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# Characterisation of drug release from cubosomes using the pressure ultrafiltration method☆

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**Abstract**

Cubosomes have been proposed as a controlled release, intravenous drug delivery system. The objective of this study was to characterise cubosomes as either a therapeutically useful, controlled release delivery system, or as a burst release carrier such as submicron emulsions. The pressure ultrafiltration method and equilibrium dialysis were used to elucidate the in vitro drug release mechanisms. On dilution of cubosomes, lipophilic compounds were released rapidly when studied by the pressure ultrafiltration method. This agrees with the behaviour predicted from simple diffusion from the bulk non-dispersed cubic phase. In contrast, equilibrium dialysis incorrectly indicated sustained drug release from cubosomes. This study illustrates that cubosomes should be classified as a burst release delivery system where drug is released by diffusion from the cubic phase matrix, and that pressure ultrafiltration may have benefits over dialysis methods for measurement of drug release from colloidal particle-based drug delivery systems.


*Keywords:* Cubosome; Cubic phase; Controlled release; Lipophilic drug; Diffusion

## 1. Introduction

Potent therapeutic agents emerging from drug discovery programs are more often poorly soluble in water. In the case of injectable drugs, the poor aqueous solubility and systemic toxicity of solvents has led to the development of lipid-based formulations such as submicron emulsions. Submicron emulsions (SMEs) allow administration of a therapeutic level of drug in a reasonable infusion volume, however, they do not retard the release of drug and as a consequence are most suitable where high levels of drug in the plasma can be tolerated.

Controlled release of poorly water-soluble drugs can reduce the frequency of administration, and avoid potential toxicity due to high transient drug levels often observed on administration of 'burst release' vehicles such as emulsions. While liposomes have provided a means for controlled release of intravenously injected hydrophilic drugs (Allen et al., 1992), there is still no delivery system that meets this need for lipophilic drugs.

For lipophilic drugs, cubosomes have been proposed as a delivery system which may provide both a solubilisation benefit (increased drug payload) and also a means for controlled or sustained release. Cubosomes are submicron particles consisting of reverse bicontinuous cubic phase (Larsson, 1997), analogous

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to liposomes being a dispersion of lamellar liquid crystalline phase. There has been intense interest in the methods of manufacture (Landh, 1994; Landh and Larsson, 1996; Gustafsson et al., 1997) and structure (Siekmann et al., 1998; Larsson, 1999; Neto et al., 1999; Monduzzi et al., 2000) of dispersed liquid crystalline particles generally, with cubosomes attracting the most interest (Andersson et al., 1995; Gustafsson et al., 1996; Spicer et al., 2001; Nakano et al., 2001; Siekmann et al., 2002).

In spite of the academic and commercial interest, there are scarce reports of studies of drug release from cubosomes. To our knowledge the only in vitro study reporting sustained release from a cubosome system is for the lipophilic drug rifampicin (Kim et al., 2000). An in vivo study which found sustained plasma levels of somatostatin from cubosomes in a rabbit model has also been reported (Engstrom et al., 1996). Oral administration of a cubic phase particulate system loaded with insulin resulted in sustained reduced blood glucose levels in rats (Chung et al., 2002). The lack of data on drug release from dispersed liquid crystalline phases was the major reason for undertaking this study.

To measure drug release from colloidal delivery systems it is necessary to dilute the dispersion and monitor subsequent release of drug from the particles into the surrounding free solution. This is often not recognised in studies where methods such as equilibrium dialysis are employed. Consequently, release is often dictated by membrane transport effects, making it difficult to reconcile the results obtained in terms of release of drug from the delivery system. Pressure ultrafiltration was utilised as the principal method in this study because it allows the colloidal dispersion to be diluted directly in the release medium, and also provides a ‘snapshot’ of drug distribution between the colloidal particles and free solution at the time of filtration (Magenheim et al., 1993).

