Characterization of the Effects of Drug Addition on the Structure of Glyceryl Monoolein/Water Gel Systems Using Low Frequency Dielectric Spectroscopy

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Abstract □ The influence of propantheline bromide incorporation on the phase structure of glyceryl monoolein/water systems has been investigated using low-frequency dielectric spectroscopy over a frequency range of 10^{-2} to 10^{6} Hz at 20 °C. The responses of glyceryl monoolein systems composed of 10% and 30% w/w were measured and the spectra modeled using an equivalent circuit based on the Maxwell–Wagner theory.[1,2] Marked changes in the dielectric responses of the systems were noted on addition of the propantheline bromide at concentrations up to 10% w/w. For the lamellar (10% w/w water) glyceryl monoolein systems, an increase in the imaginary permittivity was seen, corresponding to an increase in conductivity due to the presence of additional ionic species within the system. Evidence was also obtained for the incorporation of the drug directly into the lipid bilayers, particularly at higher concentrations (10% drug) at which dielectric behavior corresponding to bilayer disruption was seen. Incorporation of 3% and 5% w/w drug into the cubic phase systems (30% w/w water) resulted in a change to the lamellar phase. However, circuit modeling indicated that the system formed structures which showed features of both the lamellar and cubic phases at 3% w/w drug loadings. The study has therefore demonstrated that dielectric analysis may provide a novel means of studying the effects of drug incorporation on the phase behavior of complex gel systems.

Introduction

Glyceryl monoolein has attracted interest as a pharmaceutical excipient, particularly for controlled drug delivery and bioadhesive systems.[3−7] This material is known to form four mesophases depending on the water content and temperature, namely the reverse micellar (L_2), lamellar (L_α), cubic (C), and reverse hexagonal (H_{II}) phases. Among these, the lamellar and cubic systems have received particular attention within the drug delivery field. The lamellar phase is a semifluid liquid crystalline system consisting of lipid bilayers alternating with water layers, while the cubic system is a highly viscous, ordered, bicontinuous structure with curved lipid bilayers extending in three dimensions separated by water channels.

The multilayer structure and/or high viscosity of the liquid crystalline phases results in the generation of diffusional barriers; hence, there has been interest in studying drug incorporation and release from these systems. The amphiphilic nature of the lipid allows incorporation of a range of drugs of varying lipophilicity; indeed, it has been suggested that hydrophobic drugs may be trapped within the lipophilic bilayers, and hydrophilic drugs are located in the aqueous channels, while amphiphilic drugs may partition into the lipid bilayer−water interface.[8] One would therefore expect the release pattern to depend on the microstructure and physicochemical properties of the liquid crystalline phases. To date, the majority of work in the field has focused on the examination of drug diffusion and release from the liquid crystalline systems. However, it is also necessary to consider the effects of drug addition on the formation and structure of the various phases. Previous studies using polarized light microscopy have reported induced phase changes at high levels of incorporated drugs.[4−6] The study of the effects of drug incorporation has arguably been limited by the difficulties associated with effectively characterizing the physical structure of these complex systems; hence, while drug-induced phase changes are a recognized phenomena, comparatively little is known regarding the mechanisms involved.

The work presented here describes an investigation into the influence of propantheline bromide loading on the phase structure of monoolein/water systems using low-frequency dielectric spectroscopy, with a view to developing the technique as a potential means of characterizing complex pharmaceutical materials. Propantheline bromide is an antimuscarinic agent which has been previously investigated as a means of treating urinary incontinence via vaginal delivery in a monoolein/water gel system.[5] This drug is itself surface active[9] and undergoes self-association in aqueous solution via an open aggregation process, whereby no discontinuity is seen in light-scattering intensity with concentration.[10] Geraghty et al.[5] reported that monoolein/water systems with a water content of less than 15% w/w retained their lamellar structure when an increasing quantity of propantheline bromide was added. However, systems that formed a cubic phase gel in the absence of drug could only maintain their structure up to propantheline bromide loadings of less than 5% w/w. At or above this drug loading, the gels were formed in the lamellar phase. Our previous work on glyceryl monoolein/water systems[1,2] showed that low-frequency dielectric spectroscopy may be used to identify and characterize the liquid crystalline phases. Furthermore, by modeling the dielectric data in terms of an equivalent circuit, it is possible to interpret the response in terms of specific structural features of the samples. The objectives of this study are therefore to develop the use of the dielectric approach by examining a system for which the basic behavior is already known and second to gain more specific information on the effects of propantheline bromide incorporation on the phase structure of glyceryl monoolein.

