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Original Article

Interaction of insulin, cholesterol-derivatized mannan, and carboxymethyl chitin with liposomes: a differential scanning calorimetry study

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Abstract

The interaction of drugs and polymers used to incorporate in or surface modify/coat the liposomes can affect the phase transition, fluidity and other physical properties as well *as in vivo* fate of vesicles. In this study, differential scanning calorimetry (DSC) was used to investigate changes in the temperature and the enthalpy of phase transition of liposomes of various electrical charges following interaction with carboxymethyl chitin (CM-chitin) as a hydrophilic polymer, cholesterol-derivatized mannan (CHM) as a hydrophilic polymer bearing a hydrophobic moiety, and insulin as a model peptide. The results indicated that insulin incorporation or polymers caused no significant change in the phase transition temperature (T_m) of liposomes. However, reduction in the enthalpy of the transition ($\Delta H°$) following coating with CHM supports an anchoring mechanism to the bilayer by the polymer, whereas no change or little increase in the $\Delta H°$ after coating with carboxymethyl chitin suggests no significant interaction or electrostatic weak interactions of polymer with liposomes. The DSC data of liposome-polymer interaction may be suggestive of changes in membrane fluidity, drug release, and possibly the behavior of liposomes in biological milieu.

Keywords: Surface-modified liposomes; Polymer-coated liposomes; Cholesterol-derivatized mannan; Carboxymethly chitin; Differential scanning calorimetry; Phase transition

INTRODUCTION

Over the past few decades, liposomes have received much attention as model membranes or delivery systems for therapeutic drugs, vaccines and genes (1-3). Liposomes possess unique properties such as capability of entrapping hydrophilic or lipophilic agents, great biocompatibility, low immunogenicity and toxicity, and ease of preparation. However, conventional liposomes, which are mainly composed of phospholipids without any surface modification, suffer some drawbacks such as instability, inability to provide sustained drug delivery and rapid *in vivo* clearance which may limit their use as therapeutic carrier systems (4).

Many approaches have been taken to overcome these problems and/or render liposomes targetability to different cells and organs. Surface-modification of liposomes by different polymers like polysaccharides, polysaccharides derivatized with hydrophobic moieties and polyethylene glycol derivatives is the most commonly used approach to produce stealth liposomes which favor a prolonged circulating half life due to reduced uptake by mononuclear phagocytic system (4,5).

Incorporation of drugs and other additives in liposomes as well as their surface modification by polymer derivatives can alter not only the vesicles' fate *in vivo*, but also their physical properties *in vitro*; the bilayer phase transition and fluidity, the drug release behavior, and drug and liposomes stability being the most important changes to occur (6,7).

Differential scanning calorimetry (DSC) is a great tool which can be efficiently used to investigate the interaction between liposomes

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This paper is dedicated to the memory of my mentor, Professor James A. Rogers who passed away on April 1, 2011

and molecules of drugs and polymers used to incorporate in and/or surface-coat the vesicles (8-10).

Thermal studies have indicated that the transition of a pure phospholipid occurs over a narrow temperature range. The presence of drugs and other lipids may, however, broaden the phase transition or cause changes in the enthalpy of the transition (ΔH°) and/or phase transition temperature (T_m), the temperature at which the maximum peak occurs. In this study we have used thermal analysis to investigate the interaction of liposomes of variety of charges with insulin as a model peptide drug, carboxymethyl chitin (CM-chitin) as a hydrophilic polymer, and cholesterol-derivatized mannan (CHM), as a hydrophilic polymer bearing a hydrophobic anchor group. Insulin, an amphyphilic molecule, can interact with the bilayer, through both hydrophilic and hydrophobic interactions, possibly affecting physical characteristics of liposomes during and after drug entrapment. The study of interaction of CHM with liposomes has been of special interest since naturally-occurring polysaccharides like mannan, which have been derivatized with cholesterol or other hydrophobic anchors, have been widely used to modify drug release, improve physical stability and prolong circulation half life of drug liposomes *in vivo*. Thus, thermal studies on these interactions can provide us with useful information about the properties of these vesicles.

MATERIALS AND METHODS

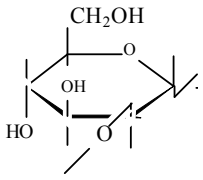
Materials

L-α-Dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylglycerol (DMPG) were obtained from Princeton Lipids, Princeton, NJ. Cholesterol (CH), stearylamine (SA), mannan and insulin were obtained from Sigma Chemical Co., St. Louis, MO and used as received. All other chemicals were of reagent grade or better.

