DIELECTRIC AND RHEOLOGICAL PROPERTIES OF COLLAGEN-DPPC LIPOSOMES

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Abstract. Interaction between liposomes and protein is important for the structure and function of cells. In the present work, the interaction between collagen and dipalmitoyl phosphatidylcholine (DPPC) liposomes was studied using dielectric spectroscopy technique and rheological measurements. Dielectric data indicated that the DPPC liposomes have strong dielectric dispersion in the frequency range of 50 Hz to 5 MHz. The conductivity and relaxation time increased after addition of collagen to DPPC liposomes. The increase in relaxation time might be attributed to increase in the localized charges distribution within the medium confirmed by the conductivity data. The increase in permittivity over the frequency range tested is an indicator of the interaction between protein and liposomal phospholipid. This is either due to the insertion of collagen into the lipid bilayer via its hydrophobic residues or to adsorption causing a protein layer to be located around liposomes. Interaction between collagen and liposomes increases the liposomal membrane rigidity and increases its microviscosity as indicated from rheological measurements. It was concluded that collagen significantly altered the physical state of liposome membrane, which may be attributed to collagen interaction with liposomes surface and/or by its incorporation within the bilayer membrane.

Key words: collagen, liposome, dipalmitoyl phosphatidylcholine, dielectric, rheological.

INTRODUCTION

Dielectric spectroscopy technique has long being known as a powerful tool in the study of heterogeneous systems such as colloidal particle dispersions [17]. When it applied to biological systems, these methods can provide valuable knowledge about different biological cell structures, their functions and metabolic activities. It has been successfully employed to investigate the structure and transport properties of cell membranes and lipid bilayers [1, 4].

Dielectric analysis of the systems consisting of phospholipids is a complicated issue in which the amphiphilic nature of these molecules is a crucial factor determining the interaction within the membrane, i.e., lipid double layer as

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well as interaction with surrounding medium (most often water). In particular, dielectric spectroscopy measurements on liposome suspensions (as a biological membrane bilayer model) have been recently advantageously used to investigate the molecular order and the reorientational motions of lipid head groups at the bilayer surface. These measurements are rapid, sensitive and noninvasive.

Liposomes are excellent models of biomembranes, in particular, to study interactions between different lipids and membrane proteins, offering the unique advantage that the lipidic composition of the bilayer can be varied in a well-defined and controlled way. Besides their structural resemblance, they have physical properties similar to those of biomembranes, including thickness, water permeability, bending rigidity, surface tension, and viscosity. Thus, they provide a unique opportunity to investigate certain physiological functions and processes in biological membranes.

Collagen is a protein with various industrial and medical applications [3]. It is a potentially useful biomaterial because it is a major structural component of many tissues such as those of the skin, bone, cartilage, tendons, and basement membranes. Several methods have been developed to improve the ability of collagen to stabilize liposomes [8, 10, 15, 16, 21]. There are numerous observations on associations between collagen fibres and lipids in both normal and pathological tissues [19]. Fonseca *et al.* [5] found that coating of liposomes with collagen results in both a higher stability *in vitro* and a selective and almost immediate accumulation of vesicles in liver Kupffer cells; therefore, these liposomes might be effective in the treatment of some infectious diseases located within macrophages.

The objective of the present experimental work is to investigate the electrical permittivity (ε'), conductivity (*s*) and loss factor (tanδ) for DPPC and collagen-DPPC liposomes in the frequency range of 50 Hz to 5 MHz at room temperature. In addition to, the effect of collagen on the rheological properties of DPPC liposomes at room temperature was investigated.

MATERIALS AND METHODS

MATERIALS

L-α-Dipalmitoyl phosphatidylcholine (DPPC) specified 99% pure, and type-I collagen from bovine, were purchased from Sigma (St. Louis, Mo, USA). Organic solvents (acetic acid, chloroform, and ethanol) were of analytical grade and obtained from Merck. Water used was double distilled in a glass apparatus (final distillation over alkaline $KMnO_4$).

PREPARATION OF LIPOSOMES

The liposomes used in this work were prepared from DPPC using Bangham method [2]. The lipids must first be dissolved and mixed in chloroform/ethanol (5:1, v/v) to ensure a homogeneous mixture of lipids. The organic solvent should be removed by rotary evaporation to obtain a thin lipid film formed on the sides of a round bottom flask. The lipid film is thoroughly dried to remove residual organic solvent by placing the flask on a vacuum pump overnight. Hydration of the dry lipid film is accomplished simply by adding a Hebes buffer solution (10 mM, pH 7.4) to the container of the dry lipid film and agitating at a temperature above the phase transition temperature of the lipid.