Materials and Methods

A single batch of Myverol 18-99 (Eastman Chemical Co.), a distilled monoglyceride with 60.9% glyceryl monoolein content, was

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used throughout the study. Propantheline bromide (Sigma Co.) was used as supplied. All mixes were prepared using water with a resistivity greater than 18 MΩ/cm, obtained from an ultrahigh quality water purification system (Elga Co.). Two sets of gel samples containing 10% and 30% w/w water and 1%, 3%, 5%, and 10% propantheline bromide were prepared. The drug was added to Myverol 18-99 at 60 °C and mixed for 2 min, followed by the addition of water at the same temperature to produce the desired (% w/w) gel composition. The mix was stirred at 2000 rpm using a Heidolph RXR50 mixer. The resulting samples were centrifuged at a speed of 3000 rpm for 20 min and then stored in sealed containers at room temperature for 7 days before use to allow equilibration of the samples, following the recommendation of Geraghty et al.[5]

The dielectric measurements were carried out using a BDC-N broad band dielectric converter (Novocontrol GmbH) and a frequency response analyzer (SI 1255, Solatron-Schlumberger) linked to a Quatro temperature control system (Novocontrol GmbH). The technique involves the application of an oscillating electrical field to a sample and the subsequent measurement of the real and imaginary components of the response over a range of frequencies (ω). This response may be expressed in terms of the complex permittivity $\epsilon^*(\omega)$, where

$$\epsilon^*(\omega) = \epsilon'(\omega) - i\,\epsilon''(\omega) \quad (1)$$

with $\epsilon'(\omega)$ and $\epsilon''(\omega)$ being the real and imaginary components at frequency ω and i being the square root of -1. These components may be measured in terms of the extrinsic parameters $C(\omega)$ and $G(\omega)/\omega$, where $C(\omega)$ is the capacitance and $G(\omega)/\omega$ is the dielectric loss, $G(\omega)$ being the conductance (representing the sum of the a.c. and d.c. contributions). The relationship between the real permittivity and the capacitance is given by

$$\epsilon'(\omega) = \frac{C(\omega)\,d}{\epsilon_0 A} \quad (2)$$

where ϵ_0 is the permittivity of free space, and d and A are the interelectrode distance and electrode area, respectively. Similarly, the imaginary permittivity is related to the dielectric loss via

$$\epsilon''(\omega) = \frac{G(\omega)\,d}{\omega\epsilon_0 A} \quad (3)$$

During a measurement, the sample was placed in a circular dielectric cell designed for liquid and semisolid samples with diameter of 20 mm and an interelectrode distance of 0.5 mm. The dielectric responses were obtained at 20 °C over a frequency range of 10^{-2} to 10^{6} Hz. At least four samples for each concentration were examined, with at least two repeat measurements being made for each sample; excellent reproducibility was found between spectra. The fitting of the data was carried out by employing a modified generalized Maxwell–Wagner equivalent circuit with dispersive RC elements, and using Winfit 2.0 program supplied by Novocontrol GmbH. The main feature of Winfit 2.0 is nonlinear curve fitting of the measured data in the frequency domain, with both real and imaginary components being fitted simultaneously. In general impedance mode, the software supports up to four single fit terms which may hold several combinations of resistance, inductance, and capacitance elements and will also incorporate power law functions for these circuit features. Microscopic observations were obtained under polarized light using an Olympus differential interference contrast (DIC) microscope at room temperature.

