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Characterization of 5-fluorouracil loaded liposomes prepared by reverse-phase evaporation or freezing-thawing extrusion methods: study of drug release

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Entrapment of the anti-tumoral drug 5-fluorouracil (5-FU) in unilamellar liposomes prepared by freeze-thawing extrusion technique (FATVET) and the reverse-phase evaporation method (REV) from natural (bovine brain) sphingomyelin (SM) and synthetic distearoylphosphatidylcholine (DSPC) phospholipids was studied. Reverse-phase evaporation vesicles obtained from DSPC sized through polycarbonate membranes of 0.2 μm pore size were found to entrap roughly double amounts of drug than did extruded liposomes (0.1 μm pore size); however, s-REV in these preparations were more heterogenous in vesicle size than FATVET. The rate of in vitro drug release from the liposomes was found to be dependent of the bilayer composition and the method used to prepare the vesicles. The permeability coefficient P obtained was approx. 10^{-11} m/s. The results suggest that 5-FU release is kinetically controlled by an interfacial process seemingly dependent on the surface activity of the drug. Also, the physical state of the bilayer determines the retention capacity of the vesicles. Thus, liposomes consisting of distearoylphosphatidylcholine whose acyl chains were in a gel state at the working temperature (37°C) retained 70% of encapsulated 5-FU after 1 h, whereas liposomes composed of natural bovine brain sphingomyelin retained only 15% over the same period.

Introduction

The use of drug carriers for improving drug therapy effectiveness relies on three distinct rationales, viz. controlled drug release, site-directed delivery and site-avoidance delivery. The drug must be released from the carrier and reach its therapeutic target to be effective. Hence the carrier must be able not only to protect the drug during delivery, but also to release it whenever needed.

Liposomes are biocompatible, biodegradable microvesicular systems consisting of at least one phospholipid bilayer. They can encapsulate water-soluble compounds in internal aqueous compartments (whether in their core or between lamellae) and/or intercalate lipophilic molecules in their bilayer(s). Liposomes can be prepared as sterile, pyrogen-free suspensions in submicron diameters in order to facilitate intravenous injection [1–3].

The in vivo behaviour of a liposomal drug delivery system can only be studied reliably after a knowledge of the in vitro release rate of an entrapped drug has been acquired. Drug release from liposomes into the host biophase has been accounted for on the basis of a number of mechanisms, one of which assumes passive diffusion of the drug through the liposome bilayer and subsequent diffusion through the cell membrane. In this respect, it is thus pertinent to determine the potential effect of some structural and physico-chemical parameters on the overall release profiles for liposomes. Because of the very nature of interactions between molecules, permeation in a lipid membrane cannot be dealt with as a physical, but rather as a physicochemical process in which interfacial and bulk proper-

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Abbreviations: 5-FU, 5-fluorouracil; SM, bovine brain sphingomyelin; DSPC, L-α-distearoylphosphatidylcholine; MLV, multilamellar vesicles; FATMLV, frozen and thawed multilamellar vesicles; SUV, small unilamellar vesicles; REV, reverse-phase evaporation vesicles; FATVET, vesicles obtained by cyclic freeze-thawing and extrusion technique; Tris, tris(hydroxymethyl)aminomethane; SCB, sodium citrate buffer; V_i, overall internal volume of the liposome suspension; DSC, differential scanning calorimetry; QELSS, quasi-elastic light scattering spectroscopy; $\langle d_h \rangle$, mean hydrodynamic diameter; PD-index, polydispersity index; S.D., standard deviation; CV, Pearson's coefficient of variation; RMS, residual mean square; R^2, determination coefficient.

ties are mutually related. Hydration of polar headgroups, bilayer curvature and phospholipid molecule packing at each monolayer making up a bilayer, in addition to the potential surface activity of the permeant, are some relevant factors which contribute to the permeability coefficient [4].

Some aspects of data processing in relation to diffusion experiments are of utmost importance for a wide variety of pharmaceutical preparations. Guy et al. [5] derived some expressions for the rate of drug release from various spherical entities such as emulsions, microcapsules and liposomes.

The aim of this work was to elucidate the permeation behaviour of two liposomal preparations obtained by the reverse-phase evaporation method (REV) and the extrusion method (FATVET). The entrapped drug was 5-fluorouracil (5-FU), a powerful hydrophilic antitumoral agent widely used in solid tumour treatment and dermatological applications. Study was carry out to the physiological temperature of 37°C. Bovine brain SM and DSPC were chosed as components of lipidic vesicles because their respectives thermotropic properties allow us to study the effect of molecular packing in bilayer at 37°C warranting at the same time drug retention during a reasonable period of time. The goodness of fit of the experimental data to reported expressions for various plausible mechanisms was determined by an analysis of variance (ANOVA).

Materials and Methods

Lipids and other reagents

Bovine brain sphingomyelin (SM) and L-α-distearoylphosphatidylcholine (DSPC) were purchased from Sigma. Their nominal purity, 99%, was checked by usual thin-layer chromatographic methods. Both substances were used without further purification. Tritiated 5-fluorouracil ([^{3}H]5-FU) was supplied by Amersham International. All other chemicals used were analytical-grade reagents and bidistilled water was employed throughout.

