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Doxorubicin eluting beads—2: methods for evaluating drug elution and in-vitro:in-vivo correlation

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Abstract DC Bead™ is a sulfonate-modified, PVA-based microspherical embolisation agent approved for the treatment of hypervascular tumours and arterio-venous malformations. The beads have previously been shown to actively sequester oppositely charged drugs, such as doxorubicin hydrochloride (dox) by an ion-exchange mechanism. In order to characterise the release kinetics and predict the in vivo behaviour of drug eluting beads (DEB), two elution methods were utilised. The first, an application of the USP dissolution method Type II - Apparatus, enables study of the complete elution of loaded DC Bead in less than 4 h, allowing relatively rapid comparison to be made between different products and formulations. Release data obtained using this method were fitted to first order kinetics ($R^2 > 0.998$) and the elution constants shown to increase with the total surface area of the beads exposed to the elution medium. Diffusion coefficients were calculated adopting the Fickian diffusion model, which predicted slow elution rates under physiological conditions. The second method involved the use of a T-Apparatus where the drug experiences an element of diffusion through a static environment. This method was developed to resemble the in - vivo situation in embolisation procedures more closely. Slow release of dox from DC Bead with half-lives over 1,500 h were predicted for all size ranges using a slow release model. A strong linear relationship was found between the release data from T-Apparatus and pharmacokinetic data obtained from patients treated with DC Bead loaded with dox in transarterial chemoembolisation (TACE) procedures. These data indicated a Level A in vitro–in vivo correlation (IVIVC) for the first 24 h post embolisation. Both systems developed were automated and good reproducibility was obtained for all samples, demonstrating the usefulness of these elution techniques for product development and comparative testing.

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Introduction

Previously we have described a microspherical embolisation device, DC Bead™, that has been approved for the treatment of hypervascular tumours and arterio-venous malformations [1, 2]. This system comprises a poly-(vinyl alcohol) (PVA) polymer hydrogel that has been modified by the addition of a sulfonic acid-containing component, and is formulated by inverse suspension polymerisation into beads ranging in size from 100 to 900 μm. The DC Bead has been shown to actively sequester oppositely charged drugs, such as doxorubicin hydrochloride (dox), by an ion-exchange mechanism making them suitable for drug delivery applications such as treatment of solid tumours (e.g. hepatocellular carcinoma (HCC)).

In traditional cancer chemotherapy the efficacy of anticancer drugs is limited by the low drug concentrations achieved in tumours, the non-specific toxicity of chemotherapeutic agents, and the development of drug resistance [3]. Local delivery of a chemotherapeutic agent increases the specific targeting of the drug at the tumour site. Furthermore, when a high dose is incorporated into a carrier

such as drug eluting beads (DEB) and injected directly into the tumour site, the exposure time of the tumour to the drug increases, as the DEB can act as a reservoir for sustained drug release. This provides high local concentrations of the drug for an extended period of time, which is associated with improved treatment efficacy and might overcome drug resistance mechanisms [4, 5]. In addition, reducing systemic levels of the toxic drug could also minimise the undesirable side effects caused by toxicity to normal tissue.

In this paper we describe methods for evaluating drug elution kinetics of DEB. The first method uses a dissolution tester Type 2 - Apparatus, as described in the US Pharmacopeia [6]. This technique allows the characterisation of the release kinetics of DEBs under accelerated conditions and provides a means to set specifications for different products. This is useful in product development and quality assurance. The ultimate purpose of an in vitro model however, is to predict in vivo behaviour. Although some models have been suggested [7, 8], there are at present no recognised in vitro release tests for drug delivery systems designed for embolisation [9]. It would, therefore, be beneficial to develop an in vitro system that more closely resembles the in vivo situation, so that release of the drug from the bead can be better modelled. This relationship is normally known as the in vitro–in vivo correlation (IVIVC) and we describe here the use of a method that employs a diffusion T-Apparatus that can be used for this purpose.

The DC Bead system loaded with dox has been evaluated in clinical studies in Europe and Asia (PRECISION clinical trials) [10]. These Phase I/II clinical studies were carried out to evaluate the safety, pharmacokinetic (PK) profile and efficacy of DC Bead in the treatment of HCC by transarterial chemoembolisation (TACE) [11]. Half of the patients in the trial participated in a dose escalation phase, which was well tolerated up to the maximum dose of 150 mg per treatment. The remaining patients were treated with the maximum dose and all patients received two treatments over a 3 months period. A number of the patients participated in a PK study, which showed that the level of dox in plasma after treatment with DEB was very low compared to historical data [12]. It was these data that were used in this study to correlate with the data obtained from the T-Apparatus method.

Experimental

Materials

DC Bead embolisation microspheres (Biocompatibles UK Ltd, Farnham, UK) were supplied in sterile vials as 2 mL hydrated bead volume, suspended in sodium phosphate solution. Doxorubicin hydrochloride (Dabur Oncology, UK) was obtained as a red powder (>99% purity) and dissolved in pure water to the desired concentrations.

