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The evaluation of crown ether based niosomes as cation containing and cation sensitive drug delivery systems

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Abstract

A novel crown ether amphiphile *N*-hexadecanoyl-2-aminomethyl-15-crown-5 (PCE) has been synthesised from hexadecanoic acid *N*-hydroxysuccinimide ester and 2-aminomethyl-15-crown-5, with the aim of developing a cation containing or cation sensitive controlled release system. Niosomes were prepared with a 1:1 molar ratio of PCE and cholesterol (CHOL) with and without the addition of 10 mol% Solulan C24 (poly-24-oxyethylene cholesteryl ether). A water-soluble fluorescent marker, rhodamine B was encapsulated within these niosomes. Rhodamine B containing PCE/CHOL niosomes of approximately 6.3 μm diameter were visualised by optical microscopy and sonicated PCE/CHOL niosomes of approximately 134 nm in size were visualised by transmission electron microscopy. The release of the fluorescent marker rhodamine B from PCE/CHOL niosomes was slightly increased by the addition of calcium ions but remained unaffected by the addition of sodium ions. This is thought to be due to a slightly greater ease of divalent cation chelation by the crown ether head groups when compared to the monovalent cation, the latter of which are more highly solvated in aqueous solution. This is the first study on the effect of ions on the release properties of crown ether based niosomes. These systems may be developed as cation containing or cation sensitive release systems for drug delivery and other industrial uses. © 1997 Elsevier Science B.V.

Keywords: Crown ethers; N-(hexadecanoyl)-2-aminomethyl-15-crown-5; Niosomes; Non-ionic surfactants; Rhodamine B; Controlled release systems

1. Introduction

Non-ionic surfactant vesicles (niosomes), analogous to liposomes are closed bilayer assemblies prepared by the aqueous dispersion of non-ionic surfactants (Florence and Baillie, 1989). Niosomes have been evaluated as drug delivery

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agents for anti-cancer drugs (Rogerson et al., 1988; Uchegbu et al., 1995) and anti-infective drugs (Baillie et al., 1986). Niosomes may be formed from a rich array of non-ionic surfactants with sugar, polyoxyethylene, polyglycerol or polyhydroxy head groups (Florence, 1993; Uchegbu and Florence, 1995). By systematically studying the physico–chemistry/biological behaviour of a variety of niosomes with different head groups, it may be possible to correlate the properties of niosomes with specific head groups. For example, the change from a poly-5-oxyethylene to a diglycerol head group resulted in a doubling of the encapsulation efficiency of a macromolecular pro-drug (Uchegbu and Duncan, 1997).

Crown ethers are known to chelate metal ions in solution—the ease of which depends on the relative size of the crown when compared to the metal ion, the nature of the solvent and the nature of substituents on the crown ether (Izatt et al., 1985). The self-assembly of aza crown ether amphiphiles in water results in the formation of micelles (Kuo et al., 1983) or niosomes (Echegoyen et al., 1988). In the presence of silver ions, C_{14} diaza crown ethers form micelles and niosomes depending on whether they are di- or mono-alkyl substituted derivatives, respectively (Monserrat et al., 1980). Crown ether amphiphiles devoid of nitrogen donor atoms have also been shown to form micelles (Kuo et al., 1983; Ozeki et al., 1990). In the present study, a new crown ether amphiphile, devoid of nitrogen donor atoms and capable of forming niosomes in the presence of cholesterol has been synthesised. The effect of metal cations on solute release from these niosomes has also been studied.

2. Materials and methods

2.1. Materials

2-aminomethyl-15-crown-5, hexedecanoic acid *N*-hydroxysuccinimide ester, cholesterol and rhodamine B were all purchased from Sigma, Dorset, UK. Solulan C24 (poly-24-oxyethylene cholesteryl ether) was a gift from Ellis and Everard, Essex, UK. Triethylamine, isopropyl alcohol, sodium chloride, calcium chloride and chloroform (HPLC grade) were purchased from BDH, Lutterworth, UK. Water was supplied by an Elgastat UHQ water system (Elga, UK)