Preparation of liposomes

Reverse-phase evaporation vesicles (REV) were prepared according to Szoka and Papahadjopoulos [6]. Lipid components were weighed in a long-neck, round-bottom quickfit flask and dissolved in 2:1 v/v isopropyl ether/chloroform. A volume of buffer solution (10 mM Tris-HCl + 140 mM NaCl, pH 7.4) containing 200 mM 5-FU (2 μCi/ml) was then added in an organic-to-aqueous phase ratio of 3:1. The flask was sealed under a nitrogen atmosphere and the mixture sonicated for 6–10 min at 40°C (SM) or 55°C (DSPC) in an Bransonic B-12 50-W ultrasonic bath. An emulsion was produced as a result from which the organic solvent was gradually removed by heating at 45°C in vacuo until its odour disappeared, after which the flask was flushed with nitrogen for 15 min. In order to remove the largest particles and hence obtain a more homogeneous liposome population, the suspensions were extruded through polycarbonate membranes of 0.2-μm pore size in a Nucleopore filtration cell with stirring at a maximum working pressure of $4.8 \cdot 10^5$ Pa [7]. Such preparations will henceforward be referred to as s-REV (sized reverse-phase evaporation vesicles). In a previous work the termothropic gel to liquid-crystalline phase transition of s-REV composed of synthetic phosphatidylcholines was monitored by DSC, fluorescence polarization of 1,3,5-diphenylhexatriene, 90°C light scattering and leakage of carboxyfluorescein. Results were compared with those of SUV and MLV of the same composition [8]. Results are in agreement with the existence of a majority of LUV structures [9,10] although it's not possible to discard the presence of a small proportion of oligolamellar vesicles.

On the other hand, the FATVET acronym denotes suspensions obtained after five freezing–thawing cycles in liquid nitrogen and warm water (50°C) prior to extrusion (10 cycles) of conventional MLV containing [^{3}H]5-FU dissolved in 10 mM Tris-HCl + 140 mM NaCl buffer of pH 7.4 at a mild pressure ($55 \cdot 10^5$ Pa) through two stacked polycarbonate membranes [11] of 0.1-μm pore size. A Lipex Biomembranes (Vancouver, Canada) extruder was used for this purpose. The initial concentration of phospholipid in the aqueous medium was 60 μmol/ml for all preparations. Others authors had demostrated that under aforementioned conditions all the liposomes are transformed into unilamellar structures with an out/in ratio of 1.0 [11].

In order to remove non-encapsulated drug, each liposomal preparation was passed through a chilled (1.5×7.0 cm) Sephadex G-75 column. Vesicles were eluted from the column and their phospholipid concentration determined according to Bartlett [12]. The amount of 5-FU entrapped by the vesicles is expressed as a percent fraction of that initially present in the medium.

Determination of the entrapped volume

The overall internal volume, V_i, of all the preparations was determined from the ratio of radioactivity (i.e., the [^{3}H]5-FU content) to the phospholipid content before and after gel exclusion chromatography. All trapped volumes are expressed in litres of overall trapped aqueous volume per mol of phospholipid.

Calorimetric determinations

Calorimetric measurements were made on a Mettler TA 3000 thermal analyser equipped with a DSC-20 measuring cell. The sample preparation and thermometric and enthalpic calibration procedures used were described in detail elsewhere [13]. Sample volumes of

20 μl containing approx. 1 mg of phospholipid were placed in 50-μl crucibles and scanned at a rate of 2 K/min. Before the temperature programme was executed, the samples were kept at the initial temperature (5°C for SM and 15°C for DSPC) for 15 min.

Determination of mean size and size distributions

The mean diameter of the liposomes and the size distribution of their suspensions were determined by quasi-elastic light-scattering spectroscopy (QELSS) using a Coulter N4 submicron particle analyser. Data were processing by the software bundled to instrument. In unimodal analysis cumulant expansion is used. In multimodal mode (SDP routine) an approximation based in Provencher's CONTIN program [14] resolved the components of polydisperse samples into discrete size subpopulations and delivered their respective proportions on no assumption of the size distribution shape. This technique has been applied to this type of system by several authors [15–19]. The measuring instrument was calibrated with monodisperse dispersions of polystyrene spheres of 91 nm and 310 nm nominal diameter. At least 10 samples of each type of liposome were measured at 20°C [15] using running times of their 200 or 2000 s and sampling times between 1.5 and 4.5 μs. The 3–3000 nm size range was used for this purpose. The scattering intensity of the dited liposomal dispersions was always between 10^5 and 10^6 cps. For further details interested readers are refered to Elorza et al. [19].

Study of drug release

(a) Experimentals. In vitro release experiments involved dispensing 0.5 ml aliquots of liposomal suspension into collodion bags (Sartorius, molecular mass cut-off = 20 kDa). The bags were transferred into test tubes of 2.5-cm internal diameter containing 10 ml of buffer which were then stoppered and immersed in a bath thermostated at 37°C. In order to ensure homogeneity in the dialysis medium, the tubes were stirred magnetically throughout the incubation period. Released drug was monitored at appropriate times (5, 10, 15, 30 and 60 min) by counting dialysate aliquots diluted in Normascint 22 cocktail in an Analytical Kontron BETAmatic I scintillation counter.

