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Evaluation of ion exchange processes in drug-eluting embolization beads by use of an improved flow-through elution method

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ABSTRACT

An improved method for evaluating drug release behaviour of drug-eluting embolization beads (DEBs) was developed utilizing an open-loop flow-through system, in which the beads were packed into an occlusive mass within the system and extracted with a flowing elution medium over time. Glass beads were introduced into the beads mass in order to ensure laminar flow, reduce dead volume and improve reproducibility by compensating for swelling phenomena. The effects of glass bead ratio, elution medium flow rate and ion concentration, DEB size and drug concentration and drug type (doxorubicin and irinotecan) were evaluated using DEB composed of a sulfonate-modified polyvinyl alcohol hydrogel (DC Bead™) as the test article. The rate and amount of drug elution from the packed beads was affected by flow rate, the bead size and initial loading dose. The raw data from the concentration profile analysis provided valuable information to reveal the drug elution behaviour akin to the pharmacokinetic data observed for embolized beads (yielding *in vitro* C_{max} and t_{max} data) which was complementary to the normal cumulative data obtained. A good correlation with historical reported *in vivo* data validated the usefulness of the method for predicting *in vivo* drug elution behaviour.

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1. Introduction

One of the most important characteristics of a novel drug-eluting system is the kinetics of the release of the active pharmaceutical ingredient (API) from the matrix or reservoir in which it is contained. A consistent, reliable and robust method carried out under controlled laboratory conditions is critical for both the development and quality control of such products. It will allow for comparative performance assessments across other similar formulations for product optimization, bench-marking purposes or bioequivalence arguments, for instance, in addition to providing key *in vitro* release testing (IVRT) data that are required for release of product for sale (Brown et al., 2011). Such testing involves subjecting the drug-eluting system to carefully controlled conditions that will facilitate drug release, with subsequent monitoring and measurement of the elution over time to obtain kinetics of the release profile and often a total amount released.

When modelling how the system may perform in the body, it is necessary to attempt to select the *in vitro* conditions that best simulate the *in vivo* environment in which the system will be located. This will vary considerably depending upon the route of administration and will need very different conditions when evaluating topical, oral, parenteral and implantable release systems for example. When being used for

quality control purposes, the method can be used to assess conformance of the system to a product specification and the stability of API release over time (Anand et al., 2011). In this case therefore, it is essential that the test conditions are selected in order to be capable of demonstrating any deviations of API elution profile from that expected for a product conforming to specification, which could be a consequence and indicative of a change in the drug-eluting system. The method is therefore an essential test for the evaluation of a system that releases API and is known as dissolution testing for oral formulations, but in recent years is also referred to as IVRT or drug elution testing for the less conventional drug delivery systems, such as drug-device combination products (Sree Lakshmi and Badarinath, 2013).

Drug-eluting embolization beads have been in clinical use for over a decade now (Lewis et al., 2006), with several types commercially available for the treatment of hypervascular tumors by occlusion of the blood supply coupled with a sustained controlled locoregional drug release (Liapi et al., 2007; Malagari, 2008; Nicolini et al., 2011). These products are made from a variety of different polymer microspheres with the ability to load and release certain drugs, usually by an ion-exchange mechanism (Anand et al., 2001; Lewis et al., 2007). The different chemical composition of the matrices means that these products have different drug loading capacities and elution kinetics which therefore translate into differences in drug pharmacokinetics and bioavailability parameters and hence may impact on their clinical performance. It is therefore becoming ever-more important to have drug elution methods

that will allow direct comparison between products, in addition to better predicting the *in vivo* performance of these systems. This is not straightforward however, as physicochemical differences in the properties of the products, such as size, morphology and density can impact on their behaviour during analysis making direct comparison in performance problematic.

The IVRT of implantable drug-device combination products can be evaluated by immersing the system, usually with stirring, in a suitable elution medium (sometimes modified with additives to help in the solubilization of particularly low-solubility drugs). The medium may be exchanged periodically to prevent saturation with drug, and is sampled periodically for analysis determination of the elution kinetics. This type of experiment can be performed with USP type II apparatus or similar, which is well-established in regulatory guidelines and the US Pharmacopeia (Chapter <711>). As an alternative to exchange of the elution medium, it may instead be circulated around or through the system depending upon its configuration (*i.e.* using a flow-through apparatus, such as a USP type IV apparatus) (Andhariya and Burgess, 2016). Drug-eluting beads have been evaluated using both of these methods; the former being more useful for quality control purposes but inappropriate for *in vivo* correlation (Gonzalez et al., 2008), whereas the latter was unable to demonstrate complete drug elution from the systems under study due to experimental limitations (Jordan et al., 2010). A T-apparatus was proposed as an alternative method that better emulates the embolization environment by provision of diffusion and convection zones representing drug diffusion from the beads through the vessel wall and surrounding tissues and then its removal in blood flowing through more distant patent vessels (Amyot et al., 2002). This method has proved useful in predicting the first 24 h of drug release into the systemic circulation, producing level A *in vitro: in vivo* correlations (IVIVC) (Gonzalez et al., 2008). The method is, however, cumbersome and not without technical limitations. Although flow-through methods have been used in the study of doxorubicin elution from ion exchange microspheres (Willmott et al., 1985; Cremers et al., 1990), detailed studies based on the factors such as flow rate, drug type and loading dose *etc.* on the mechanistic evaluation of DEB characteristics are still lacking. Moreover, there is no study of the correlation between the *in vitro* elution modelled using flow-through methods and *in vivo* drug release data. Herein we report on the development of an improved elution method based on open loop flow-through mechanism for evaluation of DEBs which overcomes some of the short-comings of previously-reported methods and will allow a better comparison between the performances of different products despite their differing characteristics.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride (Dox, Hisun, China) and irinotecan hydrochloride (Iri, ScinoPharm, China Taiwan) were obtained as pure powders (>99% purity) and dissolved in deionised water at the desired concentration to obtain stock solutions for bead loading experiments. Hydrogel beads used in the elution were DC Bead™ (Biocompatibles UK Ltd., a BTG International group company, Farnham, UK). Glass beads with size range 150–220 μm used in the experiment were purchased from Sigma. Phosphate buffered serology saline was supplied by Source BioScience (UK) and degassed using helium (BOC, UK) prior to use.

