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Non-ionic surfactant based vesicles (niosomes) in drug delivery

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Abstract

The self assembly of non-ionic surfactants into vesicles was first reported in the seventies by researchers in the cosmetic industry. Since then a number of groups world wide have studied non-ionic surfactant vesicles (niosomes) with a view to evaluating their potential as drug carriers. This article presents a summary of the achievements in the field to date. Niosomes may be formed form a diverse array of amphiphiles bearing sugar, polyoxyethylene, polyglycerol, crown ether and amino acid hydrophilic head groups and these amphiphiles typically possess one to two hydrophobic alkyl, perfluoroalkyl or steroidal groups. The self assembly of surfactants into niosomes is governed not only by the nature of the surfactant but by the presence of membrane additives, the nature of the drug encapsulated and the actual method of preparation. Methods of niosome preparation and the number of different morphologies that have been identified are detailed. The influence of formulation factors on niosome stability is also examined as are methods to optimise drug loading. In vivo these systems have been evaluated as immunological adjuvants, anti-cancer/anti-infective drug targeting agents and carriers of anti-inflammatory drugs. Niosomes have also been used in diagnostic imaging. Efforts to achieve transdermal and ophthalmic drug delivery with some formulations are also discussed.

Keywords: Non-ionic surfactants; Niosomes; Drug delivery; Self-assembly

1. Introduction

Non-ionic surfactant based vesicles (niosomes) are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures (Fig. 1). The assembly into closed bilayers is rarely spontaneous (Lasic, 1990) and usually involves some input of energy such as physical agitation or heat. The result is an assembly in which the hydrophobic parts of the molecule are shielded from the aqueous solvent and the hydrophilic head groups enjoy maximum contact with same. These structures are analogous

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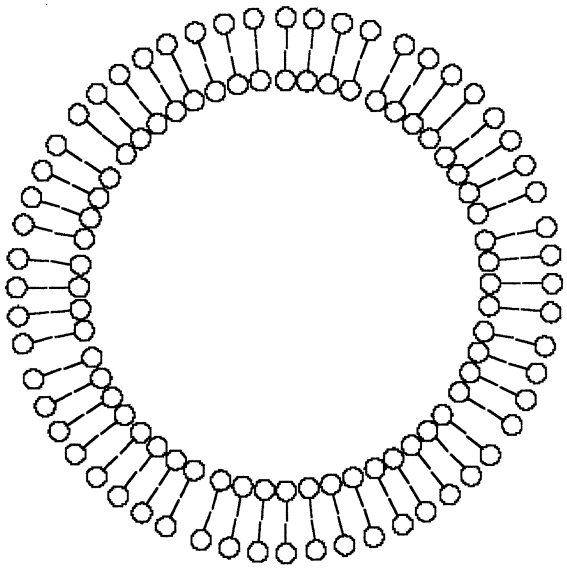

Fig. 1. Schematic representation of a niosome, ○ = hydrophilic head group, —— = hydrophobic tail.

to phospholipid vesicles (liposomes) and are able to encapsulate aqueous solutes and serve as drug carriers. The low cost, greater stability and resultant ease of storage of non-ionic surfactants (Florence, 1993a) has lead to the exploitation of these compounds as alternatives to phospholipids. Niosomes were first reported in the seventies as a feature of the cosmetic industry (Vanlerberghe et al., 1972; Handjani-Vila et al., 1979) but have since been studied as drug targeting agents. This chapter reviews the relevant data on these systems generated in our laboratories and those of others with the emphasis on the steps leading to the development of these systems as drug carriers. Areas to be covered are: non-ionic surfactant self-assembly, niosome preparation, toxicology studies, specialised systems, stability and examples of specific applications.

It is hoped that this chapter will introduce new researchers to this topic and more importantly offer the industrial community an idea of the potential utility of these systems as drug carriers.

The ultimate identity of any niosomal system and hence its properties are determined by the factors listed in Fig. 2. It is thus obvious that all these variables must be carefully controlled in the design of a niosomal drug delivery system. Invariably drug delivery design leads should always be taken from the host biology (Fig. 3). Examples of this include the use of niosomes to target the liver and spleen in leishmaniasis (Baillie et al., 1986), as

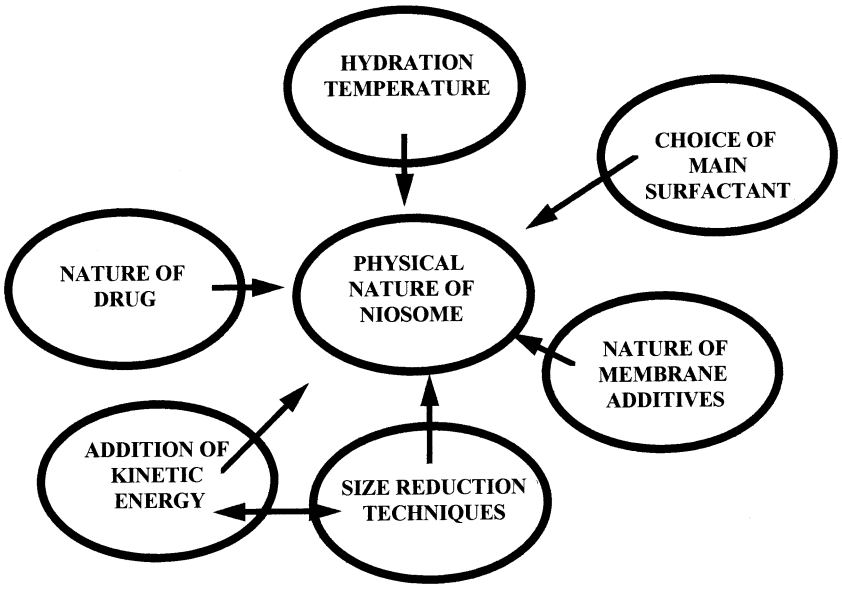

Fig. 2. Factors influencing niosome physical chemistry.

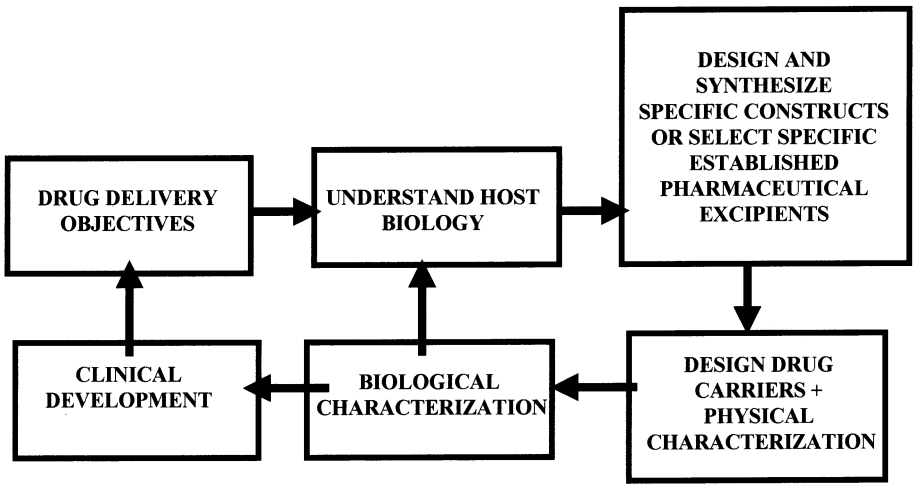

Fig. 3. Schematic flow diagram detailing the stages involved in drug delivery design.

particulate uptake by the liver and spleen is a known fact. A further example is found in anti-cancer drug targeting with niosomes (Rogerson et al., 1988; Uchegbu et al., 1995) which exploits the specific vascular architecture of tumour tissue.

It is hoped that more specific forms of targeting that incorporate molecular recognition elements may be undertaken once a correlation is made between the nature of the niosome surface and the resulting biological response. The biological response to polyoxyethylene (Blume and Cevc, 1990) coated liposomes, i.e. their reduced liver and spleen uptake has been exploited for the targeting of niosomes to tumours for example (Uchegbu et al., 1995, 1996a). Advantageously niosomes may be constructed from a variety of hydrophilic head groups (Fig. 4) and it is likely that a more specific correlation between niosome surface chemistry and niosome pharmacodynamics/pharmacokinetics may eventually emerge. The design of a hypothetical niosomal system based on a recognised biological objective would then lead either to tailored chemical synthesis or (in industrial settings where a more conservative approach prevails) the selection of tools from an existing database of approved pharmaceutical excipients (Fig. 3). Design of the drug delivery system would then be followed by stability and biological testing. Invariably yet more questions will arise from this process, but a systematic and rational evaluation scheme such as that outlined in Fig. 3 will ultimately lead to a richer understanding of the capabilities of particulate drug delivery with non-ionic surfactants.

2. Factors governing the self assembly of non-ionic surfactants into niosomes

2.1. Non-ionic surfactant structure

Theoretically niosome formation requires the presence of a particular class of amphiphile and aqueous solvent. In certain cases cholesterol is required in the formulation and vesicle aggregation for example may be prevented by the inclusion of molecules that stabilise the system against the formation of aggregates by repulsive steric or electrostatic effects. An example of steric stabilisation is the inclusion of Solulan C24 (a cholesteryl poly-24-oxyethylene ether) in doxorubicin (DOX) sorbitan monostearate (Span 60) niosome formulations (Uchegbu et al., 1995). An example of electrostatic stabilisation is the inclusion of dicetyl phosphate in 5(6)-carboxyfluorescein (CF) loaded Span 60 based niosomes (Yoshioka et al., 1994).

Previous accounts have listed the types of non-ionic surfactants that are known to form vesicles (Ozer et al., 1991; Florence, 1993b; Uchegbu, Florence, 1995). Such amphiphiles by definition

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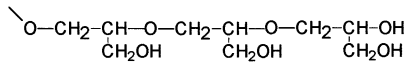

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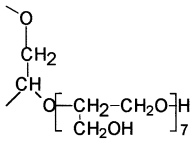

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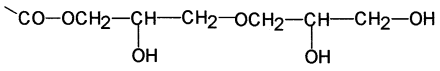

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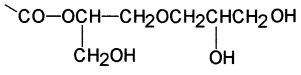

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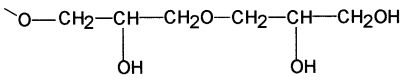

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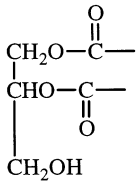

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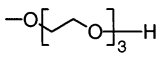

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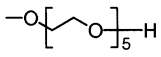

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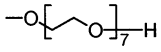

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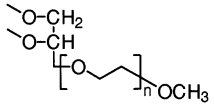

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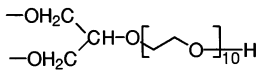
The molecular weight of n varies from 500 to 950



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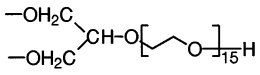

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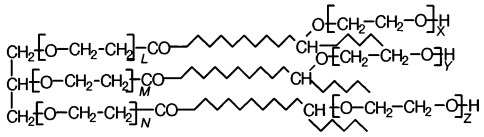

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Fig. 4.

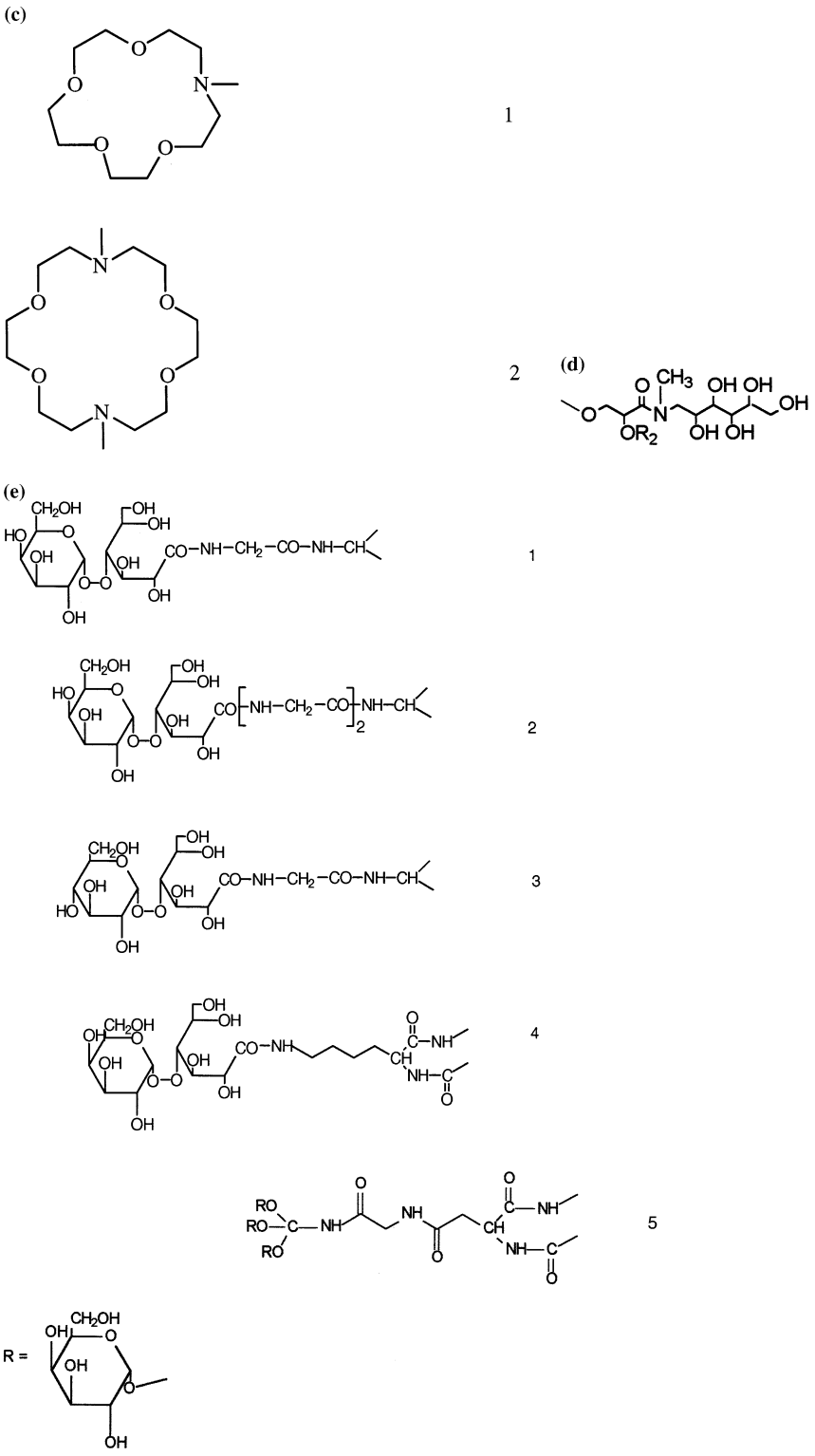

Fig. 4

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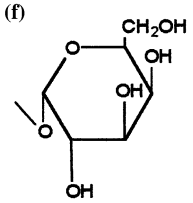

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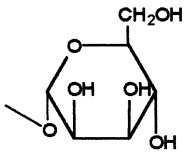

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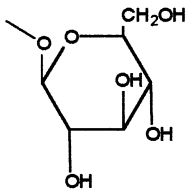

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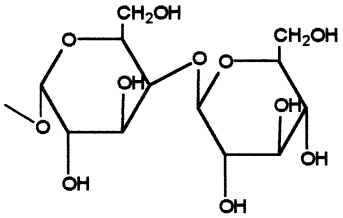

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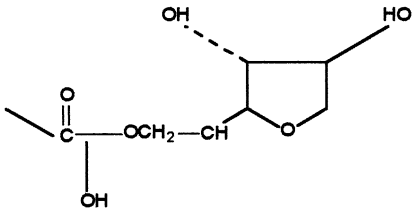

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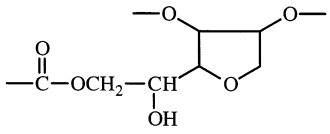

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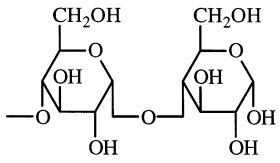

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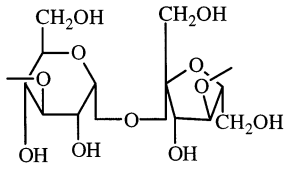

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Fig. 4. (*Continued*)

must possess a hydrophilic head group (Fig. 4) and a hydrophobic tail. The hydrophobic moiety may consist of one or two alkyl or perfluoroalkyl (Fig. 5) groups or in certain cases a single steroidal (Fig. 6) group. The alkyl group chain length is usually from C_{12}–C_{18} (Handjani-Vila, 1990; Ozer et al., 1991; Florence, 1993a; Bouwstra, Hofland, 1994; Yoshioka et al., 1994; Uchegbu, Florence, 1995). Molecules may possess one (Azmin et al., 1985; Baillie et al., 1985; Kiwada et al., 1985a,b; Rogerson et al., 1987, 1988; Stafford et al., 1988; Moser et al., 1989, 1990; Wallach, Philippot, 1993; Talsma et al., 1994; Yoshioka et al., 1994) two (Okahata et al., 1981; Azmin et al., 1985; Baillie et al., 1985; Rogerson et al., 1988; Chauhan, Lawrence, 1989; Schenk et al., 1990; Assadullahi et al., 1991; Polidori et al., 1994) or three (Tanaka, 1990) alkyl chains. Perfluoroalkyl surfactants that form vesicles possess chain lengths as short as C10 (Zarif et al., 1993; Guedj et al., 1994). Additionally crown ether amphiphiles bearing a steroidal (Fig. 6) (Echegoyen et al., 1988), C_{14} alkyl (Montserrat et al., 1980) or C_{16} alkyl (Darwish, Uchegbu, 1997) hydrophobic unit have been shown to form vesicles.

