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Optimization of a liposomal delivery system for the highly antioxidant methanol extract of stem-bark of *Schumacheria castaneifolia* Vahl

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ABSTRACT

The highly antioxidant methanol extract of stem-bark of Schumacheria castaneifolia was studied for the effect of variation of the cholesterol content on the properties of liposomes. Liposomal formulations displayed colloidal dimensions and negative zeta-potentials; and the stability varied with the lipid composition. The liposomes consisting of 9:1 and 8:2 ratio of egg yolk phosphatidylcholine to cholesterol were optimal for the highest encapsulation efficiency (86%) and loading capacity (8%) of the plant extract. The release properties of plant extract encapsulated liposomes depended on the lipid composition. Liposomal plant extract exhibited slow release over 7 h with slower release in artificial sweat than in phosphate buffered saline or deionized water. Interactions between phospholipids and cholesterol, and polyphenols of the plant extract and phospholipids appear to affect the properties of liposomes.

Key words: egg yolk phosphatidylcholine, cholesterol, encapsulation, slow release, plant extract

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INTRODUCTION

Sri Lanka is a biological hotspot consisting of flora with a high degree of potent biological activity[1,2]. Significantly, out of the 3210 flowering plants, 916 are endemic[3]. Among the non-flowering plants such as lichens endemicity and biological activity are being unraveled[4,5], highlighting the importance of evaluating their pharmaceutical potential. The endemic plant *Schumacheria castenifolia* Vahl, belonging to the genus *Schumacheria*, exhibited exceptionally high antioxidant properties[6] and moderate antimicrobial properties[7] showing its potential in cosmetics/pharmaceutical applications. The presence of the polyphenols: quercetin, kaempferol, quercetin 3-sulphate, kaempferol 3-sulphate, kaempferol 3,7-disulphate, prodelphinidin and procyanidin in *S. castaneifolia*, all of which may function as antioxidants soluble in methanol, was reported in 1981[8]. Accordingly, recent studies on the methanol extract of stem-bark of *S. castaneifolia* have shown the presence of very high polyphenol content. An advantage of the extract is its insignificant cytotoxicity[6] which favors its use in pharmaceutical, cosmetic or food industries.

Here we chose liposomal encapsulation which has been used previously as a means of enhancing skin delivery of plant-derived drugs. For example, colchicines possessing antigout properties show increased skin accumulation while catechins possessing antioxidant and anticancer properties show increased skin permeation when encapsulated in liposomes[9,10]. The cosmetic industry employs numerous plant extracts encapsulated in liposomes, notably liposomal extracts of papaya and carrot containing β-carotene[11]. In addition, numerous other benefits such as

improving bioavailability, facilitating specific-targeting, reducing dose, and/or reducing dosing frequency of encapsulated material have been reported[12-14]. Thus, we report herein the encapsulation of the methanol extract of stem-bark of *S. castaneifolia* in liposomes with a view to studying its efficacy and release.

Thus, this study was designed to evaluate the effect of lipid composition on properties such as encapsulation efficiency, loading capacity, size, zeta-potential, stability, and release, and to investigate the effect of release medium on the slow and sustained-release properties of liposomes encapsulating the methanol extract of stembark of *S. castaneifolia*. The results of this study should be useful mainly in modulating properties of liposomes encapsulating plant material.

EXPERIMENTAL SECTION

Materials

Egg yolk phosphatidylcholine (PC) (~ 60% TLC) and cholesterol (CH) (assay > 98%) were purchased from Sigma-Aldrich. Sucrose (Extra Pure) and diethyl ether were from LOBA Chemie PVT. LTD., India. Dicloromethane, ethanol and methanol were from Sigma and other chemicals were of analytical grade. Dialysis tubing (12 400 Da MWCO) was from Sigma-Aldrich while Sephadex G50 was from GE healthcare. Deionized water filtered through a 0.2 µm filter was used for all experiments.