2.2. Drug loading method

Samples of the beads under study (DC Bead™: 70–150 μm, 100–300 μm, 300–500 μm and 500–700 μm size ranges) were loaded with a target dose of the desired drug. The bead samples were first transferred from their product vials to a 10 mL measuring cylinder. 1 mL of beads was measured into a 10 mL glass vial. After as much residual

packing solution was removed as possible from the beads by using a pipette with a cotton filter on its tip, drug loading was then initiated by the addition of the drug solution at the desired concentration. The actual amount to be added was calculated based on the measured concentration of the drug loading solutions and pipetted accurately into the vial. The vial was gently agitated several times during loading and complete loading (>99%) was confirmed by UV–Visible spectrophotometric analysis of the loading solution.

2.3. Drug elution method development and set-up

Drug-loaded bead samples prepared in Section 2.2 were evaluated using an open loop flow-through system as depicted in Fig. 1. This system consists of PBS stock, a peristaltic pump (ISMATEC, Germany) with silicon tubing (ID 0.094″, OD 0.156″, Cole-Parmer, USA), a flow-through elution cell (ID 14 mm, OD 20 mm, length 125 mm), a water bath with temperature control unit (HAAKE SC100, Thermo Scientific, USA), a Varian Cary® 50 UV–Vis spectrophotometer (Agilent Technologies, Australia), and waste collection. The glass beads (150–212 μm, Sigma, USA) and drug loaded beads were mixed uniformly and sandwiched between two filter membranes (pore size 27 μm, SeFar Medifab, UK) in the elution cell. As discussed in Section 3, the introduction of glass beads is in order to create some interstitial space between beads with the aim of separating and suspending beads as well as allowing for any size changes during elution to be accommodated. In the case without glass beads, the drug-loaded beads were directly placed between the filter membranes with a 6–7 mm gap allowing beads swelling. Deionised water was used here as packing medium to avoid pre drug leaching, and during bead packing, air bubbles were carefully avoided from being introduced into the system. Further measures were adapted by using helium-sparged PBS and one more layer filter to stop any air bubble entering the elution cell.

After assembling the system, the PBS was pumped through the elution cell at 37 °C by using selected speeds between 0.5 and 45 mL/min. The eluted drug was passed through UV spectrophotometer, and monitored at 483 nm for Dox and 369 nm for Iri, respectively. Due to the high extinction coefficient of Iri and its relatively high initial rate of elution, eluted irinotecan was passed through a quartz cuvette (Hellma Analytics, Germany) with 0.50 mm path length (Fig. 1) for concentration measurement.

2.4. Doxorubicin elution through a packed bead column along the direction of flow

The study of doxorubicin distribution during flow-through elution was carried out by packing 0.6 mL of beads containing 37.5 mg/mL doxorubicin into three silicon tubes with a diameter of 3 mm and length of 150 mm, respectively. The initial packed length of drug loaded beads was 4.4 cm. The end of the silicon tubing was installed with a 10 μm filter to hold the beads during the elution. The bead packed tubing was immersed into 37 °C water bath and saline was driven through it using a peristaltic pump. At different predetermined time points, the tubes were sequentially sectioned into four equal parts according to the total length of the packed section using a sharp blade. The sectioned beads were transferred into a pipette column and extracted by DMSO-NaCl solution (NaCl concentration 1%). The extracting solution was diluted with deionised water and measured by UV spectrophotometry at 483 nm. The data were compared to a standard curve obtained under the same conditions to obtain the drug concentration in beads within each section.

The data collected were processed using Excel. Firstly the absorbance data were converted to concentration by comparing to the standard solution. Then the area under curve (AUC) and cumulative amount of drug eluted were calculated against time in minutes. The average elution curve was drawn by taking the mean of three replicate
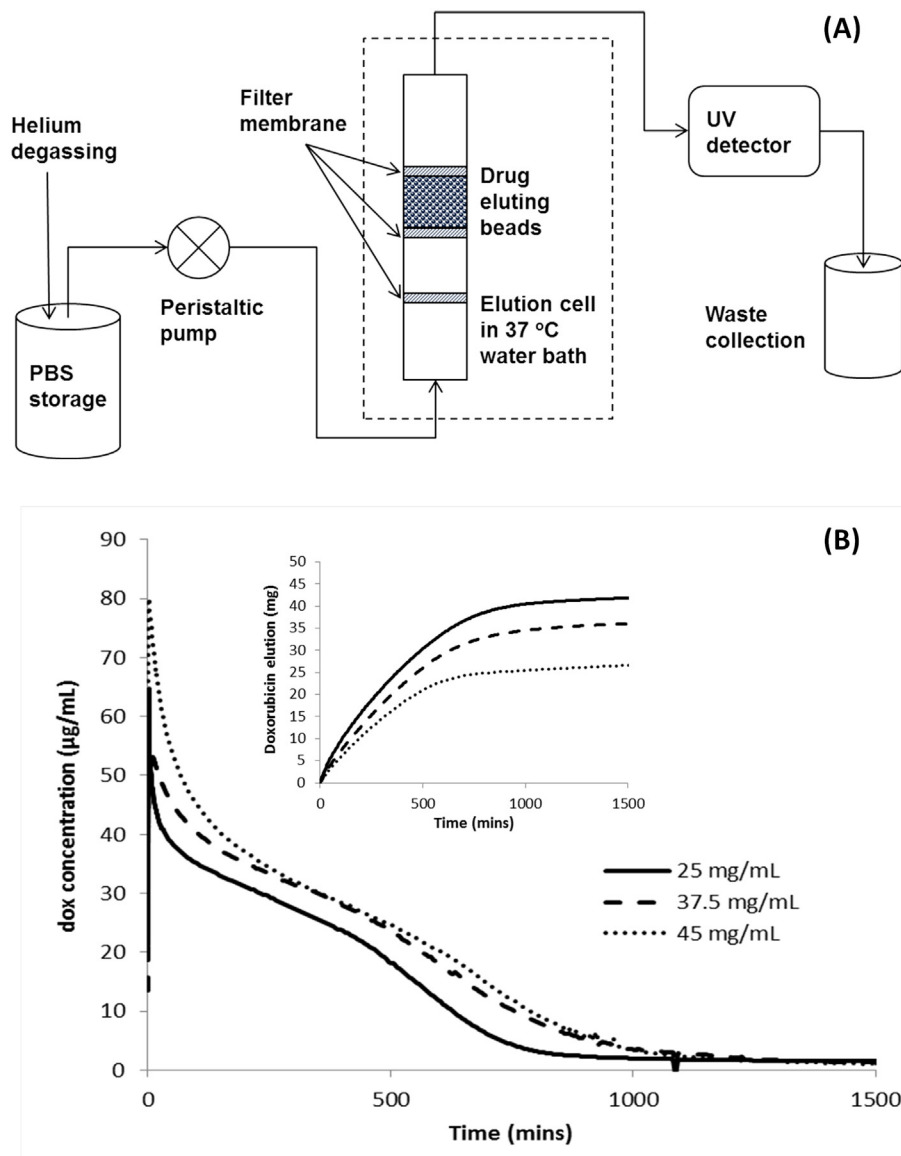
Fig. 1. Experimental set up of the open-loop drug elution method (A) and typical concentration curves and cumulative elution data generated through processing UV measurement *vs* time (B).

measurements, and error bars were generated from calculation of the standard deviation.

