Characterization of Micellar Systems by the Use of Acoustic Spectroscopy

GIULIA BONACUCINA, MONICA MISICI-FALZI, MARCO CESPI, GIOVANNI F. PALMIERI

Department of Chemical Sciences, University of Camerino, via S. Agostino 1, 62032 Camerino (MC), Italy

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ABSTRACT: Acoustic spectroscopy affords a new and unique way to characterize concentrated suspension and emulsion while avoiding the limitations imposed by dilution, an undesirable step, particularly with highly structured samples. This study sought to illustrate the potentialities of this technique by using it to characterize the self-assembling behaviour of Poloxamer 407 systems (3–25%, w/v), both alone or after the addition of various amounts of hydroxypropyl β-cyclodextrin (5–20%, w/v). Particle size and the microrheological extensional moduli (G' and G'') of the systems were determined from acoustic parameters such as sound attenuation and speed. By monitoring the variation of the particle size and the rheological extensional moduli at increasing temperatures, it was possible to define and outline the Poloxamer 407 transitions and the effect of the HP β-CD on them. Poloxamer 407 micelle formation due to progressive dehydration occurred within a temperature interval of 15°C (including gelation) and was dependent on poloxamer concentration. Particularly, particle size of the aggregates changed within this interval. Mean diameters were 600 nm at the onset of micelle formation and decreased after the thermogel formation to more or less 75 nm. The presence of HP β-CD changed the basic self-assembling mechanism of Poloxamer 407 by increasing micelle formation and particularly thermogelation temperatures. The results confirm that acoustic spectroscopy offers a powerful method for characterizing heterogeneous systems, thus indicating its potential for applications in the pharmaceutical field. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association
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INTRODUCTION

Poloxamers are triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) available in different molecular weights and PPO/PEO ratios. The presence of PEO and PPO blocks in a single polymer chain gives rise to essentially amphiphilic molecules whose self-assembling properties display a wide range of phase behaviour. This ability to form micelles and liquid crystalline phases is strongly temperature dependent,[1] since increasing temperature allows self-association, decreasing the critical micelle concentration (cmc).

In a previous study the aggregation and the phase behaviour in water of several Poloxamers was investigated with the aid of different techniques such as static and dynamic light scattering (DLS), small angle neutron scattering (SANS), polarization microscopy, differential scanning calorimetry (DSC), and ^{1}H-nuclear magnetic resonance (NMR). The sequence of phase behaviour observed at increasing concentrations appeared analogous to that shown by normal surfactants, for

Correspondence to: Giovanni F. Palmieri (Telephone: 737-402289; Fax: 737-637345;
E-mail: gianfilippo.palmieri@unicam. it)

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example, isotropic solution, cubic phase, hexagonal phase and lamellar phase. At the same time, a significant difference compared to normal surfactant was detected, since the phase transition also outlined clearly thermotropic behaviour. In fact, a sequence of different mesophases and phase transitions were also detected at constant concentration but at increasing temperatures.[2,3]

Other techniques were then utilized to determine the hydrodynamic radius of micelles, such as pulsed gradient spin echo (PGSE) NMR and fluorescent spectroscopy.[4,5]

Micellization phenomenon is related to the dehydration of PPO chains, which drives unimer aggregation to form micelles. PPO is in fact soluble in the temperature range of 2–15°C, while for higher temperatures a precipitation cloud point exists.[6] Thus it is assumed that the hydrophobic PPO is located in the core of the micelle, while the PEO lyophilic block forms the outer corona, as assessed by the use of electron paramagnetic resonance[7,8] (EPR) and the above-mentioned techniques.

An important property of these aggregates is their ability to incorporate hydrophobic substances that are usually insoluble or poorly soluble in aqueous or hydrophilic environment, enhancing their solubility. The presence of water insoluble solutes can promote a change from spherical to rod or lamellar shape, thus affecting micelle size and aggregation number, as well as various factors involved in drug delivery.[9]

Another important property of Poloxamers is their thermogelling behaviour: in fact, water dispersions of some of these polymers generally are liquid at low temperature, but give rise to a strong gel at increasing temperatures.[10–12] This sol/gel transition has been correlated to intrinsic changes in the micelles properties or to entropic variation in the ordered water molecules close to the PPO segments or to the possibility of formation of a crosslinked and three-dimensional structure able to entrap water in its network.[12–15]

Thus, both micellization and gelation depend on three different factors, temperature, polymer concentration and PEO block length.[16]

The aim of this study was to investigate the potential of acoustic spectroscopy in characterizing concentrated colloid phases, such as those formed by poloxamers, for pharmaceutical use.