2.2. Synthesis of *N*-palmitoyl-2-aminomethyl-15-crown-5 (PCE)

2-aminomethyl-15-crown-5 (500 mg) was dissolved in chloroform (5 ml). To this was added triethylamine (0.8 ml) followed by hexadecanoic acid *N*-hydroxysuccinimide ester (0.71 g) dissolved in chloroform (10 ml). The solution was stirred for 48 h at room temperature, protected from light. After which the organic solvent was removed under reduced pressure at 50°C. The product was then washed with copious amounts of water ($\approx$ 500 ml) on a filter funnel, leading to some loss of the product. The insoluble residue was frozen in liquid nitrogen and lyophilised over night (Edwards Freeze dryer, UK) in order to obtain a dry product. The white powder obtained was characterised by mass spectrometry (fast atom bombardment -FAB, ZAB-SE mass spectrometer, VG instruments) and the melting point determined (SMP 1 melting point apparatus—Stuart Scientific, UK).

2.3. Preparation of PCE/cholesterol (CHOL) niosomes

Vesicles were prepared from: (a) PCE, CHOL and Solulan C24 (poly-24-oxyethylene cholesteryl ether; molar ratio = 45:45:10); or (b) PCE and cholesterol (molar ratio = 50:50) by hydrating a thin surfactant/lipid film with aqueous solutions of rhodamine B (5 mM) or water at 60°C. A portion of the dispersion was sonicated for 4 min (MSE PG100 probe sonicator) with the instrument set at 60% of the maximum output and the sonicated dispersion left to cool slowly. For niosomes prepared encapsulating rhodamine B, unentrapped material was removed by ultracentrifugation (150 000 $\times g$ for 1 h).

2.4. Assay of PCE niosomes for rhodamine B

Niosomes were disrupted by the addition of 10 $\times$ the volume isopropyl alcohol followed by an assay for rhodamine B by fluorimetry (excita-

tion = 553 nm, emission = 614 nm; Perkin–Elmer LS-3 Fluorimeter) after suitable dilution in distilled water. Standard rhodamine B solutions were prepared in the relevant isopropyl alcohol, water mixtures.

2.5. Sizing ofthe vesicles

Sizing was carried out on a Malvern Mastersizer or Malvern Autosizer (Malvern Instruments, UK).

2.6. Optical microscopy

Photomicrographs were taken with a Nikon Microphot FXA light microscope.

2.7. Transmission electron microscopy

The Vesicle dispersion was pipetted onto a precoated copper grid (400 mesh) with a support film of 1% collodion in isoamylacetate and the sample stained with 1% phosphotungstic acid. Sample grids were viewed and photographed with a Philips 201 transmission electron microscope at an accelerating voltage of 80 kV.

2.8. In-vitro release study

1 ml of the rhodamine B niosome suspension + (a) 1 ml water; (b) sodium chloride (2 M); or (c) calcium chloride (2 M) was placed in dialysis tubing (Visking, molecular weight cut off = 12–14 kD). The niosome containing dialysis tubing was then placed in 20 ml of distilled water and the whole mixture incubated (with shaking) at 37°C. At various time intervals 0.5 ml of the dialysate was sampled and the fluorescence measured. The dialysate was replenished with 0.5 ml of fresh water after each sample had been withdrawn.

2.9. Statistics

One way ANOVA tests were conducted at each time point, followed by Bonferroni post tests. Statistical significance was defined by $P < 0.05$.