Methods

Collection and extraction of *S. castaneifolia* methanol extract

Plant materials of *S. castanei folia* were collected from Thummodara region in Ratnapura, Sri Lanka. The specimen was identified by Dr. Siril Wijesundara of the Royal Botanic Gardens, Peradeniya, Sri Lanka, where a sample specimen has been deposited. The stem-bark was air dried and ground into particles and sequentially extracted into dichloromethane and methanol at room temperature using a bottle shaker and the extract was evaporated under reduced pressure using a rotary evaporator at room temperature.

Evaluation of antioxidant activity

The antioxidant activity of the methanol extract of stem-bark of *S. castaneifolia* was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay and was expressed as IC_{50} values[15]. The final concentration of DPPH in the test mixture was maintained at 1×10^{-4} mol dm^{-3} in methanol. As the positive control, α-tocopherol was used. The IC_{50} values were determined using a concentration series. After 30 min, the absorbance of the test mixture was measured at 517 nm, using a spectrophotometer (Shimadzu, UV-1800); all the tests were carried out in triplicate.

The following equation was used to calculate percent antioxidant activity:

$$Percent\ antioxidant\ activity\ = \frac{A_i\ - A_f}{A_i} \times 100$$

where, A_i is the initial absorbance of the test mixture and A_f is the final absorbance of the test mixture.

Preparation of liposomes

Free unloaded liposomes were prepared following the reverse phase evaporation method[16]. First, an emulsion was made using diethyl ether (8 ml) in which the lipids (100 mg) were dissolved, and to this mixture water (2.4 ml) was added. The ratios of PC to CH used to prepare liposomes were: 10:0, 9:1, 8:2, 7:3 and 6:4. Liposomes were obtained by evaporation of the organic phase. The total volume of each liposome solution was adjusted to 20 ml using deionized water which was then sonicated.

Using the method described above, the plant material was encapsulated in liposomes, where an aqueous solution (2.4 ml) of plant extract was used in place of water. Unencapsulated plant material was separated from the loaded liposomes either by centrifugation or gel-filtration. Separately, plant extract loaded liposomes were prepared in the presence of 2.5 % (w/v) sucrose using the same lipid ratios as above. Preparation of each liposomal formulation was carried out in triplicate.

Determination of particle size and zeta-potential

Particle sizes of liposomes were determined using a Malvern zetasizer NanoZS (Malvern instruments, UK), fitted with a red laser of 633 nm, which operates on the dynamic light scattering technique. The liposomal suspensions

were diluted in deionized water, filtered with a 0.2 µm filter, and the scattering intensity was measured at an angle of 173˚ relative to the incident radiation after equilibrating the samples at 25 ˚C. The values reported are the z-average diameters of liposomes. Zeta-potentials of the same liposomal suspensions described above were measured using a Malvern zetasizer which operates on the Laser Doppler Electrophoresis technique. The values reported are the z-average zeta-potentials of liposomes. In order to determine the stability variation with time, the liposomal suspensions were stored at 4 ˚C for 2 months and the particle size and zeta-potential were measured after each month. The concentration of lipids in solution during storage was 5 mg / ml for all liposomal formulations.

Determination of loading capacity (LC)
Purified loaded liposomes were freeze dried and 5.0 mg of dry liposomes were disrupted using a mixture of ethanol and methanol (75:25). This procedure was repeated using purified unloaded liposomes.

The loading capacity was determined spectrophotometrically by measuring the absorbance of disrupted plant extract encapsulated liposomes against disrupted unloaded liposomes at 224 nm.

The formula used to calculate the loading capacity is given below:

$$LC = \frac{Mass\ of\ encapsulated\ plant\ extract}{Mass\ of\ plant\ extract\ encapsulated\ liposomes} \times 100$$

Determination of encapsulation efficiency (EE)
The encapsulation efficiency determined spectrophotometrically was calculated using the formula given below:

$$EE = \frac{Mass\ of\ encapsulated\ plant\ extract}{Mass\ of\ plant\ extract\ initially\ introduced} \times 100$$

In vitro release study
Liposomes of five different lipid compositions were used for release studies carried out using the dialysis bag method in deionized water. Release from liposomes with PC : CH ratio of 9:1was performed in three different media: deionized water, phosphate buffered saline (pH 7.4) and artificial sweat (pH 4.7). Aliquots withdrawn from the release medium at predetermined time intervals over a period of 7 h were replenished immediately with fresh medium. The released plant extract was quantified spectrophotometrically by measuring absorbance at 210 nm- the most prominent peak of the plant extract absorbing UV radiation.