Acoustic spectroscopy offers several advantages over traditional methods. In fact, ultrasound can propagate through concentrated dispersions,[22] allowing correct and complete characterization of a wide range of systems with volume fraction of the dispersed phase up to 40% without dilution. The determination of parameters such as attenuation frequency spectra, sound speed and colloid vibration current makes it possible to calculate particle size from 5 nm to 1000 μm and to determine ζ-potential with high precision. Ultrasound measurements are nondestructive and can act as an extensional microrheometer, providing information on the microstructure of the dispersed systems. Thus, acoustic spectroscopy provides a suitable technique for characterizing colloidal systems and also for studying their stability, with the additional possibility of distinguishing between aggregation and flocculation phenomena for both solid rigid particles and soft particles (lattices, emulsions and microemulsions). Despite its potential for the analysis of different systems such as cells, paint, cosmetic preparations, ceramics, minerals, pigments, oxides and chemical-mechanical polishing material, this technique is practically unknown in the pharmaceutical field.

In the present work, Poloxamer 407 phase transitions (a very interesting case study) and the effect of HP β-CD on them are examined in order to indicate possible usefulness of this technique in the pharmaceutical field.

The hydrodynamic diameter of the micelles of Poloxamer 407 in the concentration range of 3–25% (w/v) was investigated by measuring the attenuation and propagation velocity of the ultrasound in the medium at different temperatures (acoustic spectroscopy). Next, different amounts of hydroxypropyl β-cyclodextrin (HP β-CD) (5–20%, w/v), which is widely used in oral and parenteral pharmaceutical dosage forms to improve stability and solubility of poorly water-soluble drugs through the formation of inclusion complexes, were added to the Poloxamer 407 water systems, and the effects monitored. Previous studies have demonstrated that the addition of different glycols and polyalcohols[17] as well as the addition of HP β-CD[18] influenced both the gelation and micellization temperature of Poloxamer 407, outlining a shift of these parameters towards higher values. In this paper, acoustic spectroscopy allowed a better characterization of the microstructure and behaviour of these systems at increasing temperatures.

MATERIALS AND METHODS

Poloxamer 407 (LUTROL F127, BASF Chem. Trade Gmbh 91593 Burgbernheim, l Germany),

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hydroxypropyl β-cyclodextrin (KLEPTOSE HP, Roquette, France), deionised water obtained from an ion-exchange system MF3 (San Salvatore di Cogorno, Genoa, Italy).

Gel Preparation

Poloxamer 407 and Poloxamer 407-hydroxypropyl β-cyclodextrin samples were prepared for simple dispersion of the materials in the required amount of degassed and deionised water using the "cold" procedure. To aid solvation, cyclodextrin was added before the Poloxamer. The investigated Poloxamer 407 concentrations were in the range of 3–25% (w/v) while hydroxypropyl β-cyclodextrin concentrations ranged from 5% to 20% (w/v). Samples were then stored at 4°C for at least 24 h before being analysed.

Acoustic Spectroscopy Measurements

Theory

The ultrasound technique can provide useful information in colloid characterization such as particle sizing, rheology and electrokinetics by measuring the ultrasound's attenuation and velocity of propagation in a medium. In fact, not only do acoustics serve as a particle sizing technique, but they also provide insights about the microstructure of a dispersed system, acting as a microrheometer.

The operating principle of acoustic spectroscopy is based on the measurement of the ultrasound pulse intensity and phase after its propagation through the sample. During the passage through the sample, sound is attenuated by the presence of the liquid medium and any particles in dispersion, and thus by applying the appropriate theories, we can exploit sound attenuation to obtain information about particle properties.

There are six mechanisms of sound interaction with a dispersed system: *viscous* (related to the shear waves generated by the particles oscillating in the acoustic pressure field due to the difference in the densities between particle and medium), *thermal* (related to the temperature gradients generated near the particles surface), *scattering* (through the same principle that is active in light scattering), *intrinsic* (involving losses of acoustic energy due to the interaction of the sound wave with the particles and the medium as a homogeneous phases), *structural* (caused by the oscillation of a network of particles; this mechanism is specific for structured systems such as concentrated colloid dispersions), and *electrokinetic* (ultrasound/double layer interactions). The electrokinetic losses are negligible in terms of the total attenuation, making it possible to separate electroacoustic spectroscopy from acoustic spectroscopy. Total attenuation is mainly the sum of the first five contributions:

$$\alpha = \alpha_{\mathrm{vis}} + \alpha_{\mathrm{th}} + \alpha_{\mathrm{sc}} + \alpha_{\mathrm{int}} + \alpha_{\mathrm{st}} \tag{1}$$

