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A novel USP apparatus 4 based release testing method for dispersed systems

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ABSTRACT

A novel dialysis adapter has been developed for USP apparatus 4 for *in vitro* release testing of dispersed system dosage forms. This USP apparatus 4 method was optimized and compared with currently used dialysis and reverse dialysis sac methods. Optimization studies for the USP apparatus 4 method showed that release from solution, suspension and liposome formulations was not flow rate limited and was not affected by change in the dialysis adapter sample volume from 250 μl to 500 μl. The USP apparatus 4 method could discriminate between solution, suspension and liposome formulations of dexamethasone. On comparing the different methods, only the USP apparatus 4 method provided discrimination between dexamethasone release from extruded and non-extruded liposomes, as well as among non-extruded DMPC, DPPC and DSPC liposomes. The dialysis sac method could not discriminate between the release profiles of non-extruded DMPC and DPPC liposomes. The reverse dialysis sac could not discriminate between the release profiles of extruded and non-extruded DMPC liposomes. In addition, the USP apparatus 4 method provided the highest release and the smallest variation in the data. This novel adapter might address the problem of the lack of a compendial apparatus for *in vitro* release testing of dispersed system dosage forms.

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1. Introduction

The last decade has witnessed a rapid development in the area of novel drug delivery systems such as microspheres, liposomes, nanosuspensions, and microemulsions (Kostarelos, 2003). The advantages of these systems include: (1) controlled/modified delivery, (2) targeted delivery, (3) localized delivery, (4) decreased dose, (5) reduced toxicity, and (6) protection of labile drugs (such as proteins) from degradation prior to and after administration. Eleven microsphere and 14 liposome formulations have already been approved by the United States Food and Drug Administration (FDA). With the advances in protein and gene therapeutics, the number of such products is likely to continue to increase.

In order to assure the performance and safety of these novel delivery systems, as well as to assist in the product development process, *in vitro* testing methods must be developed. *In vitro* release is an important indicator of *in vivo* product performance. Accordingly, *in vitro* release tests are used for: (1) routine assessment of process quality control, (2) formulation optimization in product development, and (3) development of *in vitro*–*in vivo* relationships (IVIVR). In addition, *in vitro* release method(s) can also be applied for evaluation of scale-up and post approval changes (SUPAC) (Shah et al., 2002; Siewert et al., 2003).

At present, there is a lack of standard pharmacopeial/regulatory tests for controlled release parenteral products, and this poses a major obstacle in their development and regulatory processes. A number of workshops have been conducted by the American Association of Pharmaceutical Scientists (AAPS), the International Pharmaceutical Federation (FIP), the European Federation of Pharmaceutical Scientists (EUFEPS), the Controlled Release Society (CRS), the United States Pharmacopeia (USP), the European Pharmacopeia (EP), the US FDA and the European Agency for Evaluation of Medicinal Products (EMEA), in order to develop standard quality and performance parameters for controlled release parenteral formulations (Burgess et al., 2002, 2004; Shah et al., 2002; Siewert et al., 2003; Martinez et al., 2008). In particular, the need for standards for *in vitro* release methods, for guidance on *in vivo* release testing and *in vitro*–*in vivo* relationship/prediction has been emphasized. While *in vitro* release testing methods have been recommended for some controlled release formulations, suitable compendial methods have not yet been identified for liposomes (Martinez et al., 2008).

A variety of methods have been used for *in vitro* release testing of controlled release parenterals (Washington, 1990; Clark et al., 2005). Currently used methods for *in vitro* release testing from these dosage forms can be broadly divided into three categories: (1) membrane dialysis methods (such as dialysis sac (Glavas-Dodov et al., 2002; Sezer et al., 2004; Ruozi et al., 2005), reverse dialysis sac

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(Chidambaram and Burgess, 1999), micro-dialysis (Hitzman et al., 2005), and Franz-diffusion cells), (2) sample and separate methods (vial/tube/bottle method with centrifugation or filtration after sampling (Vemuri et al., 1991; Kokkona et al., 2000; Xiao et al., 2004)), and (3) flow-through cell methods (USP apparatus 4 (Kaiser et al., 2003; Zolnik et al., 2005)). These techniques are required to isolate the dosage form from the release media for analytical purposes. An agar gel method (Peschka et al., 1998) has also been reported in which liposomes are embedded in agar gel for separation from release medium. However, none of these methods use official USP dissolution/release apparatus, except the flow-through method with USP apparatus 4. In addition, the procedures and apparatus used vary among laboratories. As a result of the lack of a standard method, results from different sources are usually not comparable. Moreover, some of the methods used are subject to high variability and have limitations such as violation of sink conditions.

The FIP/AAPS report on *in vitro* release testing of novel dosage forms (Siewert et al., 2003) emphasized the need to avoid unnecessary proliferation of equipment and method design and states that compendial method(s) should be the first approach for *in vitro* release testing. This report also suggests that if a compendial method is not suitable (such as for colloidal dosage forms), modifications of compendial method(s) can be considered. Development of a modified USP dissolution apparatus 4 method for *in vitro* release testing of microsphere formulations has been reported in an earlier study (Zolnik et al., 2005). It has been shown that for microsphere formulations, USP apparatus 4 offers advantages over conventional release testing methods such as sample and separate (Zolnik et al., 2005) and USP dissolution apparatus 2 (Voisine et al., 2008). However, colloidal disperse systems such as liposomes, microemulsions or nanosuspensions, could either block the filter in USP apparatus 4 or pass through it. Moreover, liposomes present a unique challenge in that they can be designed to release their contents: immediately; in a sustained manner; after uptake in macrophages; or following a trigger mechanism such as change in pH or temperature (Martinez et al., 2008). Therefore, it may not be possible to develop a single *in vitro* release testing method for these different types of liposomes. In addition, liposomes given by different routes (IM, SC or IV) may require different release testing methods that can simulate the different *in vivo* conditions for IVIVC purposes. However, in product development and quality assurance, a method that can discriminate between different formulation variables may be sufficient.