The purpose of this study was to characterise the release of lipophilic drugs from cubosomes. Diazepam was used as the principal model lipophilic compound and its release from cubosome dispersion was investigated, and compared to release obtained with the equilibrium dialysis method. To further probe the release of lipophilic compounds from the cubosome dispersion, a range of other lipophilic compounds of varying physicochemical properties were studied by pressure ultrafiltration. Diazepam release from a submicron emulsion (SME) was also conducted for comparison with the cubosome results, and to further establish the pressure ultrafiltration method.

## 2. Materials and methods

### 2.1. Materials

Drugs utilised in this study were diazepam (Alphapharm, Australia), griseofulvin (Chifeng Pharmaceutical Factory, Chifeng, China), propofol (Archimica, Origgio, Italy) and rifampicin (Sigma, Australia), which were used as received. Myverol 18-99 (Bronson & Jacobs, Australia), Lutrol F127 (BASF AG, Ludwigshafen, Germany) and Kathon CG (Rohm & Haas, Australia), an antimicrobial agent, were also used as received. Type 1 water was obtained from an AlphaQ Type 1 water unit (Millipore, Australia).

### 2.2. Preparation of drug delivery systems

Cubosomes were prepared according to the “hot-melt” method (Gustafsson et al., 1997) by dispersing a precursor solution containing Myverol 18-99, Lutrol F127 and the drug of interest into Type 1 water containing 0.1% (v/v) Kathon CG at 80 °C (Ultraturrax, 11,000 rpm, 80 °C), followed by high pressure homogenisation (HPH, Avestin C5 Emulsiflex, 10,000 psi, 60 °C, 5 passes). The hot dispersion was allowed to return to room temperature with magnetic stirring. Final concentration of Myverol 18-99 and Lutrol F-127 were 10 and 1% (w/w), respectively. Final concentrations of model drugs in the cubosome dispersions were as follows: diazepam, 4 mg/ml; griseofulvin, 0.07 mg/ml; rifampicin, 0.08 mg/ml and propofol, 2 mg/ml.

SME was prepared by dispersing a precursor into Type 1 water containing 0.1% (v/v) Kathon CG by magnetic stirring. The precursor consisted of surfactants, a long chain triglyceride and diazepam at various concentrations. SMEs containing drug at 1, 2 and 4 mg/ml were prepared. Final concentration of lipidic excipients was 10% (w/w).

Simple drug solutions were obtained by saturation of water with drug at room temperature, and subsequent dilution as required.

Table 1
Particle size and polydispersity index for submicron dispersions measured by PCS ($n = 3$, average ± S.D.)

| Dispersion | Particle size (nm) | Polydispersity index |
|---|---|---|
| Submicron emulsion | 176 ± 2 | 0.106 ± 0.009 |
| Cubosome (griseofulvin) | 141 ± 1 | 0.124 ± 0.012 |
| Cubosome (diazepam) | 227 ± 1 | 0.053 ± 0.013 |
| Cubosome (propofol) | 157 ± 1 | 0.147 ± 0.008 |
| Cubosome (rifampicin) | 197 ± 1 | 0.045 ± 0.015 |

Particle size in the diluted formulations was determined by PCS (Malvern Zetasizer 3000, Malvern Instruments, Malvern, Worcs., UK), and presented as an average of three separate determinations in Table 1. The presence of cubosomes with reverse bicontinuous cubic phase internal structure was confirmed (data not shown) by comparison of cryo-transmission electron microscopy structure and the small angle X-ray scattering pattern with previous reports (Gustafsson et al., 1996; Spicer et al., 2001; Siekmann et al., 2002).

Drug content in dispersions and ultrafiltrate/dialysate was determined by reverse phase HPLC in all cases.