Different acoustic theories have been developed and the ECAH theory, whose acronym comes from the names of its creators (Epstein, Carhart, Allegra and Hawley),[19,20] is probably the most wellknown. It is constructed in two stages. The first one is called "single particle theory" since it attempts to account for all the ultrasound disturbances surrounding just a single particle. This stage relates the microscopic properties of both the fluid and the particle to the system properties at a single particle level. The second stage, referred to as the "macroscopic theory", relates this single particle level to the macroscopic level at which the experimental raw data are obtained. At this stage, the total attenuation is regarded as a superposition of the contributions from each particle, and particle–particle interactions are neglected. Thus this theory presents some limitations but can be surely be considered valid for moderately concentrated samples when "thermal losses" are the dominant mechanism of attenuation, as in emulsion or latex systems. Two different parameters play an important role, "viscous depth" and "thermal depth". These two parameters are a measure of the decay of the shear and thermal waves in the liquid. They are actually involved in the differences observed between emulsions and solid particle dispersions since particles oscillating in the sound wave generate these shear and thermal waves, which damp in the particle vicinity.

"Viscous depth" (δ_{v}) is the characteristic distance for the shear wave amplitude to decay, while "thermal depth" (δ_{t}) is the corresponding distance for the thermal wave

$$\delta_v = \sqrt{\frac{2\nu}{\omega}} \tag{2}$$

$$\delta_{\mathrm{t}} = \sqrt{\frac{2\tau_{\mathrm{m}}}{\omega\rho_{\mathrm{m}}C_{\mathrm{p}}^{\mathrm{m}}}} \tag{3}$$

where ν is the kinematic viscosity of the medium, ω the sound frequency, ρ_{m} the medium density,

τ_m the medium heat conductance and C_p^m is the heat capacity at constant pressure of the medium.

The ratio between δ_v and δ_t is called depth ratio and is equal to 2.6 for an aqueous dispersion.[21]

The fact that for most liquids (like water), "thermal losses" are much less sensitive than "viscous losses" to "particle–particle" interactions, makes ECAH theory valid for emulsions or other soft dispersed systems characterized by a considerable volume fraction of dispersed phase.[22]

Briefly, in the cell measure, a piezo-electric transducer converts an input electrical tone burst to an ultrasound pulse of a certain frequency and intensity and launches it into the sample. The intensity of this pulse decreases as it passes through the sample due to the interaction with the fluid. A second piezo-electric transducer converts this weakened acoustic pulse back to an electric pulse and sends it to the electronics for comparison with the initial input pulse. The total loss and time delay from the input to output transducer for each frequency and gap can be considered the "raw data" from which further interpretation is done.

It is convenient to present these raw data in terms of an attenuation coefficient $\boldsymbol{\alpha}$ defined as

$$\alpha = \frac{10}{f\,(\mathrm{MHz})L\,(\mathrm{cm})}\log\frac{I_{in}}{I_{out}} \tag{4}$$

where f is the frequency of the pulse, L the distance between transmitter and receiver and I_{in} and I_{out} are the intensities of the emitted and received pulse, respectively.

The sound speed c is obtained from $c = L/t$, t being the delay time between emitting and receiving the pulse.

An attenuation frequency spectrum, typically in the range of 1–100 MHz, and sound speed are the usual experimental outputs of an Acoustic Spectrometer.

Such experimental data can be used either for empirical correlations with other properties of the system under investigation, or for further theoretical treatment. This second step is particularly interesting, as already said, for the determination of the particle size distribution. In fact, starting from the ECAH, several other theories (rather complex but demonstrated and experimentally verified) allow the calculation of each single contribution to the whole acoustic attenuation and the transformation of these data into particle size. For further insight into these complex theories, the reader is invited to consult specific treatises on this subject.[23]

As already mentioned, the acoustic spectrometer can also act as an extensional, or longitudinal microrheometer, taking into account the fact that in this case "longitudinal" viscoelastic properties are measured because the stress is not tangential (as for an oscillation experiment in a rotational rheometer), but normal.

Thus, it is possible to relate measurements of ultrasonic absorption and velocity to the real and imaginary part of the complex modulus.[24]

