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Insights into Carbopol gel formulations: Microscopy analysis of the microstructure and the influence of polyol additives

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ABSTRACT: Carbopol is a family of high molecular weight cross-linked poyacrylic acids widely used as thickening agents in several industrial applications. In this work, the structure of Carbopol 974P NF was investigated at pH 7.4 by means of capillary viscometry, dynamic rheology, fluorescence microscopy, and electron microscopy in the dilute and semi-dilute (hydrogel) state. Especially, high-pressure freezing technique has proved to be a technique of choice to visualize the Carbopol hydrogel microstructure through cryo-scanning electron microscopy analysis without artifacts related to the formation of ice crystals. It has been shown that the Carbopol hydrogel has a hierarchical structure made of large spherical entities of a few micrometers in size packed together and composed of much smaller agglomerated microgel particles. In addition, fluorescence and electron microscopies have pointed out that the presence of polyol additives in the gel formulation favors the formation of more dense or jammed structure, probably through hydrogen bonding.
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INTRODUCTION

Carbopol polymers or carbomers are widely used as thickening, suspending, and emulsifying agents to modify rheological properties of liquid or gel formulations.[1–3] Their transparency, stability, and nontoxicity make them popular as additives for pharmaceutical and cosmetics applications.[4–6] Carbopol polymers are high-molecular-weight polymers of acrylic acid obtained by precipitation polymerization in ethyl acetate and cross-linked with polyalkenyl ethers or divinyl glycol. Their molecular weight is in the billion range because of the interlinkage of many polymer chains.[7,8] Carbopol comes in the form of a powder with grains of a few micrometers in size, which are made of agglomerated particles of cross-linked polymer chains. When dispersed in water at pH above the pKa of carboxylic functions ($pKa = 6.0 \pm 0.5$),[9] the ionization of the polymer results in a net outward osmotic pressure due to the presence of mobile counterions, and, consequently, Carbopol grains can swell up to 1000 times their original volume (10 times their original diameter).[10] As they swell, the Carbopol particles can jam and form a gel above a given concentration,. The swelling of particles is also favored by the presence of electrostatic repulsion between anionic charges, which makes the chains stiffer at $pH > 6$.

The gel microstructure has been thoroughly studied by Frisken and coworkers[10,11] as function of pH and concentration by combining light scattering and rheological analysis. The authors showed that Carbopol is a dilute dispersion of relatively noninteracting swollen microgel particles at low polymer concentration and low pH, has a percolated structure of gel particles with significant void space at intermediate concentration and has a space-filling structure composed of compressed microgel particles at high concentration or high pH. Besides, a few works have reported on the use of microscopy to image the microstructure of the Carbopol gel. Results obtained by scanning electron microscopy (SEM) are often not satisfactory because of artifacts related to the presence of ice crystals.[1,12] By using fluorescence confocal microscopy, Gutowski *et al.*[11] have revealed the artifact-free structure of Carbopol gel at pH between 3 and 4 and at different concentrations. Although authors did not perform a quantitative analysis, they showed that the Carbopol

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particles are a few microns in size and quite polydisperse. At 0.05 wt %, one can clearly distinguish pools of solvent in the gel structure, whereas at 0.5 wt % the gel has a nearly space-filling structure. However, they did not investigate the Carbopol structure at neutral pH, which would have been of interest regarding the pharmaceutical applications of the Carbopol in general.

The aim of the present work was to provide new information about the structure of the Carbopol 974P NF (C974P), the oral pharmaceutical grade of the carbomers, which has been only little addressed in the literature to date. The C974P is used in many oral and mucosal contact applications such as controlled release tablets, oral suspension, and bioadhesive formulations.[13–17] The C974P is cross-linked with allyl pentaerythritol, but no precise information about the degree of cross-linking is available to our knowledge. Herein, the physicochemical properties of C974P formulations adjusted at neutral pH will be studied both in dilute solution by capillary viscometry and in the gel state by dynamic rheology analysis in linear and nonlinear regime. The gel microstructure will be investigated in same conditions of pH by combining fluorescence confocal microscopy and cryo-SEM. In addition, the role of two polyol additives, sorbitol and the mannitol, which are commonly used in pharmaceutical formulations to adjust the osmolarity to a physiological level, will be studied as well.[18]

MATERIALS AND METHODS

Materials

Carbopol 974P NF (C974P) was provided by Lubrizol Corporation (Cleveland, OH). Sorbitol was supplied by Roquette under the trade name Neosorb P20/60. D-Mannitol was provided by Fluka (St. Louis, MO). Sodium hydroxide solution was prepared from 0.1*M* standard volumetric solutions (Roti®VOLUM, Roth, Karlsruhe, Germany). Alexa Fluor 488 hydrazide was provided by Life Technologies (Carlsbad, CA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and *N*-hydroxysuccinimide were supplied from Alfa Aesar (Ward Hill, MA) and used as received. Blue Dextran (MW = 20,000 g mol^{-1}) was provided from Aldrich (St. Louis, MO).

