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Rheological behavior of aqueous mullite-albumin-methylcellulose systems

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ABSTRACT

In this work, the thermogelling behavior of aqueous mullite-bovine serum albumin (BSA) suspensions was studied by dynamic rheology in order to determine the experimental conditions that must be used to form mullite green bodies by thermal consolidation. Viscoelastic properties (G' and G'') as a function of temperature (30–95 °C) and time were determined by temperature sweep tests and time sweep tests, respectively. On the other hand, the influence of methylcellulose (MC) (2 wt%) as a binder on the viscoelastic properties of the aqueous mullite-protein system as a function of both experimental parameters (temperature and time) was also studied. In addition, shear flow properties of aqueous mullite (40 vol%; 0.45 wt% of polyacrylic polyelectrolyte as a dispersant)–BSA (10 and 15 vol%)-MC (2 wt%) suspensions were analyzed to obtain information on the rheological behavior of the suspensions at room temperature. The results obtained showed that the presence of mullite particles and MC changed the onset temperature of gelation of the protein and increased the gelation time. Thus, both the mullite particles and methylcellulose intervened in the formation of the developed protein gel.

1. Introduction

Mullite ($2SiO_2{\cdot}3Al_2O_3$) is a powerful candidate material as much for conventional ceramics as for advanced structural and functional ceramics due to not only to its good mechanical properties at high temperature but also its low thermal conductivity, low thermal expansion coefficient and good chemical stability under severe chemical environments [1,2]. These properties make the development of mullite materials useful in several applications, such as dense bodies (e.g. structural components and microelectronic industry devices, among others) or highly porous bodies (e.g. thermal insulators, catalyst supports, combustion burners) [3,4]. The importance of this material, particularly as a porous ceramic, in both the scientific and technological fields has been demonstrated by the large amount of studies that have been published over the last several decades and its continued performance today. In recent years, however, the increased demand for porous ceramics with controlled specific microstructures and properties adequate for new applications in diverse technological fields has been notable [1]. For this reason, the development of novel processing methods and the design of different modifications to the processing routes conventionally used are critical aspects currently being discussed at length [5].

Among the several processing methods employed to prepare porous ceramics, the direct consolidation methods combined with the sacrificial template method are considered highly promising. This is due to the fact that a ceramic suspension consolidates inside non-porous molds through the formation of a physical or chemical gel on cooling or on heating, without compaction or removal of solvent, and the porosity is generated after a burning process at temperature. In this context, an innovative non-contaminant colloidal processing of porous ceramics with a cellular microstructure, denoted as “protein casting”, combines the direct-foaming technique with on-site forming by thermogelling a ceramic-protein suspension [6–8]. This method is based on thermal consolidation at temperatures lower than 90 °C by gelling an aqueous ceramic suspension foamed with globular proteins and the formation of a macro-cellular ceramic structure after burn-out (removing organic additives) and sintering treatments at high temperature. The globular proteins have the ability to reduce the surface tension of gas-liquid interfaces and, in consequence, stabilize the gas bubbles formed within the suspension and form a gel in water after heating at 70–80 °C. It is worth noting that this method, in general, requires the design of strategies to allow the consolidation of the ceramic suspension by protein gelation to occur before the foam destabilization processes begin, which will culminate in rupturing the bubbles . Thus, increasing the suspension viscosity is a possible solution to reduce the drainage rate of the liquid phase (drainage is one of destabilization mechanisms of wet protein foams), although the viscosity should not be so high as to reduce the foaming capacity of the ceramic suspension. In addition, the presence of both the ceramic particles and processing additives, particularly thickener agents, which can be included in order to

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improve the foamed suspension's stability, also influences the protein gelation process and its microstructural features. The protein gelation process, which is associated with its denaturation, formation of aggregates and cross-linking to generate a three-dimensional polymeric network, implies the occurrence of changes in the global rheological behavior and viscoelastic properties of the aqueous ceramic-protein suspensions, which also are affected by the ceramic particles [7].

Based on the above-mentioned, it can be deduced that a rigorous study of both the viscoelastic properties and shear flow of aqueous ceramic-protein suspensions will provide useful information with which to establish the experimental conditions that allow cellular ceramic bodies with controlled and homogeneous microstructures to be obtained.

Therefore, in this work, the rheological behavior of aqueous mullite–bovine serum albumin (BSA) suspensions was studied by dynamic rheology as a function of temperature and time. From these tests, the influence of mullite particles on the gelation process of the protein was also analyzed. In addition, the impact caused by adding methylcellulose (MC) as a binder on viscoelastic behavior of the aqueous mullite–BSA suspensions was also studied. Furthermore, shear flow properties of aqueous mullite–BSA–MC suspensions were analyzed to obtain information on the rheological behavior of the suspensions at room temperature.

2. Experimental data

2.1. Starting materials

A high-purity commercial mullite powder (MULS, Baikowski, Annecy, France) was used as ceramic raw material. A complete characterization of the mullite powder has been published in a previous work by the authors [9]. The main characteristics of this powder are shown in Table 1.

A commercially available high-purity (> 98%) bovine serum albumin (BSA; A7906, Sigma-Aldrich, USA) and methylcellulose powder (MC; M6385-Sigma-Aldrich, USA) were also employed. The globular protein exhibited a density of 1.27 g/cm^3 measured by He-pycnometry (Multipycnometer, Quantachrome Co., USA), 583 amino-acids, a molecular weight of 66.5 kDa [10] and an isoelectric point (IEP) of approximately 4.8–5.2 [11,12]; as for the polysaccharide, it exhibited a density of 1.28 g/cm^3 measured also by He-pycnometry (Multipycnometer, Quantachrome Co., USA), 1.7 of DS (average number of substituted hydroxyl groups per glucose) and a molecular weight of 17 kDa.

2.2. Rheological behavior of aqueous mullite-BSA suspensions and aqueous mullite-BSA-MC suspensions

The thermogelling behavior of aqueous mullite-BSA suspensions was studied by dynamic rheology with the aim of determining the experimental conditions that should be used for forming mullite green bodies by thermal consolidation. Thus, viscoelastic properties (storage and loss moduli, G' and G'', respectively, and the phase shift, δ=arc tan G''/G') of aqueous mullite–BSA (10 and 15 vol%) suspensions as a function of temperature (30–95 °C) and time were determined by temperature sweep tests at a heating rate of 2 °C/min and time sweep tests at different temperatures (64–70 °C), respectively. From these tests, the onset temperature of gelation ($T_{G'}^{O}$: temperature from which G' abruptly increased) and the gelation time (tG'^{O}: time corresponding to the abrupt increase of G') were determined. Temperature values slightly lower than the $T_{G'}^{O}$ values obtained for each system were selected in order to carry out the tests under isothermal conditions. In addition, a time period of 3000 s was considered as the maximum time of duration of these tests. The influence of the mullite particles on these parameters was studied, and thereby, the gelation process of the ceramic-protein system was analyzed.