In a viscoelastic medium the compressional part of the stress P and the compressional strain S can be related by the compressional modulus K while the shear part of the stress T_{ij} and the shear part of the strain S_{ij} are linked by the shear modulus G by the following equation:

$$P = -KS \tag{5}$$

$$T'_{ij} = GS'_{ij} \quad \text{where } i \neq j \tag{6}$$

The equation below represents the propagation of an acoustic wave in a viscoelastic medium where G and K are different from zero[24]:

$$\rho\frac{\partial^2 X_1}{\partial t^2} = \left[K + \left(\frac{4}{3}\right)G\right]\frac{\partial^2 X_1}{\partial x_1^2} \tag{7}$$

where X_1 is the displacement of a point in the medium. If X_1 is proportional to $\exp\{i\omega(t - x/\beta)\}$ it obtains that:

$$V^2 = \frac{K + (4/3)G}{\rho} \tag{8}$$

where V is the velocity of an acoustic wave in an elastic medium and is calculated from the compressional and shear moduli linearly combined, while ρ is the density. It is also possible to define a complex longitudinal modulus G^* as

$$G^* = G' + iG'' = K + \left(\frac{4}{3}\right)G \tag{9}$$

Assuming X_1 proportional to $\exp\{i\omega\tau - (\alpha + i\omega/V)x\}$, where α is the absorption coefficient a relation within G', G'', α and V is possible to define. From the separation of the real and imaginary part, there emerge two equations:

$$G'' = \rho V^2\left[\frac{1 - (\alpha V/\omega)^2}{[1 + (\alpha V/\omega)^2]^2}\right] \tag{10}$$

$$G'' = \frac{2\rho V^2(\alpha V/\omega)}{[1 + (\alpha V/\omega)^2]^2} \tag{11}$$

The value of $(\alpha V/\omega)^2$ is often small in the MHz frequency range, so

$$G' = \rho V^2 \tag{12}$$

$$G'' = \frac{2\rho V^3 \alpha}{\omega} \tag{13}$$

Acoustic Measurements

The cell of the acoustic and Electroacoustic spectrometer DT-1200 (Dispersion Technology, USA) was filled (in triplicate) with 15 mL of a Poloxamer 407 dispersion (with or without HP β-CD) and the sound attenuation and speed were monitored. Analyses were performed in the gap interval of 0.325–20 mm, in the frequency range of 3–100 MHz, and in the temperature range of 12–30°C. Particle size and rheological G′ and G″ moduli were calculated over that range of temperatures. This allowed us to follow and identify sample transitions (micellization and gelation) at increasing temperatures from the variations of the parameters described above.

The density of the liquid systems to analyse by acoustic spectroscopy was obtained between 12 and 30°C with an Anton Density Metre (DMA) 55 (Anton Paar, Graz, Austria), which is based on the oscillating U-tube principle. Analyses were performed in triplicate.

RESULTS AND DISCUSSION

Acoustic spectroscopy offers a unique opportunity to characterize concentrated samples without dilution. It is well known that dilution affects system rheological properties, with a possible variation of particle size and surface chemistry, especially for structured samples.

In this study, concentrated Poloxamer 407 and Poloxamer 407-HP β-CD samples were analysed at different temperatures, because Poloxamer 407 behaviour is strongly dependent on both concentration and temperature. In this way it was possible to observe the evolution of phase transitions that follow one another for this copolymer.

In Figure 1 the mean particle diameter at different copolymer concentration is plotted against the temperature. The evolution of the particle size allows the identification of the different phase transitions. At low temperatures, very small values of mean diameter can be associated to the presence of unimers species in solution, which then aggregate, forming micelle structures at increasing temperatures.

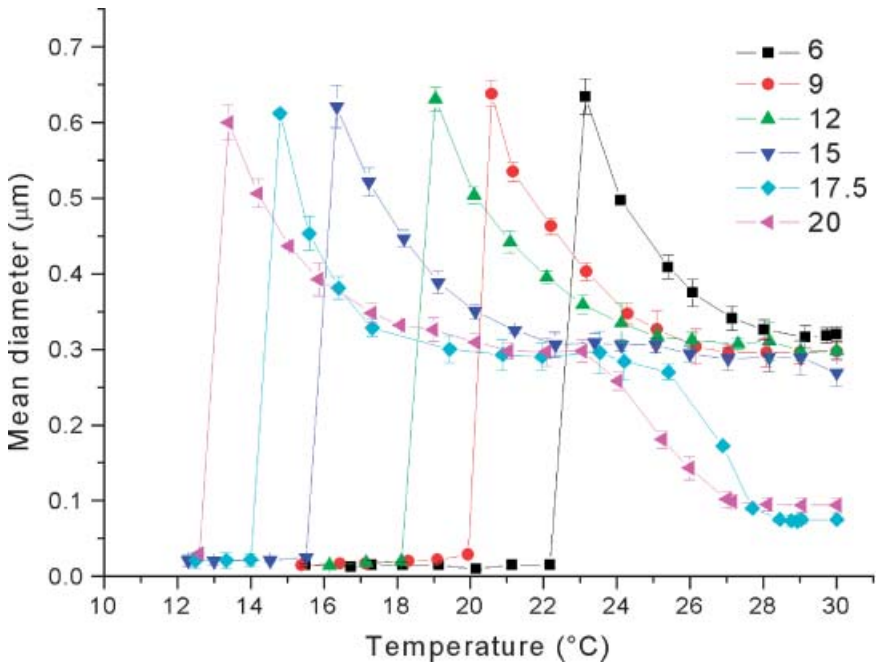

Figure 1. Particle size versus temperature plots of Poloxamer 407 samples at different concentrations (6–20%, w/v).

