International Journal of Biological Macromolecules 48 (2011) 495–500

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Rheological properties of ovalbumin hydrogels as affected by surfactants addition

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ARTICLE INFO

Article history:
Received 22 November 2010
Received in revised form 4 January 2011
Accepted 13 January 2011
Available online 22 January 2011

Keywords:
Gels
Complexation
Ovalbumin
Hydrogenated surfactants
Perfluorinated surfactants

ABSTRACT

The gel properties of ovalbumin mixtures with three different surfactants (sodium perfluorooctanoate, sodium octanoate and sodium dodecanoate) have been studied by rheological techniques. The gel elasticities were determined as a function of surfactant concentration and surfactant type. The fractal dimension of the formed structures was evaluated from plots of storage modulus against surfactant concentration. The role of electrostatic, hydrophobic and disulfide SS interactions in these systems has been demonstrated to be the predominant. The viscosity of these structures tends to increase with surfactant concentration, except for the fluorinated one. Unfolded ovalbumin molecules tend to form fibrillar structures that tend to increase with surfactant concentration, except for the fluorinated one. This fact has been related to the particular nature of this molecule.

1. Introduction

Ovalbumin (OVA), a major component of egg white proteins, has important implications in food systems improving sensory perception [1,2]. This protein is grouped into the serine proteinase family inhibitors, which controls serine proteinases involved in diverse physiological reactions, because of the close similarity in the primary and tertiary structures. It has a molecular weight of 45,000 Da and consists of a single chain of 385 amino acids with 105 titrable residues [3], containing a single disulfide bond (interconnecting two parts of the chain) and a glycosylation (mainly mannose) site. Three different ovalbumin phosphate forms containing two, one or zero phosphate groups per molecule (85:12:3 ratio respectively) occur in egg white. This protein presents a globular shape with a radius of ≈3 nm in an aqueous medium and an isoelectric point at pH 4.6 [4]. When stored for extended periods of time, or by heat processing, the native R-ovalbumin converts to S-form that is more resistant to denaturation by heat, urea, or guanidine [5]. The ovalbumin globules are also known to make a self-organized mesoscopic structure, which is governed by several factors: temperature, concentration or electrolyte [6]. As a result, solutes are often added to regulate these transformations during the processing of proteins or proteins-containing food materials. To effectively control and optimise this food processing, a deep understanding of how added solutes influence protein morphology is essential.

Studying the interactions of OVA with surfactants has been a hotspot these days [7,8]. Interaction of proteins with surfactants is of great importance in the fields of industrial (cosmetic, paints, food), biological and pharmaceutical sciences. Due to the existence of nonpolar and ionic amino acid side chains in protein molecules, the formations of these complexes is driven by electrostatic interactions between the charged headgroups of the surfactants and the oppositely charged units of the proteins, as well as by hydrophobic interactions between the alkyl chains and different parts of the proteins [9–11]. Concerning the interest of the industry in ovalbumin hydrogels range from emulsifying activity (it is higher at acidic pH) or drug delivery systems. Also, acid-sensitive microgels for the development of protein-based vaccines under acidic conditions, like those found in the phagosomes of antigen-presenting cells, are highly demanded [12].

In the present study, we have investigated the effect of sodium perfluorooctanoate, sodium octanoate, and sodium dodecanoate, on the properties and structure of the ovalbumin gels. The ability to form a gel is an important function of proteins in food systems. Most food protein gels are formed by denaturation, aggregation, and gelation during heating process. In flurry of studies, researches have focused on gel formation upon protein heating. However, few studies have dealt with protein gel formation without heating or have analyzed the effect of surfactants on protein gel properties. The studied surfactants allow us to analyze how the presence of fluorine atoms in the alkyl chain affects the gel structure. For this purpose several samples, covering a range of concentrations of surfactant from 1 mM to 10 mM, were prepared. These concentrations guarantee the non existence of micelles [13]. The physicochemical properties of these samples were evaluated by using rheological

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doi:10.1016/j.ijbiomac.2011.01.015