(b) Processing of data. We have adopted the theoretical model proposed by Guy et al. [5] for drug release from spherical particles. The model assumes that the size of the liposomes, which are considered to be spheres, remains unchanged over time, and that the diffusion coefficients are concentration-independent. So, fairly simple solutions to the differential equations correspondig to the Fick's second law (expressed in spherical coordinates) are obtained. Table I lists the equations obtained on imposing two different boundary conditions.

TABLE I

Solutions to the differential equation of Fick's second law for the theoretical model of Guy et al. [5] (rate of drug release from spherical particles with or without phase boundaries)

Equation	Application
	A. Diffusion from a sphere without phase boundaries:
1 $M_t/M_\infty = 6\pi^{1/2}kt^{1/2}$	(1) Short-time approximation
2 $M_t/M_\infty = (1-(6/\pi^2)\exp[-\pi^2 kt])$	(2) Long-time approximation
	B. Diffusion from a sphere with phase boundaries: — Slow interfacial kinetics ($\kappa \ll 1$)
3 $M_t/M_\infty = 3k\kappa t$	(3) Short-time approximation
4 $M_t/M_\infty = (1-\exp[-3k\kappa t])$	(4) Long-time approximation

In those equations M_t is the amount of drug which diffuses out of the sphere over time t and M_∞ is that which would be released after an infinite time (and hence the amount contained in the sphere at time zero). Also, $k = D/r^2$, where r is the sphere radius and D the diffusion coefficient of the drug.

In the absence of a phase boundary between the membrane and the aqueous phase, the concentration at the sphere surface remains zero. In the presence of a phase boundary at the sphere surface, a slow interfacial transfer is the rate-determining step for drug release. Such a step can be schematized as

$$C_{membrane} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_{aqueous}$$

where k_1 is the kinetic rate constant of drug transfer from the membrane (usually the organic phase) to the aqueous environment. The weight of the interfacial process relative to that of diffusion through the membrane can be expressed via a relation between k_1 and D using the following dimensionless quantity: $\kappa = k_1(1/D)$.

The cumulative [^{3}H]5-FU release results, expressed as percent fractions of the amounts initially present in the vesicles, were analysed statistically by linear fitting of Eqns. 1 and 3 and non-linear fitting of Eqns. 2 and 4 using the BMDP AR software [20]. The goodness of fit of the functions was statistically compared via an analysis of variance (ANOVA) for the regression. Based on the residual mean squares (RMS) and determination coefficients (R^2) obtained the best fit was provided by the following equation

$$A_i = A_{eq}(1-e^{-k_{ov}t_i}) + \epsilon_i \qquad (5)$$

which corresponds to a first-order kinetic process. In this equation, A_i denotes cumulative release, expressed as a percent fraction of time t_i; A_{eq} the maximum fraction released at equilibrium ($t_i = \infty$); k_{ov}

the overall rate constant (viz. a first-order rate constant); and ϵ_i a random error such that $\Sigma(\epsilon_i) = 0$ and $V(\epsilon_i) = \sigma_i^2$.

The ratio of the flux J to the concentration difference ΔC between the inner and outer solutions defines the phenomenological permeability coefficient, $P = J/\Delta C$, from which the following expression can be derived

$$P = \left(\frac{V_{aq}}{S_m}\right) k_{ov} \tag{6}$$

V_{aq} being the volume of the liposomal aqueous core, S_m the surface area of the vesicle membrane and k_{ov} the overall release rate constant. Application of the spherical structure hypothesis, irrespective of the bilayer thickness, simplifies Eqn. 6 to

$$P = \left(\frac{r}{3}\right) k_{ov} \tag{7}$$

where r is the vesicle radius.

Because of the heterogeneity of s-REV dispersions, the radius values used to calculate P and k_i were obtained from mean values determined by multimodal analysis of QELSS data rather than from unimodal analysis results.

Results and Discussion

Size distribution

Clearance of liposomes from the blood stream is influenced by their surface composition and particle diameter (the largest ones are cleared first). For this reason, it is of utmost importance to determine the mean diameter and size distribution of liposomal dispersions. Table II shows the mean hydrodynamic parameters obtained for the various liposomal preparations studied in this work as calculated by unimodal analysis of QELSS data. It also gives the polydispersity index for the preparations.

s-REV liposomes are the largest; also, DSPC liposomes are approx. 25% larger in diameter than the SM

