ELSEVIER

International Journal of Pharmaceutics 105 (1994) 1–6

international journal of pharmaceutics

Research Papers

Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85)

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(Received 9 September 1993; Accepted 6 October 1993)

Abstract

Formation of multilamellar vesicles (niosomes) of a series of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan trioleate (Span 85) has been studied using a mechanical shaking technique without sonication. 5(6)-Carboxyfluorescein (CF) was used as a model solute to investigate entrapment efficiency and release. For Span 80, cholesterol and dicetyl phosphate (DCP) in the molar ratio 47.5 : 47.5 : 5.0, entrapment efficiency increased linearly with increasing concentration of lipid. Entrapment efficiency per mmol lipid, however, was constant at about 34%, independent of the lipid concentration. Entrapment efficiency increased with increasing cholesterol content when vesicles were prepared by changing the molar ratio of non-ionic surfactant to cholesterol. Most efficient entrapment of CF occurred with Span 60 (HLB 4.7). Mean size of the un-sonicated niosomes showed a regular increase with increasing HLB from Span 85 (HLB 1.8) to Span (HLB 8.6). The release rate of CF from vesicles depended on the surfactant used in the preparation of the vesicles.

Key words: Niosomes; Carboxyfluorescein; Sorbitan ester; Entrapment efficiency; HLB; Release rate

1. Introduction

Non-ionic surfactant vesicles (or niosomes) are now widely studied as an alternative to liposomes. An increasing number of non-ionic surfactants have been found to form vesicles, capable of entrapping hydrophilic and hydrophobic solutes (Florence and Baillie, 1989). These non-ionic surfactant vesicles appear to be similar in terms of their physical properties to liposomes, being prepared in the same way and, under a variety of conditions, forming unilamellar or multilamellar structures. They may be regarded either as inexpensive alternatives, of non-biological origin, to liposomes, or perhaps in vivo as a carrier system physically similar to the liposome.

In previous studies (Baillie et al., 1985; Rogerson et al., 1987, 1988; Florence et al., 1990) we

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SSDI 0378-5173(93)E0296-3

have used non-ionic polyglycerol alkyl ethers as the basis of niosome carriers. We, like Chandraprakash et al. (1990), observed that some sorbitan esters (Spans) formed liposome-like vesicles. Therefore, in order to obtain a system for patient use composed of surfactants generally regarded as safe, the Span series was investigated as sorbitan esters are widely used as emulsifiers in food and pharmaceutical. In this study, vesicles were prepared by mechanical shaking of the lipid dispersions in the presence of hydrating fluid, without subsequent sonication, to study the influence of surfactant structure and HLB on entrapment efficiency of CF, vesicle size and in vitro release.

2. Materials and methods

2.1. Materials

The non-ionic surfactants used as vesicle-forming materials were technical grades of sorbitan monolaurate, monopalmitate, monostearate, mono-oleate and tri-oleate, respectively, Span 20, 40, 60, 80 and 85 (Fluka Chemika, Germany) used as received. Cholesterol was purchased from Sigma Chemicals, U.K. and used without further purification. Dicetyl phosphate (DCP) was obtained from Fluka Chemica, Germany. 5(6)-Carboxyfluorescein (CF) was obtained from Eastman Kodak and was purified using a Sephadex LH20 hydrophobic column before use. All organic solvents were obtained from BDH, U.K.

2.2. Methods

Preparation of non-ionic surfactant vesicles

Multilamellar vesicles (MLV) were prepared by standardised mechanical shaking of the various lipid dispersions in the presence of hydrating fluid without subsequent sonication. Surfactants and DCP with or without cholesterol were dissolved in 15 ml chloroform in a 100 ml round-bottomed flask. The organic solvent was removed at a temperature of 60°C under reduced pressure on a Heidolph rotary evaporator to form a thin film on the flask wall. The excess organic solvent was then removed with oxygen-free nitrogen for 10 min and film was dried completely. The dried lipid film was hydrated with 6 ml of CF solution by shaking with a mechanical shaker in a water bath at 60°C for about 1 h. It was essential to prepare the vesicles at a temperature above the gel-liquid transition temperature of the non-ionic surfactants; Span 60 has the highest phase transition temperature of about 50°C. Therefore, all vesicle preparations were carried out at about 60°C. The resulting multilamellar non-ionic surfactant vesicle dispersion was then left to cool. Vesicles were sized on a Malvern (series 2600) droplet and particle sizer (M4.4) using phosphate-buffered saline (PBS) pH 7.4 as the diluent.