The present work attempts to address the problem of lack of standard *in vitro* release method(s) for liposomes and other colloidal dosage forms. A novel dialysis adapter that can be used with the compendial USP dissolution apparatus 4 (flow-through) was designed, developed and evaluated. This adapter will render USP apparatus 4 suitable for *in vitro* release testing of colloidal dosage forms such as nanosuspensions, liposomes, and emulsions. Optimization and evaluation studies were performed with solution, suspension and liposome dosage forms of dexamethasone to analyze the feasibility of this novel dialysis adapter. Development of liposome formulations of hydrophobic drug, dexamethasone, with different release kinetics has been reported in an earlier study (Bhardwaj and Burgess, 2010). The discriminatory ability of this novel USP 4 based method was tested using these different formulations. In addition, *in vitro* release of dexamethasone from these liposomes formulations was also investigated with two commonly used methods, dialysis sac (DS) and reverse dialysis sac (RDS), for comparison with the novel USP 4 method.

A dialysis-based method was selected since it is more suitable for deformable formulations such as liposomes. Sample and separate methods pose the following two limitations. First, an artificially higher release might result from disruption and/or fusion of vesicles as a consequence of the separation process (high speed centrifugation or filtration). Second, an erroneous release would also result if the separation method is of the same time scale as the release study (Washington, 1990).

2. Materials and methods

2.1. Materials

Dexamethasone, sodium azide, sodium dodecyl sulfate (SDS) and HEPES, sodium salts were purchased from Sigma–Aldrich (St. Louis, MO). 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Maxidex® ophthalmic suspension of dexamethasone (0.1%, w/v) was purchased from Alcon Laboratories (Fort Worth, TX). Chloroform, acetonitrile and methanol were purchased from Fisher Scientific (Pittsburgh, PA). Spectra/Por DispoDialyzer (50 kDa molecular weight cut off (MWCO); volume, 2 ml) and Spectra/Por Biotech (50 kDa MWCO) cellulose ester dialysis membranes were purchased from Spectrum Labs (Rancho Dominguez, CA). Nanopure™ quality water (Barnstead, Dubuque, IA) was used for all studies.

2.2. Preparation of liposomes

A thin-film hydration method was used to prepare dexamethasone-loaded liposomes as reported previously (Bhardwaj and Burgess, 2010). Briefly, a chloroform solution of lipid, and a methanol solution of dexamethasone were mixed in a pear-shaped flask and evaporated in a Büchi® rotary evaporator at a temperature above the phase transition temperature(s) (Tm) of lipids to form a thin-film (lipid:drug ratio, 1:0.2 M). This film was dried overnight under vacuum for complete removal of the solvents. The lipid film was then hydrated in 10 mM HEPES buffer, pH 7.4 (with 0.1% (w/v) sodium azide as a preservative; at $T > \mathrm{Tm}$) followed by vortexing for 2 min (final lipid concentration 1.2 mg/ml). These vortexed vesicles were used as large multilamellar 'non-extruded' liposomes (referred to as 'non-extruded liposomes' henceforth). For preparation of small 'extruded' liposomes, non-extruded liposomes were sonicated (for 4 min) using an Avanti Ultrasonic Cleaner® bath sonicator ($T > \mathrm{Tm}$) followed by extrusion (11 times) through a 400 nm polycarbonate membrane ($T > \mathrm{Tm}$) using an Avanti MiniExtruder® for size homogenization (referred to as 'extruded liposomes' henceforth). Non-entrapped drug was removed from liposomes as described previously (Bhardwaj and Burgess, 2010).

2.3. In vitro release studies

2.3.1. Dialysis sac method

The pore size of the dialysis membrane can limit diffusion across the membrane. Therefore, a 50 kDa MWCO (Spectra®/Por CE DispoDialyzer) dialysis membrane was selected after screening different MWCO dialysis membranes for diffusion of dexamethasone. Liposome suspensions (1.3 ml) were added to Spectra®/Por CE DispoDialyzer 50 kDa MWCO membranes (total volume of 2 ml; exposed surface area of 1360 mm^2). The dialysis sacs containing the liposome suspensions were placed in glass tubes (Kimax® glass culture tubes; 25 mm × 200 mm) containing 50 ml HEPES buffer maintained at 37 °C in a shaker water bath (New Brunswick, Edison, NJ) and rotated at 50 rpm. One milliliter aliquots were withdrawn at each time point for release estimation and replaced with fresh buffer. Sink conditions were maintained throughout the experiment. Dexamethasone was analyzed using the HPLC method as described below. In case of incomplete release or if a plateau was

reached, SDS was added to a final concentration of 0.5% (w/v) to disrupt the liposomes and confirm complete recovery. Addition of SDS is indicated by an arrow in all figures. The results were reported as mean ± SD ($n = 3$).

2.3.2. Reverse dialysis sac method

Release was performed in glass tubes (Pyrex®; 38 mm × 200 mm) containing 125 ml HEPES buffer maintained at 37 °C in a shaker water bath and rotated at 50 rpm. Spectra®/Por CE Dispo-Dialyzer 50 kDa MWCO dialysis sacs (total volume of 2 ml; exposed surface area of approximately 1360 mm^2) containing HEPES buffer were placed in each glass tube. Liposome suspensions (2 ml) were added to the media outside of dialysis sacs. At each time point, a dialysis sac was removed from each tube and 1 ml aliquot was withdrawn from interior of the dialysis sac for release estimation. The buffer inside the dialysis sac was replenished with fresh buffer after sampling. Sink conditions were maintained throughout the experiment. In case of incomplete release or if a plateau was reached, SDS was added to a final concentration of 0.5% (w/v) to disrupt the liposomes and estimate complete recovery. Dexamethasone was analyzed using an HPLC method (see below). The results were reported as mean ± SD ($n = 3$).

