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Development of novel topical tranexamic acid liposome formulations

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Abstract

The aims of this study were to develop novel liposome formulations for tranexamic acid (TA) from various lipid compositions {neutral (hydrogenated soya phosphatidylcholine and cholesterol), positive (stearylamine) or negative (dicetyl phosphate) charged lipid}, and to investigate the effects of concentrations of TA (5 and 10% in DI water) and charges on the physicochemical properties of liposomes. Liposomes were prepared by chloroform film method with sonication. The physical (appearance, pH, size, morphology) and chemical (drug encapsulation efficiency, transition temperature, enthalpy of transition) properties of liposomes were characterized. The TA contents were determined spectrophotometrically at 415 nm, following derivatization with 2,4,6-trinitrobenzosulfonic acid. The charged liposomes demonstrated better physical stability than the neutral liposomes. The percentages of TA entrapped in all liposome formulations varied between 13.2 and 15.6%, and were independent of TA concentrations and charges of liposomes. Charges affected the physical stability, pH and size of liposomes. The particle sizes of negative blank and positive liposomes (with and without the entrapped drug) were ~ 10 times larger than the negative liposome with the entrapped TA. The multilamellar 7:2:1 molar ratio of hydrogenated soy phosphatidylcholine/cholesterol/dicetyl phosphate entrapped with 10% TA liposome (10%TA, −) was selected for further release study, due to its high physical stability, small particle size and relatively high drug encapsulation efficiency.

Keywords: Tranexamic acid; Topical liposomes; Hydrogenated soya phosphatidylcholine; Cholesterol; Dicetyl phosphate; Stearylamine

1. Introduction

Liposomes have been widely investigated for their properties as model membranes and potential drug delivery systems (Bangham et al., 1974; Gregoriadis, 1988; Lasic, 1992). They have become a valuable experimental and commercially

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important drug delivery system, owing to their biodegradability, biocompatibility, low toxicity and ability to entrap lipophilic and hydrophilic drugs. Unfavorable pharmacokinetic profile of a certain drug can be altered by entrapping the drug in liposomes (Weissmann et al., 1977; Lopez-Bersetin et al., 1984). The tissue distribution of drug in liposome formulations can be controlled, by varying the particle size and composition of lipid, as well as modifying the surface charges of liposomes. The application of liposomes has been extended to drug efficacy and potency studies (Mayer et al., 1993; Mayer and St-Onge, 1995; Gabizon et al., 1996; Jin-Chul et al., 1997), reduction of the toxicity of encapsulated drugs (Rahman et al., 1990; Bally et al., 1994; Jin-Chul et al., 1997), targeting to specific tissue sites (Li et al., 1992; Kawakami et al., 2001), control the timing and the amount of drug released (Soltan et al., 2000), and the enhancement of penetration of drug into the skin with the slow release and moisturizing effect (Mezei, 1991). Thus, liposome is an excellent novel formulation as drug and cosmetic carriers.

Tranexamic acid (TA) is a hydrophilic drug used as an antifibrinolytic agent (Borea et al., 1993; Tibbelin et al., 1995; Waly, 1995; Mitsuhiro et al., 1997; MIMS Annual, 1998; Drug Facts and Comparison, 1999). This drug has also been claimed to exhibit anti-inflammatory (Martindale, 1996) and whitening effects for topical use (Maeda and Naganuma, 1998). The current commercially available preparations of TA are tablets and injections (British Pharmacopoeia, 1998). However, there is none in liposome formulations. It has been demonstrated that TA may cause irritation and allergy (Martindale, 1996). TA entrapped in multilamellar liposomes can potentially reduce the irritation and improve moisturizing effect with prolonged action, resulting from the lipid in liposome formulations. TA which is a water soluble compound will be incorporated in the aqueous layers of liposomal membrane. The present study reports the development of multilamellar liposome formulations for TA, prepared by a chloroform film method with sonication, and the effects of various concentrations of TA (5 and 10% solutions in deionized/DI water) entrapped in liposomes and charges on the physicochemical properties of liposomes. The best formulation, evaluated from the appearance, pH, size, morphology, the percentages of entrapment of drug, transition temperature and enthalpy of transition of liposomes, was selected for further release study.

