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Niosomes containing *N*-(2-hydroxypropyl)methacrylamide copolymer-doxorubicin (PK1): effect of method of preparation and choice of surfactant on niosome characteristics and a preliminary study of body distribution

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Abstract

PK1 is an *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-doxorubicin conjugate currently in early clinical development. Niosome encapsulation is a means to increase PK1 blood residence time, potentially promote tumour uptake and produce a slow, sustained release of active drug. Factors effecting encapsulation efficiency and size of PK1-niosome formulations were studied. Five surfactants were used to prepare PK1-niosomes; hexadecyl poly-5-oxyethylene ether ($C_{16}EO_5$); octadecyl poly-5-oxyethylene ether ($C_{18}EO_5$); hexadecyl diglycerol ether ($C_{16}G_2$); sorbitan monopalmitate (Span 40) and sorbitan monostearate (Span 60). All were mixed in equimolar ratio with cholesterol and varying amounts of Solulan C24 (a cholesteryl poly-24-oxyethylene ether) (9–39 mol%). Dicetylphosphate (DCP) was also added (2 mol%). Passive association of PK1 with preformed $C_{16}G_2$ and Span 60 vesicles was low (3–4%) while subsequent dehydration (freeze drying) followed by rehydration of the formulation increased the entrapment to 61% in the $C_{16}G_2$ formulation. Transmission electron microscopy revealed that these niosomes had an electron dense core, evidence of intravesicular concentration of PK1. Increasing Solulan C24 content resulted in decreased PK1 entrapment after freeze drying, and the vesicle size was also decreased. Solulan C24 (39 mol%) caused pronounced vesicle aggregation on freeze drying, whereas at lower levels (9 mol%), PK1 appeared to act as a cryoptrotectant and the mean size of $C_{16}G_2$ niosomes was 235 nm. A PK1:surfactant/lipid ratio of 0.3 (11.2 mg ml^{-1} doxorubicin) was achieved with Span 60 niosomes. This formulation, and the $C_{16}G_2$ niosomes, did not induce red blood cell lysis at the proposed dose for in vivo use. Preliminary in vivo biodistribution studies showed PK1-$C_{16}G_2$ niosomes to be mainly taken up by the liver and spleen. After 24 h, 25 and 3% of dose administered was present as free doxorubicin in these organs respectively. © 1997 Elsevier Science B.V.

Keywords: Niosomes; HPMA copolymer-doxorubicin; PK1

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1. Introduction

N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-doxorubicin is a novel polymeric antitumour agent currently in early clinical development (Vasey et al., 1996). The conjugate contains doxorubicin bound to the polymer backbone by a glycine-phenylalanine-leucine-glycine peptidyl spacer (PK1) (Fig. 1), which is designed for intracellular cleavage by the lysosomal thiol-dependent proteases following fluid-phase pinocytic uptake (Duncan et al., 1992). PK1 shows improved pharmacokinetics when compared to doxorubicin (Seymour et al., 1990) and tumour tropism in mice (Seymour et al., 1994). In Phase I clinical trial, a 4-fold increase in the maximum tolerated dose (relative to doxorubicin) and antitumour activity have been observed (Vasey et al., 1996). The disorganised vasculature of tumours (Skinner et al., 1990) is responsible for the preferential capture of macromolecules and liposomes by tumour tissue (Nugent and Jain, 1984; Gerlowski and Jain, 1986), and the combination of increased uptake via the leaky tumour vasculature and reduced lymphatic drainage in tumour tissue has been termed the enhanced penetration and retention (EPR) effect (Matsumara and Maeda, 1986).

Preferential tumour uptake of PK1 is accompanied by rapid renal excretion of most of the remaining conjugate (Seymour et al., 1990; Pimm et al., 1996). This is due to its relatively low molecular weight ($Mw \sim 30\,000$). Increasing the molecular weight of the HPMA copolymers has been shown to prolong blood residence time and improves further the extent of polymer accumulation in the tumour (Seymour et al., 1995). Since increasing the molecular weight of the HPMA copolymer main chain is not practicable due to the non-biodegradable nature of the polymer backbone, it was thought that the encapsulation of PK1 in non-ionic surfactant vesicles (niosomes) would increase polymer blood residence time sufficiently to allow maximum tumour accumulation. In addition, doxorubicin liberation would be further prolonged. Here we describe a novel approach to cancer chemotherapy which combines two emerging drug delivery technologies, namely the use of polymer drug conjugates and niosomes, are combined in an attempt to capitalise on the unique attributes of both systems.