Doxorubicin loading into beads

A measured volume of beads was immersed into a drug solution of the desired concentration. In order to avoid competition arising from ion-exchange effects the sodium phosphate solution was removed from the beads using a syringe fitted with a 20G filter needle (B. Braun Medical, UK) just prior to addition of the drug solution. Residual drug remaining in the depleted loading solution was determined by measuring the absorbance at 483 nm using a Perkin Elmer Lambda 25 UV–visible spectrophotometer and a standard curve constructed from solutions of known concentration of drug. Detailed study on dox loading and distribution into the beads has been reported elsewhere [1, 2].

Elution of doxorubicin (Free-flowing model)

DC Bead loaded with dox was eluted into 250 mL of media (e.g. water, plasma, different salt solutions) at room temperature (RT) on a roller mixer. In all cases, 1 mL of hydrated DC Bead was loaded with 25 mg of dox just prior to elution. The UV absorbance of the dox solution was measured at 483 nm and concentrations were calculated from a standard curve in the corresponding media. The stability of the drug was assessed following the USP HPLC method for doxorubicin. HPLC analyses of the solutions were performed after loading and elution of dox from DC Bead and compared to standard dox controls.

Elution of doxorubicin using the USP Type 2 - Dissolution apparatus

DC Bead loaded with dox were eluted at 25 °C and quantified using a USP Type 2 - Dissolution Apparatus and a Perkin Elmer UV spectrophotometer. The UV absorbance of the dox solution was measured at 483 nm and concentrations calculated against a standard curve. All different commercial size ranges of DC Bead (100–300, 300–500, 500–700 and 700–900 μm) were loaded with dox (1 mL of hydrated DC Bead loaded at 37.5 mg/mL, $n = 3$) and eluted into 900 mL of medium. The dissolution media used was 30:70 water (HPLC grade): ethanol (SA grade) with 20% (w/v) potassium chloride (Aldrich). The release solution was continually sampled from the dissolution vessel to the UV spectrophotometer by peristaltic pump (Watson–Marlow) at a flow rate of 50 mL/min.

Calculation of diffusion coefficient

The elution data obtained using the USP Type 2 - Dissolution Apparatus were used to calculate the diffusion coefficients (D) for DC Bead. Values of D were calculated using the Fickian diffusion model, following two mathematical approximations, described by Richards [13] when the fraction eluted (F) is >0.6 and Chretien [9] when the fraction eluted (F) is <0.85.

In summary, for $F > 0.6$, the values of M_t/M_∞ at each time point were calculated for all three sizes. These data were fitted to Eq. 1:

$$F = 1 - 6/\pi \exp\ (-Bt), \quad (1)$$

and the values for the constant (B) were calculated. Once the constant B was calculated, the diffusion coefficient (D) was obtained using Eq. 2:

$$B = \pi^2 D/r^2 \quad (2)$$

where D is the diffusion coefficient and r is the average radius of the particle.

For $F < 0.85$, Bt was calculated for each size range for each point using Eq. 3:

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

and their values plotted versus time (t). The value of the slope is defined as B. Once the value of the slope was calculated for each size range, the value of D was calculated using Eq. 2.

Elution of dox using the T-Apparatus

As a second method, DC Bead loaded with dox were eluted into 200 mL of phosphate buffer solution (PBS) (Inverclyde Biologicals), pH 7.4 for 24 h at 37 °C and quantified using a T-Apparatus [1] connected to a Perkin Elmer UV spectrophotometer. The UV absorbance of the dox was measured at 483 m concentrations of dox in solution calculated using a standard curve. Elution of 500–700 μm DC Bead loaded with dox at different doses (6.25, 12.5, 18.75, 25 and 37.5 mg/mL of beads, (n = 3, in all cases) was performed. Additionally, elution profiles of different commercially available size ranges of DC Bead (100–300, 300–500, 500–700 and 700–900 μm (n = 3, in all cases)) loaded with dox at 37.5 mg/mL of hydrated DC Bead were obtained. The dox eluted was continually sampled from the dissolution vessel to the UV machine by peristaltic pump (Watson–Marlow) at a flow rate of 50 mL/min.

The release data obtained from this method were fitted to various kinetic models and found to be best represented by a slow release model ($m_0 - m = kt^{1/2}$), which was used to calculate the half-life for dox release. In this model, m_0 is the drug loading in beads, m is the drug left within beads after time t, k is a constant which is relevant to initial drug concentration in beads, diffusion coefficient and drug solubility [14]; from the data the constant and correlation coefficient were calculated for each sample. The value of k is an average due to the packing of beads in the elution cell.

In vivo drug release from DC Bead loaded with dox human study

Dox loaded beads were administrated via a catheter during TACE treatment of 15 patients with hepatocellular carcinoma. This PK study was part of a phase I/II clinical trial (PRECISION clinical trial) conducted in the Queen Mary's Hospital, Hong Kong, under the direction of Dr. Ronnie Poon. The patient's demographics showed a mean age of 64 ± 10 years, a mean body weight of 64.4 ± 7.3 kg and with a mean body height of 166 ± 6 cm. Patients received dox loaded beads (size range = 500–700 μm) in a dose escalation study, using doses of 6.25, 12.5, 18.75, 25 and 37.5 mg/mL of hydrated beads (n = 3, in each case). At predetermined time points following chemoembolisation, blood samples were collected from the patients. Samples were stored at –70 °C prior to analysis. The measurement of plasma concentrations of dox used an HPLC method previously validated for use at CentraLabS Clinical Research. The analytical procedures followed were based upon the Guidance for Industry on Bioanalytical Validation, FDA (May 2001). The study was performed in accordance with the guidelines of the Declaration of Helsinki and under an approved protocol from the ethics committee of Queen Mary's Hospital, Hong Kong.