In addition, the variation of viscoelastic properties of mullite–BSA systems with 2 wt% of methylcellulose (MC) as a function of temperature was also studied. Moreover, , the dynamic rheological behavior of BSA (10 and 15 vol%) solutions and BSA (10 and 15 vol%)–MC (2 wt %) solutions as a function of the temperature and time was also investigated for comparative purposes. The preliminary results indicated that for the selected MC concentration, the complete development of the methylcellulose gel should not occur.

The results obtained from these studies were taken into account for analyzing the impact of the polysaccharide on the gelation of aqueous ceramic-protein systems. It should be noted that the free water loss due to evaporation during the oscillatory isothermal tests (a typical experimental problem for rheological tests at temperature associated with heating using dry air), which are performed using small suspension volumes, and the additional formation of hydrogen bonds among water molecules and hydroxyl groups of MC, contributed to the instability of the mullite concentrated suspension and inhibited the development of the gel into the aqueous mullite–BSA–MC suspensions. For these reasons, the study of the rheological behavior of the mullite–BSA–MC systems as a function of time could not be carried out.

All the dynamic measurements were carried out using a rotational rheometer (Physica MCR 301, Anton Paar GmbH, Ostfildern, Germany) in the oscillatory mode and operated with a 25 mm-diameter parallel-plate geometry, a gap of 1 mm and a frequency of 1 Hz (6.28 rad/s). Preliminary measurements were conducted in order to obtain the linear viscoelastic range. A strain of 0.4% was used to ensure that all the temperature and time sweep tests were within the linear region. In all the measurements, a thin layer of low viscosity silicone oil was spread on the surface of the sample exposed to the atmosphere to minimize the evaporation of water from the suspension.

Additionally, shear flow properties at room temperature of aqueous mullite–BSA (10 and 15 vol%)-MC (2 wt%) suspensions were also studied in order to analyze the rheological behavior at room temperature in steady state. The obtained apparent viscosity vs. shear rate curves were compared with those recorded for aqueous mullite [13] and mullite-BSA suspensions as reported in previous work [9]. Tests were done using the afore mentioned rheometer for the oscillatory measurements along with the experimental conditions reported in previous work for the aqueous mullite suspension [13]. In this case, the rotational rheometer was operated under controlled-rate operating modes with a coaxial cylinder sensor (DIN 53019, Anton Paar GmbH) and a gap of 1 mm. Flow curves were obtained with a three-stage measuring program with a linear increase of shear rate from 0 to 1000 s^{-1} in 300 s, 60 s at 1000 s^{-1}, and a final decrease to zero shear rate in 300 s.

The optimum colloidal stability conditions for preparing the aqueous mullite suspension and mullite–BSA suspensions were previously determined by measuring zeta potential and shear flow properties [9,13]. Thus, stable and homogeneous aqueous mullite suspensions

Table 1
Characteristics of commercial mullite powder.

Commercial mullite powder	
Purity level	> 99.8 wt%
Phases	Mullite 3/2[a], α-alumina[+], θ-alumina[+], cristobalite[+] and non-crystalline silicate phases[+]
Pycnometric density	3.07 g/cm^3
Mean particle diameter (D_{50})	1.46 μm
Specific surface area	13.5 m^2/g
Particle morphology	Equiaxial three-dimensional and faceted particles

[+]Secondary phases.
[a] Main phase.

(40 vol% of total solids, pH=8.7) with 10 and 15 vol% of BSA with respect to the water amount of the suspension were prepared by mixing (with an impeller mixer with flat blades) the mullite powder with 0.45 wt% of commercial ammonium polyacrylate solution (Dolapix CE-64, Zschimmer & Schwarz, Germany) as a dispersant, and by homogenization in a ball mill for 4 h to stabilize the suspension. Subsequently, the required amount of BSA was dissolved in the aqueous mullite suspension obtained.

With regard to preparing the aqueous mullite–BSA–MC suspensions, a determined amount of methylcellulose powder was dissolved in the stable mullite suspension in order to obtain concentrations of 2 wt %, and then the resulting suspension was homogenized in a ball mill for 20 min. Finally, the corresponding amount of BSA required to obtain protein concentrations of 10 and 15 vol% was dissolved in the aqueous mullite–methylcellulose suspension. BSA, MC and BSA–MC solutions, which for the viscoelastic properties analysis as a function of temperature and time were considered for comparative purposes, were prepared using the same experimental conditions as for the aqueous ceramic suspension (pH=8.7 and 0.45 wt% of Dolapix CE-64). The selection of the MC concentration was made taking into account the following issues: (a) the apparent viscosity should be sufficiently high to promote the stability of the suspension (i.e. the foam´s ability to retain the gas for a certain time period), but not so high as to reduce both the foam´s initial volume (i.e. the "foaming capacity") and the ability to pour the suspension into molds, and (b) the methylcellulose should act mainly as a binder of the ceramic particles.

3. Results and discussion

3.1. Viscoelastic behavior of aqueous mullite–BSA suspensions and aqueous mullite–BSA–MC suspensions

First, before the presentation and discussion of the results obtained related to the viscoelastic behavior of the studied aqueous systems (BSA solutions, mullite-BSA suspensions and mullite-BSA-MC suspensions), a brief comment needs to be made about the thermal gelation process undergone by globular proteins like BSA.

It is well known that the gelation of these types of proteins (globular proteins), which is a process dependent on temperature and time, first requires that certain changes occur in their secondary and tertiary structures. These changes have to lead to the partial or total unfolding of the polypeptide segments of the chain (denaturalization process for which the hydrophobic groups of the molecule are exposed to the solvent), and consequently, the alteration of protein-protein interactions in particular (i.e. hydrogen bonds, ionic and hydrophobic interactions, Van der Waals interactions and disulphide covalent bonds) [14]. The BSA thermal gelation is in itself a complex process and includes a series of consecutive events, which is in agreement with the accepted kinetic models [15,16]: the unfolding of native protein chains, the aggregation of unfolding chains, and inter- and intramolecular reactions of aggregates. After the structure unfolds, it is essential that bonds form among the denaturalized molecules so that the sol-gel transformation occurs. Specifically, the BSA aggregation process is determined by at least three mutually dependent factors: (a) fluctuations in concentration associated with a liquid-liquid depletion process, (b) conformational changes to their structures, and (c) crosslinking. The globular proteins, like BSA, can be aggregated in two different ways. When the unfolding protein chains are highly charged and this charge is partially exposed, the formation of bonds is limited. In this case, the creation of linear fibrillar-type structures, also denoted as β-aggregates, are constituted by β-sheet structures which interact among themselves through intermolecular bonds. The second way in which the proteins can be aggregated is by random aggregation, which leads to the formation of more disordered structures.

Based on the above, it is clear that the processes jointly involved in the thermal gelation of the globular proteins will determine the significant changes in the rheological properties of the protein solution, and should be studied when planning the ceramic forming.

