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Identification of thermotropic phase transitions of glyceryl monoolein–water systems by low frequency dielectric spectroscopy

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Abstract

Thermotropic phase transitions of glyceryl monoolein–water systems have been investigated using dielectric spectroscopy over a frequency range of 10^{-2}–10^{6} Hz and a temperature range of 15–100°C, and also using differential interference contrast (DIC) microscopy fitted with a hot stage as a support technique. The frequency and temperature dependencies of the dielectric permittivity of systems undergoing transitions between lamellar, cubic, reverse hexagonal and L_2 phases are reported. It was demonstrated that the frequency dependent response is highly sensitive to changes in phase and a model is described which allows the dielectric behaviour to be interpreted in terms of an equivalent circuit diagram, thereby allowing the response to be related to specific structural features within the sample. Similarly, it was demonstrated that the phase transition temperatures and ranges could be identified using the dielectric technique, yielding results which were in good agreement with those obtained using DIC microscopy for those systems whereby the phase changes are optically identifiable. The study therefore suggests that low frequency dielectric analysis is a potentially highly useful tool for the identification of thermotropic phase changes. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Dielectric; Differential interference contrast microscopy; Glyceryl monoolein; Liquid crystal

1. Introduction

Glyceryl monoolein, a water-insoluble swelling lipid, is known to form at least four distinct mesophases depending on the water content and temperature, these being the reverse micellar (L_2), lamellar (L_α), cubic (C) and reverse hexagonal (H_{II}) phases (Larsson, 1989). These phases differ in both structure and physical properties. In the dry crystal form the lipids exist as a stack of planar molecular bilayers containing closely

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packed hydrocarbon chains with the polar heads forming the outer surfaces. The reverse micellar phase is an isotropic fluid with flow characteristics similar to a thin oil, while the lamellar phase is a semi-fluid liquid crystalline system consisting of lipid bilayers alternating with water layers which have a long range order in one dimension. The reverse hexagonal phase is periodic in two dimensions and consists of water cylinders separated by lipid bilayers. Finally, the cubic phase has a highly viscous and ordered bicontinuous structure with curved lipid bilayers extending in three dimensions separated by water channels. Recently, monoolein–water systems have received considerable attention within the pharmaceutical field as potential drug delivery and bioadhesive vehicles (Engstrom, 1990; Engstrom and Engstrom, 1992; Geraghty et al., 1996).

The present knowledge base regarding the physical properties and phase transitions of monoolein–water systems is essentially based on polarized light microscopy, X-ray diffraction and differential scanning calorimetry studies. The phases and their corresponding macroscopic textures may be observed by polarized light microscopy, the molecular dimensions of the liquid crystals can be determined by X-ray diffraction, and the temperature and enthalpies of phase transitions during melting and crystallization may be measured by differential scanning calorimetry (DSC). However, each of these approaches has concomitant disadvantages; microscopic techniques are not able to distinguish between the optically isotropic phases, while X-ray diffraction data may be difficult to interpret for complex or heterogeneous systems. Similarly, DSC cannot identify the different phases between transitions. There is therefore a need to identify and develop novel approaches with which the phase behaviour of these systems can be characterised. Previous work has shown that low frequency dielectric spectroscopy is a suitable technique with which to study the heterogeneous microstructures of a range of systems of pharmaceutical interest (Dissado et al., 1987; Rowe et al., 1988; Craig and McDonald, 1995; Hill and Cooper, 1995; Sutananta et al., 1995; Tamburic and Craig, 1995; Hill and Cooper, 1996), including gel and emulsion systems, glycerides, self emulsifying systems and bioadhesive gels. Furthermore, recent work (He and Craig, 1998) has indicated that the method may be used to characterise the different phases of glyceryl monooleate. The dielectric 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 $\varepsilon^*(\omega)$, where

$$\varepsilon^*(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega) \tag{1}$$

with ε' and ε'' 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

$$\varepsilon'(\omega) = \frac{C(\omega) \cdot d}{\varepsilon_0 A} \tag{2}$$

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

$$\varepsilon''(\omega) = \frac{G(\omega)}{\omega} \cdot \frac{d}{\varepsilon_0 A} \tag{3}$$

Our previous work on glyceryl monoolein–water systems showed that the frequency responses of the various phases exhibited marked differences, both in terms of shape and magnitude, depending on the composition and temperature. Circuit analysis, whereby a theoretical circuit is constructed which would give an equivalent response to that of the sample, allowed the dielectric response to be interpreted in terms of specific structural features of the samples. Therefore, while the dielectric method has been widely used as a means of characterizing the electrical properties of materials, it is also possible to relate those properties to the physical structure of samples such as liquid crystalline systems. However, our studies thus far have involved isothermal frequency sweeps, hence while we have studied the behaviour of the systems in various phase states we have not yet investigated the thermotropic phase transitions themselves. The work outlined here describes the novel use of dielectric temperature sweeps in order to identify these transitions, hence facilitating development of the method as a means of characterising liquid crystalline phase behaviour.