Niosomes have been used as targetable carriers of antitumour agents encapsulating methotrexate (Azmin et al., 1985; Chandraprakash et al., 1990), vincristine (Parthasarathi et al., 1994) and doxorubicin (Kerr et al., 1988; Rogerson et al., 1988; Uchegbu et al., 1995, 1996b). There is evidence that they also target to solid tumours (Rogerson et al., 1988; Uchegbu et al., 1995), probably by the EPR effect. Liposomal doxorubicin formulations have been evaluated clinically (Cowens et al., 1993; Gabizon et al., 1994) and liposomal doxorubicin and daunomycin have recently been granted product licences for use in the treatment of Kaposi's sarcoma.

Previously we have described a PK1 niosomal formulation composed of sorbitan monostearate, cholesterol, Solulan C24 (a poly-24-oxyethylene cholesteryl ether) (45:45:10) (Gianasi et al., 1996; Uchegbu et al., 1996a) which had a maximum encapsulation efficiency of 49% and a mean vesicle size of 583 nm. The formulation showed good stability under conditions of storage and also in plasma in vitro. Free doxorubicin was liberated slowly during incubation of these PK1-niosomes with a mixture of lysosomal enzymes in vitro. Vesicle size, drug carrying capacity and biocompatibility are the essential features which determine suitability for in vivo use. Thus before in vivo evaluation it was necessary to optimise these parameters. Here we have systematically studied ten formulations to understand the effect of different membrane surfactants (hexadecyl poly-5-oxyethylene ether ($C_{16}EO_5$); octadecyl poly-5-oxyethylene ether ($C_{18}EO_5$); hexadecyl diglycerol ether ($C_{16}G_2$); sorbitan monopalmitate (Span 40) and sorbitan monostearate (Span 60)), the effect of the polyethoxylated compound Solulan C24 (poly-24-oxyethylene cholesteryl ether content, and the method of preparation on vesicle size and PK1 encapsulation efficiency. In general, the ratio of the non-ionic surfactant to cholesterol was maintained at 1:1, while the level of the

polyethoxylated compound Solulan C24 was altered. As high levels of Solulan C24 are potentially haemolytic, the ability of two candidate formulations to induce red blood cell lysis was studied in vitro. Preliminary biodistribution studies were also carried out after intravenous (i.v.) administration of PK1-$C_{16}G_2$ niosomes to BALB/C mice using an HPLC technique to detect PK1-bound or free doxorubicin.

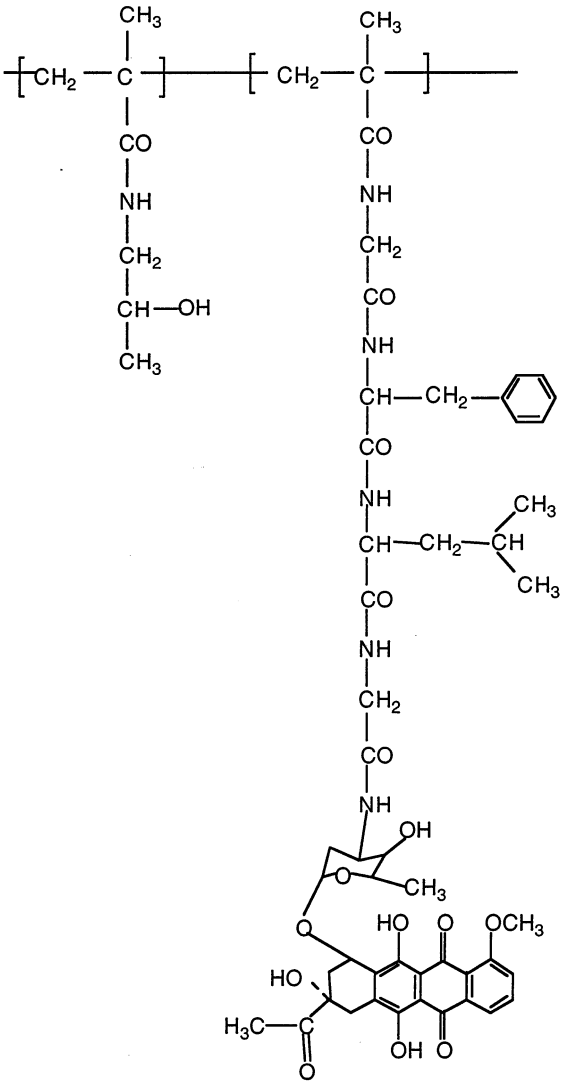


Fig. 1. Structure of PK1 an *N*-(2-hydroxypropyl)methacrylamide copolymer conjugate containing doxorubicin. Weight average molecular weight approximately 30 000, doxorubicin content 8 wt%.