Statistical analysis
All data are presented as mean ± standard deviation (S.D.) of three parallel experiments (n = 3). Microsoft Office Excel 2007 was used for the above calculations. One way ANOVA was conducted using MINITAB 14 software to compare the results and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Antioxidant activity
The antioxidant activity of the methanol extract of stem-bark of *S. castaneifolia* (IC_{50} 9.8 ± 0.3) was comparable to that of *dl*-α-tocopherol (IC_{50} 10.9 ± 4.3) which is a highly potent antioxidant. Methanol extracts of plants possessing high polyphenol content usually exhibit high antioxidant activity due to their affinity for scavenging free radicals. Accordingly, this plant extract, which has a total polyphenol content of 68.5 mg/g of gallic acid equivalents in dry plant material[7], exhibited very high antioxidant activity in the DPPH radical scavenging assay. The high antioxidant activity observed may be due to synergistic interactions among polyphenols of the extract.

Encapsulation efficiency and loading capacity
The encapsulation efficiency and loading capacity were determined by using a spectrophotometric method and the values are shown in Table1.

Table 1. Encapsulation efficiencies and loading capacities of liposomal formulations of different lipid compositions (each value is the mean of values obtained from three independent trials ± S.D.)

Liposomal formulation PC:CH	Lyoprotectant	Loading capacity (%)	Encapsulation efficiency (%)
10:0	x	6.26 ± 0.03	66.81 ± 0.38
	✓	6.73 ± 0.07	
9:1	x	7.88 ± 0.02	85.59 ± 0.26
	✓	7.99 ± 0.02	
8:2	x	7.95 ±0.02	86.41 ± 0.25
	✓	8.04 ± 0.03	
7:3	x	5.58 ± 0.03	59.05 ± 0.32
	✓	5.21 ± 0.03	
6:4	x	4.27 ± 0.03	44.55 ± 0.29
	✓	4.48 ± 0.04	

Our results indicated that the encapsulation efficiency and loading capacity of conventional liposomes containing the methanol extract of stembark of *S. castaneifolia* are dependent on the lipid composition. Increasing the percentage of cholesterol from 0 % to 10 %, increased the encapsulation efficiency from 66.81 % to 85.59 %. However, when the percentage of cholesterol was increased from 10% to 20 %, the encapsulation efficiency increased only marginally. The encapsulation efficiency decreased drastically from 86.41% to 44.55% when the percentage of cholesterol was further increased. Thus, the optimum ratios of phosphatidylcholine to cholesterol for the encapsulation of the highly antioxidant methanol extract of stem-bark of *S castaneifolia* are 9:1 and 8:2.

The increase in size of liposomes upon incorporation of cholesterol may have contributed to the observed increase of encapsulation efficiency and loading capacity of liposomes with increasing cholesterol content. Since the methanol extract of *S. castaneifolia* is highly soluble in hot water, it is expected to reside mainly in the aqueous interior of liposomes. Therefore, an increase in the size of liposomes may correspond to an increase in the encapsulation efficiency and loading capacity. Interestingly, our results are consistent with the results of Cagdas and coworkers demonstrating that incorporation of cholesterol in the lipid bilayer is essential for successful encapsulation of water soluble species in liposomes[17]. The results of this study indicate that the encapsulation of water-soluble plant material may be increased by incorporating cholesterol in the lipid bilayer of phosphatidylcholine liposomes.

The variation of loading capacity with the cholesterol content exhibited a similar pattern to that of encapsulation efficiency. Therefore, according to the loading capacity too, the optimum ratios of PC to CH are 9:1 and 8:2. Loading capacity of liposomes prepared in the absence of the lyoprotectant, sucrose, ranged from 4.2 % to 8.0 % while that of liposomes prepared in the presence of sucrose ranged from 4.5 % to 8.0 %, showing that the presence of lyoprotectants has no significant effect on the loading capacities of the extract encapsulated liposomes. Thus, lyoprotectants may be utilized to improve the stability of plant extract loaded liposomes upon lyophilization for storage.