2. Materials and methods

A single batch of GMOrphic-80 glyceryl monoolein (Eastman) was used throughout this study. This material is a distilled monoglyceride with a high monoolein content. All mixes were prepared using water with a resistivity of greater than 18 MΩ/cm, obtained from an ultra-high quality water purification system (Elga). Three sets of gel sample containing 10, 22 and 30% (w/w) water were prepared. A known weight of GMOrphic-80 was gently heated to 60°C, 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. It was found that for samples containing 10% water, uniform systems were obtained using 10–15 min stirring, while for samples containing 22 and 30% water, a highly viscous gel was produced after only 10–15 s high speed mixing. Longer mixing times were found to produce no noticeable effect on the properties of the gels. The resulting samples were centrifuged at a speed of 3000 rpm for 20 min, then stored in sealed containers at room temperature for at least 24 hours before use to allow equilibration of the samples.

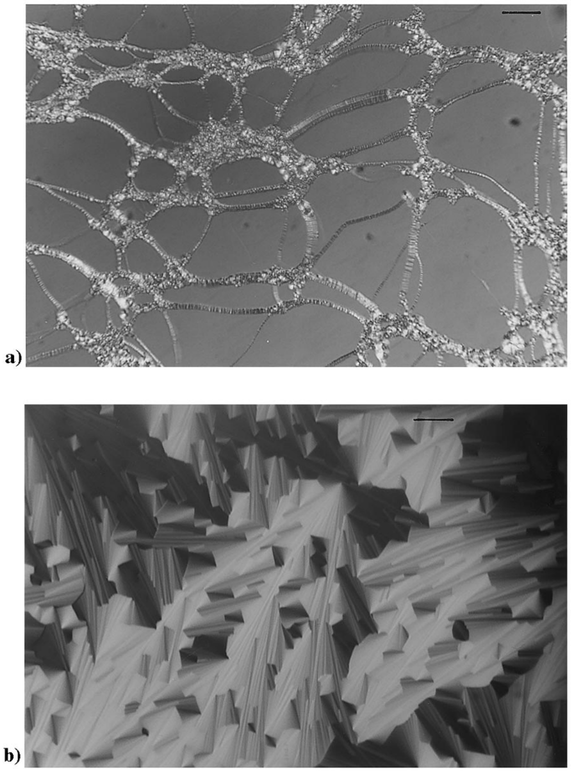

Fig. 1. DIC photographs for the 22% (w/w) water sample at (a) 20°C, and (b) 75°C, showing lamellar and reverse hexagonal phases respectively. Bar represents 40 μm.

The dielectric measurements were carried out using a BDC-N broad band dielectric converter (Novocontrol) and a frequency response analyser SI 1255 (Solatron-Schlumberger) linked to a Quatro temperature control system (Novocontrol). The sample was placed in a circular dielectric cell designed for liquid and semi-solid samples with diameter of 20 mm and an interelectrode distance of 0.5 mm. The frequency responses were obtained over a frequency range 10^{-2}–10^{6} Hz, while the temperature dependence was measured at stated frequencies over a temperature range 15–100°C, using a heating rate of 1°C/min with a precision of ± 0.1°C. The cell was cleaned thoroughly between measurements, with repeats showing satisfactory reproducibility over the spectral range. Hot stage polarizing microscopy was performed using an Olympus differential interference contrast (DIC) microscope with Mettler hot-stage facilities using a heating rate of 1°C/min.

3. Results and discussion

3.1. Isothermal phase behaviour of the 22% (w/w) systems

Previous studies (He and Craig, 1998) have related the frequency dependent dielectric behaviour of 15, 20, 30 and 35% (w/w) systems to the phase. The 22% (w/w) systems were chosen in this study as, according to the phase diagram suggested by Larsson (1989), this concentration may be