While the number of hydrophobic permutations is at present limited, there have been a wide variety of hydrophilic head groups in vesicle forming surfactants (Fig. 4) and it is in this area of vesicle forming surfactant design that considerable scope for new formulations still exist. The two portions of the molecule may be linked via ether, amide or ester bonds (Fig. 4).

We have observed that a parameter like the hydrophilic lipophilic balance (HLB) is a good indicator of the vesicle forming ability of any surfactant. With the sorbitan monostearate (Span) surfactants, a HLB number of between 4 and 8 was found to be compatible with vesicle formation (Yoshioka et al., 1994; Uchegbu, Florence, 1995). The guidance offered by the HLB number is useful as apart from the theoretical methods of estimating HLB number in which the relative proportions of both the hydrophilic and hydrophobic portions of the molecule are assessed, practical methods of HLB number determination have been reported (Trapani et al., 1995). These studies may be useful in the evaluation of new classes of compounds for their vesicle forming ability. The water soluble detergent polysorbate 20 (Fig. 7) also forms niosomes in the presence of cholesterol (Saettone et al., 1996; Santucci et al., 1996; Carafa et al., 1998). This is tucci et al., 1996; Carafa et al., 1998). This is despite the fact that the HLB number of this compound is 16.7 and it appears on first inspection to be too hydrophilic to form a bilayer membrane. However with an optimum level of cholesterol, it seems that niosomes are indeed formed from polysorbate 20 (Santucci et al., 1996)

Fig. 4. Hydrophilic head groups found in vesicle forming surfactants: (a) glycerol head groups (Vanlerberghe et al., 1972; Handjani-Vila et al., 1979; Ribier et al., 1984; Azmin et al., 1985; Baillie et al., 1985, 1986; Rogerson et al., 1987; Kerr et al., 1988; Rogerson et al., 1988; Stafford et al., 1988; Cable, 1989; Florence, Baillie, 1989; Moser et al., 1989; Lesieur et al., 1990; Moser et al., 1990; Seras et al., 1992; Uchegbu et al., 1992; Seras et al., 1994; Uchegbu et al., 1994; Arunothayanun et al., 1996; Bernard et al., 1996; Seras et al., 1996; Uchegbu et al., 1996a,b; Dimitrijevic et al., 1997; Uchegbu, Duncan, 1997) (b) ethylene oxide head groups (Okahata et al., 1981; Chauhan, Lawrence, 1989; Tanaka, 1990; Hofland et al., 1992; Yoshida et al., 1992; Wallach, Philippot, 1993; Hofland et al., 1994; Niemec et al., 1994; Schreier, Bouwstra, 1994; Talsma et al., 1994; Lawrence et al., 1996; Vanhal et al., 1996; Gianasi et al., 1997) (c) crown ether head groups (Montserrat et al., 1980; Echegoyen et al., 1988; Darwish, Uchegbu, 1997) (d) polyhydroxy head groups (Assadullahi et al., 1991) (e) sugar head groups + amino acids 1,2,4&5 = galactose derivatives, 3 = glucose derivative (Zarif et al., 1993; Guedj et al., 1994; Polidori et al., 1994) (f) sugar head groups 1 = galactose, 2 = mannose, 3 = glucose, 4 = lactose, 5 and 6 = sorbitan esters, 7 = maltose, 8 = sucrose (Kiwada et al., 1985a,b; Schenk et al., 1990; Chandraprakash et al., 1993; Naresh et al., 1993; Reddy, Udupa, 1993; Udupa et al., 1993; Parthasarathi et al., 1994; Polidori et al., 1994; Yoshioka, Florence, 1994; Yoshioka et al., 1994; Jain and Vyas, 1995a,b; Uchegbu et al., 1995; Yoshioka et al., 1995; Murdan et al., 1996; Naresh, Udupa, 1996; Duncan et al., 1997; Gianasi et al., 1997; Uchegbu, Duncan, 1997).

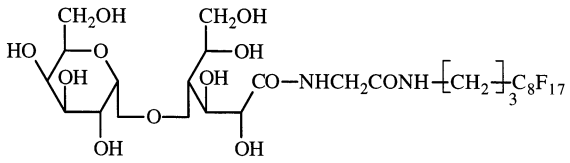

Fig. 5. Vesicle forming fluorinated surfactant (Zarif et al., 1993).

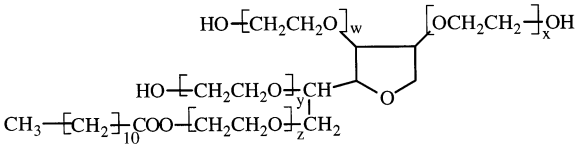

Fig. 7. Polysorbate 20.

Although a particular membrane surfactant may be chosen by combining the hydrophilic moieties given in Fig. 4 with an appropriate hydrophobic group. Established molecules may also be chosen from those mentioned in a few earlier reviews (Ozer et al., 1991; Florence, 1993b; Bouwstra, Hofland, 1994; Uchegbu, Florence, 1995). Some of these surfactants such as the Span and Brij surfactants are already established pharmaceutical excipients.

Recently neutron reflectivity data obtained from glycerol and polyoxyethylene surfactant monolayers has been used to calculate the area per molecule and degree of monolayer hydration (Barlow et al., 1995). These calculated values have in turn been used to provide computer simulations of the three dimensional form of the proposed surfactant vesicles. Whether such niosome modelling software will be used as a predictive tool in the future is unknown at the present time. However a software generated feature that will be particularly useful for vesicular drug delivery is a database incorporating all the relevant experimental data on surfactants which will in turn predict the vesicle forming behaviour of hypothetical compounds or compound mixtures.

In time it is envisaged that such an Expert System will emerge that will hold details not only on the optimum conditions required to induce vesicle formation on an amphiphile by am-

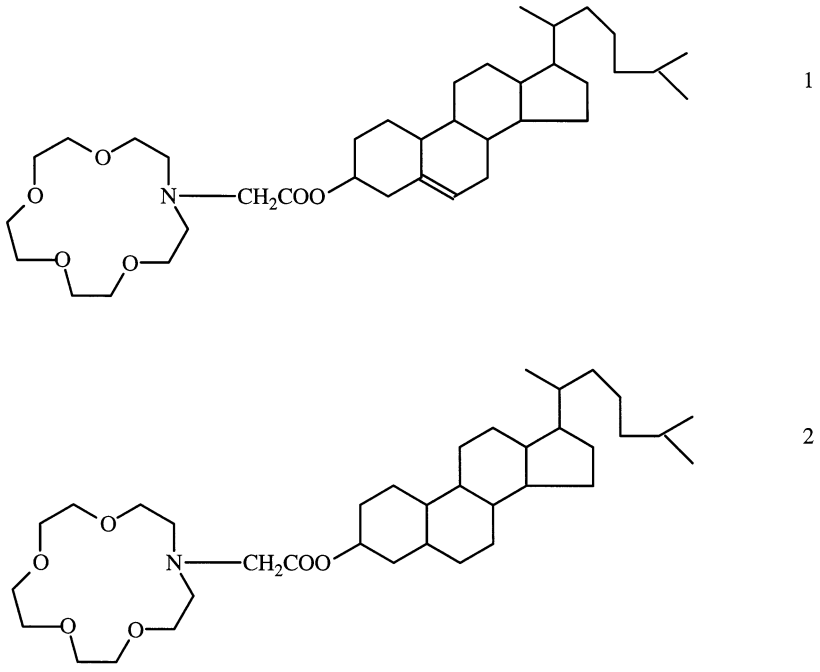

Fig. 6. Vesicle forming crown ether surfactants 1 = cholestanyl derivative, 2 = cholesteryl derivative (Echegoyen et al., 1988).

phiphile basis but also on predicted vesicle size, drug-loading capabilities and expected pharmacology. Ideally such a system should be interfaced with regulatory, intellectual property, chemical assay and compound physical stability databases.

2.2. Membrane additives

Unfortunately the prediction of vesicle forming ability is not a simply a matter of HLB numbers and chemical structure and various other factors come into play. It is generally accepted that the parameters for self-assembly laid own by Israelachvili (Israelachvili, 1985) in which a critical packing parameter (CPP) was defined, largely hold true today.

$$\mathrm{CPP} = v/l_c a_0$$

where v = hydrophobic group volume, l_c = the critical hydrophobic group length and a_0 = the area of the hydrophilic head group (Fig. 8). A CPP of between 0.5 and 1 indicates that the surfactant is likely to form vesicles (Israelachvili, 1985). A CPP of below 0.5 (indicating a large contribution from the hydrophilic head group area) is said to give spherical micelles and a CPP of above 1 (indicating a large contribution from the hydrophobic group volume) should produce inverted micelles, the latter presumably only in an oil phase, or precipitation would occur.

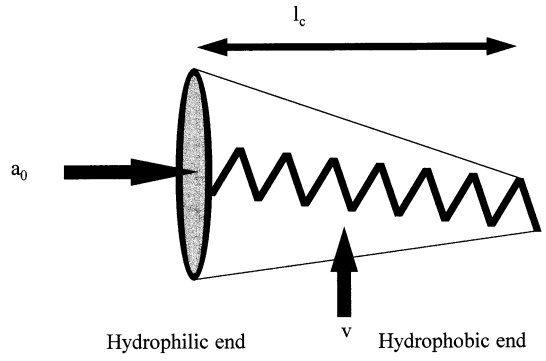

Fig. 8. Schematic representation of an amphiphile, a_0 = hydrophilic head group area, v = hydrophobic chain volume, l_c = hydrophobic chain length.

Often various additives must be included in the formulation in order to prepare stable niosomes. Fig. 9 illustrates the number of different morphologies, all with different permeability and stability properties, which may be formed by the manipulation of the membrane forming agents in a typical system (Uchegbu et al., 1996b). The most common additive found in niosomal systems is cholesterol. Thus in cases where a mixture of surfactants or cholesterol is used to prepare niosomes, the operational CPP values will be those of the entire components.

The bilayer membrane is an ordered structure and may exist in the gel state (L_β or $L_{\beta'}$—the latter indicating a situation where the alkyl chains are tilted at a slight angle with respect to the plane of the bilayer) or the liquid crystalline state—sometimes called the lamellar phase (L_α). The difference between these two phases is the degree of order, with the gel state being the most ordered structure and the liquid crystal state being less ordered. In the liquid crystal state there is lateral diffusion of bilayer material whereas in the gel state the alkyl chains are crystallised or otherwise less mobile.

For any system the liquid crystalline state exists at a higher temperature than the gel state. The increase in temperature (T) although yielding an increase in the enthalpy term (ΔH) also results in an increase in entropy (ΔS) and thus a lowering of the free energy (ΔG) of the system and it is the application of heat that is the driving force for this transition. The phase transition for hexadecyl diglycerol ether ($C_{16}G_2$): Solulan C24 niosomes (91:9) (Uchegbu et al., 1997) and 1,2-dialkyl glycerol polyoxyethylene ether surfactants (Lawrence et al., 1996) has been recorded using differential scanning calorimetry and on $C_{16}G_2$ and hexadecyl poly-5-oxyethylene ether ($C_{16}EO_5$) using phase fluorimetry (Ribier et al., 1984). With the latter technique the mobility of a membrane fluorescent probe is monitored as a function of temperature (Ribier et al., 1984). This change from the gel to liquid phase (analogous to melting) has been adequately documented for liposomal systems (New, 1990).

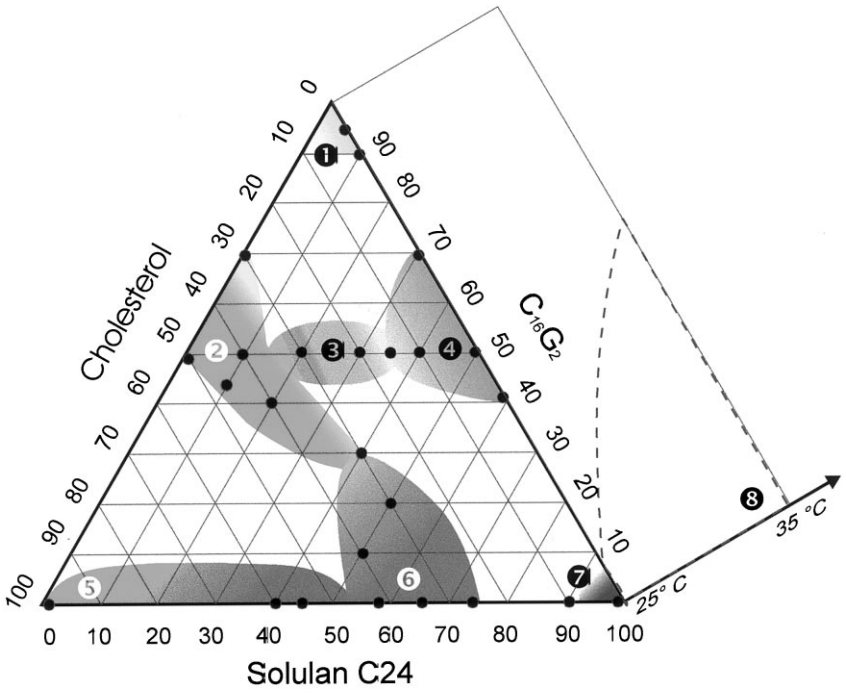

Fig. 9. The hexadecyl diglycerol ($C_{16}G_2$)-cholesterol-Solulan C24 ternary phase diagram. Total surfactant/lipid content = 60 mM. Region 1 = polyhedral niosomes (5 μm), region 2 = spherical, helical and tubular niosomes (2–10 μm), region 3 = discomes (12–60 μm), large niosomes (12–40 μm), spherical, helical and tubular niosomes (2–10 μm), region 4 = discomes and possibly Mixed micelles, region 5 = cholesterol crystals, region 6 = spherical niosomes (2–10 μm) and region 7 = a clear liquid probably consisting of mixed micelles, region 8 = mixed micelles formed on elevation of temperature (Uchegbu et al., 1996a).

Cholesterol is known to abolish the gel to liquid phase transition of liposomal (New, 1990) and niosome systems (Fig. 10) (Cable, 1989) resulting in niosomes that are less leaky (Rogerson et al., 1987). Span 60 niosomes prepared without cholesterol formed a gel and only on the addition of cholesterol was a homogenous niosome dispersion obtained (Yoshioka et al., 1994). Cholesterol is thus usually included in a 1:1 molar ratio in most formulations. However even after the addition of cholesterol, the intrinsic phase transition behaviour of vesicle forming surfactants still influences the properties of the dispersion: notably the membrane permeability, encapsulation efficiency, bilayer rigidity, ease of rehydration of freeze dried niosomes, toxicity, etc. all of which are discussed below.

Often niosomes must be stabilised by the addition of a charged molecule to the bilayer such as dicetyl phosphate as mentioned above. Dicetyl phosphate prevents the aggregation of $C_{16}G_2$ niosomes (Cable, 1989) and is also added to $C_{16}G_2$ niosomes encapsulating haemoglobin in order to achieve an electrophoretic mobility similar to that of erythrocytes (Moser et al., 1989).

