A Novel In Vitro Release Method for Submicron-Sized Dispersed Systems

Submitted April 5, 1999; Accepted: July 30, 1999; Published: August 31, 1999.

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ABSTRACT Sink conditions are often violated when using conventional release methods for dispersed systems. A novel reverse dialysis bag method was designed to overcome this problem. Model drug transport rates from submicron emulsions obtained using the conventional diffusion cell method and this novel method were compared. In the side-by-side diffusion cell method, emulsions were placed in the donor chamber and surfactant/buffer solutions in the receiver chamber. In the novel dialysis bag method, emulsions were diluted infinitely in the donor phase and surfactant/buffer solutions were placed in the receiver phase (dialysis bags). Slow release rates and linear release profiles were obtained using the side-by-side diffusion cell method apparently due to limited model drug solubility in the donor chamber resulting in violation of sink conditions. Biphasic release profiles were obtained using the dialysis bag method apparently due to an initial rapid release of free and micellar solubilized model drug from the donor to the receiver chambers followed by slow release from the oil droplets. Using both release methods, an initial increase and latter decrease in release rates were observed with increase in surfactant concentration. The initial increase was considered to be due to a decrease in the model drug oil-in-water partition coefficients and the subsequent decrease in release rates was due to micellar shape change (spheres to rods) causing a decrease in diffusion rates. Sink conditions were violated using the side-by-side diffusion cell method but were maintained in the dialysis bag method since emulsions were diluted infinitely in the donor phase.

KEY WORDS: dialysis bag, submicron emulsions, in vitro release, dispersed systems, sink conditions

INTRODUCTION

Oil-in-water (O/W) submicron-sized emulsions are commonly used for parenteral applications (1-7). In order to be an effective and reliable dosage form, submicron-sized emulsions should maintain particle size distributions of less than a micron in diameter throughout their storage time and they should have predictable drug release profiles. It is technically difficult to characterize in vitro drug release from

submicron-sized emulsions due to the physical obstacles associated with separation of dispersed and continuous phases (8). Submicron droplets have a large surface area compared to their volume, which can lead to rapid transport from the oil to the continuous phases and the potential for violation of sink conditions in the environment adjacent to the droplets (9,10). Various techniques such as the sample-and-separate method, the membrane barrier method, in situ methods, and continuous flow methods have been used to characterize in vitro drug transport kinetics from submicron-sized emulsions. However, problems are associated with each of these methods.

Sample-and-separate techniques are not ideal since it is difficult to preserve the physical integrity of emulsion droplets during the separation process. For example, to separate the released drug present in the continuous phase from the releasing source (dispersed phase droplets), filtration and centrifugation are used (11). These techniques involve the application of external energy, which can result in emulsion destabilization and, hence, erroneous results. Membrane barrier techniques include cell diffusion and dialysis bag equilibrium methods (12,13), where the dispersed phase is separated from the receiver phase by a semi-porous membrane. Barrier techniques can lead to a violation of sink conditions, due to the limited membrane surface area available for transport from the donor to the receiver chambers compared with the surface area available for transport from the dispersed phase droplets to the continuous phase. An additional constraint of the barrier methods is the limited volume of continuous phase available to solubilize the released drug in the donor chamber (14-16). In situ methods involve analysis of the released drug without separating from the releasing source. Another advantage of in situ methods is that the emulsions can be diluted infinitely so that violation of sink conditions is not a problem. However, not all compounds are suitable candidates for in situ

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techniques, as a method of analysis must be available that does not suffer interference from the dispersed phase droplets. Suitable drug candidates include molecules with fluorescent or phosphorescent moieties (17). Continuous-flow methods involve the addition of dispersed phase material to a filtration cell, which is linked in series to an analysis cell (18,19). The sink phase is continuously circulated through the filtration and analysis cells. A limitation of this technique is clogging of the filter, which causes alteration in media flow and emulsion destabilization, both of which can affect release rates.

As a consequence of the limitations of the above methods, investigators have attempted to account for the different constraints of these methods mathematically (12,15,20). The most commonly used technique for assessing in vitro transport from submicron emulsions is the side-by-side diffusion cell (barrier) method. As stated previously, the major limitation of this method is the potential for violation of sink conditions. Therefore, the objective of the present study is to develop a method for characterizing in vitro drug release from submicron-sized emulsions without violation of sink conditions. A novel in vitro bulk equilibrium reverse dialysis bag technique has been developed, and in vitro drug transport kinetics determined using this technique were compared with those determined using the conventional side-by-side diffusion cell technique. Phenylazoaniline (PAA; Aldrich Chemical Co Inc, Milwaukee, WI) and Benzocaine (BZ; Sigma Chemical Co, St. Louis, MO) were used as model drugs since they have different lipophilicities and partition coefficients despite their structural similarities (both contain an aromatic ring).