Results and Discussion

Two sets of Myverol 18-99/water systems with waters content of 10% w/w and 30% w/w and propantheline bromide concentration of 1% w/w, 3% w/w, 5% w/w, and 10% w/w were investigated. According to the phase diagram determined by Geraghty et al.,[5] the 10% w/w water system is expected to be lamellar phase, while the 30% w/w water system is predicted to be cubic phase at 20 °C in the absence of drug.

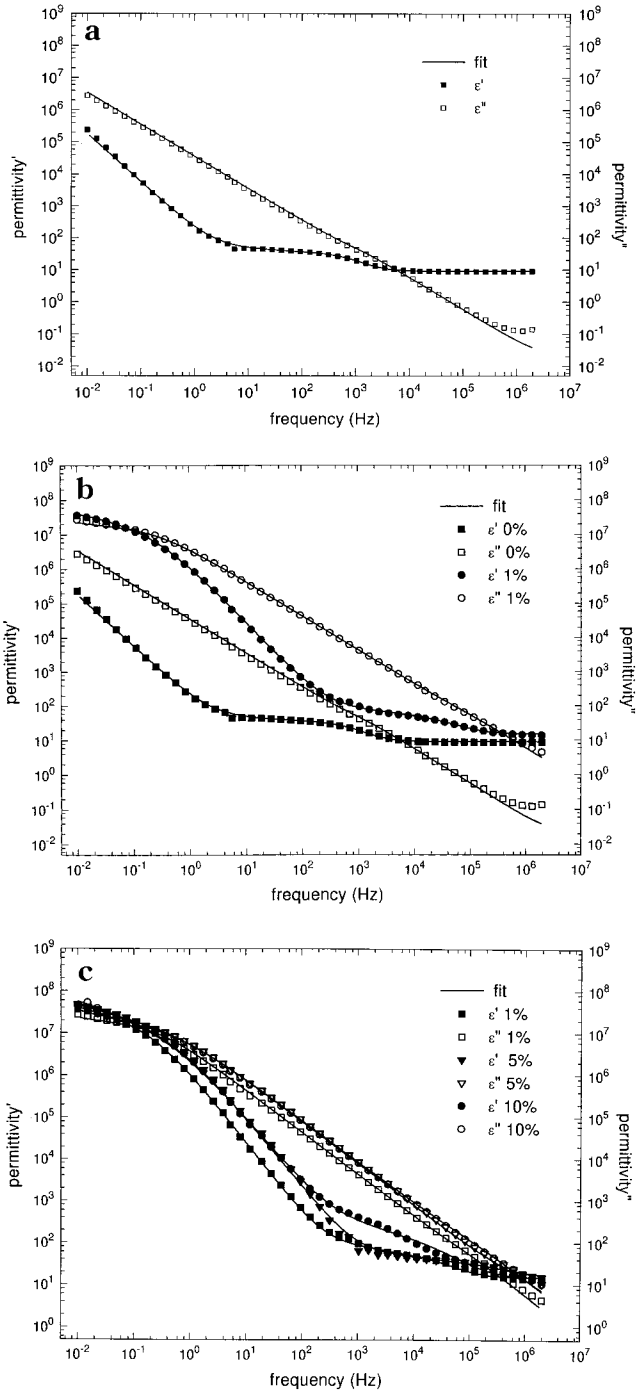

Figure 1—Low-frequency dielectric spectra of Myverol 18-99/water/propantheline bromide systems containing 10% w/w water with propantheline bromide concentration of (a) 0%, (b) comparison of 0% and 1%, and (c) comparison of 1%, 5%, and 10%. Solid lines indicate curve-fitted data.

Influence of Drug Loading on Lamellar Phase System—Both microscopy observations and dielectric spectroscopy measurements confirmed that the monoolein/water system containing 10% w/w water without addition of propantheline bromide was lamellar phase at 20 °C. The dielectric response, as shown in Figure 1a, exhibited the expected lamellar phase spectrum[1] with three principle dielectric processes: a high frequency process, corresponding to the bulk response, which is seen as a frequency independent real permittivity value of approximately 8, together with an inverse frequency dependent imaginary component, $\epsilon'' \propto (\omega)^{-1}$, reflecting a frequency independent value of $G(\omega)$ (see eq 3). A relaxation process in the Hz

