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Enhanced Liposomal Encapsulation of Ascorbic Acid by the Liquid Crystal β-sitosteryl-β-D-glucopyranoside

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β-Sitosteryl-β-D-glucopyranoside (LC) was synthesized by reacting penta-O-acetyl-β-D-glucopyranoside with β-sitosterol, followed by deacetylation with sodium methoxide. Both lyotropic and thermotropic liquid crystal properties were observed under polarized light. Its critical micellar concentration (CMC) was 8.5 × 10^{-6} M. The calculated HLB value was 6.24. Ascorbic acid was encapsulated in liposomes constructed with lecithin and cholesterol resulting in encapsulation efficiency (EE) of 44.1% and drug loading (DL) of 78%. The addition of LC to liposomes increased the EE to 63.0% and DL to 82%. Therefore, LC has good co-surfactant properties and could be used as encapsulation enhancer in liposomes.

Keywords Liposome; Ascorbic acid; β-sitosteryl-β-D-glucopyranoside; Critical micelle concentration; Encapsulation efficiency; Drug loading

Introduction

In carbohydrate chemistry, it is common practice to use acetylated saccharides as starting material to synthesize naturally occurring glycosides [1, 2]. Many methods are available for preparing acetylated saccharides; however one of the commonest methods is reaction of saccharide with acetic anhydride in the presence of sodium acetate, as a catalyst. Acetylated glucose such as penta-O-acetyl-β-D-glucopyranoside is a promising reactant for synthesizing many glycolipids. Penta-O-acetyl-β-D-glucopyranoside can be linked to cyclic or acyclic alcohols by using a Lewis acid such as anhydrous aluminum chloride [$AlCl_3$] or Boron trifluoride diethyl etherate [BF_3 . $O(C_2H_5)_2$]. Boron trifluoride diethyl etherate is a useful Lewis acid; however, it is important to maintain the inert reaction medium [1, 2]. The product can also very easily be separated using gravity column. Most promising method for deprotection of saccharide group is to react with sodium methoxide in an inert medium [1, 2].

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Carbohydrate based surfactants belong to the class of non-ionic surfactants [3, 4] consisting of a polar head and a nonpolar tail. Polar head is represented by the saccharide part and the nonpolar tail is usually a cyclic or acyclic alkyl moiety. The Hydrophile–Lipophile Balance (HLB) value is the key parameter which describes the fundamental properties of a surfactant by which its applicability can be determined [3, 4, 5]. Its value gives an indication about the solubility of the surfactant and relates it to its function as an emulsifier, stabilizer etc. In general, surfactants with low HLB are more lipophilic and can stabilize water-in-oil emulsions. Larger HLB values are indicative of greater hydrophilicity of the surfactant and their preference for stabilizing oil-in-water emulsions [5].

Most surfactants possess liquid crystal properties. Liquid crystals exhibit mesophase behavior due to closest packing or microphase separation. Mesophase behavior occurs due to two main reasons, either by imposing order in one or two dimensions or by allowing the molecules to have a degree of translational motion. Closest packing molecules maintain an ordered packing due to thermodynamic stability [6,7]. Lyotropic liquid crystals and thermotropic liquid crystals are the two major classes of liquid crystals. Lyotropic liquid crystal phase appears when immiscible phases exist while thermotropic liquid crystals are sensitive to temperature and are generally reproducible with heating or cooling. Three different classes of lyotropic liquid crystal phases are found. They are the lamellar, the hexagonal columnar and the cubic phase. Classical lamellar phase consist of a stack array of amphiphilic bilayer sheets separated by a solvent. The hexagonal columnar phase consists of long cylindrical rods of amphiphilic molecules arranged on a lattice. There are two different kinds of hexagonal phases exhibited by surfactants; one lies between the lamellar and hexagonal phase known as the bicontinuous cubic phase, the other appears between the hexagonal phase and micellar solution. The surfactant molecules arrange themselves in spherical or non-spherical micelles, which in turn form a cubic lattice called the micellar cubic phase [6, 7].

