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Monitoring the aggregation behaviour of self-assembling polymers through high-resolution ultrasonic spectroscopy

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ABSTRACT

Poloxamer 407 is a well-known self-assembling polymer with a wide range of temperature- and concentration-dependent phase behaviour, such as micellization and gelation. This work was carried out to demonstrate the potential of high-resolution ultrasonic spectroscopy in evaluating aggregation–deaggregation behaviour of self-assembling polymers. In order to achieve this objective, six different concentrations of Poloxamer 407 water dispersion were prepared and analysed between 5 and 35 °C using ultrasonic spectroscopy. For comparison, the same samples were also analysed by the DSC technique. The results showed that polymer aggregation process can be successfully monitored using both ultrasonic parameters of sound speed and attenuation. Furthermore, good agreement with DSC data was observed in terms of characteristic transition temperatures and also in terms of micellization kinetics and related parameters.

1. Introduction

During the past decade, polymeric drug carriers have proven to be useful in drug delivery, and several formulations have been studied in clinical trials (Duncan, 2003). Among all the different polymeric nano-carriers, micelles are currently recognized as one of the most promising drug delivery systems (Kataoka et al., 2001; Lavasanifar et al., 2002; Nishiyama and Kataoka, 2006). Micelles provide considerable advantages, since they can solubilise and thus increase the bioavailability of poorly soluble drugs. Furthermore, because of their size, micelles can accumulate in body regions with leaky vasculature and, if specific ligands are used, they can target the outer of particular cells.

Another important benefit is that drugs loaded in a micellar system are protected from possible inactivation caused by biological agents, thus limiting possible side effects (Bonacucina et al., 2009).

Micelles are amphiphilic colloids with particle diameter within the range of 5–100 nm, formed by amphiphilic or surface-active agents that are composed of two distinct regions with opposite affinities towards a given solvent (Mittal and Lindman, 1991). These amphiphilic molecules exist at low concentration in solution as unimers, while at increasing concentrations aggregation takes place and the final aggregates (micelles), often spherical in shape, usually include several dozen of these units. The critical micelle concentration (CMC) is defined as the concentration of a monomeric amphiphile at which the micelles appear. In some cases (block copolymers), the amphiphile solvation is strongly dependent on temperature, hence another important parameter to take into account is the critical micellization temperature (CMT) (Alexandridis and Hatton, 1995). According to the literature, together with the "traditional" micelles, composed by homopolymers, there is also a separate class of polymeric micelles, which are formed by block copolymers consisting of hydrophilic and hydrophobic units respectively (Kwon and Okano, 1996; Moffitt et al., 1996).

In previous studies, the aggregation and the structural characteristics of micelles were investigated with the aid of different techniques, such as static and dynamic light scattering (DLS), small angle neutron scattering (SANS) (Mortensen, 1996; Artzner et al., 2007), transmission electron microscopy, laser doppler anemometry (Govender et al., 2001) and ^{1}H nuclear magnetic resonance (NMR) (Hagan et al., 1996). While all these techniques are necessary for deeper understanding of the systems, they are quite complex and required highly specialized researchers and dedicated instruments. For more general characterization of the basic behaviour of systems, for example, to determine the CMC and the

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CMT, researchers use more common and widespread methodologies such as spectroscopy techniques (Alexandridis et al., 1994; Alexandridis and Hatton, 1995; Gaisford et al., 1998), light scattering (Yoshida, 2003; Sharma et al., 2007), surface tension (Rodriguez et al., 2007; Sharma et al., 2007) and in particular, differential scanning calorimetry (DSC) (Alexandridis et al., 1994; Wanka et al., 1994; Paterson et al., 1997; Artzner et al., 2007; Trong et al., 2008).

The aim of this paper was to assess the potential of low-intensity ultrasound spectroscopy (with power levels lower than 1 W/cm^2) as a valid tool for general characterization of the micellization process in a wide range of experimental conditions (temperature and polymer concentrations). The authors decided to use high-resolution ultrasonic spectroscopy, a relatively new technique, which allows high-resolution measurement of ultrasonic velocity and attenuation in different samples during a temperature scan (Buckin et al., 2002). Several previous papers used sound speed measurements in order to derive some thermodynamic parameters (isentropic compressibility and apparent molar isentropic compressibility) commonly applied during the analysis of aggregation processes (Wen and Verrall, 1997; Senkow et al., 2002; Roux et al., 2005; Fisicaro et al., 2006). However, the direct use of the ultrasonic parameters obtained during a temperature scan is rarely reported for the monitoring and characterization of copolymer self-assembling properties (Wen and Verrall, 1997).