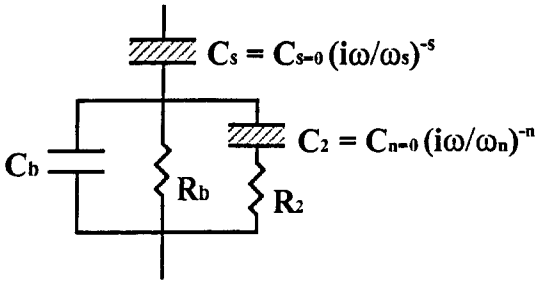

Figure 2—Modified generalized Maxwell–Wagner equivalent circuit employed to model the dielectric responses.

range was indicated by a discontinuity in the real component between frequencies of 5 Hz and 5 kHz which is thought to be caused by the relaxation of weakly bound dipoles and charge transport processes which are partially impeded by the lamellar network.[1,2] In the sub-Hz frequency range, the values of both the real and imaginary components increased, indicating the presence of a low-frequency dispersion caused by adsorbed barrier layers on the electrodes. Such layers have been described in a number of previous studies (e.g.,[11,12,13]) and are discussed in more detail in the context of these systems in a previous publication.[1]

On adding 1%, 5%, and 10% propantheline bromide to the 10% water sample, the systems remained in the lamellar phase, as indicated by the low viscosity and "oil streak" seen using microscopy.[5] The polarized light microscopy studies were unable to unequivocally distinguish between the various drug-containing systems, although the viscosity of the 10% w/w drug systems was visibly lower. However, the dielectric spectra for these systems exhibited marked differences in terms of both shape and magnitude. In comparison to the dielectric response for the 10% w/w gel system alone, the inclusion of 1% w/w drug resulted in a marked increase in imaginary permittivity of approximately 2 orders of magnitude over the frequency range of 10^{-1} to 10^{6} Hz, while the crossover frequency shifted from approximately 5 kHz to 400 kHz (Figure 1b). This crossover frequency (ω_τ) represents the inverse bulk relaxation time ($1/\tau$) of the system at $\epsilon' = \epsilon''$, given by

$$\omega_\tau = G(\omega)/C(\omega) = 1/\tau \qquad (4)$$

Therefore, the bulk relaxation time of the 1% sample decreased by approximately 2 orders of magnitude. The second relaxation process seen in the real component which is characteristic of the lamellar phase was, however, retained and shifted from a frequency of approximately 400 Hz to 400 kHz.

The responses of the 5% and 10% w/w drug systems are shown in Figure 1c in comparison to the 1% response. The spectrum of 5% drug sample showed a further slight increase in overall magnitude, but the change in spectral shape was less marked, indicating the system remained as a lamellar structure with only a slight increase of conductance. The response of the 10% drug sample had similar values of real and imaginary permittivity as the 5% material over the majority of the spectrum, although a marked increase in the magnitude of the real permittivity for the second relaxation process was seen in over a frequency range of 200 Hz to 50 kHz.

The above spectra were modeled in terms of a modified generalized Maxwell−Wagner equivalent circuit which was developed for these systems in a previous study[1] based on work by Hill and Pickup[14] (Figure 2). The circuit consists of a fractional power law dispersive capacitor, C_s, representing a layer formed at the electrode surface, in series with a parallel RC circuit (C_b and R_b), corresponding to the principal bulk processes; these are long range phenomena which are dispersed spatially in a uniform manner throughout the system. A series connection (C_2 and R_2) was employed to model the additional bulk polarization behavior, which represents the effect of the substructure within the sample. Previous studies[1,2] have related this circuit feature to the structure of the lamellar network within the system. The C_2 element is a dispersive capacitor showing power law behavior described by

$$C_2 = C_{n=0}(i\omega/\omega_n)^{-n} = C_{n=0}(\omega/\omega_n)^{-n}[\cos(n\pi/2) - i\sin(n\pi/2)] \qquad (5)$$

where $C_{n=0}$ is the capacitance when $n = 0$ and ω_n is a characteristic frequency. The exponent n is related to the extent of cooperativity between the relaxing dipoles and the immediate environment and is therefore a reflection of the degree of ordering of the system. When $n = 0$, C_2 is frequency independent and the dipoles may be considered to be totally coupled to their surroundings, while when $0 < n < 1$, the dipoles are uncoupled and the value of $C_2(\omega)$ is complex. More details of the physical significance of the exponent n may be found in a number of texts (e.g.,[14,15]).