The beginning of the micellization phenomenon can be identified with the sudden increase of size, according to previous calorimetric studies.[18]

Different hypothesis on the mechanism of micelle formation may be advanced to explain this behaviour. The high mean diameter values following the onset of micelle formation may be due to the presence of large and mild aggregates, which are characterized by a high-solvation degree. Actually, this is an initial stage of the phase separation with regions that are very rich in Poloxamer and regions that abound more in water. Subsequently, size decreases until a plateau value is achieved. This rather short plateau interval and the onset of the micellization as well depend on polymer concentration and are attributable to a further desolvation process of the PPO part, which leads to unimer aggregation and to a progressive formation of micelles close to each others, to form clusters. In fact, it is thought that the hydrophobic portion of PPO forms the "core" of the micelle, while the hydrophilic chains of PEO form the outer corona. These results are in agreement with previously reported data, which demonstrated the presence of large micelle clusters after the micellization occurred.[25]

For the 17.5% and 20% (w/v) concentrations, plots show a further decrease in the mean diameter value starting respectively from the temperatures of 25 and 23°C, and ending with the final equilibrium, which is reached at thermogelation. According to previous rheological and calorimetric studies[18] these temperatures can be identified with the sol/gel transition. Two different explanations are possible. Firstly, as stated in a previous study,[26] during gelation, the partial collapse of the PEO chains in the micellar mantle lead to the formation of a tighter structure. The micelles in the gel structure are blocked in the formation of the three-dimensional network and thus probably only the free micelles that are able to move and are not involved in the formation of the gel network, because of their smaller size, are detected. Since the instrument used for this study is equipped only to work up to 30°C, it was not possible to identify the sol/gel transition for Poloxamer concentrations lower than 17.5% (w/v), as gelation was present only at temperatures over than 30°C. In any case, below a poloxamer concentration of 15%, thermogelation no longer occurs.

The presence of 10% of HP β-CD brings about a remarkable change in sample properties. Figure 2 shows the significant modification in the shape of the mean diameter versus temperature curves. First of all, a shift (of about 2°C) towards a higher temperature of the micellization phenomenon for all the studied concentrations can be detected, confirming the results previously obtained with the DSC analysis.[18] For all the Poloxamer 407

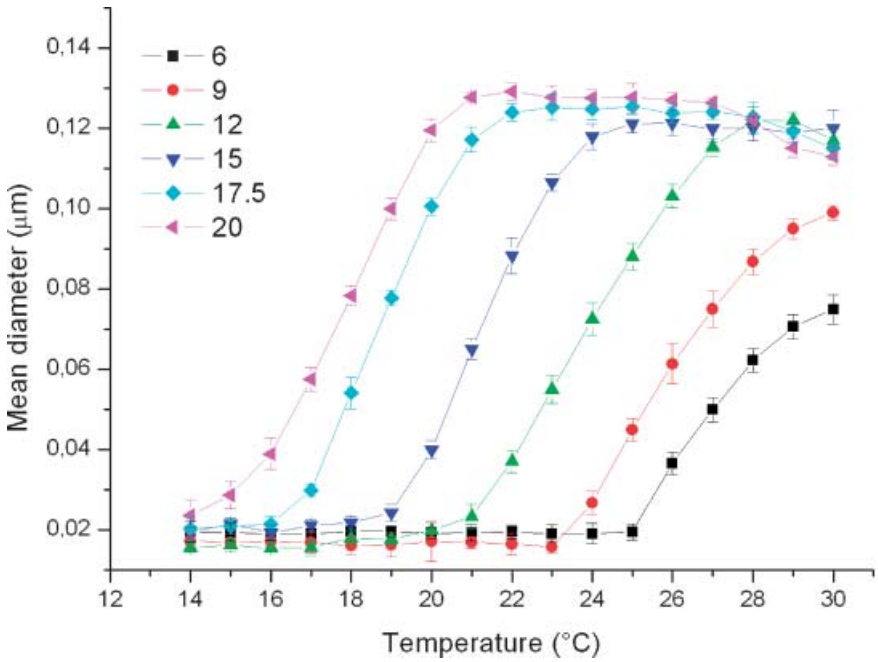

Figure 2. Particle size versus temperature plots of Poloxamer 407 samples at different concentrations (6–20%, w/v) containing the 10% (w/v) of HP β-CD.

concentrations it is possible to observe that HP β-CD slows and defers both micellization and gelation so that the 6% and 9% samples at 30°C do not reach the plateau value, which corresponds to the completion of micelle formation.