2.3.3. USP dissolution apparatus 4 method

2.3.3.1. Design of dialysis adapter for USP apparatus 4. A novel dialysis adapter was designed for USP apparatus 4 to be used in conjunction with 22.6 mm sample cells. Fig. 1A is a schematic of the dialysis adapter design and Fig. 1B shows the placement of the adapter in USP apparatus 4. The design of the dialysis adapter is a hollow cylinder and the base and top of the cylinder are made of circular Teflon with groves for O-rings seals. The top and base are supported by three metallic wires that provide the framework for the adapter. The Teflon top has an opening that can be closed with a screw. A dialysis membrane is placed over this frame and sealed with O-rings at the top and bottom. The adapter cell with a dialysis membrane was fixed on a cross shaped platform which fits the 22.6 mm USP apparatus 4 cell dimensions. This final assembled

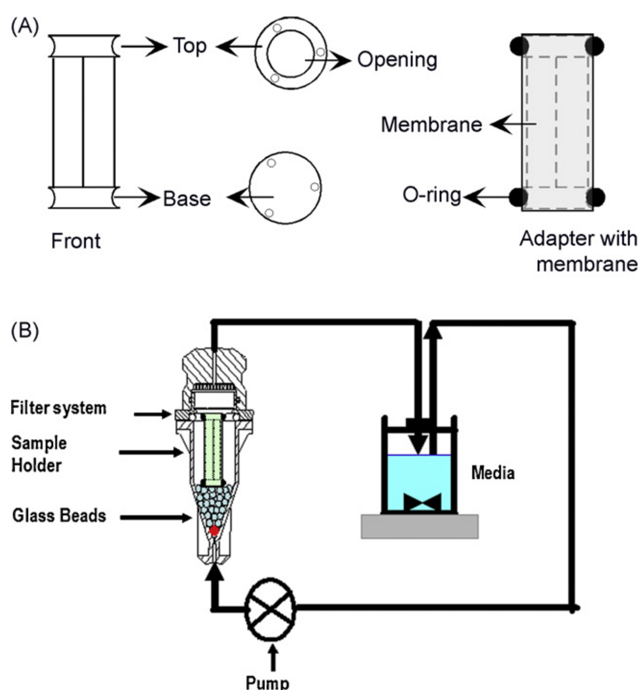

Fig. 1. (A) Schematic of the dialysis adapter design. (Left) the front of the dialysis adapter, (middle) top and bottom parts, (right) adapter with dialysis membrane sealed with O-rings. (B) The placement of the adapter in USP apparatus 4.

adapter is placed in the upright position inside the USP apparatus 4 sample cells. The apparatus 4 can be operated in both the open and closed configurations and the flow rate varied as required. The specifications of the dialysis adapter are: height, 33 mm; diameter, 9 mm; top and base thickness, 3.5 mm; total volume, 1.7 ml; and exposed surface area, ~832 mm^2.

2.3.3.2. Release studies. For the USP 4 method, a Sotax™ CE7 USP apparatus 4 equipped with 22.6 mm diameter cells was used at 37 °C. A ruby bead (5 mm diameter) was placed at the base of the 22.6 mm sample cell and 4 g of 1 mm diameter glass beads were added to fill the bottom conical part of the sample cell. Formulations (solution, suspension, or liposomes) were added to the dialysis adapter and the opening was sealed with a screw. For release studies, ~1.4 ml of liposome suspensions was added to the dialysis adapter. 250 μl and 500 μl of Maxidex® suspension were used to evaluate effect of sample volume in dialysis adapter (Fig. 3). The adapter was placed in the USP 4 sample cell as shown in Fig. 1B for release studies. 100 ml of HEPES buffer maintained at 37 °C was used as release media in these studies. The effect of flow rate on drug release from suspension and liposome formulations was evaluated by varying the flow rates between 8 ml/min and 16 ml/min. USP 4 release studies conducted at a flow rate of 16 ml/min were used for comparison of the USP 4 dialysis adapter method with the dialysis and reverse dialysis sac methods. At each time point, 1 ml samples were withdrawn from the media reservoir containers of the USP apparatus 4. The samples were replenished with fresh media. Sink conditions were maintained throughout the experiment. Dexamethasone was analyzed via HPLC (see below). The results were reported as mean ± SD ($n = 3$).

2.4. Dexamethasone analysis

Dexamethasone was analyzed using an HPLC method as described previously (Bhardwaj et al., 2007). In brief, HPLC was performed using acetonitrile/water/phosphoric acid (35:65:0.5, v/v/v) mobile phase with a Zorbax® Rx C_{18} column (4.6 mm × 15 cm) at a flow rate of 1 ml/min. Dexamethasone was detected at 242 nm using a Perkin-Elmer 785 UV-Vis detector.

3. Results

3.1. Optimization studies for the USP 4 dialysis adapter

Increase in the flow rate from 8 ml/min to 16 ml/min and 20 ml/min did not have any significant effect on the diffusion of dexamethasone solution from the dialysis adapter to the bulk media with most of the drug diffusing out in 4 h (Fig. 2A). Increase in the flow rate from 8 ml/min to 16 ml/min also did not have any significant effect on dexamethasone release from the Maxidex® suspension (Fig. 2B) or the non-extruded DMPC (Fig. 2C) and DPPC liposomes (Fig. 2D). Release from the non-extruded DMPC liposomes was faster compared to that from the non-extruded DPPC liposomes at 37 °C.

