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An investigation into the distribution of lecithins in nanosuspension systems using low frequency dielectric spectroscopy

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Abstract

A range of nanosuspensions comprising a model drug (RMKP 22) with varying concentrations of Phospholipon 90 were prepared using high pressure homogenization and analyzed using low frequency dielectric spectroscopy as a novel means of characterizing the distribution of Phospholipon 90 within the suspensions. A corresponding range of aqueous Phospholipon 90 suspensions were also studied for the purpose of comparison. The dielectric responses were interpreted using a modification of the Maxwell–Wagner approach, whereby the systems were considered to comprise a high frequency response corresponding to the bulk layer serially connected to a lower frequency response corresponding to an electrode barrier layer. The low frequency responses of both the Phospholipon 90 dispersions and the nanosuspensions were found to be essentially independent of phospholipid concentration, indicating the presence of a barrier layer covering the electrode surfaces. In contrast, the high frequency (bulk) loss response was found to increase with Phospholipon 90 concentration for the surfactant suspensions while a maximum in response was seen with Phospholipon 90 concentration for the nanosuspensions; this behaviour was attributed to the presence of impurities within the phospholipids. Based on this investigation, a model is proposed with which the dielectric response may be related to the surface coverage of the suspended drug particles. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Nanosuspension; Dielectric spectroscopy; Nanoparticle-liposome; High pressure homogenization; Phospholipid

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1. Introduction

Poor bioavailability is a commonly encountered problem in the development of pharmaceutical dosage forms, particularly when the active ingredient has a low solubility in water. An alternative formulation strategy for i.v. administration of

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poorly soluble drugs (Müller and Peters, 1998; Müller et al., 1995) as well as for oral application with increased bioavailability (Peters and Müller, 1996) is the nanosuspension, an aqueous suspension containing drug particles with a mean diameter of approximately 200–900 nm in varying drug concentrations, stabilized by surfactants. This paper describes the novel use of low frequency dielectric spectroscopy as a means of investigating the distribution of Phospholipon 90 within RMKP 22 systems, thereby leading to new insights into the mechanisms associated with the stabilization effects of this surfactant.

Dielectric spectroscopy involves the application of an alternating electric field and the measurement of the response in terms of the real and imaginary components over a range of frequencies, from which information on the structure and behaviour of the material may be obtained (Craig, 1995). Aqueous suspensions of phospholipids have previously been investigated using the technique for the purpose of determining macroscopic properties such as the dielectric permittivity of lipid membranes (Schwan et al., 1970; Redwood et al., 1972), obtaining information regarding the dynamics of zwitterionic head group relaxation processes in the polar region of lecithin bilayers (Kaatze et al., 1979; Kaatze and Henze, 1980; Göpel, 1982) and diagnostic information concerning the composition of the bilayer and the effects of the particle size of the liposomes, the nature of the head group and the optical activity of the phospholipid (Barker et al., 1994).

One of the principal difficulties associated with the early studies of the dielectric behaviour of phospholipids was the lack of availability of high purity materials. In the frequency region <1 MHz dielectric measurements prove to be very sensitive to certain classes of impurities in suspensions found in commercial lecithin such as fatty acids and fusogenic substances (Kremer et al., 1977). Uhlendorf (1984) investigated aqueous vesicle suspensions prepared from a commercial synthetic ester and the purer and chemically more stable ether lecithins. In addition, the ether and ester lecithins were purified by an ion-exchange procedure in methanol. A dielectric dispersion was found in the spectrum below 20 MHz down as far as 5 kHz with unpurified ester and ether lecithin; even purified ester lecithin showed a low frequency dispersion, albeit smaller than that of unpurified samples. In the case of purified ether lecithins, however, there was no indication of an α-dispersion. Following the addition of fatty acid to the ether lecithin, the system displayed a large low frequency dielectric dispersion with a similar spectrum to that of the unpurified sample. Uhlendorf (1984) suggested an interpretation of the low frequency dispersion in terms of lateral diffusion of small amounts of impurities such as lysolecithins, fatty acids and other contaminants to the vesicle shell.

