Higuchi and the Korsmeyer–Peppas models for all data points with < 60% triamterene release. Coefficients of correlation (r^2), kinetic constants and the exponent n are given in Table V. Good linearity

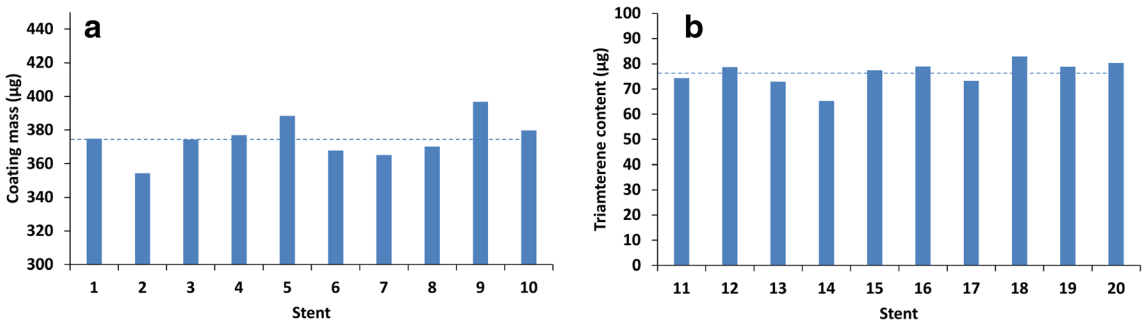

Fig. 4. Coating mass (**a**) and triamterene content (**b**) of the observed stents. Average coating mass and content, respectively, marked by dashed line

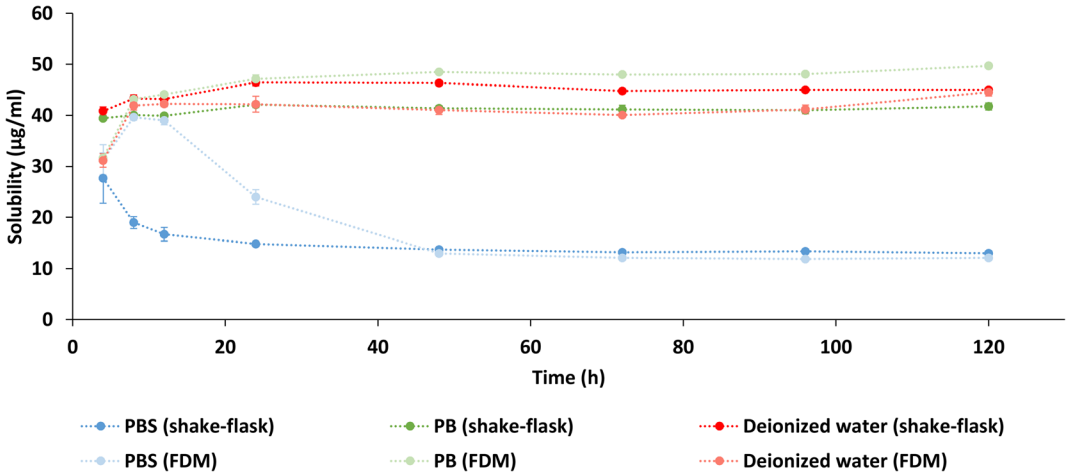

Fig. 5. Solubility of triamterene in different media obtained from the conventional shake-flask method and the facilitated dissolution method (FDM) ($n = 3$, means ± SD)

was achieved for the Higuchi kinetic (r^2 of > 0.95) indicating a diffusion-based drug release. The exponent n derived from the Korsmeyer–Peppas plot of < 0.5 indicates a release mechanism based on Fickian diffusion as well. Since the exponent n is lower than the exponents described in the literature (34), other drug release mechanisms might be considered as well. Furthermore, the geometry of the polymer film around small struts of the stent differs considerably from a thin film and a cylindrical or spherical sample to which the Korsmeyer–Peppas model is applicable.

