Rheol Acta (2016) 55:781–787
DOI 10.1007/s00397-016-0953-4

ORIGINAL CONTRIBUTION

Rheology of the ultrasound-induced gelation in poloxamer aqueous solutions

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Received: 28 March 2016 / Revised: 30 May 2016 / Accepted: 27 June 2016 / Published online: 18 July 2016

Abstract The rheological behavior of the ultrasound-induced gelation of poloxamer aqueous solutions (Pluronic F-127, Sigma-Aldrich) is analyzed in this work. A new rheometric technique is proposed in which ultrasonic pulses are applied to the sample under shear flow. An ultrasonic transducer was adapted to the lower plate of a controlled-stress rheometer with a parallel-plate fixture (AR-1000 TA Instruments). Solutions were tested under linear oscillatory-shear flow at constant angular frequency in time sweeps with and without ultrasound. The mechanical response of the ultrasound-formed gels was evaluated under linear oscillatory and instantaneous stress relaxation tests. Results suggest that these solutions produce "weak gels" according to the Winter-Chambon criterion.

Keywords Ultrasound · Weak gels · Rheometry · Ultrasound-induced gelation · Winter-Chambon gel criterion

Introduction

Ultrasound-induced gelation of organic liquids has been recognized as a new method to elaborate products with novel molecular architectures (Bardelang 2009). Recently, several gels made of organic liquids have been successfully produced (Lu et al. 2015; Mahapatra and Dey 2015; Isozaki et al. 2007), for instance, ultrasound-induced gelation has been used to obtain nano-structured hydroxyapatite (Lu et al. 2015).

Diseases of the ocular surface are usually treated with particular pharmaceutical dosages and methods (Greaves and Wilson 1993). Conventional ophthalmic-topical delivery systems often result in poor bioavailability and therapeutic response due to eye protective mechanisms (Edsman et al. 1998). Tears and blinking cause rapid drainage of the formulation, and the corneal contact is very short so that the bioavailability of the active agent is low. As a result, the suitability for ophthalmic treatment is reduced, because a more-frequent dosing and higher concentrations of the drug are required to prevent rapid dilution (Greaves and Wilson 1993; Gratieri et al. 2010).

Transparent-gel delivery (in-situ) systems last longer in the eye than conventional eye drops, and they have better tolerance than inserts and ointments (Greaves and Wilson 1993; Preetha et al. 2010). However, as it is the case with ointments, gels are also difficult to produce (Preetha et al. 2010). It has been suggested that manufacturing problems can be overcome using in-situ gelling systems. They may be produced primarily as a solution, wherein the gel is formed as a result of a physicochemical change induced by external methods such as ultrasound waves (Edsman et al. 1998; Gratieri et al. 2010). Ophthalmic in-situ gels show advantages, such as the ease of administering accurate and reproducible quantities, in contrast to already-formed gel formulations which would require proper shaping for different uses (Preetha et al. 2010). Therefore, it is important to search for free-flowing liquid formulations at environmental conditions that would fill correctly the eye surface and readily undergo a post-administration phase transition to form a semi-solid gel. Moreover, the formulation

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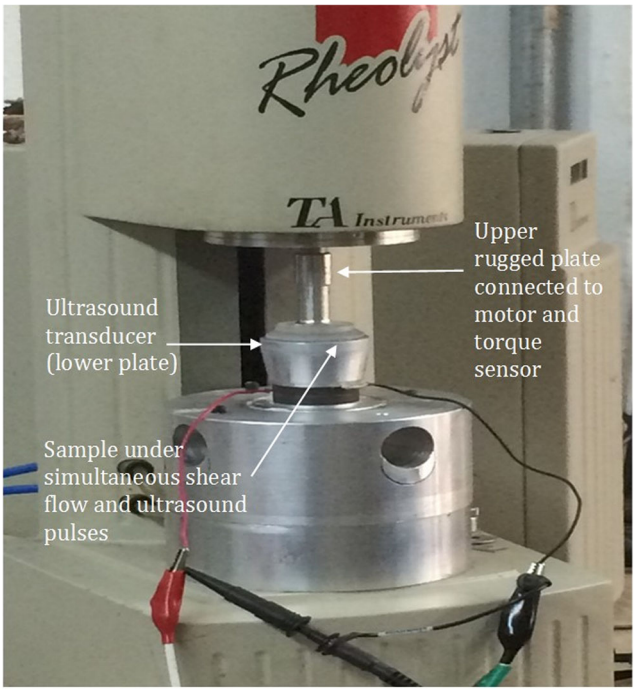

Fig. 1 Modified rheometer with fitted ultrasonic transducer

should be able to withstand shear forces and support drug release under ocular conditions.