For this reason, the sol/gel transition is not so evident for the 17.5% and 20% concentrations even if an onset of a decrease in the particle size values is visible at 28–29°C and can be identified as the beginning of this phase transition.

Furthermore, the particle size of the systems containing HP β-CD is about half of that of the corresponding Poloxamer 407 samples alone. These results allow us to suppose that HP β-CD interacts with micelles, reducing the formation of clusters. This phenomenon can also explain the shift of the gelation temperature, which is due to micelle–micelle interactions. Thus, the HP β-CD apolar cavity may interact with the more hydrophobic portion of Poloxamer when the polymer starts the dehydration process, slowing down micelle formation. In addition, the hydroxyl groups present in the HP β-CD structure may aid the formation of hydrogen bonds, slowing down the process of desolvation of the polymeric chains. Finally, the size of the cyclodextrin can physically hinder the formation of micelles and particularly, the formation of clusters. Thus, the effect on the critical micelle temperature (cmT) can be actually a consequence of these mechanisms together. Regarding the sol/gel transition temperature (sgT), this is caused by a further dehydration, also involving the hydrophilic portion of the micelles. As a consequence, PEO chains of different micelles give rise to the formation of hydrogen bonds. The hydroxyl groups of HP β-CD can compete with the PEO chains for the formation of the PEO/PEO hydrogen bonds, making necessary greater desolvation (and higher temperature) for the PEO–PEO interactions. At the same time, the steric hindrance of the HP β-CD also reduces the micelle–micelle interactions.

The same transitions can be outlined from the analysis of the rheological parameters, which are strictly related (see Acoustic Spectroscopy Measurements) with sound speed (G') and attenuation data (G''). Both moduli aid the identification of the system phase behaviour, showing a different trend depending on the temperature of analysis. It is necessary to make clear that the dynamic moduli obtained from acoustic spectroscopy measurements are not comparable with those derived from rotational rheometers working in the oscillation mode, since the applied stress is not tangential, as in an oscillatory experiment, but "normal", and the tested frequencies are much higher.

G' modulus for the Poloxamer 407 (Fig. 3) decreases during micellization until reaching a plateau value. This trend is more evident in the concentrated systems, while it is practically undetectable for the dilute ones. For the 17.5% and 20% samples it is also possible to identify a slight inflexion after the plateau, which may be

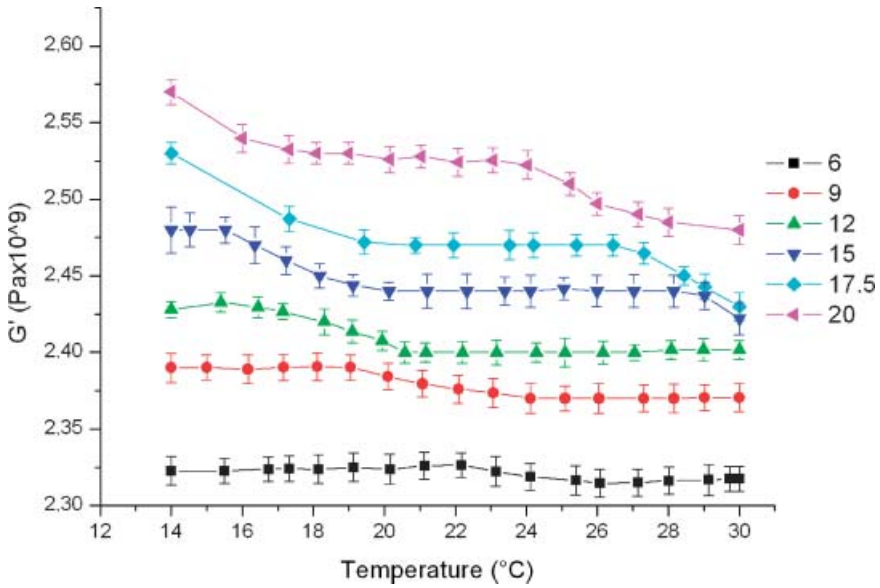

Figure 3. G' values of Poloxamer 407 samples at different concentrations (6–20%, w/v) at different temperatures calculated at the frequency of 50 MHz.

identified with the sol/gel transition since the correspondent values of temperature are in agreement with those determined rheologically and by thermal analysis.[18]

The decrease in the G' modulus values with micellization and thermogelation may be explained by the reduction, during this process, of the micelle/water interaction, which leads to the presence of an increased amount of "free" water in the system. Thus, desolvation gives rise to a reduction of "sound speed," since sound speed in free water is lower than that of water engaged in the interaction with micelles. This sound speed reduction is connected with increasing micelle rigidity and a decrease in their compressibility. This change in G' modulus is not considerable, since the water involved in the desolvation process is only a part of the total water present in the system. Besides, the difference in the sound speed between the "free" water and water interacting with the Poloxamer is scant.