3. Results

3.1. Overcoming swelling artefacts by dilution of the drug-eluting bead embolus with glass beads and the effect of drug-eluting bead:glass bead ratio

Flow-through drug elution devices have been designed to either hold the drug particles on a support matrix, such as a typical USP 4 device to allow drug particles or carriers placed on a bed of glass beads, or allow particles floating in liquid phase (Jordan et al., 2010; Wähling et al., 2011), or particles contained in a dialysis adapter (Upkar Bhardwaj, 2010). Both ways could lead to situations where drug particles are able to move around in the elution device due to particle size or density change, thus compromising the reproducibility of test results. In the geometry of the current flow-through device, the diameter of the sample holding tube was fixed as 14 mm, and the position of the bed of beads was placed in one third of the tube. The focus of the study was to examine the effect of the embolic type, size, packing with glass beads, drug dose, and elution medium flow rate on drug elution. 1 mL of

doxorubicin-loaded DC Bead™ was initially placed on a filter membrane allowing flow of PBS medium around and between the beads to facilitate ion exchange. A second filter membrane was placed on the top of the beads with about a 6–7 mm gap. The recorded drug concentration profile is shown in Fig. 2 (dash-dotted line). The resultant elution curve was irregular and difficult to reproduce as a consequence of the bed of packed beads being constantly disrupted under the flow conditions. Moreover, as the elution process began the beads started to swell and dimensional instability was introduced as the packed volume increased, a consequence of the decreased drug-bead interaction as drug is lost from the system (Lewis et al., 2007). In order to minimize these fluctuations in the elution curves, 3 and 5 mL of glass beads were uniformly mixed with 1 mL of drug eluting beads, and the top filter membrane was compressed in order to sandwich the bed of mixed beads, fixing it in position throughout whole elution period whilst maintaining the flow of elution medium despite swelling effects. Furthermore, the introduction of glass spheres appeared to reduce the likelihood of gas bubble generation in the elution cell. Fig. 2 shows the resultant effect with much smoother and reproducible curves obtained (dotted line and solid line represent the mixture with 3 mL and 5 mL glass beads, respectively). A 5 mL volume of glass beads was selected
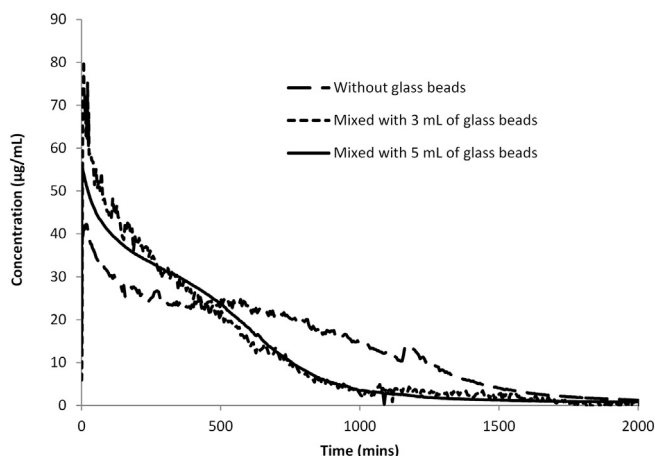
Fig. 2. Elution of doxorubicin from DC Bead™ mixed with and without glass beads at different ratios. Conditions: 1 mL of hydrogel beads (100–300 μm) loaded with 37.5 mg/mL of doxorubicin, glass bead size: 160–220 um, PBS elution medium, flow rate: 1.4–1.6 mL/min, 37 °C.

Table 1
Parameters of doxorubicin elution of DEB under different flow rate and salt concentration.

Test condition					
Flow rate (mL/min)	NaCl concentration (%)	C_{max} (μg/mL)	t_{max} (min)	$t_{50\%}$ (min)	k ($\times 10^{-3}$ min^{-1})
0.5	0.9[a]	47.5 ± 2.1	22.0	1099	0.65 ± 0.02
1	0.9[a]	46.5 ± 6.3	6.0	538	1.24 ± 0.13
2	0.9[a]	49.6 ± 10.9	8.0	258	2.79 ± 0.06
5	0.9[a]	39.6 ± 3.3	5.0	137	5.72 ± 0.05
10	0.9[a]	31.8 ± 1.9	3.0	94	7.27 ± 0.39
20	0.9[a]	28.2 ± 3.5	2.0	64	10.55 ± 1.51
45	0.9[a]	14.7 ± 0.7	2.1	47	14.58 ± 3.76
2	0.45[b]	16.5 ± 2.3	8.0	568	1.18 ± 0.07
2	0.9[b]	46.6 ± 3.0	9.0	155	4.82 ± 0.67
2	1.8[b]	65.2 ± 3.9	10.0	87	7.71 ± 0.46
2	4.5[b]	83.5 ± 1.9	4.0	77	8.50 ± 0.34

[a] 1 mL of DEB mixed with 5 mL of glass beads.
[b] The salt concentration tests were carried out by using 0.5 mL of DEB with 2.5 mL of glass beads.

for all further experiments to create a more even distribution of beads and compensate the swelling effect.

