Effect of Cholesterol Content and Surfactant HLB on Vesicle Properties of Niosomes

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Effect of cholesterol content on entrapment efficiency and influence of surfactant HLB on the vesicle size has been studied on sorbitan esters vesicles also known as niosomes prepared using a mechanical shaking technique without sonication. Primaquine phosphate was used as a model drug. Entrapment efficiency increased with increasing cholesterol content. Mean size of unsonicated niosomes showed a regular increase with increasing HLB from Span 85 (HLB 1.8) to Span 20 (HLB 8.6).

Niosomes are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures. These structures are analogous to phospholipid vesicles (liposomes), capable of entrapping hydrophilic and hydrophobic solutes[1] and serve as drug carriers. The low cost, greater stability and resultant ease of storage of non-ionic surfactants[2] has lead to the exploitation of these compounds as alternatives to phospholipids.

Cholesterol is one of the vital constituents in most of the non-ionic surfactant vesicles and addition of this substance was found to stabilize their structure by making them less leaky[3]. Niosomes have been prepared from several classes of non-ionic surfactants with varying HLB e.g., polyglycerol alkylethers[4], glucosyl dialkyethers[5], crown ethers[6], and polyoxyethylene ethers and esters[7]. Surfactant HLB is a good indicator of vesicle forming ability of any surfactant. Both cholesterol content and surfactant HLB tends to effect the important vesicular properties, which is to be optimized in their terms for better therapeutic efficacy, site targeting and for the use of vesicles in pharmaceutical application. In this study, vesicles were prepared by mechanical shaking of the lipid dispersion in the presence of hydrating fluid, without subsequent sonication, to study the influence of cholesterol content on entrapment efficiency of drug and surfactant HLB on vesicle size taking primaquine phosphate, an antimalarial agent as a model solute.

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Span 20, 40, 60, 80 and 85 were purchased from Fluka Chemika, Germany and used as received. Cholesterol and dicetyl phosphate (DCP) were obtained from Sigma Chemicals, USA. Primaquine phosphate was a gift sample from IPCA Laboratories Ltd., Mumbai. All other reagents used were of analytical grade.

Multilamellar vesicles (MLV) were prepared by standardized lipid layer hydration method. Briefly, surfactants, cholesterol and DCP were dissolved in 10 ml of chloroform in a 100 ml round bottom flask. The organic solvent was completely evaporated under controlled condition of temperature (60±4°) and pressure (15±2 psi) on a rotary evaporator to form a thin film on the flask wall. The organic solvent was then removed with oxygen free nitrogen for 30 min and film was dried completely. The dried film was hydrated with 6 ml of primaquine phosphate solution in phosphate buffered saline (PBS) pH 7.4 by agitating in a mechanical shaker at 60° over 1 h. The resulting non-ionic surfactant vesicle dispersion was then left to cool. Vesicles were sized on a Malvern (Series 2600) droplet and particle sizer (M 4.4) using PBS (pH 7.4) as the diluent.

The removal of unentrapped solute from the vesicles was accomplished by gel chromatography. One milliliter aliquot of the vesicle suspension was applied to a Sephadex G-50 column and the vesicles were fractionated using PBS (pH 7.4) as eluent[8]. To 100 μl of the vesicles dispersion obtained from the gel chromatography procedure was added 4.9 ml PBS (pH 7.4) containing 0.05% Triton X-100 to break the vesicles. The resulting solution was diluted with PBS

and analyzed spectrophotometrically at 260 nm against appropriate blank for the drug content.

The effect on the entrapment efficiency of Span 60 and Span 80 vesicles after the incorporation of cholesterol into the bilayer structure was investigated. Total lipid and DCP concentration were fixed at 400 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.1. Span 60 vesicles could be prepared without cholesterol but the resulting vesicle suspension was too viscous to be separated from unentraped drug at room temperature. Entrapment of primaquine phosphate in vesicles increased with increasing cholesterol content. The increased drug entrapment when cholesterol was included in these Span formulations was most likely to be the result of increased vesicle size and increase in the width of lipid bilayers. This behavior is similar to lecithin/cholesterol mixtures and X-ray diffraction methods have demonstrated that cholesterol increases the width of phospholipid bilayers[9]. Due to the increase in bilayer thickness, a direct proportionality between vesicle volume and entrapment efficiency could not be established. Entrapment efficiency of Span 80 formulations was much less than those of Span 60 niosomes. Span 60 and Span 80 have the same head group but Span 80 has an unsaturated alkyl chain. Introduction of double bonds into the paraffin chains causes a marked enhancement in the permeability in liposomes[10], this might be the reason for lower entrapment efficiency of the Span 80 systems.

A series of Span vesicles were prepared so that its influence on vesicle properties could be investigated. Vesicles were composed of Span, cholesterol and DCP in the molar ratio of 47.5:47.5:5.0[11]. The results are listed in Table 1. Vesicle size of Span 85 (HLB 1.8) formulations was found to be 1.1 μm, the least when compared with other surfactant preparations. The size of the vesicles prepared by mechanical shaking is dependent on the hydrophilic-lipophilic balance of the Span used, the lower the HLB the smaller the initial size of the vesicles, a result which might be due to decrease in surface free energy with increasing hydrophobicity[12].