Results

Elution of DC Bead loaded with dox (Free-flowing model)

HPLC analysis of the released dox showed a single primary peak with a similar retention time for that of pure drug that has not been loaded into the beads. No additional peaks were detected in the eluted samples when compared to the control, suggesting that there is no detrimental effect on dox during loading and release from DC Bead. It has been previously reported than ion-exchange based loading processes do not affect the chemical structure of the drugs [15, 16].

The release profiles of dox loaded DC Bead (25 mg/mL of hydrated beads) show that the drug was not released into

water, but it was slowly released in PBS and plasma. Release rate was media dependent with the amount of drug eluted in plasma in 1 h being more than twice that eluted in PBS (Fig. 1A). Figure 1B shows that dox is initially released until a plateau is reached. No further drug is released until the medium is replaced with fresh solution.

Elution in different ionic strength solutions

The dox loaded DC Bead (900–1,200 μm size range) were eluted in 250 mL of different NaCl aqueous solution (0%, 0.45%, 0.9%, 4.5% and 9% w/v) at room temperature (RT). Figure 2A shows a direct relationship between ion concentration and elution rate of dox from the beads. This elution model, where the total volume of elution is limited to 250 mL, has the limitation that the elution rate is affected by the solubility of dox into each medium. At low salt concentrations (0.45% and 0.9% w/v) only a small amount of drug is eluted from the beads and the amount of dox solubilized has less effect on the elution rate. Conversely, for the samples eluted at high salt concentration, the elution rate is influenced by the amount of drug solubilized in each media. Both effects can be observed by plotting the amount of drug eluted against the salt concentration at each time point. Figure 2B and C show attempts to linearly correlate the salt concentration and the amount of drug eluted at each time point. For low salt concentrations (0–0.9% w/v), $R^2 = 0.992$, 0.988 and 0.979 at times 1, 5 and 24 h, respectively, demonstrating a fair fit to linear relationship. This relationship however, is not maintained when the elution data from the media with higher salt concentration are included in the plot (0–9% w/v).

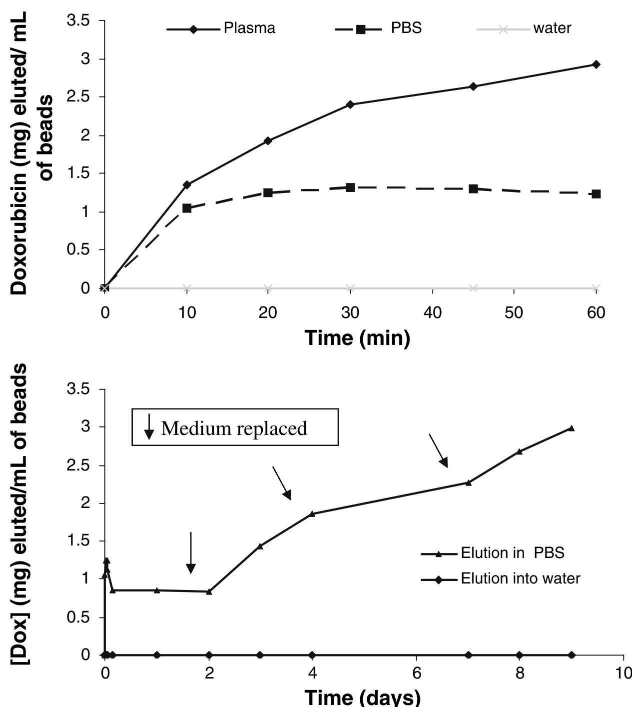

Fig. 1 (**A**) Elution profile of 1 ml of dox loaded DC Bead (25 mg/mL) into 50 mL of plasma, PBS and water. (**B**) Elution profiles of 1 mL dox loaded beads over 9 days into PBS and water at RT. The medium was replaced for fresh when equilibrium was reached