Liposomes are artificial globular shape structures which may be bilayer or multilayer. Bilayers are formed by lipids with amphipathic properties. Lecithin and cholesterol are the common components used in liposome formation. Recently many researchers have proven that liposomes provide better solutions specifically in the medical field as drug carriers, in gene therapy and many cosmetic and pharmaceutical applications. There are several methods available for the formation of liposomes; however the reverse phase evaporation method was used to achieve higher encapsulation efficiency of water soluble drugs [8].

Vitamin C (ascorbic acid) was selected as the model drug for encapsulation. Vitamin C is the most commonly used supplement all over the world, because it provides a wide range of health benefits. Due to the antioxidant and anti-inflammatory effects, ascorbic acid has potential to strengthen the immune system against foreign pathogens [9]. The main drawback is that only 7–8% of total intake is absorbed by the healthy human body [9]. Scientist revealed that high dose of vitamin C in blood has great effect for variety of illnesses. It is capable of reducing redness due to UV burning 50% faster than untreated areas. Psoriasis and eczema have also shown clinical improvement with vitamin C. In addition, vitamin C has been found to stimulate collagen synthesis and to reduce dark pigmentation of the skin (e.g., age spots). Thus, vitamin C is also considered as anti-aging ingredient [9, 10].

Materials and Method

All chemicals were purchased from BDH Chemicals Ltd, and all solvents were distilled before use. Melting points of compounds were determined by melting point apparatus (SMP1,

Stuart Scientific, UK) and the TLC was performed on silica gel 60F-254 (Merck) and visualized with UV light and anisaldehyde spray reagent. Infrared spectra were recorded on an IRPrestige-21 (P/N 206–72010) Shimadzu Fourier transforms infrared spectrophotometer. HPLC-HP 1100 series with C_{18} column was used for HPLC analysis.

Synthesis of Penta-O-acetyl-β-D-glucopyranoside

Anhydrous finely powdered sodium acetate (33.0 mmol) was added to acetic anhydride (277.0 mmol) in a 100 ml round bottom flask. The mixture was refluxed in a water bath at 90°C, for five minutes until nearly all the sodium acetate was dissolved and finely powdered D-glucose (27.0 mmol) was added. After 3 hours, it was poured into ice water with vigorous stirring. The mixture was allowed to stand for twenty minutes at room temperature, filtered and recrystallized from methylated spirit. Compound penta-O-acetyl-β-D-glucopyranoside was obtained as white cotton like solid. Yield was 32.00%. Its IR spectrum showed absorption peaks in the regions of 3000–2900 cm^{-1} (CH_2-CH bending), 1790 cm^{-1} (carbonyl) and 1490 cm^{-1} (C-O stretching).

Synthesis of β-Sitosteryl -2, 3, 4, 6-Tetra-O-Acetyl- β-D-Glucopyranoside

Penta-O-acetyl-β-D-glucose (2.2 mmol) and β-sitosterol (3.0 mmol) were dissolved in anhydrous dichloromethane (50.00 ml) and boron trifluoride diethyl etherate (0.5 ml) was added via syringe. This was stirred under nitrogen atmosphere and the system was sealed. The mixture was again stirred at room temperature for 24 h and formation of product was monitored by thin layer chromatography every three hours. After the 24 hour period elapsed, the mixture was diluted with chloroform (150.0 ml) and washed with saturated sodium bicarbonate solution (50.0 ml) followed by two portions of deionized water. The organic layer was separated and dried with magnesium sulfate. After the solvent was removed under reduced pressure, solid residue was used for column chromatography and final product was recrystallized with 95% methanol. β-sitosteryl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside was obtained as a white solid. Yield was 59%. IR spectrum showed absorption peaks in the regions of 3200–3550 cm^{-1} – (−OH group), 1790 cm^{-1} – (carbonyl), 1460 cm^{-1} – (−C−O stretching), 1225 cm^{-1} (C−H stretching) and 1110 cm^{-1} – (C−C bending).