The fitted parameters for the 10% water content samples with 0, 1%, 5%, and 10% propantheline bromide are given in Table 1. Without drug incorporation, the monoolein/water gel exhibited relatively high resistance values for both R_b and R_2, low values for the bulk capacitance C_b and the frequency dependent C_2, and a value for the exponent n of approximately 0.1. By adding 1% propantheline bromide, the resistance R_b and R_2 decreased by approximately 2 orders of magnitude which may be at least partially interpreted in terms of the increase in drug and counterion concentration. However, in addition to this effect a 10-fold increase in the $C_{n=0}$ value and a 2-fold increase in the value of n were observed, both indicating that the presence of the drug is causing disruption of the lamellar bilayer. Consequently, in addition to charge addition effects the observed decrease in bulk resistance may be a function of changes to the structure of the lipid lamellae. As the drug concentration increased to 5%, the R_b and R_2 values further decreased, while at the highest drug concentration of 10% the resistances R_b and R_2 showed only small decreases compared to the 5% value. However, at this drug concentration marked changes in $C_{n=0}$ and n were seen, indicating that the drug may be causing further alterations to the substructure of the system.

Overall, therefore, the incorporation of the drug appears to maintain the system within the lamellar phase but increases the conductance of the gel, probably due to a combination of ion addition effects and lamellar disruption. However, the increase of the conductance with increase of drug loading was nonlinear, with considerably less marked changes seen for the 5% and 10% drug systems. This could be a consequence of the surface active properties of the drug, with a decreased change in conductance seen due to the formation of assemblies which make a relatively small contribution to the charge-carrying properties of the system as a whole. In addition to this effect, however, the drug also appears to alter the substructure of the gel in a concentration dependent manner. At 1% and 5% levels, the changes in $C_{n=0}$ and n provide evidence for the presence of the drug within the lamellar bilayers themselves, while at 10% w/w the drug appears to cause substantial changes to the bilayers, with the marked increase in the exponent n reflecting disruption of the uniformity of the charge transport path. Taken together with the observation that the 10% drug systems were visibly less viscous than the

Table 1—Values of the Fitted Equivalent Circuit Parameters Derived from the Experimental Data Obtained for Myverol-80/Water/Propantheline Bromide Systems Containing 10% w/w Water Using the Model Circuit Given in Figure 2

drug concn, %	R_b (Ω)	C_b (p)	R_2 (Ω)	$C_{n=0}$ (*F*)	*n*	$C_{s=0}$ (F)	*s*
0	7.1×10^5	5.1×10^{-11}	1.2×10^6	3.1×10^{-10}	0.101	8.7×10^{-5}	0.478
1	5.9×10^3	8.5×10^{-11}	1.5×10^4	2.1×10^{-9}	0.194	1.3×10^{-4}	0.277
5	3.2×10^3	8.9×10^{-11}	2.6×10^3	1.5×10^{-9}	0.191	1.3×10^{-4}	0.349
10	3.3×10^3	8.4×10^{-11}	5.1×10^3	3.0×10^{-8}	0.321	1.4×10^{-4}	0.387

lower concentration drug samples, the data indicate that the drug is incorporated into the bilayer, causing disruption which in turn alters the viscous properties of the gels.