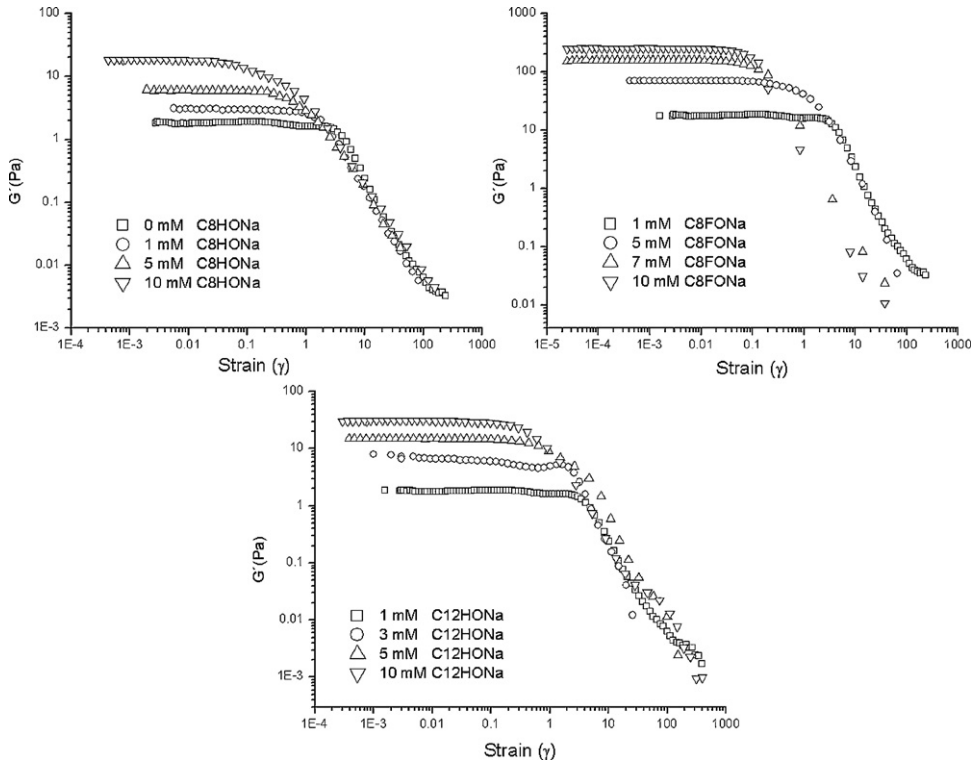

Fig. 1. The dependence of storage modulus (G') of ovalbumin surfactant mixtures on strain (γ) for different concentrations of the three surfactants under study. Ovalbumin concentration, 40 g/l.

measurements that are easy to conduct, especially in the high concentration range of proteins [14].

2. Experimental

2.1. Materials

Ovalbumin (Albumin, Chicken Egg, product A-5253, Sigma Chemical Company) was used without further purification. Sodium octanoate (C8HONa) and sodium perfluorooctanoate (C8FONa) were obtained from Lancaster Synthesis Ltd. Sodium dodecanoate (C12HONa) with purity over 99%, were obtained from Sigma Chemical Co. The protein was dissolved in water, the pH was adjusted to pH 3.0 with HCl 0.1 M. All materials were of analytical grade and solutions were made in double distilled water.

2.2. Rheology

Rheological experiments were performed on a Bohlin CS-10 stress-controlled rheometer. A Couette geometry with a cup of 27.5 mm diameter and a bob type Mooney cell was used. The cell was heated by a reservoir of fluid circulating from a Julabo thermostated bath. The sample was equilibrated for at least 20 min prior to each experiment. Both steady and dynamic rheological experiments were performed at each temperature. Dynamic frequency sweep measurements were performed in the linear viscoelastic regime of the samples, as determined previously by dynamic stress sweep measurements. For the steady-shear experiments, an equilibration time of 90 s was given at each shear stress. All experiments were performed at 25 °C.