Reducing the sample volume of the Maxidex® suspension, from 500 μl to 250 μl, in the dialysis adapter did not have a marked effect on the release rates as evident on comparing the normalized release profiles (Fig. 3). However, concentration *vs.* time release profiles showed that equilibrium was reached by 12 h for the 250 μl and by 24 h for 500 μl sample (secondary *y*-axis; Fig. 3).

3.2. Discrimination between different formulations using the USP 4 method

Dexamethasone release profiles (at 16 ml/min) from the solution, suspension and non-extruded DPPC liposomes were com-

Fig. 2. Effect of different flow rates on dexamethasone release from (A) solution, (B) suspension, (C) non-extruded DMPC liposomes and (D) non-extruded DPPC liposomes using the USP 4 dialysis adapter in 10 mM HEPES buffer, pH 7.4 at 37 °C. The addition of SDS is indicated by an arrow. Each value represents mean ± SD (n = 3).

pared to investigate the discriminatory ability of the novel method. Distinct release profiles were observed from the three formulations (Fig. 4). Drug release from the solution was the fastest (Fig. 4). Dexamethasone release from the Maxidex® suspension was slower than that from the solution and released over a period of 24 h. Release from the non-extruded DPPC liposomes was fast initially (12 h) and then a slower release phase was observed.

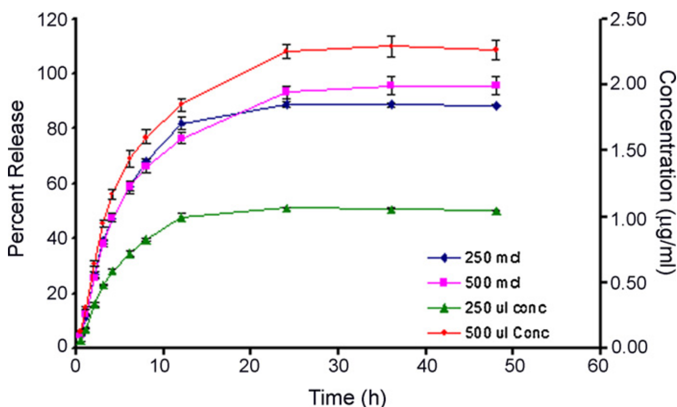

Fig. 3. Effect of different sample volumes on release from the Maxidex™ suspension in the USP 4 dialysis adapter. Release profiles were evaluated in 10 mM HEPES buffer, pH 7.4 at 37 °C at a flow rate of 16 ml/min. Each value represents mean ± SD (n = 3).

3.3. Evaluation of discriminatory ability of different release methods for liposome formulations

The discriminatory ability of the dialysis sac, reverse dialysis sac and dialysis adapter based USP 4 method was evaluated using non-extruded and extruded liposome formulations of phospholipids DMPC, DPPC and DSPC. The physico-chemical properties of

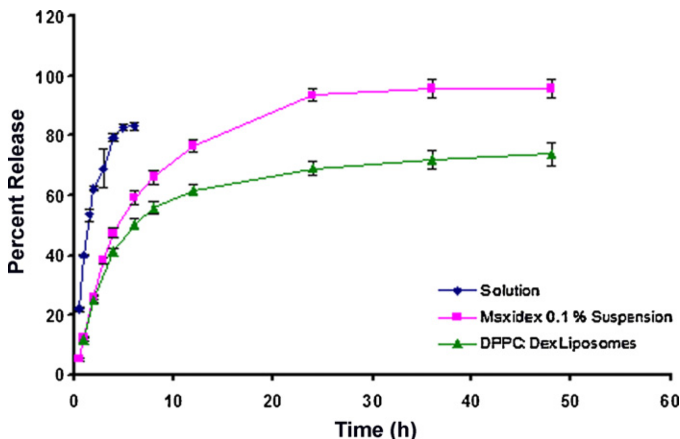

Fig. 4. Discrimination between release profiles from different formulations using the USP 4 dialysis adapter. Release profiles were evaluated in 10 mM HEPES buffer, pH 7.4 at 37 °C and a flow rate of 16 ml/min. Each value represents mean ± SD (n = 3).

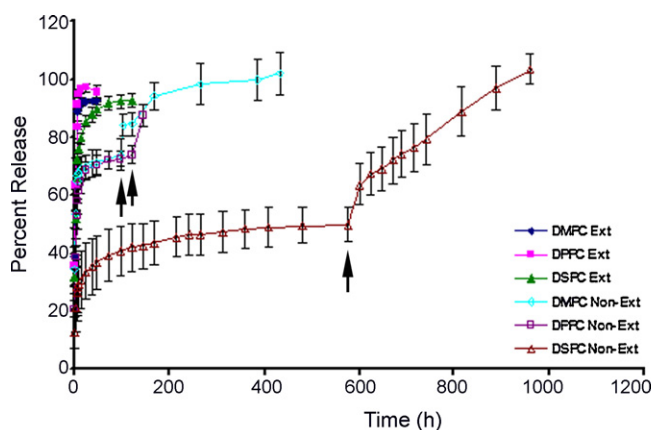

Fig. 5. Discrimination between release profiles from the extruded and non-extruded liposome formulations of DMPC, DPPC and DSPC using the dialysis sac method. Release profiles were evaluated in 10 mM HEPES buffer, pH 7.4 at 37 °C at a flow rate of 16 ml/min. The addition of SDS is indicated by an arrow. Each value represents mean ± SD (n = 3).