2.3. Nature of the encapsulated drug

Another factor often overlooked is the influence of an amphiphilic drug on vesicle formation. While sorbitan monostearate (Span 60) niosomes containing dicetyl phosphate formed homogenous dispersions when encapsulating CF (Yoshioka et al., 1994) this system formed an aggregated dispersion when encapsulation of the amphipathic drug DOX was attempted (Uchegbu, 1994). A steric stabiliser Solulan C24 (poly-24-oxyethylene

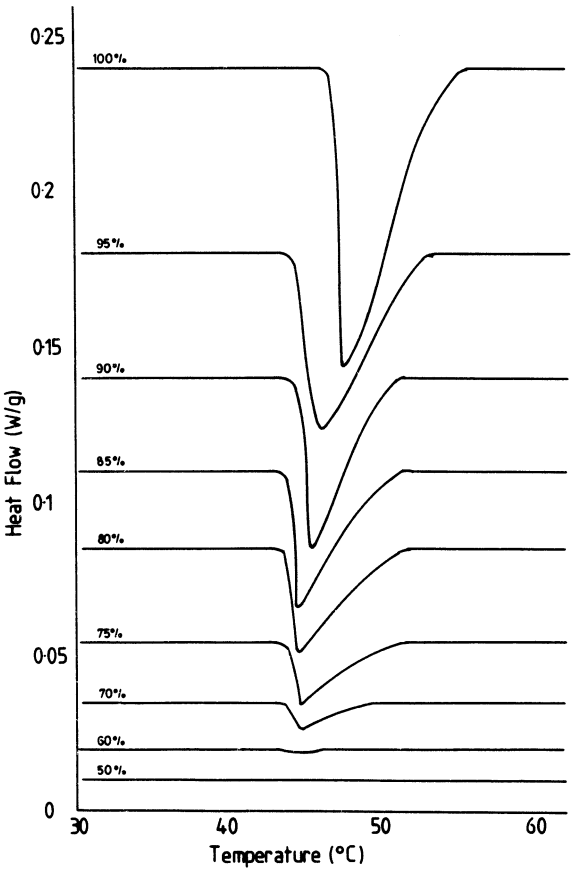

Fig. 10. Differential scanning calorimetry (DSC) scans of niosomes containing the mol% $C_{16}G_2$ shown and cholesterol (Cable, 1989).

cholesteryl ether) must be added to the formulation to ensure a homogenous formulation devoid of aggregates (Uchegbu, 1994). DOX (Fig. 11) has been shown to alter the electrophoretic mobility

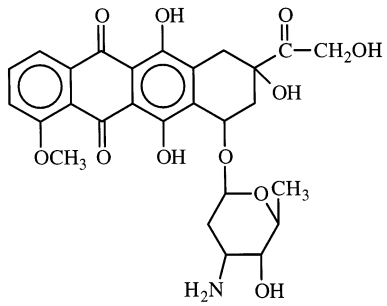

Fig. 11. Doxorubicin.

of hexadecyl diglycerol ether ($C_{16}G_2$) niosomes in a pH dependent manner (Cable, 1989), an indication that the amphipathic drug is incorporated in the vesicle membrane. From the foregoing, it is clear that Israelachvili's CPP of a potential niosome system must take into account the presence of amphipathic or hydrophobic drugs as both these substances will be incorporated into the vesicle membrane.

2.4. Surfactant and lipid levels

The level of surfactant/lipid used to make niosomal dispersions is generally 10–30 mM (1–2.5% w/w) (Okahata et al., 1981; Baillie et al., 1985; Lesieur et al., 1990; Seras et al., 1992; Uchegbu et al., 1992; Zarif et al., 1993; Lawrence et al., 1996; Saettone et al., 1996; Santucci et al., 1996; Seras et al., 1996; Uchegbu et al., 1996a). Altering the surfactant:water ratio during the hydration step may affect the system's microstructure (Tanaka, 1990) and hence the system's properties. However increasing the surfactant/lipid level also increases the total amount of drug encapsulated, as discussed below, although highly viscous systems result, if the level of surfactant/lipid is too high.

2.5. Temperature of hydration

The hydrating temperatures used to make niosomes should usually be above the gel to liquid phase transition temperature of the system.

3. Niosome preparation

The formation of vesicular assemblies requires the input of some form of energy (Lasic, 1990) and all the experimental methods surveyed consist of the hydration of a mixture of the surfactant/lipid at elevated temperature followed by optional size reduction to obtain a colloidal dispersion. This is followed by the separation of the unentrapped drug from the entrapped drug by either centrifugation, gel filtration or dialysis. Only one method (Novasome®) could be found in the literature on the preparation of niosomes on an industrial scale (Wallach, Philippot, 1993). This

involves the injection of the melted surfactants/lipids into a large volume of well-agitated heated aqueous solutions. Although a method involving the addition of an aqueous solution to a solid mixture of lipids and surfactants (Handjani-Vila et al., 1979) is said to be suitable for the handling of 'large quantities—kilograms' of dispersions.

3.1. Hydration techniques

The more commonly used laboratory methods of niosome preparation and drug loading identified in the literature are listed below.

1. The injection of an organic solution of surfactants/lipids in an aqueous solution of the drug to be encapsulated which is heated above the boiling point of the organic solvent (ether injection) (Baillie et al., 1985).
2. The formation of a surfactant/lipid film by the evaporation of an organic solution of surfactants/lipids. This film is then hydrated with a solution of the drug (hand shaking) (Azmin et al., 1985; Baillie et al., 1985). This method was previously described by Bangham and others (Bangham et al., 1965) for the preparation of liposomes.
3. The formation of an oil in water (o/w) emulsion from an organic solution of surfactants/lipids and an aqueous solution of the drug. The organic solvent is then evaporated to leave niosomes dispersed in the aqueous phase. In some cases, a gel results which must be further hydrated to yield niosomes. (reverse phase evaporation) (Kiwada et al., 1985a), previously described by Szoka and Papahadjopoulos (Szoka and Paphadjopoulos, 1978) for the preparation of liposomes.
4. The injection of melted lipids/surfactants into a highly agitated heated aqueous phase in which presumably the drug is dissolved (Wallach, Philippot, 1993) or the addition of a warmed aqueous phase dissolving the drug to a mixture of melted lipids and hydrophobic drug (Niemec et al., 1994).
5. The addition of the warmed aqueous phase to a mixture of the solid lipids/surfactants (Handjani-Vila et al., 1979).

Methods 4 and 5 do not require the use of organic solvents, which are expensive, difficult to remove in their entirety and hazardous.

6. Niosomes may also be formed from a mixed micellar solution by the use of enzymes (Chopineau et al., 1994). A mixed micellar solution of $C_{16}G_2$, DCP, polyoxyethylene cholesteryl sebacetate diester (PCSD) converts to a niosome dispersion when incubated with esterases. PCSD is cleaved by the esterases to yield polyoxyethylene, sebacic acid and cholesterol. Cholesterol in combination with $C_{16}G_2$ and DCP then yields $C_{16}G_2$ niosomes.
7. The homogenisation of a surfactant/lipid mixture followed by the bubbling of nitrogen gas through this mixture (Talsma et al., 1994). Apparently the homogenisation step may be omitted from the procedure with out affecting particle size, although a longer 'bubbling' time was required.

3.2. The reduction of niosome size

Niosomes prepared as described above are usually in the micron size range (Handjani-Vila et al., 1979; Azmin et al., 1985) although some of the methods (Baillie et al., 1985; Wallach, Philippot, 1993; Talsma et al., 1994) produce niosomes in the sub-micron (≈ 300 nm) size range.

Often a size reduction step must be incorporated into the niosome production procedure, subsequent to the initial hydration step as vesicle size has an important bearing on vesicle biodistribution. For example sub-200 nm phospholipid vesicles have been shown to avoid splenic but not liver uptake (Litzinger et al., 1994). A reduction in vesicle size may be achieved by a number of methods.

1. Probe sonication (Azmin et al., 1985; Baillie et al., 1985) which yields $C_{16}G_3$ niosomes in the 100–140 nm size range.
2. Extrusion through 100 nm Nucleopore filters (Stafford et al., 1988) which yields sodium stibogluconate $C_{16}G_3$ niosomes in the 140 nm size range.
3. In some instances the combination of sonication and filtration (220 nm Millipore® filter) has been used to achieve DOX loaded Span 60 niosomes in the 200 nm size range (Uchegbu et al., 1995).

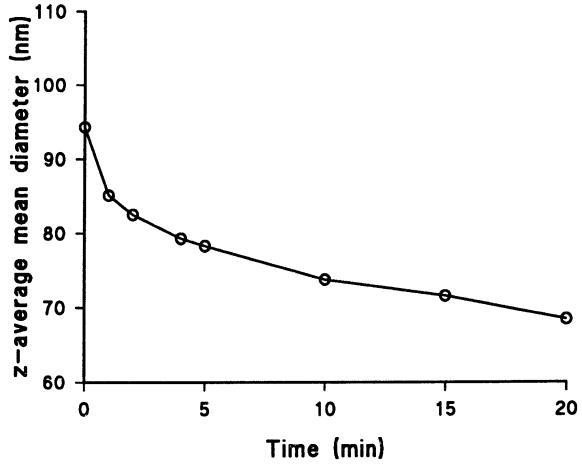

Fig. 12. The effect of microfluidization at a pressure of 0.55 mpa and a flow rate of 48 ml min^{-1} on the size of $C_{16}G_3$, cholesterol, dicetyl phosphate (47.5:47.5:5) niosomes. One cycle = 35 s.

4. The achievement of sub-50 nm sizes is possible by the use of a microfluidizer (Fig. 12).
5. High-pressure homogenisation also yields vesicles of below 100 nm in diameter although drug loading is ultimately sacrificed to achieve this small size (Fig. 13).

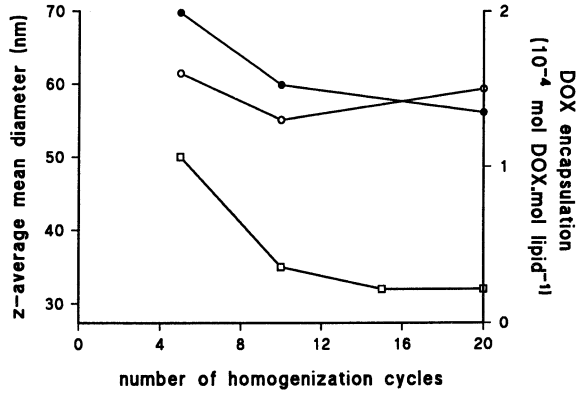

Fig. 13. The effect of homogenisation of doxorubicin multilamellar niosomes at 60 mpa pressure on niosome size and encapsulation efficiency, ● = $C_{16}G_2$, cholesterol, Solulan C24 (45:45:10)-niosome size, ○ = $C_{16}G_2$, cholesterol, Solulan C24 (45:45:10)-niosome encapsulation efficiency, □ = $C_{16}G_2$, cholesterol, Solulan C24 (30:30:40)-niosome size.

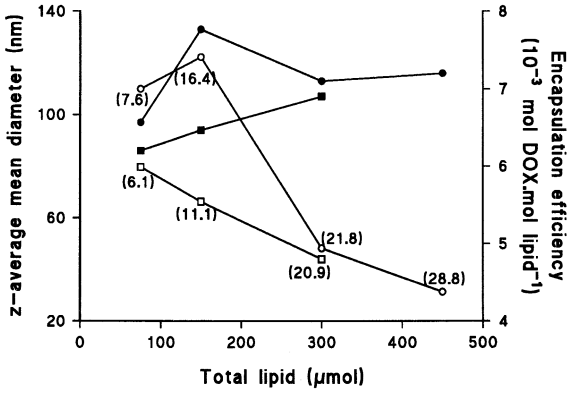

Fig. 14. The effect of total amount of surfactant/lipid used with the fixed level of DOX (6.9 μmoles) on the size and encapsulation efficiency of doxorubicin $C_{16}G_2$ niosomes. ● = $C_{16}G_2$, cholesterol, Solulan C24 (45:45:10)-niosome size, ○ = $C_{16}G_2$, cholesterol, Solulan C24 (45:45:10)-niosome encapsulation efficiency, ■ = $C_{16}G_2$, cholesterol, Solulan C24, dicetyl phosphate (42.75:42.75:9.5:2)-niosome size, □ = $C_{16}G_2$, cholesterol, Solulan C24, dicetyl phosphate (42.75:42.75:9.5:2)-niosome encapsulation efficiency. Figures in parenthesis represent % encapsulation values.

3.3. Drug loading optimisation

3.3.1. Units for the reporting of drug load

As expected drug loading is a crucial factor in the formulation of niosome delivery systems. However before a discussion on drug loading can begin, it is important to emphasise that due care and attention must be paid to the units used to quote drug-loading values. For example, drug-loading values are often quoted as the % drug encapsulated. However for these values to have any meaning the initial drug, surfactant/lipid ratio must be stated. A simple study in which the amount of DOX encapsulated was measured as a function of the initial level of surfactant/lipid, showed that this initial surfactant/lipid ratio determines ultimately the % encapsulation (Fig. 14). It was found that although the % encapsulation values steadily increased the final ratio of drug to surfactant/lipid decreased steadily. In a similar study a surfactant/lipid concentration ranging from 50–1000 μM showed no change in the final molar ratio of CF to surfactants/lipids although the '% encapsulation' increased steadily (Yoshioka et al., 1994).

Another value often quoted in the literature is the litres of drug solution encapsulated per mole of surfactant/lipid (l mol^{-1}). This value assumes no change in the concentration of the encapsulating solvent from the hydration step through to the separation and analysis steps, yet evidence abounds to show that niosome membranes are permeable to low molecular weight compounds and do release encapsulated solutes with time. It is conceivable that this release will be increased once a sufficient concentration gradient is achieved across the vesicle membrane, providing the vesicle membrane is not absolutely impermeable. It is obvious that a sufficient concentration gradient will be achieved during the separation procedure and thus drug solute leakage will commence before the start of analysis for drug encapsulation.

Clearly encapsulation efficiency once given in % encapsulation must be qualified with details on the initial ratio of drug to surfactant lipid. In our opinion the most useful value to any formulator will be the ratio of drug to surfactant in the final formulation in (g g^{-1}) or (mol mol^{-1}). This gives adequate information on the level of excipient that must be administered at each dose level.

3.3.2. Effect of bilayer constituents

The chemical nature of the niosome membrane may be manipulated to increase drug loading, by altering the nature of the hydrophilic head group and/or the hydrophobic moiety. When a series of C_{16}-sugars were examined the encapsulation efficiency for the aqueous solute [^{14}C]sucrose followed the trend glucose > mannose > galactose > lactose (Kiwada et al., 1985b). These differences may be attributed to the different levels of hydration of these sugars, a parameter that would affect their vesicle forming abilities. The encapsulation of DOX *N* (2-hydroxypropyl) methacrylamide copolymer (PK1) in $C_{16}G_2$ niosomes was twice as high as in hexadecyl poly-5-oxyethylene ($C_{16}EO_5$) niosomes under identical conditions of preparation (Uchegbu, Duncan, 1997). The decreased membrane fluidity identified in $C_{16}G_2$ bilayer membranes when compared with $C_{16}EO_5$ bilayer membranes (Ribier et al., 1984) is thought to be responsible for this increased entrapment (Uchegbu, Duncan, 1997).

The nature of the hydrophobic alkyl chain affects the encapsulation efficiency of CF (Yoshioka et al., 1994) and DOX (Uchegbu, Florence, 1995) by unsonicated Span surfactant niosomes. Span 60 (C_{18}) and Span 40 (C_{16}) gave the greatest encapsulation efficiency for CF niosomes and were the least leaky niosomes due to the fact that these Span surfactants had the highest phase transition temperature (Yoshioka et al., 1994). Also unsonicated Span 60 niosomes gave the greatest encapsulation efficiency for DOX (Uchegbu, Florence, 1995). With sonicated DOX Span surfactant niosomes the encapsulation efficiency followed the trend $C_{18} > C_{16} > C_{12}$. (Uchegbu, Florence, 1995). In the same study sorbitan monoleate was found to have the lowest encapsulation efficiency. A leaky membrane due to unsaturation in the oleic side chain (Yoshioka et al., 1994) was thought to be the cause of this. The encapsulation efficiency of C_nEO_5 niosomes for PK1 was higher for $C_{18}EO_5$ than $C_{16}EO_5$ niosomes and the former were also more rigid and less deformable (Uchegbu, Duncan, 1997). These effects are attributed to the higher membrane phase transition temperature (Bouwstra, Hofland, 1994) for C_{18} systems when compared with C_{16} systems. The ease of rehydration of freeze-dried niosomes also decreased as expected with increase in surfactant hydrophobicity.