2. Materials and methods

2.1. Materials

TA was obtained from Asahi Chemical Industry Co., Ltd. (Japan). Hydrogenated soya phosphatidylcholine (Emulmetik 950®) (HSC) was a gift from JJ-Degussa (T) Ltd., Bangkok. Cholesterol (CHL), dicetyl phosphate (DCP), stearylamine (SA), 2,4,6-trinitrobenzosulfonic acid and boric acid were obtained from Sigma Chemical Company (St. Louis, MO). Triton-X 100 was purchased from BDH Ltd. (Poole, England). Chloroform and potassium dihydrogen orthophosphate were of analytical reagent grade, and obtained from commercial sources.

2.2. Preparation of liposomes

Liposome dispersion samples were prepared by a conventional chloroform film method with sonication, with the total lipid concentration of 25 mg/ml. The lipids used were neutral (HSC and CHL) and charged lipids (SA for positively charged lipid and DCP for negatively charged lipid). Liposome formulations composed of HSC/CHL = 7:2; HSC/CHL/SA = 7:2:1(+) and HSC/CHL/DCP = 7:2:1(−) at molar ratios, with and without the entrapped TA were prepared. The concentrations of TA incorporated into the liposome formulations were 5 and 10% (in DI water) (Table 1). For each lot, an amount of 60 ml of each liposome dispersion sample was prepared.

The lipid mixture was dissolved in 60 ml of chloroform, and vacuum-desiccated (500 psi, 65 °C) using a rotary evaporator (R-124 Buchi, Switzerland) for 90 min. A formed thin film layer was flushed under a stream of nitrogen for 1 min. The thin film was re-suspended in either 60 ml of

Table 1
Compositions of nine different liposome formulations

Compositions	Formulations								
	7:2	7:2(5%TA)	7:2(10%TA)	7:2:1 (+)	7:2:1(5%TA, +)	7:2:1(10%TA, +)	7:2:1(−)	7:2:1(5%TA, −)	7:2:1(10%TA, −)
HSC	1.3125	1.3125	1.3125	1.2540	1.2540	1.2540	1.2015	1.2015	1.2015
CHL	0.1875	0.1875	0.1875	0.1815	0.1815	0.1815	0.1740	0.1740	0.1740
SA	–	–	–	0.0645	0.0645	0.0645	–	–	–
DCP	–	–	–	–	–	–	0.1245	0.1245	0.1245
TA	–	3.0000	6.0000	–	3.0000	6.0000	–	3.0000	6.0000
Molar ratio	7:2	7:2	7:2	7:2:1	7:2:1	7:2:1	7:2:1	7:2:1	7:2:1
Expected charges	None	None	None	(+)	(+)	(+)	(−)	(−)	(−)

The content of each compound was expressed as gram per 60 ml of DI water.

5 or 10% TA solution in DI water (for liposomes with the entrapped drug), or DI water alone (for liposomes without the entrapped drug). The dispersion was weighed and swelled by swirling in a water bath (80 °C, 200 rpm) for 30 min. Small multilamellar vesicles were obtained following the sonication of large multilamellar vesicles for 10 min, using a microtip probe sonicator (Vibracell, Sonics & Materials, Inc., Danbury, CT). The liposome dispersion samples were kept at 4 °C and protected from light, prior to use.

Liposome dispersion sample (5 ml) was put into a glass vial and stored at −80 °C for 24 h. The glass vial was then put into a freeze dryer (Model Lioalfa 10, Telstar, Spain), with freezing condensor at −46 °C for 48 h, to obtain the freeze-dried liposome powder. The following pre-freezing conditions: at −25 °C for 90 min, −32 °C for 90 min and −36 °C for 60 min, were employed in this study.

2.3. Physical properties determination of liposomes

The physical appearances of various liposome formulations, DI water, 5 and 10% TA solutions in DI water following storage at 4 ± 1 °C for 24 h were visually observed, and the pH of fresh liposome samples was measured by a pH meter (HORIBA, Ltd., Kyoto, Japan).

A small aliquot of liposome dispersion sample was used to characterize the particle size and size distribution, using a Light Scattering Particle Analyzer (Mastersizer S Long Bed Ver. 2.11, Malvern Instruments Ltd., Malvern, UK), 10 days after sample preparation (kept at 4 °C). The particle size range was set between 0.05 to 800 μm, with the beam length at 2.40 nm, dispersant refractive index at 1.3300, and the polydisperse model analysis was used. This study was performed in six replicates.