DISCUSSION

The coating process *via* fluidised-bed technology yielded homogeneous surfaces with fairly uniform coating masses and drug content (Fig. 3). The fluorescent microscopic examination also indicated uniform distribution of the model drug triamterene. Few coating defects were observed at the endings of some stents; however, such defects have also been observed in commercially available stents (35). Thus, a

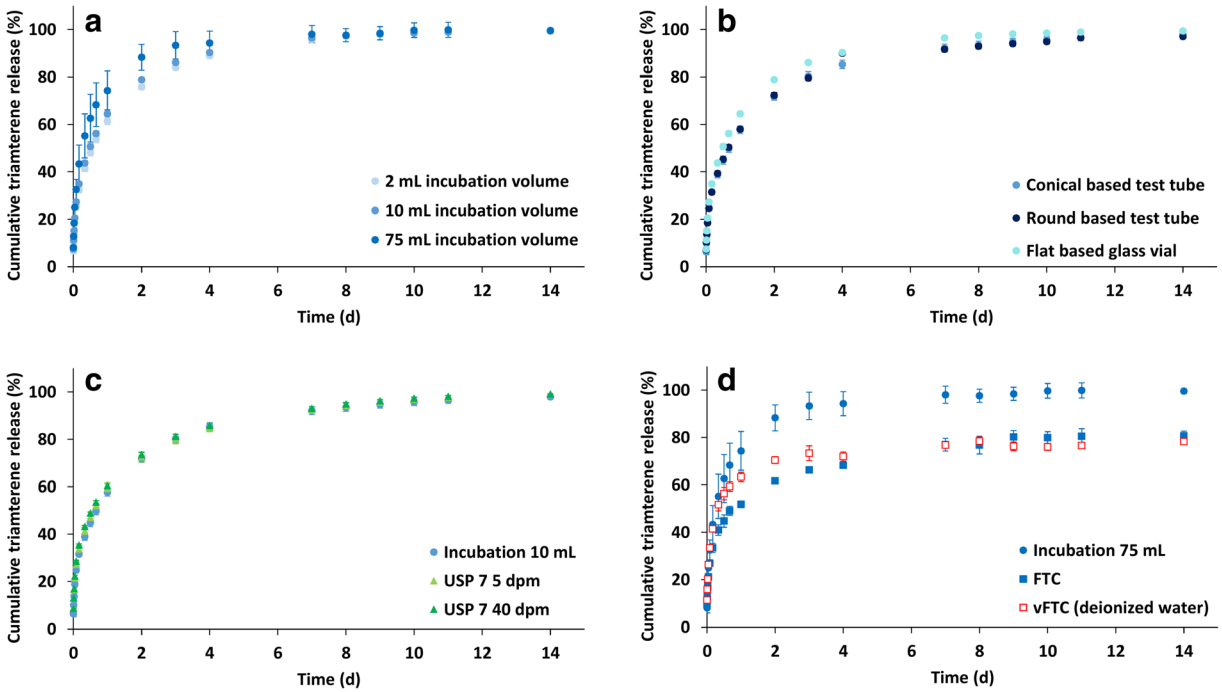

Fig. 6. Comparison of cumulative release into media obtained **a** in incubation test setups with different dissolution medium volumes carried out in 4 and 12 mL glass vials and 100 mL glass flasks, respectively; **b** in incubation test setups in different dissolution vessels with varying geometry carried out in 10 mL dissolution medium; **c** from two USP Apparatus 7 methods with a dip rate of 5 and 40 dpm compared to an incubation test setup in screw cap polypropylene test tubes with conical base and an incubation volume of 10 mL; and **d** from two flow-through methods compared to an incubation setup carried out in 100 mL glass flasks. Flow-through cells were equipped with an agarose hydrogel based on deionised water (vFTC (deionised water)) or run without a second compartment (FTC). Release is expressed as percentage of total triamterene recovery ($n = 6$, $n = 5$ for round-based vessels, $n = 3$ for flow-through methods, means ± SD)

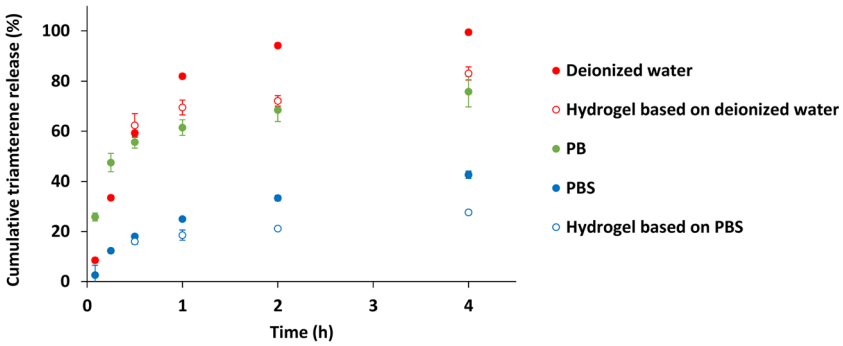

Fig. 7. Comparison of cumulative release profiles in different dissolution media (deionised water, phosphate-buffered saline (PBS) and phosphate buffer without sodium chloride (PB) as well as hydrogels based on these media) obtained in short-term experiments. Release is expressed as percentage of total triamterene recovery ($n = 3$, means ± SD)

suitable number of stents for the experiments was coated in one batch within a coating time of only 30 min, which makes the fluidised coating of stents an extremely efficient process compared to single stent coating, which is often performed.