3.2. Influence of Eluent Flow Rate on Drug Elution Characteristics and its Correlation with Salt Concentration

Flow rate of the elution medium is an important parameter in the evaluation of drug release from embolics and in understanding the elution mechanism. Fig. 3A shows the profiles of doxorubicin concentration recorded by UV at 483 nm under different flow rates. From the figure, change in doxorubicin concentration followed a similar trend in which an initial burst occurred by reaching the maximum in a short period (donated here as the experimental C_{max}), followed by a slower decrease with time. Table 1 lists the C_{max} values which are around 46

to 50 μg/mL at low flow rate tests (0.5–2 mL/min), whilst increasing flow rate from 5 to 45 mL/min resulted in a C_{max} decrease from ~40–14 μg/mL. The experimental t_{max} (time to reach C_{max}) decreased with increasing flow rate, i.e. a longer time was required to reach peak concentration at a lower flow rates (22 min observed at 0.5 mL/min, but decreasing gradually from 10 to 2 min as flow rate was increased). Elution curves generated at low flow rates displayed slower decrease in drug concentration, whilst the higher flow rates displayed a more rapid drop in concentration.

Fig. 3B shows the calculated cumulative doxorubicin elution based on the data shown in Fig. 3A. When flow rate increased from 0.5 mL/min to 45 mL/min, the drug elution rate also increased rapidly in accordance. Under low flow rates, drug elution took 112.8 h to achieve 97% of total elution at 0.5 mL/min, and 71.1 h to achieve 99% at 1 mL/min; whereas at the highest flow rate of 45 mL/min, 100% of drug elution was achieved within 6.5 h. A comparison of flow rate impact on elution is also given in Table 1 by using the time for 50% total dose eluted ($t_{50\%}$).
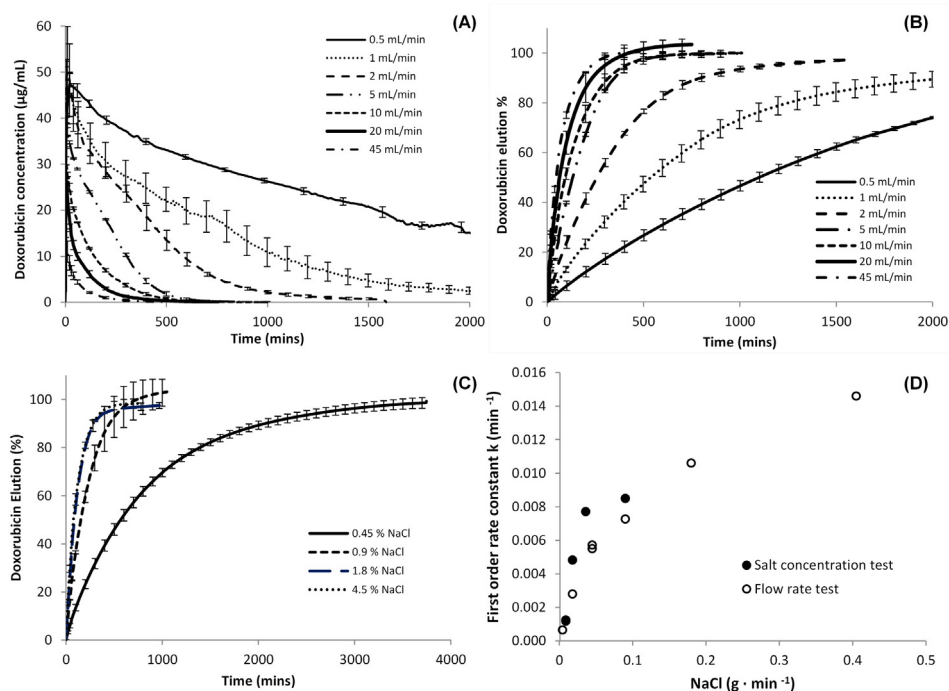

Fig. 3. The effect of flow rate and elution medium salt concentration on doxorubicin elution from 100 to 300 μm DC Bead™. (A) PBS flow rate of 0.5, 1, 2, 5, 10, 20, 45 mL/min on drug elution, NaCl concentration 0.9%. (B) Concentration profiles of doxorubicin elution under different flow rate listed in (A). (C) NaCl concentration in 0.45%, 0.9%, 1.8% and 4.5% in PBS elution medium, flow rate 2 mL/min. (D) The correlation between first order rate constant k and the flow of NaCl quantity passed through the bed of beads. The k values could be extrapolated to a linear fit for low flow rate data.

It shows that under the same salt concentration conditions, $t_{50\%}$ values decreased (from about 1099 min to 47 min) with increasing flow rate. Although a lower drug concentration was measured in the faster compared to slower flow rate tests, the flux of doxorubicin released through the bed of packed beads was much higher under the fast flow rate, according to the elution profiles. Doxorubicin cumulative release profiles generated from experiments of different salt concentrations are shown in Fig. 3C, which is intended to demonstrate the salt effect on drug release under a fixed flow rate of 2 mL/min. It appears that the drug elution rate increased as the NaCl concentration in the elution medium was increased from 0.45% to 4.5%.

To describe the drug elution property in a packed column, the cumulative doxorubicin elution was fitted with a simple and empirical first order rate law, which is given below.

$$\frac{M_{Drugeluted}}{M_{Totaldrugloaded}} = 1 - e^{-kt} \tag{1}$$

Here k is a rate constant with unit min^{-1}, which is determined by the bead and drug intrinsic properties and experimental conditions, and t is time in minutes. The fit of the doxorubicin elution profiles shown in Fig. 3B and C are provided in the supplementary information (Fig. S1 and S2). From the fitting, Eq. (1) well described the time related drug elution in the current tests with all the correlation coefficients above 0.998; values of k were obtained, which were related to the conditions of mass transfer in the ion exchange process.

To build the correlation between the effect of flow rate and NaCl concentration on drug elution, the flow of NaCl quantity per unit time was calculated for each elution test in both Figs. 3B and C by multiplying the medium flow rate with the salt concentration (%) and medium density ($Mass_{NaCl} = Flow\ rate \times Density_{medium} \times Concentration_{NaCl}$). Fig. 3D shows the plot of k values against the flow of NaCl quantities. The data points obtained from the flow and salt concentration tests basically follow the same trend, a reflection that the same mechanism of ion exchange is in operation. This plot also suggests that the increasing flow rate of the elution medium is equivalent to increasing of salt concentration flowing through the bed of beads.