3. Results and discussion

In order to understand the effect of surfactant type and concentration on structural properties of ovalbumin gels, the storage modulus (G'), loss modulus (G''), and phase angle were recorded. However, both G' and G'' developed similarly and the G' values were considerably greater in magnitude than the G'' values for all gels, suggesting predominantly elastic behavior. Therefore, at this point, only G' values are discussed. Fig. 1 shows the effect of surfactant type and concentration on the strain dependent elastic modulus, G'. A first general point to note is the wide variation in large deformation rheological behavior exhibited by the different systems. A second more specific point is that, irrespective of whether the gelation is triggered by surfactant type, the extent of the linear viscoelastic region, the range of constant G', is much shorter for the surfactant–ovalbumin gels that for the pure ovalbumin gels.

As can be observed, gel strength is dependent on both parameters tested: surfactant type and surfactant concentration. At lower surfactant concentrations, rigidity values were much lower than at the higher values and variations in rigidity values with different surfactants are significant. At this pH, the protein is not close to its isoelectric point of 4.9, and as a result protein has a positive net charge. Increased interactions due to the presence of surfactants result in increased rigidity presumably because the interactions involved contribute to structure development. It would appear that the ability to promote aggregation was higher for the surfactants under study despite the fact that probably the three surfactants did not bind to the same extent. The highest rigidity values were observed for the fluorinated surfactant, followed by sodium dodecanote and sodium octanoate. The fact that for both sodium perfluorooctanoate, and sodium dodecanoate, higher gel rigidity were obtained demonstrates that the type of atoms and the alkyl chain length present can affect this relationship. The lowest G' values obtained with sodium octanoate reveals that electrostatic interactions resulting from surfactant binding at this pH were not sufficient to overcome the net repulsive force associated with the protein. Consequently, this dramatic difference can be seen in gel rigidity values for this system.

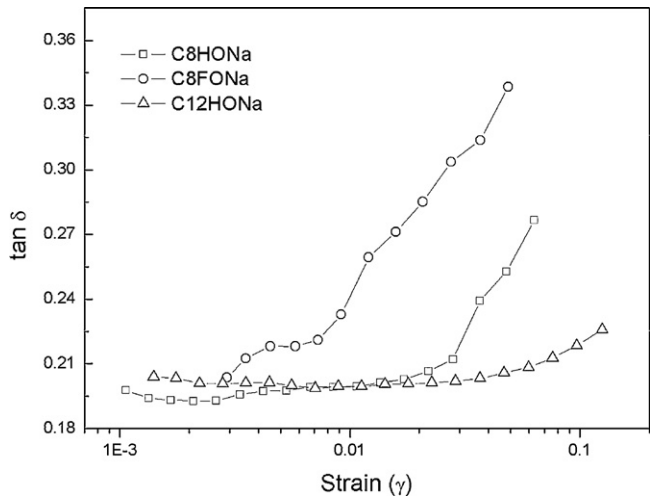

Fig. 2. Loss tangent (tan δ) versus strain (γ) for the different surfactant under study. Surfactant concentration 10 mM. Ovalbumin concentration, 40 g/l.

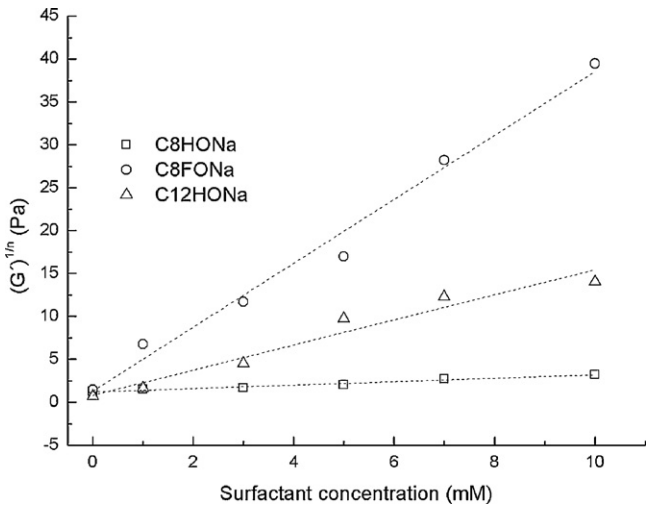

Fig. 3. Surfactant concentration dependence of G' values of ovalbumin gels.