Release of the model drug triamterene was evaluated in various test setups. Since sink conditions were maintained throughout all incubation setups, the use of different media volumes resulted in only minor differences with slightly faster dissolution in the highest volume (Fig. 6a). This could be attributed to higher mechanical stress due to the magnetic stir bar used in the 75 mL glass flask setup, which also caused deformation of the stent backbone. Furthermore, incubation in different geometric vessels as well as the use of USP Apparatus 7 with varying dip rates yielded similar dissolution profiles compared to a simple incubation (Fig. 6b, c) suggesting that the dissolution vessel geometry had no influence on the dissolution behaviour and hydrodynamic conditions in incubation setups and USP Apparatus 7 might be comparable. These findings are in contrast to the results obtained for the CYPHER™ stent (Fig. 1) where release was significantly faster in test setups using the USP 7 and considerably higher after incubation for 7 days in 2 mL compared to higher media volumes. Differences regarding the coating composition (type of polymer and drug) might explain varying susceptibility to modifications of the test setups.

However, dissolution decreased considerably using test setups based on flow-through cells (Fig. 6d), which is in accordance with previous findings for the CYPHER™ stent. One explanation might be that stents were theoretically exposed to a continuous laminar flow in contrast to turbulent conditions in all other setups possibly leading to lower shear stress (36-38) even though a fairly high flow of 35 mL/min was used in the flow-through cell. The introduction of a hydrogel based on deionised water as a second compartment was expected to result in a further decrease in triamterene release into the release medium due to a certain amount of triamterene that is released into the hydrogel. Furthermore, release from the abluminal side of the stent, where the stent is solely in contact with the hydrogel, might be considerably slower taking into consideration that distribution of the drug from this side of the stent can only occur *via* diffusion leading to non-sink conditions. However, initial release into the media was even faster and more drug had been released from the stents after 14 days when the amount released into the hydrogel was quantified (Fig. 9). This finding led to further experiments with different media and hydrogels based on different media.

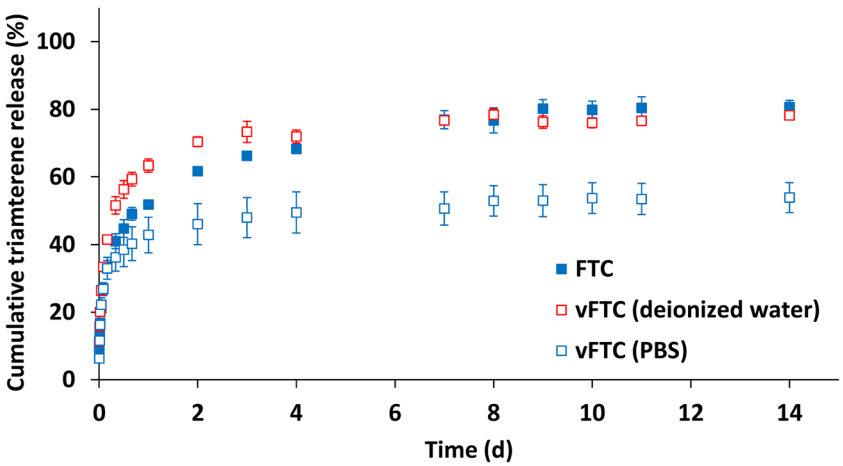

Fig. 8. Comparison of cumulative triamterene released into media obtained in different flow-through setups. Flow-through cells were equipped with agarose hydrogels based on on deionised water (vFTC (deionised water)) or PBS (vFTC (PBS)) or run without a second compartment (FTC). Release is expressed as percentage of total triamterene recovery ($n = 3$, means ± SD)

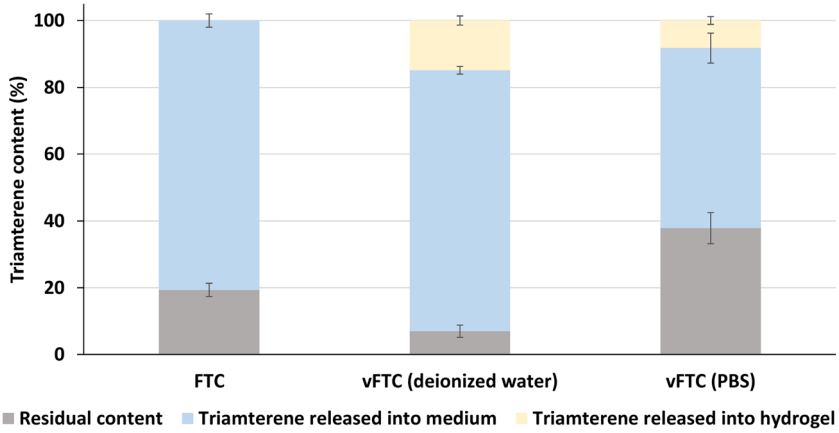

Fig. 9. Amount of triamterene recovered from stent, medium and hydrogel, respectively, after 14 days obtained in the three flow-through methods. Release is expressed as percentage of total triamterene recovery ($n = 3$, means ± SD)

