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Encapsulation, Stability and In-vitro Release Characteristics of Liposomal Formulations of Colchicine

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Abstract

The severe toxicity and low therapeutic index of colchicine limit its therapeutic use. Encapsulation in liposomes might reduce these toxic effects. The objective of this study was to determine the factors influencing encapsulation of colchicine in liposomes and to optimize the encapsulation parameters.

Colchicine was encapsulated in multilamellar liposomes and large unilamellar liposomes prepared using various phospholipids. The effects of method of preparation, type of vesicle, charge, and concentration of cholesterol on encapsulation of colchicine in liposomes were investigated. Also, stability of colchicine under stress conditions and at various temperatures, and in-vitro release characteristics were determined. A significant difference in encapsulation of colchicine in multilamellar liposomes was observed when prepared by two different methods. Induction of charge on the liposome surface increased encapsulation of colchicine in multilamellar liposomes, but did not affect large unilamellar liposomes. The liposome preparations could withstand simulated transport conditions and frequent changes in temperature. Particle size and concentration of colchicine did not change significantly during storage at various temperatures for six months. In order to retain encapsulated colchicine in liposomes, storage at or below room temperature was found to be suitable. In-vitro release of colchicine from large unilamellar liposomes was biphasic and was influenced by two rate-limiting barriers, the dialysis membrane and the liposome bi-layers.

For optimum encapsulation and stability of colchicine liposomes were prepared from a mixture of 1,2-distearoyl-sn-glycero-3-phosphocholine, cholesterol and either stearylamine or dicetyl phosphate.

Colchicine has long been the drug of choice in the treatment of gouty arthritis (Fliege 1995). Colchicine was shown to be effective in the prophylaxis of acute attacks of Familial Mediterranean fever (Familial paroxysmal polyserositis) (Levy & Eliakim 1977). Its use was extended to the treatment of immune inflammatory diseases such as Behcet's syndrome (Mizshima et al 1977), primary biliary cirrhosis (Kaplan et al 1988), recurrent pericarditis (Milliare & Ducloux 1991) and systemic scleroderma (Alarcon-Segovia et al 1979). The discovery of new indications for colchicine in these immunological diseases, aside from its use for gouty arthritis, has gained renewed interest in the drug. Colchicine is, however, a highly toxic drug and the therapeutic margin very narrow–as little as 7 mg colchicine has proved fatal (Goodman & Gilman 1980). Its dose, is therefore, is still monitored by the balance between the occurrence of side effects and clinical efficacy. So far it has proven impossible to avoid toxicity of colchicine upon chronic administration.

Liposomes have been successfully used as delivery vehicles to improve therapeutic efficacy and reduce toxic effects. They have been investigated as potential carriers for the site-specific delivery of enzymes, macromolecules, antimicrobial compounds, anti-tumour agents and biological response modifiers (Parker et al 1982; Patel et al 1982). The reduction of the toxicity of amphotericin-B (Lopez-Berestein et al 1985; Szoka et al 1987), doxorubicin (Mayer et al 1990; Kanter et al 1993) and valinomycin (Dauod & Juliano 1986) upon encapsulation in liposomes has been very well established. Encapsulation in liposomes might reduce the toxic effects of colchicine. The diversity in the design of liposomes such as composition, structure, and size makes it possible to tailor a drug-delivery system which is more efficacious than use of the free drug. The objective of this study was to determine the factors influencing encapsulation of colchicine in liposomes and to optimize encapsulation parameters to achieve delivery systems suitable for further in-vitro and in-vivo investigations.

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Materials and Methods

Materials

Colchicine, dicetyl phosphate, stearylamine, cholesterol, and protamine were obtained from Sigma (St Louis, MO). [^{3}H]Colchicine was purchased from Du Pont NEN Products (Boston, MA). 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and egg glycero-3-phosphocholine were purchased from Avanti Polar Lipids, (Alabaster, AL). Acetonitrile, chloroform and methanol were purchased from Fisher Scientific (Fairlawn, NJ). Polycarbonate membrane filters were obtained from Costar Scientific Corporation (Cambridge, MA). Scintillation cocktail (Ultima Gold) was obtained from Packard (Meriden, CT).