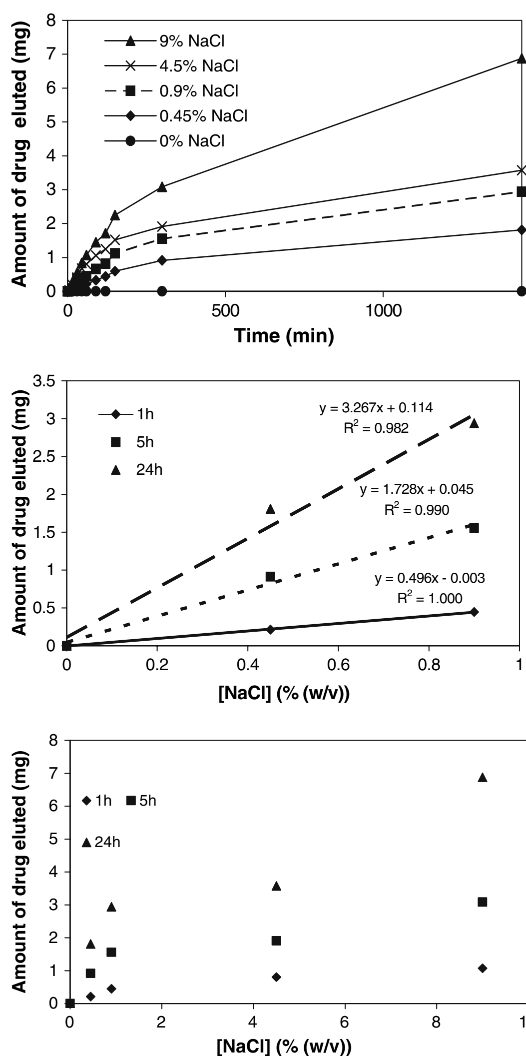

Fig. 2 (**A**) Elution of 900–1,200 μm dox loaded DC Bead into 250 mL of different [NaCl] solutions. (**B**) Amount of Dox eluted from DC Bead versus [NaCl] at different time points for low salt concentrations. (**C**) Amount of Dox eluted from DC Bead versus [NaCl] at different time points for low and high [NaCl]

Doxorubicin elution using the USP Type 2 - Dissolution Apparatus

The elution model described in Sect. 'Elution in different ionic strength solutions' has several limitations and only a small amount of drug is eluted from the beads. In order to characterize the elution kinetics more fully and allow

evaluation between different products, a new method was developed using the USP Type 2 - Dissolution Apparatus. A number of different elution media were evaluated and a 30:70 potassium chloride (20% w/v):ethanol mixture was found to be the most suitable. This solution was chosen because it was able to elute all of the dox from the DEB in less than 4 h for all size ranges with no detrimental effect on the drug. This medium contains very high levels of salt (excess), which keeps the [K^+] constant during elution, to minimise the effect of salt concentration on the elution rate.

The drug release profile obtained from the Dissolution Apparatus was modelled to first order kinetics ($m_0 - m = m_0(1 - e^{-kt})$), where m_0 is the drug loading in beads, m is the drug left within beads after time t, k is a first order constant. Correlation coefficients of $R^2 > 0.998$ for all size ranges demonstrates an excellent fit of the data to first order kinetics for dox elution from all size ranges of DC Bead. k values were calculated for each size range (Fig. 3A). These values were plotted against the average diameter of each size range (inset). The elution constant, k, was shown to decrease as the diameter of the DC Bead increases, demonstrating that the elution rate is inversely proportional to bead size. Moreover, as the same volume of loaded DC Bead was eluted in each case, the surface area exposed to the elution medium is larger for the smaller beads. Therefore, elution constant, k, was shown to increase with the total surface area of DC Bead (Fig. 4).

Calculation of coefficient of diffusion

Using the elution data generated in Sect. 'Doxorubicin elution using the USP Type 2 - Dissolution Apparatus' together with two mathematical approximation methods

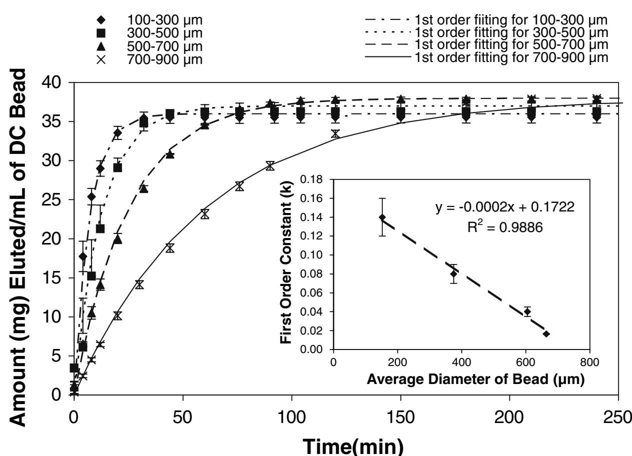

Fig. 3 DC Bead loaded with Dox elution using the USP-type II Dissolution apparatus tester and fitting to first order release model. Inset. Relation between diameter of the beads and first order kinetic constant

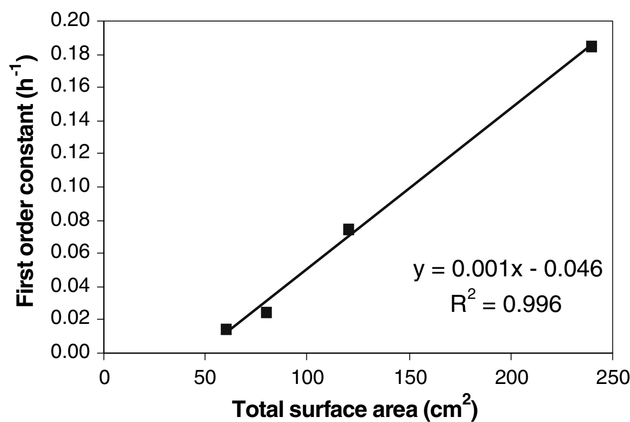

Fig. 4 Relation between total surface area of DC Bead and first order kinetic constant.