A drop of liposome dispersion sample was applied on a 300-mesh formvar copper grid on paraffin and left for 10 min, to allow some of the liposome powder to adhere on the formvar. The remaining dispersion was removed and a drop of 2% aqueous solution of uranyl acetate was applied for 5 min. The remaining solution was then removed and the sample was air dried, prior to measurement with a Transmission Electron Microscope (TEM 1200S JEOL, JEOL Ltd., Tokyo, Japan), to observe the morphology and lamellarity of liposomes.

An amount of 3–7 mg of HSC, CHL, SA, DCP, TA and the freeze-dried liposome powder were placed on sample pans and then properly sealed. All samples were scanned at the rate of 40 °C/min from 20 to 500 °C by a Thermogravimetric Analyzer (Perkin–Elmer TGA 7, Perkin–Elmer Ltd., Norwalk, CONN), to determine the glass transition temperature.

The transition temperature (T_c) and enthalpy of transition (ΔH) were determined from the thermogram, generated by a Differential Scanning Calorimeter (Perkin–Elmer DSC7). HSC, CHL, SA, DCP, TA and the freeze-dried liposome powder (3–7 mg) were put on sample pans and properly sealed. An equal amount of DI water was placed on the reference pan. The temperature ranging from 20 to 200 °C with scan rate of 5 °C/min was employed in this study. Indium standard and water were used to calibrate the calorimeter. This experiment was performed in six determinations.

2.4. Determination of drug encapsulation efficiency in liposomes

The encapsulation efficiencies of TA in the 7:2:1(5%TA, +), 7:2:1(10%TA, +), 7:2:1(5%TA, −) and 7:2:1(10%TA, −) liposomes were determined spectrophotometrically. Each liposome dispersion sample (1.5 g) was mixed with 1.5 g of DI water, and centrifuged at $150{,}000 \times g$ (4 °C) for 90 min in a Centrikron T-1180 ultracentrifuge (Kontron Instruments, Milan, Italy). The supernatant was removed and diluted (100 times) with DI water, prior to the determination of the amount of the unentrapped TA. The pellet containing liposomes was re-suspended in 5 ml of 10% Triton-X 100 solution and sonicated for 20 min. This solution was further diluted (10 times) with Triton-X 100 solution, prior to the determination of the amount of entrapped TA in liposomes. An amount of 0.1 ml of the above samples (unentrapped and entrapped TA) was withdrawn

and derivatized, following the procedures as described in the analytical method section, prior to assay by spectrophotometer. The loading of TA in liposomes was also calculated. Each sample was prepared in two lots and the experiments were performed in duplicates.

2.5. Analytical method for the determination of TA content

The TA content in liposome formulations was determined spectrophotometrically at 415 nm (Milton Roy Spectronic 1001 Plus, Rochester, NY), following derivatization with 2,4,6-trinitrobenzosulfonic acid (Atmaca, 1989). The color reagent used was 1.68% (w/v) 2,4,6-trinitrobenzosulfonic acid solution in DI water, freshly prepared and protected from light, prior to use. Each 0.1 ml of working standard solutions or samples was spiked with 0.25 ml of 0.025 M disodium tetraborate solution (pH 10) and 0.25 ml of color reagent, prior to standing at 25 °C for 30 min. The solution was then diluted to 5.0 ml with 0.1 M potassium dihydrogen phosphate solution (pH 4.5). The solution mixture in the absence of TA was used as a blank. A calibration graph was constructed, by plotting the concentrations of TA against the absorbance values. The linearity of assay was determined from five working standard solutions of TA in DI water (concentrations: 4.0–20.0 μg/ml), prepared in triplicates. The correlation (r^2), intercept and slope of the calibration graph were calculated. The absorbance values of samples were observed and compared with those obtained from the calibration graph, to determine the amount of TA in samples. This experiment was performed in duplicates of three lots of samples.