So, at a microrheological level, the interaction with water at the water/micelle interface is definitely lower compared to the unimer interactions before micellization, giving rise to a less structured interface. Therefore, even though the G' is a "bulk" parameter, it is also strictly connected with the phenomena present at the interface and for this reason it tends to decrease with micellization and thermogelation.

In presence of the 10% of HP β-CD, the phase transitions (Fig. 4) appear less defined. At low Poloxamer concentrations, the micellization phenomenon is not detectable. Only at elevated Poloxamer 407 concentrations it is possible to observe the cmT, which is shifted towards higher temperature values while the sgT is practically undetectable in the range of the tested temperatures. However, also in this case, the decrease in G' value for the 12–20% (w/v) Poloxamer concentrations can be identified with the micellization.

More evident are the results obtained from the analysis of the G'' curves (Fig. 5). As already mentioned, this parameter is strictly related with the variation of sound attenuation. In this case, the increase in the modulus values corresponds to the unimers/micelle transition, which is accompanied by an initial increase of G'' then followed by a plateau at the end of the transition. This can be explained with the microrheological modification undergone by the system, related to a lower interaction with water at micelle interface. It causes a destructuration of the sample and an increase of "free" water. Also, the gelation visible for the 20% (w/v) sample can be identified with an increase in the G'' modulus, in agreement with the reduction of the G' modulus previously described.

The HP β-CD makes it more difficult to identify both micellization and gelation temperatures (Fig. 6) since the slowing of both transitions caused a less marked change in the obtained curves. It is in any case possible to confirm a shift of the cmT, which confirms the fact that the HP

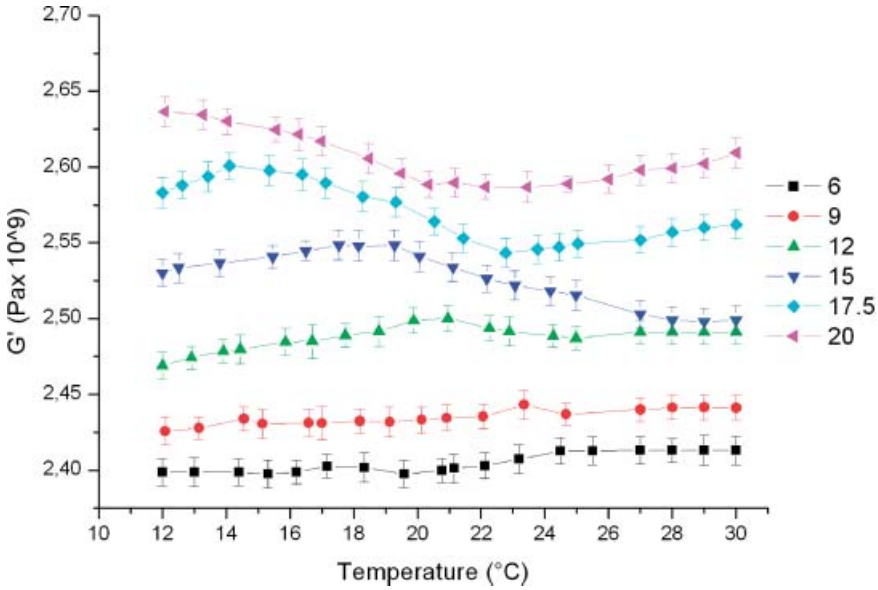

Figure 4. G' of Poloxamer 407 samples at different concentrations (6–20%, w/v) containing the 10% (w/v) of HP β-CD calculated at the frequency of 50 MHz.