Table 1 Values for the coefficient of diffusion of DC Bead loaded with dox

DC Bead size range (μm)	Diffusion coefficient (cm^2/s) (for $M_t/M_\infty > 0.6$)	Diffusion coefficient (cm^2/s) (for $M_t/M_\infty < 0.85$)
100–300	$2.2–5.8 \times 10^{-8}$	3.0×10^{-8}
300–500	$6.3–9.4 \times 10^{-8}$	8.4×10^{-8}
500–700	$4.5–7.3 \times 10^{-8}$	6.0×10^{-8}
700–900	$3.3–6.1 \times 10^{-8}$	3.8×10^{-8}

from the Fickian diffusion model, the diffusion coefficient of DC Bead (D) was calculated. Results for D using both methods can be found in Table 1.

The values for D of different bead sizes, calculated by both methods, were similar and overlapping indicating no differences between different bead sizes. This outcome is not unexpected, as D depends of the binding constants between the drug and the beads and the medium in which it is determined.

Elution studies using the T-Apparatus

To better simulate in vivo conditions, DC Bead loaded with dox at different doses was eluted using the T-Apparatus. The values obtained were fitted to a slow release model ($R^2 > 0.994$) (Fig. 5). After 24 h, 0.78, 0.93, 0.98, 1.32 and 1.48 mg of dox were eluted from the DC Bead loaded at 6.25, 12.5, 18.75, 25 and 37.5 mg/mL of hydrated beads, respectively.

In a second experiment, different sizes of DC Bead loaded with dox at a single dose of 37.5 mg/mL of beads were eluted using this method and their release profiles were modelled to a slow release kinetic model ($R^2 > 0.989$). After 24 h, the amount of dox eluted from 100–300, 300–500, 500–700 and 700–900 μm DC Bead beads was 1.78, 2.01, 1.32 and 1.72 mg, respectively.

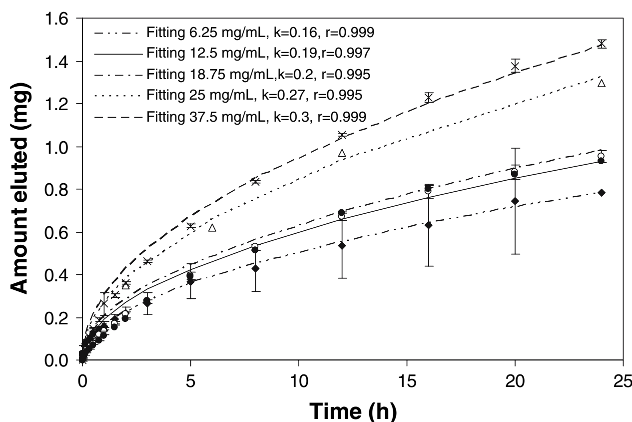

Fig. 5 Elution in of DC Bead loaded at different doses of doxorubicin using a T-Apparatus fitting to slow release model. 6.25 mg/mL (◆), 12.5 mg/mL (■), 18.75 mg/mL (□), 25 mg/mL (△), 37.5 mg/mL (×)

Using this model, the half-life ($t_{1/2}$) of dox elution was predicted for the different DC Bead size ranges and doses, as shown in Table 2. As expected, the $t_{1/2}$ increased as the concentration of dox loaded in the beads increased. However, the $t_{1/2}$ was small for the smaller bead size but did not increase proportionally to the surface area of the beads.

In-vivo in-vitro correlation (IVIVC)

Level A IVIVC, which represents a point-to-point relationship between an in vitro method and an in vivo input rate, was investigated. The amount of dox released from DC Bead using the method in Sect. 'Elution studies using the T-Apparatus' was correlated to the areas under the curve (AUC) of 15 patients treated with DC Bead loaded with dox in the PRECISION clinical study. The AUC were calculated at the different time points, using the trapezoidal method, and plotted against the release profiles of DC Bead loaded with dox using the T-Apparatus (Fig. 6).

For all doses, a strong linear point-by-point correlation (R^2 ranged from 0.991 to 0.995) between in vitro and in vivo model was obtained over a 24 h period, suggesting that the elution in the T-Apparatus can be used to develop a Level A IVIVC model and could also be useful for predicting initial in vivo release behaviour following a chemoembolisation procedure.