Preparation and characterization of liposomes

Multi-lamellar liposomes. For initial investigations two methods were employed for the preparation of multi-lamellar liposomes. For all preparations the concentration of phospholipid used was kept constant at 5% w/v. Because colchicine is

photosensitive, all procedures were performed out with minimum exposure to light and formulations were stored in amber, air-tight glass containers.

Method I. Multilamellar liposomes were prepared by the conventional thin film hydration method (Bangham et al 1965). In brief, weighed quantities of lipid and cholesterol were dissolved in chloroform in a round-bottom flask. The solvent was evaporated under reduced pressure and the flask was stored overnight under vacuum to remove traces of solvent. The film was hydrated at a temperature just above the phase transition temperature (T_c) of the lipid by using a solution of colchicine (1% w/v) in phosphate-buffered saline (PBS; 50 mM, pH 7·4) with added [^{3}H]colchicine (0·5 μCi mL^{-1}).

Method II. Multilamellar liposomes were prepared by the modified solvent-evaporation method (Mezei & Nugent 1984). Lipid and cholesterol (and stearylamine or dicetyl phosphate, if required) were dissolved in a 2:1 (v/v) mixture of chloroform and methanol in a round-bottom flask with a volume 50 times the batch volume. Glass beads weighing 20 times the weight of lipid were added to the mixture. The solvent mixture was evaporated under reduced pressure. The thin film formed on the glass beads and flask wall was dried for 2 h and then stored overnight under vacuum to remove traces of the solvent. The film was hydrated at a temperature just above the phase transition temperature of the lipid by using a solution of colchicine (1% w/v) in pharphate buffered saline (PBS) with added [^{3}H]-colchicine (0·5 μCi mL^{-1}). This hydration of the lipid film was performed for 45 min by use of a Büchi rotary evaporator at a speed of 15-20 rev min^{-1}. Liposomes were then separated from the glass beads by simple filtration through a Buchner funnel.

Large unilamellar liposomes

Large unilamellar liposomes were prepared by extruding multilamellar liposomes five times under nitrogen through a stack of two polycarbonate membrane filters (pore size 0·2 μm) using an extruder (Lipex Biomembrane, Vancouver, Canada). The temperature of the extruder was kept above the phase transition temperature of the lipid used by circulation of hot water.

Determination of encapsulation efficiency

For multilamellar liposomes, the liposome suspension was centrifuged at 35 000 rev min^{-1} for 15 min. Supernatant was siphoned with a Pasteur pipette and the pellet was washed with PBS. Washings were mixed with supernatant. Scintillation fluid was added and radioactivity in the supernatant was determined by liquid scintillation counting. Encapsulation of colchicine in large unilamellar liposomes was determined by the protamine-induced aggregation method (Kulkarni et al 1995a).

In-vitro release of colchicine from large unilamellar liposomes

For in-vitro release studies, approximately 500 μL of large unilamellar liposome suspension was placed in a 1·8 × 10 cm dialysis bag (MWCO 3500; Spectra/Por, Spectrum Medical Industries, Houston, TX). One end was sealed with a plastic clip and other end with a metal clip (to sink the bag). The bag was suspended in 500 mL PBS in a USP type II dissolution test apparatus. The temperature of the test solution was kept constant at 37°C and the solution was stirred at 25 rev min^{-1} by use of a paddle. Samples were withdrawn at pre-determined times and replaced with equal volumes of PBS. Scintillation fluid was mixed with the samples and radioactivity was determined by liquid scintillation counting. Each experiment was performed in triplicate.

Particle size determination

The particle size of large unilamellar liposomes was determined by large-angle dynamic light scattering (Brookhaven Instruments, Model BI-90). Each sample was diluted to the appropriate concentration with filtered saline and measurements were obtained at 25°C, assuming the viscosity of the medium to be 0·01 P and its refractive index 1·333.

Stability studies

For stability studies, a protocol developed by Weiner et al (1989) was adopted with some modifications. Two large-unilamellar-liposome preparations (DSPC–cholesterol–dicetyl phosphate, mole ratio 1:0·5:0·3 and DSPC–cholesterol–stearylamine, mole ratio 1:0·5:0·3) were studied for their long-term stability.

Liposome preparations were stored at 4 ± 1°C, room temperature (approximately 25 ± 1°C) and 37 ± 1°C for 6 months. Samples were withdrawn at pre-determined times and analysed for: changes in particle size; leakage of encapsulated colchicine; and the chemical stability of colchicine.