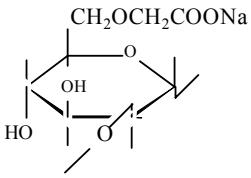
Methods

Preparation of CHM

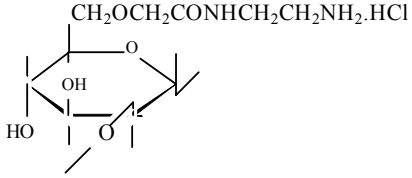
Mannan was derivatized with CH in three steps according to a previously reported procedure (11), with some modifications. Molecular structures of mannan, CHM, and intermediate products are depicted in Scheme. 1. In first step carboxymethylation of mannan (CM-mannan) was achieved by reaction of 1.2 g. mannan with 0.12 g sodium hydride (NaH) in dry dimethyl sulfoxide (DMSO) for 4 h followed by reaction with 0.15 g bromomethyl acetate for 12 h at room temperature (RT). Subsequently, the addition of absolute ethanol caused precipitation of the methylester derivative of CM-mannan, which was then separated by filtration. The precipitate was washed with



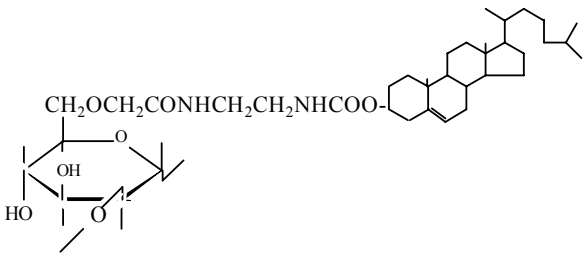
a) mannan



b) carboxymethyl-mannan



c) AECM-mannan



d) cholesterol-AECM-mannan (CHM)

Scheme 1. Chemical structures of mannan and its derivatives

ethanol, then converted to CM-mannan with stirring in a 0.2 M NaOH solution at RT for 4 h. Purification of the product was carried out by dialysis of the mixture against toluene-saturated water for 2 days and against MilliQ® water (Millipore®, Millford, MA) for 1 day. Infrared spectroscopy indicated the presence of carbonyl group at 1740 cm^{-1} as evidence of carboxymethylation. The second step CM-mannan (1 g) was reacted with ethylenediamin hydrochloride (3.2 g) at pH 4.7 for 12 h in the presence of 1-ethyl-3-(3-dimethylamino) propylcarbodiimide (0.3 g) as a coupling agent to form aminoethylcarbomethyl-mannan (AECM-mannan) which was purified by dialysis against 0.2 M NaOH solution for 2 days and MilliQ® water for 1 day. CHN elemental analysis indicated the substitution degree of 8.8% AECM spacer (8.8 spacer moiety per 100 saccharide units).

In the third step, AECM-PS was dissolved in dry DMSO and reacted with cholesterol chloroformate in dry DMF in the presence of pyridine. The mixture was stirred at 80°C for 12 h and cooled to RT. Cholesterol-derivatized mannan (CH-AECM-mannan) produced was precipitated by absolute ethanol and filtered. The precipitate was washed with ethanol and ether, dried under vacuum for 2 days, and stored in a refrigerator. The degree of substitution of CH was 2.3% (i.e. 2.3 cholesterol moieties per 100 saccharide units). This was estimated from ^{1}HNMR spectra, where methyl and methylene protons of CH molecules produced signals between 0.2 and 2.4 ppm (Fig. 1). Such signals were absent in the ^{1}HNMR spectra of the underivatized polysaccharides.