Removal of unentrapped solute

The removal of unentrapped solute from the vesicles was accomplished by gel chromatography. A 2.0 ml aliquot of the vesicle suspension was applied to a Sephadex G-50 column (55 × 1.5 cm) and the vesicles were fractionated using PBS (pH 7.4) as eluent.

Entrapment efficiency

To 10 μl of the vesicle dispersion obtained from the gel chromatography procedure described above was added 3.99 ml PBS (pH 7.4) containing 0.025% Triton X-100 to disrupt the vesicles. The resulting solution was diluted 100-fold with PBS and its fluorescence measured (Perkin Elmer LS-3 fluorescence spectrometer) at an excitation wavelength of 486 nm and emission wavelength of 514 nm. In the same manner the fluorescence of the hydrating solution was measured. Entrapment efficiencies were expressed as a percentage of the total amount of CF used initially.

In vitro release

Dialysis tubing (Visking 20/32) was washed several times with distilled water and left soaking in distilled water. 5 ml of each of the vesicle suspensions or CF solution was pipetted into a bag made of Cuprophan tubing and sealed. Each of the samples contained the same concentration of CF. The Cuprophan bag containing the vesi-

cles was placed in 250 ml of buffer solution (pH 5.0) in a 300 ml conical flask with constant shaking at 25°C. At various time intervals, fluorescence of the buffer solution was measured.

3. Results and discussion

3.1. Electron micrographs of Span vesicles

The freeze-fracture replicates of non-ionic surfactant vesicles composed of Span 80, cholesterol and DCP in the molar ratio 47.5:47.5:5.0, is shown in Fig. 1. They are similar in appearance to electron micrographs of phospholipid vesicles and show a multilamellar structure. The size distribution of vesicles trends to be fairly wide, although this can be modified by altering the hydration time and degree of shaking.

3.2. Effect of total lipid concentration

The effect of total lipid concentration on the entrapment efficiency of CF in the preparation of

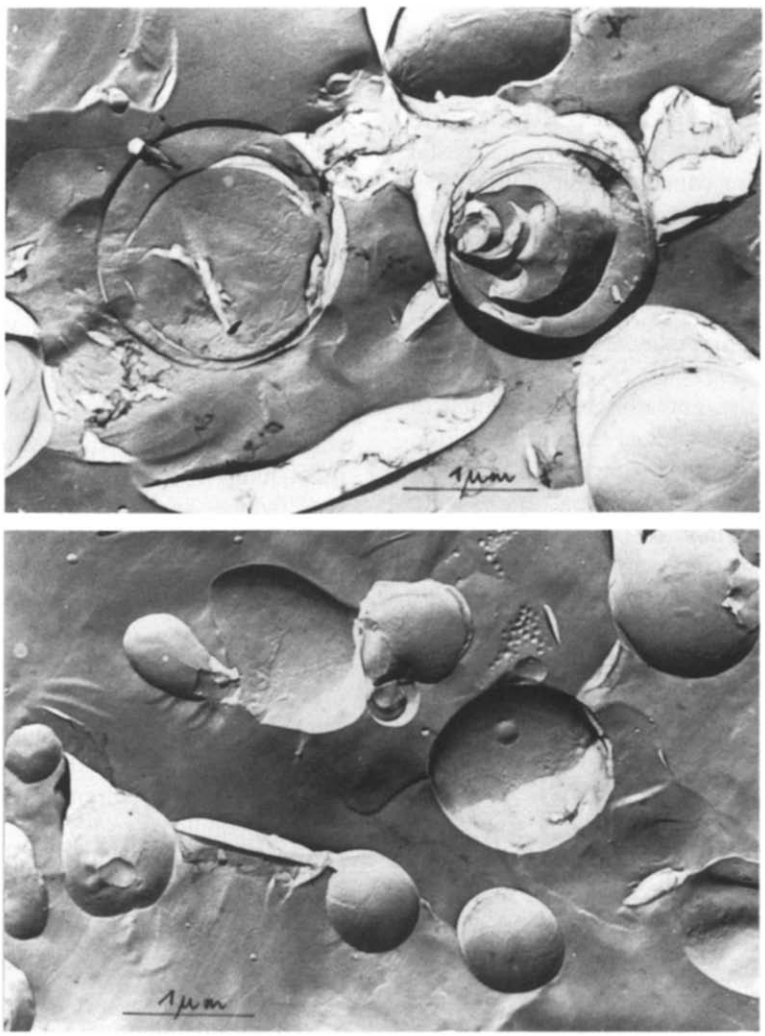

Fig. 1. Freeze-fracture replicates of non-ionic surfactant vesicle suspension prepared from Span 80, cholesterol and dicetyl phosphate in the molar ratio 47.5:47.5:5.0.