Poloxamers are non-ionic bi-functional tri-block copolymers (Gratieri et al. 2010) comprising a central block of relatively-hydrophobic polypropylene oxide attached on both sides to blocks of relatively hydrophilic polyethylene oxide (Gratieri et al. 2010; Patel et al. 2009). Poloxamer solutions at low temperatures are liquids, but when temperature approaches the critical micelle temperature, interaction of methyl groups with water molecules is weakened by heat (Dumortier et al. 2006; Escobar et al. 2006). As temperature increases, the proportion of dehydrated methyl groups increases. In addition, a phase of hexagonal-packed cylinders (formed by multi-micellar aggregates) leads to gel formation at higher temperatures (Artzner et al. 2007). This molecular conformation can be achieved by taking advantage of the properties of ultrasonic waves to promote gelation.

Poloxamer gels are characterized by the ability to be drug-carriers of significant amounts of particular drugs while being bio-degradable, non-toxic, stable, and suitable controlled-release agents (Patel et al. 2009; Baloglu et al. 2011; Dumortier et al. 2006; Escobar et al. 2006). They are used in pharmaceutical formulations as surfactants, emulsifying agents, and dispersing agents (Patel et al. 2009; Escobar et al. 2006; Domb et al. 1998). In this context, rheological measurements are a very useful tool to characterize both flow and structural properties of these solutions under stress or strain tests (Edsman et al. 1998).

