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Vesicle (niosome)-in-water-in-oil (v/w/o) emulsions: an in vitro study

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Abstract

A new vesicular formulation is described in which non-ionic surfactant vesicles (niosomes) are dispersed in an aqueous phase which is then emulsified in an non-aqueous continuous phase. The resultant vesicle-in-water-in-oil (v/w/o) system allows the delivery of vesicles in a non-aqueous vehicle. The formulation, stability and characteristics of several v/w/o systems are described. The non-ionic surfactants used to prepare the vesicles (niosomes) are also employed in the emulsification step to minimize surfactant redistribution. The relation between the HLB of the non-ionic surfactant used for preparation of the v/w/o systems and the in vitro release of 5(6)-carboxyfluorescein (CF) from the vesicles in the system was investigated, controls being water-in-oil (w/o) emulsions and a vesicle suspension. A range of v/w/o systems was prepared from niosomes made from non-ionic surfactants (sorbitan monoesters, Span 20, 40, 60 and 80) in the size range 600 nm–3.4 μm dispersed in water droplets of around 5–25 μm themselves dispersed in an oil (octane, hexadecane, isopropyl myristate). The in vitro release rate of CF showed a decrease in the order free solution > vesicle suspension > w/o emulsion > v/w/o emulsion. The rate of release of CF entrapped in the vesicles in the v/w/o system depends on the nature of the surfactants used. The hydrophobicity of the Span surfactant for the preparation of vesicles and the v/w/o emulsion had a significant influence on the release rate. In the Span 60 formulation, the release rate was the slowest, because Span 60 has the highest phase transition temperature and the v/w/o formulation gelled at both 25 and 37°C. The nature of the oil phase affected release as might be expected from the partitioning behaviour of CF. With increasing temperature, release rates increased in Span 40 and Span 80 systems, but were unaffected in the Span 60 system due to the maintenance of the gel phase.

Key words: Niosome; Non-ionic surfactant vesicle; Sorbitan ester; Emulsion; Release rate

1. Introduction

Liposomes and niosomes are generally administered as aqueous dispersions for therapeutic applications (Alving et al., 1978; Azmin et al., 1985; Hunter et al., 1988; Rogerson et al., 1988). In exploring formulations which maintain normal vesicular structure in an aqueous phase, but allow administration or application in an external non-aqueous phase, we have developed a vesicle-in-water-in-oil (v/w/o) emulsion system. In earlier

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studies on water-in-oil-in-water systems (Florence et al., 1989) one of the factors compromising stability was the migration of stabilizing surfactant from one interface to another after formation of the system. To minimise this effect in the complex surfactant dispersion which is the subject of this paper, the non-ionic surfactants of the sorbitan monoester (Span) series used to prepare the vesicles were also used to stabilise the water-in-oil emulsion formed in the second stage of the manufacture of the system.

After the completion of this work, our attention was drawn to a patent which describes a very similar system (Albert et al., 1992). In this, the preparation of lipid vesicle containing water-in-oil emulsions for the application of cosmetics is described briefly.

In this paper, we describe the preparation of niosome-in-water-in-oil systems using three oils (octane, hexadecane and isopropyl myristate) as the non-aqueous external (continuous) phase. Systems were characterised by conventional microscopy, and by determination of the rate of release of carboxyfluorescein encapsulated in the vesicles. The release of entrapped solutes (such as drugs or antigens) can be controlled by formulation. This is important in the development of the system for use as a vaccine adjuvant for parenteral or oral use. Oral administration of the v/w/o system where the oil phase is a biodegradable oil might allow release of the vesicles in the gastrointestinal tract, preserving their integrity in the stomach.

2. Materials and methods

2.1. Materials

The non-ionic surfactants, sorbitan monolaurate (Span 20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60), sorbitan monooleate (Span 80), and dicetyl phosphate (DCP) were obtained from Fluka Chemie, Germany. Cholesterol was purchased from Sigma Chemicals, U.K. and used without further purification. 5(6)-Carboxyfluorescein (CF) was obtained from Eastman Kodak and was purified using a Sephadex LH20 hydrophobic column before use. Octane, hexadecane and isopropyl myristate were purchased from Fluka Chemie, Germany. All organic solvents were obtained from BDH, U.K.