3. Results

3.1. Physical appearances and pH of liposomes

The appearances and pH of various liposome formulations, DI water, 5 and 10% TA solutions in DI water are shown in Table 2. The neutral 7:2, 7:2(5%TA) and 7:2(10%TA) liposomes showed sedimentation, following storage at 4 °C for 24 h (Table 2). Thus, the charged liposomes {7:2:1(+), 7:2:1(5%TA, +), 7:2:1(10%TA, +), 7:2:1(−), 7:2:1(5%TA, −) and 7:2:1(10%TA, −)} demonstrating favorable physical appearances (no sedimentation) were selected for further experiments (Table 2). The pH values of both positively and negatively charged liposome formulations with the entrapped TA (pH 6.9–8.0) were higher than

Table 2
Physical appearances and pH of various liposome formulations, DI water, 5 and 10% TA solutions in DI water

Formulations	Appearances	pH[a]
7:2	Translucent, white dispersion with sedimentation	ND[b]
7:2(5%TA)	Translucent, white dispersion with sedimentation	ND[b]
7:2(10%TA)	Translucent, white dispersion with sedimentation	ND[b]
7:2:1(+)	Translucent, white dispersion, no sedimentation	7.59 ± 0.06
7:2:1(5%TA, +)	Translucent, white dispersion, no sedimentation	7.92 ± 0.02
7:2:1(10%TA, +)	Translucent, white dispersion, no sedimentation	7.86 ± 0.03
7:2:1(−)	Translucent, white dispersion, no sedimentation	3.4 ± 0.1
7:2:1(5%TA, −)	Translucent, white dispersion, no sedimentation	6.90 ± 0.09
7:2:1(10%TA, −)	Translucent, white dispersion, no sedimentation	7.12 ± 0.006
DI water	Clear solution	6.30 ± 0.06
5% TA in DI water	Clear solution	7.51 ± 0.03
10% TA in DI water	Clear solution	7.57 ± 0.01

Physical appearances of samples were visually observed, following storage at 4 °C for 24 h. pH was measured immediately after sample preparation.

[a] Experimental data represent the mean ± SD of three determinations.

[b] ND, not determined.

Table 3
Particle sizes of six liposome formulations

Formulations	Particle size (μm)[a]
7:2:1(+)	17.9 ± 0.5
7:2:1(5%TA,+)	17.5 ± 0.5
7:2:1(10%TA,+)	35.8 ± 0.5
7:2:1(−)	24.78 ± 0.04
7:2:1(5%TA, −)	2.76 ± 0.02
7:2:1(10%TA, −)	2.05 ± 0.03

Particle sizes of liposome formulations were measured 10 days after sample preparation (kept at 4 °C).

[a] Experimental data represent the mean ± SD of six determinations.

that of DI water (pH 6.3), except the blank negative liposome {7:2:1(−)} (pH 3.4, Table 2). Thus, the incorporation of TA in liposomes may increase pH of the system.

3.2. Liposome size, morphology and lamellarity of liposomes

The particle sizes of six liposome formulations are presented in Table 3. The 7:2:1(+), 7:2:1(−), 7:2:1(5%TA, +) and 7:2:1(10%TA, +) liposomes demonstrated bimodal distribution of particle size and larger particle size (~ 10 times) than the negatively charged liposomes with the entrapped drug. The smallest size of 2.05 μm was observed in the 7:2:1(10%TA, −) liposome. All liposome formulations demonstrated log-normal distribution of particle size, ranging between 2.0 and 35.8 μm (Table 3). The transmission electron micrographs of the blank 7:2:1(+), 7:2:1(5%TA, +) and 7:2:1(5%TA, −) liposomes, showing the morphology and lamellarity of liposomes are shown in Fig. 1. The lamellarity of about 8–15 bilayers (multilamellar vesicles) was observed in all liposome formulations (Fig. 1).

3.3. Glass transition temperature, transition temperature and enthalpy of transition

The glass transition temperatures of HSC, CHL, SA, DCP, TA, 7:2:1(10%TA, +) and 7:2:1(10%TA, −) liposome formulations are presented in Table 4. The results indicated that all samples were decomposed at high temperature (> 200 °C), except SA which decomposed at lower than 200 °C. The T_c and ΔH values of HSC, CHL, SA, DCP, TA, 7:2:1(+), 7:2:1(5%TA, +), 7:2:1(10%TA, +), 7:2:1(−), 7:2:1(5%TA, −) and 7:2:1(10%TA, −) liposome formulations are summarized in Table 5. Under the experimental conditions, there was no peak of TA observed in the DSC thermogram.