Liposome suspensions were subjected to eight heat-cool cycles (by storing alternately at 5 and 50°C for 48 h). The encapsulation of colchicine in liposomes was determined before and after the treatment. Liposome suspensions were placed on a reciprocating shaker at 100 cycles min^{-1} for 48 h. The encapsulation of colchicine in liposomes was determined before and after the treatment.

Analytical techniques

For liquid scintillation (LSC) analysis samples were treated with scintillation fluid and counts min^{-1} were recorded using a Beckman LS 5000 TD scintillation counter (Beckman Instruments, Inc., Fullerton, CA). For HPLC analysis, a Varian 9010 solvent delivery system coupled with a 9095 autosampler and a 9050 variable wavelength UV detector were used (Varian Associates, Sunnyvale, CA). The column used was 150 × 4·6 mm, reversed phase (C18) column (Microsorb MV, Rainin Instrument Co., Emeryville, CA). Data integration was performed by use of an analogue to digital interface module and Macintosh-based software (Macintegrator, Rainin Instrument Co.). The HPLC method used was adopted from that of Fernandez et al (1993). The mobile phase was 205:75:220 (v/v) methanol–acetonitrile–phosphate buffer pH 7·4. The flow rate was 0·5 mL min^{-1} and detection was performed at 254 nm.

Results and Discussion

Table 1 shows the effect of various phospholipids and of charge on the encapsulation of colchicine in multilamellar liposomes. Colchicine was best encapsulated by multilamellar liposomes prepared using DSPC, i.e. as fatty acid chain length increased, the encapsulation of colchicine in liposomes increased. When the drug is oppositely charged its encapsu-

Table 1. Encapsulation of colchicine (%) in multi-lamellar liposomes prepared using different lipids The phospholipid concentration was 5% w/v and the phospholipid/cholesterol molar ratio was 1:0·5. The concentration of colchicine was 1% w/v. Dicetyl phosphate and stearylamine (0·3 mol) were added to provide negative and positive charge, respectively.

Lipid	Neutral		Positive		Negative	
	Method I	Method II	Method I	Method II	Method I	Method II
Egg glycero-3-phosphocholine	4·298 ± 2·134	15·252 ± 1·362	11·524 ± 2·224	26·152 ± 2·015	12·227 ± 1·035	27·493 ± 2·224
1,2-Dimyristoyl-sn-glycero-3-phosphocholine	4·050 ± 1·782	13·891 ± 1·811	9·782 ± 2·773	24·264 ± 1·425	12·726 ± 1·811	26·616 ± 2·008
1,2-Dipalmitoyl-sn-glycero-3-phosphocholine	6·228 ± 1·826	15·332 ± 1·021	13·668 ± 1·725	26·452 ± 2·113	15·889 ± 1·227	28·137 ± 1·878
1,2-distearoyl-sn-glycero-3-phosphocholine	7·335 ± 2·836	19·672 ± 2·103	15·934 ± 1·225	29·842 ± 2·324	18·745 ± 1·443	32·722 ± 2·423

lation increases because of electrostatic interaction between the drug and the bi-layers (Taylor et al 1990). The encapsulation of colchicine was increased ($P < 0·05$) both in positively charged and in negatively charged liposomes—because colchicine is a neutral drug, its encapsulation was unaffected by the nature of charge (positive or negative) and so the increase in encapsulation was solely a result of electrostatic repulsion between the adjacent bi-layers which caused an increase in the volume of the aqueous compartment. It also appears that method II is better than method I for preparation of multilamellar liposomes, because the addition of glass beads results in a greater surface area. Slower hydration in method II also facilitates efficient encapsulation of colchicine. When liposomes are prepared with lipid alone, encapsulated drug is prone to leak during storage. Cholesterol is, therefore, incorporated into the lipid bi-layers to impart rigidity and reduce leakage. It has also been observed that cholesterol improved the encapsulation of steroids in liposomes (Kulkarni & Vargha-Butler 1996). Half a mole of cholesterol per mole of lipid was used to prepare liposomes because a lower concentration (0·1 or 0·2 mol) led to a risk of leakage during storage and a higher concentration (1 mol) caused problems during extrusion (such as an increased pressure requirement and retention of cholesterol on polycarbonate membrane filters) in the preparation of large unilamellar liposomes.