2.2. Methods

2.2.1. Preparation of non-ionic surfactant vesicles (niosomes)

Multilamellar non-ionic surfactant vesicles (niosomes) were prepared by a hand-shaking method (Baillie et al., 1985; Yoshioka et al., 1994). 300 μmol of surfactant (Span 20, 40, 60 or 80), cholesterol and dicetyl phosphate (giving a molar ratio of 47.5:47.5:5.0) were the lipid ingredients dissolved in 15 ml of chloroform in a 100 ml round-bottomed flask. The organic solvent was evaporated under reduced pressure at a temperature of 60°C using a 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 the film was dried completely. The dried lipid film was hydrated with 6 ml of 5(6)-carboxyfluorescein (CF) solution by shaking with a mechanical shaker in a water bath at 60°C for 1 h. It was essential to prepare the vesicles at a temperature above the gel-liquid transition temperature of non-ionic surfactants; Span 60 has the highest phase transition temperature of about 50°C. Therefore, all vesicle preparations were carried out at 60°C. The resulting multilamellar non-ionic surfactant vesicle dispersion was then left to cool. The CF-entrapped vesicles were separated from the unentrapped material by gel chromatography.

2.2.2. Preparation of v/w/o system

The same surfactant as that used to form vesicles was added to the oil and dissolved completely at 60°C, and then one part of the resulting vesicle suspension was added to four parts of an oil containing 5% of the surfactant at the same temperature. Emulsification was carried out using a vortex mixer to produce the v/w/o emulsion. Excessive shear is avoided to prevent micronisation of the aqueous phase.

2.2.3. In vitro release kinetic study

Cuprophan tubing size 5 (Visking 20/32 in) (Medicell International, London) was washed several times with distilled water and left soaking in distilled water overnight before use. 5 ml each of CF solution, the relevant vesicle suspension (v/w), w/o emulsion or v/w/o emulsion were pipetted into a bag made of Cuprophan tubing and sealed. Each of the samples contained the same concentration of CF. The Cuprophan bag was placed in 250 ml of buffer solution (pH 5.0) in a 300 ml conical flask with constant shaking at 25 or 37°C. At various time intervals, the fluorescence of the buffer solution was measured (Perkin Elmer LS-3 fluorescence spectrometer) at an excitation wavelength of 486 nm and emission wavelength of 514 nm.

3. Results and discussion

3.1. Structure of v/w/o emulsion

The general structure of v/w/o emulsions prepared with Span 80 and octane is shown in Fig. 1. Although all the v/w/o emulsions had vesicles in the aqueous disperse phase, their size is a reflection of the kind of Span used and mode of preparation of vesicles. Vesicle size is dependent on the HLB value of the Span used when hand-shaking is used. The mean size of the niosomes showed a regular increase with increasing HLB from Span 80 (HLB 4.3) to Span 20 (HLB 8.6) (Yoshioka et al., 1993). As can be seen in the microphotograph in Fig. 1, the v/w/o emulsion comprised vesicles dispersed in water droplets of around 5–25 μm dispersed in the oil phase. Encapsulated drug or marker is released first into the aqueous disperse phase, then by transport across the continuous oil phase into the receptor phase.

3.2. Effect of surfactant

The effect of the particular Span surfactant on the release of CF was investigated by preparing niosomes and their equivalent w/o dispersions using the Span series. The results of dialysis experiments are shown in Fig. 2, together with those for free CF solutions, v/w suspensions and w/o emulsions, for comparison. The release of CF was the slowest from the v/w/o emulsion. The release rate of CF decreased in the order: free solution > vesicle suspension > w/o emulsion > v/w/o emulsion, as anticipated, regardless of the Span used. However, the rate of re-