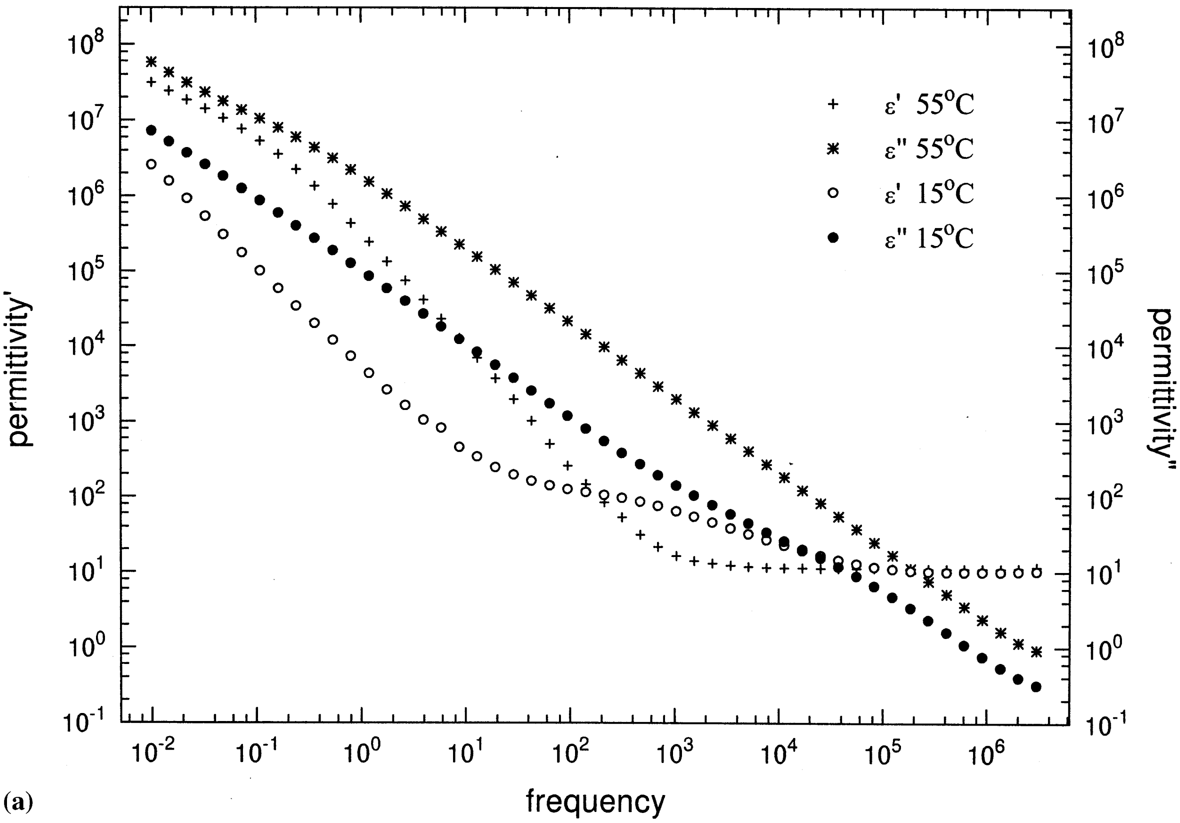

Fig. 2. Low frequency dielectric spectra of GMOrphic-80–water systems containing 22% (w/w) water (a) obtained at 15 and 55°C, and (b) at 55, 68 and 95°C.

expected to exist in all four of the main phases, depending on the temperature, thereby making it a suitable model with which to study thermotropic phase changes.

The proposed phase behaviour of the 22% (w/w) systems was investigated using hot stage microscopy, with systems at 15, 55, 68, and 95°C having appearances which were compatible with the presence of the lamellar, cubic, reverse hexagonal and reverse micellar phases respectively. The lamellar structure consisting of planar lipid bilayers with alternating water layers could be identified by the ‘oily streaks’ appearance, as shown in Fig. 1a, which previous workers have associated with this phase (Stegemeyer, 1994). The reverse hexagonal phase could be identified by a fan-shaped appearance (Fig. 1b), which is a consequence of the prevention of uniform alignment due to the rapid growth of several nuclei at the phase transition (Stegemeyer, 1994). The cubic and reverse micelles phases were optically isotropic, and could only be distinguished by their viscosity.

The frequency dependent dielectric spectra of the samples with water contents of 22% (w/w) at 15, 55, 68, and 95°C are showed in Fig. 2a,b. Clear differences are seen between samples at 15°C (lamellar phase) and 55°C (cubic phase), as previously reported for other concentrations (He and Craig, 1998); the samples at 55, 68 and 95°C (cubic, H_{II} and L_2) showed differences in magnitude but less change in spectral shape.

The spectra may be interpreted in terms of a modification of the generalised Maxwell-Wagner model (Hill and Pickup, 1985; He and Craig, 1998), whereby the dielectric behaviour is considered to consist of a bulk response, seen in the high frequency region, in series with a barrier (or electrode) response whereby material adsorbed onto