Preparation of liposomes by dehydration-rehydration (DRV) method

Liposomes were prepared according to Kirby and Gregoriadis (12) with some modifications. Briefly, DPPC, CH and a charged adduct like SA or DMPG were dissolved in chloroform in specified mole ratios (m.r.) to give a final lipid concentration of 15 mM. The lipid solutions of of DPPC, DPPC:CH (7:2 m.r.), DPPC:SA (7:1 m.r.), DPPC:DMPG (7:0.5 m.r.) in chloroform were dried in a round-bottom flask by rotary evaporation. The flask was flushed with N_2, then stored overnight in a vacuum desiccator at 40°C. The lipid film was hydrated with water at 10°C above the phospholipid T_m for 5 min, followed by probe-sonication for 1 min at RT. Liposomes were centrifuged at 5000 x g to separate titanium particles released from the sonicator probe. The supernatant containing

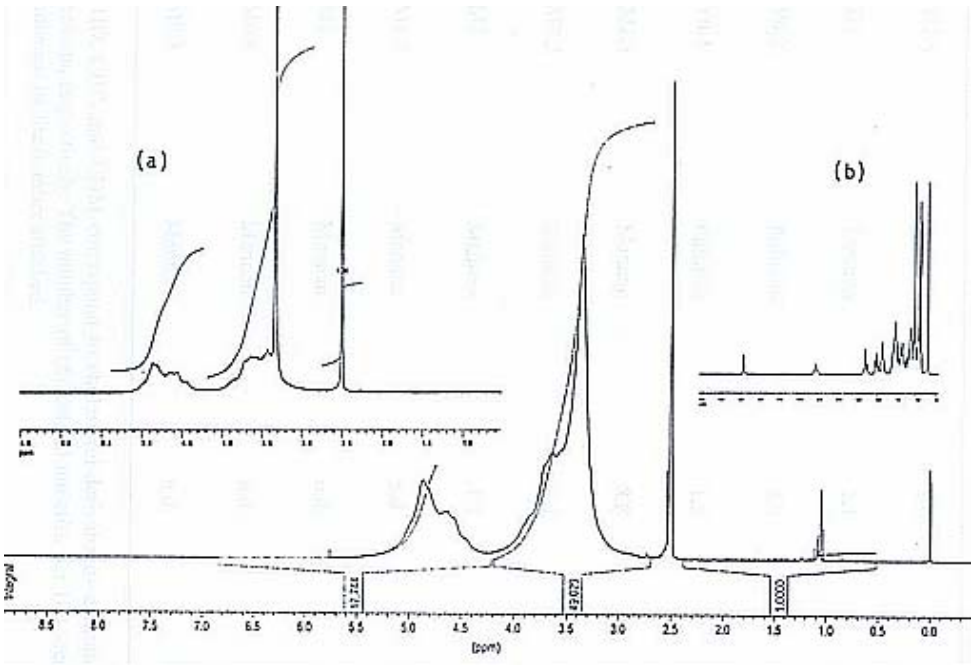


Fig.1. ^{1}HNMR spectra of CHM in DMSO-d_6 at room temperature. Inset (a) and (b) are mannan and cholesterol spectra, respectively. Protons of mannan from CHM appeared at 2.6-6.6 ppm and of cholesterol at 0.2-2.4 ppm as evidence of derivatization.

small unilamellar vesicles (SUV) was freeze-dried and a small volume of insulin solution (16 mg/ml) in 10 mM phosphate buffer saline (PBS) was used to rehydrate the lyophilized SUVs (~0.4 ml/100 mg lipid). To prepare empty liposomes, the freeze-dried liposomes were hydrated with buffer solution. After 30 min, liposomes were warmed at 10°C higher than T_m for 5 min in the rotary evaporator. After cooling to RT, liposomes were diluted with 10 mM PBS (pH 7.4) and left at RT for 3 h. In one experiment, an insulin solution was prepared in 5 mM PBS (pH 7.4) and added to SUVs before freeze-drying. Lyophilized liposomes were then rehydrated with 10 mM PBS solution of the same pH and treated as before.

Polymer-coating of liposomes

Liposomes were incubated with an equal volume of 0.5% CM-chitin or 0.5% CHM solutions in 10 mM PBS (pH 7.4) to form polymer-coated liposomes and stored in the refrigerator overnight. The weight ratio of polymer to lipid was one. Uncoated and polymer-coated liposomes were centrifuged at 10,000-130,000 x g for 15-30 min at 4°C (Beckman Ultracentrifuge, Floor Model: L8-55). The supernatant containing free insulin and/or free polymer was removed and the pellet was resuspended in PBS solution. This procedure was repeated twice.

Differential scanning calorimetry

Calorimetric measurements were performed with a Seiko thermal analyzer; model SSC/5200 (Seiko Instruments Inc, USA). The system was configured for data analysis using a Hewlett Packard® workstation model 712. Indium, palmitic acid, and benzoic acid were used to calibrate the instrument.