2. Materials and methods

2.1. Chemicals

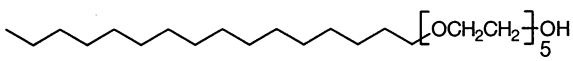
The surfactant structures are shown in Fig. 2. $C_{16}EO_5$, $C_{18}EO_5$, Span 40, Span 60 and cholesterol were all from Sigma, UK. $C_{16}G_2$ was a gift from L'Oreal, France. All organic solvents were from BDH (UK), and were of analytical grade. PK1 was prepared as previously described (Duncan et al., 1989). Solulan C24 was from D.F. Anstead, UK. Dicetyl phosphate (DCP) was from Fluka, Germany.

2.2. Preparation of PK1-niosomes

2.2.1. The vesicles were prepared by three methods

2.2.1.1. Method (A). A thin film of the surfactant/lipid mixture (150 μmol total lipid/surfactant) was hydrated with 2.5 ml water at 60°C and the resulting dispersion probe sonicated for 4 min (MSE PG100 150 W). The sonicated dispersion was centrifuged (Haraeus Varifuge 3.ORS) at $3700 \times g$ for 10 min to remove titanium particles that may have arisen from the sonication step, and the supernatant incubated for 16 h with 1 ml PK1 (10 mg ml^{-1}). Encapsulated material was then separated from unencapsulated material by ultracentrifugation (2×1 h at $145\,000 \times g$; Beckman L8-55).

2.2.1.2. Method (B). This method was a modification of the dehydration rehydration vesicles (DRV) technique (Kirby and Gregoriadis, 1984). Surfactant/lipid films (150 μmol total lipid/surfactant) were hydrated with 2.5 ml water and probe sonicated for 4 min. Samples were centrifuged at $3700 \times g$ for 10 min as above. The resultant supernatant was incubated with 1 ml PK1 (10 mg ml^{-1}) for 16 h. One ml aliquots were subsequently rapidly frozen in liquid nitrogen and the formulation freeze dried overnight. One ml of water was added to the freeze dried cake which was resuspended with vortexing. Freeze dried material was filtered (0.45 or 0.22 μm filters) and the unentrapped material separated by ultracentrifugation (2×1.5 h at $145\,000 \times g$; Beckman L8-55).



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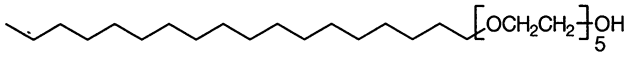
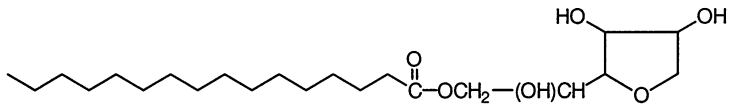


$C_{18}EO_5$



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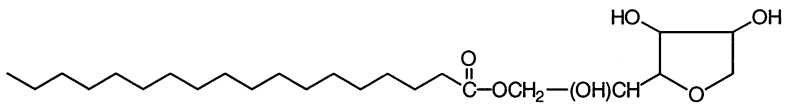

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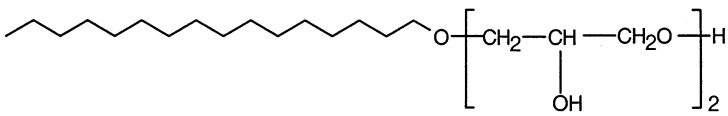
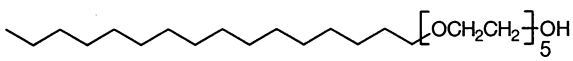
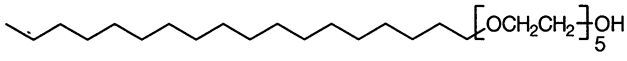


$C_{16}G_2$

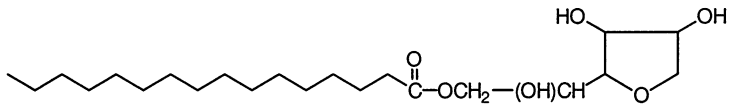
Fig. 2. Structure of the surfactants, $C_{16}EO_5$, $C_{18}EO_5$, Span 40, Span 60, $C_{16}G_2$, Solulan C24.



$C_{16}EO_5$



$C_{18}EO_5$



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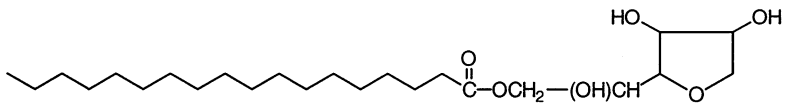
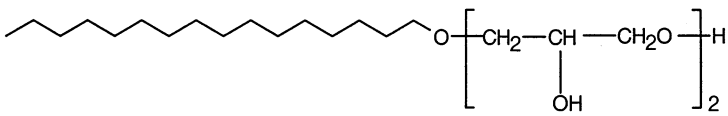


SPAN 60



$C_{16}G_2$

Fig. 2. Structure of the surfactants, $C_{16}EO_5$, $C_{18}EO_5$, Span 40, Span 60, $C_{16}G_2$, Solulan C24.