Influence of Drug Loading on Cubic Phase System—The monoolein/water system containing 30% water formed an optically isotropic cubic phase. The dielectric response of this phase, as shown in Figure 3a, consisted of a high-frequency bulk process with greater magnitudes for the imaginary component (i.e. a higher conductance) than for the lamellar phase, and a sub-Hz barrier process caused by an adsorbed layer at electrode. The high conductivity and lack of substructure relaxation processes reflect the homogeneity and bicontinuous structure of the system.[1]

On incorporating 1% drug into the system, the gel still had the appearance of the cubic phase (both visually and using microscopy), while the low frequency dielectric response still showed the same basic shape as the system with no added drug. An increase in imaginary permittivity of approximately 2 orders of magnitude was seen, which again may be attributed to the ionization of the propantheline bromide within the aqueous phase. However, the addition of further propantheline bromide was shown to promote the formation of the lamellar phase. The samples containing 3% and 5% drug concentrations were clearly of lower viscosity, and, under polarized light microscopy, exhibited similar "oily streak" textures. Examination of the dielectric responses (Figures 3b and 3c) revealed marked differences between the two higher concentration systems. The spectrum for the 3% gel showed a second relaxation process which was seen in the real component of the permittivity over a frequency range of 10^5 to 10^6 Hz, while the imaginary component of permittivity maintained similar values to that of the 1% drug sample. Consequently, the system showed features characteristic of the lamellar phase but did not exhibit the decrease in imaginary permittivity associated with the change from the cubic to lamellar phases. The dielectric spectrum of the 5% drug sample, however, showed typical lamellar behavior with the three dielectric processes outlined earlier. In addition, the values of the imaginary permittivity were 1−2 orders of magnitude lower than for the 1% and 3% drug systems over the majority of the response.

The values of the parameters obtained from fitting to the circuit model shown in Figure 2 for the 30% water samples with 0, 1%, 3%, and 5% propantheline bromide are given in Table 2. The cubic structure of the pure monoolein/water system was characterized by a low bulk resistance R_b (2 orders of magnitude lower than the bulk resistance of pure lamellar gel). The R_2 value was also low, while C_2 was frequency independent ($n = 0$) and showed similar values to that of the bulk capacitance, C_b. These features reflect the homogeneous structure of the cubic phase and are in good agreement with findings from the earlier studies.[1,2]

For the sample containing 1% drug, the bulk resistance R_b decreased approximately 30-fold, almost certainly as a result of ionization of the drug, while C_b and R_2 showed little change, implying that the system was still in the cubic phase. However, the 10-fold increase in $C_{n=0}$ and a change of the value of the exponent *n* from 0 to 0.14 reflect the effect of incorporation of drug molecules on interfaces leading to local inhomogeneity. For the 3% drug sample,