The liposomal pellets resuspended in 3 ml PBS were divided into equal volumes in microcentrifuge tubes, then centrifuged at 14,000 rpm at RT for 5 min. The supernatants were discarded and the pellets were resuspended in 50 µl PBS and transferred to 100 µl aluminum crucibles for analysis. A sealed pan containing 50 µl PBS solution was used as a reference. The samples were scanned at a rate of 1°C/min from 23-75°C.

RESULTS

The DRV liposomes obtained were multilamellar vesicles, as observed by an optical microscope, ranging in size from 1.73 to 3.08 µm (data not shown). DSC studies were conducted to obtain evidences of the

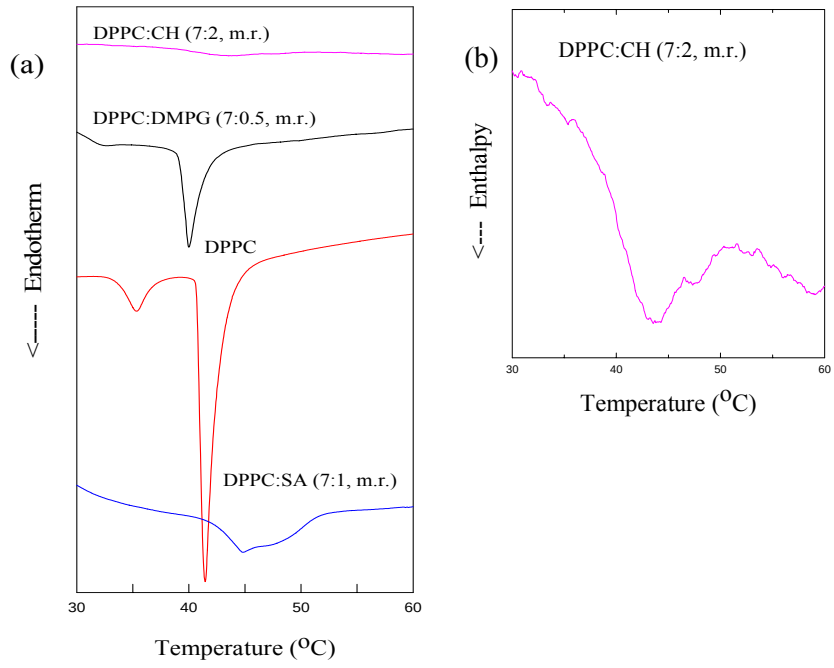


Fig.2. Effects of CH and charged adducts (SA or DMPG) on DSC thermograms of DPPC liposomes. Inset (b) shows enlargement of the thermogram of DPPC:CH (7:2 m.r.) liposomes from Fig.2.a.

coating of liposomes by polymers. Representative DSC scans shown in Fig. 2 illustrate the effects of CH and the charged adducts SA and DMPG, on the T_m of DPPC. A T_m of 41.3°C and a pre-transition temperature of 34.7°C were observed for DPPC liposomes (Fig. 2a). The T_m of DPPC increased to 43.5°C but its enthalpy of transition (ΔH°) decreased drastically to 9.1 mJ/mg from 48.7 mJ/mg with 22 mol percent CH incorporation (Fig. 2b and Table 1). The presence of 12.5 mole percent SA increased the T_m of DPPC from 41.3 to 44.7°C and also slightly increased the ΔH° from 48.7 to 52.0 mJ/mg. Incorporation of 6.7 mole percent DMPG decreased the T_m from 41.3-40.1°C and the ΔH° from 48.7 to 40.4 mJ/mg.

The effects of insulin incorporation and polymer interaction on the thermal behavior of DPPC liposomes, DPPC:SA (7:1 m.r.) liposomes, and DPPC:DMPG (7:0.5 m.r.) liposomes are illustrated in Fig. 3 and the details are presented in Table 1. The

Table 1. Thermodynamic characteristics of DPPC liposomes[a]

