Small-angle neutron scattering studies of novel non-ionic surfactant vesicles

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ABSTRACT

Small angle neutron scattering (SANS) studies were carried out on potential drug delivery systems formed from aqueous dispersions of PEGylated lipids (**1**-**3**), below and above the phase transition temperature of each amphiphile. The mean size of the liposome is smallest for the shortest hydrophobic chain lipid and it increases with the increase in the length of the chain. The bilayer thickness of the vesicles has been found to be same (40±2 Å) for all the three non-ionic surfactants. There is significant decrease in the mean size of vesicles above the transition temperature as compared to that below the transition temperature.

INTRODUCTION

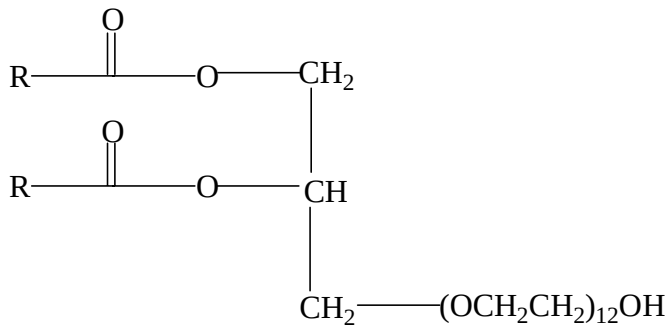
Vesicles are widely used as adjuvants and drug carrier systems in many areas of pharmaceutical technology and controlled drug delivery [1-2], for designing reaction specific catalyst [3], and in the photochemical solar energy conversion [4]. Vesicles have been found to enhance bioavailability for not only lipophilic drugs (which presumably possess some affinity for the hydrophobic bilayer) but also for polar drugs [5] and macromolecules including peptides and proteins.

The efficacy of drug carriers including vesicles can be improved by coating them with certain polymers, thus increasing their longevity and stability in the circulation, improve their biodistribution and achieve a targeting effect [6-7]. Polyethylene glycol (PEG) is an inert biocompatible polymer and is frequently used to build hydrophilic coating, since this polymer is known to be well soluble, highly hydrated and able to serve as an efficient steric protector for vesicles, micelles, nanoparticles and nanocapsules [8,9,10,11]. When administered for the purpose of drug delivery, polyethylene glycol head group in the vesicles act as shielding to reduce interactions with serum components and the reticuloendothelial system (RES) [12].

In our efforts to develop long circulating targeted pharmaceutical carriers for drugs and diagnostics that share the advantages, but none of the disadvantages of related systems termed “Stealth” vesicles [13], we have synthesized a series of synthetic polyethyleneglycolated (PEG-ylated) surfactants with general structure 1,2-diacyl-*rac*-glycerol-3-polyoxyethylene glycol (Fig.1). These lipids do not contain the typical phosphate ester head group (and are hence nonphospholipidic) and are non-ionic. When in presence of an active ingredient (drug or a cosmetic ingredient), the drug was encapsulated as the surfactants formed vesicles spontaneously [14]. Such spontaneous vesiculation, has earlier been observed with certain other lipids that do not contain a

polyethylene glycol (PEG) head group [15,16,17,18,19]. We have also shown that skin penetration profile for drugs encapsulated in these vesicles are significantly different from those in conventional vesicles or emulsions [20].

Small-angle neutron scattering (SANS) studies have been performed to investigate the three dimensional structures of the vesicles formed from these nonionic surfactants (**1**-**3**). The three amphiphiles used in this study, differ in their hydrophobic chain length. The lipids used in this work are 1,2-distearoyl-*rac*-glycerol-3-dodecaethylene glycol, **1** (GDS-12), 1,2-dioleoyl-*rac*-glycerol-3-dodecaethylene glycol, **2** (GDO-12) and 1,2-dimyristoyl-*rac*-glycerol-3-dodecaethylene glycol, **3** (GDM-12). SANS is an ideal method to study the structure of vesicles. It is suitable to study the averaged structure of the vesicles over a much larger sample volume and under actual sample conditions [21]. Herein, we present the characterization of individual vesicular suspensions prepared from lipids **1-3** using small-angle neutron scattering (SANS) studies that has been used as a technique to investigate aggregation properties of phospholipid suspensions [22,23], gemini surfactants [24], as well as other PEGylated lipids [25].