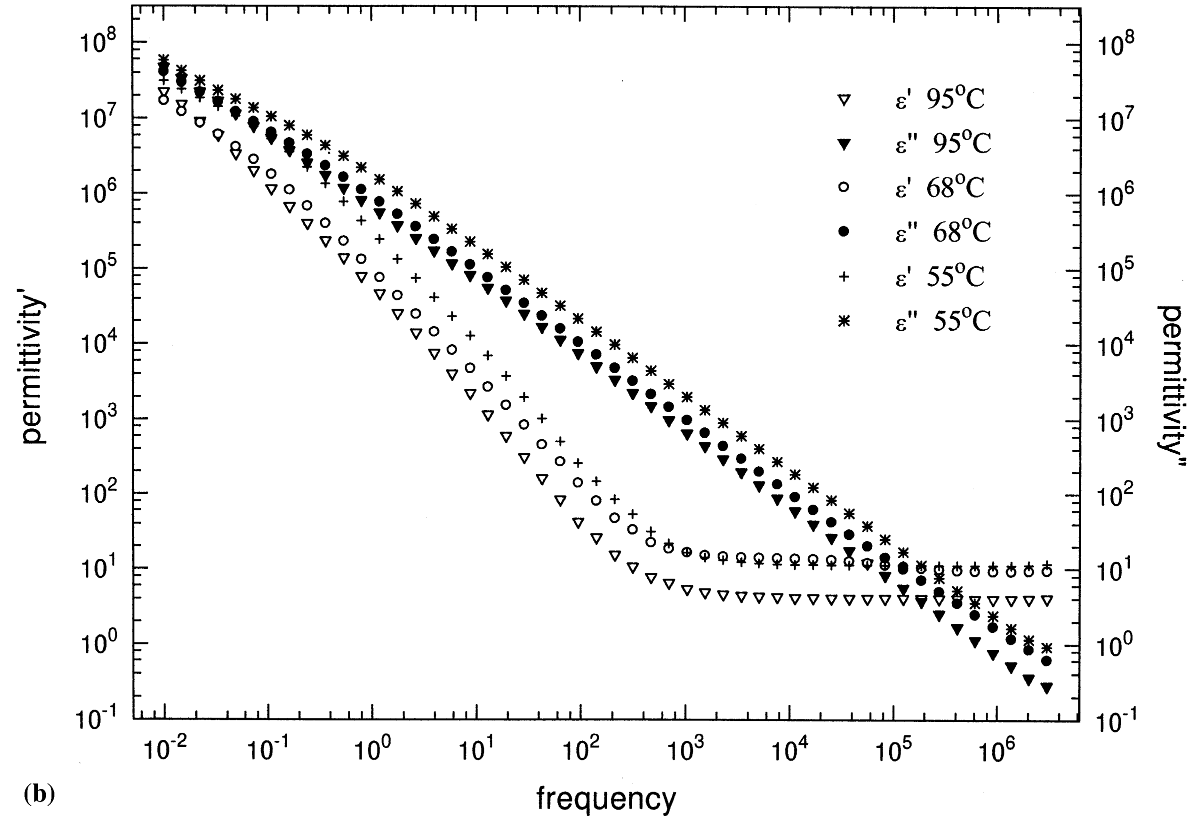

Fig. 2. (*Continued*)

the electrode surfaces make a contribution to the low frequency (sub-Hz) behaviour. The circuit model developed in our previous study is shown in Fig. 3 and the corresponding values for the various circuit elements given in Table 1. The dielectric response of the lamellar phase showed three principal processes. A high frequency process, corresponding to the major bulk response, which is seen as a frequency independent real permittivity value of approximately 10, together with an inverse frequency dependent dielectric loss component, $\varepsilon'' \propto (\omega)^{-1}$; examination of Eq. (3) indicates that such a relationship is indicative of a frequency independent d.c. conductivity process. The second process was indicated by a broad peak in the real component between frequencies of 10 and 10^4 Hz and a slight dispersion from $\varepsilon'' \propto (\omega)^{-1}$ in the imaginary component, 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. 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 barrier layers on the electrodes (Hill and Pickup, 1985).

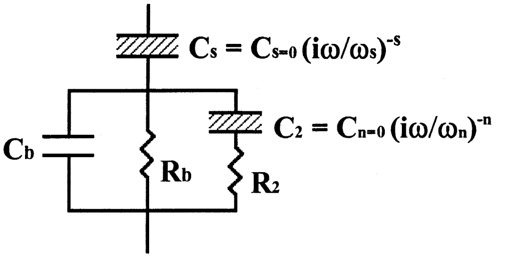

Fig. 3. Modified generalised Maxwell-Wagner equivalent circuit employed to model the dielectric responses in Fig. 2.

The response of the cubic phase consisted of a high frequency bulk process, as seen for the lamellar phase, with greater magnitudes for the imagi-

Table 1
Values of the fitted equivalent circuit parameters derived from the expeimental data obtained for GMOrphic-80–water system containing 22% (w/w) water using the model circuit given in Fig. 3

T(°C)	R_b (Ω)	C_b (p)	R_2 (Ω)	$C_{n=0}$ (F)	n	$C_{s=0}$ (F)	s
15	2.6×10^{5}	5.9×10^{-11}	5.9×10^{4}	4.6×10^{-9}	0.286	2.2×10^{-5}	0.521
55	1.2×10^{4}	3.5×10^{-11}	<10	3.6×10^{-11}	0	1.2×10^{-4}	0.397
68	3.1×10^{4}	5.1×10^{-11}	5.6×10^{4}	1.9×10^{-11}	0	7.2×10^{-5}	0.459
95	3.5×10^{4}	1.2×10^{-11}	<10	1.3×10^{-11}	0	1.2×10^{-4}	0.427

nary component (i.e. a higher conductance) and a sub-Hz electrode process. The high conductivity and lack of a sub-structure relaxation process reflected the homogeneity of this three dimensional ordered, bicontinuous structure. Compared to the cubic phase, both the real and imaginary responses of the reverse hexagonal phase were reduced, and a small peak of a second relaxation in the real permittivity existed at circa 10^4 Hz. This behaviour may be related to the change from three to two dimensional periodicity, causing twisting of the charge conduction pathway, thus leading to higher bulk resistance and an additional relaxation process due to the discontinuity of charge transport. Finally, the spectra of the isotropic reverse micellar phase showed a response which was similar in shape to the cubic phase but which yielded lower real permittivity for the high frequencies bulk process.

The dielectric data were modelled in terms of a modified generalised Maxwell-Wagner equivalent circuit, as shown in Fig. 3. This circuit consists of a fractional power law dispersive capacitance, C_s, representing a layer of lipid formed at the electrode surface, in series with a parallel RC circuit (C_b and R_b) which corresponds to a major bulk process. A series connection (C_2 and R_2) was employed to model the additional polarisation behaviour seen in the lamellar and H_{II} phases. C_2 is a dispersive capacitance showing power law behaviour described by

$$\begin{aligned} C_2 &= C_{n=0}(i\omega/\omega_n)^{-n} \\ &= C_{n=0}(\omega/\omega_n)^{-n}[\cos(n\pi/2) - i\sin(n\pi/2)] \end{aligned} \tag{4}$$

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 cooperation 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 surrounding, but when $0<n<1$, the dipoles are uncoupled and the value of $C_2(\omega)$ is complex and must be considered in terms of the real and imaginary components, which is represented by a parallel RC circuit with $C_n \propto \omega^{-n}$ and $R_n \propto \omega^{n-1}$.