Particle size and zeta-potential

In this study, the effect of cholesterol on the size and zeta-potential of unloaded liposomes and plant extract encapsulated liposomes was evaluated. Unloaded liposomes and plant extract encapsulated liposomes were compared to evaluate the effect of encapsulated plant materials on the properties of liposomes. The measurements on variation of size and zeta-potential with time of all liposomal formulations were performed immediately after preparation, after one month, and after 2 months.

Variation of particle size with cholesterol content

Fresh unloaded liposomes had hydrodynamic diameters ranging from 150 nm to 270 nm as shown in Table 2. The size increased upon increasing the cholesterol content from 0 % to 20 %. A decrease in the curvature of the bilayer due to cholesterol incorporation may be the cause for the observed increase in size. Further increase in cholesterol content decreased the size. The apparent decrease in the average size of liposomes can be attributed to the incompatibility of high cholesterol content in the lipid bilayer, which may have caused cholesterol to form other structures with smaller sizes. The hydrodynamic diameters measured after one month and those measured after two months exhibited a similar variation with cholesterol content as the unloaded freshly prepared liposomes (Table 2).

Table 2.The variation of diameter and zeta-potential of unloaded liposomes of different lipid compositions over a period of 2 months. (Each value if the mean of three independent trials ± S.D. In each column, statistically different values are indicated by different superscripts and vice versa)

Liposomal formulation PC:CH	Diameter ± S.D (nm)			Zeta-potential ± S.D (mV)		
	Fresh	1 month	2 months	Fresh	1 month	2 months
10:0	194.5 ± 45.0^{a}	138.1 ± 5.6^{a}	149.3 ± 7.2^{a}	-31.6 ± 6.1^{a}	-54.2 ± 14.8^{a}	-52.9 ± 4.4^{a}
9:1	264.3 ± 60.3^{a}	174.5 ± 56.5^{a}	323.9 ± 91.3^{a,b}	-43.0 ± 2.6^{a,b}	-64.4 ± 4.1^{a}	-58.3 ±1.6^{a,b}
8:2	272.7 ± 22.1^{a}	231.6 ± 47.2^{a}	434.7 ± 196.7^{b}	-42.8 ± 6.9^{a,b}	-52.6 ± 0.6^{a}	-61.9 ± 3.5^{b}
7:3	263.7 ± 54.6^{a}	203.5 ± 48.2^{a}	263.5 ± 80.5^{a,b}	-45.2 ± 3.7^{b}	-61.7 ± 3.4^{a}	-60.7 ± 2.4^{b}
6:4	154.0 ± 18.8^{a}	196.4 ± 80.5^{a}	198.8 ± 45.6^{a,b}	-35.2 ± 0.4^{a,b}	-60.9 ± 3.7^{a}	-65.0 ± 2.0^{b}

Like in the case of unloaded liposomes, an increase in the size to reach a maximum followed by a drastic decrease in size was observed with fresh liposomes encapsulating plant extract (loaded liposomes), upon increasing the cholesterol content (Table 3). As explained previously, the initial increase of size may be due to the decrease in the curvature of the bilayers while the subsequent decrease of average size may be due to the formation of cholesterol rich structures with low hydrodynamic diameters. These results indicate that the effect of cholesterol on the size of unloaded liposomes and loaded liposomes is similar. In fact, the effect of cholesterol on size was more evident in loaded liposomes. Also, one month old liposomes and two month old liposomes showed a gradual increase in size with increasing cholesterol content.