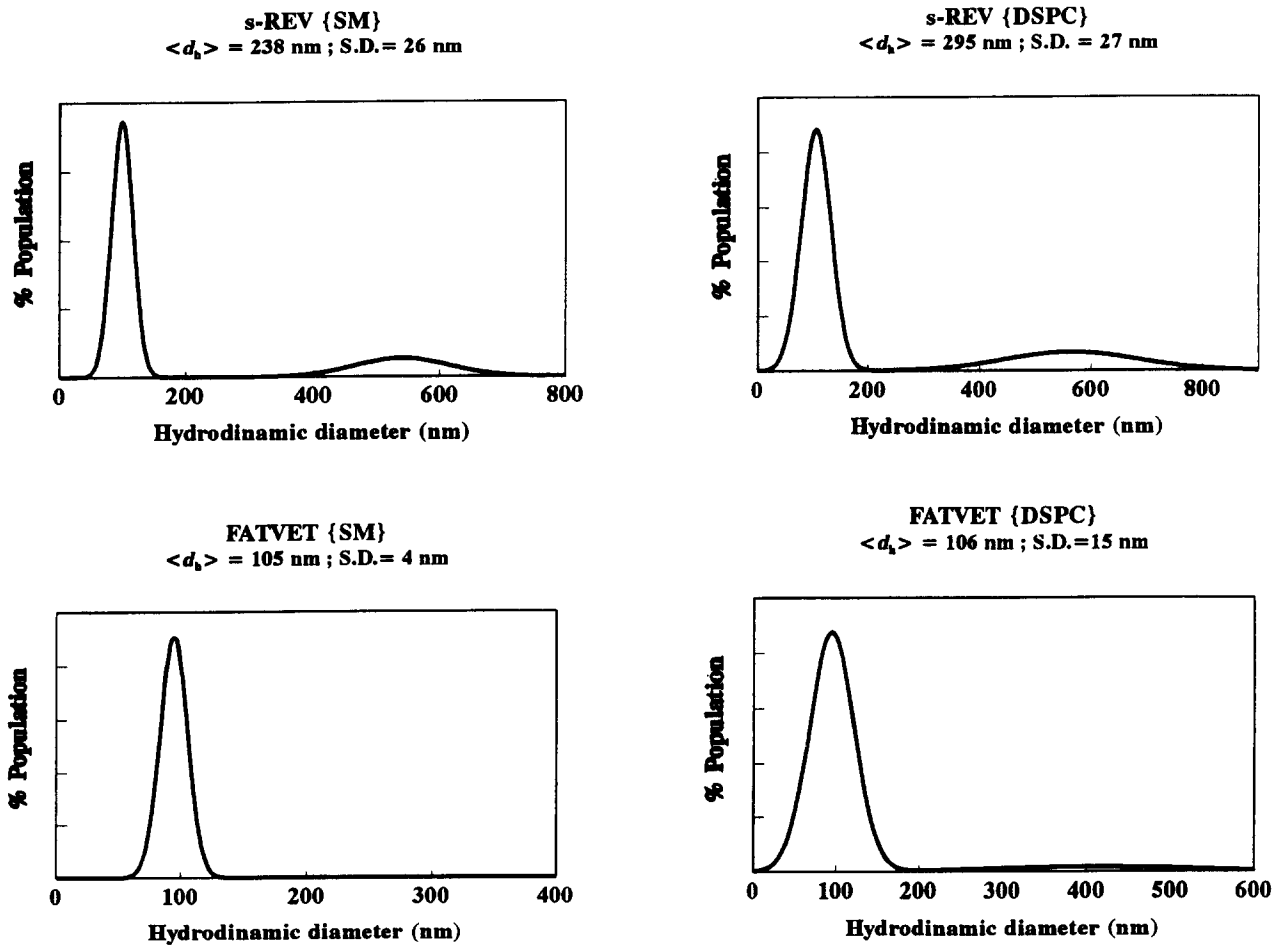

Fig. 1. Plot of fraction of particles against the hydrodynamic diameter of the equivalent sphere for various liposomal preparations. A Gaussian size distribution was assumed for the mean hydrodynamic diameter and standard deviation of each subpopulation as obtained by multimodal analysis by means of SDP routine. The functions were normalized in such a way that the area under the curves was proportional to the statistical weight of each population. $\langle d_h \rangle$ and S.D. figure labels are the values obtained by unimodal analysis for comparison.

TABLE II

Mean hydrodynamic diameters ($\langle d_h \rangle$) and polydispersity indices (PD-index) as calculated from cumulant analysis of QELSS data, and overall internal volumes (V_i) for various liposomal preparations

Data represent means ± S.D. for ten independent determinations.

Liposome type	$\langle d_h \rangle$ (nm)	PD index	V_i (l/mol)
s-REV			
SM	238 ± 26	0.352 ± 0.010	1.2 ± 0.2
DSPC	295 ± 27	0.432 ± 0.024	1.6 ± 0.3
FATVET			
SM	105 ± 4	0.133 ± 0.024	1.0 ± 0.2
DSPC	106 ± 5	0.256 ± 0.060	1.2 ± 0.1

liposomes. In addition, s-REV liposomes feature high polydispersity indices, so they are highly heterogeneous in size, even after extrusion at small presure of the original suspensions. On other hand, extrusion at high presures of FATMLV produced a highly homogeneous population of liposomes with a mean diameter of approx. 100 nm, which coincides with that obtained by Hope et al. [11] using electron microscopy. Their polydispersity index was approx. 0.1, so this type of preparation can be classed as monodisperse [21].