Table 2 Values of slow release constant and half-life for different size and different doses of DC Bead loaded with dox

	Slow release constant (mg × $h^{-1/2}$)	Correlation factor or coefficient of determination (R^2)	Half life ($t_{1/2}$) (h)
Dose of 500–700 μm DC Bead loaded with dox (mg/mL)			
6.25	0.15–0.17	0.999	381
12.50	0.17–0.20	0.997	1082
18.75	0.18–0.21	0.995	2197
25	0.26–0.28	0.995	2403
37.50	0.28–0.31	0.999	3658
Size of DC Bead loaded at 37.5 mg/mL			
100–300 μm	0.29–0.43	0.983	1505
300–500 μm	0.34–0.42	0.998	2478
500–700 μm	0.28–0.31	0.999	3658
700–900 μm	0.35–0.41	0.999	2557

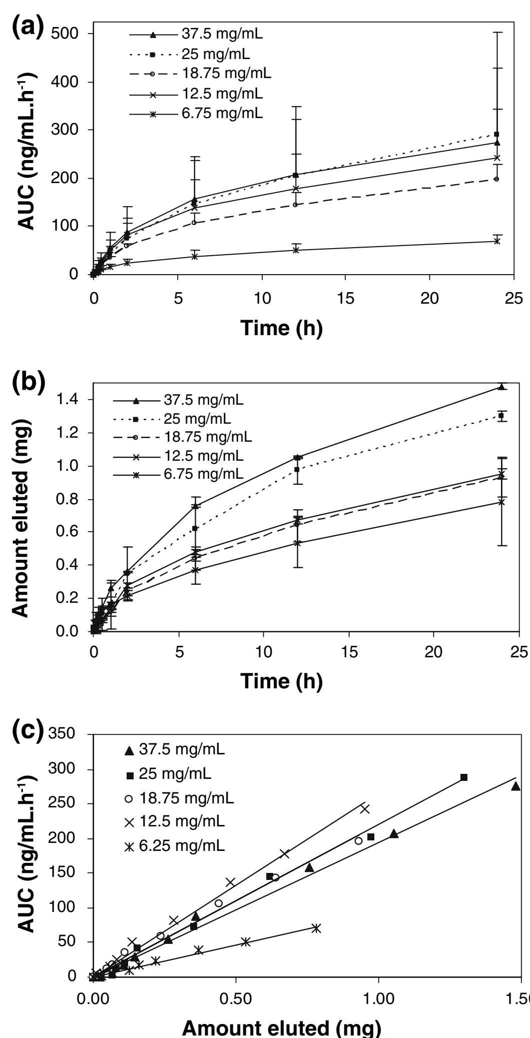

Fig. 6 (**A**) Accumulative areas under the curve of plasmatic concentrations of patients treated with DC Bead loaded with dox at different doses. (**B**) Dox eluted from DC Bead using a T-Apparatus. (**C**) IVIVC from accumulative areas under the curve of patients treated with DC Bead loaded with dox at different doses and dox eluted from DC Bead using a T-Apparatus

Discussion

As discussed in our previous papers [1, 2], DC Bead are PVA sulfonate-modified microspheres with a high loading capacity (up to 40 mg of dox per mL of beads) and a high loading efficiency for dox (>99 %). The high loading efficiency reduces wastage of drug and the high loading capacity make it possible to deliver a very high local dose by use of a small quantities of beads.

The necessary presence of ions in any elution medium suggests that an ion-exchange mechanism is the primary factor controlling the release of the dox from the beads. A suitable counter ion is required to release the bound drug. In pure water there are no available ions, explaining the absence of drug release. The main counter ion present in PBS is Na^+, whereas plasma has higher levels of sodium, potassium and calcium salts [17] and explains why the elution rate is faster in the latter.

The ion-exchange mechanism is also consistent with the respective charges of the beads and the drug. DC Bead is composed of PVA modified with sulfonate groups, which are negatively charged, whereas the dox has a primary amine that is protonated when the drug is in the form of the hydrochloride salt producing a positive charge. Electrostatic interaction between the oppositely charged species is the main mechanism of binding.

We observed that the ionic strength of the release medium has a significant effect on the rate and extent of dox release from DC Bead. The effect of salt concentration, however, is less significant with very high concentration of salt (9% NaCl). These data show the elution rate is not only determined by the salt concentration but also by the maximum solubility of the drug in each medium. Refreshment of release media is necessary to desaturate the solution and promote further drug release. This effect of concentration gradient as a driving force for diffusion has been previously reported for other authors [15, 18]. In our system, this phenomenon plays a major role in the release kinetics of the drug. In order to allow the characterisation of our products and overcome limitations of the previous method, more sophisticated elution models were necessary.

A modified elution model was therefore developed using an ideal elution medium, (30:70) potassium chloride (20% w/v):ethanol mixture. This model was based on the USP dissolution method Type II-Apparatus. The volume and the medium used in this method was selected to allow complete elution of dox from DC Bead in less than 4 h for all size ranges with no detriment to the drug.

In the ion-exchange processes, the rate controlling mechanism can be determined by chemical exchange, particle diffusion and film diffusion mechanisms. These are sequential steps and the slowest of the three will be the rate determining factor [19]. Depending on the concentration in solution of the displacing ion, a clear distinction can be made between a particle diffusion mechanism (exchange rate independent of concentration in solution) and a film diffusion or chemical mechanism (rate initially proportional to solution concentration). Often the reaction rate at the ion-exchange site is assumed to be faster than the rate of solute diffusion. Thus, the reaction is considered to be instantaneous [20]. Boyd et al. [21] investigated the kinetics of ion-exchange and concluded that for very low concentrations (<0.003 N) in solution, film diffusion is the controlling mechanism, whist at higher concentrations (>0.1 N) particle diffusion is rate controlling. They also pointed out that the qualitative effect of variation of the particle size is usually sufficient to confirm or eliminate a chemically controlled rate process.