COLLAGEN DPPC LIPOSOMES

Collagen (0.8 g) was dissolved in 1% (w/v) acetic acid solution (100 mL) by using a magnetic stirrer at room temperature to obtain a collagen solution of concentration 0.8%, which was used throughout the study. Collagen solution was to DPPC liposomes under magnetic stirring at room temperature. After addition of the collagen, the mixture was left under stirring for approximately 1 hour then incubated overnight at –4 ^{o}C.

THE ELECTRICAL PARAMETERS

The electrical parameters were measured in the frequency range of 50 Hz up to 5 MHz using a Wayne Kerr precision component analyzer, model 6440 B (UK). The sample cell has two squared platinum black electrodes each having an area of 1 cm^2 with an inner electrode distance of 1 cm. The measurements were performed at 20°C. For a dielectric material placed between two parallel plate capacitors, the measured values of capacitance (C) and resistance (R) were used to calculate the real (ε') and imaginary part (ε'') of the complex permittivity $\varepsilon^* = \varepsilon' - j\varepsilon''$, while conductivity ($s$) and the relaxation time (τ) were calculated using the following equations [6]:

$$\varepsilon' = \varepsilon_0 C_k; \quad k = 1 \text{ cm}^{-1} \tag{1}$$

where k is the cell constant which depends on the cell dimensions.

Loss tangent:

$$\tan\delta = \varepsilon''/\varepsilon' = 1/2\pi fRC \text{ so, } \varepsilon'' = \varepsilon' \tan\delta \tag{2}$$

The conductivity:

$$s = k/R \ (\Omega^{-1}\text{m}^{-1}) \tag{3}$$

$$\text{Relaxation time} = 1/2\pi f_c \quad (4)$$

where f_c is the critical frequency corresponding to the midpoint of the dispersion curve.

If any dielectric material is introduced between the two plates, the corresponding response to a sinusoidal field will be characterized by dielectric properties (dielectric permittivity ε' and conductivity *s*) which vary with frequency [7, 9, 14, 18]. The charge and current densities induced in response to an applied electric field is an example of an idealized parallel plate.

RHEOLOGICAL MEASUREMENT

These rheological parameters were measured using Brookfield LVDV-III programmable rheometer (cone-plate viscometer, Brookfield Engineering Laboratory, Incorporation, Middleboro, USA, supplied with circulating water bath controlled by a computer. The rheometer was guaranteed to be accurate within ±1% of the full scale range of the spindle/speed combination in use reproducibility is within ± 0.2%. Triplicate samples of DPPC and collagen-DPPC liposomes were analyzed at room temperature using CP.40 cone-plate geometry (cone angle = 0.8º and diameter = 2.4 cm). The range of shear rate from 35 to 485 sec^{-1} was used. Rheological parameters were measured at room temperature of 30 °C. The flow curves were plotted between shear stress (dyne/cm^2) and shear rate (s^{-1}) for each liposomal sample. Plastic viscosity, yield stress and flow index were calculated from the linear fitting of the flow curves [11–13, 20].

RESULTS AND DISCUSSION

Figure 1 shows the variation of dielectric permittivity (ε') as a function of frequency in the range of 50 Hz to 5 MHz for DPPC liposomes samples in the presence and absence of collagen. The presented dielectric data indicates that DPPC liposomes have strong dielectric dispersion corresponding to the alpha relaxation region in the examined frequency range which identified as anomalous frequency dispersion. The dispersion is considered to be related to the ionic species around the vesicle surface. A rapid increase in the dielectric permittivity for collagen-DPPC liposomes may be attributed to the tendency of dipoles in phospholipids to orient themselves in the direction of the applied field in the low-frequency range. However, in the high-frequency range the dipoles will hardly be able to orient themselves in the direction of the applied field and hence the value of the dielectric permittivity is nearly constant. Moreover, the conductivity (*s*) of DPPC liposomes and collagen-DPPC liposomes increased as the frequency increased (Fig. 2). At high frequency, the conductivity increased after incubation of DPPC liposomes with collagen.

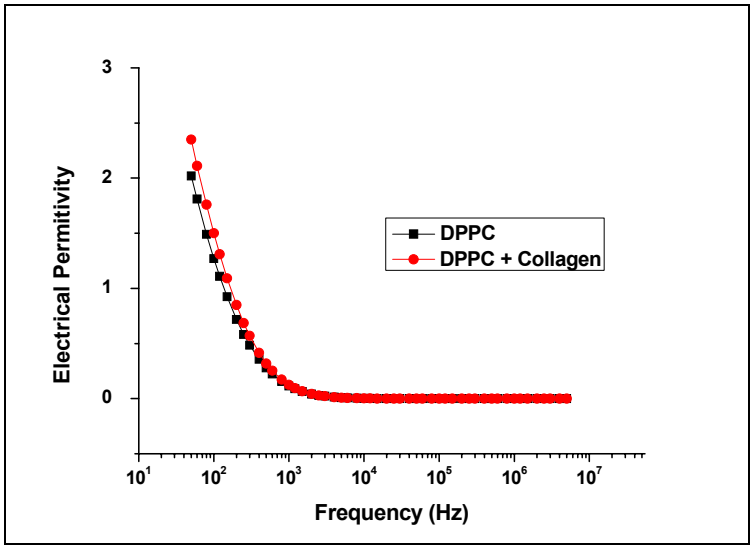

Fig. 1. Electrical permittivity ε' as function of the applied frequency in the range of 50 Hz to 5 MHz for DPPC (■) and collagen-DPPC liposomes (●).