From the above, it appears that the less fluid the bilayer (higher the gel to liquid phase transition temperature) the higher the encapsulation efficiency. This is despite the fact that cholesterol, which presumably abolishes the phase transition endotherm, is included in a 1:1 molar ratio in all the above named formulations. The intrinsic membrane gel to liquid phase transition temperature has a fundamental influence on the encapsulation efficiency and as discussed later—the toxicity of these systems even when cholesterol is included in the bilayer. However other factors must also influence the encapsulation of solutes as the examination of a series of glucosides hydrated with solutions of [^{14}C]sucrose showed that, C_{16}-glucoside niosomes had a higher encapsulation efficiency than both C_{14} and C_{18} compounds (Kiwada et al., 1985a) for reasons that are not entirely apparent.

The inclusion of dicetyl phosphate in the formation of C_{16}-glucoside niosomes was also found to increase the encapsulation efficiency of [^{14}C]sucrose (Kiwada et al., 1985b).

3.3.3. Methods of drug load enhancement

Various techniques may be used to optimise drug load and this is especially important in industrial settings where there is limited scope for the chemical modification of excipients due to regulatory concerns. One such method is the dehydration–rehydration vesicle (DRV) technique first described by Kirby and Gregoriadis (Kirby and Gregoriadis, 1984) which was found to increase the encapsulation efficiency of PK1 in $C_{16}G_2$ niosomes from 3.3 to 64.4% (Uchegbu, Duncan, 1997). Unfortunately niosome size was also doubled, increasing from 151 to 380 nm. A final PK1 to surfactant ratio of 0.3 was achieved with these DRV formulations. It was noted that the ease of rehydration of these freeze-dried dispersions was directly proportional to the phase transition temperature of the non-ionic surfactant. Other methods used to maximise drug loading include the use of pH gradients (Mayer et al., 1986). In this method a pH differential exists across the niosome membrane with a lower pH inside the niosome. The amine drug is then added external to the niosome and crosses the membrane barrier in the unionised state. Once inside the niosome the drug becomes protonated and is unable to leave the niosome. The acid pH within the niosome interior thus acts as an intra-vesicular trap. This method has been employed in the formulation of vincristine sulphate niosomes (Parthasarathi et al., 1994) using citrate buffer (pH 4.0) followed by the addition of vincristine sulphate and the upward adjustment of the pH to 7.1. Once the pH has been adjusted upwards, the formulation is heated above the phase transition temperature (60°C) of the membrane in order to increase vesicle permeability. A similar method utilising Tris buffered saline (TBS) has been reported for the loading of DOX $C_{16}G_2$ niosomes (Uchegbu et al., 1994).

An alternative intra-vesicular trap has also been developed for DOX vesicles using ammonium sulphate (Haran et al., 1993). The presence of ammonium sulphate within the vesicles apparently causes DOX to form a gel within the vesicles. Using this method for DOX Span 60 niosomes, the entrapped ratio of drug to surfactants/lipids increased from 0.014 to 0.035 mol mol^{-1} (Uchegbu et al., 1996b) and the niosomes were also less leaky at 37°C (Fig. 15).

While these remote loading procedures have been used for amine drugs, similar strategies have not been used to increase the niosome encapsulation of acidic drugs.

3.4. Separation of entrapped material

The hydration of surfactant/lipid mixtures rarely leads to the entire drug being encapsulated, regardless of the drug loading optimisation steps taken. It is thus often a requirement that unencapsulated drug be removed by various means. Although it may be argued that the use of systems in which half of the drug is encapsulated and half is external to the niosome may eventually yield systems with a beneficial biphasic biodistribution profile. This drug delivery system would give an initial burst to initiate therapy followed by a

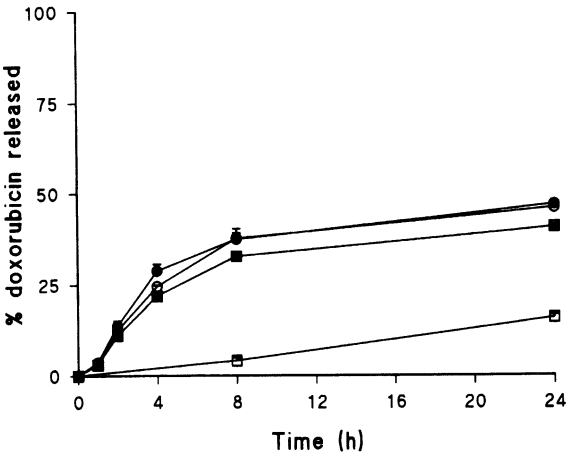

Fig. 15. The release of doxorubicin from niosomes prepared utilising a transmembrane proton gradient, niosomes were prepared utilising: ● = transmembrane proton gradient (200 μg ml^{-1} doxorubicin), ○ = transmembrane proton gradients (194 μg ml^{-1} doxorubicin), ■ = transmembrane proton gradients (134 μg ml^{-1} doxorubicin), □ = transmembrane ammonium sulphate gradients (195 μg ml^{-1} doxorubicin) (Uchegbu et al., 1996b).

Table 1
The advantages and disadvantages of the different methods of separation of the entrapped from the unentrapped drug

Separation method	Advantages	Disadvantages
Exhaustive dialysis	Suitable for large vesicles >10 μm	Extremely slow (5–24 h)
	Suitable for highly viscous systems	Large volumes of dialysate required-(may not be suitable for drugs requiring specialised disposal)
	Inexpensive	Dilutes the niosome dispersion
Centrifugation (below $7000\times g$)	Quick ($\sim$30 min)	Fails to sediment the sub-micron niosomes
	Inexpensive instrumentation	May lead to the destruction of fragile systems
	Concentrates the niosome dispersion	
Ultracentrifugation ($150\,000\times g$)	Sediments all size populations	Expensive Instrumentation
	Concentrates the niosome dispersion	Long centrifugation times (1–1.5 h)
		May lead to the destruction of fragile systems
		May lead to the formation of aggregates
Gel filtration	Quick (4–5 min with Sephadex G50)	Slow (1–2h when using Sepahrose 2B/4B for macromolecule separation)
		Gels are expensive if not reused
		Dilutes the niosome dispersion
		Not suitable for highly viscous formulations
		Not suitable for formulations with a large particle size (>10–20 μm)

sustained maintenance dose. This is demonstrated by the improved activity against *Leishmania donovani* seen with alkyl polyglycerol or alkyl polyoxyethylene based sodium stibogluconate niosomes when unentrapped drug was not removed when compared with niosomes in which the unentrapped drug had been removed (Williams et al., 1995). These former formulations were also superior to the use of the free drug.

The methods that have been used for the removal of unentrapped material include:

1. Exhaustive dialysis (Baillie et al., 1985, 1986).
2. Separation by gel filtration (Sephadex G50) (Uchegbu et al., 1994; Yoshioka et al., 1994).
3. Centrifugation ($7000\times g$ for 30 min) for DOX $C_{16}G_3$ niosomes prepared by hand-shaking and ether injection methods (Rogerson et al., 1987),
4. Ultracentrifugation ($150000\times g$ for 1.5 h) for PK1 niosomes (Duncan et al., 1997; Gianasi et al., 1997; Uchegbu, Duncan, 1997).

All these methods have their advantages and disadvantages as given in Table 1. The choice of method must take all these factors into account and for industrial purposes it may be more worthwhile to concentrate efforts and resources on the achievement of high levels of drug loading so as to avoid these separation steps altogether or to consider systems in which the unentrapped drug serves as a specific priming dose as described above.

4. Osmotic activity

The osmotic activity of niosomal dispersions is estimated by a change in niosome size when dis-

persed in a hypertonic or hypotonic medium. In some systems osmotic activity may not be observed because there is initial aggregation of the vesicles as the ions in the disperse phase shield the electrostatic charge on the vesicle surface (Cable, 1989). However the incorporation of steric stabilisers in the vesicle membrane such as polyoxyethylene compounds prevents this aggregation and enables a study of the osmotic activity of these systems.

Niosomes prepared from $C_{16}G_2$ which contain the polyoxyethylene compound Solulan C24 (Arunothayanun et al., 1996) and both $C_{16}G_3$ and $C_{16}C_{12}G_7$ (Fig. 16) (Baillie et al., 1985) are osmotically active when cholesterol is incorporated into the bilayer in a 1:1 molar ratio. Cholesterol free niosomes were found to show less change in size (Baillie et al., 1985; Arunothayanun et al., 1996) due to the fact that these niosomes are more permeable to solutes (Rogerson et al., 1987; Arunothayanun et al., 1996). Niosomes prepared from dialkyl glycerol polyoxyethylene ethers were reported not to be osmotically active at room temperature (Lawrence et al., 1996) although

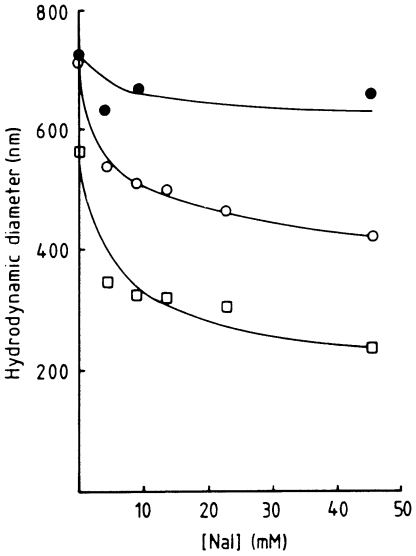

Fig. 16. The osmotic shrinkage of alkylglycerol ether niosomes, ● = hexadecyl triglycerol ether ($C_{16}G_3$) (100), ○ = $C_{16}G_3$, cholesterol (50:50), □ = hexadecyldodecyl heptaglycerol ether ($C_{16}C_{12}G_7$), cholesterol (50:50) in the presence of NaI solution (Baillie et al., 1985).

some of the higher phase transition surfactant niosomes were osmotically active at elevated temperature (50°C). The authors conclude that this lack of observed osmotic activity is due to the hydration of the polyoxyethylene head groups on the membrane surface. Membrane permeability studies were however not reported.

5. Toxicity studies

Unfortunately not too many niosome toxicity studies abound in the literature, despite the fact that often the lack of a toxicological profile is fundamental to any regulatory objections. More studies examining the toxicology of these systems can do nothing but advance the science and presumably provide opportunities for commercial exploitation. However the authors acknowledge that data demonstrating the drug delivery advantage of these systems over more established systems must first be produced to encourage the diversion of resources to toxicology studies.

In vitro studies on a ciliotoxicity model to estimate the toxicity of alkyl polyoxyethylene (C_nEO_x) niosomes on the nasal mucosa revealed that an increase in alkyl chain length was accompanied by a decrease in toxicity while an increase in the polyoxyethylene chain length caused an increase in ciliotoxicity (Hofland et al., 1992). In general an increase in chain length increases the gel to liquid transition while an increase in the length of the polyoxyethylene chain decreases the gel to liquid phase transition (Hofland et al., 1992). This study concluded that gel state niosomes are less ciliotoxic than the liquid state vesicles. Although it is worthwhile to note that the authors state that the HLB number had no influence on the toxicity of the compounds, the more hydrophobic compounds were obviously less toxic.

Neither the length of the polyoxyethylene chain or the alkyl chain had any influence on the skin toxicity of alkyl polyoxyethylene niosomes as assessed by the cell proliferation of human keratinocytes in vitro (Hofland et al., 1991, 1992). The latter is considered to be a measure of the irritability. However the nature of the linkage (ether or

ester) was a determining factor in this model and the more labile ester bond was found to be more toxic than the ether bond.

The parenteral administration of niosomes usually proceeds via the intravenous route and as surfactants are used in the formulation, it is important to conduct haemocompatibility studies. Less than 5% haemolysis was detected in vitro after 5 h of incubation of $C_{16}G_2$ and Span 60 niosomes (containing 10 mol% Solulan C24—a soluble surfactant) with rat erythrocytes at a similar surfactant level as would be achieved immediately after the administration of 10 mg kg^{-1} DOX in the form of PK1 niosomes (Uchegbu, Duncan, 1997). This level of haemolysis is not considered significant as less than 2% of the injected dose of these particular $C_{16}G_2$ niosomes is still present in the plasma 5 h after dosing (Uchegbu, Duncan, 1997) and less than 2% of the injected dose of Span 60 niosomes is present in the plasma 4 h after dosing (Uchegbu et al., 1995). By all indications Span 60 niosomes containing 10 mol% Solulan C24 are not haemotoxic. This soluble surfactant—Solulan C24 was also found to be toxic to Caco-2 cells in vitro (Dimitrijevic et al., 1997). However when incorporated into niosomes (10 mol%), this toxicity was drastically reduced. There was an increase in the toxicity when the level of Solulan C24 in niosomes was increased above 10 mol% (Dimitrijevic et al., 1997) due to the fact that above 10 mol%, the soluble surfactant Solulan C24 is not incorporated into the membrane of $C_{16}G_2$ niosomes and is thus present in solution as monomers or micelles.

The intraperitoneal injection of DOX $C_{16}G_2$ niosomes led to an inflammatory response in the lung and ultimate fatalities within 24 h (Uchegbu et al., 1994). This response was dose related and not found on the administration of these niosomes by the intravenous route. The effect was also not observed after the intraperitoneal injection of empty (not drug loaded) $C_{16}G_2$ niosomes or DOX solution (Uchegbu et al., 1994). It is possible that DOX niosomes are transported away from the peritoneum by the lymphatics via the thoracic duct allowing a higher dose in the main veins emptying into the heart (Uchegbu et al., 1994). However the exact reason for this response is unclear as although DOX is a known cardio-toxic agent (Calabresi and Chabner, 1996), heart levels of DOX were similar irrespective of whether the drug was administered intraperitoneally or intravenously (Uchegbu et al., 1994). The intraperitoneal administration of methotrexate niosomes also results in a large percentage of the dose (56%) being found in the thoracic lymph 3 h after dosing compared with 12% of the dose when methotrexate was administered in solution (Jain and Vyas, 1995a).

The toxicity of certain non-ionic surfactants may be modulated by the incorporation into niosomes. An example is the modulation of the toxicity of free Solulan C24 to Caco-2 cell monolayers by the incorporation of this soluble surfactant in niosomes (Dimitrijevic et al., 1997) or the less weight loss and bone marrow suppression observed on the intraperitoneal administration of sucrose ester niosomes in comparison with that observed on the administration of sucrose ester alone (Schenk et al., 1990).

One of the main aims of these specialised drug delivery systems is to modulate drug toxicity. This has been achieved with certain niosomal formulations. The encapsulation of vincristine within niosomes of unspecified composition reduced the neurological toxicity, diarrhoea and alopecia associated with the intravenous administration of vincristine and increased vincristine anti-tumour activity in S-180 sarcoma and Erlich ascites mouse models (Parthasarathi et al., 1994). However bone marrow suppression was similar after the administration of free or niosomal drug (Parthasarathi et al., 1994).

6. Specialised systems

6.1. Vesicle in water in oil systems

Span surfactant niosomes have been dispersed in an oil in water emulsion to yield a vesicle in water in oil system v/w/o using the same surfactant that was used to make the niosomes (Yoshioka, Florence, 1994). The release of CF from these systems followed the trend v/w/o < water in oil (w/o) emulsions < niosome dispersions. The

difference between the v/w/o and w/o formulations was minimal. The release of CF encapsulated within these niosomes was influenced by the emulsion oil following the trend, isopropyl myristate > octane > hexadecane and by the nature of the surfactant, following the trend Span 20 > Span 40 > Span 60. Span 80 v/w/o systems had a rather faster release rate due to the unsaturation in the oleyl alkyl chain, which leads to the formation of a more leaky membrane.