Table 1
Niosome formulations studied

Code	Non-ionic surfactant		Cholesterol (mol%)	Sollulan C24 (mol%)	DCP (mol%)
	Type	(Mol%)			
1	$C_{16}EO_5$	44.5	44.5	9	2
2	$C_{16}EO_5$	39.5	39.5	19	2
3	$C_{16}EO_5$	29.5	29.5	39	2
4	$C_{18}EO_5$	39.5	39.5	19	2
5	$C_{18}EO_5$	29.5	29.5	39	2
6	$C_{16}G_2$	44.5	44.5	9	2
7	$C_{16}G_2$	39.5	39.5	19	2
8	$C_{16}G_2$	29.5	29.5	39	2
9	Span 60	39.5	39.5	19	2
10	Span 60	29.5	29.5	39	2
11	Span 40	29.5	29.5	39	2

2.2.1.3. Method (C). This method was essentially similar to the DRV method (Kirby and Gregoriadis, 1984). Sonicated preformed niosomes, obtained as described above, were added to a solution of PK1 and immediately rapidly frozen in liquid nitrogen and freeze dried overnight thus omitting the 16 h incubation step. Filtration, subsequent to freeze drying and the separation procedures (ultracentrifugation) were as described above.

2.3. Characterisation of PK1-niosomes

Niosome suspensions were diluted in 2 parts isopropanol and centrifuged for 10 min at $3700 \times g$. To quantitate the efficiency of entrapment of PK1 the absorbance of the supernatant was read at 478 nm. The supernatant from the ultracentrifugation process described above was also assayed in a similar manner to measure free PK1.

Niosomes were sized by photon correlation spectroscopy on a Malvern autosizer. One hundred μl of sample was diluted to 4 ml with filtered (0.22 μm) water and sized.

2.4. Measurement of the ability of niosomes to lyse red blood cells

Niosomes were prepared as described above except that after the preparation of the empty niosomes (encapsulating water) the suspension was equally divided and to half the suspension (1.25 ml) was added 0.5 ml PK1 (40 mg ml^{-1}). Both suspensions were then frozen in liquid nitrogen and freeze dried. The samples were not filtered after rehydration and separation from unentrapped material was by ultracentrifugation as described above. Evaluation of red blood cell lysis has previously been described (Duncan et al., 1991). Briefly, blood was obtained by cardiac puncture of Sprague–Dawley rats and immediately erythrocytes were separated from other blood products by centrifugation at $1000 \times g$ for 10 min. Erythrocytes were washed three times in phosphate buffered saline (PBS), pelleting each time. Pelleted erythrocytes were weighed and a 2% w/w solution of erythrocytes in PBS was prepared. To 1 ml of this solution was added 1 ml of the niosome suspension (encapsulating water). As a control, erythrocytes were incubated in either PBS alone, or to obtain 100% haemoglobin release in PBS containing Triton-X 100 (1%). Samples were incubated for 5 h at 37°C. Released haemoglobin was separated from erythrocytes by centrifugation at $3700 \times g$ for 10 min and the supernatant diluted with an equal volume of isopropanol prior to measuring the absorbance at

Table 2
Effect of method of preparation on niosome size and PK1 loading

Formulation	Method of preparation	Size (nm ± S.D.)	PK1 entraped (% ± S.D.)
(**6**)	Passive association[a]	151 ± 13	3.31 ± 0.59
$C_{16}G_2$:Chol:Sol 24:DCP 44.5:44.5:9:2	Passive association[b] + freeze drying	381 ± 105	60.86 ± 4.05
	Co-freeze dried[c]	369 ± 114	64.44 ± 1.74
(**9**)	Passive association[a]	128 ± 18	4.20 ± 0.66
Span 60:Chol:Sol.24:DCP 39.5:39.5:19:2	Passive association[b] + freeze-drying	236 ± 21	38.24 ± 2.39
	Co-freeze dried[c]	284 ± 33	32.46 ± 2.39

[a] Method (A); [b] Method (B); [c] Method (C).

550 nm. The results are expressed as percentage haemoglobin release relative to the Triton-X 100 (100%) and PBS (0%) controls.

2.5. Preliminary evaluation of body distribution

Niosomes were prepared from $C_{16}G_2$, cholesterol, Solulan C24, dicetyl phosphate (44.5:44.5:9:2) using method C. Briefly the surfactant lipid mixture was hydrated with water and the resulting dispersion probe sonicated and centrifuged at $3700 \times g$ for 20 min to remove titanium particles. The supernatant was mixed with a solution of PK1 in water, flash frozen in liquid nitrogen and freeze dried overnight. Finally the freeze dried cake was rehydrated with water and the entrapped PK1 separated from the unentrapped material by ultracentrifugation ($150\,000 \times g \times 1.5$ h). Sizing and the assay of PK1 niosomes for PK1 was carried out as previously described.