Synthesis of β-Sitosteryl-β-D-Glucopyranoside

β-Sitosteryl-2, 3, 4, 6-tetra-O-acetyl-β-D-glucopyranoside (1.3×10^{-3} mmol) was added to a 100 ml round bottom flask which was under nitrogen atmosphere, 15% sodium methoxide (20.00 ml) was added to it and stirred 24 h. After completion of the reaction, mixture was quenched with 1 g of ice. The white residue obtained was recrystallized from acetone. Yield was 41%. IR spectrum showed absorption peaks in the regions of 3600–3400 cm^{-1}(-OH group), 2900–2850 cm^{-1} (aliphatic asymmetric C−H stretching), 900 cm^{-1}(out of plane C-H bending), 830–800 cm^{-1}(−C=C−H stretching).

Lyotropic Liquid Crystal Properties

A known amount of liquid crystal was placed on a glass side and a Known amount of solvent was carefully added to the liquid crystal and covered with a cover slip. The textural patterns

were observed under polarized light microscope at several concentrations by subjecting above prepared sample to both heating and cooling.

Measurement of CMC by UV-absorption Spectroscopic Method

A stock solution (0.025 mol dm^{-3}) of β-sitosteryl-β-D-glucopyranoside was prepared and diluted for measurements into a concentration range between (0.25–5.0) $\times$ 10^{-4} mol dm^{-3}. Then a saturated aqueous solution of iodine (1.50 cm^3) at 25°C was poured into each of marked and stoppered test tubes (10 cm^3) and varying amounts of stock solution were added and made up to a volume of 10.00 cm^3 with distilled water. UV spectra of all solutions were obtained from 250–500 nm at room temperature. The absorbance values at 2 wavelengths, 286.6 nm and 350.4 nm of I_2 in each solution were recorded and plotted to obtain the CMC.

Measurement of CMC by Turbidity Method

Turbidity of each solution was measured using a digital nephlo-turbidity meter in nephelometric turbidity units (NTU). The instrument was calibrated using standard solutions supplied with the instrument before taking measurements. A series of concentrations of β-sitosteryl-β-D-glucopyranoside was prepared by the dilution of each stock solution (2.00 mmol dm^{-3}) with distilled water. The concentrations of the diluted stock solutions ranged from 2.50×10^{-5} mol dm^{-3} to a maximum concentration of 1.00 $\times$ 10^{-3} mol dm^{-3}. Then turbidity of each solution was measured at room temperature in triplicate at 30 second intervals.

Preparation of Liposomes by Reverse Phase Evaporation

Pure or mixed lipids with or without glycolipids (10.0 mg) were placed in a 50 ml round bottom flask and dissolved in chloroform (15.0 ml). After that, solvent was removed under reduced pressure by a rotatory evaporator. The solidified lipids were purged with nitrogen and re-dissolved in diethyl ether. Ascorbic acid (8.2 mg) in deionized water was added and the mixture was purged again with nitrogen. Encapsulation efficiency (EE) and Drug loading (DL) were calculated using the equations given below. Standard curve was prepared for concentrations 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm using the High performance liquid chromatography (HPLC) instrument.

$$\mathrm{EE} = \left\{ \frac{[\text{Ascorbic}]\text{Initial} - [\text{Ascorbic}]\text{Supernatant}}{[\text{Ascorbic}]\text{Initial}} \right\} * 100$$

Table 1. Melting Point Data

Compound	Observed (°C)	Literature (°C)
β-D-Glucopyranoside	130–133	130–132
β –sitosterol [11]	136–140	137–140
β-sitosteryl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside	132–137	—
β-sitosteryl-β-D-glucopyranoside	311–319	314