3. Results and discussion

3.1. Synthesis of PCE

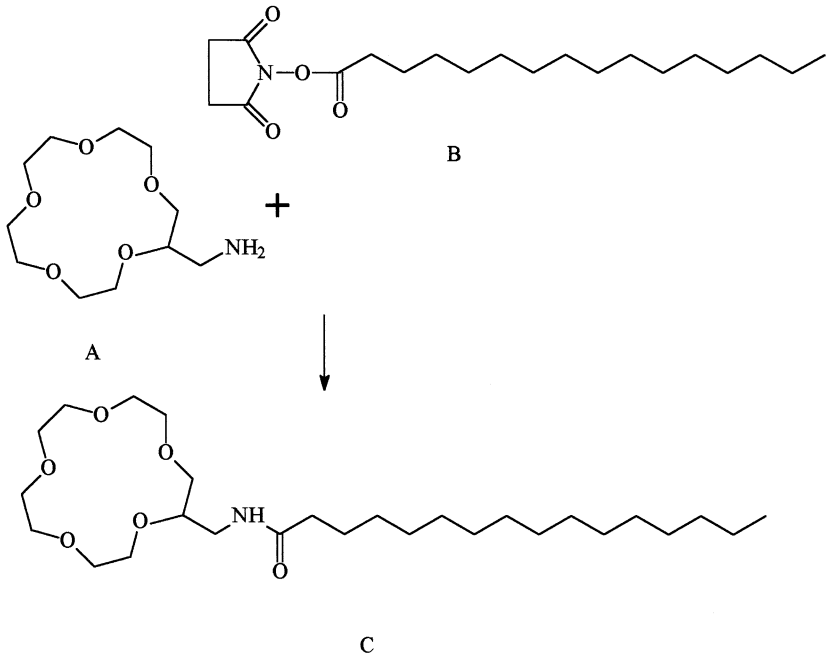
PCE (Scheme 1) was synthesised according to the method of Lapidot et al., 1967 in which activated *N*-hydroxysuccinimide esters specifically attack amino groups (Scheme 1). The product, a white powder, was insoluble but dispersible in water at room temperature. Mass spectrometry data yielded one main peak corresponding to the mass ion plus sodium and a further minor peak corresponding to the mass ion plus potassium (Table 1). The absence of the mass ion itself is not unexpected as crown ether compounds show an affinity for metal cations (Izatt et al., 1985). The melting point of PCE was 54°C.

3.2. PCE/CHOL niosomes

PCE was shown to form niosomes on hydration followed by sonication, in the presence of cholesterol with and without the addition of the steric stabiliser Solulan C24 (Fig. 1a and b, respectively). The inclusion of 10 mol% Solulan C24 altered the appearance of these sonicated niosomes. This could reflect a difference in membrane fluidity, as the addition of soluble surfactants such as Solulan C24 is known to increase the elasticity of some vesicle bilayers (Uchegbu and Florence, 1995). The encapsulation of rhodamine B by PCE niosomes could be visualised easily in unsonicated samples (Fig. 2). The encapsulation efficiency for PCE, cholesterol, Solulan C24 (45:45:10) niosomes was 0.012 moles rhodamine per mole of surfactant lipid. There was no effect on vesicle size by the encapsulation of rhodamine B (Table 2). Previous reports of crown ether based niosomes all refer to the use of aza-crown ethers (Monserrat et al., 1980; Echegoyen et al., 1988) and this is the first report of a crown ether based niosome prepared using a crown ether amphiphile not possessing nitrogen donor atoms.

3.3. In-vitro release study

The release of rhodamine B from PCE vesicles was largely unresponsive to the presence of



Scheme 1. The synthesis of PCE. A, 2-amino methyl 15-crown-5; B, hexadecanoic acid *N*-hydroxysuccinimide ester; C, PCE.

sodium ions but increased slightly in the presence of calcium ions (Fig. 3). There was a molar excess of metal cations in each case. It is possible that crown ether chelation of cations in solution would lead to a change in the character of the membrane and possibly an increase in membrane leakiness. The inclusion of the hexadecanoyl chain is not expected to alter the cation chelating ability of this macrocyclic compound (Lamb et al., 1981; Ikeda et al., 1982). The ionic radii of sodium (1.02 Å) and calcium (1.00 Å) cations are similar to the radius of the 15-crown-5 ether head group cavity (0.86–0.92 Å) and complex stability is optimised when the ratio of crown cavity size to cation diameter is at or near 1 (Izatt et al., 1985). Ratios less than 0.7 yield very low stability complexes (Izatt et al., 1985). Although the cations are too large to 'fit' into the ligand 1:2 complexes may be formed (Lamb et al., 1981). The difference in the response of PCE vesicles when in contact with sodium and calcium ions may be explained by considering the complex stability. The complex stability of calcium-PCE would exceed that of sodium-PCE, due to the higher solvation energy of sodium ions in water. The solvation energy barrier has been known to prevent crown ether chelation of highly solvated cations (Izatt et al., 1985).