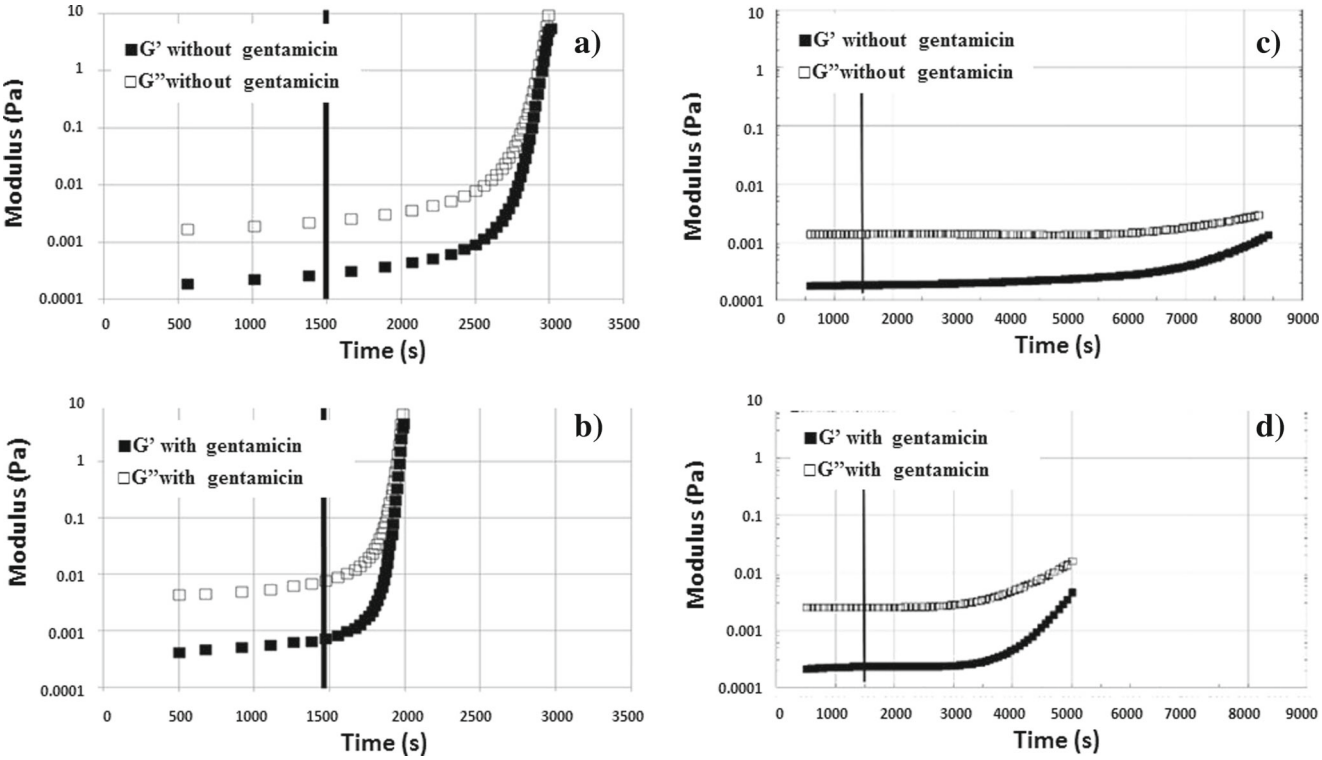

Fig. 2 **a**–**d**. Linear oscillatory time sweeps for poloxamer-407 solution without gentamicin (**a**) and with gentamicin (**b**) at 1 Hz oscillatory frequency with simultaneous ultrasound application of 40 Hz and 75 W and linear oscillatory time sweeps for poloxamer-407 solution without gentamicin (**c**) and with gentamicin (**d**) at 1 Hz oscillatory frequency with simultaneous ultrasound application of 60 Hz and 75 W

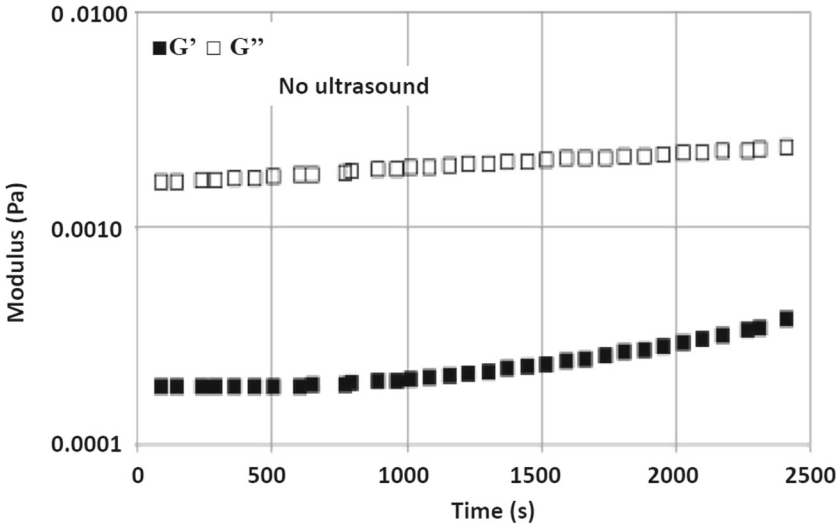

Fig. 3 Linear oscillatory time sweeps for poloxamer-407 without gentamicin solution at 1 Hz oscillatory frequency in the absence of ultrasound

Scarce studies have been reported on the effect of ultrasound on the rheological response of complex materials. Studies have been made in a curing resin under dynamical-mechanical tests in which the propagation of low-frequency ultrasound waves is used to measure the sound velocity (storage modulus) and attenuation (loss modulus) (Lionetto and Maffezzoli 2013). In the present work, ultrasound waves are used to induce a gelation reaction instead.

In this work, a new experimental set-up has been designed to simultaneously measure the rheological response of a system and the effect of the application of ultrasound in the transverse direction to the flow. The main objective of this study is the analysis of the effect of ultrasound on the gelation process of a poloxamer solution (Pluronic F-127 in water) under controlled-flow conditions. The ultrasound-formed gels are characterized by linear oscillatory flow in a conventional stress-controlled rheometer (AR-G2, TA Instruments) and under instantaneous stress relaxation tests.

Materials and methods

Gels preparation

Poloxamer 407 (Pluronic F-127®, Sigma-Aldrich) solutions were prepared by the cold method using a phosphate-buffered saline solution (0.1 M, pH 7.4) as solvent. The required poloxamer amount for each solution was slowly added to the cold solvent (at approx. 8 °C) under magnetic stirring (300 rpm) until a clear solution was obtained (the mix was kept under stirring at 5 °C for at least 24 h). Samples were sterilized by filtration through a 0.22 μm pore membrane and stored under refrigeration. Pluronic F-127 (10 wt.%) samples were prepared with a phosphate-buffered saline solution (PBS) as solvent, with and without gentamicin at 1 wt.% as active agent.