Liposome composition	Pretransition temp. (°C)	Main transition temp. (T_m) (°C)	Enthalpy change (mJ/mg)
DPPC	34.7	41.3	48.7
DPPC:CH (7:2 m.r.)	-	43.5	9.1
DPPC + CM-chitin	34.7	41.3	48.2
DPPC + CHM	-	41.4	41.5
DPPC:SA (7:1 m.r.)	-	44.7	52.0
DPPC:SA (7:1 m.r.) + Insulin	-	44.7	50.4
DPPC:SA (7:1 m.r.) + CM-chitin	-	44.7	57.3
DPPC:SA (7:1 m.r.) + CHM	-	44.8	42.7
DPPC:DMPG (7:0.5 m.r.)	32.5	40.1	40.4
DPPC:DMPG (7:0.5 m.r.) + Insulin	-	40.4	41.6
DPPC:DMPG (7:0.5) + CM-chitin	-	40.0	40.5
DPPC:DMPG (7:0.5 m.r.) + CHM	32.4	40.0	36.2

[a.] Liposomes contain no insilin, unless otherwise stated. Plus sign (+) indicates liposomes containg insulin or coated with the polymer. Values are means of 2 or 3 measurements.

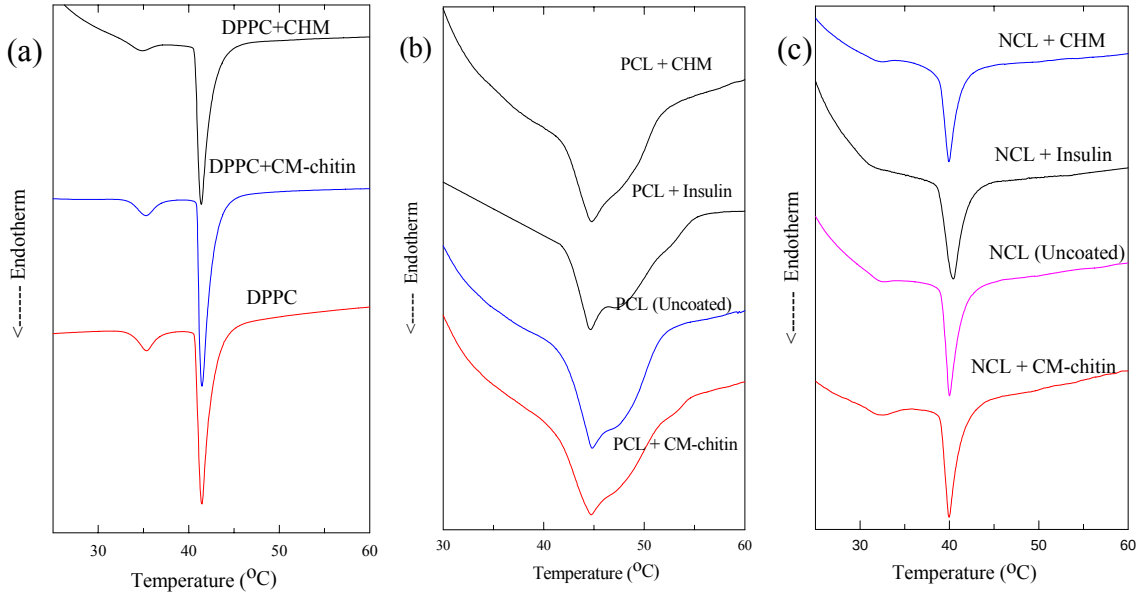


Fig. 3. Effects of insulin and polymer coating on the DSC thermograms of, a) neutral DPPC liposomes, (b) positively-charged DPPC:SA (7:1 m.r.) liposomes (PCL), and (c) negatively-charged DPPC:DMPG (7:0.5 m.r.) liposomes (NCL). Plus sign (+) indicates liposomes containing insulin or coated with the polymer.