The multimodal analysis, the results of which are shown in Fig. 1, confirms that, irrespective of their lipid composition, s-REV preparations are made up of at least two subpopulations which have similar mean diameters and occur in roughly the same proportions. The major population (73%) is centred at 120 nm, while the minor population (27%) is centred at 550 nm. On the other hand, FATVET liposomes are essentially monodisperse and have a mean diameter of approx. 100 nm (only DSPC preparations contain some 400-nm structures, which never exceed 4% as a whole). The reproducibility of these results was very good for FATVET liposomes (CV = 5%) and somewhat poorer for s-REV liposomes (CV = 15%).

On the other hand, FATVET preparations typically feature phospholipid recoveries above 90%, whereas

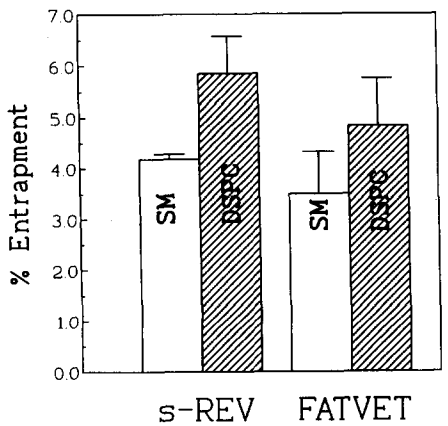

Fig. 2. Representative entrapment efficiency values expressed as percentages.

those of s-REV preparations rarely exceed 50% owing in part to filtration. This is so irrespective of the lipid composition [21] and has major industrial manufacture implications.

The various available methods for preparation of REV suspensions use widely varying volumes of aqueous and organic phases. Incomplete emulsification during sonication or a rapid loss of isopropyl ether followed by a slower removal of chloroform may be responsible for the presence of large vesicles having more than one bilayer in the final preparation [22]. This may be the source of the high variability in the

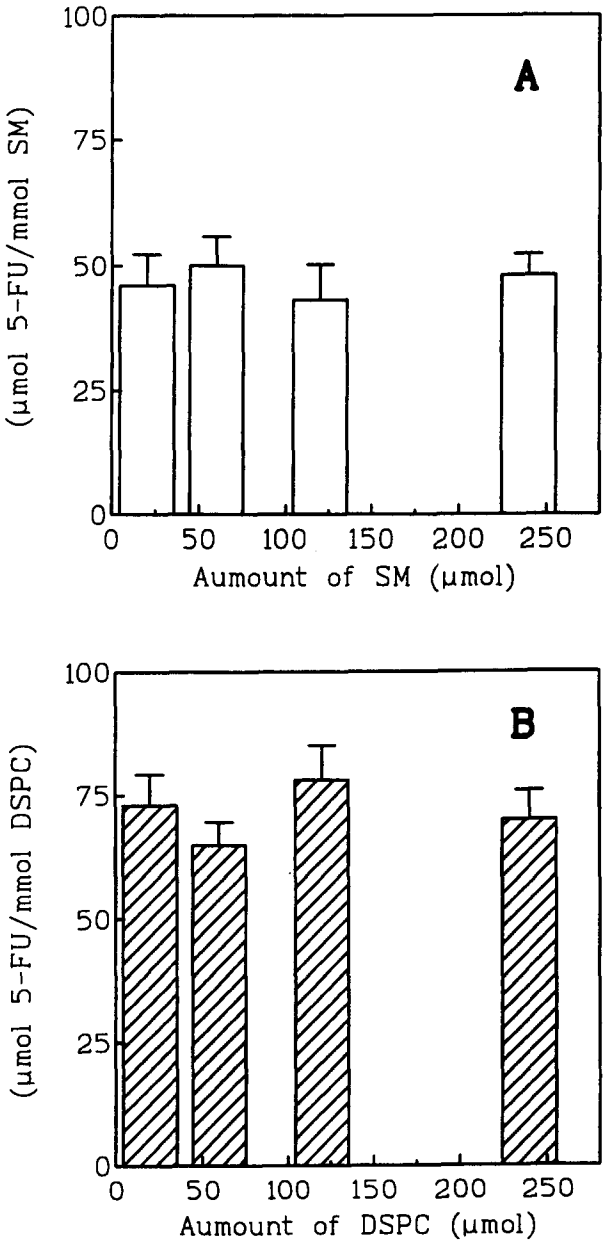

Fig. 3. Influence of the amount of phospholipid in (A) SM and (B) DSPC liposomes on the 5-FU encapsulation efficiency. Bars denote the standard deviations of duplicates from at least three different experiments.