Table 1
Characterisation of PCE

Analysis method	Results
Mass spectrometry—ZAB-SE MS fast atom bombardment (FAB)	526 (19%, $M^+ + K$) 510 (100%, $M^+ + Na$)
Melting point	54°C

The inclusion of dicyclohexano-18-crown-6 in vesicles prepared from sodium 4-(1′heptyinonyl)benzene sulfonate increased the permeability of these vesicles to potassium and sodium tracer molecules (Hamilton and Kaler, 1987). Other changes in self assembled crown ether systems on the addition of metal cations include an increase in the critical micelle concentration (CMC) of octyl 15-crown-5 on the addition of sodium ions due to the formation of a

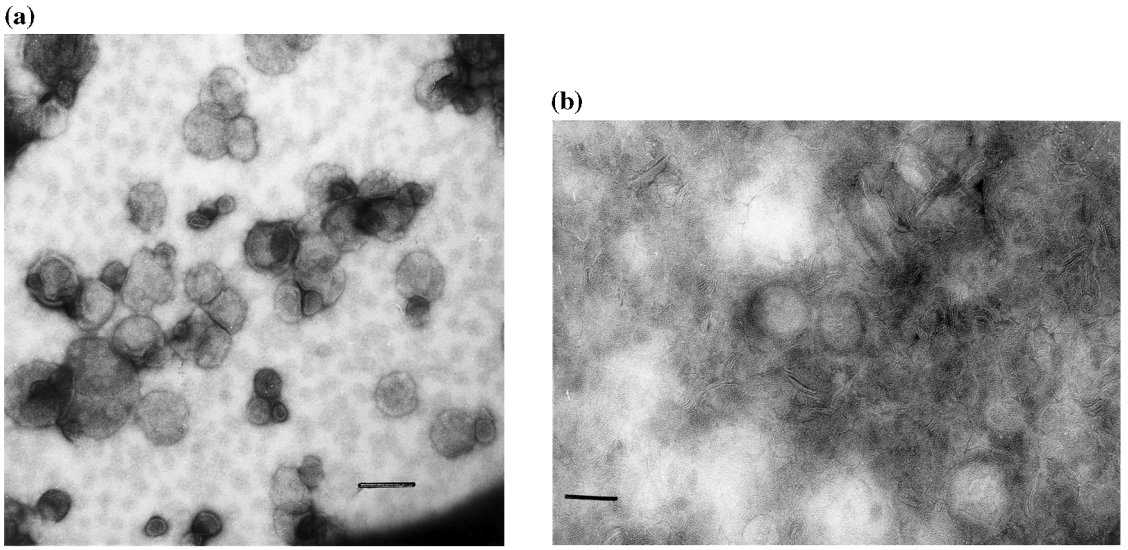


Fig. 1. X103 000 MAG. Transmission electron micrograph of: (a) PCE, cholesterol, Solulan C24 (45: 45:10) niosomes; (b) PCE, cholesterol (50:50) niosomes; bar, 100 nm.

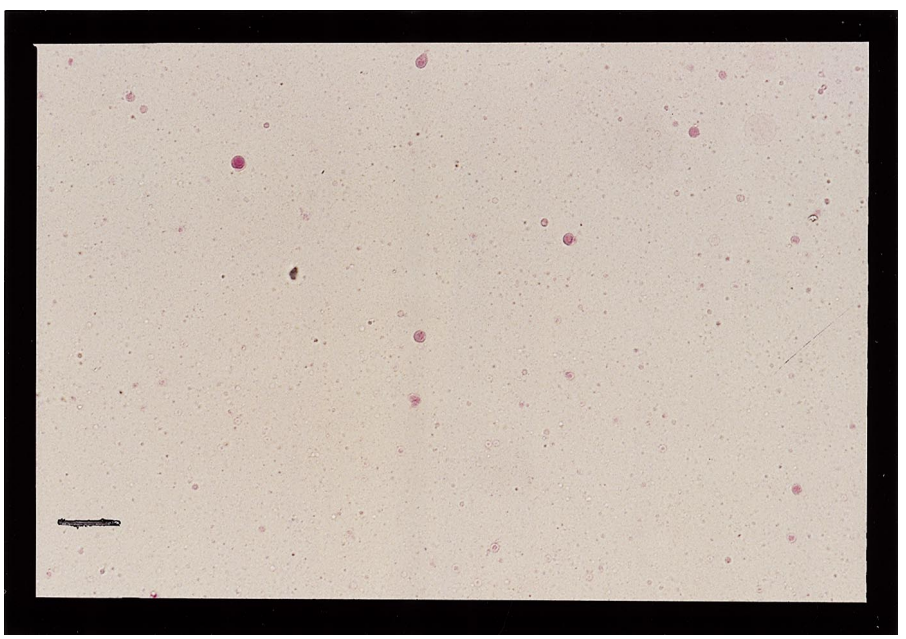


Fig. 2. X250 MAG. Optical micrograph of PCE, cholesterol, Solulan C24 (45:45:10) niosomes encapsulating rhodamine, bar = 40 μm.