Another parameter that provides some information on network structure is the loss tangent value, tan δ, the ratio of the loss modulus or viscous component to the storage modulus or elastic component. The tan δ value can give indication of the type of structure formed [15]. Low tan δ values are normally associated with a well crosslinked network where the elastic component is high compared to the viscous component. Meanwhile, high tan δ values are associated with a lack of crosslinking due to either excessive aggregation or excessive solubilization of the protein. For all our systems, variations in tan δ values show decrease with increasing surfactant concentration. This supported the G' data suggesting that the inclusion of surfactant served to counteract the electrostatic repulsion by providing a bridge between protein molecules. The increased protein gel network should theoretically arise from physical cross-links instead of chemical cross-links. For a fixed surfactant concentration tan δ values are markedly higher for C8FONa than for the hydrogenated surfactants, Fig. 2. Interestingly, this dramatic difference was not seen in gel rigidity values. The influence of hydrogenated surfactants on network formation appeared to give a more elastic product, but this did not translate into increased gel rigidity.

Various experimental techniques in rheology have been applied to elucidate fractal structures in aggregates or proteins gels [16–19]. In the framework of fractal theories, the network structure of protein gels is considered as closely packed fractal flocs with the fractal dimension of n [14]. The elastic properties of a floc are dominated by its effective backbone, which can be approximated as a linear chain of springs. The elastic constant (K) of the individual flocs is inversely related to their size (ξ), by means of $K \sim K_0/\xi^{2+x}$ where K_0 is the local bending constant between two adjacent springs that belong to the effective backbone of a floc and x is the fractal dimension of the elastic backbone. Since fractal flocs are considered scale invariant, the size of the flocs is related to the volume fraction (ϕ) as $\xi \sim \phi^{1/(n-3)}$. When the links between the neighboring flocs have a higher elasticity than those in the flocs, which is the so-called strong-link regime, the macroscopic elastic constant of the gel (G') is dominated by K. Since G' of a system of size L can be related to K as $G' \sim (L/\xi)K$, the dependence of G' on ϕ is derived as $G' \sim \phi^{(3+x)/(3-n)}$.

Later, several works based on percolation and fractal theories found a generic description of gel elasticity [20,21]. In this case, G', can be written as a power law according to $G' \sim (c - c_p)^n$, where c is the concentration of monomers in the system, c_p is the critical percolation threshold concentration, and n is a scaling exponent, which depends on the type of Hamiltonian that describes the network elasticity. In contrast, the fractal models assume $c_p = 0$ and predict a power law behavior written as $G' \sim c^w$, where the exponent w is related to the fractal dimension of so-called flocs that form the network. The fractal dimension depends on the interaction between the monomers. Finally, van der Linden et al. [22] found that most data can be described by one percolation model and the scaling exponent (n) can be determined by their graphical method. This method uses plots of $(G')^{1/n}$ versus concentration, c, and extrapolates these plots to 0. This procedure makes use of the fact that independent of the value of n, the curves must all intersect the concentration axis at the same value. When the assumed value for n is close to the actual value, the plot will be linear. If n is too small or too large the lines are curved. From the plots of $(G')^{1/n}$ versus c for various n, those n values that give an approximately straight line were selected.

Returning to our systems, we analyzed the surfactant concentration dependence of storage modulus of the ovalbumin plus surfactant gels. The value for G' was obtained in the linear regime, where it is independent of the applied strain. The experimental points and the corresponding selected fits are plotted in Fig. 3. Values of n obtained for our systems were 1.3, 1.5 and 2.5 for dodecanoate, perfluorooctanoate and octanoate, respectively. As we have previously pointed out, the exponent n is a function of the gel structure. High values, $n > 3$, suggest that network is composed by branched linear particles or large cluster with a low density. Meanwhile, low values indicate that structures are larger, compact and homogeneous [23]. These results correlate well with those found by Veerman et al. for ovalbumin [24], β-lactoglobulin [25] and bovine serum albumin [26] at low pH.