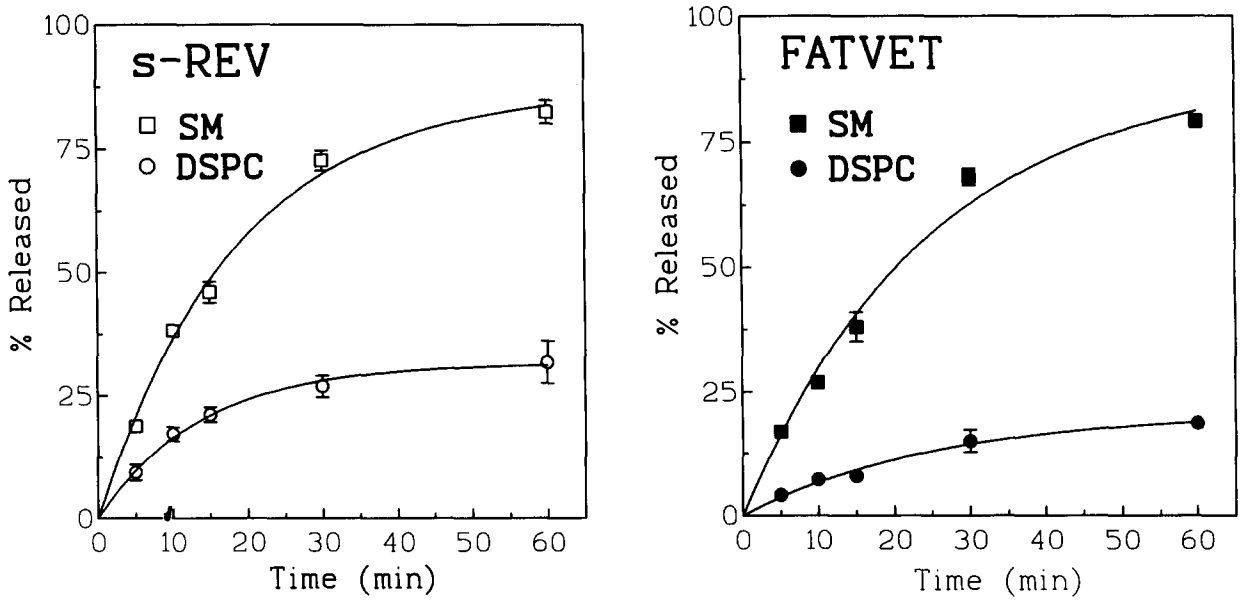

Fig. 4. Experimental data for 5-FU release from vesicles at 37°C and matching fitting curves (Eqn. 5). Bars denote S.D. values for eight individual experiments.

mean sizes and entrapped aqueous volumes reported by some authors [6,23]. Our overall internal volume (V_i) values were similar in all instances, the highest volumes being measured for DSPC that formed bilayers (Table I). These values are quite satisfactory compared to previously reported [24–27]. Singer [27] and Egerdie and Singer [28] showed that the volumes captured by multilamellar liposomes (i.e., internal aqueous spaces) depend on their lipid structure. In addition to the structural differences, one should consider the dependence of size distribution on lipid composition (Fig. 1).

Fig. 2 reflects the dependence of liposomal 5-FU entrapment on the composition of the lipid bilayer and the preparation method used. The scarce entrapment achieved (3–6%) is typical of these liposomal systems and drug molecules with similar solubility properties [24–26]. The fact that DSPC liposomes entrap larger amounts of drug than do SM liposomes may be related to a different 'fluidity' state of the bilayer [24].

Even though 5-FU is a hydrophilic drug, it is very sparsely soluble in water. The partition coefficient for 5-FU in octanol/0.01 SCB at 20, 30 and 37°C is 0.15, 0.20 and 0.26, respectively [26]. Accordingly, the drug has a slight affinity for the lipid liposomal phase. However, such an affinity does not seem to be high enough to allow 5-FU to remain in the bilayer. The 5-FU entrapment efficiency can be increased by increasing the amount of phospholipid used; however, the encapsulation yield in terms of the mol of encapsulated drug/mol phospholipid ratio is not increased as a result (Fig. 3). Therefore, encapsulation of 5-FU in liposomes bears no relationship to the partition coefficient of the drug, but only to the overall volume of aqueous phase that is encapsulated during liposome formation [26].

TABLE III

Non-linear regression parameters for the fitting of experimental 5-FU release data to Eqn. 5, regression determination coefficients (R^2) and residual mean squares (RMS)

Data represent means ± S.D. for eight independent experiences.

Liposome type	A_{eq}	β	$\bar{\epsilon}_i$	R^2 ($p > 0.05$; df = 39)	RMS
s-REV					
SM	90.0 ± 1.81	0.06 ± 0.003	0.05 ± 0.02	0.988	$1.78 \cdot 10^{-8}$
DSPC	31.1 ± 1.91	0.07 ± 0.010	0.02 ± 0.07	0.955	$5.23 \cdot 10^{-7}$
FATVET					
SM	90.7 ± 2.07	0.05 ± 0.004	−0.05 ± 0.03	0.990	$1.06 \cdot 10^{-7}$
DSPC	20.9 ± 1.05	0.04 ± 0.006	0.03 ± 0.04	0.938	$1.08 \cdot 10^{-8}$

TABLE IV

Overall rate constants for the efflux process (k_{ov}), phenomenological permeability coefficients (P) and kinetic rate constants (k_i) for the interfacial process (drug transfer from the membrane phase to the bulk aqueous phase) in various liposomal preparations

Liposome type	k_{ov} (s^{-1}) ($\times 10^4$)	P (m/s) ($\times 10^{11}$)	k_1 (s^{-1}) ($\times 10^{18}$)
s-REV			
SM	10	3.4	3.5
DSPC	12	4.4	4.7
FATVET			
SM	8.3	1.5	0.8
DSPC	6.7	1.2	0.6

In vitro release of 5-FU from liposomes

Fig. 4 shows the results obtained by monitoring the in vitro release of 5-FU from s-REV and FATVET liposomes in addition to the best fitting function (Eqn. 5). The fitting parameters and statistical results for the full regression are given in Table III, while overall release rate constants, permeability coefficients and rate constants for the interfacial process are listed in Table IV.