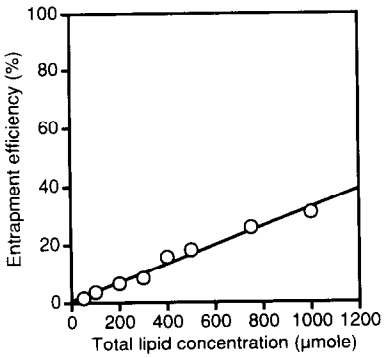

Fig. 2. Effect of the total lipid concentration on the entrapment efficiency.

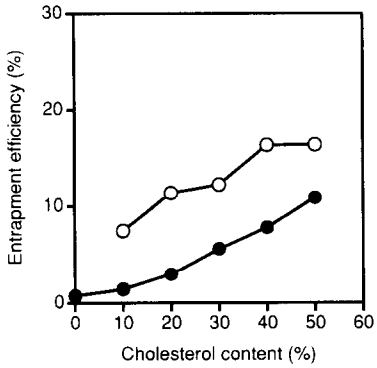

Fig. 4. Effect of the cholesterol content on the entrapment efficiency. (○) Span 60, (●) Span 80.

Span vesicles is shown in Fig. 2 and Fig. 3. For Span 80, cholesterol and DCP in the molar ratio 47.5 : 47.5 : 5.0, entrapment efficiency increased linearly with increasing concentration of lipid (Fig. 2). However, entrapment efficiency per mmol lipid has the same value, about 34%, independent of the lipid concentration (Fig. 3).

3.3. Effect of cholesterol content

The effects on the entrapment efficiency of Span 60 and Span 80 vesicles of the incorporation of cholesterol into the bilayer structure were examined. Total lipid and DCP concentrations were fixed at 300 and 15 μmol, respectively, and vesicles were prepared by changing the molar ratio of non-ionic surfactant to cholesterol. The results are shown in Fig. 4. Span 60 vesicles could be prepared without cholesterol at 60 °C, but the resulting vesicle suspension gelled at this temperature, and vesicles could not be separated from unentrapped CF at room temperature. Entrapment efficiencies increased with increasing cholesterol content. X-ray diffraction methods have demonstrated that cholesterol increases the width of phospholipid bilayers (McIntosh, 1978). The increased CF entrapment when cholesterol was included in the these Span formulations was most likely to be the result of increased vesicle size, whilst an increased bilayer thickness was probably responsible for the lack of direct proportionality between vesicle volume and entrapment. Entrapment efficiencies of Span 60 formulations were higher than those of Span 80 formulations. Span 60 and Span 80 have the same head group but Span 80 has an unsaturated alkyl chain. De Gier et al. (1968) demonstrated that the introduction of double bonds into the paraffin chains causes a marked enhancement in the permeability in liposome, possibly explaining the lower entrapment efficiency of the Span 80 systems.

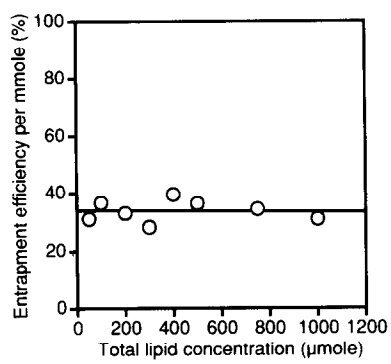

Fig. 3. Effect of the total lipid concentration on the entrapment efficiency per mmol lipid.