Viscosity curves are reported in Fig. 4 and show the trend of viscosity as a function of the shear rate for all investigated samples. All measurements were performed controlling the shear stress, so that the shear rate range depends on the sample viscosity. Initial experimental values are 1 s^{-1} in all cases.

In general, one common data pattern can be concluded: under steady shear flows, no Newtonian regions were observed either at the high or low limiting shear rates (the low shear Newtonian region can lie outside the shear rate range accessible to the instrument). Meanwhile, a strong shear thinning in the intermediate shear rate region was found. Generally, solutions of interacting colloidal particles at high concentrations tend to order into crystalline lattices at rest. When shear is applied, the flow concentrates stress above all at lattice dislocations where particles are loosely trapped [27]. Under flow, the microstructure of the solution can be mod-

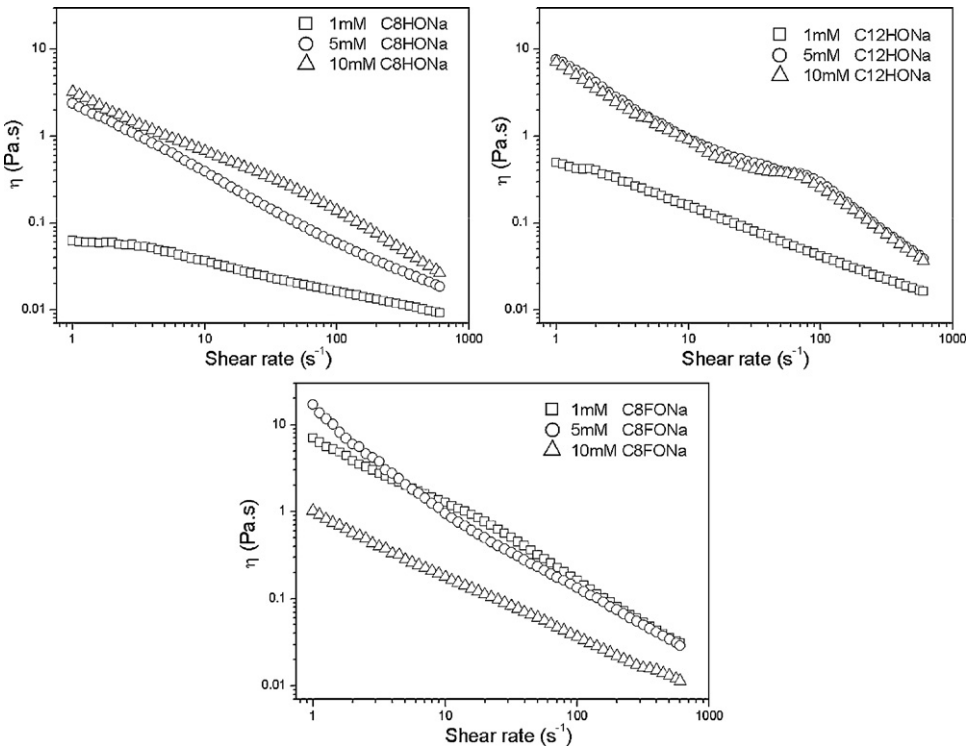

Fig. 4. Experimental viscosity as a function of shear rate for samples of ovalbumin gels in the presence of different concentration of surfactants. Ovalbumin concentration, 40 g/l.

eled as a solid ordered phase coexisting with a fluid disordered phase and when shear rate increases the fluid disordered phase rises above the solid ordered one. This is, the shear destroys part of the ordered structure (disruption of entanglements and other intermolecular interactions) and the so-called shear-thinning behavior is observed. Finally, the non existence of a Newtonian region at high shear rates suggests that these systems are not capable of maintaining part of its initial overall structure. It can be seen that the amplitude of the shear-thickening is not strongly dependent on the surfactant concentration.