The circuit was found to yield an excellent fit to the experimental data, as was observed for the other concentrations in a previous study (He and Craig, 1998). The values of the parameters from the fitting data of the four concentrations are given in Table 1. These fitted data may be considered in terms of the structure of the phases. At 15°C, the lameller phase exhibits high resistance values for both R_b and R_2 and a high value for $C_{n=0}$ with a positive value of n, reflecting the dispersive relaxation of weakly bound dipoles and charge transport process across the lipid bilayer due to the discontinuous bilayer structure. At 55°C, the cubic phase shows low R_2 and $C_{n=0}$ values, the latter being similar to that of the bulk capacitance, C_b, indicating the disappearance of the lamellar network and the formation of an homogeneous structure. At 68°C, the H_{II} phase shows a relatively high R_2 value, resulting from the discontinuity of charge transport across the lipid bilayers. At 95°C, R_2 decreases again, reflecting the homogeneous open structure of the L_2 phase. The low C_b and $C_{n=0}$ values corresponds to the low dielectric permittivity of the oil medium.

3.2. Thermotropic phase transitions

Given that the isothermal frequency responses

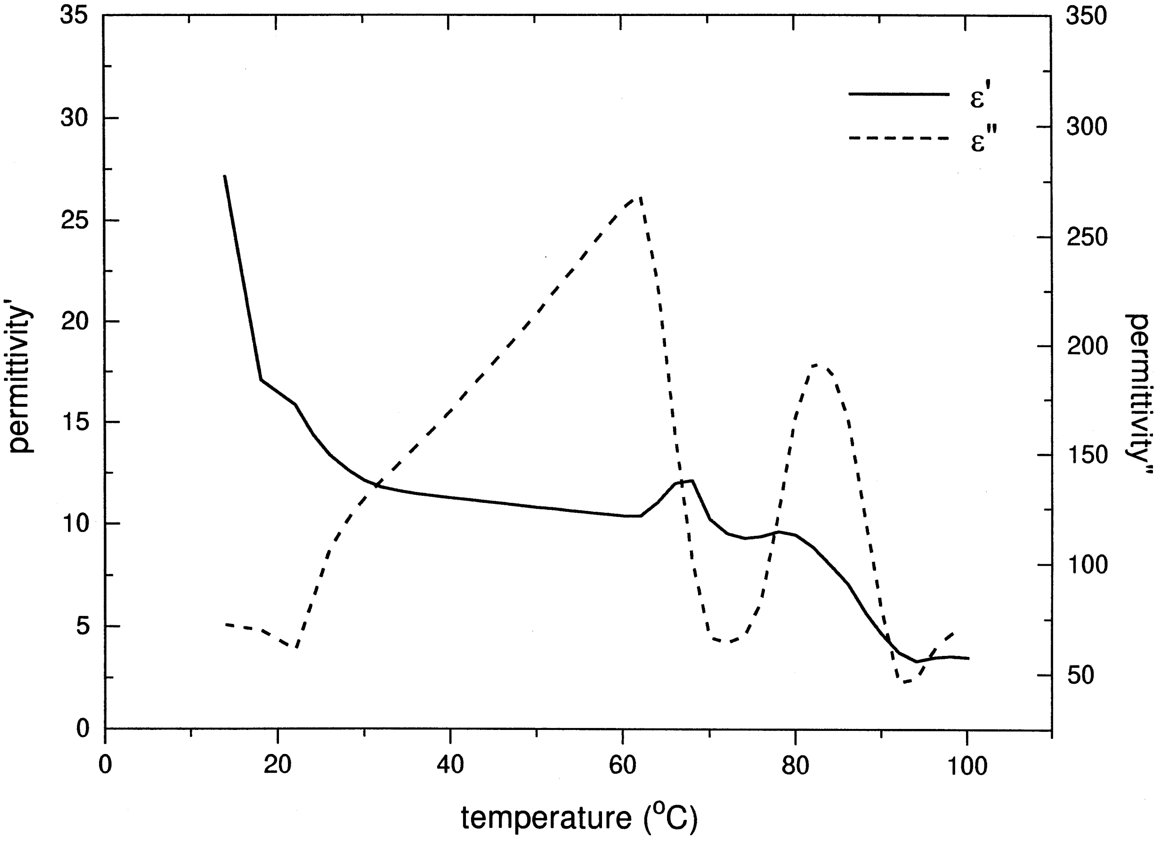

Fig. 4. Temperature dependence of the permittivity at 10 kHz for the 22% (w/w) water sample over the temperature range 15–100°C.

at specific temperatures showed changes dependent on the phase behaviour, it is logical to suggest that temperature sweeps may allow identification of the transitions between these phases. The isothermal runs described above allowed selection of the most appropriate frequency for the dielectric measurements to be chosen and these are stated for each study.