Barker et al. (1994) based their investigations of liposome suspensions on theories arising from the Dissado–Hill theory (Dissado and Hill, 1979) and in particular the Hill and Pickup (1985) modification of the Maxwell–Wagner model (described below). The present study concerns the application of the Hill and Pickup (1985) approach to study the stabilizing effect of Phospholipon 90 on nanosuspensions, with particular emphasis on the interaction with the nanoparticle surface and the mobility of the lecithin molecules in the aqueous medium. The range of techniques available with which nanoparticulate systems may be studied is somewhat limited, hence it is intended that over and above the specific information regarding the suspensions under study the investigation will establish the applicability of the dielectric approach in general terms.

2. Theoretical: the Hill and Pickup modification of the Maxwell–Wagner theory

The dielectric response of a material at any single frequency may be described by

$$C^*(\omega) = C'(\omega) - iC''(\omega) \tag{1}$$

where C^* is the complex capacitance at frequency (and C' and C'' are the real and imaginary components respectively, with i being the square root of -1. The real component C' is referred to as the capacitance, while the imaginary component is referred to as dielectric loss C'' and is equal to the total conductance (G) divided by the angular frequency ω. The dielectric loss takes account of any ac or dc conductivity processes taking place within the system.

The Hill and Pickup (1985) model is applicable to heterogeneous systems and assumes the presence of a high capacitance adsorbed layer on the electrodes (the barrier layer) generated either by charge injection or by adsorption of solutes or other material from the sample, in series with a conductive bulk layer; the response may therefore be considered to be a modification of the Maxwell–Wagner model. The barrier and bulk layers may be represented by parallel resistance/capacitance circuits connected in series, whereby the resistance and capacitance elements may be dispersive.

In an ideal case, the two parallel circuits are composed of components which show no intrinsic frequency dependence. This is represented in Fig. 1a where the subscripts b and s refer to the bulk and surface layers, respectively; it may be seen that the surface and bulk responses are seen at