In order to validate the sensitivity and accuracy of ultrasonic parameters, the results obtained by high-resolution ultrasonic spectroscopy were compared to those observed by analysis of the same samples using DSC, a well-established method.

The authors chose one of the best-known self-assembling polymers, Poloxamer 407, as a model. This material is a triblock copolymer of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) whose self-assembling properties display a temperature-dependent phase behaviour (micellization and also gelation) (Alexandridis et al., 1994).

Ultrasound spectroscopy, a relatively new technique, is quickly advancing in the market, especially in the food, chemical and pharmaceutical fields, because its positive features make it a rapid, non-destructive and suitable method for analysing concentrated and opaque samples (McClements, 1995). The main concern about this methodology is probably the difficulty of interpreting the acquired parameters with the exclusive use of the fundamental laws of sound wave transmission. Thus the authors' goal was not only to demonstrate the potential of ultrasound technique as a valid alternative to standard methods of analysis, but also to show how the transition observed in ultrasound parameters can be easily interpreted, at least in the case of the aggregation process for self-assembling polymers.

2. Materials and methods

2.1. Materials

Poloxamer 407 (Pluronic F127, BASF Chem. Trade Gmbh 91593 Burgbernheim, l Germany) was used as received. The molecular weight of Poloxamer 407 is about 12,500 g/mol. Deionised water was obtained from an ion-exchange system GAMMA 3 s.n.c. (Castelverde, CR).

2.2. Preparation of samples

Poloxamer samples were prepared by simple dispersion of the materials in the required amount of degassed and deionised water using the "cold" procedure. The concentrations of the investigated polymers were in the range of 5–30% (w/w).

Samples were then stored at 4 °C for at least 24 h before being analysed.

2.3. Thermal analysis

The calorimetric studies were carried out with a micro DSC III (SETARAM) using in 1 ml batch Hastelloy cells. 0.5 g of Poloxamer aqueous solutions and an equal amount of deionised water as reference were loaded in the calorimetric cells at 1 °C, and then analysed by the following procedure: (A) isothermal step for 30 min at 1 °C, (B) heating ramp from 1 to 35 °C at 0.25 °C/min, and (C) cooling ramp from 35 to 1 °C at 0.25 °C/min.

All samples were run in triplicates.

2.4. Ultrasonic measurements

Ultrasonic parameters (ultrasonic velocity and attenuation) were measured using an HR-US 102 high resolution spectrometer (Ultrasonic Scientific, Ireland) fitted with two 1 ml ultrasonic cells, one filled with water as reference and the other with the different Poloxamer 407 samples in the concentration ranges of 5–30% (w/w) at the selected frequency of 5.2 MHz. The limiting resolution was 0.2 mm/s for ultrasound velocity and 0.2% for attenuation. Temperature control was achieved with a HAAKE C25P water bath. The heating/cooling rate was 0.25 °C/min, going from 1 to 35 °C. Samples were left at 1 °C for at least 30 min to allow system equilibration before the acquisition. Measurements were performed in triplicate without stirring to ensure that experimental conditions were similar to those of thermal analysis.

2.5. Micellar molecular weight determination

The molecular weight was determined according to the following equation (Pandya et al., 1993; Zhou and Chu, 1994; Nolan et al., 1997):

$$R_{\mathrm{h}}^3 = \frac{3[\eta]\mathrm{Mm}}{10N\pi} \tag{1}$$

where R_{h} is the micelles radius, Mm is the micelles molecular weight, N is the Avogadro's number and $[\eta]$ is the intrinsic viscosity.

Intrinsic viscosity was graphically calculated from specific viscosity values (η_{sp}), according to the equation:

$$[\eta C] = \lim_{c\to 0} \frac{\eta_{\mathrm{sp}}}{C} \tag{2}$$

where C is the polymer concentration (%, w/w).