Preparation of Carbopol Gels

Non-neutralized gels of 0.25% w/v C974P were prepared by progressively adding the polymer powder during 30 min into water while continuously stirring the solution. Sorbitol or mannitol is premixed at the solid state with Carbopol powder before addition into water. After full solubilization, the solution is kept under stirring during at least 2 h. Then, the pH is adjusted to 7.4 with 0.1*M* NaOH using a WTW inoLab pH 730 pH meter with a glass/platinum combined electrode (N6000A, Schott).

Capillary Viscosimetry

Flow time measurements were performed using a Ubbelohde 0c (0.46 mm internal diameter) capillary viscometer. The viscometer was placed in a thermostat bath (Schott CT52), and the temperature was maintained at 20°C for all measurements. Diluted solutions of Carbopol were prepared from a Carbopol 974P NF stock solution. Sorbitol or mannitol was added to the Carbopol solutions in such a manner that the polyol to Carbopol mass ratio was 14. In the range of Carbopol concentrations investigated (0.005–0.015 wt %), the viscosity of the polyol is negligible.[19] The pH of all solutions was adjusted to 7.4 with 0.1*M* NaOH.

Rheometric Measurements

Rheological properties of aqueous solutions were characterized at 20°C using an Anton Paar Physica MCR301 stress-controlled rheometer equipped with a cone plate geometry (50 mm diameter and 2° angle). Strain sweep tests were done to determine the transition from linear to nonlinear regime at an angular frequency of 10 rad s^{-1}. Dynamic analyses were then performed in the linear domain from 0.1 to 100 rad s^{-1}. It was verified by using pre-shear tests followed by resting periods that the sample is not sensitive to shear history (no thixotropy).

Cryo-Scanning Electron Microscopy

The samples were prepared by two different methods. In the first method, a drop of Carbopol hydrogel is directly immersed in liquid nitrogen at −180°C (preliminary freezing), and then transferred to the cryo-chamber (−100°C), where the frozen sample was fractured. After sublimation of ice (−50°C, 15 min), the sample was coated with gold–palladium for SEM imaging. Observations with a JEOL 6700F scanning electron microscope were done in the cold stage at −160°C with 5 kV accelerating voltage. The second method is based on the high-pressure freezing technique, where artifacts related to the formation of ice crystals can be eliminated. The technique takes advantage of the higher viscosity and lower freezing point of water temperature at 2100 bars to considerably reduce the nucleation of ice crystals. It means that very high freezing rates (>10,000°C s^{-1}) are not required with this technique. Practically, a drop of Carbopol sample was introduced in a freeze fracture planchette (Leica Microsystems, Vienna, Austria) and then assembled with a second planchette to create a sandwich that was loaded in a HPM 100 high-pressure freezing machine (Leica Microsystems). The sample was frozen within 5 ms at 2100 bars. The frozen sandwich was longitudinally fractured in liquid nitrogen, and one side of the fractured sample was mounted in a cryo-transfer stage (Quorum Technologies, UK) at −195.8°C. Ice in the frozen specimen was partially sublimed away at −95°C at 2.10^{-7} Bars. A layer of platinum with a thickness of 2–3 nm was sputtered on the specimen surface. The specimen was then transferred and examined under different areas in a FEI Quanta 250 FEG SEM, with an accelerating voltage of 5 kV in high vacuum. The sample was kept at −140°C during imaging.