3.4. Tranexamic acid entrapped in liposomes

The calibration graph of TA solution in DI water was shown to be linear ($r^2 = 0.9937$), over the concentration range 4.0–20.0 μg/ml. The regression equation was as follows: $y = 20.7903x - 0.1942$, where y is the concentration of TA (μg/ml) and x is the absorbance of the derivative of TA formed with 2,4,6-trinitrobenzosulfonic acid (mAU*s). The percentages of TA recovered from the liposome formulations with the entrapped drug, using spectrophotometric assay varied between 91.4 and 104.7%. The other components in liposome formulations neither reacted with the color reagent nor demonstrated significant absorption at 415 nm. The percentages of TA entrapped in liposomes, the free drug and the loading of TA in liposome formulations are shown in Table 6.

4. Discussion

The charged liposomes with and without the entrapped TA, showing no sedimentation following storage at 4 °C for 1 day, may indicate better physical stability than the neutral liposomes (Table 2). This may be associated with the effects of charges on the surface of liposomes. TA is a synthetic amino acid, which has amino and carboxylic groups. When TA dissolves in water, it will ionize in equilibrium Eq. (1):

$$C_8H_{15}NO_2 + H_2O \rightleftarrows C_8H_{16}NO_2^+ + OH^- \quad (1)$$

The pH of samples increased when TA was incorporated into the liposome systems (Table 2). This may be due to the effect of concentration of

hydroxyl ion (Eq. (1)). TA in DI water appeared to have weak positive charges (Eq. (1)). The positively charged liposomes with the entrapped TA demonstrated higher pH values than those without the entrapped drug (Table 2). The entrapped TA may provide more hydroxyl ions to the liposome dispersion sample (Eq. (1)). Similarly, the negatively charged liposomes with the entrapped TA showed higher pH values than the blank negative liposome (Table 2).

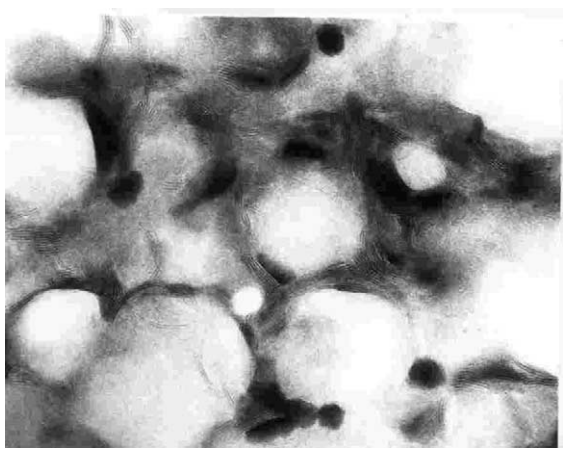
SA and DCP are amphiphilic compounds, and they may ionize when incorporated into the liposomes and aqueous environments, as shown in the following equations:

$$C_{18}H_{39}N + H_2O \rightleftarrows C_{18}H_{40}N^+ + OH^- \quad (2)$$

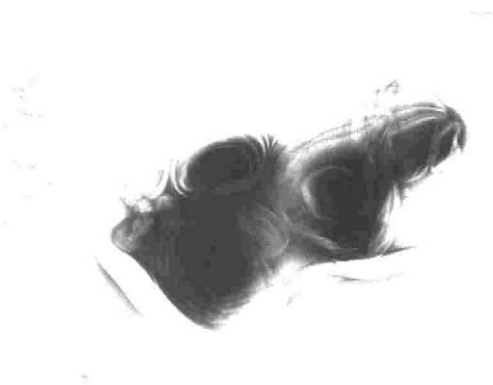
$$C_{32}H_{67}O_4P + H_2O \rightleftarrows C_{32}H_{66}O_4P^- + H_3O^+ \quad (3)$$

The positively charged liposomes received hydroxyl ion from SA (Eq. (2)), whereas the negatively charged liposomes received hydronium ion from DCP (Eq. (3)). Thus, all positively charged liposomes (pH 7.6–7.9) exhibited higher pH values than all negatively charged ones (pH 3.4–7.1, Table 2). Hydronium ion from DCP (Eq. (3)) may be neutralized by hydroxyl ion, resulting from the ionization of TA (Eq. (1)).