3.3. Effect of drug-eluting bead size range on elution

Fig. 4 shows the results of a series of doxorubicin elution studies using the flow-through model packed with the beads of different sizes, 70–150 μm, 100–300 μm, 300–500 μm and 500–700 μm. The insert in Fig. 4A is the initial 50 min elution profiles. The elution from the smallest size range, 70–150 μm, had the highest C_{max} 58.9 ± 8.4 μg/mL, which decreased with increasing bead diameter: 100–300 μm = 46.6 ± 3.0 μg/mL; 300–500 μm = 30.9 ± 1.2 μg/mL; 500–700 μm = 27.0 ± 0.8 μg/mL. It follows the trend of beads of smaller average diameter having faster drug elution rate than the larger sized beads, due to the increased surface area to volume ratio for small beads which facilitates more efficient ion exchange. This was further evidenced by the relatively higher doxorubicin concentration of larger size beads after 300 min. Fig. 4B shows the cumulative doxorubicin elution with smaller beads having the faster elution rate as for the obeservation in Fig. 4A.

3.4. Effect of doxorubicin dose on elution

Different doses of doxorubicin were loaded into the same volume of beads, and the dose effect on drug elution in the flow through model was examined. Fig. 5 shows the doxorubicin elution profiles of two doses, 25 and 37.5 mg/mL, of beads. The flow through model test indicates that the concentration of 37.5 mg/mL dose drug was always higher than the low dose beads, and the amount of eluted drug from high dose beads was more than the low dose throughout the whole period of elution. It is known that the doxorubicin loading into DC Bead™ can cause bead volume shrinkage due to the interaction between doxorubicin stacking and increased bead hydrophobicity (Lewis et al., 2006; Lewis et al., 2007). In this test the loaded bead volume for 25 and 37.5 mg/ mL dose is about 0.7 and 0.6 mL, respectively. Under the current test conditions therefore, the result suggests that the volume difference is negligible and the dose effect is the predominant factor.

3.5. Evaluation of irinotecan drug-eluting bead by the flow-through method

In addition to the loading and elution of doxorubicin into DC Bead™ for treating primary liver cancer, irinotecan hydrochloride can alternatively be loaded and eluted following the same ion-exchange mechanism as doxorubicin, as is adopted in the clinic for treating colorectal cancer metastases to the liver (Aliberti et al., 2006; Fiorentini et al., 2012; Martin et al., 2011; Taylor et al., 2007; Vogl et al., 2009). Fig. 6 shows the irinotecan elution profiles of two samples, 70–150 μm and 100–300 μm (0.5 mL each) loaded with 25 mg of irinotecan, and one sample of 100–300 μm beads (1 mL) loaded with 50 mg of drug. Compared to the doxorubicin, irinotecan elution was much quicker, eluting the loaded amount within about 2 h. The C_{max} of the 0.5 mL of 70–150 μm and 100–300 μm size beads are 926.8 ± 76.3 μg/mL and 887.1 ± 41.4 μg/mL, respectively, which appeared at almost the same time point around 9 min (Fig. 6A). The C_{max} of 50 mg dose beads was 1071 ± 38.1 μg/mL at 10 min, and the drug concentration was higher than the 25 mg dose samples, similar to the doxorubicin elution observed in Section 3.5. A shoulder before the drug peak was observed in the irinotecan elution profiles. The area corresponds to 7.8% and 4.3% of the total dose in 70–150 μm and 100–300 μm beads, respectively. It was considered to be due to either residual irinotecan in the solution after loading or a pre-displacement of loaded irinotecan by the
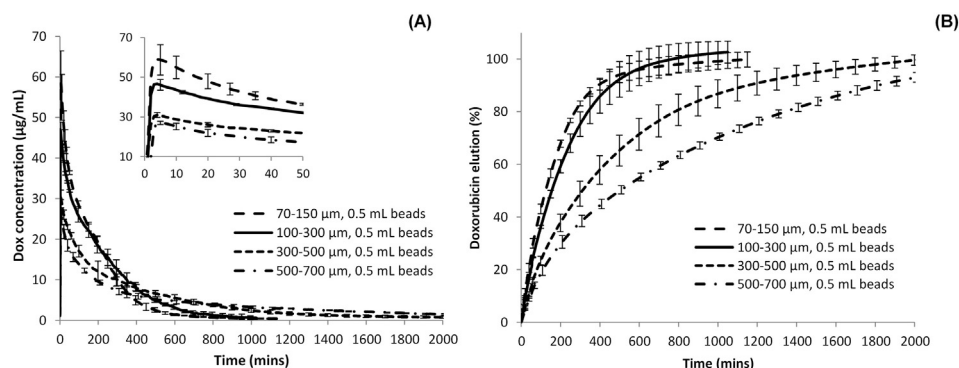

Fig. 4. Profiles of doxorubicin elution from loaded beads (37.5 mg/mL dose) of different size ranges. (A) Concentration profiles *vs* time. The insert is an enlargement of the initial phase to show the maximum peak concentration and time; (B) cumulative elution profiles. Elution condition: 0.5 mL of beads used for loading doxorubicin to achieve 37.5 mg/mL dose, PBS as elution media, flow rate 2 mL/min, 37 °C.
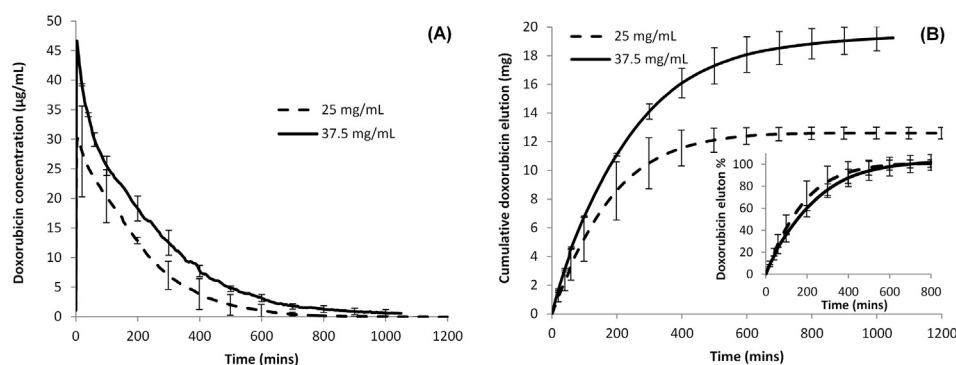
Fig. 5. Comparison of doxorubicin elution from 100 to 300 μm beads with different loading dose, 25 and 37.5 mg/mL mixed with 2.5 mL of glass beads, respectively. (A) Concentration profile. (B) Cumulative doxorubicin elution in mass *vs* time. The insert is elution in percentage. Condition: glass bead size range in 100–200 μm and packed in a flow-through tube. Flow rate was 2 mL/min at 37 °C.

front of saline and deionised water mixture. UV spectrophotometric analysis of the solution of deionised water washed beads were consistent with the aforementioned levels.