In order to evaluate if differences in hydrogel composition regarding the use of buffer or deionised water as a base might be an explanation for the varying dissolution behaviour of the triamterene stents, short-term experiments were conducted in deionised water, PBS, PB and hydrogels based on deionised water and PBS (Fig. 7). As expected, dissolution in hydrogels was slower than in the stirred liquid media. This can be attributed to the additional drug transport *via* convection in the stirred liquid media, whereas drug transport in hydrogels occurs only *via* diffusion due to the concentration gradient of the drug. Additionally, the use of deionised water either as dissolution medium or as hydrogel base led to a considerably faster triamterene release than the use of PB or PBS. The differences in solubility of triamterene between the three liquid media observed in the solubility studies in this work might explain the faster dissolution in deionised water and hydrogels based on deionised water compared to PBS and PBS-based gels. Most significantly, solubility of triamterene was considerably lower in PBS than in PB and deionised water. On the one hand, this could be attributed to the "salting-out" effect of strong electrolytes like sodium chloride on organic compounds in aqueous solutions leading to increased polarity of the solvent and therefore reducing the solubility of non-polar solutes like triamterene (39, 40). On the other hand, lower solubility in PBS might be the result of a common ion effect. It is well known that hydrochloride salts usually have a low solubility in chloride containing media due to the suppression of the solubility product equilibrium (41-43). Anyway, since triamterene is a weak base with a p*K*s of 6.2 and therefore only 6% might be converted to hydrochloride salt at pH 7.4 (44), this might only slightly affect the solubility (45). The empirical Setschenow constant expresses the extent of salt effects described above on the solubility on non-electrolytes. The determined K_{salt} of 3.72 ± 0.08 in PBS indicates a "salting-out" effect as well and is in accordance with Setschenow constants previously determined for other weak bases as papaverine in sodium chloride solution (43). Since K_{salt} in PB was quite similar, phosphate ions might contribute to the "salting-out" effect as well. Solubility in water was in accordance with Dittert *et al.* (42) but was fairly higher than proposed by Watanabe *et al.* (46) who determined the solubility after a very short equilibration period. The conventional shake-flask method and the facilitated dissolution method yielded mostly similar results. Slightly higher solubility after 120 h in PB using FDM might be attributed to increased solubility in octanol overcoming poor wetting of triamterene by aqueous systems. Due to the slow distribution of solute from octanol into the aqueous phase, initial solubility after 4 h was lower using FDM compared to the shake-flask method (Fig. 5).

However, decreased dissolution rate in PBS compared to deionised water may also be explained considering the coating polymer Eudragit® RS 30 D. Permeability of this polymer is controlled by anion exchange as suggested by Wager *et al.* (47): Chloride as the counter ion of the quaternary ammonium groups (QAG) in the insoluble Eudragit® RS is replaced by anions from the dissolution medium dependent on the attraction of the anions to the QAG (Fig. 10). As anions are exchanged, a water flux is induced due to the oscillation of the anions resulting in the release of the incorporated drug.

In media with high chloride content, the equilibrium is shifted to the left side of the reaction. Therefore, exchange of chloride anions is reduced and the water flux and the drug

Table V. Kinetic Parameters of Fitted Release Curves in Two Different Test Setups

Test setup	First order		Higuchi		Korsmeyer–Peppas		
	r^2	k_1 (day^{-1})	r^2	k_H ($day^{-0.5}$)	r^2	n	k_{KP} (d^{-n})
Incubation in screw cap polypropylene test tubes with conical base	0.9380	0.7686	0.9847	0.5369	0.9958	0.3863	0.6017
Flow-through cell	0.8707	0.6315	0.9593	0.4626	0.9955	0.3115	0.5586