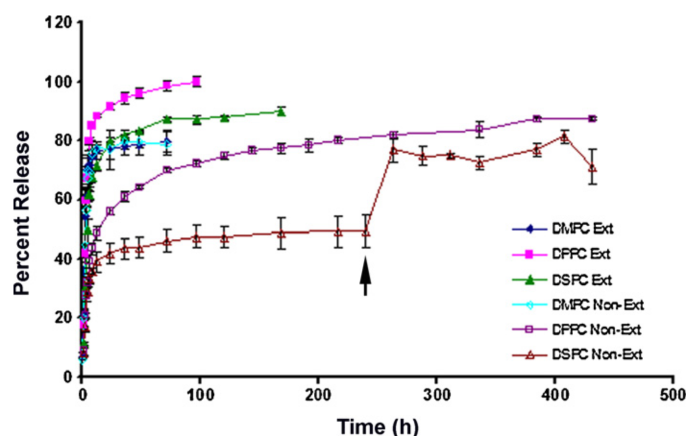

Fig. 6. Discrimination between release profiles from the extruded and non-extruded liposome formulations of DMPC, DPPC and DSPC using the reverse dialysis sac method. Release profiles were evaluated in 10 mM HEPES buffer, pH 7.4 at 37 °C at a flow rate of 16 ml/min. The addition of SDS is indicated by an arrow. Each value represents mean ± SD (n = 3).

these liposomes have been reported earlier (Bhardwaj and Burgess, 2010). The non-extruded liposomes showed slower release compared to the extruded liposomes of the same phospholipid. In addition, the phase transition temperatures of DMPC, DPPC and DSPC are ~23.5 °C, 41.4 °C and 54.5 °C, respectively (Bhardwaj and Burgess, 2010). Therefore, they are expected to have different release properties at 37 °C. A reliable *in vitro* release testing method should be able to distinguish between these formulation variants.

3.3.1. Dialysis sac

The dialysis sac method could discriminate between the non-extruded and extruded liposomes of the same lipid (Fig. 5). The release profiles of the extruded liposomes were faster compared to the non-extruded liposomes for all three lipids. Release profiles of the extruded DMPC and DPPC liposomes were similar and DSPC was slightly slower (Fig. 5). Release from all the extruded liposomes was complete within 72 h. The non-extruded liposomes showed an initial faster release followed by a slower release phase (Fig. 5).

Among the non-extruded liposomes of the three lipids, the dialysis sac method was not able to discriminate between the release profiles of the DMPC and DPPC liposomes (Fig. 5). At 12 h, 68.8% and 64.2% release was observed from the non-extruded DMPC and DPPC liposomes, respectively (Table 1). Release from the non-extruded DSPC liposomes was the slowest (30.5% in 12 h; Table 1). The dexamethasone release profiles plateaued for all the non-extruded liposomes. To achieve complete release, SDS at a final concentration 0.5% (w/v) was added to disrupt the liposome membranes (Fig. 5).

3.3.2. Reverse dialysis sac

The reverse dialysis sac method was not able to discriminate between the release profiles of the non-extruded and extruded DMPC liposomes (Fig. 6 and Table 1). At 12 h, release from the non-extruded and extruded DMPC liposomes was 76.4% and 77.6%, respectively. However, discrimination was observed between the release profiles of the non-extruded and extruded DPPC and DSPC liposomes (Fig. 6 and Table 1). For DPPC and DSPC, release from the extruded liposomes was much faster (within 72 h) compared to the non-extruded liposomes.

The reverse dialysis sac method was able to discriminate among the release profiles of the non-extruded liposomes of three lipids. The dexamethasone release from the non-extruded liposomes using the reverse dialysis sac method was faster for DMPC liposomes (within 24 h), while DSPC liposomes showed the slowest release (39.2% in 12 h) (Fig. 6 and Table 1). A plateau was reached for the non-extruded DSPC liposomes after 168 h. The addition of SDS increased the release from the non-extruded DSPC liposomes. Release from DPPC liposomes was intermediate (48.6% in 12 h), releasing slowly after day 3 until completion (Fig. 6). Unlike the dialysis sac method, release from the non-extruded DPPC liposomes was slower than the non-extruded DMPC liposomes using the reverse dialysis sac method.

3.3.3. USP apparatus 4 method

The USP 4 method was able to discriminate between the non-extruded and extruded liposomes of the same lipid (Fig. 7). Unlike the reverse dialysis sac method, release from the non-extruded DMPC liposomes (70.4% at 12 h; Table 1) was slower than that from the extruded liposomes (83.5% at 12 h; Table 1) using the USP 4 method. A faster release of dexamethasone was observed from the extruded liposomes (Fig. 7) with most of the drug released in the first 12 h (Table 1).

The USP 4 method was also able to discriminate among the release profiles of the non-extruded liposomes of the three lipids. The rank order of the release from the non-extruded liposomes was DMPC > DPPC > DSPC. At 12 h, 70.4%, 61.1% and 43.8% drug was released from the non-extruded DMPC, DPPC and DSPC liposomes, respectively. The non-extruded DPPC and DSPC liposomes did not release all their contents and reached a plateau by day 4 (Fig. 7).

Table 1
Percent release at 12 h from the extruded and non-extruded DMPC, DPPC, and DSPC liposomes (lipid:drug – 1:0.2 M).

Liposomes	Dialysis sac		Reverse dialysis sac		USP apparatus 4	
	Extruded	Non-extruded	Extruded	Non-extruded	Extruded	Non-extruded
DMPC	92.1 ± 1.2	68.8 ± 4.3	76.4 ± 2.5	77.6 ± 2.0	83.5 ± 1.9	70.4 ± 3.9
DPPC	96.3 ± 1.2	64.2 ± 3.6	88.2 ± 0.6	48.6 ± 2.2	92.9 ± 0.6	61.1 ± 1.5
DSPC	79.5 ± 1.7	30.5 ± 10.0	71.8 ± 1.8	39.2 ± 3.2	81.2 ± 2.4	43.8 ± 2.6

Each value represents mean ± SD (n = 3).