η_{sp} values were obtained from dynamic viscosity values, measured using two Ubbelohde (size 75 and 150) suspended level capillary viscometers (CANNON Instruments Company) and a densimeter (Mettler TOLEDO DA-100M). Measurements were done in the range of micellization temperatures.

The micellar radius had been previously calculated (Bonacucina et al., 2007).

3. Results and discussion

3.1. Differential scanning calorimetry

Differential scanning calorimetry is one of the most commonly used techniques to analyse the micellization process. DSC easily detects the endothermic phenomena of aggregation characterized by polymer desolvation. Thermal analysis made it possible to identify the onset, peak temperature, and enthalpy associated with the micellization of the different Poloxamer 407 concentrations. The thermograms and all the relevant parameters (calculated through

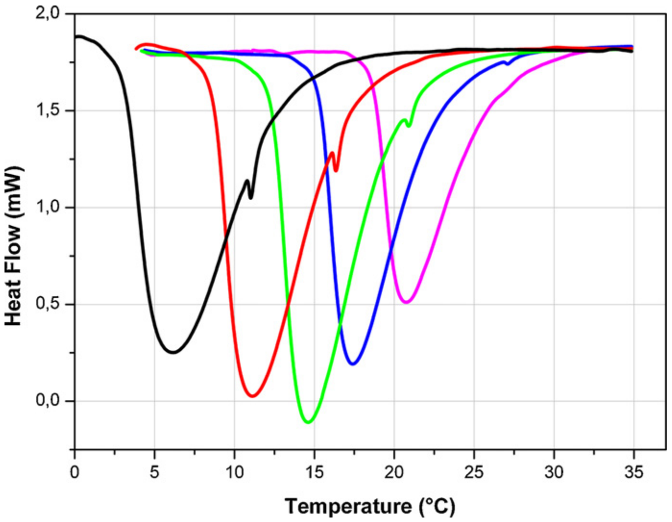

Fig. 1. Heat flow (exo up) during heating ramp of aqueous solution of Poloxamer 407 at different concentrations (▬ 30%, ▬ 25%, ▬ 20%, ▬ 15%, and ▬ 10%, w/w) as function of temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

integration of the extrapolated onset and endset (Artzner et al., 2007)) are reported in Fig. 1 and Table 1.

The copolymers show a broad endothermic peak centred at CMT, which corresponds to the formation of the polymeric micelles. Moreover, it is possible to observe a second, smaller transition, which occurs at a higher temperature and is superimposed on the end of the first peak. According to several studies in the literature comparing DSC data with rheological or SANS analysis, this smaller, endothermic peak corresponds to micelle rearrangement during gelation (Mortensen and Talmon, 1995; Artzner et al., 2007; Bonacucina et al., 2007; Trong et al., 2008). The gelation peak is visible only for polymer concentrations higher than 15% (w/w).

As expected from studies in the literature (Alexandridis and Hatton, 1995; Bonacucina et al., 2007; Trong et al., 2008), the results reported in Fig. 1 and Table 1 show that increasing Poloxamer 407 concentrations lead to more energetic transitions (higher enthalpy values both for micellization and gelation) occurring at lower temperatures (lower CMT and CGT).

3.2. Ultrasonic spectroscopy

Ultrasonic spectroscopy is based on the measurement of sound speed (U) and ultrasonic attenuation (N). When a sound wave travels through a system, it loses part of its energy, depending on the structure of the system itself. In a liquid system, ultrasound velocity is related to adiabatic compressibility (β_s) and to medium density (ρ) by the Laplace equation:

$$U = \frac{1}{\sqrt{\beta_s \cdot \rho}} \tag{3}$$

Table 1
Summary of differential scanning calorimeter transition data.

Concentration (%, w/w)	CMT (°C)	ΔH (CMT) (J/g)	CGT (°C)	ΔH (CGT) (J/g)
10	20.75	2.56		
15	17.39	3.91	27.13	0.004
20	14.63	4.83	20.96	0.017
25	11.09	5.49	16.34	0.026
30	6.09	6.54	11.06	0.031

As a consequence, this parameter is directly related to the high frequency elasticity of the medium and is extremely sensitive to the intermolecular interaction and composition of the sample.