3.4. The influence of surfactant structure on vesicle properties

A series of Span vesicles was prepared so that the influence of surfactant structure on vesicle properties could be investigated. Vesicles were

Table 1
Entrapment of CF and mean size of Span vesicles

Span	Type	HLB	% CF	Size (μm)
20	sorbitan monolaurate	8.6	11.4	3.4
40	sorbitan monopalmitate	6.7	16.2	1.1
60	sorbitan monostearate	4.7	16.4	0.96
80	sorbitan mono-oleate	4.3	10.9	0.90
85	sorbitan tri-oleate	1.8	9.1	0.65

composed of Span, cholesterol and DCP in the molar ratio 47.5:47.5:5.0. The results are listed in Table 1. Entrapment efficiencies in Span 40 and Span 60 formulations were higher than those in other Span formulations. Span 40 and Span 60 are solid at room temperature and showed the higher phase transition temperature (T_c). The results reflect the effect of the phase transition temperature. The Span having the highest phase transition temperature provides the highest entrapment. As for vesicle size, increasing hydrophobicity of the surfactant monomer led to smaller vesicles (Fig. 5), a result which might be anticipated since surface free energy decreases with increasing hydrophobicity (Wan and Lee, 1974a,b).

3.5. *In vitro release study*

Results of an in vitro study on the release of CF from the Span vesicles are shown in Fig. 6. As expected, the rate of release of CF across the dialysis membrane, for all CF loaded vesicles, was slower than that for free CF. 'Release' after 6 h was approx. 70% from the free CF solution but only 30% from the Span 40 or Span 60 containing vesicles. The CF flux from the free solution was linear over the first 3 h, then began to plateau, whereas, release from all Span vesicles was linear for 6 h. The release rates from Span 40 and Span 60 formulations were slower than those from other Span formulations. At 25°C molecules of Span 40 and Span 60 are in the ordered gel state, but those of Span 20, Span 80 and Span 85 are in the disordered liquid-crystalline state. The addition of cholesterol appeared to disrupt the ordered array of the hydrocarbon chains in the gel phase (Ladbrooke et al., 1968). Below the T_c cholesterol made the membrane less ordered, which above the T_c it made the membrane more ordered (Papahadjopoulos et al., 1973). In liposomes prepared from lecithin and cholesterol containing 50 mol%, all the chains were found to be in the fluid state (Ladbrooke et al., 1968). Moreover, liposomes prepared from the mixture of DMPC with equimolar portions of cholesterol are more leaky than those prepared from DPPC-cholesterol mixtures (Taylor et al., 1990). The introduction of double bonds into the paraffin chains and decreasing chain length causes a marked enhancement of vesicle permeability (De Gier et al., 1968).

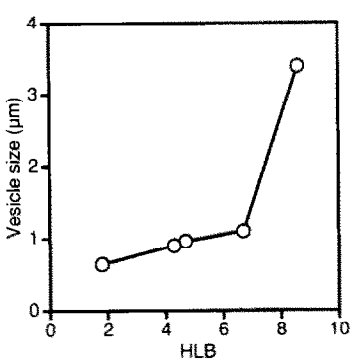

Fig. 5. Effect of HLB on vesicle size.

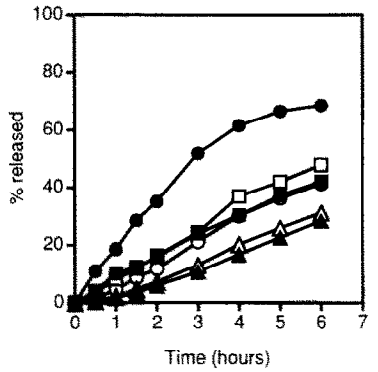

Fig. 6. Release of CF from a solution of free CF and CF loaded vesicles composed of Span:cholesterol:DCP in the molar ratio 47.5:47.5:5.0 at 25°C; (●) free CF solution; (○) Span 20; (△) Span 40; (▲) Span 60; (□) Span 80; (■) Span 85.

4. Conclusions

Sorbitan monoesters of the Span surfactant series form, with cholesterol, multilamellar vesicles which entrap solutes such as carboxyfluorescein. Entrapment efficiency depends on the total lipid concentration, although entrapment per mmol lipid is constant, increases with increasing cholesterol content in the bilayers and increases with the increasing phase transition temperature of the Span used. The size of the vesicles prepared by mechanical shaking is dependent on the hydrophile-lipophile balance of the Span used, the lower the HLB the smaller the initial size of the vesicles. The fact that the Span surfactants are used widely in foodstuffs paves the way for the use of these vesicles in pharmaceutical formulations.

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