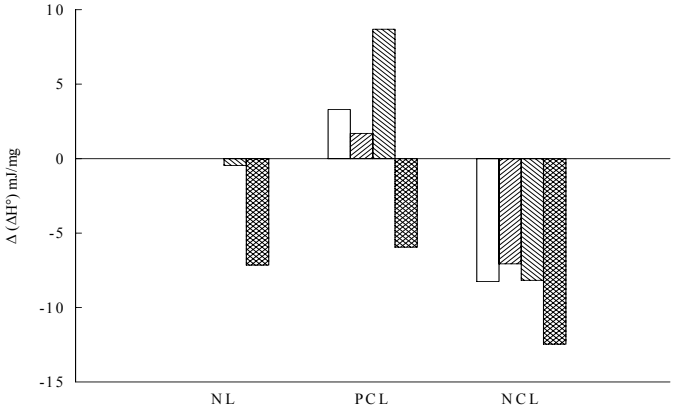


Fig. 4. The effect of insulin incorporation or polymer-coating on the enthalpy change (ΔH°) of the main transition endotherms of neutral liposomes (NL), positively-charged liposomes (PCL), and negatively-charged liposomes (NCL), composed of DPPC, DPPC:SA (7:1 m.r.), and DPPC:DMPG (7:0.5 m.r.), respectively. For the sake of comparison, the enthalpy changes of the transitions of all systems were subtracted from that of liposomes composed of DPPC only (horizontal line at zero). Values was calculated from Table 1. ▭, empty and uncoated; ▨, insulin-encapsulated and uncoated; ▧, CM-chitin-coated; and ▩, CHM-coated.

differences in the phase transition enthalpies of DPPC liposome following insulin incorporation, SA or DPMG addition, or polymer coating are depicted in Fig.4. The effect of insulin on T_m and ΔH° was negligible. CM-chitin increased ΔH° of DPPC:SA liposomes to 57.3 mJ/mg, but had no effect on the T_m. The thermal behavior of all other liposome systems was not affected by coating with CM-chitin. Coating with CHM had no effect on T_m, however, ΔH° decreased (Table 1).

DISCUSSION

DSC has been widely used to obtain values of T_m, the temperature at which the maximum peak occurs, i.e. the transition is half completed, ΔH°, and, hence, entropy change of the transition (ΔS°) of various lipid systems.

Liposomes composed of DPPC, as the only constituent, had a sharp transition mid-point T_m of 41.3 (Table 1). This value was lower than the values of 43, 44, 44.58 reported by Ikeda and coworkers (13), Boggs and coworkers (14) and Langer and coworkers (15); but in good agreement with the value of 41.5 and 41.3 obtained by Lo and Rahman (16), and slightly higher than the value of 40.5 reported by Seki and Tirrell (17). These differences may be attributed to various factors related to instrument or sample variability (18). Sensitivity of the DSC instrument as well as the nature and number of standards used to calibrate the instrument play important roles in the accuracy and reproducibility of results (16). Indium, with a T_m of 156.6, is often used to calibrate the conventional DSC, which is more suitable for compounds with melting points above 100°C. In contrast, multiple standards are routinely used to calibrate a high-sensitivity DSC. This can be advantageous in producing more reliable measurements in the desired temperature range. Thus, the high sensitive DSC instrument used in this study, which was calibrated with two standards having the melting points of 28.2°C and 75.9°C, better and more accurately indicated the thermal behavior of phospholipids with melting points below 100°C.

With regards to the heating, an increase in heating rate increases the onset and midpoint of transition temperatures (13,18). Another important factor is sample characteristics. In the case of DPPC liposomes it has been shown that where they were prepared as multilamellar vesicles (MLVs) a T_m of 41.5 (19) or 41.3 (20) was obtained, while in the form of SUV the T_m was shifted to almost 4°C lower temperatures (19,20). Therefore, the variation in aggregation state of liposomes may be partly responsible for the differences in T_m previously mentioned (21).

The T_m of DPPC decreased from 41.3 to 40.1 upon inclusion of 6.7 mole percent DMPG Since the acyl chains of these phospholipids are not identical, two transitions of gel-to-

liquid crystalline states were expected to occur somewhere between two T_m values. However, a DMPG peak was not detected because of low content in the mixture. The shifting of T_m of DPPC, upon incorporation of 12.5 mole percent of SA, to 44.6°C can be explained by the presence of longer acyl chains (i.e. stearyl) in the bilayers. It also caused broadening of the transition peak, indicating lower chain cooperativity in the bilayer.

The thermal behavior of the biomembrane phase transition is affected by the presence of foreign molecules. The current study was conducted to evaluate the thermodynamic changes of neutral, negatively-charged, or positively-charged liposomes composed of DPPC (as the main phospholipid in the formulation) upon their interaction with polymers or insulin. Interactions between macromolecules (peptides) and phospholipids have been classified by Papahajopoulos *et al*. into three major groups: (1) surface adsorption only, (2) partial embedding into the lipid bilayer, and (3) penetration into the core of anionic or zwitterionic lipid bilayers (19). The first group interaction resulted in an increase in the ΔH° accompanied by either an increase or no change in the T_m, while processes of the second and third class resulted in reduction in the transition enthalpy with a decrease or no change in T_m. Some contradictions to Papahajopoulos *et al*.'s classification scheme have been reported (16,22,23) and some modifications have been suggested (16).