The other parameter, attenuation, reflects the energy loss due to scattering and absorption phenomena typical of heterogeneous samples. It provides information on the physicochemical properties of the systems (McClements, 1995).

Relevant changes in both the above parameters in a polymeric dispersion suggest structural modifications in the system, for example, aggregation or de-aggregation phenomena. However, one must take into account how modifications of the experimental conditions may affect the ultrasonic parameters of the pure solvent. For example, water sound speed changes according to the temperature, following a well-known polynomial series. Therefore, interpretation of ultrasonic data during a temperature scan should always take into account the behaviour of the pure solvent. For this reason, ultrasonic parameters will be reported as relative parameters, obtained by subtracting the contribution of the pure solvent from the total attenuation or sound speed ($\Delta U = U_{\text{sample}} - U_{\text{solvent}}$).

According to Pasynski (1938), desolvation should manifest with an increase of compressibility and therefore with a reduction of sound speed. Several authors report that compressibility of solutes and their closely associated water molecules are negligible (Aeberhardt et al., 2005; Van Durme et al., 2005; Burakowski and Glinski, 2007; Kushare et al., 2007), thus a desolvation process leads to an increase of free water in the system and consequently to higher compressibility.

The relative sound speed for each Poloxamer concentration is reported in Fig. 2A. As would be expected given the observations above, the ΔU values rise as the polymer's concentration increases, as a consequence of the lower amount of free water in the samples.

Furthermore, this approach is also able to explain the shape of the obtained curves. The relative sound speed decreases as the temperature increases, and on the graph this is shown in three different regions, an initial zone and a final linear one, linked by a nonlinear middle part. A linear trend of the sound speed is usually due to the effect of the temperature on the whole system, while an abrupt deviation depends on a modification in the amount of water interacting with the polymer. In particular, the curve shape suggests the presence of a desolvation process that can be associated with a transition from unimers to micelles. ΔU data clearly show the presence of a transition, but the detection of the characteristic temperatures (onset, peak and endset) is not an easy procedure. This can be addressed by using the first derivate (Fig. 2B), which displays the transitions and the relative temperatures more clearly, so much so that data look like DSC thermograms with well defined peaks.

In a system, the attenuation of a sound wave depends on several mechanisms, commonly grouped in two categories: absorption and scattering. Any variation of the size or thermal and viscous properties of any dispersed material modifies the total recorded attenuation (Allegra and Hawley, 1972; McClements, 1995; Dukhin and Goetz, 2002). For all the analysed samples (Fig. 2C), the attenuation rose when the unimers started to aggregate, as a consequence of the increase of sample heterogeneity, due to the presence of different entities (unimers, micelles and water molecules) with different characteristics. The 30% and, in a less evident way, the 25% systems showed a second peak at higher temperatures that could be attributed to the gelation process. In this case, the further increase of attenuation could be explained by the "structural" mechanism, which occurs when particles are organized in some kind of network (Dukhin and Goetz, 2002). Moreover, modifications in the sample viscosity could also aid the attenuation increase. It is important to highlight that the increase in consistency and the final gel strength depended more markedly on polymer concentration than on micellization. In fact, samples with a polymer concentration