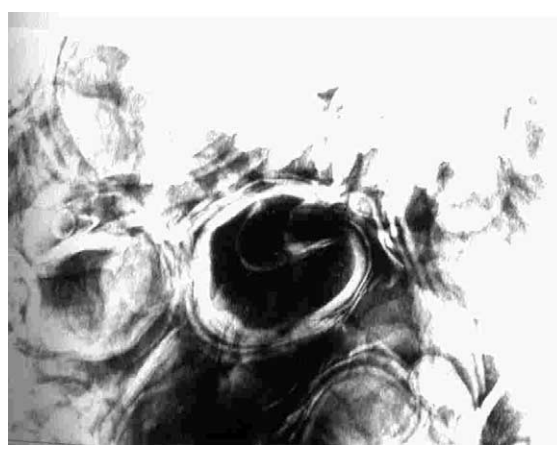
SA and DCP incorporated into the liposomal bilayer membranes render the surface electrically



(a)



(b)



(c)

Fig. 1. Transmission electron micrographs of the: (a) blank 7:2:1(+) liposomes (6000 ×); (b) 7:2:1(5%TA, +) liposomes (5000 ×); (c) 7:2:1(5%TA, −) liposomes (6000 ×).

Table 4
Glass transition temperatures of HSC, CHL, SA, DCP, TA, 7:2:1(10%TA, +) and 7:2:1(10%TA, −) liposome formulations

Formulations	Glass transition temperature (°C)
HSC	257.77
CHL	246.66
SA	162.22
DCP	218.47
TA	223.63
7:2:1(10%TA, +)	222.22
7:2:1(10%TA, −)	229.63

like charges. This results in a repulsion and hence, an increase in the distance between different bilayers (Benita et al., 1984). Similar charges of lipid bilayers to the entrapped TA can increase the distance between bilayers, as observed in the positively charged liposomes with large particle size (17.5–35.8 μm, Table 2). In contrast, the negative charges of lipid bilayers, opposite to the charges of entrapped TA will decrease the distance (liposome size: 2.0–2.8 μm), due to the neutralization of charges (Table 2). Thus, the smallest particle size was observed in the negative liposome with the entrapped TA concentration of 10% (Table 2).

Table 5
Transition temperatures and enthalpy of transition of HSC, CHL, SA, DCP, TA, 7:2:1(+), 7:2:1(5%TA, +), 7:2:1(10%TA, +), 7:2:1(−), 7:2:1(5%TA, −) and 7:2:1(10%TA, −) liposome formulations

Formulations	Transition temperature (°C)	Enthalpy of transition (J/g)
HSC	61.2 ± 0.4	38.5 ± 9.8
CHL	149.7 ± 0.2	66.6 ± 1.0
SA	57.1 ± 0.6	293.7 ± 53.0
DCP	79.3 ± 0.2	187.0 ± 1.8
7:2:1(+)	77.8 ± 0.4	50.0 ± 7.3
7:2:1(5%TA, +)	76.2 ± 0.4	11.8 ± 0.3
7:2:1(10%TA, +)	77.1 ± 1.1	8.3 ± 2.4
7:2:1(−)	70.0 ± 0.5	45.4 ± 10.6
7:2:1(5%TA, −)	71.3 ± 0.5	14.7 ± 2.8
7:2:1(10%TA, −)	70.8 ± 0.5	6.3 ± 3.2

There was no peak of TA observed in the DSC thermogram. Experimental data represent the mean ± SD of six determinations.

The T_c values of the positively and negatively charged liposome formulations with and without the entrapped TA were broadly comparable (70.8–77.1 °C vs. 70.0–77.8 °C). However, the ΔH values of blank positively and negatively charged liposome formulations were higher (45.4–50.0 J/g) than those of the respective formulations with the entrapped drug (6.3–14.7 J/g, Table 5). This may indicate that TA was entrapped in the aqueous layer of the charged liposomes. TA may interfere with the formation of lipid bilayers, thereby reducing the ΔH values of liposome formulations. The higher the entrapped TA concentration in liposomes, the more marked reduction of ΔH was observed (Table 5). TA did not exhibit T_c and ΔH, when scanned in the range of 20–200 °C, indicating high thermal stability of this drug.