Colchicine is highly soluble in water, methanol and chloroform. It has been well established that for hydrophilic drugs large unilamellar liposomes are a desirable delivery system and multilamellar liposomes are suitable for hydrophobic drugs (Kulkarni et al 1995b). Table 2 lists the amount of colchicine encapsulated in large unilamellar liposomes obtained by extrusion of multilamellar liposomes prepared by methods I and II. No significant difference ($P < 0·05$) in encapsulation of colchicine was observed between the large unilamellar liposomes prepared by either method. The reorganization of lipid bi-layers during extrusion was not affected by the method of preparation used, as all the lipid was already hydrated. The encapsulation of colchicine was found to be higher ($P < 0·05$) in neutral large unilamellar liposomes when compared with neutral multilamellar liposomes. This effect was not, however, as significant among charged multilamellar liposomes and charged large unilamellar liposomes, because when there is no charge large unilamellar liposomes enclose a larger aqueous volume than multilamellar liposomes.

In-vitro release studies are often performed to predict how a delivery system might work in ideal situations which might give some indication of its in-vivo performance. Fig. 1 shows the in-vitro release of colchicine encapsulated in charged and uncharged large unilamellar liposomes prepared using DPPC or DSPC. The simple solution of colchicine needed approximately 2 to 4 h to cross the dialysis barrier whereas 6 to 8 h were required for colchicine encapsulated in DPPC large unilamellar liposomes and 10 to 12 h for colchicine encapsulated in DSPC large unilamellar liposomes. The duration of release of colchicine is longer from DSPC liposomes because the phase transition temperature of DSPC is higher than that of DPPC; this restricts the leakage of the encapsulated drug. No significant difference was, however, observed when release from large unilamellar liposomes prepared using the same lipid, but with different charges, was compared ($P < 0·05$). The in-vitro release of colchicine encapsulated in liposomes was driven by the concentration gradient and limited by two bar-

Table 2. Encapsulation of colchicine (%) in large unilamellar liposomes prepared using different lipids. The phospholipid concentration was 5% w/v and the phospholipid/cholesterol molar ratio was 1:0·5. The concentration of colchicine was 1% w/v. Dicetyl phosphate and stearylamine (0·3 mol) were added to provide negative and positive charge, respectively. Large unilamellar liposomes were obtained by extrusion of multilamellar liposomes through 0·2 μm polycarbonate membranes.

Lipid	Neutral		Positive		Negative	
	Method I	Method II	Method I	Method II	Method I	Method II
Egg glycero-3-phosphocholine	16·298 ± 1·634	18·253 ± 2·136	25·556 ± 1·673	26·223 ± 1·928	26·894 ± 1·627	28·192 ± 2·451
1,2-Dimyristoyl-sn-glycero-3-phosphocholine	21·150 ± 1·342	22·542 ± 2·671	24·996 ± 2·114	25·489 ± 2·365	27·176 ± 1·225	27·785 ± 1·982
1,2-Dipalmitoyl-sn-glycero-3-phosphocholine	26·138 ± 1·246	24·151 ± 2·342	26·645 ± 1·459	27·775 ± 2·035	30·769 ± 1·376	29·128 ± 2·366
1,2-distearoyl-sn-glycero-3-phosphocholine	28·335 ± 1·136	27·463 ± 2·167	29·783 ± 2·225	31·101 ± 1·998	32·765 ± 1·263	34·266 ± 2·876