A more detailed description of the plots allows us to discover some divergences among the systems. At this level, significant differences arise from the varied nature of the surfactant and their interactions with the protein network. For both hydrogenated surfactants at the lowest concentration, the shear stress was less dependent on the shear rate on decreasing the rate to 3 s^{-1}, indicating that an apparent yield stress might exist, as has been observed in some colloidal systems [28]. On the other hand, for C8HONa and C12HONa viscosity increased with surfactant concentration, whereas for C8FONa the lowest viscosity was found for the highest concentration, for all shear rate range. The differences in these flow behaviors among the surfactants may reflect the differences in nature of the formed aggregates, for example, the extent in flexibility of fibrillar aggregates. Former studies have demonstrated that upon heating, ovalbumin molecules denature and aggregate into thin strands (linear aggregates) or more dense particles (random aggregates) depending on the conditions used for the heat treatment: pH, ionic strength or protein concentration. Under conditions of high electrostatic repulsions, far from p*I*, denatured ovalbumin mainly formed linear semi-flexible aggregates of approximately 5–12 nm while their level of branching considerably increased when the electrostatic repulsions are screened [29]. The nonlinear dependence of the viscosity with surfactant concentration reflects that linear structures are not perfect, probably upon addition of surfactants protein is unfolded and different interactions between complexes of polypeptide–surfactant arise [30]. It is well known that the formation of protein surfactant complexes is driven by electrostatic interactions between the charged headgroup of the surfactants and the oppositely charged units of the protein, as well as by hydrophobic interactions between the alkyl chain of the surfactants and the nonpolar protein residues. In aqueous media surfactants ions compete with water molecules for binding sites on the protein, for which in the case of ovalbumin it was found an average number of 33 mol water bound per mole of ovalbumin [31]. On the other hand, the overall gelation process requires that the proteins unfold or undergo some conformational changes initially and that the second aggregation step proceeds relatively more slowly than the first to allow the denatured protein molecules to orient themselves and interact at specific points, thus forming a three dimensional network [32]. Arntfield et al. [33] investigated the role of disulfide (SS) bonds in heat-induced networks from ovalbumin, and found that SS bonds may contribute to the elasticity and strength of protein networks. The nature of crosslinks in protein gels has been discussed by several authors [34]. The consensus view is that with the exception of SS bonds in some protein gels, the molecules are held together by a combination of weak intermolecular forces, i.e., hydrogen bonds, electrostatic forces, van der Waals forces, and hydrophobic interactions. These results implicate essentially all of the potential interaction sites or functional groups of the molecules in the gelation process, including –SH groups. Also, it was suggested that critical number of sulfhydryl groups can be introduced beyond which the microstructure of the aggregates transforms from fibrillar into amorphous. Rheological studies further suggested that covalent networks, once formed, do not have the possibility to rearrange, reducing the possibility to attain a stronger network. These results show that, even though aggregation of ovalbumin may be primarily driven by physical interactions, formed disulfide bonds are important to determine the resulting aggregate morphology and rheological properties [35]. From our results it can be inferred that there is an initial electrostatic interaction between surfactant and protein. This interaction unfolds partially protein structure, enhancing SS interactions