1: R = $(CH_2)_{16}$-CH_3
2: R = $(CH_2)_7$-CH═CH-$(CH_2)_7CH_3$
3: R = $(CH_2)_{12}$-CH_3

Fig. 1

MATERIALS and METHODS

The nononionic surfactants were used as synthesized by Global Seven Inc. (Paterson, NJ, USA). To prepare the surfactant dispersions, 10 mg of the surfactant was taken in a vial and dispersed in 5 ml of D_2O (99.9 %D). These surfactant dispersions (2 mg/ml) were gently shaken to give isotropic dispersions. For mixed lipid systems, 7 mg of a commercial phospholipid (Precept, SPI Group, San Leandro, CA, USA) and 3 mg of the nonionic surfactants were taken in a vial and 5 ml of D_2O was subsequently added

and shaken gently to yield a mixed dispersion. The use of D_2O instead of H_2O provides better contrast in neutron experiments. Small-angle neutron scattering experiments were carried out using SANS diffractometer at the Swiss Spallation Neutron Source SINQ, Paul Scherrer Institut [21]. The wavelength of the neutron beam was 8 Å and the experiments were performed at three different samples to detector distances of 2, 6 and 18 m to cover a wide Q range 0.002 to 0.37 $Å^{-1}$. The scattered neutrons were detected using a two-dimensional 96 cm × 96 cm detector. All measurements were made at two different fixed temperatures below (30 °C) and above (55 °C) the phase transition temperature of the surfactants. The samples were held in a quartz sample holder of thickness 1 mm. The measured SANS distributions after standard corrections and normalizations are shown in Fig 2-3.

SANS ANALYSIS

In small-angle neutron scattering, one measures the coherent differential cross section (dΣ/dΩ) per unit volume. For a system consisting of monodisperse unilamellar vesicles, dΣ/dΩ can be expressed as [26]

$$\frac{d\Sigma}{d\Omega}(Q,R) = n(\rho_v - \rho_s)^2 \left[\frac{4}{3}\pi R^3 \frac{3J_1(QR)}{QR} - \frac{4}{3}\pi (R+t)^3 \frac{3J_1[Q(R+t)]}{Q(R+t)} \right]^2 \qquad (1)$$

where n denotes the number density of the vesicles, ρ_v and ρ_s are, respectively, the scattering length densities of the vesicle bilayer and the solvent. R is the radius of the vesicle and t is the thickness of the bilayer. $J_1(x)$ is the first order Bessel function and is given by

$$J_1(x) = \frac{\sin x - x\cos x}{x^2} \qquad (2)$$

In the case of polydisperse solution, the eqn. (1) can be written as

$$\frac{d\Sigma}{d\Omega}(Q) = \int \frac{d\Sigma}{d\Omega}(Q,R)\, f(R) dR \qquad (3)$$

where $f(R)$ gives the size distribution of the vesicles. Using Schultz distribution, the polydispersity is given by [27]

$$f(R) = \left(\frac{Z+1}{R_m} \right)^{z+1} R^Z \exp\left[-\left(\frac{Z+1}{R_m} \right) R \right] \frac{1}{\Gamma(Z+1)} \qquad (4)$$

where R_m is the mean of the distribution and Z is the width parameter. The relative spread in the size distribution is given by

$$\frac{\Delta R}{R_m} = \frac{1}{(Z+1)^{1/2}} \qquad (5)$$

In actual SANS experiments however, the measured scattering [$I(Q)$] is a convolution of the true scattering function ($d\Sigma/d\Omega$) of the system with the resolution function of the instrument and therefore it is calculated as [28]

$$I(Q) = \int \frac{d\Sigma}{d\Omega}(Q')R(Q-Q')dQ' \qquad (6)$$

where $R(Q\text{-}Q')$ is the instrumental resolution function centered at Q.