Table 3.The variation of diameter and zeta-potential of plant extract containing liposomes of different lipid compositions over a period of 2 months. (each value is the mean of three independent trials ± standard deviation. In each column, statistically different values are indicated by different superscripts and vice versa)

Liposomal formulation PC:CH	Diameter ± S.D (nm)			Zeta-potential ± S.D (mV)		
	Fresh	1 month	2 months	Fresh	1 month	2 months
10:0	168.2 ± 7.9^{a,b}	149.2 ± 32.4^{a}	151.4 ± 16.4^{a}	-58.2 ± 4.3^{a}	-45.0 ± 7.1^{a}	-38.7 ± 8.9^{a}
9:1	189.5 ± 8.4^{a,b}	179.5 ± 24.6^{a}	175.1 ± 6.8^{a}	-69.0 ± 9.1^{a}	-51.7 ± 7.1^{a}	-47.0 ± 2.9^{a,b}
8:2	206.9 ± 6.9^{a,b}	208.5 ± 30.8^{a}	239.7 ± 20.5^{b}	-65.3 ± 3.8^{a}	-60.1 ±0.1^{a}	-49.5±6.8^{a,b}
7:3	228.1 ± 36.2^{a}	319.5 ± 14.4^{b}	376.5 ± 14.1^{c}	-65.2 ± 0.6^{a}	-58.5 ± 3.7^{a}	-53.4 ± 3.2^{a,b}
6:4	156.8 ± 37.5^{b}	364.8 ± 71.7^{b}	455.7 ± 35.7^{d}	-65.8 ± 4.5^{a}	-54.4 ± 5.5^{a}	-54.1 ± 5.4^{b}

Thus, the size of plant extract loaded liposomes may be modulated by varying the lipid composition, specifically the ratio of phosphatidylcholine to cholesterol. Since the size affects other properties of liposomes, including encapsulation efficiency, release kinetics, skin permeation and biodistribution,[18] size modulation of loaded liposomes may enable fine tuning of liposomes with respect to other properties.

Variation of zeta-potential with cholesterol content

The average zeta-potentials of fresh unloaded liposomes ranged between -32.0 mV and -45.0 mV (Table 2). These results indicate that unloaded liposomes, stable in solution, can be prepared using PC and CH in the given ratios. However, it was expected that liposomes with zeta-potentials close to zero would form since both phosphatidylcholine and cholesterol are neutral at physiological pH[18]. Negative zeta-potentials observed in this study may be due to the presence of other negatively charged phospholipids such as phosphatidic acid, phosphatidylserine and phosphatidylinositol, in addition to neutral phosphatidylcholine, in the phospholipids that was used for the preparation of liposomes. Besides, PC used in this study was only of 60 % purity. Zeta-potentials gradually became more negative with increasing cholesterol content. This information suggests that interactions between PC and CH favour the phosphate group of phosphatidylcholine to be exposed to the external environment, under the conditions used to measure the zeta-potential. Indeed, this observation is consistent with the report by Xia and Xu who suggested that the hydroxyl group of CH may interact with the choline group of PC, thereby exposing the phosphate group of PC to the external environment[19]. Although zeta-potentials of unloaded liposomes gradually decreased to reach a minimum upon increasing the cholesterol content, further increase of the cholesterol content resulted in a slight increase of the zeta-potential. Formation of cholesterol rich structures with less negative or zero zeta-potential may be the cause for the observed increase of the zeta-potential. Although one month old liposomes did not show variation of zeta-potential with cholesterol, two month old liposomes, stable in solution, demonstrated the ability of cholesterol to favour orientation of the phosphate group of phosphatidylcholine to the external environment probably via hydrogen bonding with the choline group.

The zeta-potentials of fresh loaded liposomes were not significantly different from each other (Table 3). The same was true for zeta-potentials of liposomes remaining in suspension after one month of preparation. However, the zeta-

potentials of loaded liposomes remaining in suspension after two months of became gradually more negative with increasing cholesterol content. This variation of zeta-potential may be due to the interactions of cholesterol and choline group of phosphatidylcholine to expose the phosphate group to the external solution, as explained previously. Basically, zeta-potentials of plant material encapsulated liposomes indicate that encapsulated liposomes stable in solution can be prepared using phosphatidylcholine and cholesterol.

Variation of particle size with time

The stability of unloaded liposomes in solution was investigated by measuring the size and zeta-potential with time for a period of two months (Table 2). All liposomal formulations showed precipitation of lipidic structures with time, most probably due to fusion and / or aggregation of those structures. Table 2 gives the sizes and zeta-potentials of liposomes remaining in the suspension. Unloaded liposomes did not show a significant variation of size with time. Thus, our results indicate that unloaded liposomes stable over a period of 2 months may be prepared using phosphatidylcholine and cholesterol.