Laser Scanning Confocal Microscopy

The Carbopol 974P NF was covalently labeled with Alexa Fluor 488 as follows. The Carbopol was first dispersed in water at 0.43% (w/v), and the pH adjusted to 4.8 with 0.1*M* NaOH. Then, Alexa Fluor 488 hydrazide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, and *N*-hydroxysuccinimide were added to Carbopol in molar ratios of 0.001 : 0.01 : 0.002, with regards to a mole unit of carboxyl group. The final concentration in Carbopol was 0.33% (w/v). The reaction was performed during 4 h in dark at room temperature at pH 4.8. At

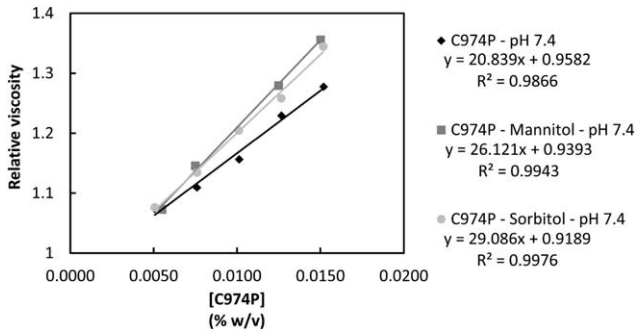

Figure 1. Relative viscosities of solutions of Carbopol 974P NF (C974P) at 20°C, pH 7.4 with and without addition of mannitol or sorbitol. The mannitol (sorbitol) to carbopol mass ratio is 14.

the end of reaction, the pH was adjusted to pH 7.4, and the gel diluted to a concentration of 0.25% (w/v). Laser scanning confocal microscopy analysis of Alexa-labeled Carbopol gel was performed using a LEICA TCS SP5 II microscope. The sample was observed at a 488 nm excitation wavelength from an Ar laser. The emitted light is collected on the spectral range from 500 to 600 nm by an ultrasensitive hybrid photomultiplier.

RESULTS AND DISCUSSION

Capillary Viscosimetry

The Carbopol 974P NF was first studied in dilute aqueous solution at pH 7.4 at concentrations below than 0.02% (w/v) where it is expected that the Carbopol behaves as a dispersion of relatively noninteracting microgel particles.[11] Dynamic light scattering analysis failed to provide a reliable value of hydrodynamic size of microgels because of the presence of some large particles in the formulation (results not shown). Hence, a rough estimation of the characteristic microgel size was achieved through measurements of relative viscosities at various Carbopol concentrations by using the Einstein's viscosity law:

$$\frac{\eta}{\eta_0} = 1 + 2.5\phi \quad (1)$$

where, ϕ is the volume fraction of the microgel and $\frac{\eta}{\eta_0}$ the relative viscosity, that is, the ratio of the sample viscosity to the solvent viscosity. The microgel radius can be then deduced from the volume fraction by considering that

$$\phi = \frac{4\pi R_G^3 N_A c}{3M} \quad (2)$$

where, R_G is the gyration radius, N_A the Avogadro number, c the polymer mass concentration, and M the molar mass, which is in the 10^9 g mol^{-1} range according to the manufacturer.[8] The evolution of the relative viscosity as function of the polymer concentration (Figure 1) could be reasonably fitted with Einstein's viscosity law, although the value at zero concentration is not exactly 1. Radii of gyration of 690, 750, and 770 nm were obtained for C974P, C974P + mannitol, and C974P + sorbitol, respectively, with a polyol to Carbopol mass ratio of 14. These values are only rough estimations of the true microgel size, as the molecular weight given by the manufacturer is not that accurate, and the Einstein law theoretically applies for hard spherical particles whereas microgels are an assembly of soft polymer blobs.[10] Nonetheless, a small but significant increase in microgel size was obtained in the presence of polyol, which suggests the existence of some physical interaction between the polyacrylic acid residues and the polyols, which could be—although we do not have direct experimental evidence of that—hydrogen bonding, considering the molecular structures involved.

Rheometric Analysis

Dynamic rheology measurements were performed with Carbopol 974P NF at pH 7.4 at a higher concentration, $c = 0.25\%$ (w/v),where the Carbopol is in the gel state (Figure 2).[11] Measurements of the elastic (G') and loss (G'') modulus were performed at first as function of frequency at 0.2% strain in the linear viscoelastic regime. The elastic moduli were constant around 100 Pa, whereas the loss moduli values were an order of magnitude lower and goes through a minimum around $\omega = 1$ rad s^{-1}