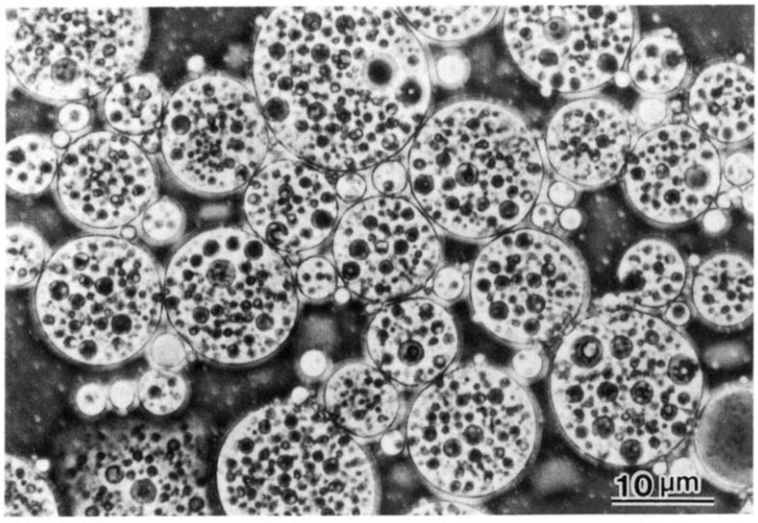

Fig. 1. Photomicrograph of a v/w/o emulsion formulated with large niosomes prepared with Span 80, and 5% of Span 80 in the oil phase, octane.

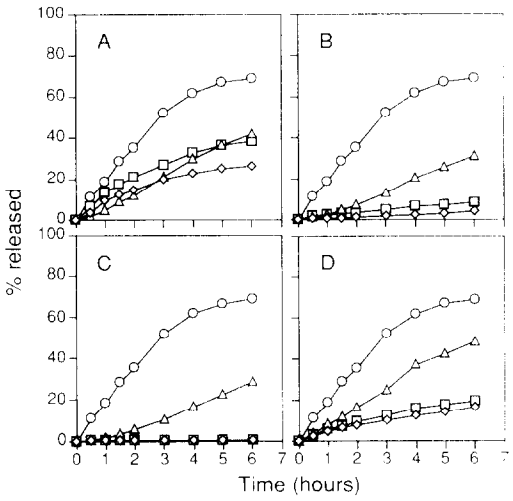

Fig. 2. In vitro release profile of CF from various formulations prepared with (A) Span 20, (B) Span 40, (C) Span 60 and (D) Span 80. (○) CF solution, (△) vesicle suspension, (□) w/o emulsion, (◇) v/w/o emulsion.

lease of CF entrapped into the vesicles in the v/w/o system was found to change depending on the nature of the surfactants used both in niosome preparation and as the secondary emulsifier. Among the components of the w/o emulsions and v/w/o emulsions, the Span surfactant is common to both and may be responsible for the different release patterns. The degree of hydrophobicity of Span surfactant had a significant influence on the release rate. In the Span 60 formulation, the release rate was the slowest, the most likely explanation being that Span 60, and to a lower extent Span 40, caused the gelation of

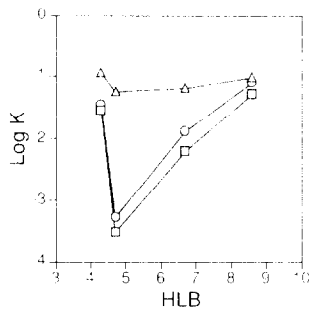

Fig. 3. First-order release rate constants of various formulations prepared with different surfactants plotted as a function of surfactant HLB. (○) w/o emulsion, (△) vesicle suspension, (□) v/w/o emulsion.

the oil phase at 25°C. The gelation was most likely caused by the crystallization and solidification of the excess Span 60 in the oil phase, although the process has not been studied in detail.