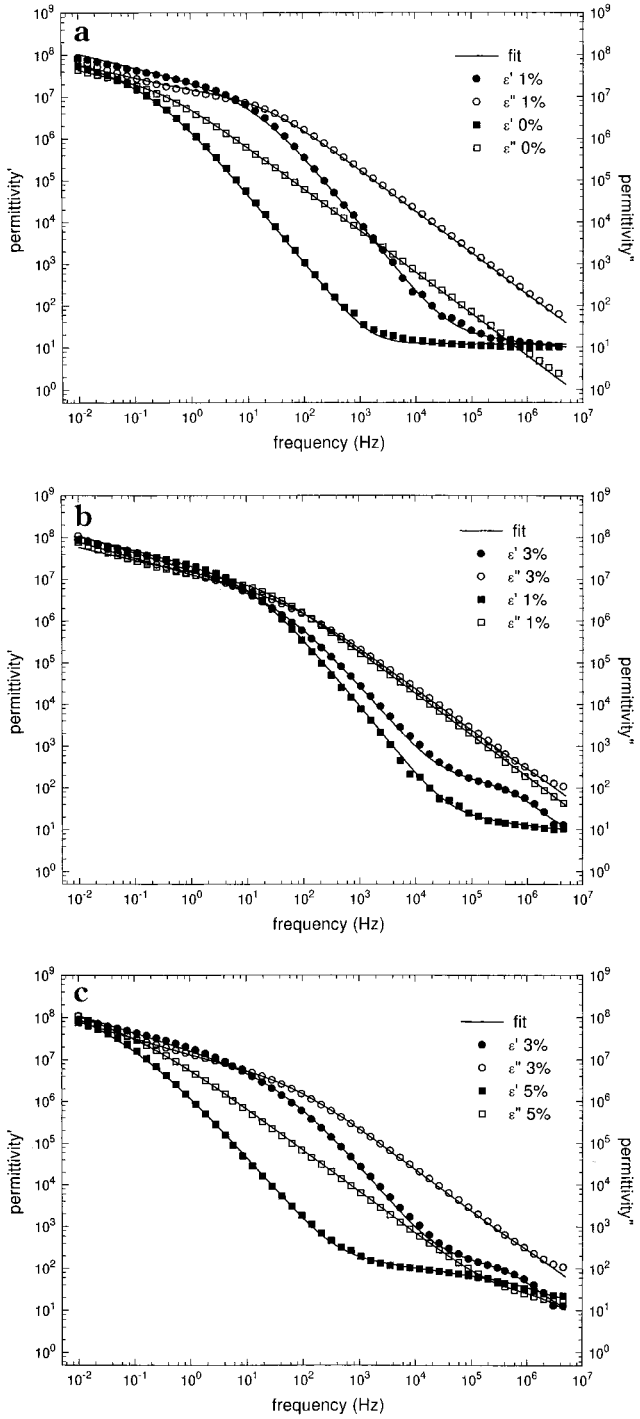

Figure 3—Low-frequency dielectric spectra of Myverol 18-99/water/propantheline bromide systems containing 30% w/w water with propantheline bromide concentration of (a) 0 and 1%, (b) 1% and 3%, and (c) 3% and 5%. Solid lines indicate curve fitted data.

Table 2—Values of the Fitted Equivalent Circuit Parameters Derived from the Experimental Data Obtained for GMOrphic-80/Water/Propantheline Bromide Systems Containing 30% w/w Water Using the Model Circuit Given in Figure 2

drug concn, %	R_b (Ω)	C_b (p)	R_2 (Ω)	$C_{n=0}$ (*F*)	*n*	$C_{s=0}$ (*F*)	*s*
0	4.5×10^3	3.2×10^{-11}	<10	3.5×10^{-11}	0	1.4×10^{-4}	0.347
1	1.6×10^2	3.5×10^{-11}	<10	3.4×10^{-10}	0.140	2.7×10^{-4}	0.320
3	1.1×10^2	4.3×10^{-11}	2.6×10^2	1.0×10^{-8}	0.180	2.5×10^{-4}	0.394
5	4.1×10^3	8.9×10^{-11}	4.8×10^2	4.8×10^{-9}	0.203	2.6×10^{-4}	0.450

the values of the substructure elements R_2 and $C_{n=0}$ showed marked increases while the bulk resistance R_b and capacitance C_b remained largely unchanged compared to the 1% systems. As the drug concentration increased up to 5%, all bulk and substructure elements R_b, C_b, R_2, and $C_{n=0}$ exhibited similar values for lamellar systems shown in Table 1.

These data indicate that the incorporation of propantheline bromide causes the system to change from the cubic to the lamellar phase at 20 °C, as reported by Geraghty et al.[5] However, the dielectric study has allowed further information to be obtained regarding the transformation. In particular, while the systems containing 0 and 5% drug show spectra typical of the cubic and lamellar phases, respectively, the gels containing 1% and 3% drug show features of both systems which indicate that these systems have structures which appear to be intermediate between the two phases.

Conclusions

The objectives of this study were to explore the use of dielectric analysis as a means of characterizing glyceryl monoolein gel systems containing a model drug and to gain further information on the effects of that drug on the phase behavior of the gel systems. The technique has been shown to be capable of detecting the phase behavior of the gels by matching the spectra of drug-containing systems to those of the cubic and lamellar phases, yielding trends which are in good agreement with those previously reported.[5] However, in addition to gross identification of the phases present the technique also appears to allow a more sophisticated analysis of the effects of incorporated drug than has been previously possible. In particular, the circuit modeling has yielded information on the location of the drug within the gel as well as identifying structures which are intermediate between the lamellar and cubic phases. Such findings may have important implications for understanding not only the drug release properties of these gels but also their mechanical and bioadhesive behavior.

Acknowledgments

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