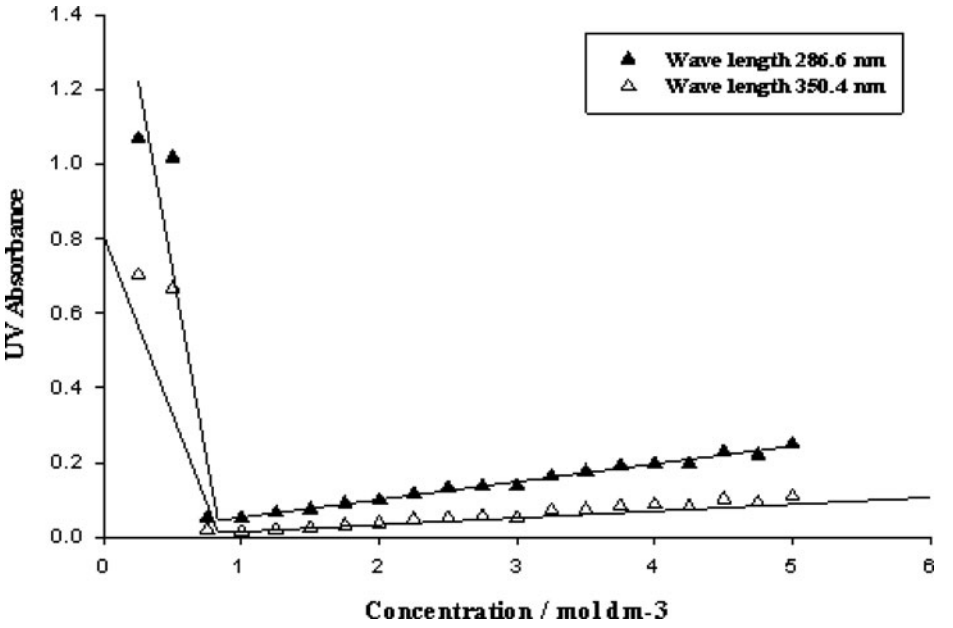

Figure 1. Determination of CMC value of β-sitosteryl-β-glucopyranoside by UV-Visible spectroscopy (286.6 nm and 350.4 nm), turning point gives the critical micelle concentration. Graph created by sigmaplot® 10, significant level $P < 0.05$.

$$\mathrm{DL} = \left\{ \frac{[\text{Ascorbic}]\text{Initial} - [\text{Ascorbic}]\text{Supernatant}}{\text{Weight of lipids}} \right\} * 100$$

Results and Discussion

The low product yield of the deacetylated compound ($41.0 \pm 0.2\%$) may be due to 2 main reasons. They are incomplete deacetylation or sensitivity of the glycosidic linkage to the basic conditions required for deacetylation.

Table 1 shows measured melting points of the glycolipid synthesized and intermediate compounds. The measured melting points of the intermediate compounds, tally well with literature values.

CMC value of 8.5×10^{-6} mol dm^{-3}obtained from both UV- absorption spectroscopic method (Fig. 1) and turbidity method (Fig. 2) are in the range for non-ionic surfactants. The CMC values obtained from both methods are summarized in Table 2. The HLB value of 6.24 obtained is compatible with values for nonionic surfactants and this indicates its potential application as a surfactant or as a wetting agent.

The values for critical micelle concentration determined by both UV-visible spectroscopy and turbidity measurements agrees well with each other.

HLB value for β-sitosteryl-β-D-glucopyranoside was calculated as,

$$\mathrm{HLB} = \left\{ \frac{\text{Mass of Hydrophilic portion}}{\text{Total mass of the molecule}} \right\} * 20$$

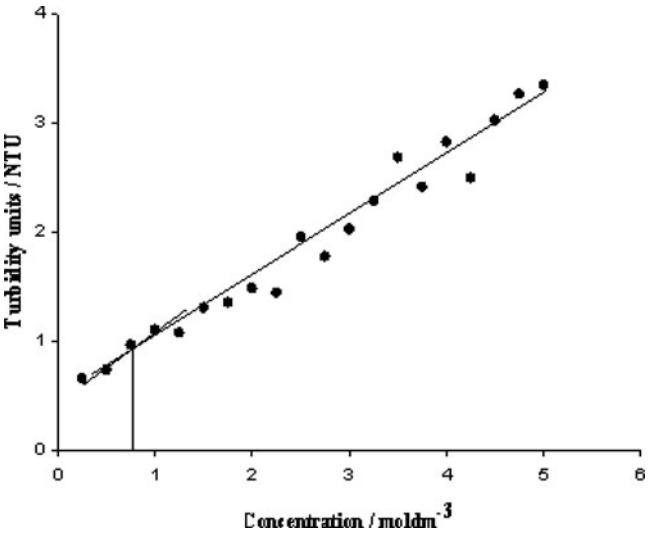

Figure 2. Determination of CMC value of β-sitosteryl-β-glucopyranoside by Turbidity units.