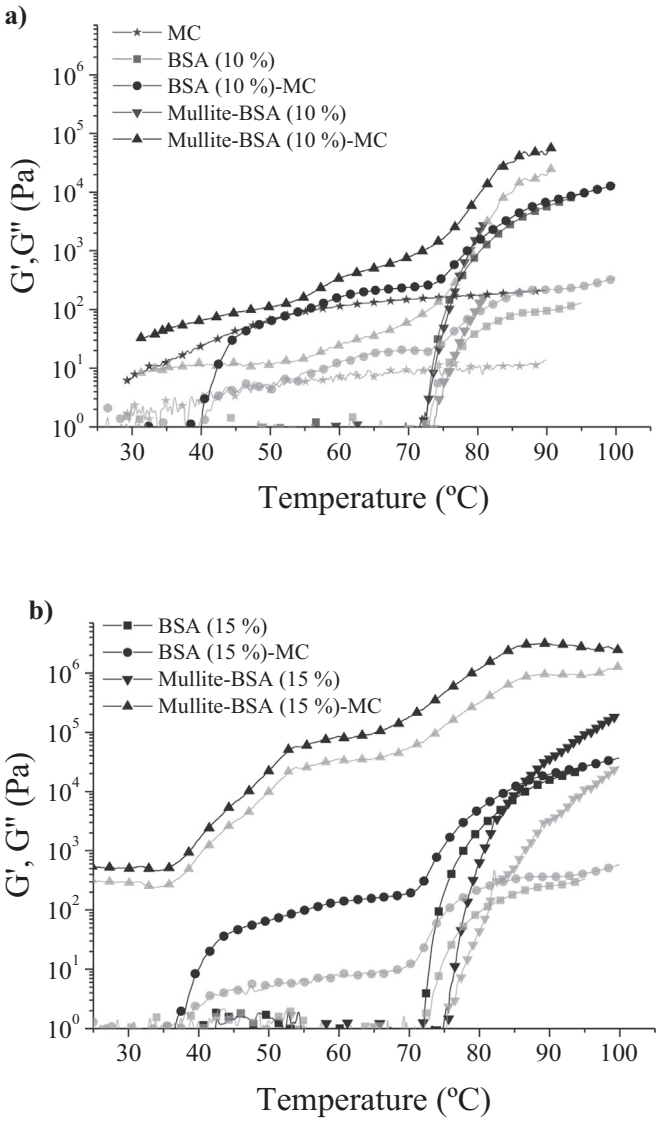

Fig. 1. G' and G'' vs. temperature for: (a) aqueous mullite–BSA (10 vol%) and mullite–BSA (10 vol%)–MC suspensions, and BSA (10 vol%), MC and BSA (10 vol%)–MC solutions, and (b) aqueous mullite–BSA (15 vol%) and mullite–BSA (15 vol%)–MC suspensions, and BSA (15 vol%) and BSA (15 vol%)–MC solutions; G' (black) and G'' (grey).

3.1.1. Effect of temperature

The variation of G' and G'' as the temperature increased for the aqueous mullite–BSA (10 and 15 vol%) suspensions and mullite–BSA (10 and 15 vol%)–MC (2 wt%) suspensions are shown in Fig. 1. In addition, G' and G'' vs. temperature curves corresponding to aqueous solutions of BSA (10 and 15 vol%), MC (2 wt%) and BSA (10 and 15 vol %)–MC (2 wt%) are also included in this figure for comparative purposes since the protein is the gelling agent which is able to consolidate the ceramic suspension, provided that the polysaccharide is the additional additive used as a binder. Note that the variation of both parameters with regard to temperature was recorded in linear logarithmic scale because this allows a clearer appreciation of the complete curve and the onset temperature of gelation at which G' abruptly increases.

In Table 2, the following characteristic temperatures and selected viscoelastic parameters are presented: onset temperature of gelation ($T_{G'}{}^{O}$), storage and loss moduli corresponding to a temperature which

Table 2
Characteristic temperatures and viscoelastic parameters of aqueous mullite–BSA and mullite–BSA–MC suspensions, and aqueous BSA and BSA-MC solutions.

Aqueous systems	$T_{G'}^{O}$ (°C)	G'_{T1O} (KPa)	G''_{T1O} (KPa)	δ_{T1O} (°)
BSA (10 vol%)	72	1.77	0.06	2
Mullite-BSA (10 vol%)	73	6.05	0.30	3
BSA (10 vol%)-MC	74	3.15	0.15	3
Mullite-BSA (10 vol %)-MC	75	33.00	10.50	18
BSA (15 vol%)	72	3.97	0.13	2
Mullite-BSA (15 vol%)	75	7.25	0.45	4
BSA (15 vol%)-MC	72	6.89	0.25	2
Mullite-BSA (15 vol %)-MC	68	850.0	225.8	15

is 10° higher than $T_{G'}^{O}$ (G'_{T1O} and G''_{T1O}), and the phase shift (δ). $T_{G'}^{O}$ values were determined from the point of intersection between tangent lines drawn before and after the point of discontinuity of the curves [15].

In both protein solutions, G' significantly increased once a certain temperature was reached, namely the onset temperature of gelation ($T_{G'}^{O}$), whose value did not depend on the protein concentration (10 or 15 vol%). In addition, the loss modulus (G'') exhibited a pattern qualitatively similar to that obtained for G' as a function of temperature, remaining slightly below the latter parameter in the whole temperature range analyzed. Moreover, the phase angle (δ) was below 45° for the thermally treated protein systems. In short, the global behavior of both G' and G'' moduli with the temperature is attributed to the formation of the protein gel. After gelation begins, the loss modulus registered at 10° above $T_{G'}^{O}$ was one order of magnitude smaller than the storage modulus, so that the phase angle ended up being closer to 0° than 90°, which indicates that the BSA gels behave mainly as elastic solids. Based on data reported in the literature [10,17–19], from 40 to 50 °C, conformational reversible changes of the BSA molecule (reorganization of the secondary and tertiary structures, in which the hydrophobic groups are exposed to the solvent, resulting in the formation of new conformations with a short half-life) begin to occur and lead to its denaturalization. Then, by increasing the temperature up to 60 °C, different events occur that increase the proportion of disordered structure: (a) reversible and incomplete unfolding of the α-helix structures of the protein, which helps cause some specific regions such as hydrophobic sites or SH-free groups to become more exposed to new intermolecular interactions, and (b) generation of secondary β-sheet structures. From 60 °C, the irreversible unfolding of the BSA molecules progresses and the formation of β-aggregates (fibrillar structure constituted by intermolecular β-sheet structures) begins. Above 70 °C, a three-dimensional gel network begins to grow, which in turn results in an increase in the system's viscosity. The network develops even more as the temperature increases, which is reflected in an additional increase of G' with respect to G''.

According to the onset temperature of gelation values ($T_{G'}^{O}$) recorded for the solutions with different BSA content (Table 2), and in agreement with data reported in the literature [13] for highly concentrated solutions, $T_{G'}^{O}$ did not decrease when the protein concentration was increased (note that a strong increase of $T_{G'}^{O}$ was recorded for concentrations lower than the concentrations used in this work [20]). Moreover, both viscoelastic moduli increased, especially G', when the BSA concentration was increased, although the phase shifts corresponding to protein solutions with 10 and 15 vol% were equal and close to zero, indicating that both systems behave as elastic solids (the phase angle was below 45°). The values displayed for the studied viscoelastic parameters were in the range of values reported for BSA gels [21]. The obtained results indicated that the analyzed protein solutions were able to form a three-dimensional structure by action of the temperature. This fact was also determined by ocular inspection of the material when heating the protein solution, and subsequently to the end of the rheological test at temperature.