more ionic species (Kuo et al., 1983). However micelle formation was promoted by the addition of barium ions to an aqueous dispersion of the octadecyl 18-diazacrown-6 amphiphile (Le Moigne et al., 1977).

It must be emphasised that the differences be-

Table 2
PCE niosome size

Composition of PCE niosomes	Particle size (sonicated niosomes; nm)	Particle size (unsonicated niosomes; μm)
Empty niosomes (PCE/CHOL; 50:50)	124	9.0
Empty niosomes (PCE/CHOL/Solulan C24; 45:45:10)	134	6.7
Rhodamine B niosomes (PCE/CHOL/Solulan C24; 45:45:10)	—	6.3

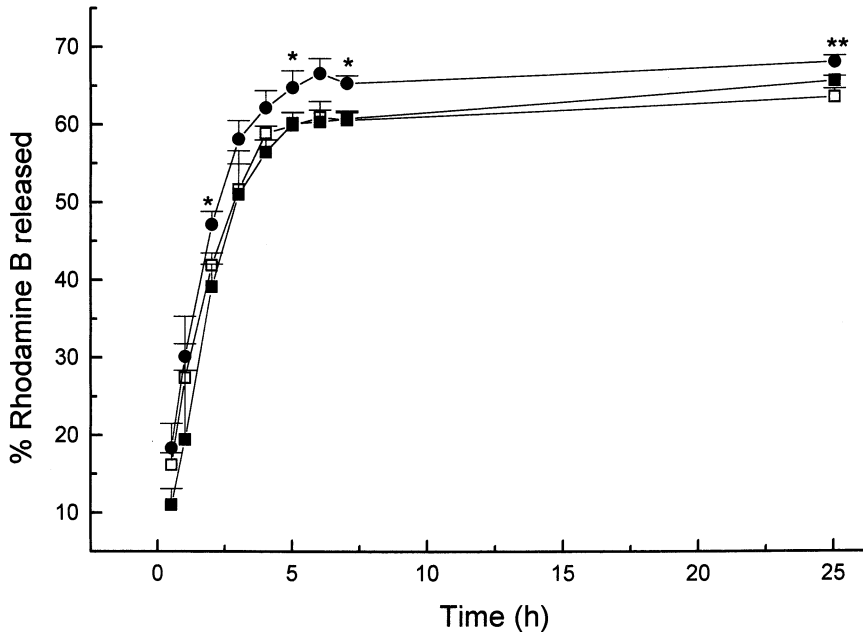


Fig. 3. The in vitro release of rhodamine B from PCE, cholesterol, Solulan C24 (45:45:10) niosomes in the presence of ●, calcium chloride (2 M); ■, sodium chloride (2 M); and □, water. Data points represent the mean ± SEM; $n = 3$. $^{**}P < 0.01$; $^{*}P < 0.05$. Statistically significant differences are seen at 2 and 4 h between calcium chloride and sodium chloride incubation mixtures and also at 5 h and 24 h between calcium chloride and control (water) mixtures. There were no differences seen between sodium chloride and control (water) incubation mixtures.

tween the rhodamine B release rates of PCE niosomes in the presence of sodium and calcium ions is only slight. This may be due to the inherent leakiness of these niosomes. In the event of this being the case the inclusion of other vesicle forming surfactants may be used to remedy the leakiness (Uchegbu and Florence, 1995).

However, the purpose of the present communication is to present crown ether based niosomes prepared from a new crown ether amphiphile—PCE and cholesterol as metal ion sensitive/containing systems. The use of systems such as these in calcium homeostasis is proposed. Also cation containing controlled release systems, once optimised, may be used simply because they present the user with a different type of charged system.