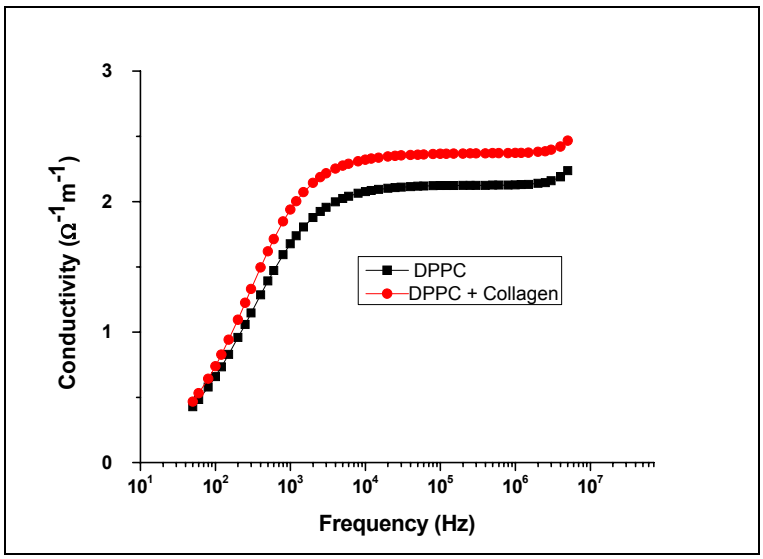

Fig. 2. Electrical conductivity (*s*) as function of the applied frequency in the range of 50 Hz to 5 MHz for DPPC (■) and collagen-DPPC liposomes (●).

The variation of loss factor (tanδ) as a function of frequency for the DPPC liposomes and collagen containing DPPC liposomes is shown in Figure (3). It is clear that DPPC liposomes show a relaxation process. The relaxation time was found to be increased from 0.637 μs for DPPC liposomes to 1.327 μs after addition

of collagen to DPPC liposomes as calculated from the data in Figure 3. The increase in relaxation time is due to an increase in the localized charges distribution within the medium, that may attributed to the redistribution of collagen molecules within liposome membrane or on its surface. Since the increase in relaxation time would cause an increase in the dipole moment of liposomal lipid molecule a higher electric conduction was expected for collagen containing liposomes due to the increase of the surface charge density of liposome lipid molecule. These results are in agreement with that obtained from the conductivity data (Fig. 3).

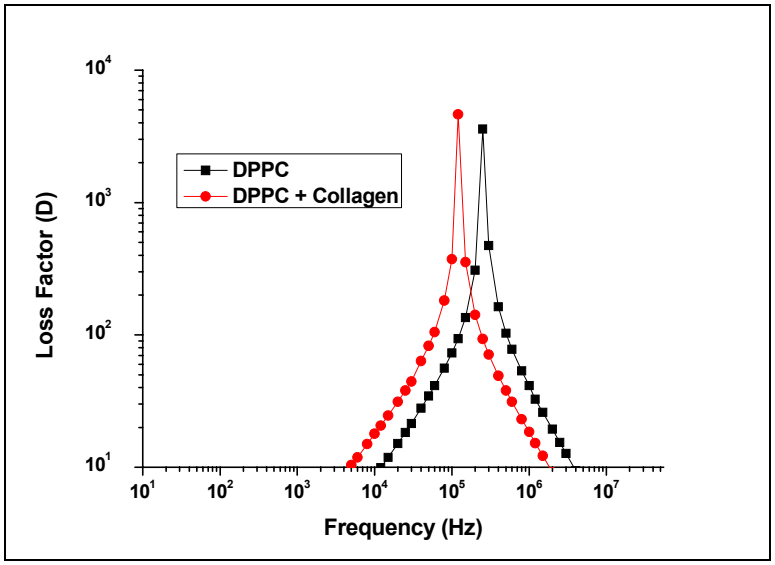

Fig. 3. The variation of loss factor (tanδ) with the applied frequency in the range of 50 Hz to 5 MHz for DPPC (■) and collagen-DPPC liposomes (●).