Rheological tests

Solutions were tested under linear viscoelastic time sweeps with simultaneous ultrasound application to monitor the gelation process. The resulting gel was characterized under linear oscillatory frequency sweeps and stress relaxation tests at room temperature (25 °C). A controlled-stress rheometer (AR-1000 TA Instruments) was modified to fit an ultrasonic transducer replacing the lower plate (Fig. 1). Rheological measurements were

Fig. 4 Gel produced on the rugged transparent plate corresponding to the sample of Fig. 3

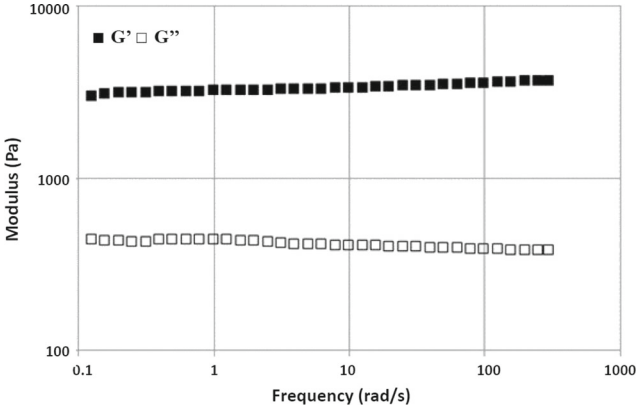

Fig. 5 Frequency sweeps of the gels produced under ultrasound (poloxamer-407 solution without gentamicin)

made by simultaneously applying ultrasonic pulses at a fixed frequency (40 or 60 Hz) with 75 W power output (Sanchez-Solis et al. 2013). A 40-mm parallel plate geometry (upper plate rugged), with a gap of 1000 μm was used. Time sweeps were performed with blank solutions with and without active agent (gentamicin) recording the elastic modulus as a function of time. No mechanical interference was observed with the torque signal during ultrasound applications. A second rheometer was used to characterize the ultrasound-formed gels (AR-G2, TA Instruments) performing instantaneous stress relaxation tests and small amplitude oscillatory shear (SAOS) measurements.

Scanning electron microscopy

Sample preparation of the poloxamer 407 (Pluronic F-127®, Sigma-Aldrich) gel for scanning electron microscopy (SEM) measurements has been described elsewhere (Medina-Torres et al. 2006). Primarily, the sample was freeze-dried (K40 8C, 50 mbar for 3 h). The lyophilized sample was placed on an aluminum slide using an electrically conductive tape (Bal-Tec, Fürstentum Liechtenstein, Germany) and coated with gold at 10 mbar for 90 s (Polaron SC-7610, Fisson Instruments, CA, USA). The images were obtained with a scanning electron microscope Leica Stereoscan S420i (Cambridge, England).