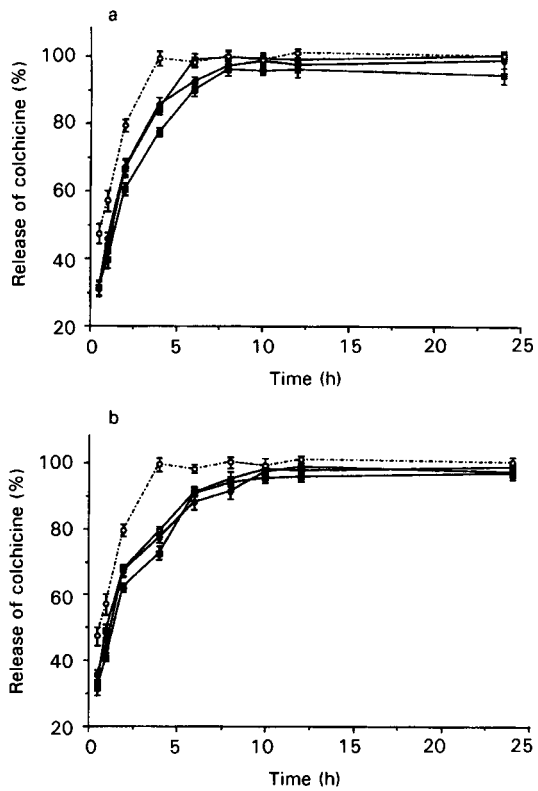

FIG. 1. In-vitro release of colchicine from (a) DPPC large unilamellar liposomes and (b) DSPC large unilamellar liposomes at pH 7·4 and 37°C. The concentration was 5% w/v for DPPC or DSPC and 1%w/v for colchicine. The lipid/cholesterol molar ratio was 1:0·5 and 0·3 mol stearylamine or dicetyl phosphate was added for positive or negative charge, respectively. □, Neutral; ■, positive; ●, negative; ○, colchicine solution.

riers, namely the phospholipid bi-layer and the dialysis membrane. About 70% of the drug was not encapsulated in the liposomes and accounted for the fast release of the drug during the first 2 h. After the first 2 to 4 h the encapsulated drug was released as a result of the difference in the concentration of drug inside liposomes and in the dialysis bag. Two preparations, one with positive charge (DSPC–cholesterol–stearylamine, 1:0·5:0·3 molar ratio) and the other with negative charge (DSPC-cholesterol-dicetyl phosphate, 1:0·5:0·3 molar ratio) were investigated for their stability at different temperatures and under various mechanical stresses. Table 3 shows the stability of these preparations after eight heating and cooling cycles and after shaking for 48 h (to mimic transport conditions). No significant change ($P < 0·05$) in encapsulation of colchicine was observed, indicating that these preparations could withstand transport stresses and sudden changes in temperature. It should be noted that these liposomes were prepared using DSPC which has a high T_c (56°C) and could, therefore, withstand such rigorous conditions.

Monitoring the particle size of a liposomal drug delivery system is an integral part of long-term stability studies. Elorza et al (1993) have reported that the polydispersity factor could give vital information about the uniformity of the particle-size distribution and concluded that when the polydispersity factor was below 0·1 the particulate system could be considered monodisperse. Table 4 shows the change in mean particle size and polydispersity factor of positively and negatively charged DSPC liposomes, when stored at various temperatures. No significant change in mean particle size or polydispersity factor was observed for either type of liposome. Fig. 2 shows the retention of colchicine in positively and negatively charged large unilamellar liposomes, when stored at various temperatures for 6 months. It was observed that at room temperature and 4°C encapsulated colchicine did not leak significantly ($P < 0·05$) from either type of liposome. Approximately 22 (± 6)% of the encapsulated drug leaked from both types of liposome when stored at 37°C for 6 months, however. Thus, in order to retain the encapsulated colchicine these liposomes need to be stored at or below room temperature (25°C). Because an increase in temperature might also increase the rate of degradation of colchicine, it was essential to determine the stability of colchicine in these preparations at various temperatures. Although at 37°C encapsulated drug leaked from both types of liposome, the amount of colchicine present in these preparations was constant and no degradation was observed at any temperature studied. A simple solution of colchicine was also kept at these temperatures and similar investigations were performed. No degradation of colchicine was observed at these temperatures (data not shown).

In summary, a stable liposomal disperse system was prepared using DSPC. Large unilamellar liposomes were obtained by extrusion of multilamellar liposomes prepared by two different methods, without any significant difference in the encapsulation of colchicine. Induction of charge on the liposome surface increased encapsulation of colchicine in multilamellar liposomes, but did not affect that in large unilamellar liposomes. The liposome preparations could withstand simulated transport conditions and frequent changes in temperatures. The particle size and the chemical stability of colchicine did not change significantly during six months of storage. In order to retain the encapsulated colchicine in liposomes, storage at or below room temperature was found to be suitable. The in-

Table 3. Effect of mechanical stress and heat-cool cycles on particle size and retention of colchicine in DSPC large unilamellar liposomes. The DSPC/cholesterol molar ratio was 1:0·5 and 0·3 mol stearylamine or dicetyl phosphate was added to induce positive or negative charge, respectively. The concentration was 5% w/v for DSPC and 1% w/v for colchicine.