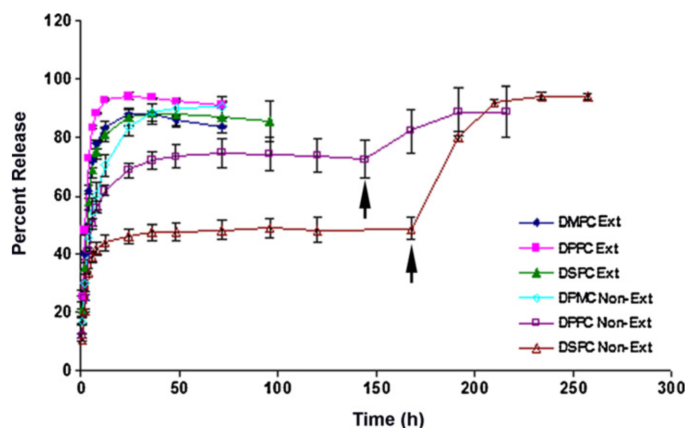

Fig. 7. Discrimination between release profiles from the extruded and non-extruded liposome formulations of DMPC, DPPC and DSPC using the USP 4 dialysis adapter. Release profiles were evaluated in 10 mM HEPES buffer, pH 7.4 at 37 °C at a flow rate of 16 ml/min. The addition of SDS is indicated by an arrow. Each value represents mean ± SD ($n = 3$).

Complete release was obtained following addition of SDS to the release medium.

3.3.4. Comparison of release from the non-extruded liposomes among different methods

The release profiles of the non-extruded liposomes of each phospholipid obtained using the three methods were plotted together for comparison between the methods (Fig. 8). Initial 12 h release from the non-extruded liposomes of the low transition temperature lipid DMPC was faster using the dialysis sac and reverse dialysis sac methods compared to the USP 4 method (Fig. 8A and Table 1), however the dialysis sac and reverse dialysis sac methods slowed down at the later time points. However, higher total release was achieved with the USP 4 method without addition of SDS. In the case of the dialysis sac method, addition of SDS was required to achieve complete release. The complete release profiles from the non-extruded DMPC liposomes were in the order USP 4 > reverse dialysis sac > dialysis sac. For the intermediate transition temperature lipid DPPC, the release profiles from the non-extruded liposomes using the dialysis sac and USP 4 methods appeared similar, while the reverse dialysis sac method showed slightly slower release (Fig. 8B). The trend was the same for the initial release (Fig. 8B and Table 1). The addition of SDS led to complete release using the dialysis sac and USP 4 methods after a plateau was reached. Complete release was observed using the reverse dialysis sac method without addition of SDS. For the high transition temperature lipid DSPC non-extruded liposomes, the overall dexamethasone release using the reverse dialysis sac and USP 4 methods was similar, while the dialysis sac method was slower and lower (Fig. 8C and Table 1). However, a similar plateau level was reached eventually for all three methods (dialysis sac ~48%; reverse dialysis sac ~49% and USP 4–48%). The addition of SDS was required for complete recovery from the DSPC liposomes using all three methods.

4. Discussion

Optimization studies for the USP 4 dialysis adapter showed that the release of dexamethasone from the solution, suspension and liposome dosage forms was not flow rate limited (Fig. 2). This