From the semi-logarithmic linear relationship of the percent drug remaining as a function of time, values of a release rate constant, K, were extracted for the systems and plotted vs surfactant HLB (Fig. 3). The release rate was the lowest at HLB values around 4.7. Span 20 and Span 80 are liquids, but Span 40 and Span 60 are solid at room temperature. Therefore, v/w/o emulsions prepared with Span 40 and Span 60 caused gelation of oil phase, although the gel in Span 40 formulations is weak. Moreover, the K values for the vesicle suspensions were almost the same, independent of the surfactants, however, those for the w/o emulsions and the v/w/o

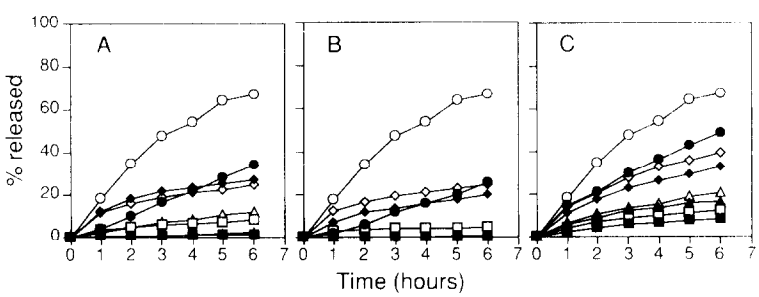

Fig. 4. In vitro release profile of CF from various vesicle and emulsion formulations prepared with different oils. (A) Span 40, (B) Span 60, (C) Span 80. (○) CF solution, (●) vesicle suspension, (△) w/o emulsion (octane), (□) w/o emulsion (hexadecane), (◇) w/o emulsion (IPM), (▲) v/w/o emulsion (octane), (■) v/w/o emulsion (hexadecane), (◆) v/w/o emulsion (IPM).

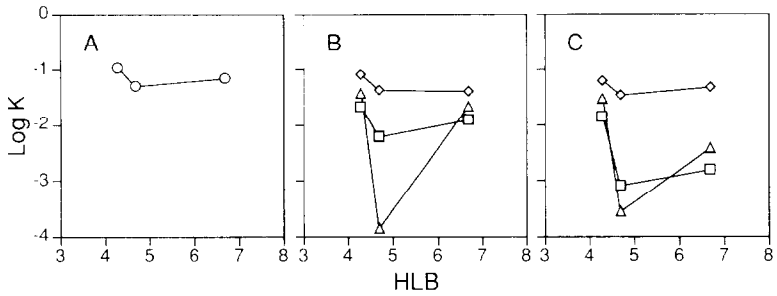

Fig. 5. HLP profile of first-order release rate constants of (A) vesicle suspension, (B) w/o emulsion and (C) v/w/o emulsion prepared with different oils. (△) Octane, (□) hexadecane, (◇) isopropyl myristate.

emulsion were different. This suggests that the rate-limiting step was not transport through the vesicle membrane but transport through the oil layer, as suggested by Omotosho et al. (1986a).

3.3. Effect of the oil phase

Using all the Spans in turn, the effect of the oil phase on release rate was examined with octane, hexadecane and isopropyl myristate. The results are shown in Fig. 4. In Span 40 and Span 60 v/w/o emulsions prepared with octane and hexadecane, CF released little, because these formulations were viscous gels. From the semi-logarithmic linear relationship of the percent drug remaining as a function of time, the K values were extracted and plotted vs HLB (Fig. 5) as before. When isopropyl myristate was used as the oil phase, the release rate constant was greater and almost same in all systems. In Span 40 and Span 60 systems, the release rate constants of v/w/o emulsions prepared with hexadecane were the almost same, but those prepared with octane varied with the surfactant. Omotosho et al. (1986b) examined the effect of the nature of the oil phase and the release of solutes from multiple w/o/w emulsions and demonstrated that the main factor in determining the differences in rates of release from the hydrocarbon w/o/w emulsions was the droplet size of the internal aqueous phase; i.e., the degree of drug leakage from the internal phase of multiple emulsion was greatest with systems of low particle size. It is reasonable to suppose that, as with conventional emulsions, the nature of the oil phase can markedly affect the behaviour of the system, and the stability of the oil membrane against leakage of the entrapped material will depend, among other factors, on the nature of the oil used in preparing the emulsion. Also, for a given internal (w) phase volume, a decrease in droplet size would increase the surface area exposed, result-