The results herein presented clearly demonstrate the existence of an interaction between monomeric type I collagen and phosphtidylcholine vesicles. This interaction results in the formation of a protein-phospholipid complex. We considered that the potential contribution of this hydrophobic domain to the interaction of collagen with phospholipids vesicles. The phospholipid vesicle-collagen complex was proposed to be maintained by electrostatic interaction between the zwitterionic polar heads of phosphatidylcholine, the phospholipids used for such a study, and the amino acid side chains of the protein. Collagen molecules were expected to penetrate into the lipid bilayer in a parallel orientation and interact with the alkyl chains of DPPC with the hydrophobic residues on the collagen molecule surface.

The rheological properties of liposomes were measured to enable evaluation of the interaction between collagen and DPPC liposomal membranes. Figure 4 shows the flow curves for DPPC and collagen-DPPC liposomal samples.

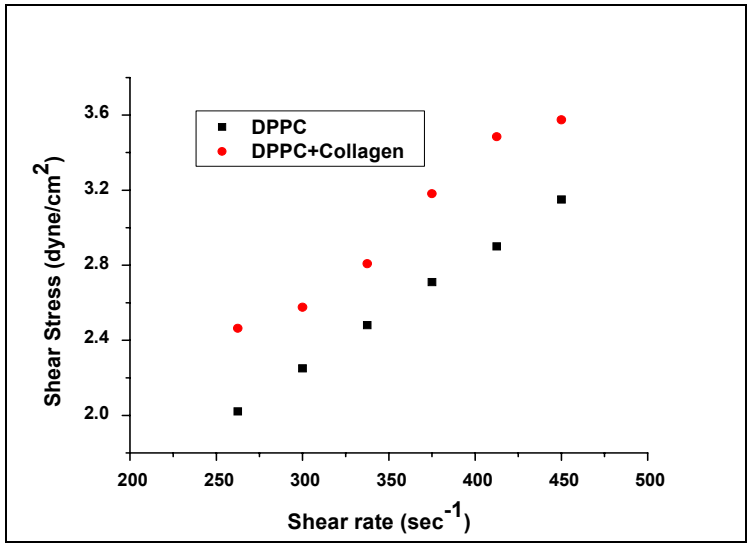

Fig. 4. The relation between shear rate and shear stress for DPPC (■) and collagen-DPPC liposomes (●) at room temperature.

Equation (5) was used to fit the experimental data from the different samples, and the rheological parameters are shown in Table 1.

$$\tau = k\,\gamma^{n} \tag{5}$$

where τ is the shear stress, k is the consistency index, γ is the shear rate, and n is the flow behavior index. In order to obtain the flow behavior index (n) and consistency index (k) values, equation (5) was used to fit the experimental data of different liposomal formulations (Table 1).

The plastic viscosity (a measure of the internal resistance to fluid flow of a Bingham plastic, expressed as the tangential shear stress in excess of the yield stress divided by the resulting rate of shear), yield stress (dyne/cm^2) (the minimum stress needed to cause a Bingham plastic to flow), consistency index k (an indication of the viscous nature of liposomes), the flow behaviour index (n) (a measure of departure from Newtonian flow) [12, 13].

The plastic viscosity of DPPC liposomes is increased for collagen-DPPC liposomes (from 0.59 to 0.65 cP), which indicates that the membrane rigidity is increased. Based on these results of liposomal rheological properties, it can be concluded that interaction between collagen and DPPC phospholipid would cause an increment of the DPPC liposomes plastic viscosity and an increase of the membrane rigidity. This may be due to an interaction (simple surface binding) between the triple helix of type-I collagen and phosphatidylcholine liposomes resulting in relative immobilization of the phospholipid molecules around the protein and consequently a decrease in their fluidity and increases the liposome

microviscosity. The flow index (n) values ranged from 0.76 to 0.77, respectively. Liposome suspension exhibited a pseudoplastic behavior because the values of the flow behavior index (n), a measure of departure from Newtonian flow, were < 1.

Table 1

Power law parameters of rheological measurements of different liposmal samples

Sample	Plastic viscosity (cP)	Yield stress (dyne/cm^2)	Flow index
DPPC	0.59	0.47	0.76±0.042
DPPC+Collagen	0.65	0.657	0.77±0.069

CONCLUSIONS

The interaction between collagen and DPPC lipid will shed light on the mechanism by which collagens will interact with lipids either in natural tissues or in artificial drug delivery systems. Dielectric and rheological measurements can be used to investigate the effects of electrostatic interactions on the lipidic structure and on the dynamics of formation of the lipid vesicles. Investigation of these effects could represent the key to understand the mechanisms that allow biological cell to control membrane organization. These results indicate that a lipid-collagen complex could help in the design and development of improved liposomal drug delivery systems either in cosmetology, clinical, biotechnological fields or pharmacology.

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