As can be seen, the experimental data fit well to Eqn. 5 although the sphericity hypothesis concerning liposome shape is very crude [29,30]. All of the in vitro patterns obtained are consistent with rapid leakage (half-lives of 10–17 min). To some authors, such a rapid release of the drug suggests that it is transported primarily via a diffusion-controlled mechanism [26]. Although our experimental data compare well with them, the fact that they conform to an equation which is formally similar to that derived for diffusion from a spherical structure with a phase boundary (viz. Eqn. 4) led us to adopt this latter model.

The overall rate constants (k_{ov}) and permeability coefficients (P) given in Table IV were similar in all instances. The k_{ov} and P values obtained in this work suggest that 5-FU can readily cross the bilayer. At pH 7.4, 5-FU (pK_a 8–13) occurs in neutral form and behaves as a surface-active drug with an estimated critical micelle concentration CMC = $8.0 \cdot 10^{-10}$ M [31]. Its moderate hydrophilicity and small size facilitate diffusion across the lipid matrix of the bilayer.

Based on the theoretical model of Guy et al. [5], k_1 small values suggest a tendency for 5-FU to remain adsorbed at the outer lipid/water interface. Srivastava et al. [31] found that 5-FU and some of its derivatives give rise to liquid membrane phenomena on adsorption onto a supporting membrane as a result of their surface activity. In addition, the liquid membranes act as barriers by altering the transport of other permeants.

An interesting observation immediately arises from our results: while k_{ov} and P values are virtually identical for s-REV and FATVET liposomes irrespective of their lipid composition (Table IV), DSPC liposomes possess a greater ability to retain 5-FU. Thus, approx. 70–80% of 5-FU remained within DSPC liposomes 1 h after the release process was started, whereas only 15–20% was still retained in SM liposomes after the same period. One further inference is that all liposome systems studied reached equilibrium as regards the release process (see A_{eq} values in Table III and Fig. 4).

Some of the differences found in 5-FU release from s-REV and FATVET (e.g., in the k_i values for the interfacial process) may have arisen from the presence of oligolamellar structures in s-REV preparations and from its size heterogeneity despite the fact that the r value in Eqn. 7 is the weighted average from r values of each subpopulation. Thus, the larger liposomes may mask the osmotic activity and permeation properties of the smaller liposomes present in the same dispersion [32]. Other differences (e.g., the retention capacity of DSPC liposomes) may be a result of the different packing of the acyl chains in the lipid matrix at the experiences temperature.

In fact, bilayer permeability is to a great extent determined by the degree of disorder of the bilayer [33]. Thus, bilayers in a liquid-crystalline state are more permeable to entrapped materials than they are in a gel state [34,35]. The loss of encapsulated material is temperature-dependent and generally greatest around the phospholipid phase transition temperature [36]. The thermotropic gel–liquid crystal phase transition in brain bovine SM bilayers occurs over the temperature range 30.4–38.4°C [32], while the main transition temperature for DSPC bilayers is approx. 52°C. Fig. 5 shows DSC curves for MLV structures consisting of bovine brain SM and synthetic DSPC. At the temperature where our experiments were conducted, 37°C, DSPC bilayers were 15°C below their transition temperature in the gel state. On the other hand, SM bilayers were at the end of their transition. In any case, this situation should not result in the erratic leakage of 5-FU (see Fig. 4) arising from mismatching in the

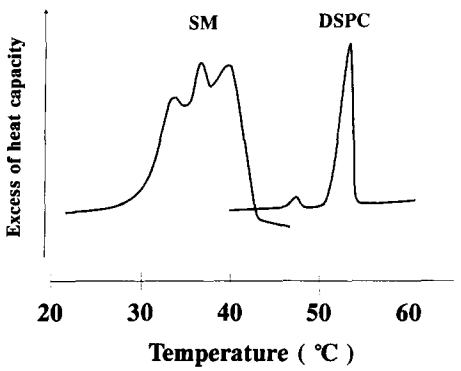

Fig. 5. DSC curves for a heating scan on natural multilamellar SM vesicles and synthetic multilamellar DSPC vesicles run at a rate of 2 K/min.

packing of lipid molecules at the ordered fluid boundaries. The thermotropic behaviour of aqueous dispersions of natural mixtures of sphingomyelins is characteristic of the mixture as a whole rather than any of the individual components. In particular, it seems unlikely that any phase separation of major components occurred [37]. Our observations on 5-FU release suggest that permeation parameters are controlled by bilayer fluctuations and transient defects [38], as well as by the surface activity of the drug rather than the partition coefficients and energy barriers involved.