Also, the evaluation of the viscoelastic properties of BSA gels developed by heating aqueous protein solutions up to 95 °C and subsequent controlled cooling to room temperature was carried out by dynamic strain sweep tests. From this test, the strain where G' equals G'' (i.e. critical deformation percentage; % ε_c) was considered as an indicator of the gel strength. Both gels showed G' values higher than G'' and phase shifts close to zero, which confirms that in a defined range of strain, the gel behaves as an elastic solid. As strain increased and the magnitude of G'' overcame G', the gel structure broke down and the system behaved as a viscous fluid. For strains lower than determined % ε_c (~150%), the values obtained for both viscoelastic moduli were higher (even more so for G'') than those obtained in the gels that developed up to 10 °C above $T_{G'}^{O}$. This behavior indicates that the gelation process progresses further during the controlled heating up to 95 °C and cooling to room temperature, and the formed gels should be more susceptible to deformation (δ=4°).

On the other hand, the mullite particles present in the protein solutions produced a slight shift of $T_{G'}^{O}$ to higher values, which indicates a delay in the beginning of the gelation process. The increase in $T_{G'}^{O}$ values was more marked for the system with 15 vol% of BSA. Furthermore, the addition of mullite into both BSA solutions caused both moduli (G' and G'') to increase by raising the temperature to a level that depended on the protein concentration (note that the increment was higher for the system with 10 vol% of BSA). In addition, for both mullite-BSA systems, the increase of G'' was notably higher than that of G'. Table 2 . However, the formed mullite-BSA gels presented quite similar phase shifts compared to the gels developed from protein solutions, being δ_{T1O} somewhat greater for the mullite suspension with 15 vol% of BSA than that with 10 vol% of BSA (Table 2). Thus, with the aim of analyzing the effect caused by mullite particles in the protein gel formation and their viscoelastic parameters, including $T_{G'}^{O}$, the following factors should be considered: (a) the decrease of free water due to the interaction of the water molecules with the mullite particles, which reduces the amount of water available to interact with ceramic particles, (b) the protein specific adsorption on ceramic particles induced by electrostatic interaction implies the partial unfolding of one part of the structure, which is a process, along with aggregation, that generates the three-dimensional structure (i.e. gel), which in part includes the ceramic particles, (c) the increase of the starting system viscosity attributed to a higher interaction between hydrated protein molecules and mullite particles, and (d) the increase of the hydrodynamic volume.

Moreover, based on results previously reported [9], the adsorption mechanism for BSA in the experimental conditions used in this work involves the formation of one side-on monolayer and the additional adsorption of protein molecules forming dimers with those from the first monolayer. This mechanism activates due to the surface charge of the particle being completely masked and the IEP cannot shift further than the IEP of the protein since from this point the process would be controlled by protein-protein interactions.

Taking the above-mentioned information into account, it can be assumed that the ceramic particles act to a greater or lesser extent depending on the protein concentration as discontinuity points in the gel, whose formation and development, even though is hindered, is definitively controlled by the network of the protein gel. The presence of these discontinuity points in the gel could be the cause of the loss of gel elasticity.

On the other hand, the presence of a lower amount of free water in the suspension with the greater content of BSA (15 vol%) could be considered the determining factor explaining why this system presented the highest shift of $T_{G'}^{O}$ with respect to the value registered for the system without ceramic particles. In addition, a lower amount of mullite respect to the BSA content, along with a greater amount of free

protein in the aqueous medium, could justify the slightest increase of viscoelastic parameters registered for the aqueous mullite–BSA (15 vol %) suspension.

As for the analysis of the influence of methylcellulose (MC) on the rheological behavior of aqueous mullite-BSA suspensions, the viscoelastic behavior of the aqueous MC solution (2 wt%) and the aqueous BSA (10 and 15 vol%)–MC (2 wt%) solutions was previously studied. The fact that aqueous MC solutions with a concentration higher than that considered critical form a reversible elastic gel during heating was what gave rise the study of this system. Based on reported studies [22,23], it is known that intermolecular hydrophobic associations among methoxyl groups play an important role in the thermoreversible gelation process of methylcellulose in water. At low temperatures, water molecules form cage-like structures to surround the hydrophobic methoxyl groups; in consequence, this causes the MC to become water-soluble, while upon heating these structures distort and fragment to expose the hydrophobic regions to medium and induce the formation of aggregates. In general, the experimental evidence has shown that the gelation process of the methylcellulose occurs in two stages during the solution heating: first, aggregates or clusters of the polymer chains with different morphology and sizes, formed by hydrophobic associations, control the process in the range of low temperatures; then, the aggregates disintegrate, and a three-dimensional network forms due to hydrophobic association, which competes with the liquid-liquid phase separation [22,24,25]. Even though the temperature between the two stages depends on the solution's concentration, it is generally situated at approximately 50 °C.

Based on Fig. 1, G' and G'' moduli recorded for the MC solution increased monotonically and slowly with the temperature before reaching a plateau above 55–60 °C. According to this variation Fig. 1 and the visual observation of the sample when the rheological test ends (i.e. the sample flowed and did not show appreciable turbidity), it can be assumed that the complete development of the methylcellulose elastic gel did not occur even though the formation of aggregates (weak elastic structures) from hydrophobic associations could have occurred for the selected MC concentration Moreover, G' always remained above G'' over the entire analyzed temperature range and the phase angle was below 45°, which would indicate that the MC solution has an elastic component higher than the viscous component.

Regarding the BSA-MC solutions, a similar viscoelastic behavior to that recorded for the MC solution up to ~55 °C can be observed in Fig. 1. Above this temperature, the G' value increased slightly up to ~73 °C (Table 2) (temperature close to the onset temperatures of gelation for BSA solutions even in presence of mullite particles). This increase could be attributed not only to the formation of BSA clusters and MC aggregates but also the generation of BSA and MC aggregates associated with the existence of interactions, mainly hydrophobic, between the protein (the protein is negatively charged because its IEP (5.0) is lower than the pH value (8.7) employed in the preparation of the suspensions) and the polysaccharide (neutral). Finally, at higher temperatures, G' increased abruptly due to the formation of the BSA gel in the presence of MC [26,27]. Regarding G'', behavior similar to that of G' was recorded over the entire analyzed temperature range, although their values were always lower than those reached by G'.

In this case, the increase in the viscoelastic properties (G', G'' and δ) of the BSA–MC gels obtained by heating to 95 °C and subsequently cooling to room temperature, with respect to those registered for the BSA-MC gels at 10 °C above $T_{G'}{}^{O}$, is consistent with the occurrence of a more advanced gelation process. Nevertheless, the presence of the methylcellulose chains in the structure of the BSA gel caused a slight decrease in the three-dimensional structure's stiffness. The observed overall behavior of G' with the temperature is consistent with that reported in the literature [26] for BSA–MC systems with similar concentrations of both biopolymers.