### 2.3. Measurement of drug release by pressure ultrafiltration

The pressure ultrafiltration method used in these studies is based closely on that described by Magenheim et al. (1993). In this study, an Amicon 8050 pressure ultrafiltration cell fitted with a Millipore YM10 membrane was used (both obtained from Millipore, Sydney, Australia). The membranes were rinsed with Type 1 water, and soaked overnight in water for conditioning prior to use. Adsorption of drug to the membrane and equipment was characterised by forcing a free drug solution through the membrane and measuring drug concentration in the ultrafiltrate. The volume of solution required to be passed through the membrane until the concentration of drug in the ultrafiltrate was within 95% of that in the cell was determined. This volume was then discarded prior to collection of the ultrafiltrate for measurement of drug concentration at each time point during release experiments. This ensured that the concentration of drug in the ultrafiltrate sample was representative of that in the free solution and not influenced by membrane binding. In these experiments, the volume required to be discarded was less than 1 ml. For the release measurements, a 50-fold dilution was used, such that 49 ml of release medium (Type 1 water) was placed into the Amicon cell, and 1 ml of the colloidal dispersion injected into the cell while stirring ($t = 0$). At set time points, pressure was applied such that the flow rate of ultrafiltrate was approximately 1 ml/min, and sample collected (after discarding the appropriate amount of solution). An equivalent amount of Type 1 water was added to the release medium at each time point to maintain the total volume at 50 ml. A sample of the initial dispersion was used to calculate the extent of drug release. All operations were conducted at ambient temperature (22 ± 2 °C).

### 2.4. Measurement of drug release by equilibrium dialysis

Equilibrium dialysis was conducted in a side-by-side horizontal diffusion cell (Permegear, Bethlehem, PA, USA), fitted with magnetic stirring in both 4.5 ml compartments. A circular piece of Spectrapor 3, 3500 MWCO membrane (Spectrum, Breda, The Netherlands) was located between the two compartments, and equilibrated overnight with Type 1 water on both sides of the membrane. The water was removed from both sides and replaced with fresh Type 1 water, and the required volume of cubosome dispersion or free drug solution was added to the donor compartment to provide the same dilution factor as for pressure ultrafiltration. Samples were removed from the receptor compartment at appropriate time intervals and replaced with an identical volume of Type 1 water. Experiments were performed at ambient temperature.

## 3. Results and discussion

### 3.1. Dispersion properties

The size and polydispersity of the cubosomes and SME produced in the course of this study are presented in Table 1. The low polydispersity indices indicate that the mean particle size is a reasonable indicator of the size of the majority of particles in the dispersions. For the cubosome dispersion, the mean particle size from PCS agreed well with the average size observed

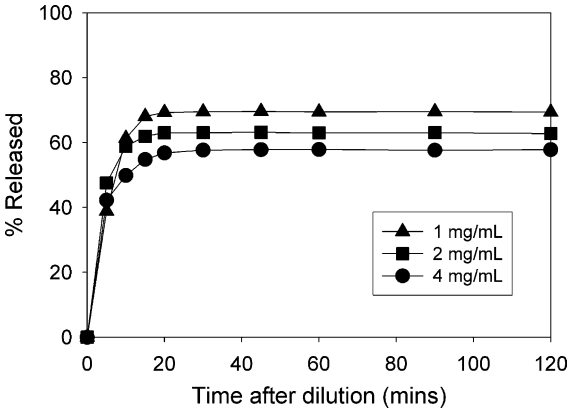


Fig. 1. Release of diazepam from a submicron emulsion using the pressure ultrafiltration method, at different levels of drug loading. Drug load in dispersions prior to dilution were 1 mg/ml (triangles), 2 mg/ml (squares) and 4 mg/ml (circles).

by cryo-transmission electron microscopy (data not shown). A small proportion of vesicular structures were also observed to co-exist with the cubosomes, which agrees with previous reports (Gustafsson et al., 1996; Spicer et al., 2001; Siekmann et al., 2002).

### *3.2. Pressure ultrafiltration method establishment using submicron emulsions*

Drug release from SMEs at the three drug concentrations was initially rapid, but reached a plateau value within 20 min (Fig. 1). The extent of release in all cases was around 60%; full release was not attained because the release medium was not an infinite sink. The drug loading did not influence the plateau value for the extent of release, which indicates that the release in these experiments is under partition control, and that the results obtained using this method are not concentration dependent. The short time taken to achieve 60% release under non-sink conditions justifies the conclusion that these systems are a burst release vehicle, and that in sink conditions the drug release would be even more rapid.