In eqn. (1), the scattering from monodisperse vesicles is characterized by a rapidly decreasing function modulated by two Q dependent oscillations: a fast oscillation which depends on the radius of vesicles with a period of $2\pi/R$ and a slower oscillation which depends on the membrane thickness with a period of $2\pi/t$ [22, 23]. SANS measurements (Figs. 2-3) do not show fast oscillations as predicted by Eq. (1). This may be due to either polydispersity in vesicle size or smearing by the instrumental resolution. We have used Eq. (6) for the analysis of data, which takes account of both polydispersity and the resolution effects. The mean radius (R_m), polydispersity ($\Delta R/R_m$) and bilayer thickness (t) are the fitting parameters in the analysis. The resolution function has been calculated using the procedure as described earlier [28].

RESULTS and DISCUSSION

Fig. 2 shows the SANS data from vesicular suspensions (2 mg/ml) of nonionic surfactants at temperatures below and above the transition temperature T_m. The characteristics of SANS data are similar to those, which have been observed in earlier SANS experiments on vesicular suspensions of phospholipid, dipalmitoylphosphatidylcholine (DPPC) [23]. In the low Q region (<0.05 Å $^{-1}$), the scattering intensity decreases as a function of Q in almost a straight line and it indicates the large sizes of vesicles in these systems. For large size vesicles, the intensity decreases as $1/Q^2$ in the low Q region. At higher Q values (>0.05 Å $^{-1}$), there is increase in the drop of the intensity and a minimum is observed, which depend on the thickness of the bilayer. The data have been analyzed in terms of Eq. (6), which takes account of both polydispersity of vesicles and the resolution effects. The fitted parameters are given in Table 1. It is found that the vesicular sizes are in the order of GDS-12> GDO-12> GDM-12. That is, the mean vesicle size is smallest for the shortest hydrophobic chain lipid and it increases with the increase in the length of the chain. It is interesting to note that the bilayer thickness of the vesicles has been found to be same (40±2 Å) for all the three non-

ionic surfactants irrespective of the different hydrophobic structure and chain length of the surfactant. This suggests the different conformation of the hydrophobic chains in their bilayer.

Fig. 3 shows the SANS data from a mixture of phosphatidyl choline (precept) and the nonionic surfactant below and above the phase transition temperatures of the amphiphiles. When the precept is added to the lipid solutions, the bilayer thickness of the vesicles remains the same (Table 2). The size of the vesicles decreases with the addition of the precept. When the lipid solutions with precept are heated to 55 ^{o}C above the transition temperature, both the bilayer thickness and the size of the vesicles remain the same.

Thus the present SANS experiments provide additional information to the earlier optical microscopic experiments on these systems. SANS is suitable over these techniques to study the averaged structure of the vesicles over a much larger sample volume and under actual sample conditions. This makes SANS technique particularly useful to study the structural changes in vesicles at the transition temperature. SANS also gives the value of the bilayer thickness for which optical microscopy is usually not sensitive. In the case of x-rays study on cast films of vesicles, the results are obtained on multilamellar vesicles in terms of the repeat distance between the bilayers, which also includes a water layer in addition to the actual bilayer thickness. However, in SANS measurements from the unilamellar vesicles, one directly obtains the value of the bilayer thickness.

CONCLUSION

SANS measurements from dispersions of new synthetic PEGylated lipids indicate formation of spontaneous vesicles upon addition of water. The vesicles formed without heat, cool or sonication methods were predominantly unilamellar and polydispersed. The SANS technique is non-invasive and provides direct and detailed information on vesicular mean diameter and bilayer widths. Applications of these lipid systems in drug and cosmetic delivery is currently being investigated.

Table 1. The fitted parameters of the vesicles of PEGylated non-ionic surfactants at below and above the transition temperature.