On the other hand, even though the presence of mullite particles in the BSA–MC solutions did not greatly modify the qualitative behavior of G' and G'' with the temperature recorded for these systems, a notable increase in the values of both moduli was recorded over the entire range of analyzed temperatures, and a change was detected in the onset temperatures of gelation (Table 2). Note that while for the mullite–BSA (10 vol%)–MC system, $T_{G'}{}^{O}$ was higher than the temperature corresponding to the MC-free suspension; for the mullite–BSA (15 vol%)–MC, the $T_{G'}{}^{O}$ value was lower than the temperature recorded for the aqueous mullite–BSA suspension without MC. This last fact can be attributed to the occurrence of water loss during heating due to the evaporation (typical experimental problem for rheological tests at temperature associated with heating using dry air) of small suspension volumes such as those used for performing these rheological tests. Based on this, the values of both moduli, in particular G', recorded for the suspension with 15 vol% of BSA could have increased not only due to the presence of mullite particles and MC but also water loss from evaporation. Moreover, this process could have led to a volume exclusion effect (the particles have to rearrange themselves into a smaller available volume) and contributed to the instability of the mullite concentrated suspension. Furthermore, gels developed from suspensions containing BSA, MC and mullite particles exhibited higher phase shifts (Table 2) than BSA-MC gels. This result indicates that the rigidity of the mullite-BSA-MC gels is lower than those without ceramic particles, with the latter behaving more like an elastic solid than the systems with mullite, where the viscous component is more pronounced. It is worth noting that the protein was incorporated into the aqueous mullite-MC suspension, previously prepared, whereby if all adsorption sites were occupied by the defloculant, MC and a part of the BSA total content, a certain amount of protein, either free or bonded to the methylcellulose by hydrophobic interactions, could have remained (especially when the BSA concentration was 15 vol%). These results seem to indicate that even though the gel formation is dominated by the protein, ceramic particles and MC aggregates, either free or associated, could remain trapped in the three-dimensional network and, in consequence, modify the protein gel structure by steric hindrance or by incorporating discontinuity points.

3.1.2. *Effect of time*

Variation of G' as a function of time for aqueous protein solutions and aqueous mullite–BSA suspensions held at different temperatures are shown in Fig. 2. G' vs. time curves obtained at different temperatures for BSA–MC solutions along with BSA solutions are presented in Fig. 3 for comparative purposes. The treatment temperatures were selected based on $T_{G'}{}^{O}$ values obtained from temperature sweep tests. In all cases, these temperatures were slightly lower than $T_{G'}{}^{O}$. For the BSA–MC solutions, the gelation time ($t_{G'}{}^{O}$) was determined from a point at which G' increased abruptly (this point can be recognized as a discontinuity in the G' vs. time curve on a log-log scale, as used in Figs. 2 and 3) [15]. When the gelation time is not be precisely detected, as occurred for BSA solutions and mullite–BSA suspensions, the time at which G' increased above a threshold value was used (in this work, the value of 2 Pa was selected). $t_{G'}{}^{O}$ values obtained for aqueous BSA solutions, BSA–MC solutions and aqueous mullite–BSA suspensions are presented in Table 3.

Even though the G'' values were not included in Figs. 2 and 3 in order to make reading easier the graphics, both moduli exhibited similar behavior as time increased, with G' always higher than G'', even from the beginning of the measurements, which exhibited large deviations attributed to low torque signals. After a certain time, both moduli increased much more rapidly, and then, in some cases, they reached a plateau.

According to the BSA solution curves (Fig. 2), the change of G' over time depended mainly on the testing temperature. The higher the temperature, the shorter the time required for the gelation process ($t_{G'}{}^{O}$) to begin, which is in agreement with the fact that this process is a kinetically controlled process. It is well known that the solution's concentration also influences the $t_{G'}{}^{O}$ values, since this effect is

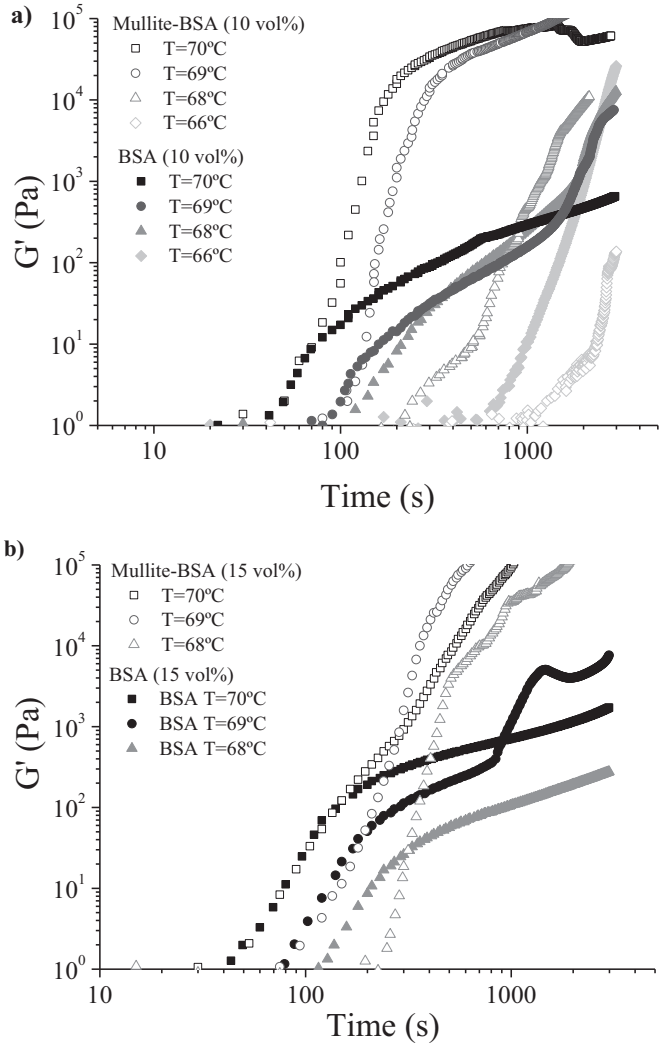

Fig. 2. G' vs. time at different temperatures for: (a) BSA (10 vol%) solutions and mullite–BSA (10 vol%) suspensions, and (b) BSA (15 vol%) solutions and mullite–BSA (15 vol%) suspensions. *Solutions (filled shapes), Suspensions (open shapes).*