Span 60 was found to cause the formation of a gel in the oil phase, which the authors attribute to the crystallisation of Span 60 within the oil phase. The net result is an extremely slow release rate from the Span 60 v/w/o formulation (Yoshioka, Florence, 1994). These gelled Span 60 systems may be stabilised by incorporation of polysorbate 20 (Murdan et al., 1996) and the resultant Span 60 v/w/o organogels (oil phase = hexadecane) were found to have a temperature dependant release profile when CF was encapsulated within the niosomes. The release rate was highest at 37°C when the gel microstructure showed the presence of 'tubules'—presumably aqueous water channels along which CF is transported and slowest at 60°C when the gel transforms to a recognisable v/w/o system. At this elevated temperature the water channels present in the gel state transform to water droplets within which niosomes are contained. The slow rate of CF transport at 60°C was presumed to be due to the presence of the oil phase completely surrounding the water droplet through which CF must traverse.

An explanation for this gel formation is sought in the phase transition behaviour of Span 60. At the elevated temperature (60°C) which exceeds the Span 60 membrane phase transition temperature (50°C) (Yoshioka et al., 1994), it is assumed that Span 60 surfactant molecules are self assembled to form a liquid crystal phase. This liquid crystal phase stabilises the water droplets within the oil. However below the phase transition temperature the gel phase persists and it is likely that the monolayer stabilising the water droplets collapses and Span 60 precipitates within the oil. This Span 60 precipitate thus immobilises the liquid oil to form a gel. Water channels are subsequently formed when the w/o droplets collapse.

This explanation is plausible as the aqueous volume marker CF was identified within these elongated water channels and non-spherical aqueous droplets were formed within the gel (Murdan et al., 1996).

These v/w/o systems have been further evaluated as immunological adjuvants as discussed below.

6.2. Niosomes in hydroxypropyl methyl cellulose

A transdermal flurbiprofen (1% w/v) formulation has been prepared from Flurbiprofen Span 60, cholesterol, DCP (46:50:4) niosomes incorporated within a hydroxypropyl methyl cellulose semi-solid base containing 10% glycerine (Reddy, Udupa, 1993). The in vitro characterisation of the formulation is not given although this formulation was evaluated in a rat inflammation model as discussed below.

6.3. Discomes

The solubilisation of $C_{16}G_2$ niosomes by Solulan C24 results in the formation of the discome phase (Uchegbu et al., 1992). This phase consists of giant vesicles of 60 μm in diameter which encapsulate aqueous solutes such as CF. These large vesicles were found to be of two types large vesicles that appear ellipsoid in shape and large vesicles that are truly discoid (Uchegbu et al., 1996a). These morphologies were confirmed by confocal laser scanning microscopy and are only formed in a very specific region of the $C_{16}G_2$ ternary phase diagram (Fig. 9) namely regions 3 and 4. The discomes found in region 3 coexist with small spherical, helical and tubular niosomes (2–10 μm) which are found in a neighbouring region—region 2. While discomes found in region 4 do not co-exist with small spherical, helical and tubular vesicle (Uchegbu et al., 1996b). On heating the discome dispersion identified in region 3, the large discomes are seen to disappear leaving only the spherical/helical and tubular structures (Uchegbu, Florence, 1995). This is a reversible process and the discomes reform on cooling although the encapsulated aqueous solute is lost to the disperse phase by this heating and cooling

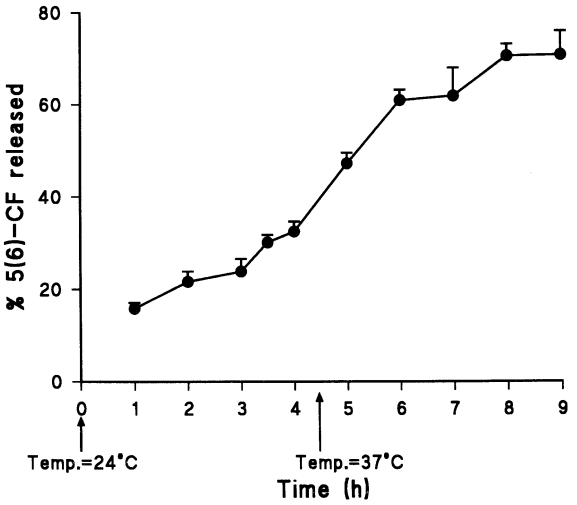

Fig. 17. The release of 5(6)-CF from discomes prepared from $C_{16}G_2$, cholesterol, Solulan C24 (50:35:15). The temperature was altered as shown by the arrows.

cycle (Fig. 17). Once the discomes are destroyed the release of CF is once again slowed and represents the release from the remaining small spherical, tubular and helical niosomes.

It is proposed that the system described by region 3 (Fig. 9) may prove useful in ophthalmic delivery as the initial instillation of the formulation in to the eye would result in the slow destruction of the discomes and release of an initial burst dose in accordance with the kinetics shown in Fig. 17. The remaining small spherical, helical and tubular vesicle would then release the rest of the dose slowly to the eye. The large size of the discomes means that clearance from the eye will be slowed down and the destruction of the discomes at 37°C results in the release of the encapsulated contents taking place over several min (Fig. 17) which would in theory allow the dose to enjoy an increased residence time within the eye.

On heating (>35°C) the discomes formed in region 4 (Fig. 9), a clear isotropic solution is obtained thought to consist of mixed micelles. Hydrophobic drugs such as paclitaxel may be solubilised by this system and no precipitation of the drug was observed on heating the formulation above 35°C (Uchegbu et al., 1996a). This paclitaxel formulation could be stored freeze dried.

6.4. Polyhedral niosomes

Polyhedral niosomes (Fig. 18) (Uchegbu et al., 1997) are formed in low cholesterol regions of the $C_{16}G_2$, cholesterol, Solulan C24 ternary phase diagram (Fig. 9). Polyhedral niosomes also encapsulate aqueous solutes such as CF. The vesicle membrane is in the gel phase (L_α) (Uchegbu et al., 1997), meaning that the hydrocarbon chains enjoy minimum mobility. This gives the vesicles the unusual angular shape. On heating these vesicles above the phase transition temperature (43°C), the angular shape is lost and a spherical morphology is observed (Fig. 19a, b, c) (Bernard et al., 1996), which on cooling results in an altered morphology (Fig. 19d). It appears that the heating and cooling cycle causes irreversible changes to the membrane.

Polyhedral niosomes were found to be thermoresponsive (Fig. 20a) (Uchegbu et al., 1997). Above 35°C, there was an increase in the release of CF from these niosomes even though the polyhedral shape was preserved until these vesicles were heated to 50°C. Since Solulan C24 free polyhedral niosomes do not exhibit this thermoresponsive behaviour (Uchegbu et al., 1997) it was thought to be due to a decrease in the interaction of the polyoxyethylene compound—Solulan C24 with water at this temperature (due to decreased hydrogen bonding) as identified by viscometry (Bernard et al., 1996). This observed thermoresponsive behaviour was used to design a reversible thermoresponsive controlled release system (Fig. 20b). Thermoresponsive liposomal systems which rely on the changing membrane permeability when the system transfers from the gel state (L_α) to the liquid crystal state (L_β) (Ono et al., 1994) are not reversible. This is not unexpected as there is a definite alteration of the membrane characteristics on proceeding through a cooling and heating cycle across the phase transition temperature (Fig. 19).

It is proposed that these thermoresponsive polyhedral dispersions may be used in dermatology as they are extremely viscous (due to the polyhedral niosome shape) (Bernard et al., 1996) and also due to the fact that at 30°C, (skin surface temperature lies between 26 and 30°C) they are

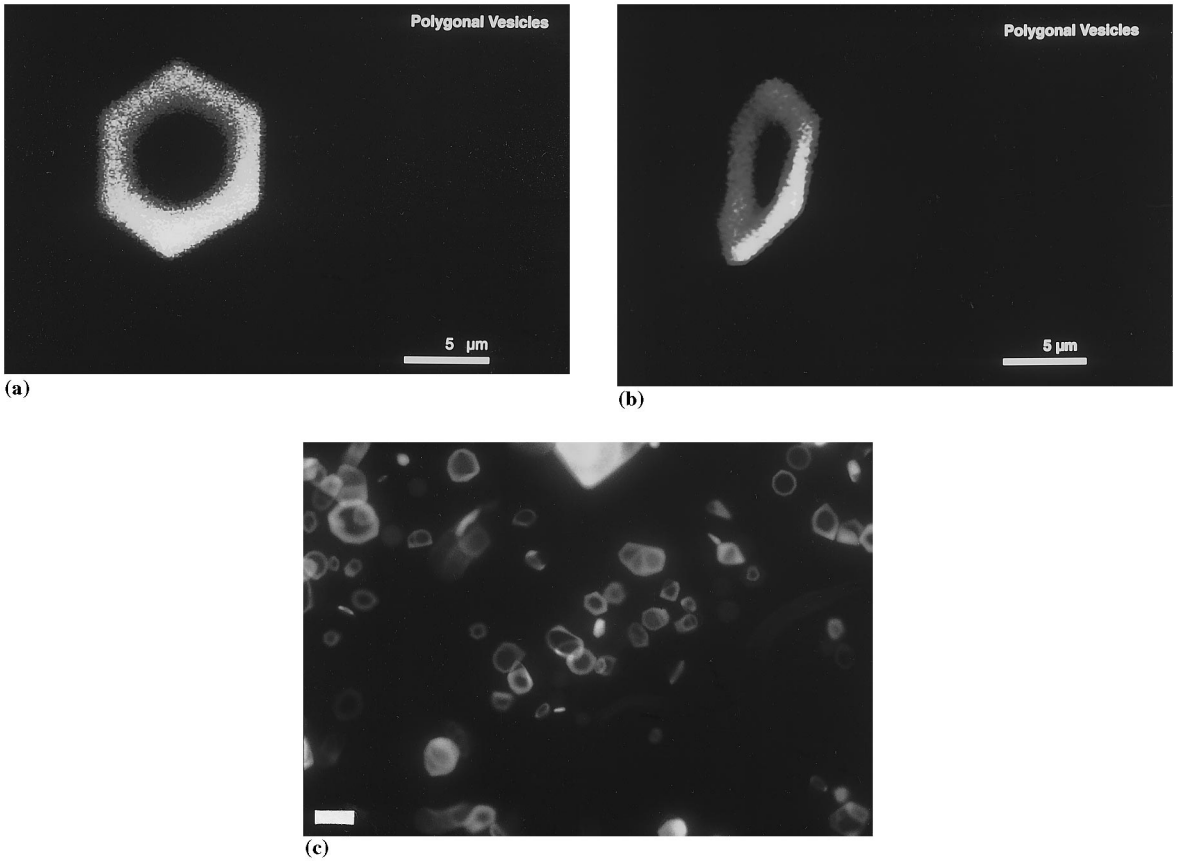

Fig. 18. a and b (× 4400) three dimensional reconstruction from a series of confocal laser scanning micrographs of polyhedral niosomes prepared from a mixture of $C_{16}G_2$, Solulan C24 (91:9) (a) viewed from the top; (b) viewed from the side; and (c) (× 1000) fluorescence micrographs of polyhedral niosomes prepared as described above and stored for 36 days, bar = 10 μm (Uchegbu et al., 1997).

non-thermoresponsive and thus are capable of releasing their encapsulated contents once either the ambient temperature increased to 35°C (e.g. in the use of photoprotective agents) or the skin temperature was raised (e.g. in inflammation).

7. Niosome stability

It would be unwise not to include a separate discussion of niosome stability in this review although it must be borne in mind that all the material presented above relate to or have a direct influence on the stability of niosomal dispersions.

A stable niosome dispersion must exhibit a constant particle size and a constant level of entrapped drug. There must be no precipitation of the membrane components, which are to a large extent not insoluble in aqueous media. Ideally these systems should be stored dry for reconstitution by nursing staff or by the patient and when rehydrated should exhibit dispersion characteristics that are similar to the original dispersion. Freeze dried dispersions of Span 60 niosomes encapsulating the DOX polymer conjugate PK1 have been prepared (Gianasi et al., 1997) but on reconstitution only 50% of the drug is encapsulated. As mentioned previously, this may not necessarily be a draw back for a drug delivery system. The solubilisation of a model hydrophobic drug paclitaxel in discomes remained unchanged after storage at 4°C as a freeze dried

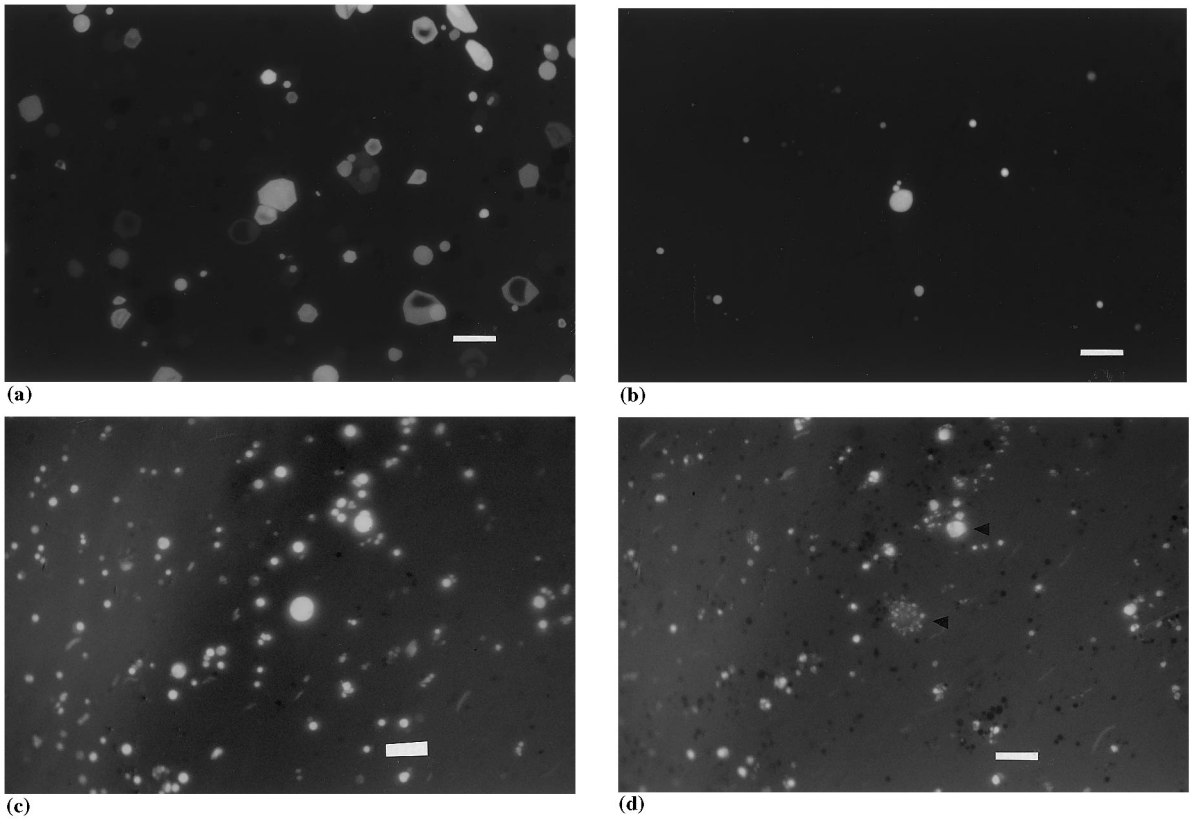

Fig. 19. (× 500) fluorescence micrographs of polyhedral niosomes prepared from $C_{16}G_2$, Solulan C24 (91:9) (a) at 28°C; (b) on heating to 50°C; (c) on cooling to 40°C; and (d) on cooling to 35°C—polyhedral vesicles are seen to reform and also an apparent splintering of the vesicles is observed (arrows) (Bernard et al., 1996) (bar = 20 μm).

formulation for 10 days (Uchegbu et al., 1996b). This was assessed by analysing the amount of paclitaxel still solubilised after rehydration of these formulations.

As insufficient data abounds on the storage of freeze dried niosomal dispersions, the rest of the discussion will be limited to the stability data available on niosomes in suspension.