Fig. 6B is the cumulative elution of irinotecan, in which the two elution curves with 25 mg dose were very similar, although the 70–150 μm beads eluted slightly faster than 100–300 μm beads. The 50 mg dose beads eluted slower compared to the 25 mg 100–300 μm beads. The very slow elution after 30 min is due to the way in which a packed column of beads allows for a process of drug absorption and desorption to occur along the flow direction of the column. The drug residue left at the top of the column corresponds to the later stage of the elution curve.

3.6. Doxorubicin elution through a packed bead column

Understanding doxorubicin transport in a packed column during the ion exchange process is of importance for the evaluation of DEB using flow-through methodologies, as it may provide insight into the mechanisms of release within blood vessels. Fig. 7 shows the set up for studying doxorubicin redistribution during the flow-through elution process (see the insert), and the doxorubicin concentration measured at different time points of the elution. As seen in Fig. 7, the initial drug concentration in the beads was 37.5 mg/mL (denoted by dashed line). At 25 min the drug concentration along the flow direction in each section were 25.9, 31.1, 34.8, and 40.8 mg/mL, and the difference between section 1 and 4 was 14.9 mg/mL. At 75 and 150 min, the difference between section 1 and 4 became larger, increased to 24.8 and 48.3 mg/mL. Visually the beads in the front section became less red and appeared a more bluish colour, the original colour of unloaded DC Bead™.
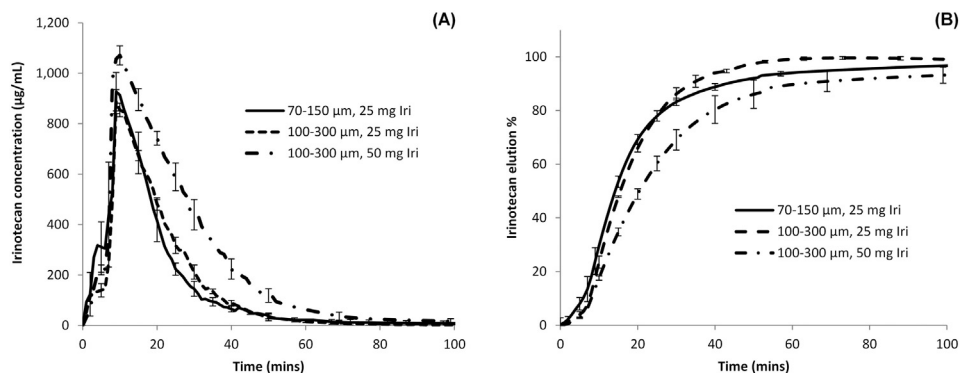
3.7. Correlation of the release kinetics with historic in vivo pharmacokinetic data in vitro-in vivo correlation (IVIVC)

Given the elution curves for doxorubicin generated under different experimental conditions in Sections 3.1–3.5, it is possible to make an attempt to correlate these release kinetic data with those described in a porcine hepatic artery embolization model in which residual drug remaining in two sizes of DC Bead™ was determined after 28 and 90 days implantation (Namur et al., 2010). This provides three data points (T = 0, 672 and 2160 h) that can be overlaid onto the extrapolated release curves based on the first order rate constant model. To estimate a flow rate matching *in vivo* data, curves of residual doxorubicin in beads *vs* time were drawn, including the *in vivo* data points of doxorubicin residue in 100–300 μm (square) and 700–900 μm (circle) beads reported in the work of Namur et al. (2010). Then under the assumption that first order rate law (1) still valid at very low flow rate, a dash-double dotted line was calculated by searching a k value using Eq. (1) to give best fit of the *in vivo* data points. In this case the k was 1.5×10^{-5} min^{-1}, and a corresponding flow rate was obtained as 0.01 mL/min, according to the correlation between the k values and low flow rate range (0.5 to 10 mL/min) in Table 1. Fig. 8 shows a plot of the elution data under different flow rates and predicts that *in vivo* elution could be emulated in this model using a flow rate of 0.01 mL/min. It is obviously not practical to carry out a 90 day experiment of this kind in this model but it is extremely useful and informative to know that by selecting a higher flow rate, accelerated elution can be achieved that is representative of the ion-exchange processes that are occurring when the beads are implanted within the liver.

Fig. 6. Irinotecan elution profiles of 70–150 μm and 100–300 μm beads loaded with drug 50 mg/mL drug (A) concentration profile and (B) cumulate amount. Solid line and dashed line were using 0.5 mL of beads with dose 25 mg, dash-dotted line was using 1 mL of beads with dose 50 mg. 2.5 mL of glass beads were used, and medium flow rate 1.6 mL/min, 37 °C.
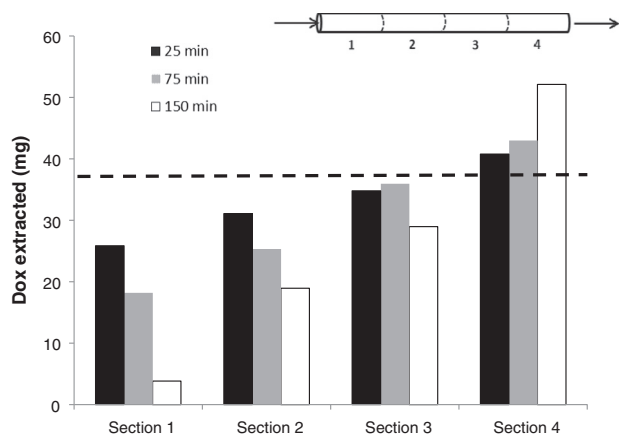
Fig. 7. Change of doxorubicin distribution in a column of 100–300 μm beads during elution. The dashed line denoted as the initial doxorubicin loading, 37.5 mg/mL. Conditions: flow rate 1.5 mL/min, saline mobile phase, 37 °C.