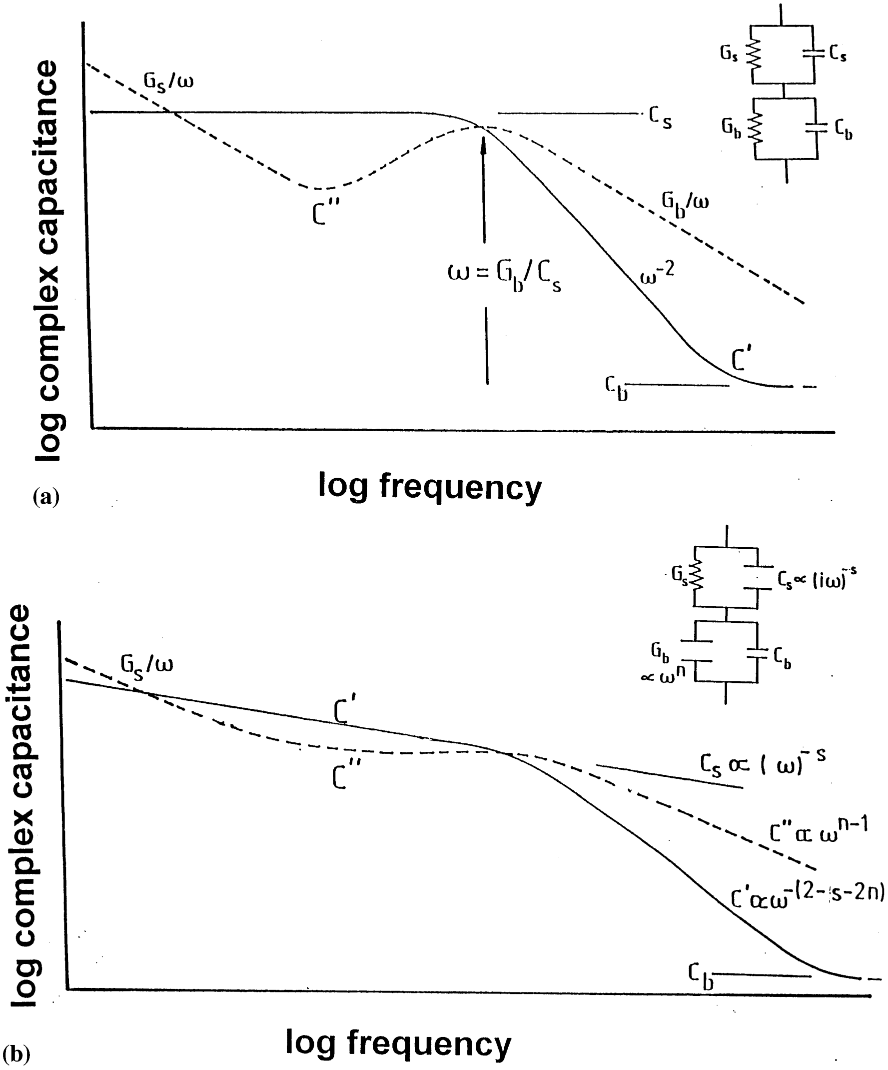

Fig. 1. Diagrammatic representation of the Maxwell–Wagner response at low frequencies in the presence of adsorbed electrode layers (a) ideal model (b) model incorporating frequency dependent (dispersive) elements. Equivalent circuits are shown inset.

low and high frequencies, respectively, due to the greater thickness of the bulk layer resulting in a lower capacitance which is seen at higher frequencies. In both the high and the low frequency regions the gradients of the $\log C'' - \log \omega$ plot are -1 (as a function of the relationship $C'' = G/\omega$), while the region of dispersion in the capacitance is characterized by an inverse square law relationship, $C' \propto \omega^{-2}$.

In practice, the response may be more complex. In the case of the modified Maxwell–Wagner response, the circuit components do not behave ideally but instead show frequency dependence; this may be described by the diagram shown in Fig. 1b. Such non-linear (power law) behaviour may be interpreted by the Dissado–Hill theory whereby the susceptibility (χ) of the sample at any frequency ε may be described in terms of two indices, m and n, via

$$\chi^2 = \frac{\chi_0}{[\omega_p/(\omega_p + i\omega)]^{n-1}} \cdot {}_2F_1\left(1-n, 1-m; 2 - n; \frac{\omega_p}{\omega_p + i\omega}\right) \qquad (2)$$

in which χ_0 is the static susceptibility, ω_p the relaxation frequency, (, ; ;) the Gaussian hypergeometric function, and $0 \leq m$, $n \geq 1$. The indices m and n describe the power law behaviour of the response and contain information concerning the structure of the material. The degree of interaction between the relaxing dipoles and their environment is measured by n, and m is a measure of the degree of cooperativity between dipoles. This approach may be applied to the response shown in Fig. 1b by considering the slope of the low frequency capacitance to be given by $-s$. The value of the exponent s reflects the structure of the barrier; $s = 0$ implies a perfect barrier which blocks the movement of charge, as was seen in the perfect Maxwell–Wagner response and $s = 1$ implies that no barrier exists. The capacitance in the barrier region is dispersive if s lies between 0 and 1, i.e. its value varies with the frequency of the applied field, with the value of 0.5 indicating the presence of a charge diffusion layer. The slope of the loss curve is described by the exponent $n - 1$, where a value of 0 indicates a direct current conductivity process. The high frequency capacitance slope is given by $-(2 - s - 2n)$, showing that, in keeping with this model, the response in the higher frequency region may be effected by the structure of the barrier layer (and hence the value of s). In the case of a bulk dc conductivity ($n = 0$), the slope of the high frequency capacitance will be $-s$; therefore, the sum of the two slopes will theoretically be -2.

The model therefore indicates that the rise in capacitance, observed when the frequency is taken down into the sub-Hz region, is a function of the electrode layer capacitance. Hitherto, such rises in capacitance were ascribed to electrode polarization effects; the Hill and Pickup model allows analysis of such low frequency effects in terms of sample structure and also allows the effects of barrier layers on the response at higher frequencies to be understood.