The USP Type-II Apparatus dissolution model used an ionic solution at high concentration (>1.3 N), therefore it is expected that the rate controlling mechanism be determined by particle diffusion or Fickian model.

The elution profile fitted to a first order kinetic model perfectly for all size ranges. This was expected, as in this model the elution rate for a selected bead size only depends on the amount of drug remaining in them at each time. This first order fitting confirms that the level of salt (excess) and the amount of drug in the elution medium are not affecting the elution rate and almost sink conditions are obtained. The elution constants, k, were shown to increase as the average diameter of the bead decreases, suggesting that the elution rate is dependent upon the total surface area exposed to the elution medium, being faster in the smaller beads. This relatively fast elution method can be used to determine the main product characteristics such as diffusion coefficient (shown in Sect. 'Calculation of coefficient of diffusion') making it possible to define parameters to set specifications for commercial products or to enable comparison between different formulations (e.g. different drugs or different types of beads). The diffusion coefficients calculated for these DEBs in a medium with an excess of ions were in the order of 10^{-8} cm^2/s suggesting that the interactions between the drug and the polymer are very strong and the release in physiological media would be very slow. A similar D was obtained for all bead sizes indicating that the bead structure, interaction between beads and dox and the cross linking in the beads are the same for the different size beads.

DC Bead loaded with dox has been evaluated in Europe as an embolic device for transarterial chemoembolisation (TACE) procedures. Embolisation is a process that consists of blocking the blood supply of the vessels feeding a tumour or pathological area in order to starve this area of blood and nutrients. To simulate these in vivo conditions an elution model, known as the T-Apparatus has been developed. This system was created taking into account the

theory and methods previously described concerning elution methods for loaded embolisation beads [8]. When one considers the consequences of an embolisation process, a reduction of the blood flow to the embolised area is expected. The drug device combination will be located within the target site and initially surrounded by a milieu of thrombus and cells. Transport of the drugs from the beads will be firstly driven by diffusion into adjacent tissues and then eventually will be carried away from the area by vessels distant to those at the embolised site. Hence, any in vitro method developed to emulate this process should control the hydrodynamic conditions such that the mass transfer process is composed of both convection and diffusion components [22].

Different sizes and doses of DC Bead loaded with dox were eluted using this elution method and their profiles shown to fit well to a slow release model, which describes diffusion-based release of a drug dispersed within a solid matrix [14]. The predicted half-life increased with both dose and total surface area, but showed no obvious trend with decrease of bead size. These data disagree with results obtained using the USP-Type II Dissolution method, where the elution rate was dependent upon the total surface area of the beads. For the T-Apparatus this effect is minimised as the elution is affected by other overruling factors. The T-Apparatus model consists of a closed loop system with a low flow rate and a low volume of elution medium to better mimic the in vivo conditions. Therefore, the amount of drug released is limited by both the solubility and the amount of the drug circulating in the elution medium.

Using the T-Apparatus with PBS as elution medium, incomplete dox release is expected as the medium has low ionic strength (0.9% NaCl). This phenomenon has been previously reported [7] and it can be attributed to the tendency for dox to self-associate at higher loading level. After the positive charge of dox is neutralized by the anionic sulfonate groups in the polymer, drug molecules will be held in close proximity to one another and can self-associate by hydrophobic interactions and assemble by stacking upon each other [23]. This drug–drug interaction is greater than the purely ionic complexation with the polymer sulfonate groups and demands extreme conditions such as high ionic strength in order to affect release of the drug from the polymer. Changes in the elution conditions set up as temperature or flow rate are insufficient to affect significant release [7].

The FDA guidance on IVIVC [24] provides general methods for establishments of IVIVC for oral formulations. However, there is very little information available on development of appropriate in vitro methods for IVIVC for non-oral forms. Although several authors [7, 25, 26] have investigated the development of in vitro methods to correlate with the drug release from non-oral formulations, no standard procedure is approved. Here, we proposed a T-Apparatus, as a method to enable emulation of the in vivo conditions post-embolisation. Level A linear correlations between the in vitro model and in vivo PK profiles were obtained for all doses of DC Bead loaded with dox over a 24 h period, suggesting that elution of samples in the T-Apparatus can be useful in the prediction of initial in vivo release behaviour following chemoembolisation procedures. Level A correlation is considered to be the most informative and is thus recommended for regulatory purposes.

This correlation was obtained using the PK data from plasma concentrations of patients and it may allow prediction of the initial levels of systemic dox that become available post TACE and hence the degree of drug related toxicity. It is generally accepted the lifetime limit of systemic exposure to dox is in the region of 600 mg total due to dose-limiting cardiac toxicity [27]. The PK study demonstrated a 2-log reduction of systemic dox for loaded DC Bead compared to conventional TACE and the IVIVC may help to predict how a local dose could be administered before systemic effects occur. Although it would be desirable to correlate between our model and the amount of drug released into tumour tissue, this measure is extremely difficult to perform in humans due to practical and ethical considerations of subjecting patients to more than one procedure over various time points (TACE followed by resection). The amount of dox released from DC Bead into tumour tissue was measured in a complementary study using a Vx-2 tumour rabbit model. The study confirmed high levels drug in the tumour over the entire period of the study (14 days) and associated widespread necrosis of the tumour tissue [28].