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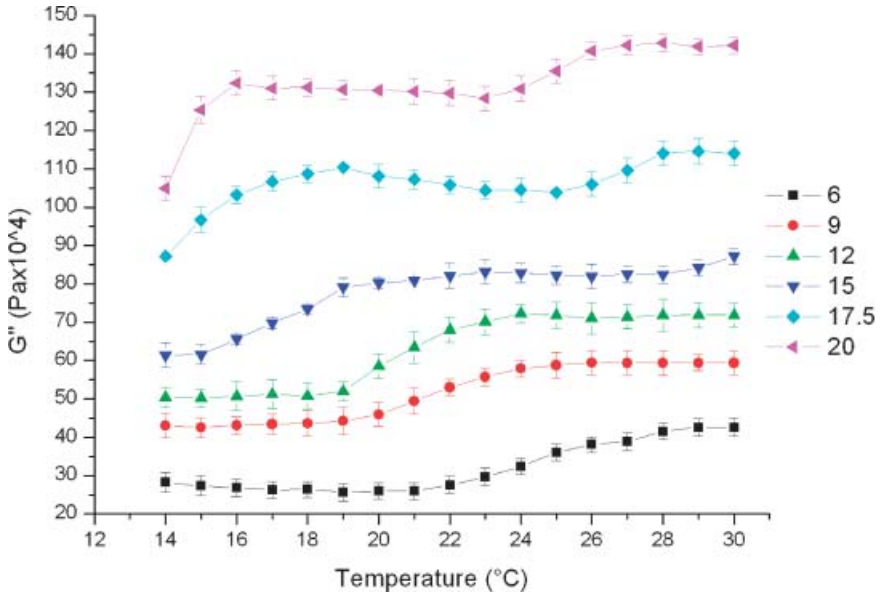

Figure 5. G'' values of Poloxamer 407 samples at different concentrations (6–20%, w/v) at different temperatures calculated at the frequency of 50 MHz.

β-CD delayed the phase transitions, shifting them towards higher temperatures, depending on the concentration of Poloxamer in the sample. In fact, it is also important to highlight that the effect of HP β-CD is less important at increasing Poloxamer concentration.

In presence of HP β-CD (in the studied range of temperatures), as observed for the G', the sol/gel transition temperature is not observed for all the investigated polymer concentrations. Thus, G' and G'' moduli are related to the microstructural changes of the investigated system. The decrease in the G' modulus in presence of two important phase transitions (micellization and gelation) is related to sound speed reduction during these phenomena. At the same time the corresponding increase in attenuation drives to an obvious increase in G'' modulus.

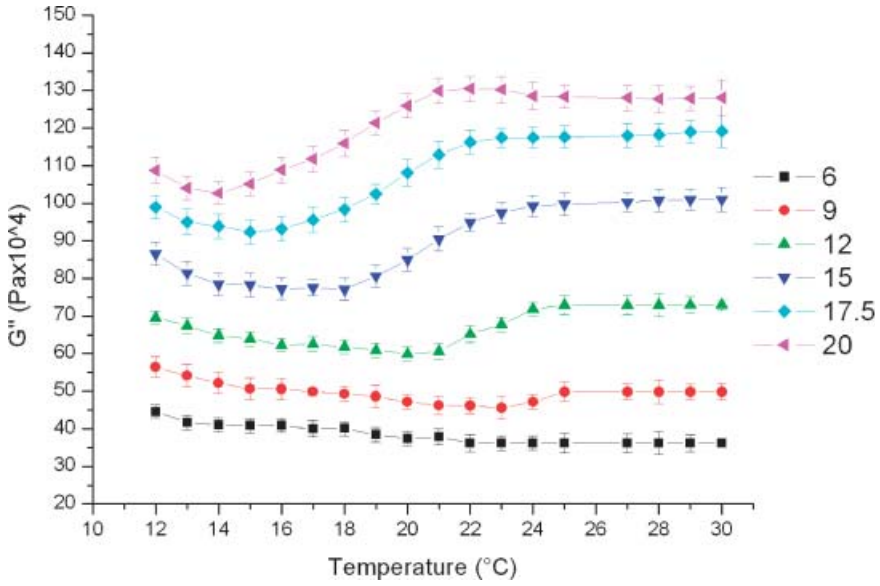

Figure 6. G'' of Poloxamer 407 samples at different concentrations (6–20%, w/v) containing the 10% (w/v) of HP β-CD calculated at the frequency of 50 MHz.

CONCLUSION

Acoustic spectroscopy offers a new and effective technique for analysing complex colloidal samples like Poloxamer 407 water dispersions. It eliminates the need for dilution and thus facilitates study of the concentrated (real) system. In fact, the micelle formation and the sol/gel transition of the real undiluted samples can be very easily pointed out by monitoring the evolution of the particle size and the microrheological parameters in function of temperature. The technique was also very sensible in defining the influence of hydroxypropyl β-cyclodextrin on the Poloxamer 407 phase transitions, and thus promises to be a prime tool for gaining insights on system microstructure.

In addition, findings obtained by acoustic spectroscopy were in agreement with previously reported data and findings reached with different analytical methods.

Thus, this technique could also be successfully used in the characterization of complex systems for pharmaceutical use, such as different types of dispersed systems.

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