All five different formulations of plant extract-loaded liposomes showed aggregation and precipitation with time. The degree of precipitation increased with increasing cholesterol content. Table 3 gives the sizes of liposomes remaining in the suspension. Although loaded liposomes with low cholesterol contents exhibited stability in size with time, those with high cholesterol contents exhibited an increase in size with time. This suggests that high cholesterol contents in plant extract loaded liposomes favor aggregation and / or fusion of liposomes.

These results indicate that only loaded liposomes with low cholesterol contents remain stable with respect to size with time, and that, interactions of polyphenols of the plant extract and polar lipids in the liposomes may lead to aggregation and/or fusion of loaded liposomes.

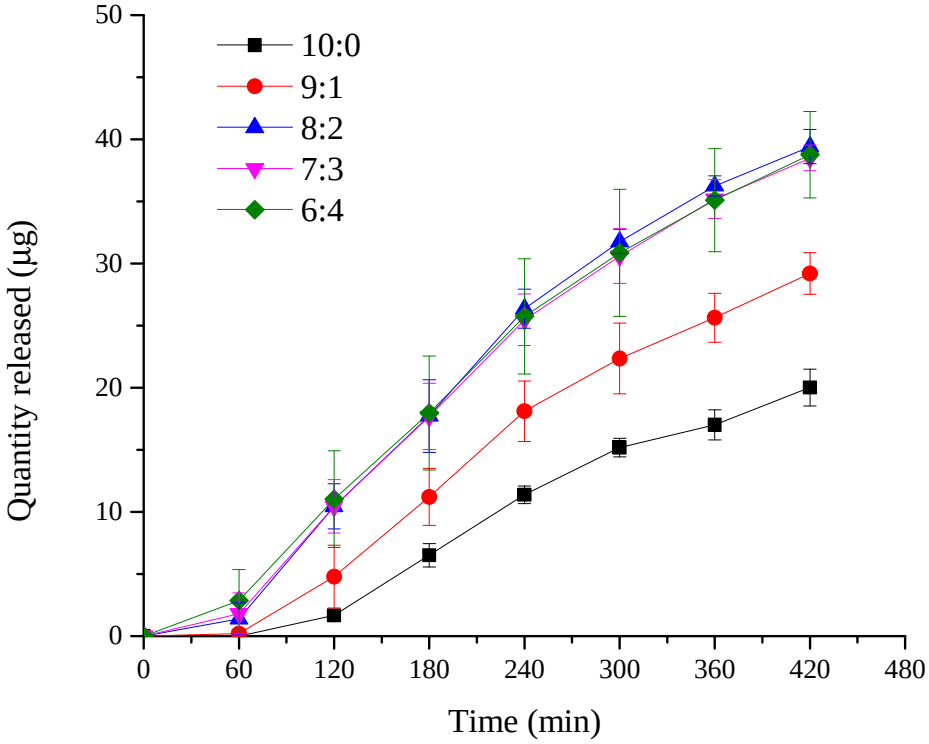

Figure 1. Release profiles of *S. castaneifolia* methanol extract from different liposomal formulations

Variation of zeta-potential with time

The average zeta-potentials of unloaded liposomes became more negative one month and two months after preparation (Table 2).This decrease in zeta-potential may be the result of the removal of less stable liposomes from solution over time. Further, this result indicates that aggregation and subsequent removal of liposomes with less negative zeta-potentials from the suspension occur mainly during the first month of storage, after which the liposomes suspending in solution remain stable over a period of two months.

In contrast to unloaded liposomes, loaded liposomes became less negative with time (see Table 3). However, precipitation of lipidic structures, most probably with less negative zeta-potentials with time, was observed even with loaded liposomes. Thus, it was expected that the average zeta-potentials would become more negative with time. This anomaly may be due to leakage of small quantities of encapsulated plant extract with time. Encapsulated polyphenols, upon leakage, have the potential to interact with phosphate groups of phosphatidylcholine through hydrogen bonding, thus shielding the negative charge of phosphatidylcholine leading to liposomes with less negative zeta-potentials which gives rise to increased aggregation.