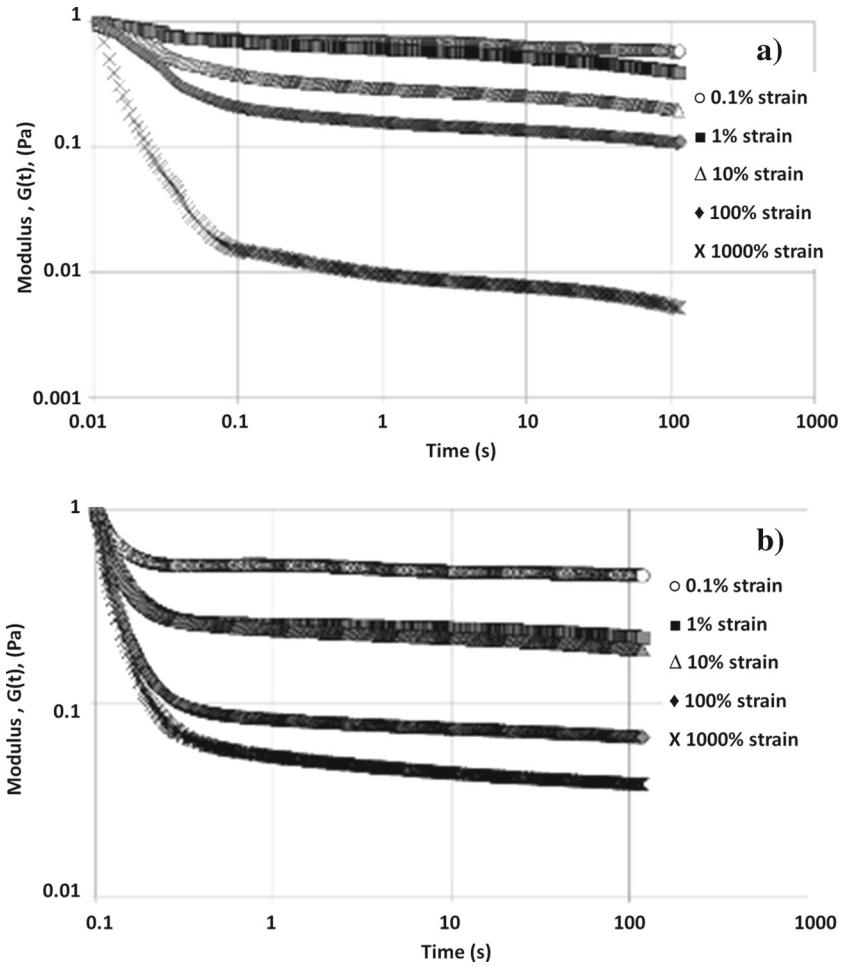

Fig. 6 **a**, **b** Instantaneous stress relaxation tests for the gels produced under ultrasound at 10 wt.% **a** without gentamicin and **b** with gentamicin

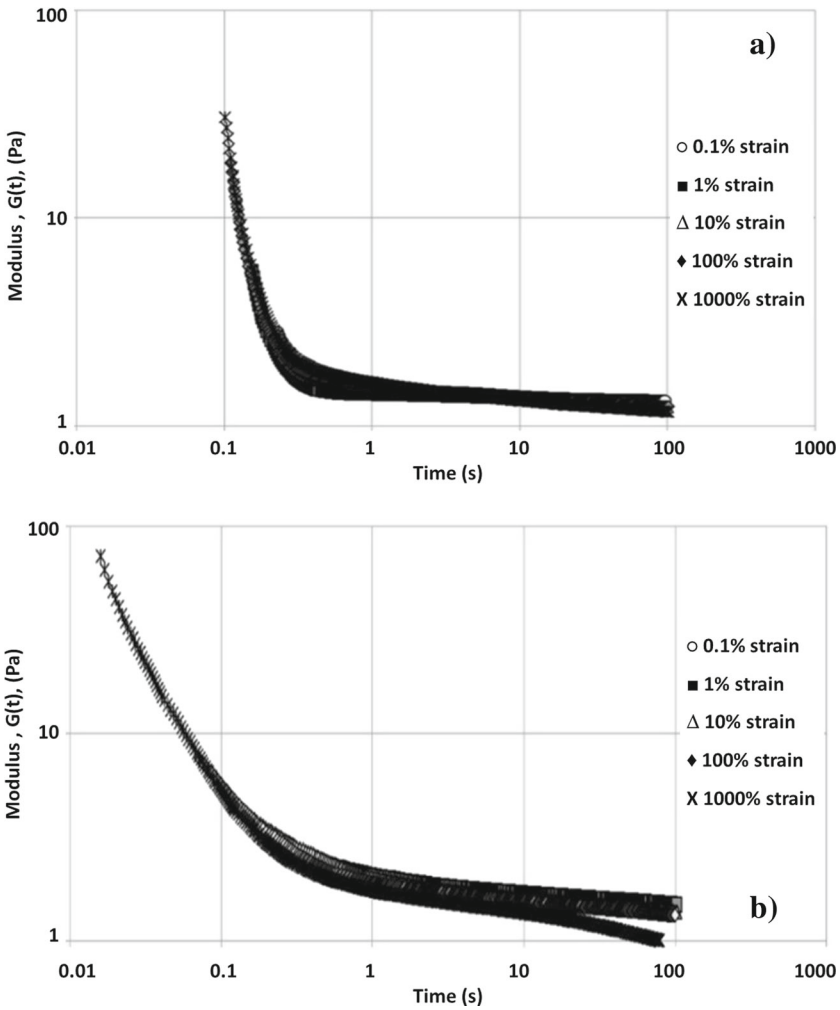

Fig. 7 **a**, **b** Master curve of the stress relaxation with various applied strains for gels produced under ultrasound **a** without gentamicin and **b** with gentamicin