3. Materials and methods

3.1. Materials

RMKP 22, a drug with a solubility in water of less than 1 mg/ml, was provided by Dr Karl Thomae GmbH (Biberach, Germany). Phospholipon 90 is a pharmaceutical grade phosphatidylcholine purified from soya lecithin. Phospholipon 90 contains 93 ± 3% phosphatidylcholine, approximately 3% lyso-phosphatidylcholine, at least 0.1% -tocopherol and further minor components such as fatty acids, cephalin and phosphatic acid. Water was double distilled and subjected to the same homogenization process as the dispersions and the nanosuspensions.

3.2. Methods

The RMKP 22 powder was dispersed in the surfactant solution using an Ultra-Turrax stirrer T 25 (Janke und Kunkel, Staufen i.Br., Germany). The obtained coarse pre-dispersion was homogenized at pressures of 1500 bar and 7 cycles using an APV Gaulin Micron LAB 40 homogenizer (Lübeck, Germany). The surfactant solutions and the distilled water as reference were homogenized applying the same conditions.

Table 1
Mean diameters obtained via photon correlation spectroscopy of Phospholipon 90 dispersions and RMKP 22 nanosuspensions[a]

Composition	Mean diameter (nm)	PI	S.D. (nm)
0.6% Phospholipon 90	83	0.388	4
1.2% Phospholipon 90	86	0.376	4
5.0% Phospholipon 90	70	0.373	6
3% RMKP 22, 0.6% Phospholipon 90	584	0.270	12
3% RMKP 22, 1.2% Phospholipon 90	558	0.298	8
3% RMKP 22, 5.0% Phospholipon 90	512	0.315	7

[a] PI, polydispersity index.

Particle size analysis was performed by photon correlation spectroscopy (PCS) (Malvern Zetasizer IV, Malvern, UK). Measurements were carried out at 25°C in distilled water for Phospholipon 90 dispersions and in drug saturated solution for the nanosuspensions. The values given in Table 1 are the mean of nine measurements.

Dielectric studies were conducted using a Dielectric Spectrometer (Dielectric Instrumentation, UK) over a frequency range of 10^{-2}–10^{5} at 19°C. The electrodes were made of stainless steel, with an area of 0.5 cm^2 and separation distance 1 mm. A signal of 0.1 V rms was generated using a frequency-response analyser and passed across the sample. The returning sample was analyzed in terms of the capacitance and the dielectric loss at each frequency. At least two measurement were made for each sample, yielding a maximum variation of 5%.

4. Results and discussion

4.1. Phospholipon 90 dispersions

Previous studies have indicated that high pressure homogenization of phospholipids results in the formation of small unilamellar vesicles. In particular, Brandl (1990) has shown that processing soya lecithin dispersions results in the formation of SUVs in the size range 50–150 nm, depending on the number of homogenization cycles. The PCS data for the vesicles produced using Phospholipon 90 alone are shown in Table 1. The diameters obtained are in good agreement with those previously reported by Brandl (1990) and Diederichs (1993).

The dielectric responses of the Phospholipon 90 vesicles and water are shown in Fig. 2. The shapes of the responses correspond to that described by Hill and Pickup (1985); comparison with Fig. 1 indicates that at low frequencies (≤ 0.1 Hz) the response corresponds to an electrode barrier layer while at the highest frequencies under study (10^3–10^4 Hz) a frequency independent capacitance is seen corresponding to C_b, with the dielectric loss indicating a conductivity process which corresponds to that of the bulk response. The principle modelling parameters are shown in Table 2.

The water sample shows a barrier region, seen at frequencies from $10^{-0.5}$–10^{-2}, probably due to small amounts of impurities which are present even in distilled water or which result from the homogenization process. In the barrier region the capacitance slope has an s value of -0.36 (Table 2), indicating an imperfect blocking layer leading to C_s exhibiting power law behaviour. In the high frequency region, the slope of the loss curves were found to be -1 in all cases, indicating a dc conductance process ($n=0$). According to the Hill and Pickup model, the linear region of the capacitance dispersion should therefore have a gradient of -1.64. The measured gradient was -1.68, indicating the validity of the Hill and Pickup model for these systems.