System	Mean Radius (Å)	Polydispersity (%)	Bilayer Thickness (Å)
GDS-12 (T=30 ^{o}C)	1500±100	50±5	40±2
GDS-12 (T=55 ^{o}C)	1200±100	50±5	40±2
GDO-12 (T=30 ^{o}C)	850±50	40±2	40±2
GDO-12 (T=55 ^{o}C)	700±50	40±2	40±2
GDM-12 (T=30 ^{o}C)	750±50	35±2	40±2
GDM-12 (T=55 ^{o}C)	650±50	35±2	40±2

Table 2. The fitted parameters of the vesicles of PEGylated non-ionic surfactants mixed with precept at below and above the transition temperature.

System	Mean Radius (Å)	Polydispersity (%)	Bilayer Thickness (Å)
GDS-12 (T=30 ^{o}C)	1100±100	50±5	40±2
GDS-12 (T=55 ^{o}C)	1100±100	50±5	40±2
GDO-12 (T=30 ^{o}C)	750±50	40±2	40±2
GDO-12 (T=55 ^{o}C)	750±50	40±2	40±2
GDM-12 (T=30 ^{o}C)	700±50	35±2	40±2
GDM-12 (T=55 ^{o}C)	700±50	35±2	40±2

FIGURE CAPTION

Fig. 1. General structure of 1,2-diacyl-*rac*-glycerol-3-polyoxyethylene glycol

Fig. 2. SANS data from 2 mg/ml vesicular solutions of PEGylated non-ionic surfactants. The measurements are made for two temperatures (30 and 55 ^{o}C) below and above the transition temperature. For clarity the data of 55 ^{o}C are shifted vertically by multiplying 10.

Fig. 3. SANS data from 2mg/ml vesicular solutions of PEGylated non-ionic surfactants mixed with precept in the weight ratio of 3:7. The measurements are made for two temperatures (30 and 55 ^{o}C) below and above the transition temperature. For clarity the data of 55 ^{o}C are shifted vertically by multiplying 10.