Condition	Positive charge		Negative charge	
	Encapsulation (%)	Particle size (nm)	Encapsulation (%)	Particle size (nm)
Initial	29·836 ± 0·726	211 ± 14	32·365 ± 0·372	189 ± 15
After shaking for 48 h	29·126 ± 0·425	222 ± 18	32·676 ± 0·635	192 ± 11
After eight heat–cool cycles	28·783 ± 1·028	206 ± 19	31·892 ± 1·112	199 ± 18

Table 4. Change in mean particle size and polydispersity factor of positively and negatively charged DSPC large unilamellar liposomes. The concentration was 5% w/v for DSPC and 1% w/v for colchicine. The DSPC/cholesterol molar ratio was 1:0·5 and 0·3 mol stearylamine or dicetyl phosphate was added for positive or negative charge, respectively.

Charge	Storage (weeks)	At 4°C		At 24°C		At 37°C	
		Particle size (nm)	Polydispersity factor	Particle size (nm)	Polydispersity factor	Particle size (nm)	Polydispersity factor
Positive	0	181 ± 3·6	0·065 ± 0·009	181 ± 3·6	0·065 ± 0·009	181 ± 3·6	0·065 ± 0·009
	4	177 ± 2·8	0·049 ± 0·008	174 ± 9·2	0·051 ± 0·011	170 ± 2·1	0·072 ± 0·005
	12	180 ± 7·1	0·068 ± 0·019	177 ± 4·9	0·066 ± 0·024	170 ± 3·5	0·051 ± 0·020
	24	181 ± 5·8	0·078 ± 0·008	182 ± 4·8	0·088 ± 0·009	173 ± 4·2	0·083 ± 0·010
Negative	0	185 ± 6·5	0·082 ± 0·003	185 ± 6·5	0·082 ± 0·003	185 ± 6·5	0·082 ± 0·003
	4	178 ± 4·9	0·064 ± 0·018	174 ± 14·2	0·090 ± 0·012	189 ± 7·1	0·059 ± 0·014
	12	171 ± 7·1	0·083 ± 0·017	184 ± 7·8	0·089 ± 0·013	173 ± 10·6	0·096 ± 0·009
	24	172 ± 4·3	0·085 ± 0·012	191 ± 6·8	0·078 ± 0·009	183 ± 5·8	0·074 ± 0·012

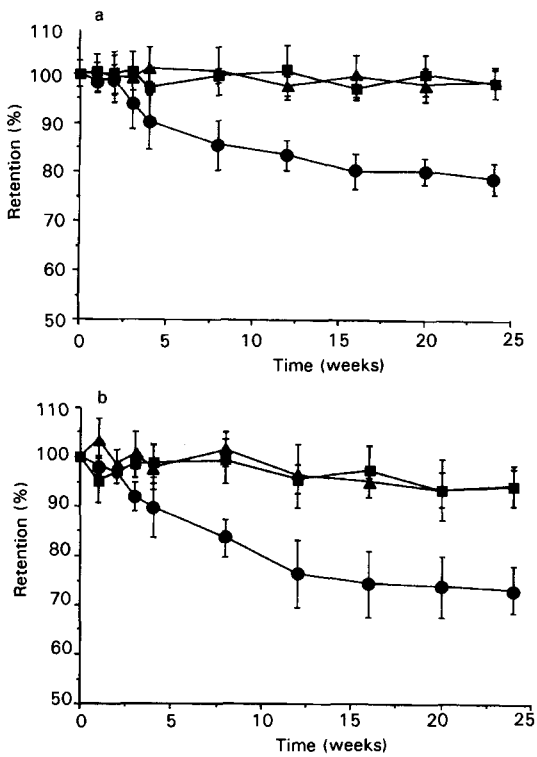

FIG. 2. Retention of colchicine in (a) positively charged and (b) negatively charged DSPC large unilamellar liposomes during long-term storage at different temperatures. The concentration was 5% w/v for DSPC and 1% w/v for colchicine. The DSPC/cholesterol molar ratio was 1:0·5 and 0·3 mol stearylamine or dicetyl phosphate was added for positive or negative charge, respectively. ■, 4°C; ▲, room temperature; ●, 37°C.

vitro release of colchicine from large unilamellar liposomes was biphasic and was influenced by two rate-limiting barriers, the dialysis membrane and the liposome bi-layers.