Male BALB/C mice (approximately 7 weeks of age, average weight 21 g) were injected iv with 5 mg/kg doxorubicin equivalent in PK1 niosomes. Mice were sacrificed in groups of 3; at 0.5, 1, 2, 5 and 24 h. The heart, liver, spleen and a blood sample were taken. Plasma was separated from blood by centrifugation at $3700 \times g$ for 10 min and the organs were frozen until assay. HPLC analysis for free doxorubicin and PK1-bound doxorubicin were conducted as previously described (Seymour et al., 1990). To assay free doxorubicin, homogenised tissue samples or plasma were added to ammonium formate buffer

Table 3
Characteristics of PK1-niosomes prepared by co-freeze drying

Formulation	Mean size (nm)	PK1 entraped (%)	PK1 content (mg ml^{-1})	Ratio of PK1 surfact/lipid (g g^{-1})
(**6**) $C_{16}G_2$:Chol:Sol24:DCP 44.5:45.5:9:2	334	—	None added	—
(**6**) $C_{16}G_2$:Chol:Sol24:DCP 44.5:45.5:9:2	235	41.38	9.8	0.27:1
(**9**) Span 60:Chol:Sol 24:DCP 39.5:39.5:19:2	401	—	None added	—
(**9**) Span 60:Chol:Sol 24:DCP 39.5:39.5:19:2	270	43.98	11.2	0.3:1

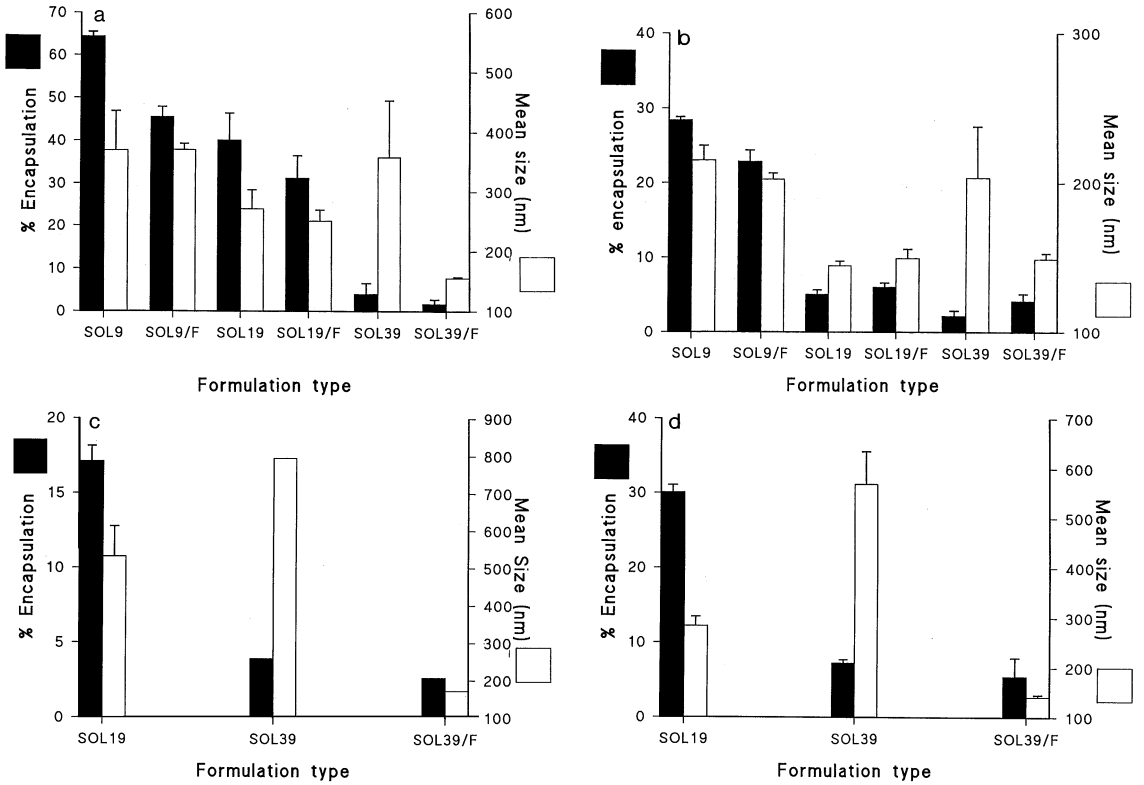


Fig. 3. Effect of Solulan C24 (SOL) content on the size and encapsulation efficiency of PK1. The ratio of non-ionic surfactant: cholesterol was 1 and the vesicles contained 2 mol% DCP. Panel (a) $C_{16}G_2$; panel (b) $C_{16}EO_5$; panel (c) $C_{18}EO_5$ and panel (d) Span 60.