Drug release from SMEs has previously been reported to occur via burst release. For example, release of diazepam (Benita and Levy, 1993) and miconazole (Magenheim et al., 1993) from a SME, determined using pressure ultrafiltration, was found to be very rapid when sufficient sink condition was utilised. The use of albumin in the release medium allowed a greater extent of release than when no albumin was used in the reported study, by providing solubilisation of drug and hence a sink condition in the aqueous release medium.

Ideally for drug release measurements, an infinite sink is employed to allow complete release of drug from particles into the surrounding solution. For lipophilic drugs under partition control, dilution by many orders of magnitude may be required in order to allow a reasonable percentage of drug to partition out of the particles into the surrounding solution. This often leads to analytical sensitivity issues and high proportions of non-specific binding. Alternative approaches, such as addition of surfactants or proteins to provide solubilisation of released drug, and hence a sink condition, have been utilised by previous workers, however, there is always a risk of interference of the added surfactants with the structure of the colloidal particles themselves. This was felt to be even more critical when dealing with colloidal liquid crystalline particles, where the presence of surfactants is likely to induce a change in the self-assembled phase that comprises the cubosome particles, and hence was not employed in this study.

### *3.3. Drug release from cubosomes by pressure ultrafiltration*

Release of a range of lipophilic drugs from cubosomes including diazepam was investigated, and in each case the release had reached a plateau value within 20 min (Fig. 2). The drugs were not completely released again because non-sink conditions were prevalent.

Based on the results obtained for the SMEs, the plateau value for extent of release is expected to depend on the partitioning of drug from the lipid regions of the cubosomes, and not on absolute drug concentration. Therefore, it is anticipated that the partition coefficient of the lipophilic drug will dictate the extent of release compared to other drugs at the same dilution. The rank order for the extent of release for the three unionised drugs, griseofulvin, diazepam and propofol was as expected upon consideration of published octanol–water partition coefficient ($\log K_{o/w}$) listed in Table 2. The lower affinity of griseofulvin for the hydrophobic phase provided the greatest extent of release, and conversely for propofol. Rifampicin

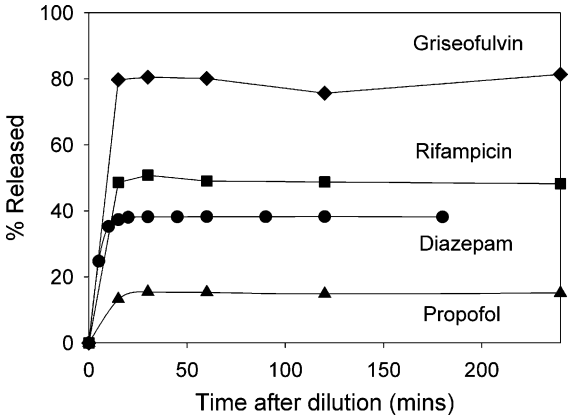


Fig. 2. Release of lipophilic drugs from cubosome dispersions by pressure ultrafiltration. Dispersions were loaded with griseofulvin (diamonds), rifampicin (squares), diazepam (circles) and propofol (triangles).

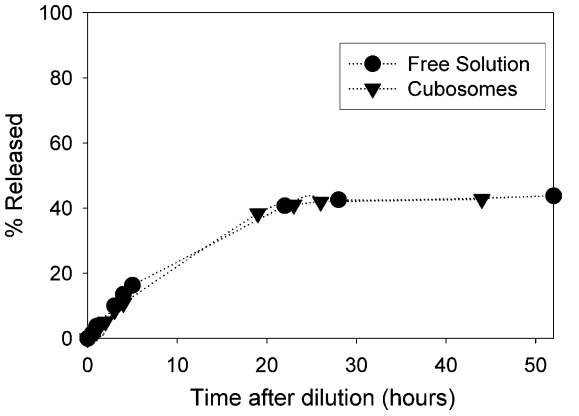


Fig. 3. Release of diazepam from cubosomes (triangles) and free solution (circles) by equilibrium dialysis.

would be expected to be ionised to some degree in the neutral dispersions ($pK_a \sim 7.9$; Merck Index, 1989), but no control over pH was employed, so it is difficult to rank the overall lipophilicity of rifampicin in these experiments relative to the other three drugs.