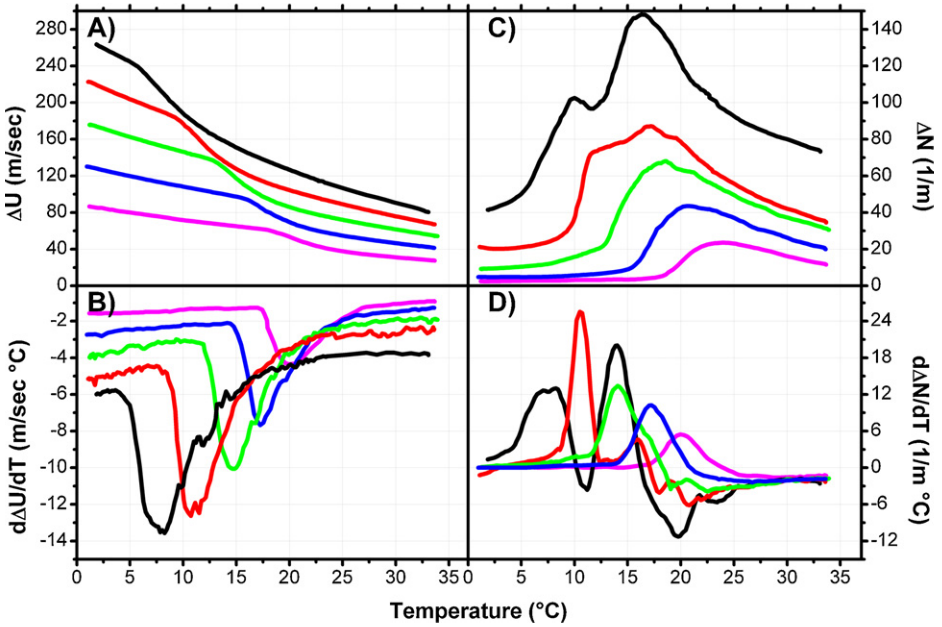

Fig. 2. Effect of temperature on the (A) relative ultrasonic velocity; (B) first derivate of relative ultrasonic velocity; (C) relative attenuation; and (D) first derivate of relative attenuation, for all the analysed samples (▬ 30%, ▬ 25%, ▬ 20%, ▬ 15%, and ▬ 10%). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

lower than 15% did not gel at all, as observed from the DSC analysis and from a previous work (Bonacucina et al., 2007). Both phase transitions occurred in a quite wide temperature range, so the systems appeared highly heterogeneous for a certain time, depending on the rate of temperature scan. The first derivate (Fig. 2D) appeared to be a more powerful method for characterizing the transitions not only for the ΔU data, but also for the ΔN data as well. In this case it proved to be more sensitive; in fact, the gel transition is also detectable for the 20% sample.

An interesting way to analyse the sound speed curves is to normalize the ΔU data for the different Poloxamer concentration. The results, reported in Fig. 3, clearly show that all the curves are superimposed at low and high temperatures, independently of the concentration, while remarkable differences are evident in the middle part, which corresponds to the micellization transition. These results suggest that differences do not exist when the polymer is totally in the unimers or in the micelles state, and that variations observed in the original data (Fig. 2A) can be exclusively attributed to the concentration. Therefore, it is possible to assert that micelle structure, in term of hydration properties and consequently in terms of size and shape, is completely independent of the original polymer concentration, at least in the analysed concentration range.

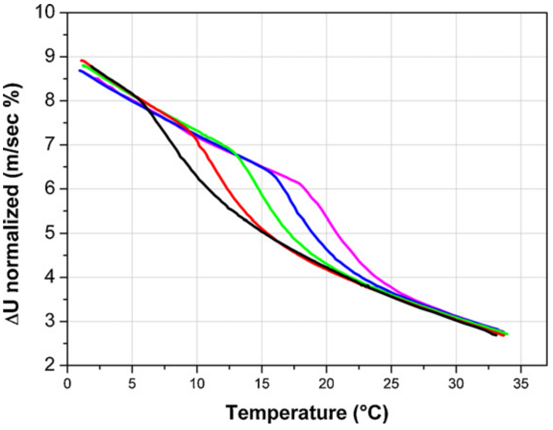

Fig. 3. Effect of temperature on the normalized relative ultrasonic velocity, for all the analysed samples (▬ 30%, ▬ 25%, ▬ 20%, ▬ 15%, and ▬ 10%). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.3. *Comparison of the two techniques*