HLB for β-sitosteryl-β-D-glucopyranoside = 6.24

Lyotropic liquid crystal properties of deacetylated compounds (Fig. 3) were observed under polarized light microscope using dichloromethane, acetone, chloroform, ethyl acetate, methanol and water as solvents.

Hexagonal columnar lyotropic liquid crystal properties were clearly observed in polar solvents such as water, methanol and mildly polar solvents such as ethyl acetate (Fig. 3F, E, and D). Deacetylated compound dissolves in polar solvents and quickly forms anisotropic solutions. In polar solvents, the polar head (glucose moiety) of the deacetylated compound orients to the outside towards the solvent and β-sitosterol (the non polar tail) orients to the inside and form the micelles (Fig. 4 A, B) also mild temperature act as a driving force for arranging in micelles. When liquid crystal concentration reaches the CMC, micelles are formed. Above the CMC, it reaches a certain thermodynamically stable phase by converting to lamellar phase. In the lamellar phase liquid crystals arrange in closest packing, therefore, certain repulsions arise. Also density is a crucial factor for stabilizing the phase. However, due to the increase in density and repulsions, Gibbs free energy of lamellar phase increases and lamellar phase suddenly converts to the hexagonal column phase (Figure 3 D, E, F). Hexagonal columnar phase was observed in different solvents and at different temperatures.

In polar solvents, hexagonal columnar liquid crystal phase appears at different concentrations and different temperatures, (Water – (8.70% (w/w), 26°C) Methanol – (1.64%

Table 2. CMC values obtained by UV-VIS spectroscopy and turbidity measurements

Method	Wavelength/nm	Concentration/mol $dm^{-3} \times 10^{-6}$
UV – VIS	286.6	8.55
Spectroscopy	350.4	8.54
Turbidity	—	8.50