The persistence of bilayer self-assembly into closed spheres was observed in niosome dispersions prepared from $C_{16}G_3$, cholesterol in a 1:1 molar ratio (+ 5% dicetyl phosphate) (Fig. 21) and $C_{16}G_2$, cholesterol in a 1:1 molar ratio (+ 5% stearylamine) after storage for 84 months at ambient temperature. Niosomes were prepared by hydrating a thin film using the hand shaking method and were presumably originally in the 1–10 μm size range. The observed difference in the nature of these particles after this length of time was the formation of near perfect spherical shaped niosomes (Fig. 21). Fig. 22 illustrates DOX niosomes of a similar morphology which had been prepared from $C_{16}G_2$, cholesterol, Solulan C24 (40:40:10) by high pressure homogenisation (600 bar × 10 cycles). These particles had been stored for 84 days and had an original particle size of 70 nm. The particle size of this dispersion increased approximately 250-fold. Sonicated niosomes prepared from these surfactant mixtures, which had also been stored for 84 months, revealed the presence of small needle shaped crystalline material, indicating a precipitation of the bilayer material. It appears that the original size of the formulation has an effect on the stability of the

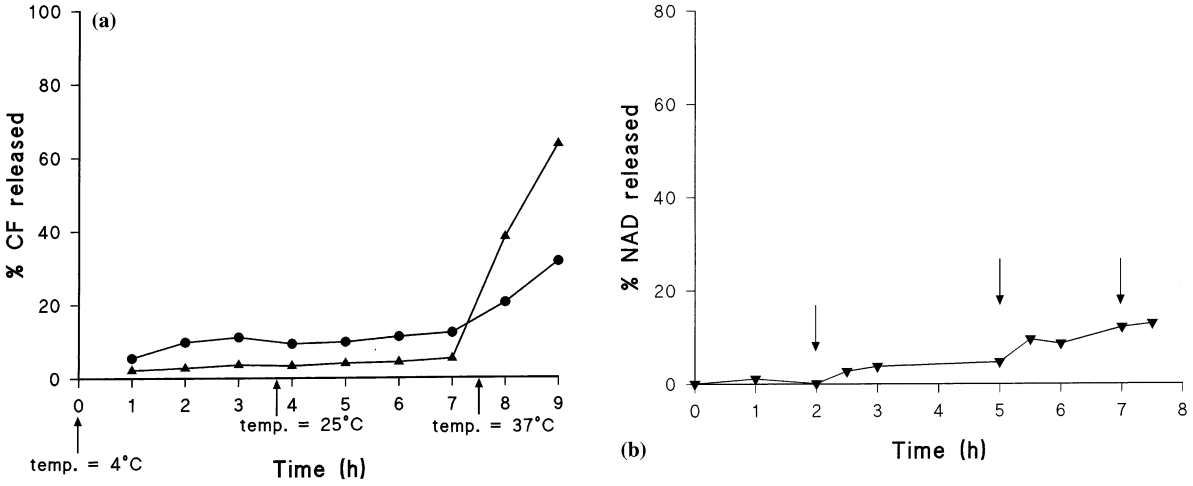

Fig. 20. (a) The release of CF from exhaustively dialysed polyhedral niosomes. The temperature of the surrounding medium was altered as shown by the arrows, ● = $C_{16}G_2$, Solulan C24 (91: 9), ▲ = $C_{16}G_2$, Solulan C24 (95:5) (Uchegbu et al., 1997). (b) The release of nicotinamide adenine dinucleotide from polyhedral niosomes prepared from $C_{16}G_2$, Solulan C24 (91:9) at 24°C. Arrows = points at which the temperature of the surrounding medium was raised to 37°C for 10 min.

system. This is in keeping with thermodynamic theory as the smaller niosomes require a higher input of energy and thus contain more excess energy and an inherently greater instability than the larger niosomes prepared by hand shaking.

A further example of how the method of vesicle formation has important bearing on the stability of these systems (Engberts, Hoekstra, 1995) is the fact that vesicles prepared by the solvent injection method (ethanol) are found to have an additional phase transition due to the presence of residual ethanol. A number of membrane properties rely on the temperature of the main phase transition such as the membrane permeability and rigidity. The introduction of membrane defects due to the presence of residual ethanol may destabilise these drug delivery systems.

Vesicles prepared from $C_{16}G_3$, Solulan C24 (50:50) which had also been stored for 84 months were also seen to be morphologically stable (Fig. 23). The large size of these vesicles coupled with their angular morphology indicates that these are analogous to the discomes formed in 3 and 4 of the $C_{16}G_2$, cholesterol, Solulan C24 ternary phase diagram (Fig. 9). Discomes prepared from $C_{16}G_2$, cholesterol, Solulan C24 (50:15:35) were morphologically stable 12 months after preparation (Uchegbu et al., 1996a). Polyhedral niosomes visualised after storage for 36 days (Fig. 18c) also showed an unaltered morphology (Uchegbu et al., 1997).

It is interesting that the surfactant molecules in all the systems described above were still self assembled into closed bilayers after prolonged periods and Figs. 21 and 23 represent the first record of niosomes and to our knowledge vesicles of any kind that had been stored for over 7 years! However although this data pointing to a persistence of surfactant self assembly is impressive, not much is revealed from the above on the stability of a drug delivery system in all its aspects after this length of time.

7.1. Influence of the surfactant/lipid nature

The choice of membrane surfactant determines the nature of the membrane and ultimately affects the stability of the system. The leakiness of CF loaded Span surfactant niosomes was found to follow the trend Span 80 < Span 20 < Span 40 < Span 60 (Yoshioka et al., 1994) and was determined by the degree of membrane fluidity The incorporation of cholesterol into these niosomal systems also decreases the leakiness of the membrane (Rogerson et al., 1987, 1989).

7.2. Influence of the encapsulated drug

The encapsulated drug could also be the major determinant of the fate of any niosomal system. 75% of the drug polymer conjugate (PK1) remained encapsulated within the vesicles 28 days after storage as the vesicle suspension at 4 and at 25°C (Gianasi et al., 1997). Vesicle size was also found to remain unchanged (Gianasi et al., 1997). The encapsulation of a polymer obviously leads to a more stable system as the membrane is sufficiently impermeable to this macromolecule.

The physical nature of the encapsulated material also affects stability. DOX loading into vesicles using an ammonium sulphate gradient is said to lead to the formation of a gel within the vesicles (Haran et al., 1993). Niosomes loaded using this technique were also less leaky (Fig. 15) (Uchegbu et al., 1996b).

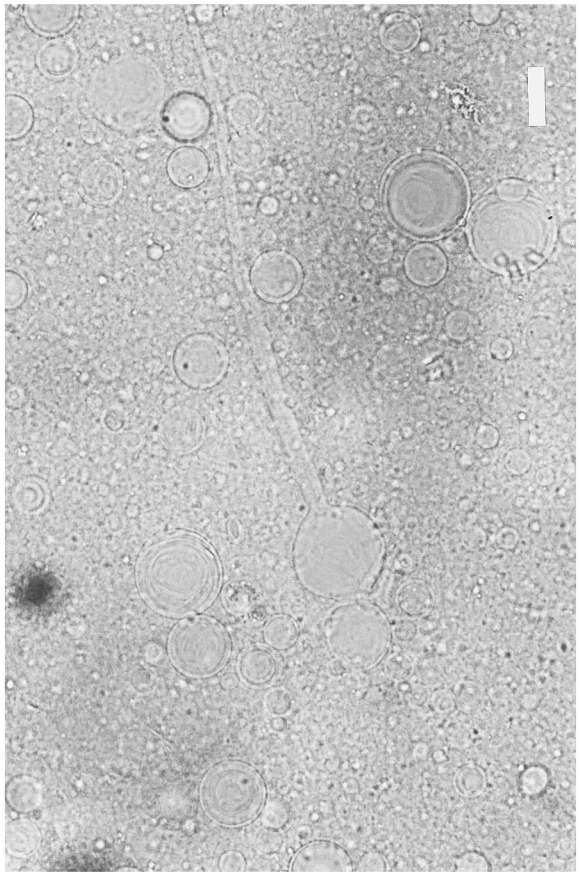

Fig. 21. (× 500) micrograph of $C_{16}G_3$, cholesterol, dicetyl phosphate (47.5:47.5:5) niosomes that had been stored for 84 months (bar = 20 μm).

7.3. Temperature of storage

The temperature of storage of these dispersions must be controlled as a change in the temperature of the system often leads to a change in the fundamental nature of the system (Tanaka, 1990) or an increase in the release of an encapsulated solute (Santucci et al., 1996; Uchegbu et al., 1997) a property which may be exploited to construct a thermoresponsive system (Fig. 20b).

7.4. Detergents

High concentrations of detergents (soluble surfactants) are incompatible with niosomal systems and cause eventual solubilisation of the vesicles to form mixed micelles and a host of intermediate aggregates (Lesieur et al., 1990; Seras et al., 1992; Uchegbu et al., 1992; Serascansell et al., 1996; Uchegbu et al., 1996a). This solubilisation has been studied for a few formulations and the destruction of $C_{16}G_2$ niosomes by octyl glucoside appears to proceed via the build up of a critical localised concentration of octyl glucoside molecules within the niosome membrane before micellisation can occur (Seras et al., 1994). The solubilisation $C_{16}G_2$ niosomes by Solulan C24 has been shown to proceed via the formation of discomes which are then converted into mixed micelles (Uchegbu et al., 1992).

7.5. Thermodynamic considerations

Hydrated bilayer systems such as liposomes and niosomes are not deemed to be thermodynamically stable and are thought to represent a metastable state in that the vesicles possess an excess of energy (Lasic, 1990). These particles are thus predicted to transform into bilayer stacks with time. To produce a system with maximal stability thus requires that these predicted transformations be slowed down to such an extent as to produce a product with a reasonable shelf life. In effect the colloidal dispersion must not show

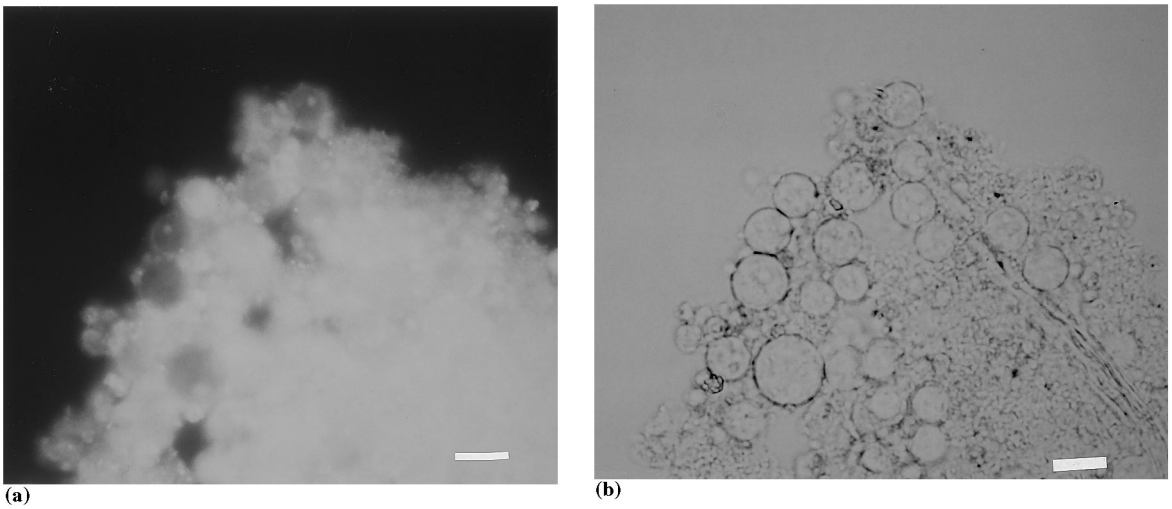

Fig. 22. (× 1000) micrographs of doxorubicin $C_{16}G_2$, cholesterol, Solulan C24 (40:40:10) niosomes that had been prepare by hand shaking and high pressure homogenisation and stored for 84 days. The original particle size of this dispersion was 79 nm. The presence of doxorubicin which is preferentially located at the membrane is confirmed by (a) the fluorescence and (b) the dark colour (bar = 10 μm).

vesicle aggregation, fusion or swelling. The entrapped material must remain entrapped and show no incompatibility with the bilayer materials.

7.6. Stability enhancement

Methods to enhance the stability of these niosomes are also found in the literature. Decreasing the air water interface may prevent the crystallisation of these self assembled surfactant monomers (Engberts, Hoekstra, 1995) and it may be possible to stabilise niosomes by a variety of methods such as the addition of polymerised surfactants to the formulation, the use of membrane spanning lipids and the interfacial polymerisation of surfactant monomers in situ (Florence, 1993a).

The inclusion of a charged molecule in the bilayer shifts the electrophoretic mobility making it positive with the inclusion of stearylamine and negative with the inclusion of DCP and also prevents niosome aggregation (Cable, 1989). In addition, as mentioned above, the entrapment of hydrophobic drugs (Uchegbu et al., 1996b) or macromolecular prodrugs (Gianasi et al., 1997) also increases the stability of these dispersions.

8. The evaluation of niosomes as drug delivery agents

Although pharmaceutical niosome formulations have yet to be commercially exploited, a number of studies have demonstrated the potential of niosomes in drug delivery.

Examination of the literature reveals that on intravenous administration of niosomes the highest drug levels are found in the liver (Azmin et al., 1985; Baillie et al., 1986; Ozer et al., 1991; Uchegbu et al., 1995). However there were exceptions. When DOX 850 nm $C_{16}G_3$ niosomes were administered, DOX liver levels were not significantly different from the administration of DOX solution (Rogerson et al., 1988) and serum levels though low ($\sim$ 0.5% of the administered dose 10 min after dosing) were higher for the niosome formulation. The cause of this non liver uptake is not apparent although smaller DOX niosomes are found to accumulate in the liver following intravenous administration (Uchegbu et al., 1995). Liver accumulation is also avoided when DOX vesicles are prepared from palmitoyl muramic acid, cholesterol, Solulan C24 (45:45:10) (Uchegbu, 1998). With these muramic acid based

vesicles 5% of the intravenously administered dose was found in the liver 5 h after dosing, compared with 18% of the intravenously administered dose still present in the liver at this time point when the drug was administered in the form of sorbitan monostearate, cholesterol, Solulan C24 (45:45:10) niosomes (Uchegbu, 1998). Splenic uptake is not avoided with the use of palmitoyl muramic acid vesicles (Uchegbu, 1998).

The intravenous administration of iopromide $C_{16}G_3$ and $C_{16}C_{12}G_7$ niosomes containing stearylamine and extruded through a 220 nm filter resulted in these niosomes being found predominantly in the kidneys (Erdogan et al., 1996). The authors conclude that the incorporation of the surface positive charge (stearylamine) enables targeting to the kidneys.

In vitro studies reveal that niosomes prepared from ester surfactants were found to be degraded to a larger extent by esterases which are normally present in the plasma although they resist degradation by phospholipase A_2 which readily degrades dipalmitoylphosphatidylcholine liposomes (Florence, Baillie, 1989). The use of ester surfactants will thus affect the stability of niosomes in the plasma and ultimately the biodistribution of the drug in niosome formulations. The intraperitoneal administration of niosomes (Span 85 based) (Jain and Vyas, 1995a) results in targeting to the lymphatics while the $C_{16}G_2$ DOX formulation (Uchegbu et al., 1994) on intraperitoneal administration acts as a depot within the peritoneum, eventually peaking in the plasma 2 h after dosing.

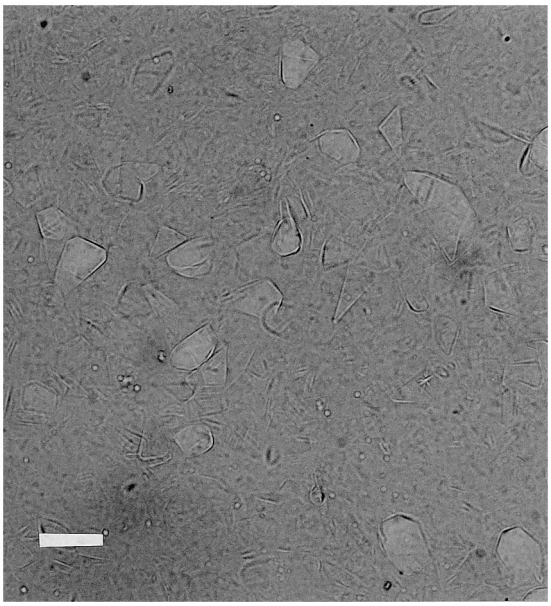

Fig. 23. ($\times$ 250) micrograph of vesicles prepared from $C_{16}G_3$, Solulan C24 (50:50) stained with toludine blue and store for 84 months. These vesicles are analogous to the discomes found in region 4 (Fig. 9) of the $C_{16}G_2$-cholesterol-Solulan C24 ternary phase diagram (bar = 40 μm).