The spectra of the Phospholipon 90 dispersions were similar to that of water in terms of the capacitances in the very high frequency region and the $n=0$ high frequency conductance processes. The s values of the low frequency capacitance (-0.31, -0.32, -0.32 for the 0.6, 1.2 and

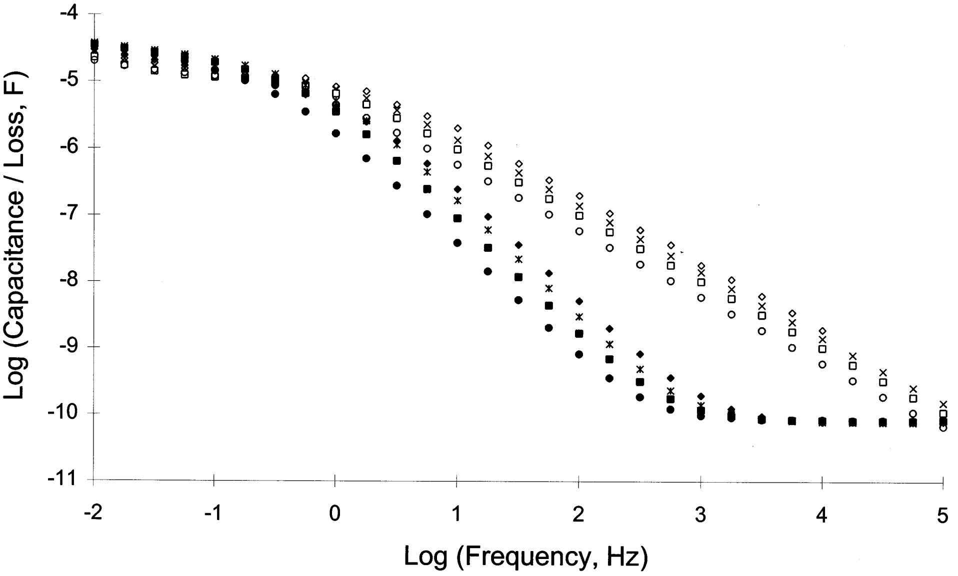

Fig. 2. Dielectric response of surfactant dispersions containing 0.6% (□), 1.2% (×) and 5.0% (◇) Phospholipon 90 in comparison to water (○). Closed symbols capacitance, open symbols dielectric loss.

5.0% Phospholipon 90 dispersion, respectively) are within the same order of magnitude as that of the water. Again, the spectra may be interpreted in terms of the Hill and Pickup model, with the sum of the capacitance slopes being -2.04, -2.06, -2.00 for the 0.6, 1.2 and 5.0% Phospholipon 90 dispersion, respectively, all of which are close to the theoretical value of -2. The similarities in the low frequency response suggest a common origin which may be related to the adsorption of one or more layers of ions or impurities onto the electrode surface. The key difference between the spectra lies in the magnitude of the loss process, in turn indicating differences in charge mobility within the bulk of the systems. The rise in loss values (and hence conductivity) observed with increasing quantities of phospholipid may reflect the presence of conducting impurities either originating from the raw materials or arising during the homogenization

Table 2
Characteristic parameters associated with the dielectric response of distilled water, Phospholipon 90 dispersions and nanosuspensions stabilized with Phospholipon 90

Sample	log C at 10^4 Hz	log C at 10^{-2} Hz	log slope of C (s) (one decade $<\omega_{\mathrm{mw}}$)	log G/ω at 10^4 Hz
Distilled water	-10.070	-4.465	0.36	-8.975
0.6% Phospholipon 90	-10.080	-4.453	0.31	-8.742
1.2% Phospholipon 90	-10.100	-4.429	0.31	-8.597
5.0% Phospholipon 90	-10.105	-4.528	0.32	-8.455
3% RMKP 22, 0.6% Phospholipon 90	-9.930	-4.352	0.32	-7.746
3% RMKP 22, 1.2% Phospholipon 90	-9.466	-4.270	0.33	-7.485
3% RMKP 22, 5.0% Phospholipon 90	-10.020	-4.385	0.35	-8.107