The plateau value for the release of diazepam from cubosomes was lower than that from the SMEs at the same dilution. A possible reason for this may be that the water-soluble surfactants used to prepare SME are providing some solubilisation of drug in the surrounding aqueous solution. This would lead to partitioning of the drug in favour of the aqueous phase at equilibrium, resulting in the greater extent of release from the SME at the same dilution.

The principal objective of this study was to discriminate between delivery systems that exhibit burst release and those that provide therapeutically useful controlled or sustained release over a period of time. If the drug release is completed within 1 h, the delivery system is unlikely to provide any real benefit from a controlled release point of view. Determination of the exact release kinetics from these systems was not necessary for this study so that although non-sink conditions were utilised, the time required for drug release to plateau still indicates whether or not a useful release mechanism prevails. Dilution of the cubosomes containing lipophilic drugs in this study displayed a burst release phenomenon, leading to the conclusion that release in all cases would be completed within an hour if an absolute sink was employed. Hence, it is unlikely that any therapeutic benefit, by virtue of controlled release, could be obtained using cubosomes as a delivery system for lipophilic drugs.

Table 2
Extent of release at equilibrium for 50-fold dilution of cubosome dispersions and literature octanol–water partition coefficients

| Drug | Extent of release (%) | $\log K_{o/w}$ (Reference) |
|---|---|---|
| Griseofulvin | 81 | 2.2 (Leo et al., 1971) |
| Diazepam | 38 | 2.9 (Taillardat-Bertschinger et al., 2002) |
| Propofol | 15 | 3.8 (Hansch et al., 1995) |
| *Rifampicin* | *48* | *4.2*[a] |

[a] c log *P* value calculated using the KowWin software program (http://esc.syrres.com).

### *3.4. Drug release from cubosomes by equilibrium dialysis*

Diazepam release from cubosomes when measured by equilibrium dialysis, was extremely slow in contrast to that measured by the pressure ultrafiltration method. Without the insight of results obtained in the previous section, Fig. 3 would appear to indicate that the release of diazepam from the cubosome particles takes place over >24 h.

In order for dialysis to reflect release of drug from the colloidal particles, the free drug concentration on the receptor side must be representative of the free drug concentration on the donor side at all times (or

at least at times much shorter than the release process itself). Washington has pointed out that dialysis methods suffer from membrane limited diffusion of the free drug from the donor compartment to the receptor compartment. The concentration of drug in the receptor compartment lags significantly behind that of the donor compartment, and hence is not useful in following drug release from colloidal particles over times shorter than days (Washington, 1989).

The final extent of release in the equilibrium dialysis experiment under non-sink conditions, should be representative of the true drug distribution between particles and free solution, even though the kinetics are not reflective of drug release over time. In order to test these effects, simple diazepam solution was placed into the donor compartment, at a concentration twice that in the receptor compartment at equilibrium in the cubosome equilibrium dialysis, which allows equilibration of drug into the receptor compartment to approximately the same concentration as that in the cubosome experiment.

It was found that the time course of equilibration of free solution between the donor and receptor compartments was almost identical to that for drug appearance in the receptor compartment in the cubosome experiment (Fig. 3). This indicates that the experiment conducted to measure drug release from cubosomes using this method actually measures equilibration of the free drug component from the donor compartment to the receptor compartment irrespective of the drug release rate, where the release rate is faster than diffusion through the membrane. In contrast, the pressure ultrafiltration method provides an instantaneous snapshot of drug distribution between the particles and free solution, and if sufficient dilution towards an infinite sink is utilised, the drug content of the ultrafiltrate is close to representing true drug release. It was significant that the extent of release at equilibrium was the same for the cubosomes in Figs. 2 and 3. This is expected because the same dilution was employed; only the apparent release kinetics differ, due to the slow transport of drug across the dialysis membrane.