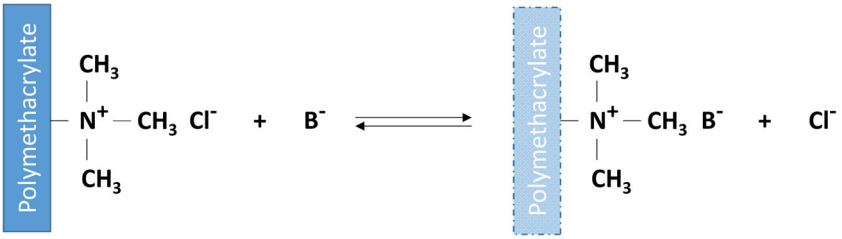

Fig. 10. Ion exchange at the polymers quaternary ammonium group in dissolution medium controlling the permeability of Eudragit® RS (B^- = buffer anion)

release decreased accordingly. Hence, triamterene release from Eudragit® RS 30 D was considerably faster in deionised water than in PBS which is characterised by a high chloride concentration. These findings are in agreement with literature data for tablets and beads coated with Eudragit® RS (48, 49).

For the CYPHER™ stent (20), the use of media based on PBS had been evaluated in a preliminary experiment to exclude the influence of the different osmolalities (data not shown), but no difference was observed compared to media based on deionised water. However, since sirolimus contains no functional groups that are ionisable in the pH range of 1–10 (50) and the polymers used in the CYPHER™ stent are PEVA and PBMA, differences between release in PBS and deionised water were not to be expected.

As a consequence of these findings, dissolution was again carried out using the vFTC, but with a hydrogel based on PBS. Figure 8 depicts that by the use of a hydrogel based on PBS, all expectations were met. Release of triamterene into the medium was considerably slower than in the vFTC based on deionised water and the FTC. Moreover, the amount of triamterene recovered from the hydrogel based on PBS was smaller than from the one based on deionised water (Fig. 9) due to the lower solubility of triamterene in PBS, which is consistent with the results depicted in Fig. 7. Therefore, residual amount of triamterene on the stent was highest using the vFTC with a hydrogel based on PBS.

Finally, comparing the results obtained in the different dissolution test setups, it can be concluded that simple test setups such as incubation and even the highly standardised USP Apparatus 7 might overestimate the release of hydrophobic drugs from stent coatings *in vivo*. The use of flow-through setups and especially the introduction of a hydrogel as a second compartment mimicking the tissue in which the stent is implanted might be a feasible approach to establish a more biorelevant dissolution test setup. Depending on the coating polymer and solubility of the drug, the composition of the hydrogel should be chosen carefully since it can highly influence the dissolution rate as well. Ideally, the composition of the hydrogel should be adapted to provide more biorelevant conditions and to simulate the situation *in vivo*. First attempts in this regard have already been described in the literature (51). Additionally, further studies should be conducted with different coating formulations in particular containing anti-proliferative agents as sirolimus in order to evaluate if the biorelevant dissolution test setups are discriminative as well. Since marketed stents as the CYPHER™ stent are known to release the drug over a period of up to 90 days (52), the duration of the dissolution tests must be extended to ensure complete dissolution of the drug or critical quality attributes must be defined.

CONCLUSION

Triamterene-coated model drug-eluting stents for *in vitro* testing were produced *via* fluidised-bed technology. A smooth coating surface and high coating uniformity with homogeneous triamterene distribution were achieved. The results indicate the excellent suitability of the fluidised-bed technology for the coating of DES.

For *in vitro* testing, different incubation setups with varying dissolution medium volumes and vessel geometry as well as the USP Apparatus 7 and two flow-through methods were used. In contrast to previous studies (20), an influence of the dissolution medium volume on the *in vitro* dissolution behaviour of DES could not be observed. Furthermore, no dependency on the vessel geometry and no difference between release in incubation setups and the USP Apparatus 7 were determined. Slightly lower release occurred in the FTC, whereas dissolution was faster when including a hydrogel compartment. It was found that this unexpected finding was related to the use of deionised water as the base for the hydrogel. Dissolution decreased considerably using the vFTC with a hydrogel based on PBS. The approach of including a hydrogel compartment might be considered as more biorelevant, whereas other test setups possibly overestimate drug release from DES based on the physiological situation. However, there is no *in vivo* data available supporting this assumption. Nevertheless, as some findings are in contrast to those obtained in previous studies, general recommendations regarding the selection of a suitable *in vitro* test setup for each DES cannot be given.

In addition, the results also show an influence of the composition of the hydrogel used in vFTC on the dissolution behaviour of the investigated DES. Since this might be attributed to the coating polymer Eudragit® RS 30D as well as the model drug triamterene, special emphasis on drug solubility in the dissolution medium and interactions of the coating polymer of DES should be taken when considering an *in vitro* dissolution setup.

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