(pH 8.5) and daunomycin added as the internal standard. They were then were extracted into a chloroform: isopropanol mixture (4:1). To assay samples for total doxorubicin content (PK1 bound and free), samples (containing daunomycin as an internal standard) were subject to acid hydrolysis to release the aglycone adriamycinone. The aglycone could then be extracted as described above. The chloroform: isopropanol mixture was evaporated to dryness under a stream of nitrogen, and the residue reconstituted in methanol before injection onto a C_{18} μbondapak column (150 × 3.9 mm). The mobile phase was an isopropanol, water mixture (29:71) with the pH lowered to 3.2 by the addition of concentrated orthophosphoric acid. A fluorimetric detection (emission wavelength 480 nm, excitation wavelength 560 nm) method was used.

3. Results and discussion

PK1-niosomes were prepared from the formulations listed in Table 1. In all cases, 150 μmol (approximately 120 mg) lipid/surfactant was mixed with 10 mg PK1. A previously described method for preparation of PK1-Span 60 niosomes (Gianasi et al., 1996) involved hydration of lipid films with a solution of PK1 at 60°C. However, it is known that PK1 precipitates from aqueous solution at 55°C (Uchegbu et al., 1996d) and this leads to a drastically reduced incorporation of PK1 into niosomes when high concentrations of PK1 (20 mg ml^{-1}) are used to hydrate lipid films. It was thus necessary to seek alternative formulation methods in order to minimise drug wastage. A comparison of three methods of vesicle preparation namely (A) passive association of PK1 with

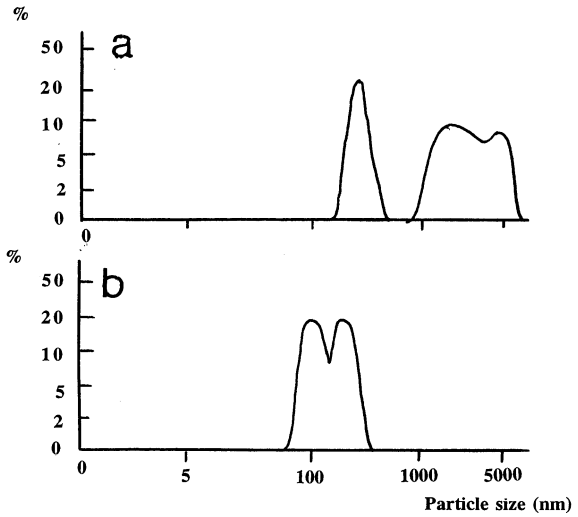


Fig. 4. The size distribution of niosomes prepared from $C_{16}G_2$, Cholesterol, Solulan C24, DCP 29:5:29:5:39:2 by method (B), either after rehydration of the dehydrated vesicles (panel (a)) or following filtration through a 0.45 μm filter (panel (b)).

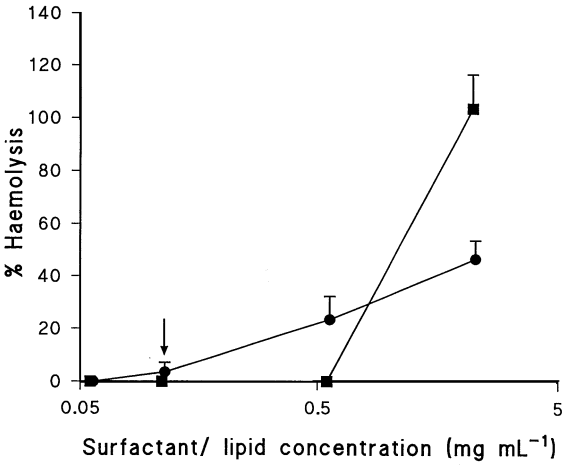


Fig. 6. Effect of niosomes (formulations not encapsulating PK1) on haemoglobin release from rat erythocytes in vitro. Effect of $C_{16}G_2$, cholesterol, Solulan C24, DCP (44.5:44.5:9.2) niosomes (●——●), (■——■) are shown. Arrows indicate the calculated level of surfactant to be administered to achieve an intravenous dose of 10 mg kg^{-1} doxorubicin.

preformed niosomes, (B) the passive association of PK1 with preformed niosomes followed by the DRV technique and (C) the DRV technique, showed that the DRV technique resulted in high incorporation of PK1 resulting in a 10–20 fold increase in encapsulation efficiency (Table 2). Passive association of PK1 with preformed niosomes is minimal (Table 2).