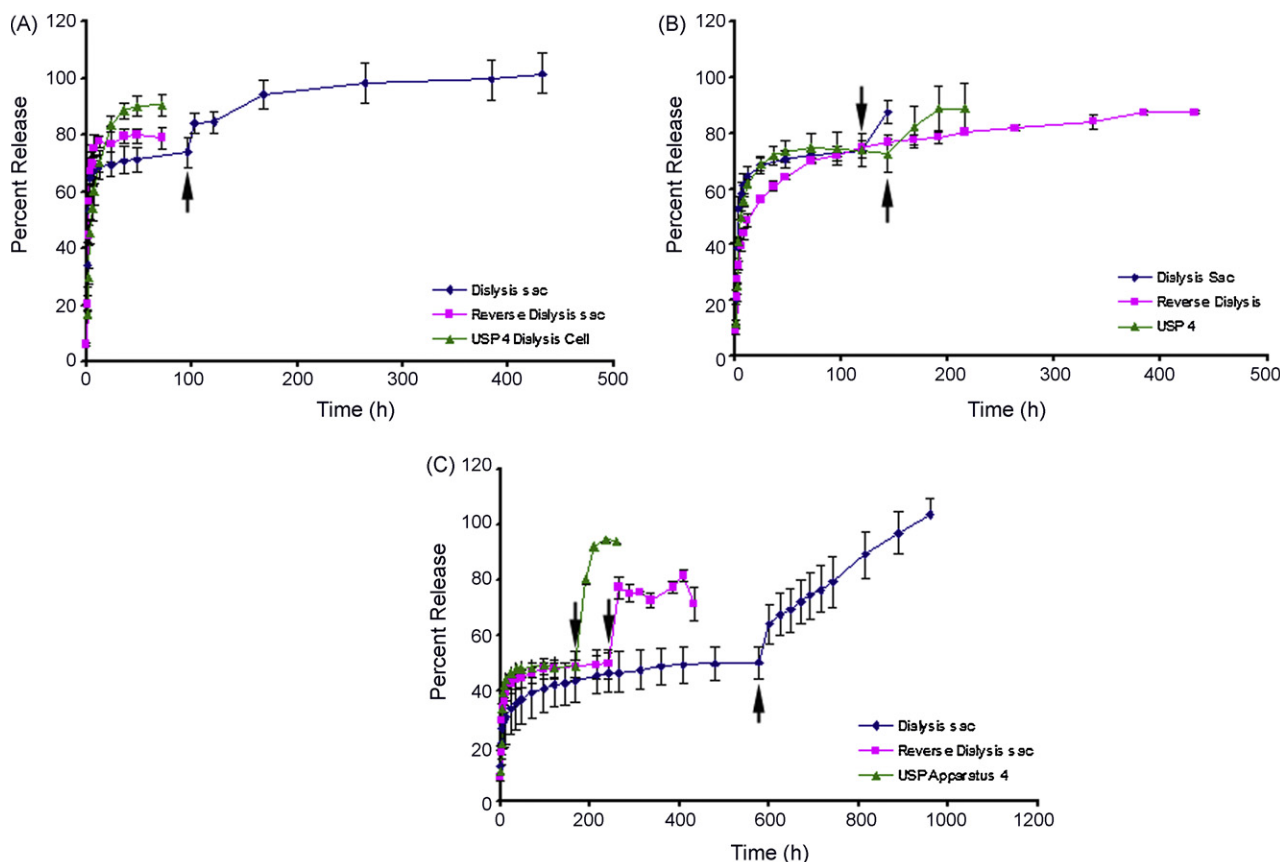

Fig. 8. Dexamethasone release profiles from the non-extruded liposomes using dialysis sac, reverse dialysis sac and USP 4 dialysis adapter methods. (A) DMPC, (B) DPPC, and (C) DSPC. Release profiles were evaluated in 10 mM HEPES buffer, pH 7.4 at 37 °C and a flow rate of 16 ml/min. The addition of SDS is indicated by an arrow. Each value represents mean ± SD ($n = 3$).

indicates that adequate agitation was obtained around the dialysis adapter in the 22.6 mm USP 4 sample cell at both flow rates. Similarly, the suspension sample volume in the dialysis adapter did not influence the percent release (Fig. 3), however the method was sensitive enough to show a difference in the time to reach the plateau concentration for the higher sample volume (Fig. 3; secondary axis). The USP 4 method was also able to distinguish drug release from the solution, suspension and non-extruded DPPC liposome formulations of dexamethasone (Fig. 4). These studies proved the feasibility of the dialysis adapter design and its utility for USP apparatus 4 for release testing of colloidal dosage forms.

For product development and quality control, an *in vitro* method should be able to discriminate between different formulation variants. Previously, it was observed that the non-extruded liposomes of DMPC, DPPC and DSPC had different physico-chemical properties compared to the sonicated and extruded liposomes (Bhardwaj and Burgess, 2010). The multilamellar non-extruded liposomes had larger particle size and approximately twice the drug encapsulation efficiency. Moreover, DMPC, DPPC and DSPC liposomes have different phase transition behavior (Bhardwaj and Burgess, 2010). Therefore, different *in vitro* drug release profiles can be expected from liposomes prepared using these three lipids at 37 °C.

Only the dialysis adapter based USP 4 method was able to discriminate among all three liposome formulations, extruded and non-extruded (Fig. 7). For each lipid used, dexamethasone release from the non-extruded liposomes was slower compared to the release from the extruded liposomes. However, the dialysis sac and reverse dialysis sac methods could not discriminate between the different liposome formulations. The dialysis sac method could not discriminate between the non-extruded DMPC and DPPC liposomes (Fig. 5). Dexamethasone release from the fast releasing DMPC liposomes appeared to be slower when using the dialysis sac method compared to the USP 4 and reverse dialysis sac methods (Fig. 8A). This may be attributed to violation of sink conditions within the dialysis sacs as the drug is released rapidly from the liposomes. The drug release from dialysis sac method is a two-step process: (1) the drug is released from liposomes to media inside dialysis sac, and (2) the drug molecule diffuses across the dialysis membrane to sink medium (where it is analyzed). The volume of media inside dialysis sac is limited (step 1). Hence, if the release is faster than diffusion across the membrane, sink conditions will not exist inside dialysis membrane slowing down the release from liposomes. This may be due to inadequate agitation in the dialysis sac method. Chidambaram and Burgess (1999) have earlier reported similar violation of sink conditions using the dialysis sac method for the *in vitro* release testing of emulsions.

The reverse dialysis sac method could not discriminate between non-extruded and extruded DMPC liposomes (Fig. 6). It appears that the higher dilution in the reverse dialysis sac method masked the difference in the physico-chemical properties of the non-extruded and extruded DMPC liposomes. Therefore, both dialysis sac and reverse dialysis sac methods might have limitations when used for *in vitro* release testing of fast releasing formulations.

Comparison of the dialysis sac, reverse dialysis sac and USP 4 methods for non-extruded liposomes prepared using the same lipid showed that the percent release for the USP 4 method was the highest or similar to the next highest method (Fig. 8). Moreover, release profiles obtained using the novel USP 4 method showed low variation among the replicates (as indicated by smaller error bars). These results underscore the robustness of flow-through USP apparatus 4 in providing adequate agitation and maintaining temperature uniformity in the sample cells. For extruded liposomes, similar release profiles were observed for all three methods using liposomes of a particular lipid. In addition, none of the methods showed a clear trend for three types of extruded liposomes studied. It was not possible to select one method over the other for extruded liposomes. This may be due to the fast release from all extruded liposomes.