Development of Bulk Equilibrium Reverse Dialysis Bag Technique

Side-By-Side Diffusion Cell Technique

The donor and receiver chambers in the side-by-side diffusion cell technique usually have equal volume capacities and are separated by a membrane of variable pore size. Submicron-sized emulsions are placed in the donor chamber and the continuous phase is placed in the receiver chamber. The rate of drug appearance in the receiver chamber is analyzed by sampling. However, the membrane surface area available for drug diffusion is small compared with the emulsion surface area, which can lead to violation of sink conditions in the donor chamber and restriction of free drug transport to the receiver chamber. Additionally, sink conditions may be violated due to limited continuous phase in the donor chamber. k_i is the rate constant for drug diffusion from the oil to the continuous phase and k_o is the rate constant for drug diffusion from the continuous to the oil phase. k_o is negligible compared to k_i since the drug concentration gradient drives diffusion from the oil to the continuous phase. k_p is the rate constant for drug transport from the continuous phase to the receiver chamber. The first-order release rate constant k can be described as

$$k = AD/h$$

where A is the surface area of the membrane, D is the diffusion coefficient across the membrane, and h is the membrane thickness. Subscripts p and i refer to the permeating membrane and the interface between the dispersed phase droplets and the continuous phase, respectively. k is therefore, the rate-limiting factor in the overall kinetic process even if D_p is much larger than D_i , since A_i is much larger than A_p (rendering the value of k_p smaller than k_i). Therefore, it can be deduced that the side-by-side diffusion cell technique has a possibility of violating sink conditions.

In the bulk equilibrium dialysis bag technique described here, emulsion is introduced inside the dialysis bag and the continuous (receiver) phase is placed outside. An advantage of this technique is the increase in the membrane surface area available for transport from the donor to the receiver phases. This increase in area, however, is insufficient to compensate for the large oil droplet interfacial area. Emulsions in the donor phase cannot be diluted infinitely due to the restricted volume inside the dialysis bag, which can also lead to a violation of sink conditions. As a consequence of these

limitations, we have developed a bulk equilibrium reverse dialysis bag technique through which it is possible to maintain sink conditions in the donor phase by infinite dilution of the emulsion into the outer vessel. Dialysis bags containing the continuous phase (receiver phase) alone are suspended in a vessel containing the donor phase (diluted emulsion) and the system is stirred. At predetermined time intervals, each dialysis bag is removed and the contents are analyzed for released drug. The bulk equilibrium reverse dialysis bag technique overcomes the shortcomings of the side-by-side diffusion cell and bulk equilibrium dialysis bag techniques by diluting the submicron-sized emulsion in the donor chamber (theoretically the emulsion can be infinitely diluted) and by increasing the surface area of the permeating membrane (dialysis bags). Consequently, violation of sink conditions can be avoided. No separation step is required since the dispersed phase does not penetrate into the dialysis bags. Thus, the possibility of emulsion destabilization during mechanical separation is avoided. Another advantage of this method is the increased efficiency in terms of staffing as a consequence of the reduction in the number of steps.

Reverse Dialysis Bag Technique

The reversed dialysis bag technique mimics the in vivo situation more closely than the side-by-side diffusion method for emulsion dosage forms and other dispersed systems introduced directly into the bloodstream (IV) or taken orally, as these systems will experience infinite dilution following administration via these two routes.

MATERIALS

Light mineral oil and cetyltrimethylammonium bromide (CTAB) were purchased from Fisher Scientific (Springfield, NJ). Dialysis tubes (Spectra/por4, diameter 5 mm, MW cutoff: 1,000, and Spectra/por6, diameter 5 mm, MW cutoff: 50,000) with closures were purchased from Spectrum Inc., CA. All chemicals were used as received without further purification.