The effectiveness of the ultrasound technique can be determined by comparison with DSC results. Both techniques clearly display Poloxamer transition from unimers to micelles and the influence of temperature and concentration. However, the proper way to evaluate the ultrasound precision is to compare the numerical values characteristic of the transition, in particular onset, peak, and endset temperatures. These typical values can be easily extrapolated for the ultrasonic parameters from the first derivative traces. Comparison of the different parameters obtained with the two techniques is reported in Fig. 4. Sound speed results show excellent correlation with DSC data for the micellization transition in terms of peak, onset, and endset temperatures. Thus they are very effective in characterizing the micellization process, but fail completely when gelation is considered (see Fig. 2). Both micellization and gelation can be considered as consecutive and superimposed dehydration processes, so that when sound speed is considered, they appear as only one transition. On the other hand, attenuation data also allow identification of the gelation process. Contrary to sound speed, the attenuation values depend on the structural characteristic of the sample itself, in term of thermal and viscous properties and also in terms of network architecture. However, despite their higher flexibility, attenuation data prove to be less accurate for identifying the characteristic temperature transitions. In particular, attenuation results are slightly inaccurate in detecting the endset micellization temperature and, in a more remarkable way, fail to pinpoint the temperature range of the gelation process, even though the peak gelation temperatures seem to be quite accurate (Fig. 4 inset).

Onset, peak, and endset temperature determination is the most common and direct way to analyse these kinds of transitions. However DSC data offer greater potential, including the possibility to

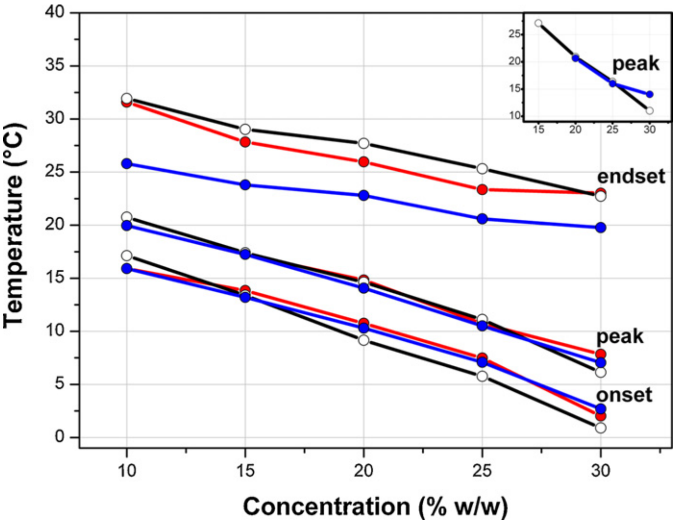

Fig. 4. Comparison of characteristic micellization transition and gelation (inset) temperatures (▬ DSC data, ▬ sound speed data, ▬ attenuation data). All the parameters were calculated using the same approach with the OriginPro 8 software (Origin Lab Corporation, USA). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

follow the kinetics of polymeric micelles formation (Trong et al., 2008).

For DSC, the concentration of micelles (Cm %, w/w) and unimers (Cu %, w/w) during the heating ramp can be determined by the following relation:

$$\mathrm{Cm} = \frac{H(T)}{\Delta Hm} \rightarrow \mathrm{Cu} = C_{\mathrm{tot}} - \mathrm{Cm} \qquad (4)$$

The same relation could be also applied to the first derivative of sound speed. However, such data are never as smooth as DSC data and the final results would be somewhat affected by error.Nevertheless, the kinetics of micelle formation can be obtained directly from the ΔU data. Fig. 3 shows that the ΔU of a dispersion composed only by unimers or micelles change linearly in function of temperature. Therefore we can suppose that for each temperature, the total DU value is determined by the sum of the DU relative to micelles and to unimers. Moreover, it should be kept in mind that the micellization process follows a sigmoid trend, as already shown in the literature (Trong et al., 2008). Thus, in the light of these assumptions, it is possible to model the DU data with the following equation:

$$\Delta U = C_U(T)\cdot(A_U + B_U\cdot x) + C_M(T)\cdot(A_M + B_M\cdot x) \qquad (5)$$

where the subscript refers to unimers or micelles, A and B are respectively the intercept and the slope of the straight line describing exclusively unimer or micelle dispersion, and $C(T)$ is the function describing the change of concentration of the single entities in function of the temperature, according to the following equation:

$$C(T) = \frac{\alpha}{1 + \mathrm{e}^{(-T-\chi\,\ln(2^{1/\delta}-1)-\beta/\chi)^{\delta}}} \qquad (6)$$

where α, β, χ and δ are the characteristic parameters of the sigmoid function $C(T)$.