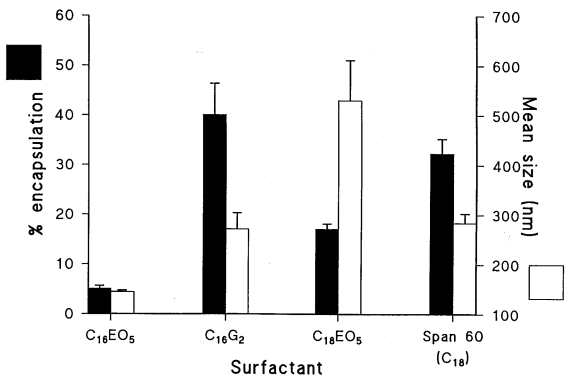


Fig. 5. The size and encapsulation efficiency of niosomes prepared from C_{16} and C_{18} surfactants. Non-ionic surfactant:cholesterol ratio was 1 and the nisomes contained 19 mol% Solulan C24 and 2 mol% DCP.

To obtain liposomes of submicron size usually requires a cryoprotectant (Crowe and Crowe, 1993), such as lactose (Vemuri and Rhodes, 1994), trehalose (Kim and Jeong, 1995), or sucrose (Ausborn et al., 1994). PK1-niosome formulations prepared from Span 60 and $C_{16}G_2$ (not filtered) were reduced in size when PK1 was added (Table 3) an indication that PK1 can itself act as a cryoprotectant. The hyroxylated polymer probably acts in the same way as sugars and HPMA copolymers may be useful more generally as a cryoprotectants when freeze drying pharmaceuticals. Filtration of formulation through a 0.22 μm filter resulted in the production of vesicles in the 100 nm size range which had an electron dense vesicle core (TEM not shown), evidence that PK1 is indeed encapsulated within the niosome interior. No differences in encapsulation efficiency or mean particle size could be detected using methods (B) and (C) (Table 2).

In general for freeze dried and unfiltered vesicles, increasing the level of Solulan C24 decreased the encapsulation efficiency (Fig. 3), despite an increase in vesicle size. Formulations containing high levels of Solulan C24 (19 and 39 mol%) were found to have two distinct size populations (Fig.

4). Examination of the dispersion by transmission electron microscopy (TEM) revealed the presence of vesicle aggregates. The incubation of $C_{16}G_2$ niosomes with Solulan C24 at high temperature results in vesicle aggregation prior to eventual micellisation (Uchegbu et al., 1996c). The longer alkyl chain surfactants gave vesicles with a higher entrapment and vesicle size. This is because larger vesicles are formed when the hydrophilic portion of the molecule is decreased relative to the hydrophobic portion (Israelachvili, 1993), leading to increased entrapment. The increase in entrapment

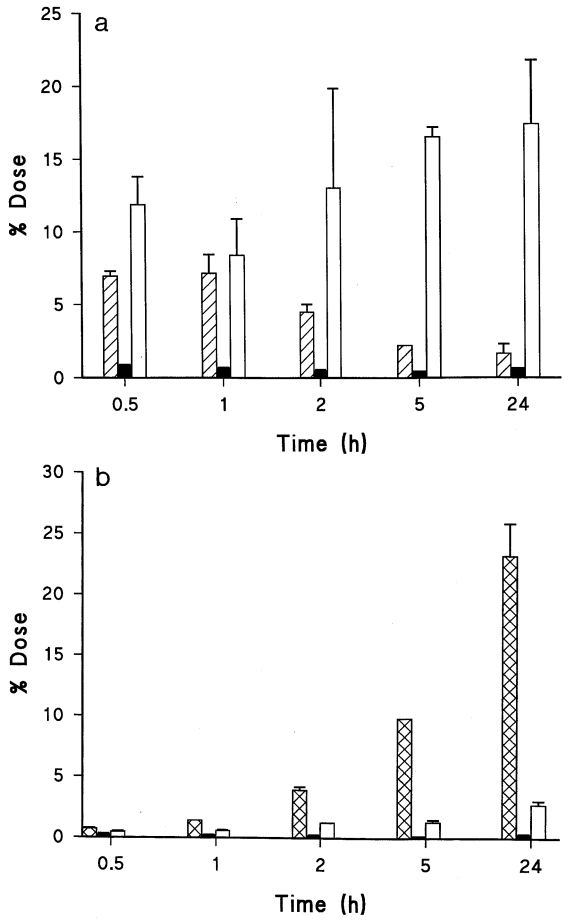


Fig. 7. The biodistribution of panel (a) total doxorubicin (polymer bound + free doxorubicin) and panel (b) free doxorubicin following the intravenous administration of PK1 $C_{16}G_2$ niosomes (5 mg kg^{-1} doxorubicin equivalent) to male BALB/C mice. (/fill), plasma; (full fill), heart; (open fill), spleen; (X fill), liver.

across a homologous series of Span surfactants has been reported previously (Uchegbu and Florence, 1995)Fig. 5.