It should be noted that, in terms of extent of release, it is coincidental that the two curves overlay one another in Fig. 3. In the case of the free solution, some binding to the membrane and apparatus is evident, as the equilibrium extent of release should be 50%, and the cubosome curve overlays this only because of the specific partition coefficient for the system chosen.

These findings have implications for previous reports of rifampicin release from cubosomes measured by equilibrium dialysis (Kim et al., 2000). The lipophilic nature of rifampicin, and the burst release illustrated in Fig. 2 using the ultrafiltration method, indicates that the conclusion that rifampicin release is slow from cubosomes may have been an artefact of the method used to study release, and not representative of the true release mechanism. While non-sink conditions were utilised in the pressure ultrafiltration experiments reported in this study, almost half the drug was released within 20 min of dilution; were an infinite sink present this would occur even more rapidly, which makes it difficult to reconcile the slow release observed previously. Attempts to monitor rifampicin release from cubosomes using equilibrium dialysis for direct comparison with previous reports were not successful due to degradation of the drug in the neutral aqueous medium over the extended time period required for the equilibrium dialysis experiment. No degradation was apparent over the relatively short time frame required for the pressure ultrafiltration experiment.

### *3.5. Anticipated drug release rates from cubosomes based on simple diffusion considerations*

Consideration of simple diffusion kinetics dictates that controlled release is unlikely from colloidal particles comprised of reverse bicontinuous cubic phase. There are numerous reports of slow release from the matrix of the bulk reverse cubic phase for a range of lipophilic and hydrophilic drugs (Engstrom, 1990; Wyatt and Dorschel, 1992; Burrows et al., 1994; Chang and Bodmeier, 1997, 1998; Helledi and Schubert, 2001) peptides and proteins (Ericsson et al., 1988; Engstrom, 1990; Ericsson et al., 1991), as well as haemoglobin (Leslie et al., 1996). Shah et al. (2001) have recently reviewed the use of cubic phase gels as drug delivery systems. Release from the cubic phase matrix in most cases has been shown to follow Higuchi diffusion-controlled kinetics (Higuchi, 1967), where diffusion out of the matrix shows a linear dependence with the square root of time according to Eq. (1):

$$Q = [D_m C_d (2A - C_d)t]^{1/2} \quad (1)$$

Table 3
Comparison of drug release from bulk cubic phase and cubosomes from available data for diazepam and somatostatin

| Bulk phase release | Diazepam | Somatostatin |
|---|---|---|
| Total volume of cubic phase ($cm^3$) | 1[a] | 1[b] |
| Surface area for release ($cm^2$) | 0.785[a] | 1[b] |
| Measured $Q$ ($mg/min^{1/2}$) | 51.2[a] | 500[c] |
| Cubosome release[d] | | |
| Total volume of cubic phase ($cm^3$) | 1 | 0.15 |
| Total surface area ($cm^2$) | $1.7 \times 10^5$ | $7.8 \times 10^4$ |
| Surface area relative to bulk release ($cm^2$) | $2.2 \times 10^5$ | $7.8 \times 10^4$ |
| $Q'$ ($mg/min^{1/2}$) | $1.1 \times 10^7$ | $3.9 \times 10^7$ |
| Mass of drug loaded (mg) | 40000 | 10.5[e] |
| Estimated 100% release time (min) | $1.2 \times 10^{-3}$ | $1.0 \times 10^{-11}$ |

Calculations are based on Eq. (1).

[a] Obtained from measurement of release of diazepam from GMO bulk non-dispersed cubic phase. Ratio of volume cubic phase:release medium was identical to cubosome experiment to allow direct comparison. $Q$ obtained from slope of mass released vs. $t^{1/2}$ (unpublished results).

[b] Assumes a 1 $cm^3$ cube of cubic phase, with one exposed face was used for the somatostatin bulk phase release study in Ericsson et al. (1988).

[c] Value for $Q$ interpreted from plot in Ericsson et al. (1988), assuming 500 ml release medium.

[d] Calculations assume cubosome dispersion to consist of monodisperse cubic particles, whose diagonal corner to corner "diameter" is 200 nm.