Conclusions

The in vitro release mechanism and kinetics of DC Bead loaded with dox have been determined, showing that ion-exchange is the main mechanism affecting the release kinetics of these systems. Two methods were designed to evaluate the release kinetics of DEBs. The first method applied the USP dissolution method Type II-Apparatus, which enables comparison between different products and formulations. Release data obtained from this model fitted first order kinetics perfectly and was used to calculate diffusion coefficients that predicted very slow release. This method could be standardised to set specifications for manufacturing processes. The second method developed, a diffusion T-apparatus, resembles more closely the in vivo situation in embolisation procedures, where the drug diffuses through a static environment. The release data obtained from this method were modelled to slow release

kinetics, predicting a half-life of elution over 1,500 h for all size ranges of DC Bead. Strong linear correlations were found between release data from T-Apparatus and PK data from patients treated with DC Bead loaded with dox in TACE procedures, suggesting that level A IVIVC models can be developed for these types of beads.

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References

1. A. L. LEWIS, M. V. GONZALEZ, B. HALL, et al., *JVIR* **17**(2) (2006) 335
2. A. L. LEWIS, M. V. GONZALEZ, S. W. LEPPARD, et al., *J. Mater. Sci. Mater. Med.* doi: 10.1007/s10856-007-3068-8
3. B. SPERKER, T. E. MURDTER, M. SCHICK, et al., *J. Pharmacol. Exp. Ther.* **281**(2) (1997) 914
4. W. J. JUSKO, *J. Pharm. Sci.* **60** (1971) 892
5. M. J. BOYER and I. F. TANNOCK, in "Cellular and Molecular Basis of Chemotherapy. The Basic Science of Oncology" (Toronto, MacGraw-Hill Health Professions Division, 1998) p. 350
6. US Pharmacopeia 28, (2005)
7. R. Y. CHEUNG, R. KUBA, A. M. RAUTH and X. Y. WU, *J. Control Release* **94** (2004) 25
8. F. AMYOT, V. BOUDY, K. JURSKI and J. COUNORD, *ITBM-RBM* **23** (2002) 285
9. C. CHRETIEN, V. BOUDY, P. ALLAIN and J. C. CHAUMEIL, *J Control Release* **96** (2004) 369
10. M. VARELA, M. I. REAL, M. BURREL, et al., *J. Hepatol.* **47** (2007) 159
11. J. M. LLOVET, M. I. REAL, X. MONTAÑA, et al., *Lancet* **359** (2002) 1734
12. P. JOHNSON, C. KALAYCI and N. DOBBS, *J. Hepatol.* **13**(1) (1991) 120
13. J. RICHARDS, in "The Role of Polymer Permeability in the Control of Drug Release. Polymer Permeability" (London, Elsevier Applied Science Publisher, 1985) Chapt. 6, p. 217
14. T. HIGUCHI, *J. Pharm. Sci.* **52**(12) (1963) 1145
15. Z. LIU, R. CHEUNG, X. Y. WU, J. R. BALLINGER, et al., *J. Control Release* **77** (2001) 213
16. A. SAWAYA, R. FICKAT, J. P. BENOIT, et al., *J. Microencapsul.* **5** (1988) 23
17. C. L. SCHAUF, M. MOFFETT and S. B. MOFFETT, in "Human Physiology. Foundations and Frontiers" (Edward. F. Murphy, 1990)
18. X. Y. WU, G. ESHUN and Y. ZHOU, *J. Pharm. Sci.* **87** (1998) 255
19. D. REICHENBERG, *J. Am. Chem. Soc.* **75** (1953) 589
20. M. ABDEKHODAIE and X. Y. WU, *Biomaterials* **27** (2006) 3652
21. G. BOYD, A. ADAMSON and L. MYERS *J. Am. Chem. Soc.* **69** (1947) 2836
22. F. AMYOT, K. JURSKI, G. DUFAUX and G. GUIFFANT, *Int. Comm. Heat Mass Transfer* **20**(9) (2002) 623
23. X. LI, D.J. HIRSH, D. CABRAL-LILLY, A. ZIRKEL, et al., *Biochim. Biophys. Acta* **1415** (1998) 23
24. GUIDANCE FOR INDUSTRY: extended release oral dosage forms: development, evaluation and application of in vitro/in - vivo correlations, Food and Drug Administration, (1997)
25. G. SCHELIECKER, C. SCHMIDT, S. FUSCHS, et al., *J. Control Release* **94** (2004) 25
26. N. CHIDAMBARAM and D. J. BURGESS, *AAPS Pharm. Sci.* **1**(3) (1999) article 11
27. G. MINOTTI, P. MENNA and E. SALVATORELLI, *Pharmacol. Rev.* **56**(2) (2004) 185
28. K. HONG, A. KHWAJA, E. LIAPI, et al., *Clin. Cancer Res.* **12**(8) (2006) 2563