Results and discussion

Ultrasound gelation

Time sweeps of the evolution of moduli G′ and G″ of the poloxamer solution with ultrasound are shown in Fig. 2. In the absence of ultrasound, the sample is initially stabilized under a linear oscillatory time-sweep at a fixed frequency of 1 Hz during 10 min. Under ultrasound, the viscous and elastic moduli are monitored throughout the entire test. In Fig. 2, a predominant viscous behavior is observed at the beginning of the test with the viscous modulus (G″) dominating over the elastic modulus (G′), as expected for a viscous liquid (see Fig. 3). After 600 s of ultrasound application (a vertical line in the figure signals the initiation of the ultrasound application process at 1500 s), a sudden increase in the moduli at approximately 2500 s (Fig. 2a) signals the onset for the gelation kinetics leading to a build-up of a gel-like network in the sample. A crossover of the curves (G′ = G″) is observed at approximately 3000 s after which G′ is expected to exceed the viscous modulus G″ (onset for *solid-like behavior*) (Winter and Chambon 1986), but gel breakage occurs caused by volume changes in the sample. The gel produced under these conditions is shown in Fig. 4.

The same protocol was followed for the poloxamer solution with gentamicin (Fig. 2b). In this case, gelation is faster since the sudden increase of the moduli begins almost immediately upon ultrasound application, and the equal-modulus point is reached at 2000 s. With regard to the applied ultrasound waves, various frequencies were tested in the range 1–100 Hz. However, gelation was only induced at 40 and 60 Hz. At 40 Hz, gelation times amount to 50 min without gentamicin (Fig. 2a) and 30 min with gentamicin (Fig. 2b),

Table 1 Stress relaxation slopes of the gels produced with ultrasound. Poloxamer-407 solution at 10 % with and without gentamicin

% strain	Slope 1 (fast)	Slope 2 (slow)
Without gentamicin	0.5859	0.1267
With gentamicin	7.58	0.1951

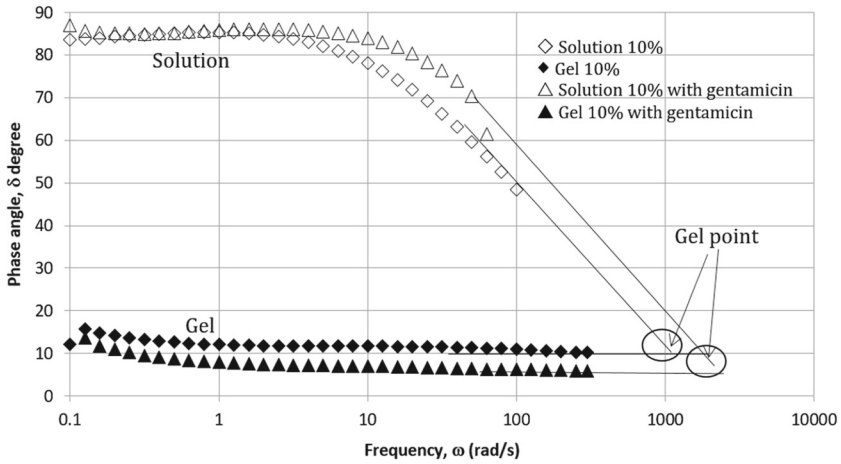

Fig. 8 Phase angle as a function of frequency obtained from SAOS data for poloxamer-407 solutions and gels produced with ultrasound

while at 60 Hz the gel reaction is slowed (gelation starts after 1 h of ultrasound application with gentamicin, see Fig. 2d) and no gelation was observed after 2 h without gentamicin (see Fig. 2c).

When the same procedure is followed in the absence of ultrasound, no gel production is observed (Fig. 3), but only a slight increase in the moduli probably due to evaporation. Effectively, gelation is solely induced by the ultrasonic waves applied to the sample during the rheological measurements.