Basically, plant extract encapsulated liposomes with low cholesterol contents are more stable than those with high cholesterol contents. However, storage of loaded liposomes in solution leads to changes in the size and zeta-potential, and thus, measures must be taken to increase the stability of those liposomes. For instance, the size of the liposomes could be maintained by incorporating liposomes in hydrogels made of carbomer[20]. Thus, incorporation of plant extract encapsulated liposomes in a gel may increase the stability.

***In vitro* release studies**

The effect of cholesterol on sustainedrelease properties of liposomes encapsulating the methanol extract of stembark of *S. castaneifolia* was evaluated using deionized water as the release medium.

Our results reveal that the release of the encapsulated plant material from liposomes made of phosphatidylcholine and cholesterol is dependent on the lipid composition of liposomes. In fact, the liposomes became progressively leaky as the amount of cholesterol was increased from 0 % (w/w) to 20 % (w/w) beyond which the release profiles overlapped. This observation is consistent with the literature showing the dependence of release of entrapped species on the lipid composition of liposomes[21]. Lipid packing and fluidity of membranes depend on the cholesterol content. Therefore, incorporation of cholesterol affects the permeability of the encapsulated material from liposomes[22]. Raffy and Teissie suggested that cholesterol increases the ordering of lipids in the membrane, thus making the membrane less leaky[23]. However, Xiang and Anderson reported that incorporation of cholesterol in phospholipid bilayers, depending on the amount incorporated, may sometimes increase the permeability of the encapsulants[24]. We observed increased release of plant extract upon increasing the cholesterol content in the lipid bilayer. The differences among loaded liposomes in this study include differences of size and loading capacity in addition to the difference of cholesterol content. These factors also may affect the release kinetics of encapsulated material from liposomes. In addition to size and loading capacity, the amount of phospholipids was different among the five liposomal formulations. Thus, as stated previously, phospholipids present in egg yolk could interact with encapsulated polyphenols through hydrogen bonding, thus retarding their release. The content of phospholipids decreases with increasing cholesterol content, resulting in a decrease of the amount of interactions between phospholipids and encapsulated polyphenols. This may be the cause for the observed increase in release of encapsulated material with the cholesterol content. In sum, the observed variation of release profiles of different liposomal formulations may most probably be due to a combined effect of size, encapsulated amount and lipid composition of loaded liposomes.

Release studies were conducted in deionized water and in two physiologically relevant buffer systems - PBS (pH 7.4) and artificial sweat (pH4.7) - in order to study the effect of media on the release kinetics of loaded liposomes. Liposomes with PC to CH ratio of 9:1, which showed intermediate release, were selected for the study.