[e] Inferred from data of Engstrom et al. (1996), 7 mg/ml dispersion containing 10% cubic phase, 1.5 ml injection.

where $Q$ is the amount of drug released per unit area of matrix, $D_m$ is the diffusion coefficient of the drug in the matrix, $A$ is the initial amount of drug in unit volume of matrix, $C_d$ is the solubility of drug in matrix and $t$ is time.

Division of the bulk cubic phase into cubosomes greatly increases the surface area through which drug diffusion can occur. The diazepam example in Table 3 shows that for the same total volume of cubic phase, the surface area in the 200 nm cubosome dispersion is over 200,000 times greater than the equivalent single, cubic particle of cubic phase, with one exposed surface. As a consequence, drug may be transported into solution at a much greater rate. Because the solubility, loading and diffusion of drug through the cubic matrix are expected to be unchanged on division of the cubic phase into cubosomes, Eq. (1) can be used to calculate the release rates based on data found for the bulk phase release.

Table 3 lists the predicted rates of release of diazepam and somatostatin from cubosomes based on available data from release from bulk cubic phase and existing cubosome studies, using simple geometry and Eq. (1). The calculations illustrate that in both cases, release would be complete within fractions of a second, which is supported by the experimental findings in this paper. In the case of somatostatin, in vivo data show an elevated plasma level over 5 h (Engstrom et al., 1996). The sustained plasma level was attributed to retention of the drug inside the cubosome structure—a conclusion difficult to reconcile on the basis of these simple diffusion measurements and data presented earlier in this paper.

Using the diffusion considerations described above, the particle size that would provide a useful release profile may be calculated. A controlled release, intravenous dosage form would be very useful if it were able to match the free drug levels of a long infusion of a solution of poorly water-soluble drug over ca. 10 h. Using the diazepam data for this purpose, a surface area of 42 $cm^2$, with a resultant particle diameter in the range of 1 mm is required, in order that the surface area provides the correct flux of drug out of the particles to match the time requirement. Thus, it is unlikely that submicron dispersions of liquid crystalline phase suitable for intravenous administration, will be able to provide useful release profiles for lipophilic drugs without some other modification to prevent drug release into the general circulation. The use of functionalised phospholipids as a possible means of retaining drug inside the cubosome has been described recently by Lynch and Spicer (2002).

The temporal resolution of the pressure ultrafiltration method is limited by the time taken to remove a sample of sufficient volume for analysis, which is of the order of a minute, so the true release rates may only be measurable by complicated spectroscopic techniques.

These findings are also important in the wider context of regulatory requirements for measuring controlled release from colloidal dosage forms. No clear guidelines exist as to the methods that should be adopted in order to qualify drug release in a research, manufacturing or quality control setting for products of this type. As recently as September 2002, at a workshop on Dissolution/In Vitro Release Testing and Specifications for Special Dosage Forms, it was concluded that "reverse dialysis systems may be appropriate" for the measurement of drug release from colloidal particles in parenteral dosage forms. Reverse dialysis, while being faster to equilibrate than regular equilibrium dialysis, still suffers from membrane limited diffusion into the receptor phase (Levy and Benita, 1990). Data obtained earlier in this lab indicates that it takes in the order of hours for lipophilic drugs in general to equilibrate across the membrane at typical concentrations used in release studies (unpublished data). Hence it is unlikely that this method is adequate for measuring drug release from colloidal particles used as an intravenous delivery system, and at this juncture, pressure ultrafiltration may provide the best approach from both a research and a commercial perspective.

## 4. Conclusions

Cubosomes and SMEs have both been shown to be burst release delivery systems for lipophilic drugs. The burst release nature of cubosomes can be shown to be inevitable based on the diffusion kinetics which govern the release of drug from the non-dispersed bulk cubic phase. Equilibrium dialysis may provide misleading results for in vitro release of lipophilic drugs from colloidal drug delivery systems. Pressure ultrafiltration is a more useful method which allows elucidation of the drug release mechanism from colloidal dispersions, as it provides an instantaneous 'snapshot' of drug distribution between the particles and free solution.

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