4. Discussion

Flow-through systems have been used extensively to evaluate different dosage forms. The flow- through device has demonstrated advantages in extended release studies under sink condition and for developing *in vitro-in vivo* correlations, by overcoming the limitations of the USP 2 type elution apparatus (D'Souza and DeLuca, 2006). The USP apparatus 4 has been used to study the release of dexamethasone from poly (lactic-*co*-glycolic acid) (PLGA) microspheres mixed with glass beads for providing laminar flow and reduce dead volume (Andhariya and Burgess, 2016; Zolnik et al., 2005) but still there is a need for flow through systems designed for testing dosage forms under a controlled hydrodynamic environment and with intraluminal hydrodynamics (Fotaki, 2011; Kakhi, 2009). Early pioneering studies have demonstrated the use of a flow-through system to study adriamycin release based on an ion-exchange mechanism by fixing microspheres on either a bed of glass beads or glass wool packed in a column (Willmott et al., 1985). In these methods, repositioning or redistribution of microspheres in the flow through cell were the main cause of compromised reproducibility (Wähling et al., 2011). Some modifications in the current study were made to address the release characteristics of beads under embolization from both a quality control (QC) assessment and *in vivo* mimicking perspective. The changes
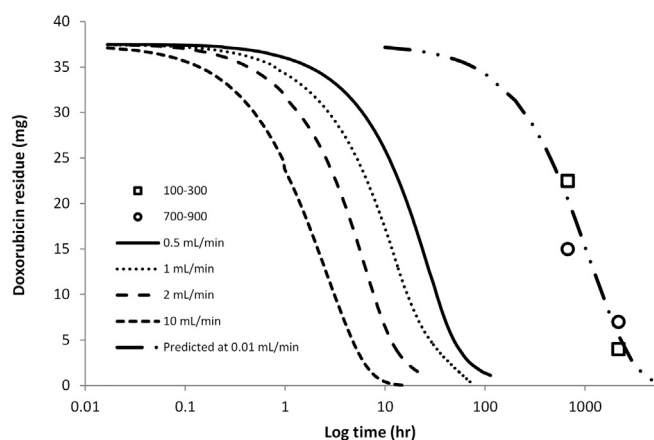

Fig. 8. Calculation to match literature *in vivo* data based on the extrapolated first order rate constant from different flow rate test. The 0.5 (solid line), 1 (small dot), 2 (long dashed) and 10 mL/min (short dashed) curves were obtained from the current flow through method. The predicted 0.01 mL/min data (dash-dotted) based on the current model fitted with the *in vivo* data of 100–300 μm (square) and 700–900 μm (circle) from literature (Namur et al., 2010).

include the addition of a uniform fibre mesh proximal to the bead mass to provide laminar flow and prevent possible air bubbles passing through. Under the flow rates set in the study, even with the fastest 45 mL/min flow, the Reynolds numbers of the elution medium flow are below 65 in the column, indicating a laminar flow regime. In addition, the mixture of glass beads and embolic beads between the two filters creates interstitial space which reduces the impact of bead swelling during elution, maintains laminar flow with uniform distribution across the diameter of the bed (Kakhi, 2009) and serves to reduce turbulence (Groth and Johansson, 1988). Air bubbles attached to the dosage forms could affect the drug dissolution and flow of medium (Degenhardt et al., 2004) and therefore the measures adopted in the current system include a procedure to avoid the bed of beads being exposed to the air and involve a pre-degassing step with helium sparging to further remove air dissolved in the elution medium.

It has been demonstrated that doxorubicin elution from DC Bead™ is an ion-exchange process (Gonzalez et al., 2008). The drug elution rate depends on the time to build cation-doxorubicin ion-exchange equilibrium and the drug solubility in elution medium. Fig. 3A showed that the doxorubicin concentration change in PBS elution medium under different flow rates followed a very similar trend. In general, the profiles could be separated into three stages: an initial steep increase; followed by a quick drop of concentration; finally a long tail representing residual drug release from beads. Following the same principle as an ion-exchange column, the initial peak in initial drug concentration is the result of accumulating doxorubicin at the sodium ion front through continuous adsorption and desorption equilibrium (Helfferich, 1962). In the study the C_{max} usually appeared with high values at low flow rate (0.5 to 2 mL/min) because of a more complete ion exchange and less dilution by slow moving medium. After the front passed, the drug concentration in the bottom of the bed reduced significantly. The drug concentration gradient along the axial direction towards the top of bed explained the quick drop of the drug concentration in the second stage. At the top of the bed, the drug concentration was maintained at a relatively high level due to the passing medium carrying concentrated drug, which led to a long period of end stage.

The distinct difference between the curves under different flow rate, size or doses is a collective effect of bead intrinsic structure, size, the pattern of packing, drug-polymer interaction and medium conditions. (Gonzalez et al., 2008; Liu et al., 2001; Glen et al., 1992). Flow rate selection in flow-through cell study depends on many considerations, which is often difficult to define (Wähling et al., 2011). With regard to the vascular embolization, *in vivo* blood flow rates vary from 200 mL/min to almost stasis after total occlusion, in which the direct drug diffusion into the contact tissue becomes more dominant. Various *in vitro* flow rates were tested to match the *in vivo* drug elution for the best reflection of *in vivo* doxorubicin elution. Doxorubicin release from dextran microspheres bearing sulfopropyl groups was tested on a flow- through column with a flow rate of 0.5 mL/h (Cheung et al., 2004) using the rate of interstitial fluid loss, 0.14–0.22 mL/h/g of tissue as reference (Butler et al., 1975). Other reports on embolization microspheres evaluated by flow-through cell have used flow rate 5.5 mL/h (Willmott et al., 1985) and 13.6 mL/min (Cremers et al., 1990). In the current study, various flow rates from 0.5 to 45 mL/min were tested for trending purpose. Through a comparison of the residual doxorubicin in beads between the *in vitro* data and the *in vivo* data of 100–300 μm and 700–900 μm beads adapted from Namur's work (Namur et al., 2010), an *in vivo* rate constant was obtained (Fig. 7), under the assumption that the first order rate law (1) is still valid at very low flow rate. Based on the correlation between the *k* values and flow rates in Table 1, the *in vivo* flow rate was estimated around 10 μL/min. This value is about five times higher than the fluid loss value, 0.14 mL/h/g of tissue. By balancing the experimental time against IVIVC considerations, subsequent elution work were mainly carried out at 2 mL/min.