METHODS

Emulsion Preparation

Emulsions were prepared in 100 ml batches. 66 mg of PAA or 40 mg of BZ were dissolved in 20 ml of mineral oil. Model drug concentrations were their maximum solubilities in mineral oil at 37°C. The oil phase was mixed with 80 mL of 2% w/v CTAB solution to form coarse emulsions, which were introduced into the reservoir cell of the microfluidizer (Model 110T; Microfluidics, Newton, MA). The emulsions were passed through the microfluidizer five times at 80 psi. The microfluidizer was fitted with cooling coils, which were surrounded by ice water to rapidly cool the eluent. Emulsions were collected and immediately used in the transport studies. Emulsions were diluted 1:1 with buffer or surfactant/buffer solutions prior to the transport studies. Emulsions containing CTAB concentrations higher than 1% w/v were prepared by the addition of excess CTAB dissolved in buffer following emulsification, resulting in a 1:1 dilution. Emulsions for which no excess surfactant was added were diluted 1:1 with buffer. Consequently, all final emulsions contained 10% v/v oil phase.

Emulsion Stability Determination

Emulsions were diluted with CTAB buffer solution (0-6% w/v) and 0.5 ml of these emulsions were sealed in 1ml ampoules and placed in temperature controlled water baths $\pm 0.5^\circ\text{C}$ at 5, 25 and 37°C. Emulsions are stored for 1 month and samples were withdrawn at predetermined time intervals and analyzed for particle size. Emulsion mean droplet diameters and size distributions were determined using a Nicomp Submicron Particle Sizer (Model 370; Particle Sizing System Inc, Santa Barbara, CA). All emulsions were prepared in triplicate; all measurements were conducted three times per sample; and mean values and standard deviations were calculated.

In Vitro Release Kinetic Experiments

Model Drug Solubility Studies

The solubilities of PAA and BZ were determined in phosphate buffer pH 7.0 as a function of CTAB concentration (% w/v) and in light mineral oil (oil phase). An excess of PAA or BZ was added to samples of each medium tested. The mixtures were equilibrated at $37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ for 48 hours (until concurrent values were obtained), filtered, and analyzed spectrophotometrically. Measurements were conducted three times per sample; mean values and standard deviations were also calculated.

Side-by-Side Diffusion Cell Technique

Water-jacketed side-by-side diffusion cells (glass chambers, with a 4-mL volume and an 11-mm diameter circular opening available for diffusion) mounted with dialysis membranes (MW cutoffs: 1 or 50 kd) were used for kinetic studies of model drug release from emulsions. The water-jacketed diffusion cells were maintained at $37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$. Drug loaded emulsions and either buffer or CTAB/buffer solutions were placed in the donor cells. The receiver cells contained drug free CTAB/buffer solutions at the same concentration of CTAB as the micellar phase of the emulsions. Each chamber was stirred using magnetic stirring bars. Samples were withdrawn from the receiver cells (2 ml) and

analyzed spectrophotometrically (-maximum of PAA and BZ in CTAB solutions were 377 nm and 286 nm, respectively) at predetermined time intervals. The same volume of buffer or CTAB solution was replaced into the receiver cells to maintain volume and sink conditions. Measurements were conducted three times per sample; mean values and standard deviations were also calculated.

Bulk Equilibrium Reverse Dialysis Bag Technique

The PAA and BZ submicron-sized emulsions (5 mL) were directly placed into 500 mL of a stirred sink solution in which numerous dialysis sacs containing 2 mL of the same sink solution were previously immersed. These sacs were equilibrated with the sink solutions for about 30 minutes prior to experimentation. At predetermined time intervals, dialysis bags were withdrawn and the contents assayed spectrophotometrically for model drug concentration. The release studies were performed at a fixed temperature of $37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ under constant stirring. Measurements were conducted three times per sample; mean values and standard deviations were also calculated.

RESULTS

Emulsion stability

The mean diameters of mineral oil-in-water emulsions stabilized with CTAB were in the range of 173 to 178 nm and they had single peak size

TABLE I: Effect of storage time on mean droplet size (volume mean diameter) of emulsions diluted with different CTAB solutions (pH 7.0, I=0.2, 37°C , mean values of three determinations)