Insulin only slightly reduced the enthalpy of transition in the amount incorporated (Table 1 and Fig. 3) while it had no effect on the T_m of DPPC of PCL or NCL. The stabilization effect of cholesterol-derivatized polysaccharides on phospholipid bilayers has been already demonstrated using surface potential or surface pressure measurements, isothermal titration calorimetry, cryogenic scanning electron microscopy and DSC (24,25). The functionalized polysaccharides have also been shown to lower the total enthalpy and entropy changes of phospholipid after interaction and yield more thermodynamically stable systems (11). The ΔH° of DPPC liposomes was found to be 48.65 mJ/mg (Table 1), in agreement with a value of 8.7 Kcal/mole (~49.55 mJ/mg) reported elsewhere (26). Interaction of CHM with neutral DPPC liposomes decreased ΔH° from 48.65 to 41.5 mJ/mg, a 14.7 % reduction (Table 1 and Fig. 3). The same pattern was also observed after CHM interaction with both PCL and NCL consisting of DPPC:SA (7:1 m.r.) and DPPC:DMPG (7:0.5 m.r.), respectively (Fig. 3). The T_m was, however, insensitive to such interactions. Similar thermotropic behaviors have also been reported for DPPC and DMPG upon interaction with alginate and α-sarcin, respectively (27,28). On this basis it is suggested that CHM interacts with liposomes according to Papahadjopoulos *et al*. (19), i.e. the polymer becomes inserted in the lipid membrane.

In contrast to CHM, CM-chitin interactions with neutral or negatively-charged liposomes did not alter ΔH° from which it is assumed that such interactions were weak. However, CM-chitin slightly increased ΔH° of positively-charged liposomes. This may be attributed to a stronger association of PCL with the oppositely-charged CM-chitin at pH 7.4. CM-chitin interaction with liposomes therefore falls into the first category in which macromolecules interacting with bilayers exhibit a strong dependence on electrostatic binding without significant penetration into the hydrocarbon region.

CONCLUSION

DSC studies indicated that polymer association caused no change in T_m of liposomes. However, the changes in the ΔH° supported an anchoring mechanism of CHM to the bilayers, whereas no changes after coating with CM-chitin was suggestive of no significant interaction. It can be, therefore, concluded that a hydrophilic polymer which are hydrophobized (like CHM) interact more strongly than a polymer lacking a hydrophobic moiety (like CM-chitin) with liposomal bilayers. Thus, information obtained from the DSC study can be used to determine liposome-polymer interaction, which it can accordingly affect membrane fluidity, drug release behavior, and possibly the *in vivo* fate of the liposomes (4-8).