4. Conclusions

A crown ether based niosome system has been formed from a new crown ether amphiphile—PCE and cholesterol. These crown ether based niosomes may be used to develop cation sensitive/

containing controlled release systems, as PCE/CHOL niosomes encapsulating rhodamine B were more leaky in the presence of calcium ions than in the presence of sodium ions.

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References

Baillie, A.J., Coombs, G.H., Dolan, T.F., Laurie, J., 1986. Non-ionic surfactant vesicles, niosomes, as a drug delivery system for the anti-leishmanial drug sodium stibogluconate. J. Pharm. Pharmacol. 38, 502–505.

Echegoyen, L.E., Hernandez, J.C., Kaifer, A.E., Gokel, G.W., Echegoyen, L., 1988. Aggregation of steroidal lariat ethers: the first example of non-ionic liposomes (niosomes) formed from neutral crown ether compounds. J. Chem. Soc. Chem. Commun. 12, 836–837.

Florence, A.T., Baillie, A.J., 1989. Non-ionic surfactant vesicles—alternatives to drug delivery?. In: Prescott, L.F., Nimmo, W.S. (Eds.), Novel Drug Delivery and its Therapeutic Application. Wiley, London, 1989, pp. 281–295.

Florence, A.T., 1993. Non-ionic surfactant vesicles. In: Gregoriadis, G. (Ed.), Liposome Technology, vol.1, CRC Press, Boca Raton, FL, 1993, pp. 157–176.

Hamilton, R.T., Kaler, E.C., 1987. Ionic permeability of synthetic vesicles. J. Colloid Interface Sci. 116, 248–255.

Ikeda, I., Emura, H., Yamamura, S., Okahara, M., 1982. Contribution of lipophilicity to the performance of crown ethers. Effect of bulk and shape of the lipophilic substituents. J. Org. Chem. 47, 5150–5153.

Izatt, R.M., Bradshaw, J.A., Nielsen, S.A., Lamb, J.D., Christensen, J.J., 1985. Thermodynamic and kinetic data for cation–macrocycle interaction. Chem. Rev. 85, 271–339.

Kuo, P., Tsuchiya, K., Ikeda, I., Okahara, M., 1983. Salt effects on the surface properties of long chain alkyl substituted crown compounds. J. Colloid Interface Sci. 92, 463–468.

Lamb, J.D., Izatt, R.M., Garrick, D.G., Bradshaw, J.S., Christensen, J.J., 1981. The influence of macrocyclic ligand structure on carrier-facilitated cation transport rates and selectivites through liquid membranes. J. Membr. Soc. 9, 83–107.

Lapidot, Y., De Groot, N., Fry-Shafrir, I., 1967. II. A general method for the preparation of acylaminoacyl–tRNA. Biochim. Biophys. Acta. 145, 292–299.

Le Moigne, J., Gramain, P., Simon, J., 1977. Fast cation transfer at a micelle subsurface: synthesis and properties of an amphiphilic macrocylce. J. Colloid Interface Sci. 3, 565–567.

Monserrat, K., Gratzel, Tundo, P., 1980. Light-induced charge injection on functional crown ether vesicles. J. Am. Chem. Soc. 102, 5527–5529.

Ozeki, S., Kojima, A., Harada, S., Inokuma, S., Takahashi, H., Kuwamura, T., Uchlyama, H., Abe, M., Ogino, K., 1990. Molecular assemblies of ((dodecyloxy)methyl)-18-crown-6 in water. J. Phys. Chem. 94, 82078212.

Rogerson, A., Cummings, J., Willmott, N., Florence, A.T., 1988. The distribution of doxorubicin in mice following administration in niosomes. J. Pharm. Pharmacol. 40, 337–342.

Uchegbu, I.F., Duncan, R., 1997. Niosomes containing *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer doxorubicin (PK1): effect of method of preparation and choice of membrane surfactant on niosome characteristics and a preliminary study of body distribution. Int. J. Pharm. 155, 7–18.

Uchegbu, I.F., Florence, A.T., 1995. Non-ionic surfactant vesicles (niosomes): physical and pharmaceutical chemistry. Adv. Colloid Interface Sci. 58, 1–55.

Uchegbu, I.F., Double, J.A., Turton, J.A., Florence, A.T., 1995. Distribution, metabolism and tumoricidal activity of doxorubicin sorbitan monostearate (Span 60) niosomes. Pharm. Res. 12, 1019–1024.