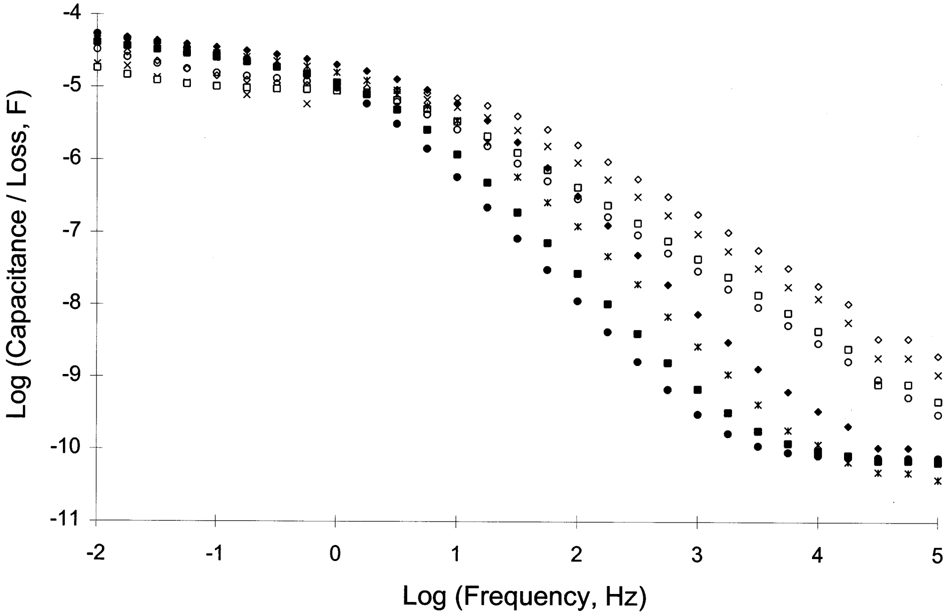

Fig. 3. Dielectric response of nanosuspensions stabilized with 0.6% (□), 1.2% (×) and 5.0% (◇) Phospholipon 90 in comparison to a surfactant dispersion containing 0.6% Phospholipon 90 (○). Closed symbols capacitance, open symbols dielectric loss.

process in addition to a smaller contribution from the vesicles themselves.

4.2. RMKP 22 nanosuspensions

The PCS data for the systems containing 3% model drug are shown in Table 1. As the concentration of Phospholipon 90 increases a decrease in the mean diameter of the nanosuspensions is seen, possibly reflecting more efficient dispersion of the drug particles at higher lipid content. Fig. 3 shows the dielectric response of the pure 0.6% Phospholipon 90 dispersion compared to that of the nanosuspensions containing 3% RMKP 22, stabilized with 0.6, 1.2 and 5.0% Phospholipon 90. The shapes of the dielectric responses are similar to that of pure Phospholipon 90 dispersions; n is close to 0 and the s values in the low frequency region are of the same order of magnitude as in the Phospholipon 90 dispersion (Table 2), implying a dispersive barrier layer. In addition, the sum of the logarithmic capacitance values is consistent with the theoretical values of -2, indicating that the behaviour of the nanosuspensions also resembles the modified Maxwell–Wagner response.

4.3. Concentration dependence of the responses

Fig. 4a and b shows the Phospholipon 90 concentration dependence of the capacitance and loss for both the aqueous phospholipid dispersions and the nanosuspensions taken at representative low and high frequencies. As stated previously, the Phospholipon 90 vesicles show little concentration dependence in the low frequency (10^{-1} Hz) region for either the capacitance or loss, while an increase in both parameters is seen in the higher frequency region, corresponding to the bulk behaviour of the sample. However, the responses of the systems containing the model drug showed markedly different concentration dependent behaviour, firstly in that the responses were considerably higher for the drug systems and secondly there was a maximum in the response seen at mid-range concentrations of phospholipid.