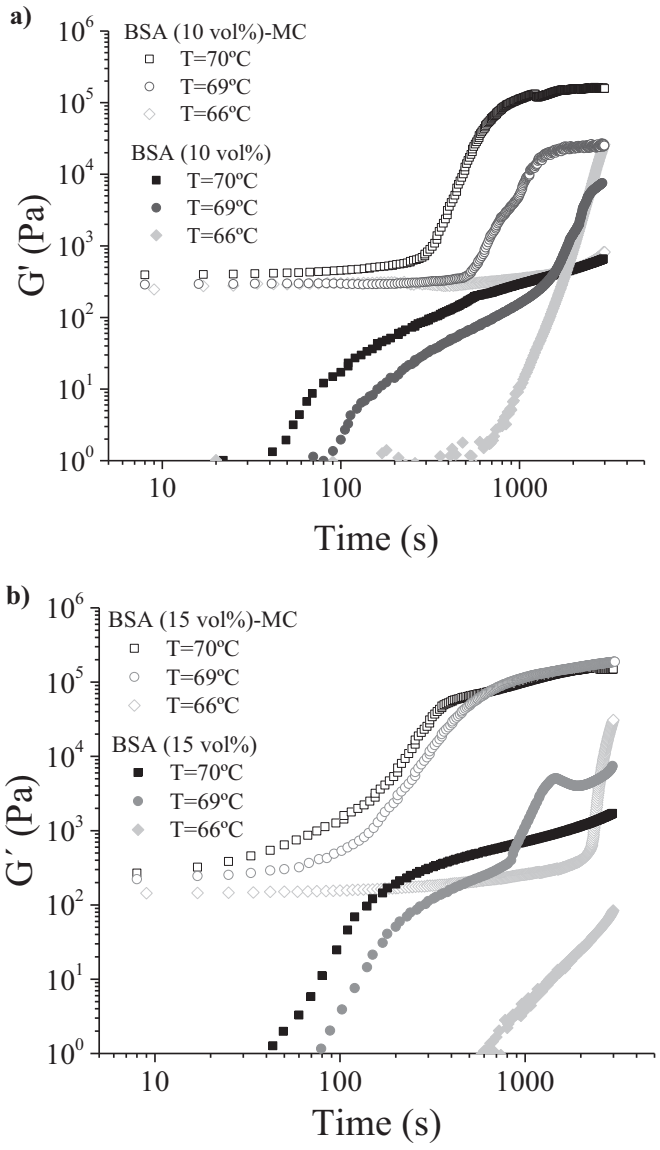

Fig. 3. G' vs. time curves at different temperatures for: (a) BSA (10 vol%) and BSA (10 vol%)–MC solutions, and (b) BSA (15 vol%) and BSA (15 vol%)–MC solutions. *BSA solutions (filled shapes), BSA-MC solutions (open shapes).*

dependent on the temperature. However, for the protein solutions studied, the influence of the protein concentration on the gelation time was not very significant. In general, $t_{G'}{}^{O}$ values for the less concentrated protein solution (10 vol%) were close to those obtained for the solution with 15 vol%. Note that if a certain amount of water is evaporated during the isothermal test, the $t_{G'}{}^{O}$ values determined for the lowest testing temperatures (tests with longer times) as well as for the highest temperatures would be underestimated ($t_{G'}{}^{O}$ smaller).

On the other hand, the presence of mullite particles in aqueous protein solutions generally increased the gelation time for each testing temperature (Fig. 2 and Table 3). This effect was stronger when the treatment temperature was lower. This behavior shows once again that the ceramic particles delay the gelation process. Note that the $t_{G'}{}^{O}$ values for the suspension with the highest protein concentration were, in general, very similar to those corresponding to the system with 10 vol% of BSA. This fact can be attributed to the evaporation effects which made $t_{G'}{}^{O}$ lower. In addition, this effect could also explain the pronounced increase in G' recorded for the mullite–BSA system treated at the highest temperatures compared to the behavior shown by the system at lower temperatures.

Despite the fact that the evaluation of the viscoelastic properties as a function of time for the aqueous mullite–BSA system with MC cannot be carried out, the results obtained from analysis of the BSA–MC solutions were relevant to clarify the effect of this polysaccharide in the thermogelling behavior of the protein. Thus, the behavior of G' with time for BSA–MC solutions was different compared to that recorded by protein solutions, particularly for the highest temperatures. At the beginning of the test (short times), G' remained relatively constant up to a certain time (in general, this time was higher than $t_{G'}{}^{O}$ corresponding to the protein solution), which depended on the testing temperature and the protein concentration, from which G' abruptly

Table 3
$t_{G'}{}^{O}$ (s) values for aqueous BSA and BSA–MC solutions, and aqueous mullite–BSA suspensions.

T (°C)	BSA (vol%)		BSA (vol%)–MC		Mullite–BSA (vol%)	
	10	15	10	15	10	15
70	50	49	295	100	51	53
69	98	88	520	160	108	95
68	130	136	—*	—*	242	250
66	730	720	2100	2020	1250	—*

—*Value not determined.

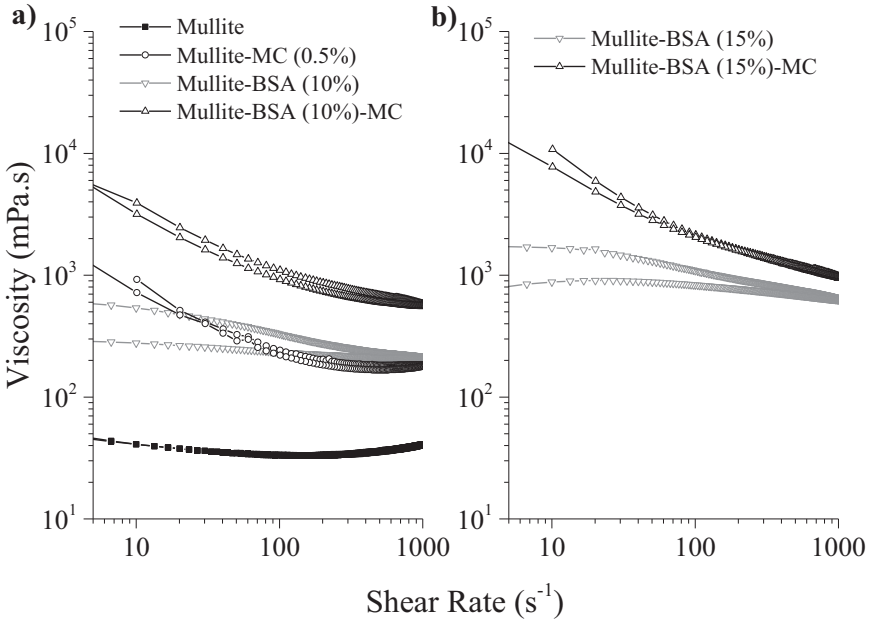

Fig. 4. Viscosity curves for: (a) aqueous mullite, mullite–MC, mullite–BSA (10 vol%)– MC (0 and 2 wt%) suspensions, and (b) aqueous mullite–BSA(15 vol%)–MC (0 and 2 wt%) suspensions.

increased. Note that for the solutions with 15 vol% of protein treated at the highest temperatures (i.e. 70 and 69 °C), the increase of G' registered at very short times was attributed to the evaporation of a certain amount of water.