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References

1 Lopez-Berestein, G. (1989) in Liposomes in the Therapy of Infections Diseases and Cancer (Fidler, ed.), Alan R. Liss, New York.
2 Mezei, M. (1988) in Liposomes and Carriers of Drugs (Gregoriadis, G., ed.), Wiley, Chichester.
3 Woodle, M.C. and Papahadjopoulos, D. (1989) Methods Enzymol. 171, 193–217.
4 Disalvo, E.A. (1985) Chem. Phys. Lipid 37, 385–397.
5 Guy, R.H., Hadgraf, J., Kellaway, I.W. and Taylor, J. (1982) Int. J. Pharm. 11, 199–207.
6 Szoka, F. and Papahadjopoulos, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4194–4198.
7 Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J. and Papahadjopoulos, D. (1979) Biochim. Biophys. Acta 557, 9–23.
8 Elorza, B. (1989) Ph. D. Thesis, Complutense University, Madrid.
9 Lichtenberg, D. and Barenholz, Y. (1988) Methods Biochem. Anal. 33, 337–462.
10 Düzgüneş, N., Wilschut, J., Hong, K., Fraley, P.C., Perry, C., Friend, D.S., James, T.L. and Papahadjopoulos, D. (1983) Biochim. Biophys. Acta 732, 289–299.
11 Hope, M.J., Bally, M.B., Webb, G. and Cullis, P.R. (1985) Biochim. Biophys. Acta 812, 55–65.
12 Bartlett, G.R. (1959) J. Biol. Chem. 243, 466–468.
13 Sainz, M.C., Chantres, J.R., Elorza, B. and Elorza, M.A. (1993) Int. J. Pharm. 89, 183–190.
14 Provencher, S.W. (1979) Makromol. Chem. 180, 201–209.
15 McCracken, M.S. and Sammons, M.C. (1987) J. Pharm. Sci. 76, 56–59.
16 Ruf, H. (1989) Biophys. J. 56, 67–78.
17 Kojro, Z., Lin, S.Q., Grell, E. and Ruf, H. (1989) Biochim. Biophys. Acta 985, 1–8.
18 Devoisselle, J.M., Vion-Dury, J., Confort-Goung, S., Coustaut, D. and Cozzone, P.J. (1992) J. Pharm. Sci. 81, 249–254.
19 Elorza, B., Elorza, M.A., Sainz, M.C. and Chantres, J.R. (1993) J. Pharm. Sci., in press.
20 Dixon, W.J. and Jennrich, R. (1989) in BMDP Statistical Sofware, User's Manual (Dixon, W.J., ed.), University of California Press, Los Angeles.
21 Elorza, B., Elorza, M.A., Sainz, M.C. and Chantres, J.R. (1993) J. Microencap. 10, 237–248.
22 Taylor, K.M.G., Taylor, G., Kellaway, I.W. and Stevens, J. (1990) Int. J. Pharm. 58, 49–55.
23 Mayhew, E., Lazo, R. and Vail, W.J. (1984) in Liposome Technology (Gregoriadis, G., ed.), Vol. 2, pp. 19–31, CRC Press, Boca Raton, FL.
24 Ozer, A.Y. and Talsma, H. (1989) Int. J. Pharm. 55, 185–191.
25 Mazumder, A. (1981) Ind. J. Biochem. Biophys. 18, 120–123.
26 Tsukada, K., Ueda, S. and Okada, R. (1984) Chem. Pharm. Bull. 32, 1929–1935.
27 Singer, M. (1982) Chem. Phys. Lipid 31, 145–159.
28 Egerdie, B. and Singer, M. (1982) Chem. Phys. Lipid 31, 75–85.
29 Clerc, S.J., Allietta, M.M. and Thompson, T.E. (1992) Biophys. J. 61, A242.
30 Mui, B.L.-S., Cullis, P.R., Evans, E.A. and Madden, T.D. (1993) Biophys. J. 64, 443–453.
31 Srivastava, R.C., Shankar, M., Tandon, A. and Nagappa, A.N. (1987) Int. J. Pharm. 38, 239–245.
32 Schullery, S.E. and Garzaniti, J.P. (1974) Chem. Phys. Lipid 12, 75–95.
33 Braganza, L.F., Blott, B.H., Coe, T.J. and Melville, D. (1983) Biochim. Biophys. Acta 731, 137–144.
34 Inoue, K. (1974) Biochim. Biophys. Acta 339, 390–402.
35 Blok, M.C., Van der Neut-Kok, E.C.M., Van Deenen, L.L.M. and De Gier, J. (1975) Biochim. Biophys. Acta 406, 187–196.
36 Papahadjopoulos, D., Jacobson, K., Nir, S. and Isac, T. (1973) Biochim. Biophys. Acta 311, 330–348.
37 Barenholz, Y. and Thompson, T.E. (1980) Biochim. Biophys. Acta 604, 129–158.
38 Nagle, J.F. and Scott, H.L. (1978) Biochim. Biophys. Acta 513, 236–243.