Hot stage microscopy studies indicated that for the 22% systems the lamellar phase was observed below 25°C, the cubic phase from 27 to 66°C, the reverse hexagonal phase from 68 to 81°C, and the L_2 phase at higher temperatures. Fig. 4 shows the real and imaginary components of the permittivity as a function of temperature for the 22% w/w water samples at a representative frequency of 10 kHz over a temperature range 15–100°C. The absolute values obtained do not comply exactly with those shown in Fig. 2 (although given that the former is scaled logarithmically the discrepancy is not great). These differences are almost certainly due to the fact that the temperature sweeps are dynamic while the frequency sweeps are equilibrium measurements with respect to temperature, hence any kinetic components to the response will result in differences between the two sets of measurements. A sharp discontinuity is seen in both the real and imaginary permittivities at around 25°C, with the loss component increasing and the real component showing a discontinuity in the temperature dependent decrease seen at lower temperatures; these correspond to the transition from the L_α to the cubic phase. The real component showed little temperature dependence within the cubic phase while the imaginary component showed a near-linear increase with temperature. At 62–70°C, the dielectric loss showed a marked decrease from

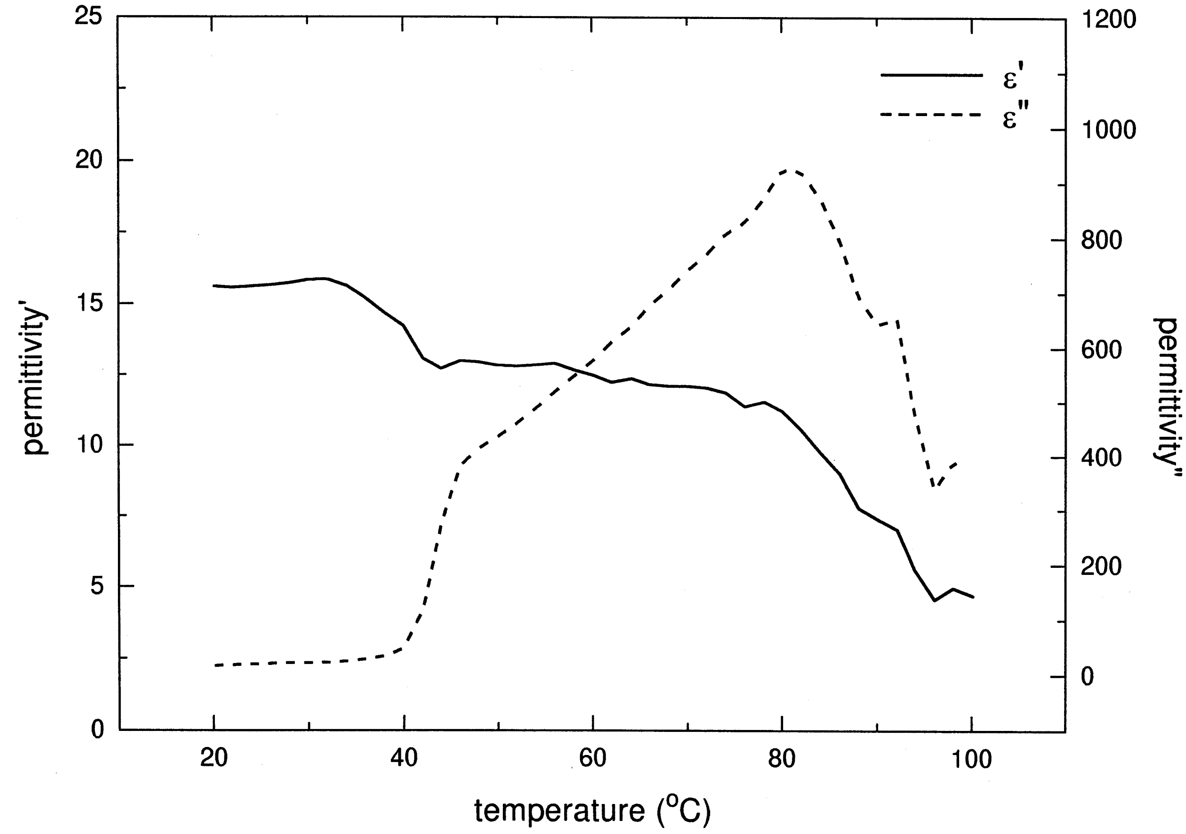

Fig. 5. Temperature dependence of the permittivity at 1 kHz for the 10% (w/w) water sample over the temperature range 20–100°C.

approximately 270 to 60 while the real component showed a small peak at approximately 68°C, corresponding to the transition from the cubic to the reverse hexagonal phase. From 75–82°C, the imaginary component increased to a maximum while the real component showed a small decrease, corresponding to the melting of the reverse hexagonal structure observed under the hot stage microscope. The onset temperatures were approximately 25, 64, and 80°C for the phase transitions from lamellar to cubic, cubic to reverse hexagonal and then to L_2 respectively, hence there is good agreement with the hot stage microscopy.

In order to further establish the validity of using the dielectric technique as a means of identifying phase transitions, the temperature dependence of the 10 and 30% (w/w) water systems were studied. The 10% systems were identified as being in the optically anisotropic lamellar (L_α) phase up to 38°C, whereupon the optically isotropic cubic phase was formed, followed by the isotropic fluid L_2 phase at approximately 80°C. Fig. 5 shows the temperature dependence of the dielectric permittivities, with a sharp discontinuity being seen, particularly in the imaginary component, as the system underwent a phase transition from the lamellar to cubic phase at approximately 38°C, and from the cubic to L_2 phase with negative steps in both components at approximately 80°C.