The only conclusion that may be drawn from these studies is that issues such as niosome surface nature, in vivo stability and vesicle size all contribute to the observed biodistribution on parenteral administration. The nature of the encapsulated drug may also affect the chemistry of the niosome surface and in this way influence biodistribution

An additional factor to be taken into account are the type of assay methods used in various reports. Methods that detect clinically relevant drug concentrations such as high performance liquid chromatography (HPLC) with specific detection parameters are more discerning than methods measuring radioactivity which detect metabolic fragments in addition to the parent drug.

The transdermal (Reddy, Udupa, 1993; Hofland et al., 1994; Schreier, Bouwstra, 1994) and oral routes (Azmin et al., 1985; Yoshida et al., 1992; Rentel et al., 1996) have also been explored and are discussed in greater detail below.

It must also be stated that drug metabolism in vivo is also altered by encapsulation within niosomes (Azmin et al., 1985; Kerr et al., 1988; Rogerson et al., 1988; Al-Angary and Halbert, 1992; Uchegbu et al., 1995)

8.1. Anti-infective agents

Indeed one of the earliest diseases for which niosomal formulations proved particularly beneficial was from the antiparasitic class, specifically in the treatment of experimental leishmaniasis (Baillie et al., 1986). The intravenous administration of sodium stibogluconate $C_{16}G_3$ niosomes or

dipalmitoyl phosphatidylcholine (DPPC) liposomes both containing 30 and 20% cholesterol, respectively, resulted in higher liver levels of antimony when compared with the administration of the drug in solution (Baillie et al., 1986). These niosomes were prepared by the ether injection method and thus are presumed to be in the 300 nm–1 μm size range. There was no apparent difference in antimony levels when liposomes or niosomes were administered. When the liver parasite burden was assessed in this study it was found that niosomal sodium stibogluconate was significantly more active in reducing parasite burden than the free drug and the effect observed after multiple dosing suggests that niosomal formulations act as a depot within the liver (Baillie et al., 1986). In addition there appeared to be no difference in activity when a series of other non-ionic alkyl glycerol ether and ester surfactants were used providing antimony dose levels were in excess of 40 μg per mouse (Hunter et al., 1988). With one formulation ($C_{16}G_3$, cholesterol 70:30) at antimony doses below 40 μg per mouse there was an inexplicable increase in the liver parasite burden (Hunter et al., 1988) not previously seen in earlier studies (Baillie et al., 1986), indicative of a variation in response with this particular formulation. This increase in parasite burden was not observed when cholesterol levels in $C_{16}G_3$ niosomes were increased to 50% (Hunter et al., 1988). The use of specified niosome formulations at a particular dose level to reduce liver *Leishmania donovani* parasite burdens thus appears proven and can be attributed to the rapid uptake of these particulate formulations by the liver on intravenous administration. However the failure of these formulations to eradicate splenic and bone marrow parasite burdens would allow for relapse of the disease (Collins et al., 1993) leading to the conclusion that formulations capable of delivery to spleen and bone marrow regions are required (Carter et al., 1988). Niosomes prepared from polyoxyethylene surfactants $C_{16}EO_2$, $C_{16}EO_4$, $C_{16}EO_6$ and the polyglycerol surfactant $C_{18}G_3$, however did suppress parasite burdens in the spleen when compared with controls (Williams et al., 1995). $C_{16}EO_6$ niosomes also suppressed the proliferation of bone marrow parasites (Williams et al., 1995). All niosomes used against leishmaniasis in these more recent studies contained in addition to the main surfactant 40% cholesterol and 10% dicetyl phosphate.

Rifampicin, an anti-tuberculosis agent, encapsulated within Span 85 (sorbitan tri-oleate) based niosomes in the 8–15 μm size range were found to accumulate in the lung of mice (Jain and Vyas, 1995b) thus offering the possibility of improved anti-tuberculosis therapy.

8.2. Anticancer drugs

8.2.1. Methotrexate

When methotrexate 100 nm $C_{16}G_3$ niosomes containing either 47.5 or 30% cholesterol were administered intravenously or orally higher levels of the drug were found in the liver—more so for the formulations administered by the intravenous route—with serum levels higher than when the drug was administered in solution (Azmin et al., 1985). A 23-fold increase in the area under the plasma level time curve was observed when Span 60 4.5 μm methotrexate niosomes were administered by the intravenous route to tumour bearing mice (Chandraprakash et al., 1993) a fact attributed to the large size of these niosomes. The area under the plasma level time curve was increased 100-fold when methotrexate Span 60 niosomes were administered following macrophage activation with muramyl dipeptide-gelatin conjugates and methotrexate tumoricidal activity was also increased (Chandraprakash et al., 1993). Earlier studies with methotrexate had found that increased levels of methotrexate were found across the blood brain barrier with $C_{16}G_3$ niosomes administered by the oral or intravenous route (Azmin et al., 1985). Unfortunately drug delivery to the brain with niosomes is not an area that has enjoyed extensive study and the potential of these formulations to deliver drugs across the blood brain barrier while indicated by these results still awaits confirmation and possible exploitation.

Metastitial cancer of the lymphatic system has been targeted by the administration of 8 μm Span 85 methotrexate niosomes intraperitoneally (Jain and Vyas, 1995b). High levels of methotrexate

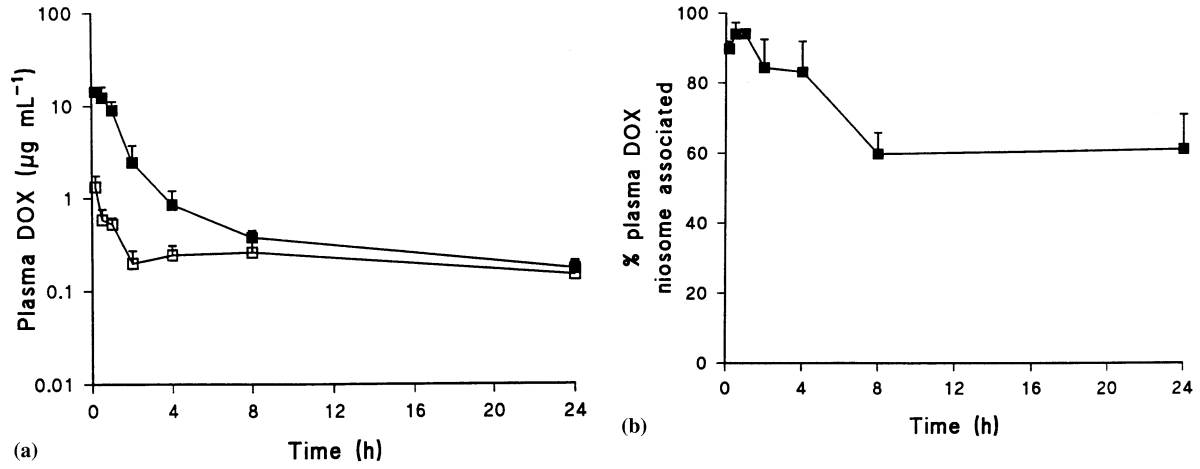

Fig. 24. (a) Mean plasma levels (± S.E.M.) of doxorubicin after the intravenous administration of doxorubicin (10 mg kg^{-1}) Span 60, cholesterol, Solulan C24 (45:45:10) niosomes to female tumour bearing mice, ■ = niosome-associated doxorubicin, □ = doxorubicin released from the niosomes in vivo (Uchegbu et al., 1995). (b) Mean% of plasma doxorubicin (± S.E.M.) still encapsulated in niosomes after intravenous administration of doxorubicin (10 mg kg^{-1}) Span 60, cholesterol, Solulan C24 (45:45:10) niosomes to female NMRI mice (Uchegbu et al., 1995).

were found in the thoracic lymph following niosomal administration by this route when compared with administration via the intravenous route and the administration of the free drug via the peritoneal route.

8.2.2. DOX

DOX administered in 850 nm $C_{16}G_3$ niosomes to tumour bearing mice resulted in increased tumour, serum and lung levels but not in an increase in liver levels of DOX (Rogerson et al., 1988). DOX 240 nm Span 60 niosomes increased plasma, liver and tumour levels (Uchegbu et al., 1995). Lung levels were also increased. Fractionation of plasma samples over a Sepharose 2B column revealed that 90% of plasma DOX was still encapsulated within Span 60 niosomes up to 4 h after intravenous dosing, falling to 50% by 24 h (Fig. 24) (Uchegbu et al., 1995). Initial plasma levels and liver levels have been found to be the highest with this formulation than any of the other DOX formulations studied (Fig. 25a, b) (Uchegbu et al., 1996a). Tumoricidal activity was increased with different DOX niosome formulations as measured by decreased proliferation of the S180 sarcoma in NMRI mice (Fig. 26) (Rogerson et al., 1988) and terminal mean tumour weight of a MAC 15A tumour in NMRI mice (Uchegbu et al., 1996a). However studies involving a human lung (Kerr et al., 1988) or human ovarian xenograft (Uchegbu et al., 1996a) revealed that in these latter models niosomal formulations had no advantage over the free drug.

It is well known that good clinical anti-tumour activity cannot be predicted by the use of animal models but it is clear from the above that the choice of animal model may prove crucial at the early in vivo screening stage (Uchegbu et al., 1996a). It is advocated that a large number of animal models are chosen to evaluate delivery systems containing these broad spectrum anti-cancer agents such as DOX. The apparent discrepancy in response offered by the mouse and xenograft tumour models may lie in differences in tumour anatomy/biochemistry.

DOX is a cardiotoxic drug and it is thus beneficial to observe that heart levels of the drug were slightly decreased with niosomal formulations (Kerr et al., 1988; Rogerson et al., 1988) and that cholesterol free niosomes decreased heart levels to a larger extent than the cholesterol containing analogues (Rogerson et al., 1988). Ultimate drug targeting requires that toxicity be minimised and that therapeutic benefit be maximised. In this

respect the administration of DOX in niosomes does offer some benefit. However drug metabolism was increased on the administration of DOX niosomes (Kerr et al., 1988; Rogerson et al., 1988; Uchegbu et al., 1995) due to the increased bioavailability of the drug.

8.2.3. DOX N(2-hydroxypropylmethacrylamide) copolymer conjugate

A DOX PK1 copolymer in which DOX is bound to *N*(2-hydroxypropyl methacrylamide) by a peptidyl spacer (Duncan et al., 1989) has been designed to enable DOX targeting to tumours. Following fluid phase pinocytic uptake of this drug polymer conjugate the active drug will be cleaved off by lysosomal enzymes (Duncan and Spreafico, 1994) and become available intracellularly to exert its pharmacological effect. Recently this polymeric prodrug has been encapsulated within sorbitan ester, alkyl polyoxyethylene ether and $C_{16}G_2$ niosomes (Duncan et al., 1997; Gianasi et al., 1997; Uchegbu, Duncan, 1997).

In vitro, this drug delivery system was found to be stable in plasma and no free DOX was detected although free DOX was detected when this system was incubated with a lysosomal enzyme preparation (Gianasi et al., 1997). This indicates that free DOX would only be released in vivo once the formulation was exposed to lysosomal enzymes. In vivo, 420 nm $C_{16}G_2$ PK1 niosomes yielded non-detectable plasma levels of DOX but high spleen levels of PK1 and low spleen levels of DOX (Uchegbu, Duncan, 1997). Liver levels of DOX were found to rise steadily with time until at the 24 h time point, 25% of the administered dose

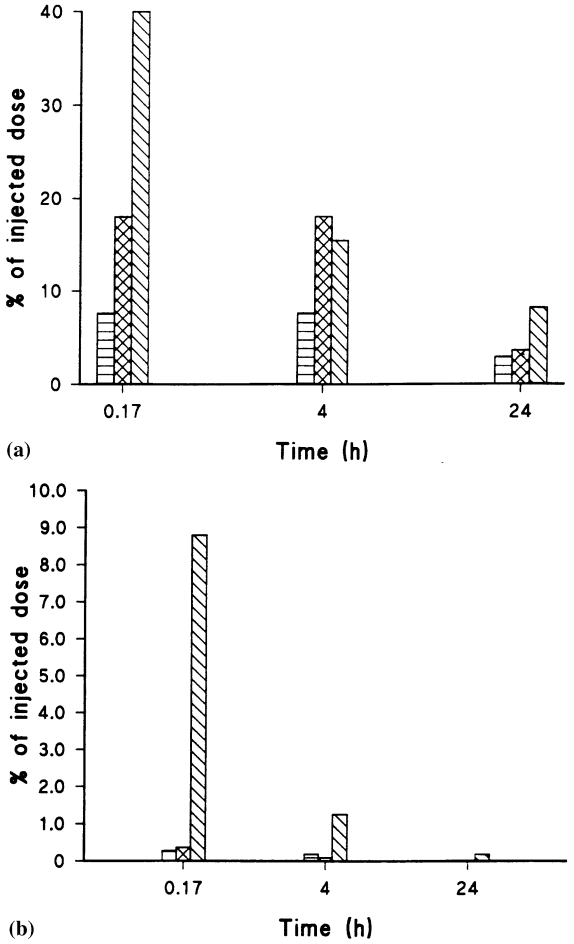

Fig. 25. Murine tissue levels of doxorubicin following the intravenous administration of doxorubicin niosomes, (—) box fill = $C_{16}G_3$ (100) niosomes, size = 850 nm, (X) box fill = $C_{16}G_3$, cholesterol (50:50) niosomes, size = 850 nm, (\) box fill = Span 60, cholesterol, Solulan C24 (45:45:10), size = 235 nm (a) = liver and (b) = plasma (Uchegbu et al., 1996a).

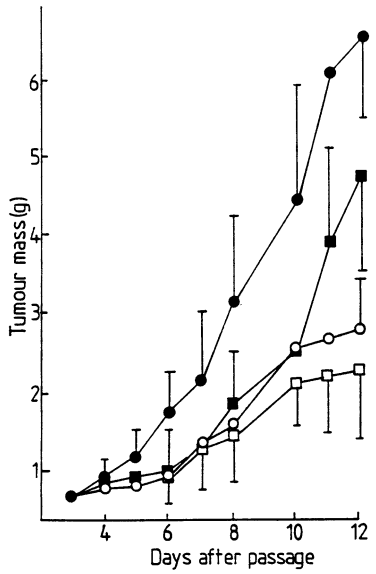

Fig. 26. The growth in the mass of implanted tumour as a function of time after the intravenous injection of (●) phosphate buffered saline pH 7.4, (■) doxorubicin solution (5 mg kg^{-1}), (○) doxorubicin (5 mg kg^{-1}) $C_{16}G_3$ (100) niosomes, (□) doxorubicin (5 mg kg^{-1}) $C_{16}G_3$, cholesterol (50:50).

was present in the liver (Fig. 27). This is the highest DOX liver level we are currently aware of with any drug targeting strategy at the 24 h time point. This unusual liver profile is due to the fact that large amounts of the encapsulated polymer prodrug are taken up by the liver and DOX is then slowly released by enzymatic cleavage. It is possible that this polymer in niosome formulation may act as a depot within the liver giving rise to a controlled release of an active substance in a defined organ. This formulation may be useful in the treatment of hepatic metastasis

8.2.4. Other agents

Vincristine Span 40 niosomes increased the vincristine anti-tumour activity in S-180 sarcoma and Erlich ascites bearing mice (Parthasarathi et al., 1994). Span 60 bleomycin niosomes also increased the tumoricidal activity of bleomycin in these two tumour models (Naresh, Udupa, 1996) However the intraperitoneal administration of sucrose palmitate stearate ester (HLB = 7) niosomes was found to result in no apparent increase in life span when mice bearing a P388 murine leukaemia were administered glyoxal-bis-guanyl hydrazone encapsulated within these niosomes (Schenk et al., 1990).

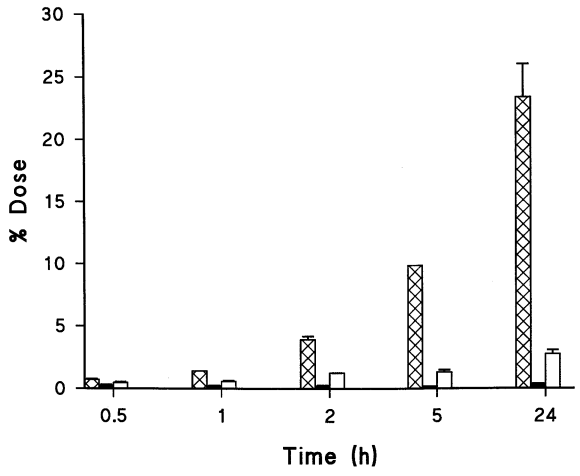

Fig. 27. The biodistribution of free doxorubicin after the intravenous administration of PK1 (a doxorubicin polymer conjugate) $C_{16}G_2$, niosomes (5 mg kg^{-1} doxorubicin) to male BALB/C mice, (solid) fill = heart, (open) fill = spleen, (X) fill = liver.