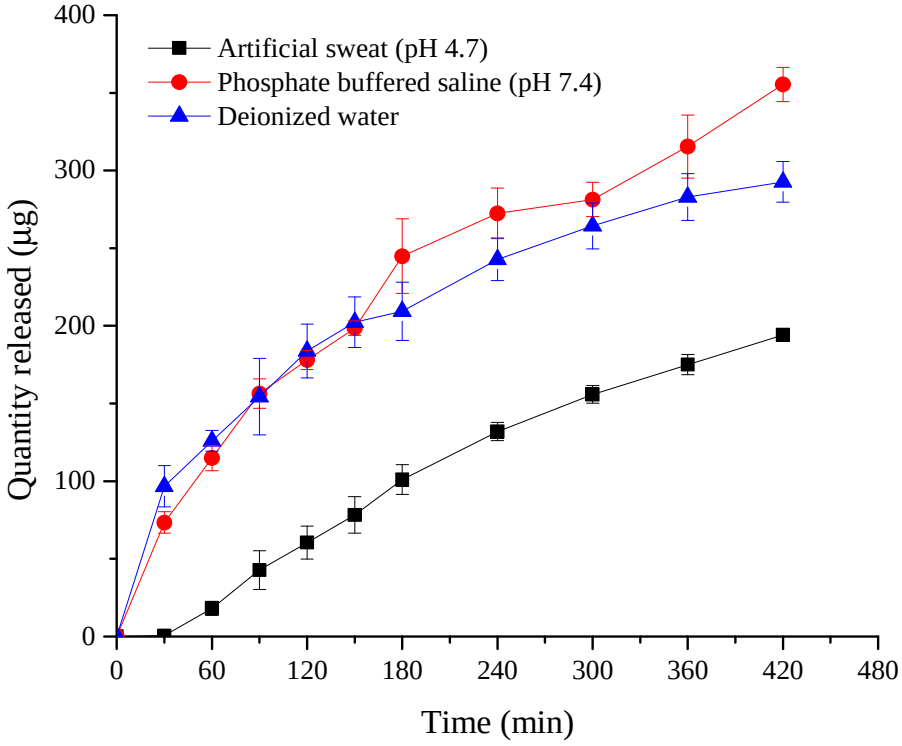

Figure 2. Release profiles of *S. castaneifolia* from liposomal formulation PC:CH - 9:1 in different media

As depicted in Figure 2, slow and sustained release of encapsulated material from liposomes occurred upto 7 h in all types of media used, beyond which release was not monitored. The release profile of the plant extract from the liposomes in deionized water and in PBS which was of higher ionic strength overlapped. This suggests that the effect of salt concentration and hence that of ionic strength on the release kinetics of chemical constituents of the methanol extract of stem-bark of *S. castaneifolia* from liposomes is negligible at least up to the ionic strength of PBS. However, the release profile of plant extract in artificial sweat was significantly different from those of above in that it was much slower.The lower pH of artificial sweat compared to other two media may be one reason for this difference. In a medium of pH as low as 4.7, a greater percentage of phenolic groups will remain protonated (un-ionized). This protonation will lead to lower solubility of polyphenols in artificial sweat than in aqueous component of liposomes. Thus, liposomal polyphenols will tend to remain in the vesicles rather than to leak out.

In addition to differences in release kinetics, we observed aggregation of liposomes during release studies in artificial sweat, which was not observed in deionized water or in PBS. The much higher ionic strength of artificial sweat than that of PBS may have contributed to the aggregation observed in artificial sweat. Indeed, it has been reported that higher concentrations of salts result in faster aggregation of liposomes[25].

These results indicate that this liposomal formulation may be used for slow and sustained release of the chemical constituents of the methanol extract of stem-bark of *S.castaneifolia* in physiological buffers. Moreover, if loaded liposomes are incorporated in topical formulations, only a small quantity of the extract will release to sweat, as indicated by the release profile in artificial sweat. However, upon penetration of liposomes through the stratum corneum to deeper layers of the skin, the extract will release faster, as indicated by the relative position of release profile in PBS. This shows the applicability of the loaded liposomes in topical applications.

CONCLUSION

The highly antioxidant methanol extract of stembark of *S. castaneifolia* was successfully encapsulated in conventional liposomes made of phosphatidylcholine (assay approx. 60 %) and cholesterol. The optimum ratios of phosphatidylcholine to cholesterol were 9:1 and 8:2 (w/w) in terms of encapsulation efficiency and loading capacity. The presence of sucrose - a lyoprotectant - does not affect the loading capacity of plant extract encapsulated liposomes.

The change in the lipid composition had mixed results on the properties of liposomes. When unloaded liposomes were examined, the change in lipid composition towards an increase in cholesterol content had minimum effect on the stability and aggregation properties of the liposomes. In addition, the size of liposomes did not show significant variation. However, loading the *S. castaneifolia* extract induced pronounced changes to the size, charge and stability with time as well as the release profile of the loaded liposomes. Thus, loaded liposomes should be either lyophilized or incorporated in a viscous medium such as a gel as means of storage in order to increase the stability of the liposomes.

Conventional liposomes encapsulating the methanol extract of stembark of *S. castaneifolia* can be used as a slow and sustained release system of the plant extract. The sustained release properties can be modulated by changing the lipid composition. Furthermore, release of encapsulated plant material depends of the medium to which the encapsulated material is released. In fact, release of encapsulated plant material in artificial sweat is much slower than in PBS or in deionized water. Thus, conventional liposomes may be suitable for topical formulations where sustained release of plant material is desired.

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