Hot stage microscopy of the 30% (w/w) water samples indicated that the system was in the cubic phase from ambient to 47°C and the reverse hexagonal phase from 50 to 92°C, followed by the L_2 phase at higher temperatures. A series of photomicrographs taken of the 30% (w/w) sample at 49, 50, 52, 54, 58, and 61°C is shown in Fig. 6, revealing the intermediate structures formed on development of the reverse hexagonal phase. It should be stressed that the visualisation of these transitions

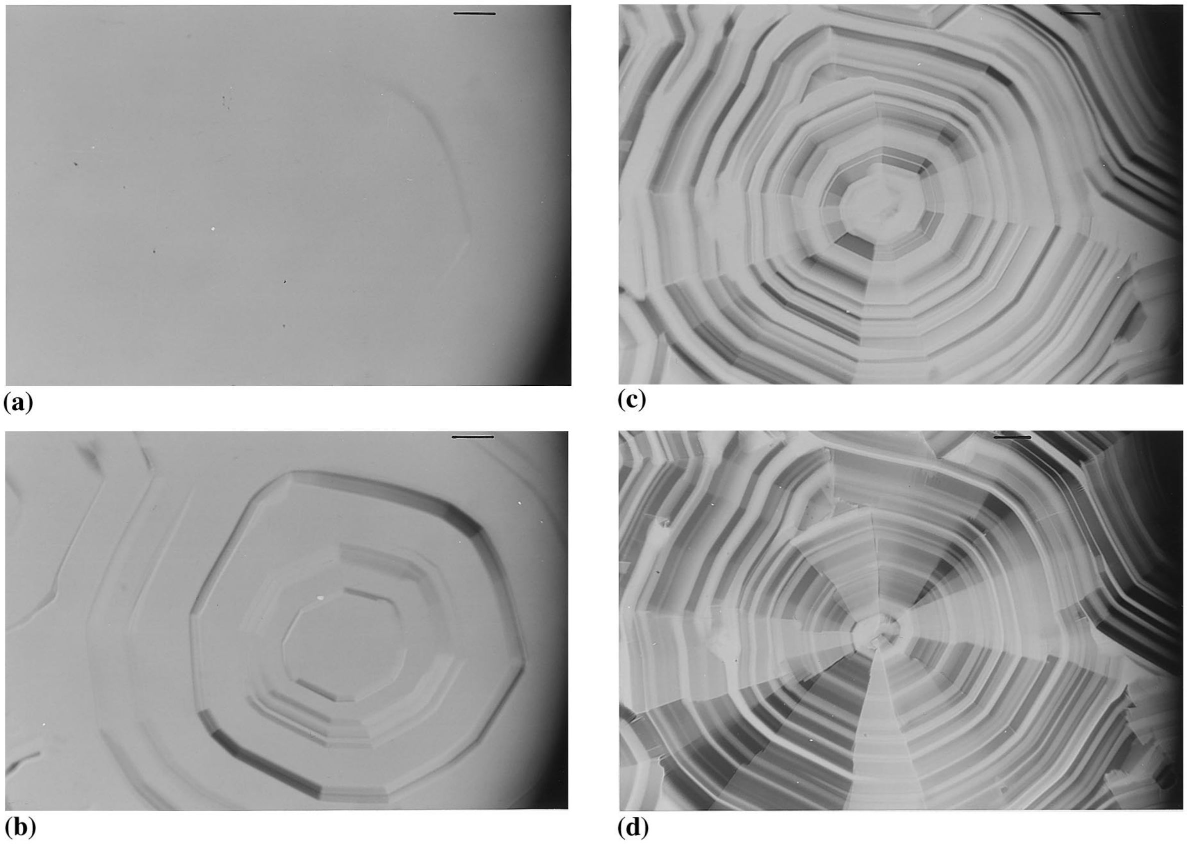

Fig. 6. A series of DIC photographs taken of 30% (w/w) water sample at (a) 49, (b) 50, (c) 52, (d) 54, (e) 58 and (f) 61°C, showing the dynamic transition process of cubic to reverse hexagonal phase. Bar represents 40 μm.

was not completely reproducible. The dielectric investigations (Fig. 7) showed marked changes in the real and imaginary permittivities at approximately 52°C, with a negative step in the imaginary component and a peak in the real permittivity as the system went from the cubic to the reverse hexagonal phase. The detection of this peak, with the concomitant implication for the presence of intermediate structures, was observed reproducibly for all samples undergoing the transition from the cubic to H_{II} phase. This is in marked contrast to the reproducibility of the phase transitions noted using DIC microscopy, with varied from sample to sample and across different regions of the slide; the dielectric technique, therefore, does appear to be more reliable in this respect. The slight increase in both components at high temperatures may represent the onset of the transition from the hexagonal to L_2 phase, although the transition could not be clearly seen within the experimental temperature range.