All liposome formulations with and without the entrapped TA exhibited an endothermic peak, with an average maximum peak of T_c (70.0–77.8 °C) higher than HSC (61.2 °C, Table 5). This may indicate that cholesterol incorporated into the liposomal membrane had its hydroxyl group oriented towards the aqueous layer, with the aliphatic chain aligned parallel to the acyl chains in the center of lipid bilayers. The presence of rigid steroid nuclei along side the first 10 or so of carbons of the phosphatidylcholine chain of HSC may reduce the freedom of movement. This may result in an enhanced stability of the liposomal membrane, and hence the high T_c value of liposome formulations.

Reaction between TA and 2,4,6-trinitrobenzosulfonic acid in alkaline condition forms a yellow color compound, with the maximum absorption spectrum of 415 nm (Atmaca, 1989). The optimum condition for the reaction to occur is as follows: pH 10 at 25 °C for 30 min. The 0.1 M potassium dihydrogen phosphate buffer solution (pH 4.5) was added to terminate the reaction. The intensity of color was relatively stable in the reaction medium, for at least 3 h when protected from light.

The percentages of TA entrapped in all liposome formulations varied between 13.2 and 15.6% (Table 6). There were no significant differences in the percentages of entrapment of TA between

Table 6
Percentages of entrapment of TA, free TA, and the amount of TA per total lipid in liposome formulations

Formulations	Form of drug	% Entrapment	Loading of TA (μg/mg lipid)[a]
7:2:1(5%TA, +)	Total drug	100.0	–
	Entrapped drug	14.7 ± 0.6	275.2 ± 13.9
	Free drug	85.3 ± 0.2	–
7:2:1(10%TA, +)	Total drug	100.0	–
	Entrapped drug	15.0 ± 0.4	627.0 ± 10.8
	Free drug	85.0 ± 0.9	–
7:2:1(5%TA, −)	Total drug	100.0	–
	Entrapped drug	15.6 ± 0.7	290.4 ± 5.7
	Free drug	84.5 ± 2.6	–
7:2:1(10%TA, −)	Total drug	100.0	–
	Entrapped drug	13.2 ± 0.3	547.3 ± 13.6
	Free drug	86.8 ± 0.3	–

Experimental data represent the mean ± SD of four determinations.
[a] Total lipid in 1 ml of liposome dispersion sample was 25 mg.

different formulations (Table 6), as analyzed by ANOVA ($P > 0.05$). This may indicate that the charges and TA concentrations did not affect the percentages of TA entrapped in liposome formulations. The negatively charged liposome which had opposite charged to TA (weak positive) may improve the entrapment of drug in the liposomes. On the other hand, the positively charged liposomes with the entrapped drug demonstrated larger particle size, and hence larger volume for the entrapment of drug than the negative liposomes (Benita et al., 1984). These phenomena may explain why the percentages of entrapment of TA in the positive and negative liposomes were broadly comparable (14.7–15.0% vs. 13.2–15.6%, Table 6).

The loading of TA in the charged liposomes increased, as the initial concentration of drug increased, from 275 μg/mg lipid {7:2:1(5%TA, +)} to 627 μg/mg lipid {7:2:1(10%TA, +)}, and from 290 μg/mg lipid {7:2:1(5%TA, −)} to 547 μg/mg lipid {7:2:1(10%TA, −)} (Table 6, Foldvari et al., 1993).

In conclusion, the liposomes composed of HSC/CHL/charged lipids at molar ratios of 7:2:1(+) and 7:2:1(−) with the entrapped TA (5 and 10% solutions in DI water) were demonstrated as multilamellar vesicles. Thus, TA was advantageous when entrapped in liposome, since this drug delivery system can potentitally reduce the skin irritation and improve moisturizing effect. The particle sizes of all liposome formulations, with and without the entrapped TA were in the range of 2.05–35.8 μm, with the smallest size was observed in the negative liposome with the entrapped TA concentration of 10%. The charged liposomes with the entrapped TA demonstrated high physical stability. The concentrations of TA appeared to affect the pH, particle size and enthalpy of transition of liposome formulations, whereas charges seemed to affect the physical stability, pH and particle size of liposomes. The best formulation was concluded to be the negatively charged 7:2:1(10%TA, −) liposome, which exhibited high physical stability, small particle size and relatively high percentage of drug entrapment. This formulation will be further evaluated for release study. Overall, it has been demonstrated that TA which is a hydrophilic drug can be favorably entrapped in liposomes.

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