REFERENCES

1. El Maghraby GM, Barry BW, Williams AC. Liposomes and skin: From drug delivery to model membranes. Eur J Pharm Sci. 2008;34:203-222.
2. Swaminathan J, Ehrhardt C. Liposomes for Pulmonary Drug Delivery. In: Smyth HDC, Hickey AJ, editors. Controlled Pulmonary Drug Delivery. New York: Springer; 2011. p. 313-334.
3. Tan ML, Choong PFM, Dass CR. Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery. Peptides. 2010;31:184-193.
4 Allen TM. Long-circulating (sterically stabilized) liposomes for targeted drug delivery. Trend Pharmacol Sci. 1994;15:215-220.
5 Moghimi SM, Szebeni J. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. Prog Lip Res. 2003;42:463-478.
6. Mady MM, Darwish MM. Effect of chitosan coating on the characteristics of DPPC liposomes. J Adv Res. 2010;1:187-191.
7 Tribet C, Vial F. Flexible macromolecules attached to lipid bilayers: impact on fluidity, curvature, permeability and stability of the membranes. Soft Matter .2008;4:68-81.
8 Chiu MH, Wan CPL, Weers PMM, Prenner EJ. Apolipophorin III interaction with model membranes composed of phosphatidylcholine and sphingomyelin using differential scanning calorimetry. Biochim Biophys Acta. 2009;1788: 2160-2168.
9. Panicker L. Interaction of propyl paraben with dipalmitoyl phosphatidylcholine bilayer: a differential scanning calorimetry and nuclear magnetic resonance study. Colloid Surf B Biointerfaces 2008;61:145-152.
10. Saunders M, Taylor KM, Craig DQ, Palin K, Robson H. High sensitivity differential scanning calorimetry study of DNA-cationic liposome complexes. Pharm Res. 2007;24:1954-1961.
11. Rosilio V, Madelmont G, Akiyoshi K, Sunamoto J, Baszkin A. Thermal behavior of hydrated dimyristoylphosphatidylcholine / cholesteryl-pullulan mixtures. J Colloid Interface Sci. 1994;162:418-424.
12. Kirby C, Gregoriadis G. Dehydration-rehydration vesicles: A simple method for high yield drug entrapment in liposomes. Biotechnology. 1984;11: 979-984.
13. Ikeda T, Ledwith A, Bamford CH, Hann RA. Interaction of a polymer biguanide biocide with phospholipid membranes. Biochim Biophys Acta. 1984;769:57-66.
14. Boggs JM, Moscarello MA, Papahadjopoulos D. Phase separation of acidic and neutral phospholipids induced by human myelin basic protein. Biochemistry. 1977;16:5420-5426.
15. Suzuki T, Komatsu H, Miyajima K. Effects of glucose and its oligomers on the stability of freeze-dried liposomes. Biochim Biophys Acta. 1996;1278: 176-182.
16. Lo YL, Rahman YE. Protein location in liposomes, a drug carrier: a prediction by differential scanning calorimetry. J Pharm Sci. 1995;84:805-814.
17. Seki K, Tirrell DA. pH-Dependent complexation of poly(acrylic acid) derivatives with phospholipid vesicle membranes. Macromolecules. 1984;17:1692-1698.
18. Gibbott P. Principles and Applications of Thermal Analysis. Oxford: Wiley-Blackwell; 2008.
19. Papahadjopoulos D, Mocarello M, Eylar H, Isac T. Effects of proteins on thermotropic phase transitions of phospholipid membranes. Biochim Biophys Acta. 1975;401:317-335.
20. Suurkuusk J, Lentz BR, Barenholz Y, Biltonen RL, Thompson TE. A calorimetric and fluorescent probe study of the gel-liquid crystalline phase transition in small. single-lamellar dipalmitoylphosphotidylcholine vesicles. Biochemistry. 1976;15:1393-1401.
21. Chiu MH, Prenner EJ. Differential scanning calorimetry: An invaluable tool for a detailed thermodynamic characterization of macromolecules and their interactions. J Pharm Bioallied Sci. 2011;3: 39-59.
22. Boggs JM, Wood DD, Moscarello MA. Hydrophobic and electrostatic interactions of myelin basic protein with lipid. Participation of N-terminal and C-terminal portions. Biochemistry. 1981;20: 1065-1073.
23. Boggs JM, Rangaraj G, Moscarello MA, Koshy KM. Interaction of myelin basic proteins and polylysine with synthetic species of cerebroside sulfate. Biochim Biophys Acta. 1985;816:208-220.
24. Morone N, Ueda T, Tsudo Y, Okumura Y, Rosilio V, Baszkin A, *et al*. Surface pressure analysis of poly(ethylene oxide)-modified fusogenic liposomes incorporated into a phospholipid monolayer. J Bioact Compat Pol. 2007;22:5-18.
25. Bai G, Nichifor M, Bastos M. Association and Phase Behavior of Cholic Acid-Modified Dextran and Phosphatidylcholine Liposomes. J Phys Chem. 2010;1:932-936.
26. Mouristen OG, Biltonen RL. Protein- lipid interactions and membrane heterogenity. In: Wattts A, editor. Protein-Lipid Interactions.Amsterdam: Elsevier; 1993. p. 1-34.
27. Cohen S, Carmen Bano M, Chow M, Langer R. Lipid-alginate interactions render changes in phospholipid bilayer permeability. Biochim Biophys Acta. 1991;1063:95-102.
28. Gasset M, Onaderra M, Martinez del Pozo A, Schiavo G-P, Usobiaga P, Gavilanes JG. Effect of the antitumour protein a-sarcin on the thermotropic behaviour of acid phospholipid vesicles. Biochim Biophys Acta. 1997;1068:9-16.