Gel rheological characterization

To characterize the ultrasound-produced gels, care was taken in switching-off the ultrasound immediately after gelation to avoid gel breakage. The gels produced were removed from the lower plate and placed in another rheometer (AR-G2, TA Instruments) to perform frequency (SAOS) and stress relaxation tests. Figure 5 shows the SAOS data of gels produced under ultrasound without gentamicin. The moduli correspond to a gel with frequency-independent behavior and with a predominant storage modulus ($G' > G''$).

Figure 6a and b showed the normalized instantaneous stress relaxation tests at various initial strains for the gel produced under ultrasound. It is interesting to note that all stress relaxation curves have similar terminal behavior at long times, i.e., all curves have the same terminal quasi-plateau which is an indication of power-law behavior as predicted by Chambon and Winter (1987). The lower curve in Fig. 6a is that corresponding to the largest strain, illustrating two different slopes, one at short times (initial slope) and the other at long times (terminal behavior). As the strain is increased, the curves show a gradual slope increase corresponding to the fast mode, indicative of potential curve-superposition. The data of the gels produced under ultrasound with gentamicin (Fig. 6b) show a similar tendency.

The curve with the largest applied strain (lower curve, Fig. 6a, b) was taken as the reference (envelope curve). Superposition is obtained (Fig. 7a, b) by shifting all curves in the vertical and horizontal directions. Two different slopes are observed in the master curves, namely, the slope corresponding to the initial relaxation mode and that of the terminal quasi-plateau behavior. The slope of the initial relaxation is characteristic of the power-law gel behavior (Ng and McKinley 2008). It is interesting to note that the terminal behavior is independent of the gentamicin content since both master curves have approximately the same slope (Fig. 7a, b). However, the initial slope illustrates a faster relaxation for the system with gentamicin, which is associated with the molecular interaction of gentamicin with the

Table 2 Frequency at the gel point from Fig. 8

Concentration	Frequency (rad/s)
10 %	1050
10 % gentamicin	1300

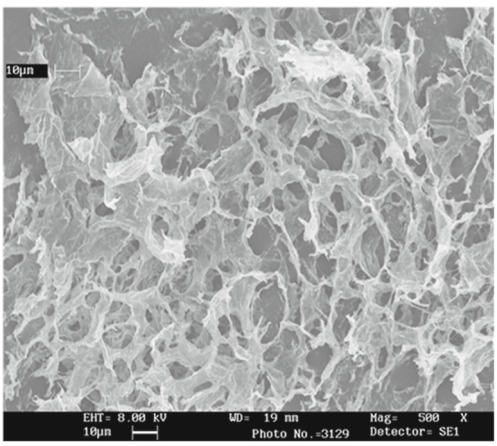

Fig. 9 SEM image of the poloxamer gel corresponding to Fig. 2a. 1 Hz oscillatory frequency with simultaneous ultrasound application of 40 Hz and 75 W

poloxamer. Values of the slopes corresponding to the relaxation master curves are reported in Table 1.

The gelation-point of the gel produced with ultrasound was revealed by extrapolation of the phase-angle curve as a function of frequency (Fig. 8) from the SAOS data. In this case, the two characteristic mechanical spectra, one obtained before ultrasound application (viscous solution behavior) and the other one measured after ultrasound application (gel behavior, Fig. 5) are compared in Fig. 8. As stated by Winter and Chambon (1986; Chambon and Winter 1987), phase-angle curves obtained from gelled systems should cross at the *gel point*. In the present analysis, it is not possible to arrest the gelation process to measure the development of the reaction as a function of time, but, instead, only the initial and final stages are sufficient to determine the gel point (Table 2).

SEM micrographs reveal that micro-phase separation is absent in the resulting gels (see Fig. 9). The gel exhibits a 3D network with homogeneous distribution of pore sizes. This is consistent with the linear viscoelastic characterization of the gels where the storage modulus, G′ is larger than the loss modulus G″. Similar observations by Nnamani et al. (2013) on gentamicin sulfate hydrogels concluded that the poloxamer 407 based-hydrogels exhibit the best in-vitro performance.

Conclusions

A novel experimental set-up consisting in a rheometer adapted with an ultrasound transducer enables the analysis of ultrasound-*induced gel behavior* in linear oscillatory time sweeps. Loss and storage modulus were monitored simultaneously as ultrasound was applied to poloxamer solutions under linear oscillatory shear flow. This new rheometric technique allows the characterization of the effect of ultrasound waves on complex materials under controlled-flow conditions.