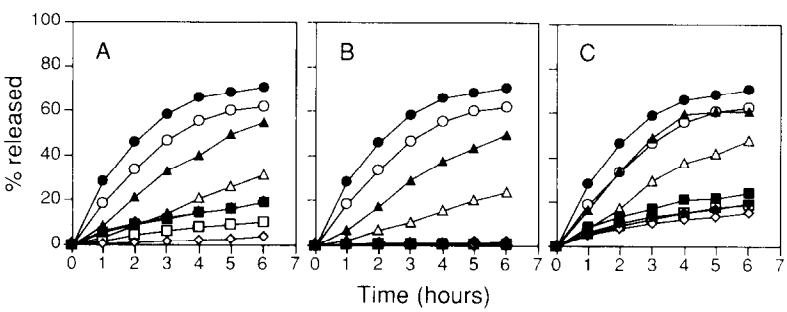

Fig. 6. In vitro release profile of CF from various formulations at different temperatures. (A) Span 40, (B) Span 60, (C) Span 80. 25°C: (○) CF solution, (△) vesicle suspension, (□) w/o emulsion, (◇) v/w/o emulsion; 37°C: (●) CF solution, (▲) vesicle suspension, (■) w/o emulsion, (◆) v/w/o emulsion.

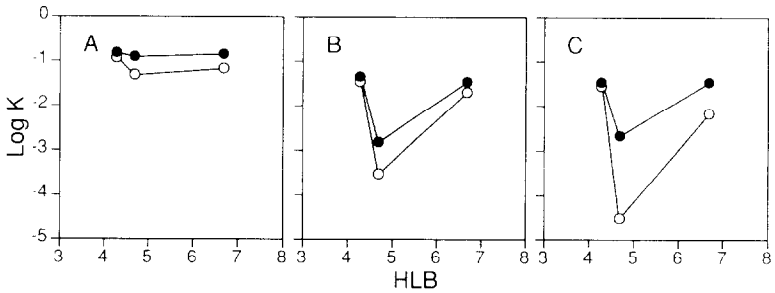

Fig. 7. HLB profile of first-order release rate constants of an aqueous vesicle suspension (A), w/o emulsion (B) and v/w/o emulsion (C) at different temperatures: (○) 25°C, (●) 37°C.

ing in an increased release rate which would explain the large differences in rates of release of CF from v/w/o emulsions prepared with octane, hexadecane and isopropyl myristate, but the over-riding differences in release rate constants appear to be due to gel formation.

3.4. Effect of temperature

The effect of temperature on the release rate of CF from various formulations was evaluated at 25 and 35°C. The temperature dependency of the release of CF to the dialysis medium is illustrated in Fig. 6: the higher the temperature, the more rapid was the release rate observed in all systems. From the semi-logarithmic plot, the release rate constants were calculated for Fig. 7. With increasing temperature of the dialysis medium the release rates increased in Span 40 and Span 80 systems, but in the Span 60 system the release rate did not increase due to the gelation of the oil phase. Generally, it is expected that the diffusion coefficients should decrease with increasing viscosity. In this experiment the viscosity in all cases appeared to decrease with increasing temperature. So, these results suggest that the release rate of CF is largely dependent on the state of the external oil phase.

4. Conclusion

Span niosomes with their multilamellar structures can be dispersed in their aqueous medium in oil to form v/w/o systems of potential use in drug delivery or as a vaccine vehicle. The release rate of CF entrapped in the vesicles has been found to change depending on the nature of the surfactants used. The degree of hydrophobicity of the Span surfactant employed had a significant influence on the release rate. With increasing hydrophobicity, the release rate decreased until HLB 4.7, and then increased. Also, the nature of the oil phase affected release. As would be expected, monitoring of the release rate of CF from various formulations at different temperatures showed in all formulations that CF was released faster at the higher temperature. These results suggest that it may be possible to control the physicochemical v/w/o emulsions properties in order to regulate the delivery rate of drugs by appropriate choice of surfactant, oil and temperature of the dialysis medium.

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