The described behavior could be associated mainly with the formation of both BSA–MC aggregates bonded by hydrophobic interactions that lasted even longer than the gelation times of the BSA solutions, and the three-dimensional network generated by the protein whose structure, which begins to develop more tardily, is different than that originating from protein solutions without MC.

With regard to the gelation times obtained for the BSA–MC solutions, the presence of methylcellulose notably increased the values of this parameter (Table 3). The results obtained for these solutions indicated that the gelation process was markedly accelerated by the increase in protein concentration, although this effect was smoothed out at the lowest testing temperature. On the other hand, by considering that the gelation time obtained for the solution with 15 vol% of BSA treated at the highest testing temperature should have been higher, the loss of water by evaporation during the test could be the main factor behind why the value for this parameter could have been sub-estimated.

Finally, according to results obtained by oscillatory rheological testing, the methylcellulose slowed the kinetics of the protein gelation process and modified the gel structure formed by BSA even though it did not act as an additional gelling agent of the studied systems. The results obtained for BSA–MC system highlight the importance of performing isothermal tests in order to elucidate the complex gelation process of the studied systems.

3.2. Shear flow properties

As was already mentioned above, the existence of associations of biopolymers among themselves (i.e. BSA–BSA and MC–MC), and among water molecules, has been extensively reported [23–28]. Thus, in the specific case of methylcellulose, three different kinds of interactions were reported: (a) hydrogen bonding between unmodified hydroxyl groups (–OH) of the cellulose chain, (b) hydrogen bonding between hydroxyl groups of the cellulose chains and water molecules, and (c) intermolecular hydrophobic associations among methoxyl groups of the MC molecules [22].

On the other hand, understanding the different protein-polysaccharide interactions in an aqueous solution continues being one of the more challenging aspects in the field of hydrocolloid research. With regard to last interactions, associations mainly of a non-covalent nature (i.e. electrostatic, hydrogen bonding, hydrophobic and steric) have been reported, although recently the existence of covalent associations between both types of biopolymers was also reported [23,27,28]. Even though electrostatic attraction is the main driving force that leads to the formation of protein-polysaccharide complexes, hydrogen bonding and hydrophobic interactions play a role, although secondary, in the stabilization of aggregates. The extent of this last type of interaction depends on the temperature since it determines the protein conformations, among others characteristics. The formation of strong protein-polysaccharide complexes occurs when the pH of the medium is lower or higher than the protein isoelectric point, so that the charged protein may thus interact with oppositely charged polysaccharides. Weaker complexes can also be formed when the solution pH is very close to the protein isoelectric point, and therefore, the protein net charge is almost zero. For this condition, the system becomes unstable, which leads to separation in two phases (thermodynamic incompatibility) since the similarly charged polymers repel each other [28].

In the specific case of a BSA–MC solution, in which methylcellulose is a neutral polysaccharide, the formation of a thermodynamically incompatible system, which can occur in a phase separation, was particularly reported for highly concentrated biopolymer solutions, in a very different condition to that used in this study. However, the methylcellulose exerts little effect on the BSA molecular behavior, so that given the nature of this polysaccharide , the influence of hydrophobic interactions and hydrogen bonds, which could modify the rheological properties of the system [23,27], is highly probable. Thus, the study of the flow behavior of the mullite–BSA suspensions with methylcellulose can be considered as a useful tool to be used in the study of the formation of BSA–MC structures in which the ceramic particles might also participate.

The variation of the apparent viscosity as a function of shear rate at room temperature for the aqueous mullite–BSA (10 and 15 vol%) suspensions and mullite–BSA (10 and 15 vol%)–MC suspensions are shown in Fig. 4. Flow curves corresponding to the aqueous mullite suspension prepared in the same experimental conditions as for the mullite–BSA suspensions and the aqueous mullite suspension with

Table 4
Apparent (η_{10} and η_{1000}) and relative (ηr) viscosities of the aqueous mullite suspension, aqueous mullite–MC suspension and aqueous mullite–BSA–MC suspensions.

Systems	η_{10} (mPa.s)	η_{1000} (mPa.s)	ηr (at 10 s^{-1})	ηr (at 1000 s^{-1})
Mullite	41	40	1	1
Mullite-BSA (10 vol%)	538	215	13.12	5.38
Mullite-BSA (15 vol%)	1680	646	40.98	16.15
Mullite-MC (0.5 wt%)	933	183	22.76	4.58
Mullite-BSA (10 vol %)-MC	3920	567	95.61	14.18
Mullite-BSA (15 vol %)-MC	10,780	999	262.93	24.98

2 wt% of methylcellulose were included for comparative purposes. Apparent viscosity values at shear rates of 10 s^{-1} (η_{10}), which were considered as initial apparent viscosities, and 1000 s^{-1} (η_{1000}) together with relative viscosities (ηr) at both shear rates (10 and 1000 s^{-1}) for studied systems, are reported in Table 4. The relative viscosity was defined as the ratio of the apparent viscosity of the mullite–BSA or mullite–BSA–MC suspensions and the apparent viscosity of the BSA-free and MC-free mullite suspension, respectively (all the suspensions were prepared with 40 vol% of total solid loading).

According to viscosity curves (Fig. 4a), the aqueous mullite suspension exhibited a slight transition from shear-thinning (pseudoplastic) to shear-thickening (dilatant) behavior for shear rates higher than 300 s^{-1}, thus behaving as a complex-fluid [29]. For this rheological behavior, the viscosity progressively decreased while being maintained at the highest shear rate, 1000 s^{-1} for 60 s, as reported in previous work [9]. Similar complex rheological behavior was also reported in previous work by the authors for a cordierite precursor mixture [30] and mullite–starch suspensions [13] as well as by other authors for mullite suspensions with similar characteristics [31,32]. The pseudoplasticity of the suspension and the hysteresis (from 170 up to 30,000 Pa s^{-1} ; values determined as the area between the up and down shear stress-shear rate curves) of the shear stress–shear rate curve, which in general is interpreted in terms of thixotropy, was increased by increasing the BSA amount in the aqueous mullite suspension.. In this case, based on results obtained from the study of the colloidal stability of the mullite–BSA system [9], this time-dependent behavior can be associated with: (a) the presence of an anionic polyelectrolyte of high molecular weight acting as defloculant of the mullite particles by an electrosteric stabilization mechanism, and (b) the formation of one protein side-on monolayer by adsorption on mullite particles, and the subsequent adsorption of proteins forming dimers with those from the first monolayer, which could also act as a binder for the concentrated ceramic suspension. The impact of sedimentation effects and demixing of the suspension with time has been ruled out since there was no experimental evidence of these processes within the time scale of the viscometric measurement. In particular, the aqueous mullite suspension with 15 vol% of BSA did not show dilatancy, which could be associated with the rearrangement of the ceramic particles in the flow direction and the extension of the protein chains, which allows the formation of new structures with a more stable configuration. In addition, the apparent viscosity of the mullite suspension increased notably when the amount of protein increased. This increase was much more marked at the lowest shear rates, as indicated by the ηr at 10 s^{-1} compared to this parameter determined at 1000 s^{-1} (Table 4). The difference between the relative viscosity values corresponding to systems with 10 and 15 vol% of BSA was constant at both shear rates.