The novel dialysis adapter utilizes the advantages of the compendial USP dissolution apparatus 4. The dialysis adapter based USP 4 method also presents a platform to mimic *in vivo* conditions easily. Release conditions can easily be changed during a run to provide biorelevant conditions such as addition of serum or enzymes, change in temperature or pH, and addition of a surfactant to trigger release. It might also be possible to use this method for formulations where a membrane dialysis based method is recommended at present (for example, semisolid topical formulations for which a Franz-diffusion cell is recommended) (Siewert et al., 2003). In addition, this method can also find application in purification of proteins (and other macromolecules) providing advantage of continuous buffer replenishment.

5. Conclusions

This study showed the feasibility and discriminatory ability of the dialysis adapter USP apparatus 4 method for *in vitro* release testing of liposomes and other dispersed system formulations. This novel USP 4 method was able to discriminate between different dosage form and between different liposome process and formulation variants. Whereas, the dialysis and reverse dialysis sac methods were not able to discriminate between all the formulation variants tested. This novel dialysis adapter method fulfills the need for a method based on a compendial apparatus for *in vitro* release testing of liposomes and other dispersed systems.

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References

Bhardwaj, U., Burgess, D., 2010. Physicochemical properties of extruded and non-extruded liposomes containing hydrophobic drug dexamethasone. Int. J. Pharm., doi:10.1016/j.ijpharm.2010.01.003.

Bhardwaj, U., Sura, R., Papadimitrakopoulos, F., Burgess, D., 2007. Controlling acute inflammation with fast releasing dexamethasone-PLGA microsphere/PVA hydrogel composites for implantable devices. J. Diabetes Sci. Technol. 1, 8–17.

Burgess, D., Crommelin, D., Hussain, A., Chen, M., 2004. Assuring quality and performance of sustained and controlled released parenterals. Eur. J. Pharm. Sci. 21, 679–690.

Burgess, D., Hussain, A., Ingallinera, T., Chen, M., 2002. Assuring Quality and Performance of Sustained and Controlled Release Parenterals: Workshop Report. AAPS PharmSci. 4, article 7.

Chidambaram, N., Burgess, D., 1999. A novel in vitro release method for submicron sized dispersed systems. AAPS PharmSci. 1, E11.

Clark, B., Dickinson, P., Pyrah, I., 2005. In vitro/in vivo release from injectable dispersed systems. In: Burgess, D. (Ed.), Injectable Dispersed Systems: Formulation, Processing and Performance. Taylor & Francis, pp. 125–157.

Glavas-Dodov, M., Goracinova, K., Mladenovska, K., Fredro-Kumbaradzi, E., 2002. Release profile of lidocaine HCl from topical liposomal gel formulation. Int. J. Pharm. 242, 381–384.

Hitzman, C., Wiedmann, T., Dai, H., Elmquist, W., 2005. Measurement of drug release from microcarriers by microdialysis. J. Pharm. Sci. 94, 1456–1466.

Kaiser, N., Kimpfler, A., Massing, U., Burger, A., Fiebig, H., Brandl, M., Schubert, R., 2003. 5-Fluorouracil in vesicular phospholipid gels for anticancer treatment: entrapment and release properties. Int. J. Pharm. 256, 123–131.

Kokkona, M., Kallinteri, P., Fatouros, D., Antimisiaris, S., 2000. Stability of SUV liposomes in the presence of cholate salts and pancreatic lipases: effect of lipid composition. Eur. J. Pharm. Sci. 9, 245–252.

Kostarelos, K., 2003. Rational design and engineering of delivery systems for therapeutics: biomedical exercises in colloid and surface science. Adv. Colloid Interface Sci., 147–168.

Martinez, M., Rathbone, M., Burgess, D., Huynh, M., 2008. In vitro and in vivo considerations associated with parenteral sustained release products: a review based upon information presented and points expressed at the 2007 Controlled Release Society Annual Meeting. J. Control Release 129, 79–87.

Peschka, R., Dennehy, C., Szoka, F.J., 1998. A simple in vitro model to study the release kinetics of liposome encapsulated material. J. Control Release 56, 41–45.
Ruozi, B., Tosi, G., Forni, F., Angela Vandelli, M., 2005. Ketorolac tromethamine liposomes: encapsulation and release studies. J. Liposome Res. 15, 175–185.
Sezer, A., Bas, A., Akbuga, J., 2004. Encapsulation of enrofloxacin in liposomes I: preparation and in vitro characterization of LUV. J. Liposome Res. 14, 77–86.
Shah, V., Siewert, M., Dressman, J., Moeller, H., Brown, C., 2002. Dissolution/in vitro release testing of special dosage forms. Dissolution Technol. 9, 1–5.
Siewert, M., Dressman, J., Brown, C., Shah, V., 2003. FIP/AAPS guidelines for dissolution/in vitro release testing of novel/special dosage forms. Dissolution Technol. 10, 6–15.
Vemuri, S., Yu, C., Pushpala, S., Roosdrop, N., 1991. Drug release rate method for a liposome preparation. Drug Dev. Ind. Pharm. 17, 183–192.
Voisine, J., Zolnik, B., Burgess, D., 2008. In situ fiber optic method for long-term in vitro release testing of microspheres. Int. J. Pharm. 356, 206–211.
Washington, C., 1990. Drug release from microdisperse systems: a critical review. Int. J. Pharm. 58, 1–12.
Xiao, C., Qi, X., Maitani, Y., Nagai, T., 2004. Sustained release of cisplatin from multivesicular liposomes: potentiation of antitumor efficacy against S180 murine carcinoma. J. Pharm. Sci. 93, 1718–1724.
Zolnik, B., Raton, J.-L., Burgess, D., 2005. Application of USP apparatus 4 and in situ fiber optic analysis to microsphere release testing. Dissolution Technol. 12, 11–14.