References

Alarcon-Segovia, D., Ramos-Niembro, F., De Kasep, G. I., Alococer, J., Perez-Tamayo, R. (1979) Long term evaluation of colchicine in treatment of scleroderma. J. Rheumatol. 6: 705–710

Bangham, A. D., Standish, M. M., Watkins, J. C. (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 13: 238–252

Dauod, S., Juliano, R. (1986) Reduced toxicity and enhanced antitumor effects in mice of the ionophoric drug Valinomycin when incorporated in liposomes. Cancer Res. 46: 5518–5523

Elorza, B., Elorza, M., Sainz, M., Chantres, J. (1993) Comparison of particle size and encapsulation parameters of three liposomal preparations. J. Microencap. 10: 237–248

Fernandez, F., Bermejo, A. M., Tabernero, M. J., Lopez-Rivadulla, M., Cruz, A. (1993) Determination of colchicine in biological fluids by reverse-phase HPLC. Variation of colchicine level in rats. Forensic Sci. Int. 59: 15–18

Fliege, K. (1995) Getting to know gout. FDA Consumer 29: 19–22

Goodman, L. S., Gilman, A. (1980) Drugs employed in the treatment of gout. In: Goodman, L. S., Gilman, A. (eds) The Pharmacological Basis of Therapeutics, 6th edn. McMillan, New York, pp 717–722

Kanter, P. M., Bullard, G. A., Pilkiewicz, F. G., Mayer, L. D., Cullis, P. R., Pavelic, Z. P.(1993) Preclinical toxicology study of liposome encapsulated doxorubicin: comparison with doxorubicin and empty liposomes in mice and dogs. In-Vivo 7: 85–95

Kaplan, M., Alling, D., Zimmerman, H. (1988) A prospective trial of colchicine for primary biliary cirrhosis. N. Engl. J. Med. 318: 1709–1711

Kulkarni, S. B., Vargha-Butler, E. I. (1996) Effect of bi-layer additives on encapsulation of steroids in MLV liposomes. Int. J. Pharm. Adv. 1: 408–413

Kulkarni, S. B., Dipali, S. R., Betageri, G. V. (1995a) Protamine induced aggregation of unilamellar liposomes. Pharm. Sci. 1: 359–366

Kulkarni, S. B., Betageri, G. V., Singh, M., (1995b) Factors affecting microencapsulation of drugs in liposomes. J. Microencap. 12: 229–246

Levy, M., Eliakim, M. (1977) Long-term colchicine prophylaxis in familial Mediterranean fever. Br. Med. J. ii: 808–809

Lopez-Berestein, G., McQueen, T., Mehta, K. (1985) Protective effect of liposomal amphotericin B against *C. albicans* infection. Cancer Drug Delivery 2: 183–189

Mayer, L., Tai, L., Bally, M. (1990) Characterization of liposomal systems containing doxorubicin. Biochim. Biophys. Acta 1025: 143–148

Mezei, M., Nugent, F. (1984) Method of encapsulating biologically active materials in multi-lamellar lipid vesicles. US Patent 4 485 054

Milliare, A., Ducloux, G. (1991) Treatment of acute or recurrent pericarditis with colchicine. Circulation 83: 1458–1465

Mizshima, Y., Matsumura, N., Mori, M. (1977) Colchicine in Behcets disease. Lancet 2: 1037

Parker, R. J., Priester, E. R., Seiber, S. M. (1982) Comparison of biodistribution of free and liposome-entrapped cytosine arabinofuranoside following intraperitoneal administration to rats. Drug Metab. Dispos. 10: 40–47

Patel, K. R., Jonah, M. H., Rahman, Y. E. (1982) In-vitro uptake and therapeutic application of liposome-encapsulated methotrexate in mouse hepatoma 120. Eur. J. Cancer Clin. Oncol. 18: 833–839

Szoka, F. C., Milholland, D., Barza, M. (1987) Effect of lipid composition and liposome size on toxicity and in-vitro fungicidal activity of liposome intercalated Amphotericin-B. Antimicrob Agents Chemother. 31: 421–429

Taylor, K. M., Taylor, G., Kellaway, I. W., Stevens, J. (1990) Stability of liposomes to nebulizations. Int. J. Pharm. 58: 57–61

Weiner, N., Martin, F., Riaz, M. (1989) Liposomes as a drug delivery system. Drug Dev. Ind. Pharm. 15: 1523–1554