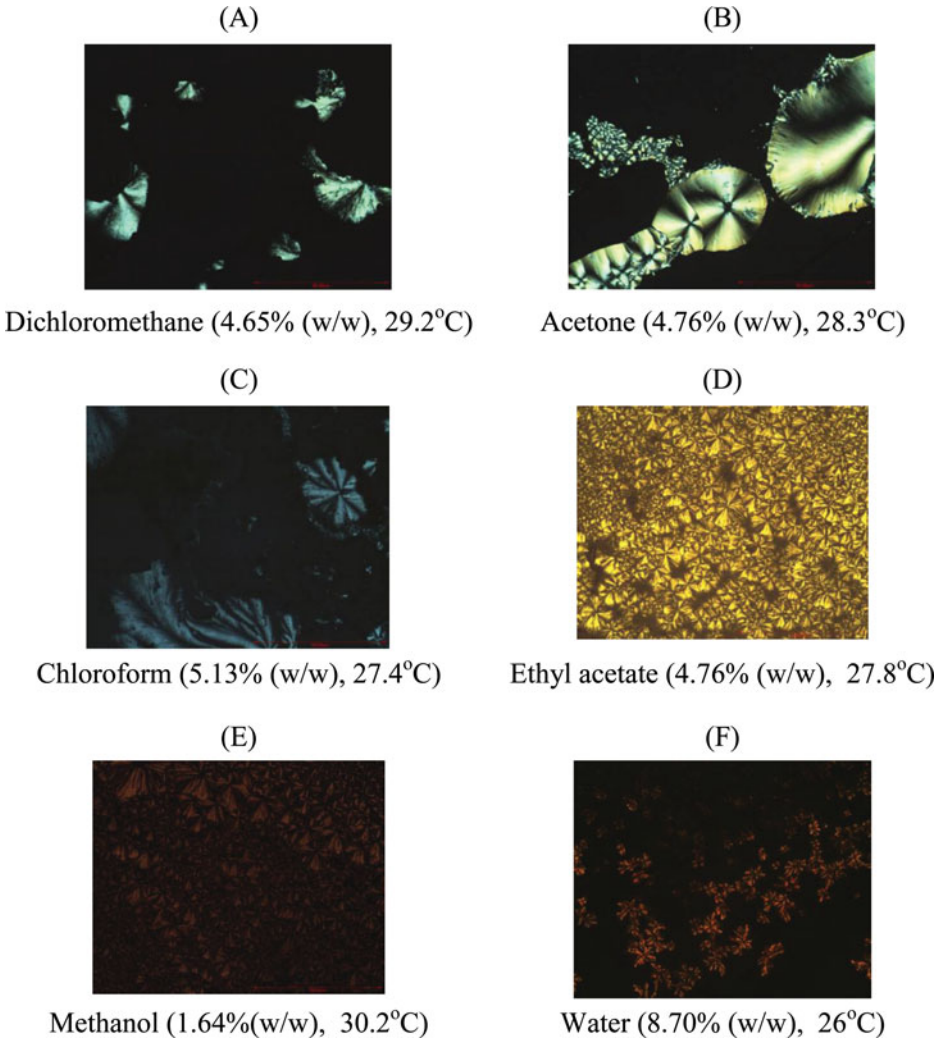

Figure 3. Optical polarizing micrographs of β-sitosteryl-β-D-glucopyranoside taken at different concentrations and different temperatures, "A to F" textures of β-sitosteryl-β-D-glucopyranoside in Dichloromethane, Acetone, chloroform, Ethyl acetate, Methanol and Water respectively.

(w/w), 30.2°C) Ethyl acetate – 4.76% (w/w), 27.8°C). Reason being the dependence on concentration and temperature. Deacetylated compound is a polar compound and very quickly makes hydrogen bonds with polar solvents; as a result, Gibbs free energy of hexagonal phase becomes comparatively lower than that in the lamellar phase. Therefore, hexagonal columnar phase is very stable and prominent. In water, a higher amount of deacetylated compound is required to form micelles at ambient temperature. However, in methanol, a lower amount of deacetylated compound is required to form micelles, 1.64% (w/w), and the required temperature is 30.2°C. A similar behavior was exhibited by the deacetylated compound in ethyl acetate. When deacetylated compound was dissolved in non-polar solvents such as dichloromethane, acetone, and chloroform hexagonal columnar liquid crystal mesophase was formed (Fig. 3A, B, C). In non polar solvents, glucose moiety

Table 3. Encapsulation efficiencies and drug loading capacities of selected liposomes and their compositions

	Composition ratios / mg					
No	Lec	LC	Chol	Beta	Encapsulation efficiency	Drug loading
1	8	0	0	2	24.70%	16.50 %
2	0	0	2	8	10.70%	7.10 %
3	2	0	0	8	33.20%	44.50 %
4	1	0	4	5	16.40%	10.90 %
5	8	1	1	0	63.00%	82.00 %
6	8	0	2	0	44.10%	78.00 %

Lec-Lecithin, LC- β-sitosteryl-β-D-glucopyranoside, Chol- Cholesterol, Beta- β-Sitosterol.

of deacetylated compound orients towards the inside and non-polar parts arrange towards the non-polar solvent (Fig. 4C, D). Therefore, these solvents facilitate formation of heterogeneous hexagonal columnar liquid crystal mesophase due to poor solubility in non-polar solvents. In non-polar solvents, polar heads of the deacetylated molecules oriented towards inside thereby, intra-molecular hydrogen bonding occurs as a result of strong ring structure appearing at the center. Number of molecules participating to form this structure depends on the density and bulkiness of the molecule; therefore, few deacetylated molecules arrange to form this structure. But, non-polar part of the deacetylated compound is somewhat bulky and there is a tendency to bend the molecules. Overall result is deacetylated molecules form thermodynamically stable intermediate state of hexagonal phase in the presence of non-polar solvents.

Liposomes consist of phospholipids and cholesterol in the lipid bilayer. Egg yolk lecithin was used as the source of phospholipids. A surfactant or co-surfactant may be added to improve the size/flexibility of the liposomes. Since the encapsulation efficiency and drug loading are dependent on the lipid composition, we aimed at replacing the surfactant with the synthesized compound and therefore formulated liposomes using lecithin, cholesterol, and β-sitosterol/β-sitosteryl-β-D-glucopyranoside.