$C_{16}G_2$ niosomes had a greater entrapment efficiency and larger size than $C_{16}EO_5$. niosomes. The poly-5-ethoxylated surfactant confers a larger head group than its diglycerol analogue leading to decreased membrane fluidity for the latter (Ribier et al., 1984) and the accompanying increased entrapment. This larger head group would also increase of membrane curvature and decrease vesicle size (Israelachvili, 1993). $C_{16}G_2$ and Span 60 niosomes containing 9% Solulan C24 did not cause significant red blood cell lysis at the surfactant level commensurate with an intravenous dose of 10 mg/kg doxorubicin in PK1 form (Fig. 6).

The body distribution, PK1 niosomes ($C_{16}G_2$, cholesterol, Solulan C24, dicetylphosphate—44.5:44.5:9:2) with a mean size of 420 nm and containing 6.29 mg ml^{-1} PK1 (0.57 mg ml^{-1} doxorubicin) was studied in Balb/C mice. Intravenous administration resulted in accumulation in the hepatosplenic tissue (Fig. 7) and after 24 h, 20% of the dose administered was in the spleen. Of this, 3% was free doxorubicin. In liver, free doxorubicin recovered at 24 h constituted 25% of the injected dose. Technical difficulties were encountered in analysis of total doxorubicin (PK1-bound and free) in liver samples, probably due to the presence of a high concentration of niosomes. Plasma clearance of the PK1-niosomes was rapid with only 7.2% of the doxorubicin (total) accounted for of the dose at 1 h, and at 24 h this fell to 1.7% (Fig. 7). The spleen has been shown to preferentially take up liposomes in the 300 nm size range in contrast to smaller liposomes (Litzinger et al., 1994), and also doxorubicin niosomes have been found to accumulate in the liver (Uchegbu et al., 1995). The incorporation of polyethylene glycol compound—Solulan C24, failed to prevent reticuloendothelial uptake.

The depot of polymeric prodrug within the liver resulted in sustained release of free doxorubicin with time and at 24 h, 25% of the dose administered was detected as the active drug. This might be a useful approach for the treatment of hepatic (primary or secondary) tumours, and is especially significant when compared with administration of

Table 4
Effect of method of preparation on the size and encapsulation efficiency of PK1-niosomes containing 39% Solulan 24

Formulation	Method A		Method B	
	Size (nm)	Entrapment (%)	Size (nm)	Entrapment (%)
(**3**) $C_{16}EO_5$:Chol:Sol24:DCP 29.5:29.5:39:2	104	1.57	203	1.89 ± 0.35[a]
(**5**) $C_{18}EO_5$:Chol:Sol24:DCP 29.5:29.5:39:2	132	3.28	794	3.96
(**8**) $C_{16}G_2$:Chol:Sol24:DCP 29.5:29.5:39:2	—	—	372	6.9 ± 4.48[a]
(**10**) Span 60:Chol:Sol24:DCP 29.5:29.5:39:2	142	2.69	568 ± 114[a]	7.35 ± 0.69[a]
(**11**) Span 40:Chol:Sol.24:DCP 29.5:29.5:39.2	129	1.41	4572	6.64

[a] Mean $\pm$ S.D. for three preparations.

doxorubicin-niosomes which resulted in less than 9% of dose in the liver of NMRI mice after 24 h (Uchegbu et al., 1996b). However, if enhanced uptake by disseminated peripheral solid tumour deposits is to be achieved it will in the future be essential to produce PK1-niosome formulations that can escape recognition by the reticuloendothelial system.

4. Conclusions

The dehydration rehydration method for preparation of Niosomes resulted in a 10–20 fold increase in the encapsulation efficiency Table 4. Inclusion of the surfactant $C_{16}G_2$ produced niosomes with the highest entrapment efficiency, 60%. Niosomes were not haemolytic in vitro at the doses selected for in vivo use. $C_{16}G_2$, cholesterol, Solulan C24, dicetylphosphate (44.5: 44.5:9:2) niosomes containing PK1 were rapidly cleared by the RES after i.v. administration to mice resulting in high levels in liver and spleen. Free doxorubicin was slowly liberated in the liver, and kinetics of active drug release recommend this approach for treatment of primary or secondary liver cancer. Niosomes containing other polymeric prodrugs could be useful for treatment of other liver-related diseases. In the context of cancer chemotherapy, future research must seek PK1-niosome formulations which can also escape reticuloendothelial uptake.

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