% of CTAB	Benzocaine (BZ) Mean droplet size $\pm$ S.D.		Poly dispersity values	Phenylazoaniline (PAA) Mean droplet size $\pm$ S.D.		Poly dispersity values
	0 day (nm)	30 days (nm)		0 day (nm)	30 days (nm)	
1.0	176 $\pm$ 2.0	190 $\pm$ 2.5	1.07	175 $\pm$ 1.5	188 $\pm$ 1.8	1.06
1.5	177 $\pm$ 1.0	184 $\pm$ 1.5	1.04	176 $\pm$ 0.8	182 $\pm$ 1.0	1.03
2.0	176 $\pm$ 2.0	185 $\pm$ 2.0	1.05	174 $\pm$ 1.0	181 $\pm$ 1.5	1.04
4.0	176 $\pm$ 1.0	188 $\pm$ 2.0	1.07	174 $\pm$ 0.6	180 $\pm$ 1.0	1.03

distributions (Table 1). No visible oil layer was observed on the surface of the emulsions. The CTAB emulsions stored at 37°C were considered to be stable over a 1-month study period, since there was no considerable change in the mean droplet size. The polydispersity values obtained from the Nicomp did not change with time. Both freshly prepared and 1-month-old emulsions had polydispersity values of approximately 1.04 (Table 1). Polydispersity values greater than 1.40 are considered to indicate polydispersity (21).

Figure 1: Release Rates of BZ through 1 KD membrane (side-by-side diffusion cells); error bars are within the symbols (pH=7.0, I=0.2, 37°C)

Figure 3: Release Rates of PAA through 1 KD membrane (side-by-side diffusion cells); error bars are within the symbols (pH=7.0, I=0.2, 37°C)

Figure 4: Release Rates of PAA through 50 KD membrane (side-by-side diffusion cells); error bars are within the symbols (pH=7.0, I=0.2, 37°C)

Side-By-Side Diffusion Cell Technique

During a 6 hour period, 6 and 10% of BZ were released through the 1- and 50-kd cellulosic membranes, respectively (Figures 1 and 2). Whereas, 2% and 3% of PAA were released over a 6 hour period through the 1-D and 50-kd cellulosic membranes, respectively (Figures 3 and 4). The release rates of BZ and PAA were linear with time as determined by the concentration of the model drugs in the receiver compartment. The release rates of BZ and PAA change with CTAB concentration, increasing initially with an increase in CTAB

Figure 2: Release Rates of BZ through 50 KD membrane (side-by-side diffusion cells); error bars are within the symbols (pH=7.0, I=0.2, 37°C)

concentration (up to 0.5% w/v) and then decreasing with further increase in CTAB concentration. A Shift in the -maximum of the UV absorbance was observed for BZ and PAA from 287 nm to 298 nm and 370 nm to 398 nm, respectively, in the presence of CTAB (Figure 5).

Reverse Dialysis Bag Technique

During a 1-hour period, 50 and 55% BZ were released through the 1- and 50-kd cellulosic membrane, respectively (Figures 6 and 7), whereas 25 and 35% PAA were released over a 1-hour period through the 1- and 50-kd cellulosic membranes, respectively (Figures 8 and 9). The release profiles are biphasic, with an initial rapid phase followed by a slower phase. An inflection point is observed in the rapid phase in the case of BZ. The release rates of BZ and PAA change with CTAB concentration as determined by the concentration of drug in the dialysis bag, increasing initially with an increase in CTAB concentration (up to 0.5% w/v) and then decreasing with further increase in the CTAB concentration.

Figure 5: UV absorbance spectra of BZ and PAA in the presence and absence of 1% w/v CTAB (pH=7.0, I=0.2)

Figure 6: Release Rates of BZ through 1 KD membrane (reverse dialysis bag); error bars are within the symbols (pH=7.0, I=0.2, 37°C)

Figure 7: Release Rates of BZ through 50 KD membrane (reverse dialysis bag); error bars are within the symbols (pH=7.0, I=0.2, 37°C).

Figure 8: Release Rates of PAA through 1 KD membrane (reverse dialysis bag); error bars are within the symbols (pH=7.0, I=0.2, 37°C)

DISCUSSION

Emulsion Stability

The emulsions appear to be stable over the 1-month study period, since the mean droplet diameter and the polydispersity values were relatively constant throughout the study. Emulsion stability was studied for 1 month since the model drug transport studies were completed in less than a month. It is important that the emulsions are stable throughout the duration of the transport studies. A change in the droplet diameter would affect the surface area, and this may have a considerable impact on the model drug transport rates from the oil droplets to the continuous phase.

In Vitro Release Techniques

The release rate of PAA is slower than that of BZ, since PAA is more lipophilic than BZ, which leads to a higher partition coefficient (Table 2) and therefore a lower driving force from the oil to the continuous phase. The linear release profiles obtained using the side-by-side diffusion cell technique for both PAA and BZ may be a result of constant release from the emulsion droplets, constant release from the micelles, saturation of the continuous phase, or restricted passage of drug entrapped in the micelles across the membrane.