4. Conclusions

This study demonstrates that low frequency dielectric analysis is sensitive to the phase structure and behaviour of glyceryl monoolein–water systems. The isothermal frequency responses allow modelling of the data in terms of an equivalent circuit, from which information on the microstructure of the system may be obtained, as discussed in more detail in a previous publication (He and Craig, 1998). However, in addition the instrument may be used in the temperature domain in order to identify phase

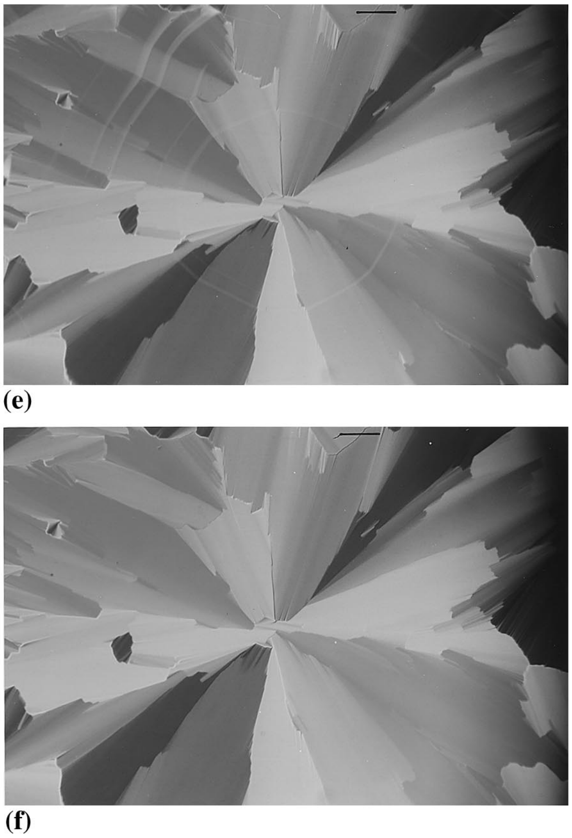

Fig. 6. (*Continued*)

transitions in a quantifiable manner. The temperature sweep data was found to be in good agreement with hot stage microscopy studies, thereby verifying the applicability of the technique as a means of detecting thermotropic structural changes in these samples. However, the dielectric technique is in many respects a more reliable approach than hot stage microscopy, as it is not dependent on the changes being between anisotropic phases and is less dependent on operator and sample variation.

These findings may be of use for a number of reasons. Firstly, the technique is clearly useful for the characterisation of glyceryl monooleate systems, which are finding increased application within the pharmaceutical sciences. More importantly, however, the study illustrates the concept of using low frequency dielectric analysis not only as a means of modelling the microstructure of complex systems but also as a means of detecting transitions which may be difficult to identify using other techniques. In this respect, therefore, the use of the technique may be applicable to a much wider range of systems and problems than have been studied to date.

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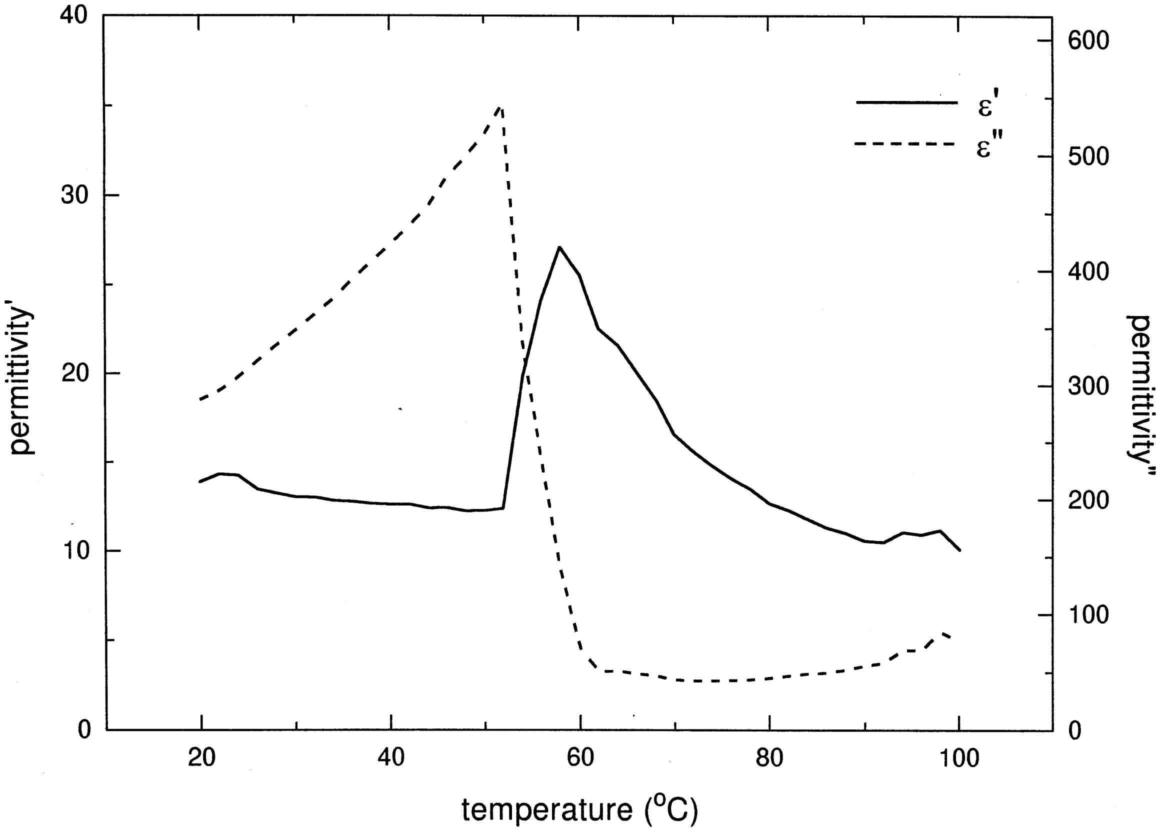

Fig. 7. Temperature dependence of the permittivity at 10 kHz for the 30% (w/w) water sample over the temperature range 20–100°C.

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