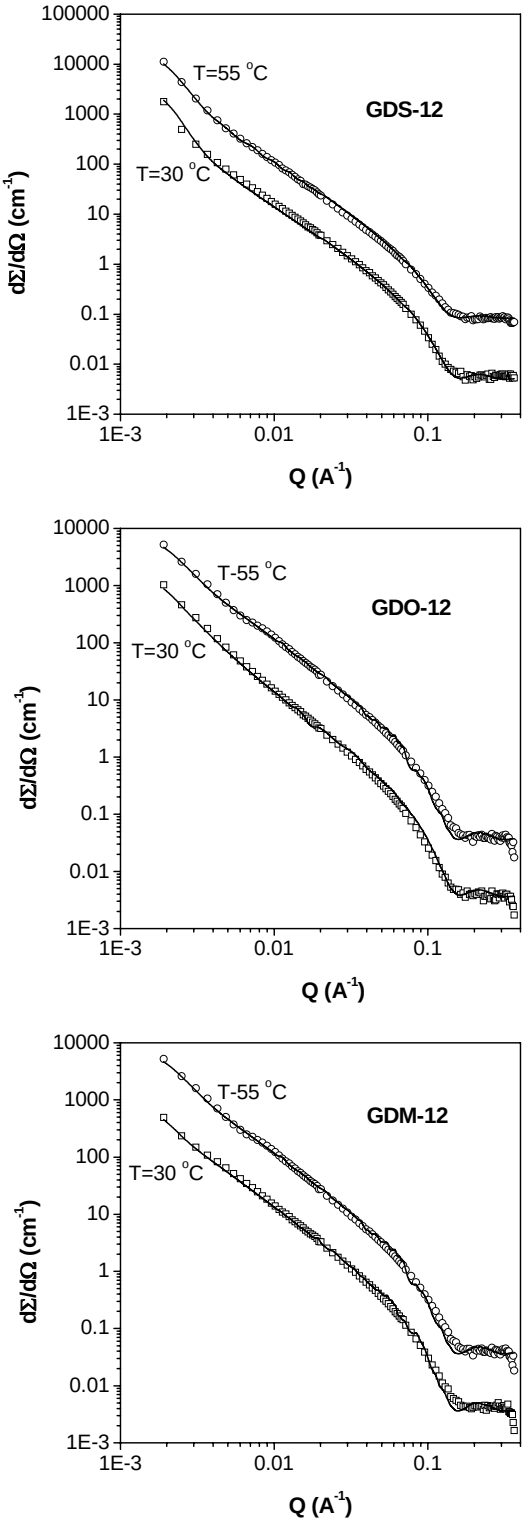

GDS-12
T=55 °C
T=30 °C
dΣ/dΩ (cm-1)
Q (A-1)
GDO-12
T-55 °C
T=30 °C
dΣ/dΩ (cm-1)
Q (A-1)
GDM-12
T-55 °C
T=30 °C
dΣ/dΩ (cm-1)
Q (A-1)

Fig. 2

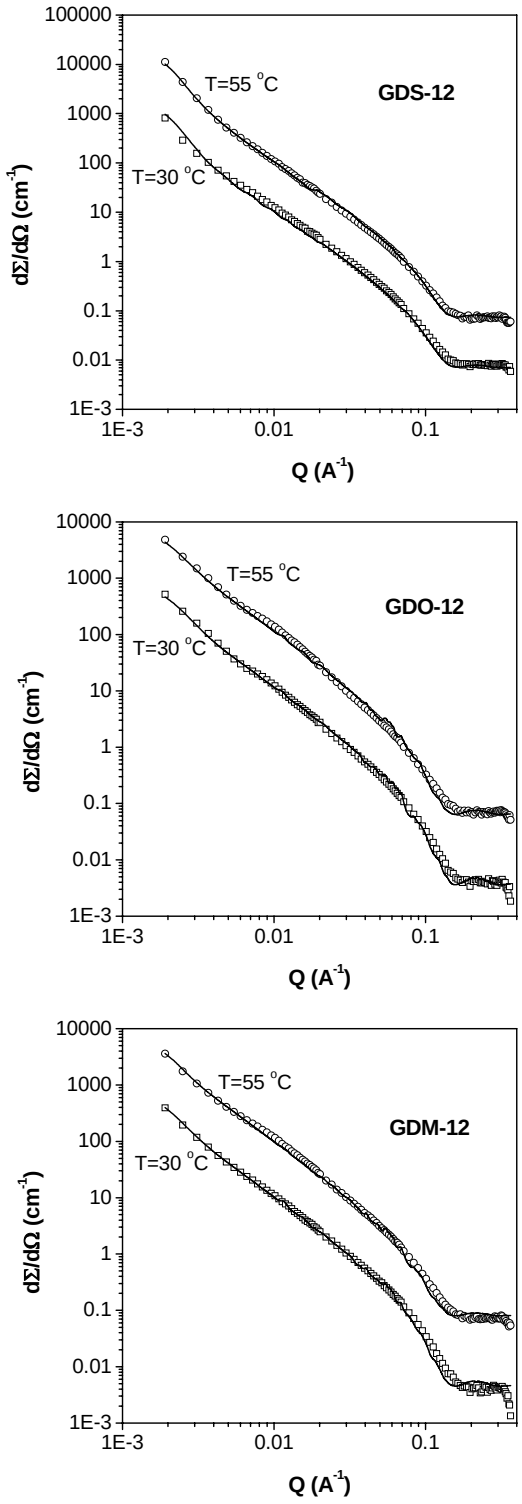

GDS-12
T=55 °C
T=30 °C
GDO-12
T=55 °C
T=30 °C
GDM-12
T=55 °C
T=30 °C
dΣ/dΩ (cm-1)
Q (A-1)
100000
10000
1000
100
10
1
0.1
0.01
1E-3
0.01
0.1

Fig. 3

REFERENCES

[1] D. Attwood, A. T. Florence (Eds.), Surfactant Systems, Chapman and Hall, London, (1983).