A shift in the UV -maximum of both model drugs occurred in CTAB solutions at CTAB concentrations above the critical micellar concentration value. This implies that the model drugs form complexes with the CTAB micelles and that released drug will be mostly associated with the micellar phase. Consequently, restricted passage of drug entrapped in the micellar phase may be responsible for the slower release rates and the linear release profiles obtained. However, when the membrane pore size was increased by 50 times to allow passage of the micelles, the release rates of BZ and PAA only increased approximately twofold. This can be compared to a tenfold increase in the release rates when the reverse dialysis bag technique was used instead of the side-by-side diffusion cell method. Therefore, it appears that restricted passage of drug entrapped in the micellar phase is not responsible for the slow release rates and linear release profiles obtained using the side-by-side diffusion cell method. The surface area available for diffusion of the model drugs from submicron oil droplets is 1,234,000 cm² (calculated based on emulsion mean droplet diameter). This is considerably larger than the cellulosic membrane surface area available for diffusion from the donor to the receiver chambers (3.80 cm²). In the reverse dialysis bag technique, the emulsion samples were diluted approximately 100

TABLE II: Effect of CTAB concentration on oil/surfactant phosphate buffer solution partition coefficient of BZ and PAA (pH 7.0, I=0.2, 37°C)

CTAB (% w/v)	Partition Coefficient	
	PAA (pH 7.0)	BZ (pH 7.0)
0	120.0	1.2
0.1	10.0	1.1
0.3	3.7	1.0
0.5	2.5	0.9
0.8	2.0	0.8
1.0	1.7	0.8
1.5	1.5	0.7
2.0	1.2	0.6
3.0	1.0	0.4

Figure 9: Release Rates of PAA through 50 KD membrane (reverse dialysis bag); error bars are within the symbols (pH=7.0, I=0.2, 37°C)

times compared to twice in the side-by-side diffusion cell method. As a consequence of the restricted membrane surface area and volume of the continuous phase in the donor chamber, saturation of the continuous phase of the donor chamber may occur. Therefore, violation of sink conditions using the side-by-side diffusion cell method may be responsible for the slow release rates and linear release profiles.

A biphasic release profile was obtained for both model drugs using the reverse dialysis bag method. The faster initial rate may be due to free and micellar solubilized drug release from the donor continuous phase to the receiver chamber. The inflection point observed in this phase in the case of BZ is probably due to the membrane lag effect. The latter slower rate may be due to drug release from the oil droplets to the receiver chamber through the continuous phase of the donor chamber. From the schematics of the proposed physical models (Figure 10), it can be observed that sink conditions are easily violated in the side-by-side diffusion cell technique due to the limited volume available for solubilization of model drugs in the continuous phase of the donor chamber. Using the reverse dialysis bag technique, the system can be diluted until sink conditions are achieved.

Figure 10: Schematic diagram for drug transport in triphasic emulsion systems

Effect of Surfactant Concentration on the Release Rate of BZ and PAA

Using both release methods, the release rates of BZ and PAA increase initially with increase in CTAB concentration (up to 0.5% w/v) and then decrease. The initial increase in the release rates is considered to be due to a decrease in the O/W partition coefficients of the model drugs with the increase in the micellar phase (Table 2). The latter decrease in the release rate is considered to be due to change in the micellar shape from spheres to rods at high CTAB concentrations (20). Consequently, the viscosity of the media is increased at high CTAB concentrations; therefore, the model drug diffusion coefficients and release rates are decreased.

Effect of Membrane Pore Size on the Release Rate of BZ and PAA

When the membrane pore size was increased by 50-fold (from 1 to 50 kd), the release rates of PAA and BZ increased by approximately twofold using the side-by-side diffusion cell technique, compared to 0.1-fold using the reverse dialysis bag method. Since sink conditions are considered to be violated in the case of the side-by-side diffusion cell method, the model drugs are saturated in the continuous phase of the donor chamber. This maximizes the driving force for drug release and may explain, in part, the differences in the change in release rates observed

Side-by-side diffusion cells

Reverse dialysis bag method

for the two release methods, on increasing the membrane pore size.

ACKNOWLEDGEMENTS

The authors acknowledge Boehringer Ingelheim Pharmaceuticals Inc. for the provision of a research fellowship to N.C.

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