Various combinations of lipids were used and the encapsulation efficiencies of ascorbic acid measured by HPLC are shown in Table 3. The combination no. 6 with Lecithin: cholesterol ratio of 8:2 shows an encapsulation efficiency of 44.1%, and loading capacity

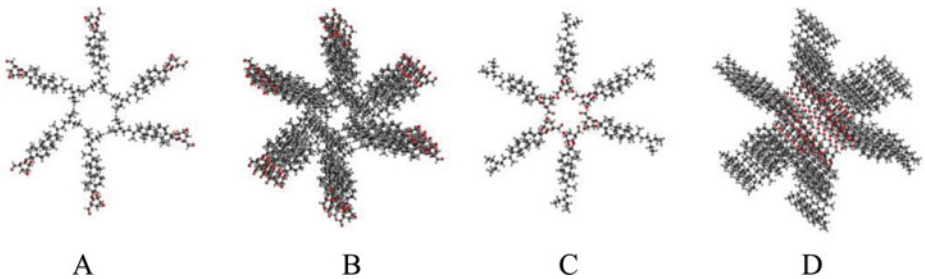

Figure 4. Hypothetical model of the arrangement of β-sitosteryl-β-D-glucopyranosidemolecules in polar solvents (A & B) and in non-polar solvents (C & D).

of 78% for ascorbic acid. However, in the presence of the glycolipid combination no. 5 with a ratio of Lecithin: cholesterol: β-sitosteryl-β-D-glucopyranoside of 8:1:1, the encapsulation efficiency of ascorbic acid was increased to 63.0%. Also, our results clearly show the increase in loading capacity when LC is incorporated to the formulation with the highest loading capacity of 82%. From the calculated HLB value and the observed CMC value, it is evident that β-sitosteryl-β-D-glucopyranoside functions as a co-surfactant, and helps to improve drug loading.

Conclusion

The compound β-sitosteryl-β-D-glucopyranoside clearly shows lyotropic liquid crystal properties in various solvents such as water, methanol, ethyl acetate, acetone, chloroform and dichloromethane, which is an added advantage for the spontaneous self-assembly of molecules. These properties are different to those observed for the acetylated precursor β-sitosteryl-2, 3, 4, 6-tetra-O-acetyl-β-D-glucopyranoside. The HLB value of β-Sitosteryl-β-D-glucopyranoside shows its potential application as a W/O emulsifier and as an antifoaming agent. The CMC value of 8.5×10^{-6} mol dm^{-3} further suggests that it can be used for W/O emulsion as a co-surfactant. Incorporation of ascorbic acid in liposomes can be enhanced by adding β-sitosteryl-β-D-glucopyranoside to the liposome.

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References

[1] Milkereit, G., Garamus, V.M., Yamashita, J., Hato, M., Morr, M., & Vill, V. (2005). *J. Phys. Chem.*, *109*, 1599.
[2] Smits, E., Engberts, J.B.F.N., Kellogg, R.M., & Doren, H.A.V. (1996). *J. Chem. Soc., Perkin Trans*, *1*, 2873.
[3] Razafindralambo, H. et al. (2012). Carbohydrate-Based Surfactants: Structure-Activity Relationships, In: *Advance in Chemical Engineering,* Nawaz, Z. and Navved, S. (Eds.), Chapter 8, InTech: Europe, 215.
[4] Kitamoto, D., Morita, T., Fukuoka, T., Konishi, M., & Imura, T. (2009). *Current Opinion in Colloid & Interface Science*, *14*, 315.
[5] Jin, X., Streett, D.A., Dunlap, C.A., & Lyn, M.E. (2008). *Biological Control*, *46*, 226.
[6] Somaratne, K., Perera, A.D.L.C., & Karunaratne, D.N. (2008). Synthesis of Glucopyranoside Carbohydrate Derived Liquid Crystal. *Proc. of SLASS*, 64.
[7] Vill, V., Minden, H.M.V., Koch, M.H.J., Seydel, U., & Brandenburg, K. (2000). *Chemistry and Physics of Lipids*, *104*, 75.
[8] Pidgeon, C., & McNeely, S. (1987). *Biochemistry*, *26*, 17.
[9] Liposomal vitamin C, The Trojan horse of supplement delivery, Sure Screen Life Sciences, UK.
[10] Lautenschläger, H.H. Medical Beauty Forum (1)14 and (2)16.
[11] Arora, M., & Kalia, A.N. (2013). *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*, *1*, 245.