Sorbitan esters of the Span surfactant series form, multilamellar vesicles, which can entrap solute like primaquine phosphate. Entrapment efficiency increases with the increasing cholesterol content in the bilayers. Mean size of the vesicles showed a regular increase with increasing surfactant HLB.

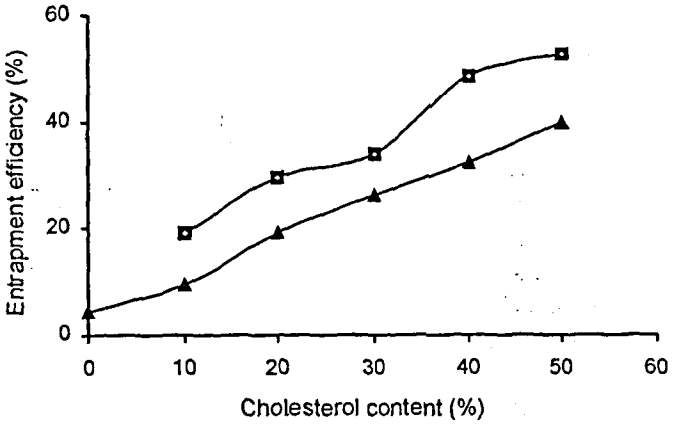

Fig. 1: Effect of cholesterol content on the entrapment efficiency.

Niosomal vesicles of (-□-) Span 60 and (-▲-) Span 80 with varying cholesterol content were prepared to study its effect on entrapment efficiency of the drug.

TABLE 1: MEAN SIZE OF SPAN VESICLES

Span	Type	HLB	Mean size (μm)
20	Sorbitan monolaurate	8.6	4.2±0.58
40	Sorbitan monopalmitate	6.7	2.8±0.31
60	Sorbitan monostearate	4.7	2.2±0.36
80	Sorbitan mono-oleate	4.3	1.6±0.22
85	Sorbitan tri-oleate	1.8	1.1±0.17

Niosome vesicles of Spans (HLB 8.6 to 1.8) were prepared to study the influence of surfactant HLB on vesicle size. Vesicles were sized on a Malvern droplet and particle sizer.

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Antimicrobial Activity of Some Indigenous Plants

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Diethyl ether and methanol extracts of dried powdered, aerial parts of *Artemesia sieversiana* (Asteraceae), *Origanum majoram* (Lamiaceae), fruit peel of *Musa paradisiaca* var *sapientum* (Musaceae) and stem bark of *Moringa pterygosperma* (Moringaceae) were evaluated for antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Cryptococcus neoformans* by cup-plate method. Both the extracts of *Moringa pterygosperma* and methanol extract of *Musa paradisiaca* have shown significant activity in comparison with the standards benzyl penicillin and streptomycin. All others except ether extract of *Musa paradisiaca* are inhibitory to the tested human pathogenic fungi.

The growing worldwide resurgence in infectious diseases due to the AIDS pandemic and the increasing resistance to the newer antibiotics have become major hurdles in the management of infections[1]. Hence there is a need for newer, antimicrobial biomolecules. This study reports the antimicrobial evaluation of four medicinal plants selected on the basis of folklore indications.

Aerial parts of *Artemesia sieversiana* Ehrh. (Asteraceae) are used as a tonic and febrifuge[2]. It's essential oil is inhibitory to plant pathogenic fungi[3] and aqueous extract is reported be amoebicidal[4]. *Musa paradisiaca* Linn. var *sapientum* (Musaceae) fruit is a laxative. Fruit peel is indicated in hypertension and used as a dusting powder on wounds[5]. Its aqueous extract is reportedly antidiabetic[6] and antiulcerogenic[7]. Aerial parts of Origanum *majoram* Moench. (Lamiaceae) are used as an anthelmintic and expectorant[8]. It's essential oil is reported to be insecticidal[9], antifungal[3] and antiviral[10]. *Moringa pterygosperma* Gaertn. (Moringaceae) stem bark is used as an anthelmintic[11]. Leaf and flower extracts are reportedly antimicrobial[12]. Extracts of the plant parts have also been shown to be abortifacient[13]

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Aerial parts of *Artemesia seiversiana*, *Origanum majoram and Musa paradisiaca* fruits were purchased from the local market. Bark of *Moringa pterygosperma* was procured from a private vegetable garden. These were authenticated in the Botany Field Research Laboratory, Madras University, Maduravoyal, Chennai.

The dried coarse powder of the aerial parts, bark and fruit peel (50 g each) were extracted exhaustively and successively with diethyl ether and methanol (150 ml each), using a Soxhlet extractor. The extracts were concentrated to dryness in a Rotary vacuum evaporator. The antibacterial and antifungal activity of the prepared extracts was studied using cup-plate method[14]. Dimethylsulphoxide was used to