Under sink conditions, increased salt concentration could increase the doxorubicin release rate as shown in Fig. 3C. The derived first

order constant k is also increased with the salt level, suggesting high sodium ion concentration favours more ion-exchange under unit time in the column. This result is consistent with literature on ion-exchange beads carried out in a closed system (Gonzalez et al., 2008; Liu et al., 2001; Biondi et al., 2013). To explore the correlation between flow rate and salt concentration effect in Fig. 3B and C, a quantity of sodium chloride flow was used to correlate the two effects, which was the flux of NaCl across a unit area of the bed of beads. Based on the stoichiometric cation-doxorubicin exchange, the consistence of first order constants from the two sets of experiment in Fig. 3D evidenced that the amount of NaCl passing the beads is the dominant factor in the flow-through test of drug release. This also is the rationale of using NaCl as the only source of the salt in this study, because in principle the flow rate variance is actually the direct result of NaCl availability for ion exchange. Through the correlation between flow rate and NaCl concentration, it is evident that the mechanism of ion exchange of doxorubicin elution is determined by the NaCl salt concentration. We have only considered NaCl in this mechanistic study, as although it has been shown that the presence of divalent cations and proteins in plasma can also have an effect on drug release from the beads (Gonzalez et al., 2008), sodium is in by far the highest concentration in extracellular fluids and therefore the predominant species available for ion exchange (136–145 mEq/L for sodium compared to ~10 mEq/L for potassium, calcium, magnesium and proteins combined (Martin, 1983)). In the current setting, the DEBs were uniformly mixed with glass beads, therefore the contact among DEBs were reduced. It is expected that the drug elution behaviour would be different from the fully packed and contacted bead aggregates without glass bead 'diluent', which was supported by the data without glass beads in Fig. 2 and other tests (unpublished data). It would be more challenging if the beads were swollen during elution and caused column blockage with reduced flow rate and increased back pressure, a situation perhaps more akin to *in vivo* embolization.

Doxorubicin elution rate from beads through an ion-exchange mechanism was controlled by the step of either particle diffusion or film diffusion or both, and the ion-exchange at the fixed ionic group site was treated as a faster 'reaction' (Cremers et al., 1990; Helfferich, 1962; Biondi et al., 2013). It has been demonstrated that the kinetics of doxorubicin elution from DC Bead™ into bulk solution under agitation was controlled by film diffusion (Biondi et al., 2013). The increased doxorubicin elution rate with increasing flow rate in the packed column support that film diffusion is the rate-determining step. The rate of exchange is in proportion to the velocity of laminar flow of the elution medium, which reduces the 'film thickness' (Harland, 1994). Fig. 3A and B also evidenced that at for a higher flow rate range of 10, 20 and 45 mL/min, a hydrodynamic value of film thickness was gradually reached. Thus the increase of doxorubicin elution rate was less pronounced. A particle diffusion mechanism was not supported by the data. From the study of drug concentration within the packed column at different time points it can be observed that ion exchange in the column under flow is a process of drug desorption and reabsorption. In the front column (sections), doxorubicin was constantly exchanged and washed away by eluent Na^+ in the saline mobile phase; in the following sections, especially the last section, doxorubicin ions in the mobile phase were able to further exchange with the Na^+ and loaded drug. Due to the higher doxorubicin concentration in mobile phase along the flow direction, at the end of the column the drug loading became more dominant. The result was that the drug concentration at the end section was reached the maximum doxorubicin loading capacity of the beads. This result demonstrates that mechanistically, the doxorubicin elution of DEB in a packed column through which residual flow is maintained is an dynamic equilibrium process with constant desorption and reabsorption of drug and salt cations along the direction of flow.

Previous work on doxorubicin loaded DC Bead™ elution showed the drug elution rate was slowed down with increase bead size in a USP-type II dissolution apparatus (Gonzalez et al., 2008). A similar trend was observed in the current flow-through study, although the beads were co-packed with glass beads in this case. This size dependent trend was found in the drug elution work with packed albumin microspheres in a column as well (Willmott et al., 1985). The reason could be the reduced surface area with increasing particle size which led to reduced ion-exchange opportunities. The increase in particle size causing increased distance for drug diffusion may also contribute under the same flow rate conditions.

The dose effect on doxorubicin elution from beads through the ion-exchange mechanism has been studied with different type beads (Gonzalez et al., 2008; Liu et al., 2001; Cheung et al., 2004; Biondi et al., 2013). For sulfopropyl dextran microspheres, the high dose of doxorubicin tended to slow down the drug elution in a closed elution medium presented in both percentage and mass forms. The cause was attributed to the increased hydrophobicity by drug-drug/drug-polymer interaction in microspheres (Liu et al., 2001; Cheung et al., 2004). In DC Bead™, beads with a high loading of doxorubicin eluted more dose than the beads with a lower amount of drug, *i.e.* the higher dose showed faster elution, despite in percentage terms the elution of lower dose beads seemed to increase faster as shown in the insert of Fig. 5B; this despite the drug initial release coefficient (k_{rel}^0, equivalent to the first order rate constant which was calculated at different doses of doxorubicin (Biondi et al., 2013)), showed a decrease with drug dose increase. As a reflection of drug concentration change against total loading dose, it is actually consistent with the trend shown by mass.

The flow-through elution system was also applied to irinotecan elution from packed beads. Due to the much weaker interaction between irinotecan molecules with sulfonate groups on DC Bead™, irinotecan elution rate was much faster compared to the doxorubicin elution. The results from both concentration profiles and cumulative curves could still differentiate the dose and size difference among the samples (Fig. 6).

5. Conclusions

A flow-through device has been tested to evaluate the elution properties of doxorubicin and irinotecan loaded DC Bead™ under a packed state and in sink conditions. By selecting different parameters, such as bead packing, elution medium flow rate, dose of loading, and bead size, it has been demonstrated that the doxorubicin elution was controlled by a film diffusion step, which was supported by the experimental observation that increasing flow rate led to increased drug elution rate. The rate and amount of drug elution from the packed beads was affected by flow rate, the bead size and initial loading dose. In addition to doxorubicin, measurement of elution of irinotecan was also possible on the device, although it has much weaker interaction with the beads. The data from the concentration profile analysis provided valuable information to reveal the drug elution behaviour akin to the pharmacokinetic data observed for embolized beads and complementary to the normal cumulative data. Thus, the flow-through system is a useful tool to evaluate DEBs both in QC and for *in vivo* mimicking studies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ejps.2016.08.020.

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