From kinetic data the aggregation number (N_{agg}) can be calculated transforming the amount of the two entities, unimers and micelles, from grams to moles, knowing their molecular weight. The N_{agg} can then be calculated according to the following equation:

$$N_{\mathrm{agg}\,T} = \frac{M_{\mathrm{U0}} - M_{\mathrm{UT}}}{M_{\mathrm{MT}}} \qquad (7)$$

where M_{UO} is the initial moles of unimers, and M_{UT} and M_{MT} are the moles of unimers and micelles at temperature T respectively.

The kinetics of the micellization process is shown in Fig. 5 for both techniques. Even though the kinetic data were obtained by completely different procedures, in terms of raw data acquisition and data processing, the results show excellent correspondence, especially after the initial steps of the process. Very good agreement between the two techniques is also confirmed when one compares the aggregation numbers (Fig. 5). Both methods reveal little variation of aggregation number (9–10) values as the polymer concentration increases, suggesting that polymeric micelles possess a similar structure independently of their concentration, as assumed from the normalized DU values (Fig. 3). Moreover, at least for the selected range, the temperature does not seem to affect the micelle structure.

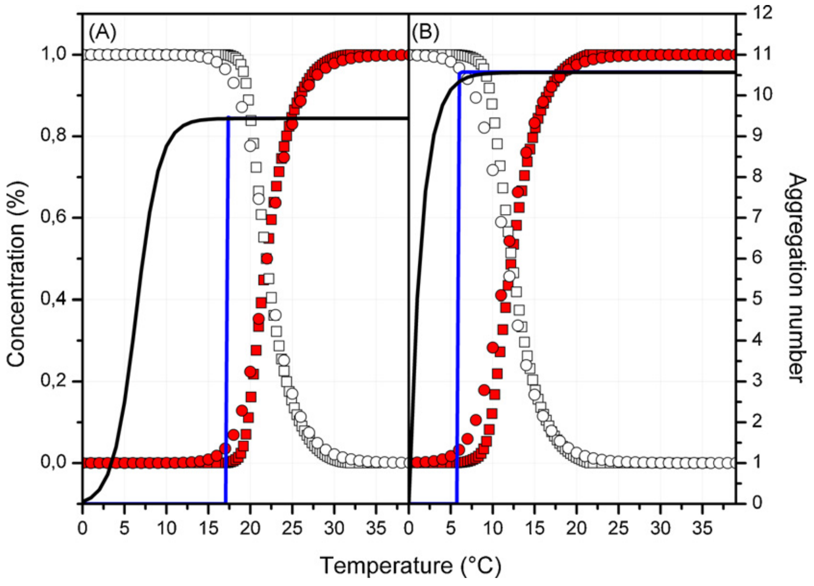

Fig. 5. Comparison among DSC and HR-US techniques on micellization process kinetics and on the aggregation number for (A) 10% polymer dispersion and (B) 25% polymer dispersion (concentration of □ unimers from DSC data, ○ unimers from HR-US data, ■ micelles from DSC data, ● micelles from HR-US data, aggregation numbers from ▬ DSC data, ▬ HR-US data). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

4. Conclusion

High-resolution ultrasound spectroscopy was used to study the micellization process of a well-known self-assembling polymer, Poloxamer 407. The ultrasound parameters of sound speed and sound attenuation proved effective in measuring the micellization process, albeit with different sensitivity. Sound speed values were very effective for analysis of the aggregation–deaggregation process, as demonstrated by comparison with DSC results; however, they failed to detect the gelation process. On the other hand, sound attenuation data were able to identify both transitions of micellization and gelation. This parameter gave a very good correlation with DSC data when the peak temperatures were considered, although small differences were observed when it was applied to the identification of the exact temperature range of the analysed processes. In any case, it is worth of interest to highlight that DSC, and consequently also ultrasound spectroscopy, do not represent the techniques of choice for gelation detection and for a more accurate analysis they should be supported by more effective techniques (i.e. rheology).

In the light of these results, high-resolution ultrasound spectroscopy can prove to be a very effective and accurate technique for monitoring temperature-induced polymer-assembling processes (micellization), thus providing an interesting alternative to DSC measurement.

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