[2] G. Gregoriadis (Ed.), Liposomes as Drug Carriers: Recent Trends and Progress, Wiley, Chichester, (1988).

[3] P. Scrimin, P. Tecilla, U. J. Tonellato, Am. Chem. Soc. 114 (1992) 5086.

[4] A. Kay, M. Gratezel, J. Phys. Chem. 97 (1993) 6272.

[5] S. Muranishi, Crit. Rev. Ther. Drug Carrier Syst. 7 (1990) 1.

[6] H. Takeuchi, H. Kojima, H. Yamamoto, Y. Kawashima, J. Control. Release 68 (2000) 195.

[7] H. Takeuchi, H. Kojima, H. Yamamoto, Y. Kawashima, J. Control. Release 75 (2001) 83.

[8] G. S. Kwon, K. Kataoka, Adv. Drug. Deliv. Rev. 16 (1995) 295.

[9] G. S. Kwon, Crit. Rev. Ther. Drug Carrier Syst. 15 (1998) 481.

[10] D. D. Lasic, F. Martin (Eds.), Stealth Liposomes, CRC Press, Boca Raton, FL, 1995.

[11] V. P. Torchilin, M. I. Papisov, J. Liposome, Res. 4 (1994) 725.

[12] A. Vertut-Doi, H. Ishiwata, K. Miyajima, Biochim. Biophys. Acta 1728 (1996) 19.

[13] M. J. Lawrence, S. Chauhan, S. M. Lawrence, D. J. Barlow, STP Pharma Sci. 6 (1996) 49.

[14] D, D. Lasic, R. Joannic, B. C. Keller, P. M. Frederik, L. Auvray, Adv. Coll. Interface Sci. 89-90 (2001) 337.

[15] P. L. Luisi, P. Walde (Eds), Giant Vesicles, Wiley Europe, 1999.

[16] S. Takajo, H. Nagano, O. Dannenmuller, S. Ghosh, A. M. Albrecht, Y. Nakatani, G. Ourisson, New J. Chem. 25 (2001) 917.

[17] T. Eguchi, K. Arakawa, K. Kakinuma, S. Ghosh, Y. Nakatani, G. Ourisson, Chem. Eur. J. 6 (2000) 3351.

[18] D. Kitamoto, S. Ghosh, G. Ourisson, Y. Nakatani, Chem. Commun. 10 (2000) 861.

[19] S. Ghosh, S. J. Lee, K. Ito, K. Akiyoshi, J. Sunamoto, Y. Nakatani, G. Ourisson, Chem. Commun. (2000) 267.

[20] S. Ghosh, R. Wester, S. Barbadillo, M. J. Choi, X. Hui, H. Maibach, Unpublished results, 2003.

[21] J. Kohlbrecher, W. Wagner, J. Appl. Cryst. 33 (2000) 804.

[22] J. Pencer, F. R. Hallett, Phys. Rev. E 61 (2000) 3003.

[23] P. C. Mason, B. D. Gaulin, R. M. Epand, G. D. Wignall, J. S. Lin, Phys. Rev. E 59 (1999) 3361.

[24] V. K. Aswal, J. Haldar, S. De, P. S. Goyal, S. Bhattacharya, Phys. Chem. Chem. Phys. 5 (2003) 907.

[25] G. Ma, D. J. Barlow, M. J. Lawrence, R. K. Heenan, P. Timmins, J. Phys. Chem. B 104 (2000) 9081.

[26] S. H. Chen, T. L. Lin, D. L. Price, K. Skold (Eds.), Methods of Experimental Physics B, Academic Press, New York, 23 (1987) 489.

[27] V.K. Aswal, P.S. Goyal, P. Thiyagarajan, J. Phys. Chem. B 102 (1998) 2469.

[28] J. S. Pedersen, D. Posselt, K. Mortensen, J. Appl. Cryst. 23 (1990) 321.