As for the effect of MC in the mullite–BSA suspension, the results obtained indicated that the global rheological behavior of the suspension was modified. Thus, the mullite–BSA suspensions with MC showed a purely shear-thinning behavior with a higher hysteresis degree (25,000–35,000 Pa s^{-1}) of the shear stress–shear rate curve than the MC-free ceramic-protein suspensions (< 30,000 Pa s^{-1}). The more favored extension of the MC chains and their orientation in the flow direction due to their structural characteristics with respect to those of the globular protein (coil structures) would explain the higher pseudoplasticity registered for the systems with MC. Even though this behavior generally resulted in being very similar to that exhibited by the mullite–MC suspension, (which is characteristic of aqueous suspensions of organic binders with high molecular weight, as is the case of the used methylcellulose), the existence of different hydrophobic associations between the protein molecules and the polysaccharide chains adsorbed to the mullite particles (mullite–MC–BSA–mullite; mullite–MC–BSA–MC-mullite), besides the hydrogen bonds with water molecules, could explain in part the greater dependence of the viscosity on time. Also, the presence of a large amount of protein, together with the polysaccharide both acting as a binder (binding of ceramic particles) for the concentrated ceramic suspension, could justify this behavior. On the other hand, the possible presence of BSA-free molecules in the aqueous medium, with some of them forming BSA-BSA aggregates, as would occur especially in the system prepared with 15 vol% of BSA, could modify the type and stability of the structures present in the system and decrease the hysteresis degree of the shear stress–shear rate curve to even below the determined value (30,000 Pa s^{-1}) for the mullite–BSA (15 vol%).

With regard to apparent viscosities measured for the aqueous mullite–BSA (10 and 15 vol%) suspensions with MC, which were notably higher than for MC-free systems, a significantly higher increase in this parameter was recorded at 10 s^{-1} compared to that obtained at 1000 s^{-1}. Moreover, this increase was more pronounced for the system with 10 vol% of BSA, and the viscosity difference between both suspensions was even higher at 1000 s^{-1}. In addition, the formation of coils and entanglement of polymer chains, which would lead to an increase of the hydrodynamic volume, could explain in part the increase in suspension viscosity. The possible presence of BSA-free in the system with 15 vol% of BSA could be the cause of the decrease in MC binding power. Of course, the relative densities followed the same tendency as showed by the apparent density values.

In conclusion, based on the above-mentioned discussions, the global rheological behavior exhibited by aqueous mullite–BSA–MC suspensions can be attributed to the overlapping of those corresponding to mullite–MC and mullite–BSA suspensions modified in part by the presence of interactions between the protein and the polysaccharide. These associations, which could generate the formation of new structures, could hinder the occurrence of some structural change associated with the creation-destruction of structures that characterizes the fluid-complex behavior.

4. Conclusions

The integral study carried out on the thermogelling behavior and flow properties of aqueous mullite–BSA–MC systems by oscillatory and rotational rheological tests is of great interest for processing porous ceramic materials. Thus, based on the results obtained from these tests, it is possible to select the adequate experimental conditions to be used when forming a cellular ceramic with a controlled microstructure by the protein casting method. In addition, the results obtained from the analysis of the influence of the ceramic particles and the methylcellulose on the protein gelation process constitutes a relevant contribution to understanding the interactions existent among components of the studied systems.

For both BSA solutions studied, the global behavior of both the G' and G'' moduli with temperature was attributed to the formation of a

protein gel which behaves mainly as an elastic solid. Moreover, as expected, both viscoelastic moduli increased, especially G', when the BSA concentration was increased, although the phase shift values for the two protein solutions were equal and close to zero, which is in agreement with the fact that both systems exhibited a higher elastic component. In addition, the influence of the protein concentration on the gelation time was not very significant.

In general, the presence of mullite particles in the protein solutions produced a slight delay (increase of both $T_{G'}{}^{O}$ and $t_{G'}{}^{O}$) in the beginning of the gelation process, which was more marked for the system with 15 vol% of BSA. In addition, the increase in both viscoelastic moduli by increasing the temperature depended to a degree on the protein concentration. Moreover, the phase shifts were quite similar to those obtained for gels developed from protein solutions. Thus, it was considered that the ceramic particles, which are in part included in the three-dimensional network of the protein gel, act as discontinuity points of the gel, whose formation and development, although hindered, is controlled by the network of the protein gel.

With regard to the influence of the methylcellulose on the protein thermogelling behavior, it was considered that, even though the complete development of the gel did not occur for the selected MC concentration, BSA clusters and MC aggregates were initially formed and BSA–MC aggregates associated with the existence of interactions mainly hydrophobic were generated later. These aggregates remained even up to times longer than the BSA clusters , and the subsequent formation of a three-dimensional network, different from that originating from the protein solutions, was generated by the protein. The inclusion of methylcellulose chains in the protein gel structure were also determined to cause a slight decrease in its stiffness. From the analysis of the viscoelastic properties as a function of time, a strong increase in the gelation time respect to that obtained in the MC-free systems was determined. Additionally, the gelation process was markedly accelerated by the increase in the protein concentration, although this effect was smoothed for the lowest testing temperature.

The presence of mullite particles in the BSA–MC solutions caused a notable increase in the values of both moduli over the entire range of analyzed temperatures and a change in the onset temperatures of gelation. The rigidity of the mullite-BSA-MC gels was lower than those without ceramic particles, with the viscous component being more pronounced.

Based on the obtained results, it was assumed that the gel formation in the aqueous mullite–BSA–MC systems is dominated by the protein, with ceramic particles and MC aggregates, free or associated among themselves, trapped into the three-dimensional network, which modifies the protein gel structure by steric hindrance or the incorporation of discontinuity points. In short, even though the methylcellulose did not act as an additional gelling agent in the studied systems, it did slow the kinetics of protein gelation process and modified the gel structure formed by BSA.

Finally, the analysis of the flow behavior of the aqueous mullite–BSA–MC suspensions was considered a useful tool to be used in the study of the formation of BSA–MC structures in which the ceramic particles could also participate. The presence of methylcellulose in the mullite–BSA system modified its global rheological behavior. These systems showed a purely shear-thinning behavior with a higher hysteresis degree of the shear stress–shear rate curve than the MC-free ceramic-protein suspensions. This behavior was attributed to the fact that the extension of the MC chains and their orientation in the flow direction were more favored due to their structural characteristics. The existence of hydrophobic associations between protein molecules and the polysaccharide chains adsorbed to the mullite particles, as well as hydrogen bonds with water molecules and the action of both additives acting as a binder of ceramic particles, were considered the factors responsible for the greater dependence of the viscosity on time. In particular, the BSA–MC associations, which could generate the formation of new structures, would hinder the occurrence of some structural change associated with the creation-destruction of structures that characterizes the fluid-complex behavior. In addition, the increase of the suspension viscosity produced by adding MC was associated with the formation of the coil and entanglement of polymer chains, which could lead to an increase in the hydrodynamic volume.

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