The data indicate different concentration dependencies of the dielectric responses of the Phospholipon 90 suspensions and RMKP 22 nanosuspensions. Given the negligible water solubility of the phospholipid, it has been suggested that the observed increase may be associated with impurities that are present in the raw material or may arise due to partial degradation during the homogenization process; such a hypothesis is in keeping with previous observations regarding the dielectric response of liposomes in this region (e.g. Uhlendorf, 1982). The question then arises as to why the nanosuspensions yield higher loss values which show a maximum with concentration. The low solubility (and high purity) of RMKP 22, along with the non-linearity in loss with concentration, indicates that the response is not a simple function of the presence of the drug. A further possibility, however, is that the phospholipid molecules will be covering the surface of the particles, resulting in decreased entrapment of impurities within liposomes, thereby increasing the dielectric loss. However, as the surface coverage becomes complete then increasing the quantity of Phospholipon 90 simply increases the number of liposomes, hence increasing the entrapment of the impurities; this in turn may lead to a decrease in dielectric loss. A similar scenario has been suggested for surfactant addition to inhalation aerosols (Craig and Taylor, 1995).

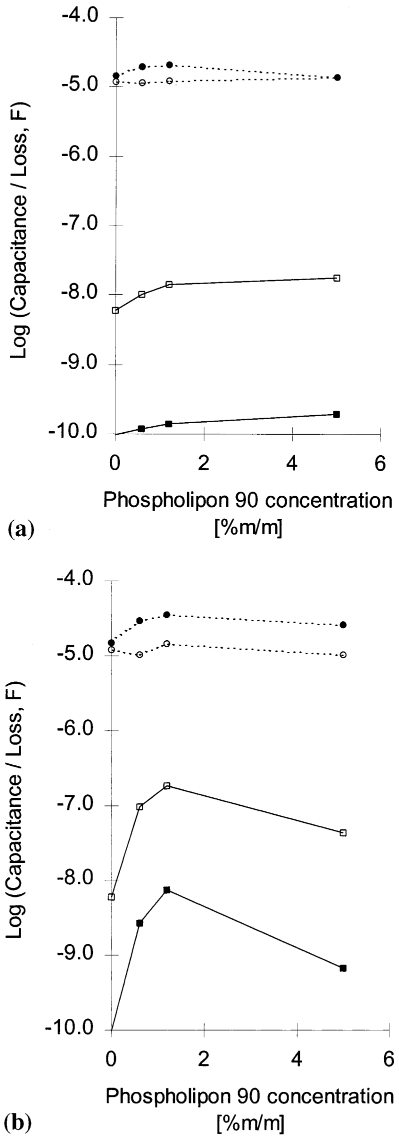

Fig. 4. Concentration dependence of the dielectric response of (a) Phospholipon 90 dispersions and (b) nanosuspensions stabilized with Phospholipon 90 at 0.1 Hz (- -○- -) and 1 kHz (—□—). Closed symbols capacitance, open symbols dielectric loss.

5. Conclusions

The low frequency dielectric response of lecithin dispersions and lecithin stabilized nanosuspensions was measured and the data analyzed by considering the system to consist of a bulk layer in series with a barrier layer, located at the electrode surface. Through investigations of dispersions and nanosuspensions with varying Phospholipon 90 concentrations a model was developed which attributed the observed dielectric behaviour to the Phospholipon 90 surface cover on the nanoparticles and the related presence of liposomes. The possibility of observing the surface cover of nanoparticles with lecithin molecules using low frequency analysis as a non-invasive method appears to be promising for the purpose of monitoring the stabilizing effect of lecithin molecules in nanosuspensions, not only with regard to the optimization of nanosuspension production but also for the purpose of detecting how the system is affected by influences such as temperature and storage time.

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