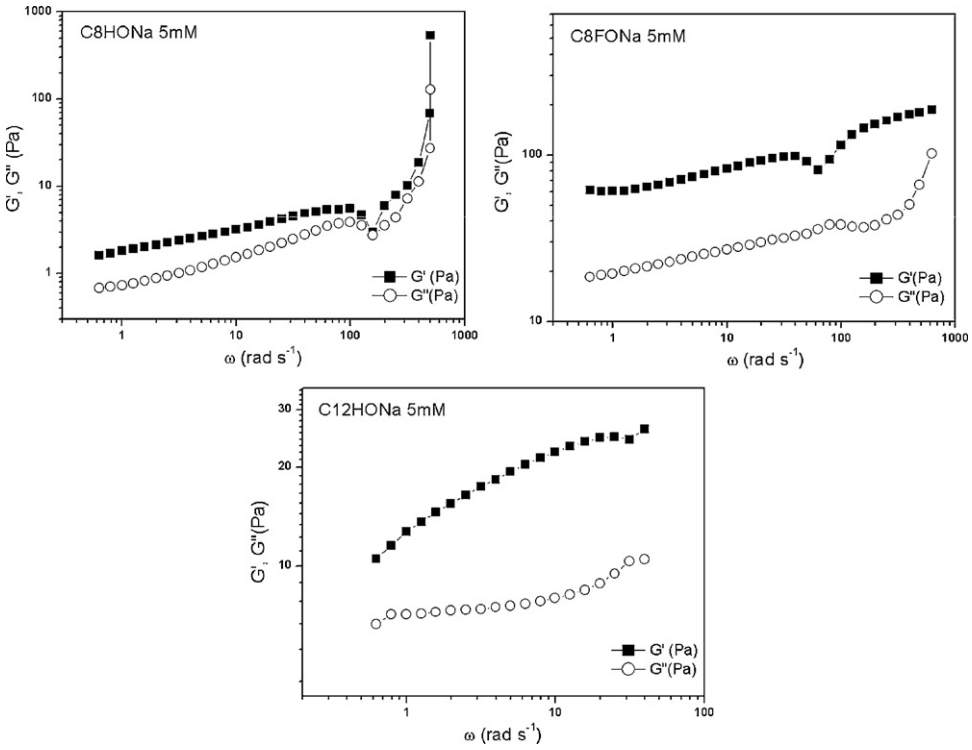

Fig. 5. Storage (G') and loss (G'') moduli as a function of frequency for ovalbumin surfactant mixture. Ovalbumin concentration, 40 g/l. Surfactant concentration 5 mM.

and gel strength. However, in the case of the fluorinated surfactant, at high concentrations there is a disruption of the network and a decrease in viscosity. This result could be simultaneously related with the high hydrophobicity of this surfactant, similar to those of the C12HONa, and the stiffer fluorinated alkyl chain, which results in lower gauche conformations that become more important when viscosity of samples is increased.

Fig. 5 shows the surfactant type dependence of the dynamic viscoelastic behavior of the gels (similar patterns were found for the other surfactant concentrations). Both the storage and the loss moduli increased with frequency. We note that, for the three surfactants, the storage modulus was always larger than the loss modulus throughout the frequency domain studied at all concentrations, which is a characteristic feature of systems exhibiting elastic behavior. This fact also suggests that frequency did not significantly change the network structures of the ovalbumin–surfactant gels, and that such network behavior was not due to the limited frequency range available.

4. Conclusions

In this work, we have provided some novel insight about the gelation of ovalbumin–surfactant mixtures. The main features from this study can be summarized as follows. Firstly, gelation of ovalbumin–surfactant systems for three different surfactants (C8HONa, C8FONa and C12HONa) at different surfactant concentration mixture ratios in the range 0–10 mM was studied, and the strength of the gel was observed to increase with increasing surfactant concentration. For the three surfactant under study gel strength follows the order C8FONa > C12HONa > C8HONa. Secondly, the surfactant concentration dependence of the storage modulus can be described by power laws. The results suggest a fibrillar structure of the gels and a fractal dimension dependent of the surfactant nature. Finally, based on viscosity measurements, the different nature of the fluorinated surfactant provokes a disruption of the gel network at high concentrations.

Acknowledgements

The authors thank the Xunta de Galicia for their financial support (Project No. 10PXIB206258PR). P.V.M. acknowledges the financial support from the Universidad Nacional del Sur, Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Concejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET) and Education, Audiovisual Culture, Executive Agency, European Commission. EMUNDUS18. V.I.D. thanks CONICET (PIP 114-200801-00090), ANPCyT (PICT-2008-00083) and PGI-UNS (PGI 24/ZQ07).

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