8.2.5. Anti-cancer niosomes and the future

Niosomes are proving useful agents in the targeting of anti-cancer drugs although problems such as the choice of suitable animal tumour models still remain unresolved.

8.3. Anti-inflammatory agents

Diclofenac niosomes reportedly prepared from polysorbate 60, cholesterol and DCP (22:73:5) and 3 μm in size were found to reduce the inflammation in rats with carageenan induced paw oedema on intraperitoneal administration to a greater extent than the free drug (Naresh et al., 1993). This increase in activity is a direct result of an observed increase in the area under the plasma time curve. In a similar study involving flurbiprofen Span 60 niosomes (Reddy, Udupa, 1993) there was also increased bioavailability and an increased reduction of carageenan induced rat paw oedema when the niosomal formulation was applied topically in a hydroxypropyl methyl cellulose semisolid base containing 10% glycerine or orally as a suspension in saline.

8.4. Diagnostic imaging with niosomes

Apart from the use of niosomes as various drug carriers one report in the literature details the evaluation of these systems as diagnostic agents. $C_{16}G_3$ and $C_{16}C_{12}G_7$ niosomes containing cholesterol and stearylamine encapsulating the radioopaque agent iopromide were found to concentrate in the kidneys on intravenous administration (Erdogan et al., 1996). This kidney targeting was attributed to the presence of the positive charge on the niosome surface although no neutral control niosomes were used in this study. The highest kidney iopromide concentration was found with the $C_{16}G_3$ niosomes which are said to form a less fluid bilayer than the $C_{16}C_{12}G_7$ niosomes (Erdogan et al., 1996). Although the niosome formulation enhanced the opacity of this contrast agent, low levels of encapsulation of the contrast agent were a problem with this system and clinically relevant enhancement of opacity was not achieved in this study.

8.5. Niosomal formulations as vaccine adjuvants

A number of surfactants have documented immunostimulatory properties (Hilgers et al., 1989) and have been used in emulsion vaccine adjuvants. The adjuvanticity of niosomes prepared from 1-mono-palmitoyl glycerol, cholesterol, dicetyl phosphate—5:4:1 has been demonstrated in mice, on subcutaneous administration of bovine serum albumin (Brewer and Alexander, 1992) ovalbumin (Brewer et al., 1996) or a synthetic peptide containing a known T-cell epitope (Brewer et al., 1996). The encapsulation of the antigen was determined to be crucial to the adjuvanticity (Brewer and Alexander, 1992). The same niosome system has also been shown to act as a vaccine adjuvant when administered intraperitoneally to severe combined immunodeficiency mice reconstituted with peripheral blood lymphocytes (PBL-SCID mice) (Walker et al., 1996). This mouse model is designed to mimic the human response to an antigen challenge (Walker et al., 1995). A vesicle in water in oil (v/w/o) emulsion (Yoshioka, Florence, 1994) prepared from Span 80 and cotton seed oil has been evaluated as an immunological adjuvant using the model antigen tetanus toxoid (Yoshioka et al., 1995). An increased secondary response (level of IgG1) was observed when the v/w/o formulation was administered by the intramuscular route in comparison with the vesicle formulation and the free antigen

8.6. Niosomes and the oral route

The oral delivery of drugs using niosomal formulations was first demonstrated by a study involving 100 nm methotrexate $C_{16}G_3$ niosomes (Azmin et al., 1985). Significantly higher levels of methotrexate were found in the serum, liver and brain of PKW mice orally administered a niosomal formulation when compared with the administration of the free drug. It thus appears that there is enhanced drug absorption with these niosomal formulations.

Recently more gut labile compounds such as proteins (Rentel et al., 1996) have been administered by this route and the intestinal transport of a niosomal formulation of the peptide 9-desglycinamide 8-arginine vasopressin has been studied in vitro (Yoshida et al., 1992). In one of the first studies of its kind sucrose ester niosomes loaded with ovalbumin were found to cause a modest but significant increase in the level of specific antibodies after oral administration (Rentel et al., 1996).

8.7. Transdermal drug delivery

Although the emergence of niosomes into the pharmaceutical arena was the result of activity in the cosmetic industry, it was only fairly recently that the transdermal delivery of drugs with niosomes was seriously considered. The enhanced delivery through the stratum corneum of niosome encapsulated drugs has been observed (Reddy, Udupa, 1993; Niemec et al., 1994; Schreier, Bouwstra, 1994; Vanhal et al., 1996) and it therefore remains to elucidate the mechanism of this delivery, especially as the stratum corneum is considered to be a particularly impermeable barrier (Junginger et al., 1991). Small (100 nm) vesicular structures have been observed between the first and second layer of human corneocytes 48 h after incubation with niosomes prepared from 'dodecyl alcohol polyoxyethylene ether' and cholesterol (Junginger et al., 1991). Penetration by niosomes of this upper layer appears plausible as these layers are only loosely packed (Junginger et al., 1991). However the same study reports the presence of vesicular structures in the deeper seemingly inaccessible areas of the skin and concludes that there was a reorganisation of the niosome membrane into individual monomers which on arriving at these deeper layers reformed into niosomes (Junginger et al., 1991). In vitro studies on the transdermal penetration (passage through pieces of excised stratum corneum) of oestradiol using high phase transition sucrose ester niosomes or $C_{18}EO_7$ niosomes and low phase transition $C_{12}EO_7$ niosomes revealed the latter to be better transdermal carriers (Vanhal et al., 1996). $C_{12}EO_7$ micelles were ineffective as drug carriers in this study. The higher flexibility of these bilayers is said to be responsible for this improved transdermal penetration (Vanhal et al., 1996) a similar explanation to that given for the

transdermal delivery of material by transfersomes (flexible liposomes) (Cevc et al., 1995; Cevc, 1996; Cevc et al., 1996). Reducing the cholesterol content of these niosomes also increased the transdermal delivery of oestradiol (Vanhal et al., 1996).

The migration of cyclosporin A from cyclosporin glyceryl dilaurate/$C_{16}EO_{10}$/cholesterol niosomes into the deeper skin strata has also been studied in vitro and it was found that factors such as dosing volume in non-occluded conditions affected the rate of uptake with smaller dose volumes giving rise to an increased uptake of the drug into deeper skin strata (Niemec et al., 1994). This is due to the time required for dehydration of the formulation, a fundamental element of the penetration process (Niemec et al., 1994). This same formulation was found to beneficial for the deposition of cyclosporin A and alpha-interferon into pilosebaceous units of the hamster ear model (Niemac et al., 1995).

Based on the above studies, it does appear that transdermal drug delivery with niosomes appears promising for hydrophobic and amphiphilic drug molecules and would require that the dose be applied in high concentration and within niosomes prepared from low phase transition surfactant mixtures. Data on the advantage of occluded over non-occluded application appears at present to be inconclusive (Niemec et al., 1994; Vanhal et al., 1996).

The method of niosome penetration between bilayers appears to lie between the highly flexible hypothesis (Cevc, 1993, 1996; Cevc et al., 1996) and the destruction/reconstruction hypothesis (Junginger et al., 1991). It is likely that the more hydrophilic surfactants that are used to make these vesicles which are capable of transdermal delivery possess a shorter residence time within the vesicle (Israelachvili, 1985) somewhere between the residence times of micelle and the more hydrophobic surfactant vesicle monomers. This may allow the passage of a more loosely associated set of monomers through the stratum corneum. On examination, this hypothesis accommodates the fact that transdermal delivery has only been reported with hydrophobic or amphiphilic molecules (Israelachvili, 1985; Reddy, Udupa, 1993; Niemec et al., 1994; Cevc, 1996; Vanhal et al., 1996) as these hydrophobic or amphiphilic molecules are more likely when compared with more hydrophilic molecules to associate with this loosely bound amphiphile aggregate.

Span 60 flurbiprofen niosomes when applied transdermally in a hydroxypropyl methyl cellulose semi solid base gave a higher area under the plasma level time curve than when administered orally in a saline suspension (Reddy, Udupa, 1993). It is likely that the use of hydroxypropyl methyl cellulose in this formulation may affect drug penetration and it is unclear as to what effect the presence of this semi-solid base had on niosome integrity.

8.8. Ophthalmic drug delivery

A single study reports on the biological evaluation of a niosomal drug delivery system for ophthalmic delivery (Saettone et al., 1996). Cyclopentolate was encapsulated within niosomes prepared from polysorbate 20 and cholesterol and found to penetrate the cornea in a pH dependant manner within these niosomes. Permeation of cyclopentolate increased at pH 5.5 but decreased at pH 7.4. Contrary to these findings, in vivo there was increased mydriatic response with the niosomal formulation irrespective of the pH of the formulation (Saettone et al., 1996). It is concluded that the increased absorption of cyclopentolate may be due to the altered permeability characteristics of the conjuctival and scleral membranes (Saettone et al., 1996).

Additionally discomes (Uchegbu et al., 1992; Uchegbu, Florence, 1995; Uchegbu et al., 1996b) have been proposed as ophthalmic drug delivery agents as detailed above.

9. General conclusions

A number of hydrophilic units may be used to synthesise vesicle forming non-ionic surfactants. While the correlation of head group chemistry with vesicle physical chemistry and biology remains to be systematically carried out, it is evident that a rich array of vesicular structures may be produced from a variety of as yet unsynthe-

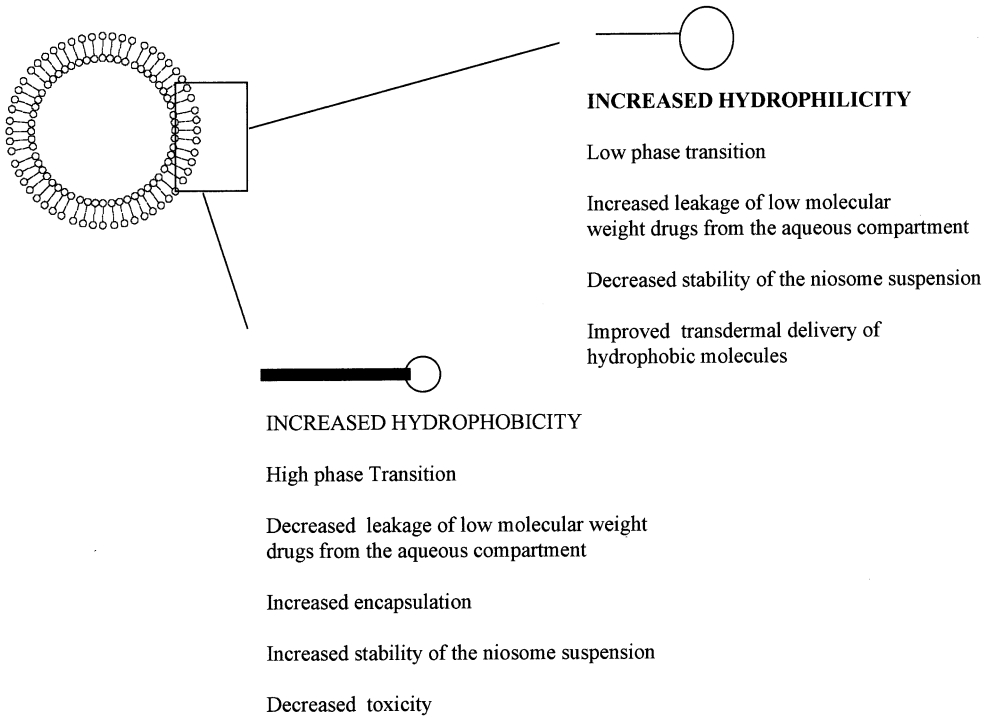

Fig. 28. The effect of the choice of niosome forming surfactant on the properties of the niosome dispersion.

sised compounds. Niosomes have been proven to be useful in the delivery of anti-infective agents, anti-cancer agents anti-inflammatory agents and fairly recently as vaccine adjuvants. These systems have been proven to target certain areas of the mammalian anatomy and may be exploited as diagnostic imaging agents. All this is supremely encouraging.

Without a doubt the potential of these systems can only be fully realised when issues such as stability, toxicology, molecular factors responsible for targeting, etc. are systematically studied. The problems of drug loading remain to be addressed and although some remote loading methods and the preparation of DRV's may go some way to alleviate these problems, it is still necessary to increase encapsulation efficiencies or to evaluate the biological potential of systems in which a proportion of the drug is encapsulated and a proportion is unencapsulated.

In making a choice of surfactant the higher phase transition surfactants appear to yield more desirable permeability and toxicity profiles (Fig. 28). Delivery by the transdermal route, however appears to show that the more fluid membranes appear to be more efficient. Vesicle size has not yet been fully characterised from a biological point of view and studies designed to systematically define the size requirements for certain pharmacodynamic objectives are sorely desired. At the present time the red blood cell may be used as the upper size limit for the intravenous route while larger vesicles are predicted to be better for ophthalmic delivery for example. The flexibility of

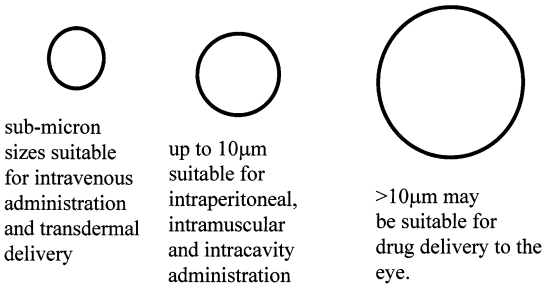

Fig. 29. Suitable niosome sizes for particular routes of administration.

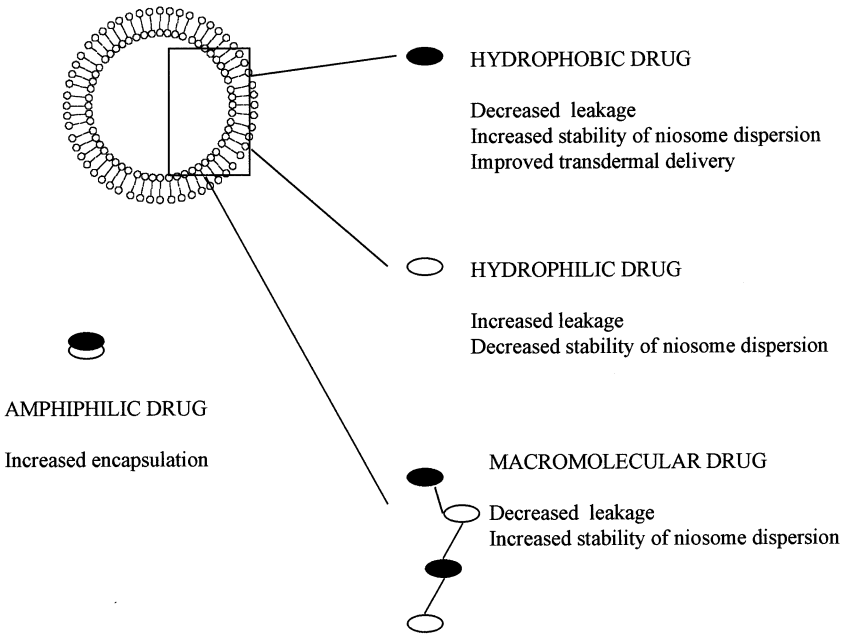

Fig. 30. The effect of the nature of the encapsulated drug on the properties of the niosome dispersion.

these vesicles should allow for larger sized particles to be instilled into the eye. Intraperitoneal injection of particle sizes of over 8 μm has been carried out and the use of vesicles of this size or greater by the intramuscular route may pose no problem (Fig. 29). In choosing a suitable drug to be delivered by niosomes, it should be borne in mind that niosomes encapsulating hydrophobic drugs and macromolecules are more stable than niosomes encapsulating low molecular weight drugs. These factors will also affect niosome stability in vivo. In addition transdermal drug delivery appears possible with hydrophobic or amphiphilic molecules (Fig. 30).

Niosomes have been formulated in oils as v/w/o emulsions and the encapsulation of polymer drug conjugates revealed rather surprising pharmacokinetics with organ targeted sustained release in vivo being realised from the unusual pharmacokinetic profile of this system.

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