The ultrasound-induced gelation is promoted when gentamicin is added to the poloxamer solution. The ultrasound-induced gel was characterized under oscillatory flow and stress relaxation tests, exhibiting a uniform microstructure with homogeneous pore-size distribution.

The gelation point and characteristic gel constants were reported for this system. Results indicate that these solutions produce weak gels according to the Chambon and Winter criterion.

Finally, this work reveals that ultrasound-induced poloxamer gels are real alternatives to classical temperature-induced gels in view of their potential in-situ application for ophthalmic drug-delivery systems. The issue concerning further applications of this technique is currently under investigation.

References

Artzner F, Geiger S, Olivier A, Allais C, Finet S, Agnely F (2007) Interactions between poloxamers in aqueous solutions: micellization and gelation studied by differential scanning calorimetry, small angle X-ray scattering, and rheology. Langmuir 23:5087–5092

Baloglu E, Karavana S, Senyigit Z, Guneri T (2011) Rheological and mechanical properties of poloxamer mixtures as a mucoadhesive gel base. Pharm Dev Technol 16:627–636

Bardelang D (2009) Ultrasound induced gelation: a paradigm shift. Soft Matter 5:1969–1971

Chambon F, Winter HH (1987) Linear viscoelasticity at the gel point of a crosslinking PDMS with imbalanced stoichiometry. J Rheol 31: 683–697

Domb AJ, Kost, J, Wiseman DM (1998) Handbook of biodegradable polymers. CRC Press

Dumortier G, Grossiord JL, Agnely F, Chaumeil JC (2006) A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Pharm Res 23:2709–2728

Edsman K, Carlfors J, Petersson R (1998) Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur J Pharm Sci 6: 105–112

Escobar JJ, López M, Naïk A, Kalia N, Quintanar D, Ganem A (2006) Applications of thermo-reversible Pluronic F-127 gels in pharmaceutical formulations. J Pharm Pharm Sci 9:339–358

Gratieri T, Martins G, Melani E, Sarmento V, De Freitas O, Fonseca V (2010) A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. Eur J Pharm Biopharm 75:186–193

Greaves JL, Wilson CG (1993) Treatment for diseases of the eye with mucoadhesive delivery systems. Adv Drug Deliv Rev 11:349–383

Isozaki K, Takaya H, Naota T (2007) Ultrasound-induced gelation of organic fluids with metalated peptides. Angew Chem Int Ed 46: 2855–2857

Lionetto F, Maffezzoli A (2013) Monitoring the cure state of thermosetting resins by ultrasound. Materials 6:3783–3804

Lu X, Xie Y, Han Y, Wang X, Dai H, Li S (2015) Ultrasound-induced albumin gelation method for the preparation of nanostructured hydroxyapatite. Mater Lett 161:128–131

Mahapatra RD, Dey J (2015) Ultrasound-induced gelation of organic liquids by L-cysteine-derived amphiphile containing poly(ethylene glycol) tail. Langmuir 31:8703–8709

Medina-Torres L, Brito De-La Fuente E, Gómez-Aldapa C, Aragón-Piña A, Toro-Vázquez J (2006) Structural characteristics of gels formed by mixtures of carrageenan and mucilage gum from Opuntia ficus indica. Carbohydr Polym 63:299–309

Ng TSK, McKinley GH (2008) Power law gels at finite strains: the nonlinear rheology of gluten gels. J Rheol 52:417–449

Nnamani PO, Kenechukwu FC, Anugwolu CL, Obumneme AC, Attama AA (2013) Characterization and controlled release of gentamicin from novel hydrogels based on poloxamer 407 and polyacrylic acids. Afr J Pharm Pharmacol 7:2540–2552

Patel HR, Patel RP, Patel MM (2009) Poloxamers: a pharmaceutical excipients with therapeutic behaviors. Int J Pharm Technol Res 1: 299–303

Preetha JP, Rekha KK, Elshafie K (2010) Formulation and evaluation of in situ ophthalmic gels of diclofenac sodium. J Chem Pharm Res 2: 528–535

Sanchez-Solis A, Manero O, Machorro R, Calderas F. (2013) Rheometer and ultrasound device performing rheological measurements and ultrasound simultaneously. Patent: MX/A/2013/011435.

Winter HH, Chambon F (1986) Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. J Rheol 30:367–382