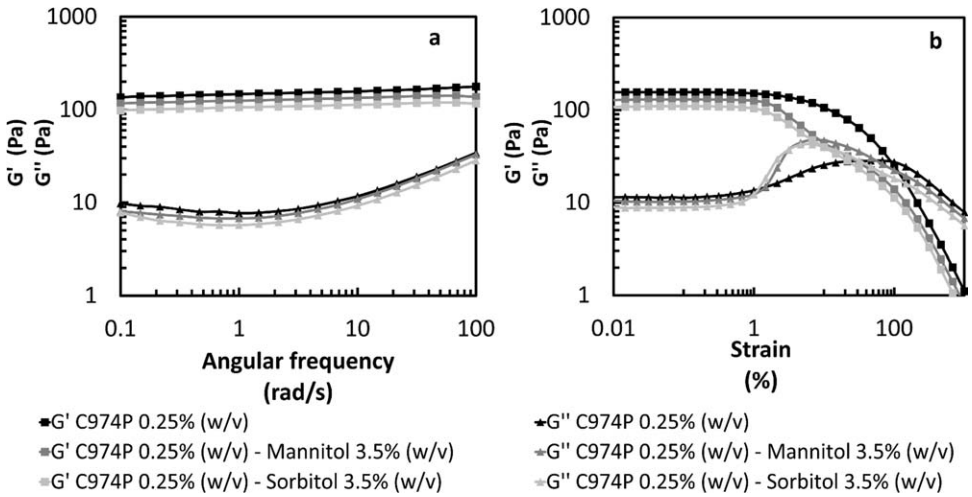

Figure 2. Rheometric measurements of 0.25% (w/v) Carbopol 974P NF (C974P) formulations at pH 7.4 alone or in the presence of sorbitol or mannitol at 3.5% (w/v). (a) Frequency sweep tests at 0.2% strain at 20°C. (b) Strain sweep tests at 10 rad s^{-1} at 20°C.

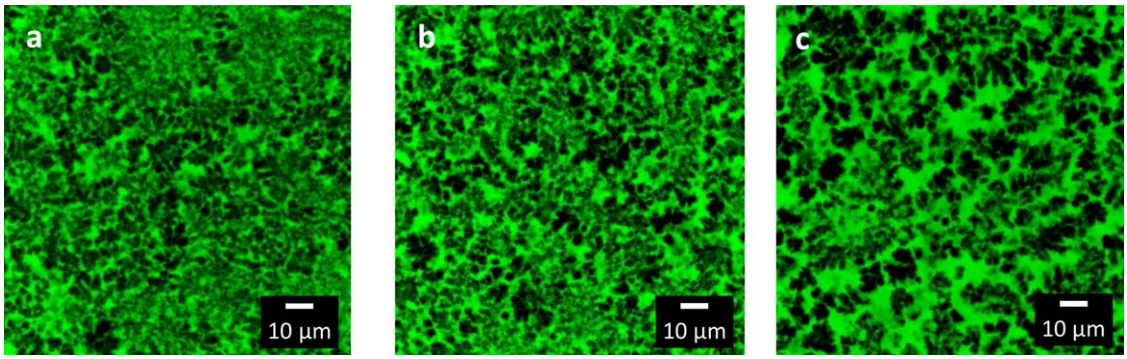

Figure 3. Confocal microscopy pictures of Carbopol 974P NF 0.25% (w/v) gel tagged with Alexa Fluor 488 at pH 7.4 (a) and in the presence of 3.5% (w/v) of sorbitol (b) or mannitol (c). The scale bar represents 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

[Figure 2(a)]. This is typical of a soft glassy material characterized by structural disorder and metastability. In such materials, the thermal motion is not enough to achieve a full structural relaxation owing to an energy barrier too high compared with typical thermal energies.[20] The fact that similar values of elastic modulus were found for Carbopol formulations with and without polyol suggests that the polyol does not modify the gel structure to a large extent. However, a significant difference of behavior in the presence of polyol was found in large-amplitude oscillatory shear (LAOS) measurement [Figure 2(b)]. Indeed, for Carbopol 974P NF at 0.25%, the transition from linear to nonlinear domain is characterized by a broad overshoot of G'' for a shear strain of about 2%–3%, whereas G' progressively decreases as reported also by Gutowski *et al.*[11] for Carbopol Ultrez 10 at pH 7. When sorbitol or mannitol was added to the formulation, the transition between the two regimes is sharper as seen by the variation of G' and G'' [Figure 2(b)]. It is interesting to note that two distinct steps can be detected in the presence of polyol: the first one is characterized by a sharp decrease of G' and an increase of G'', which goes through a maximum; the second one at higher shear strain (~200%) features a more pronounced decrease of G' while G'' is also decreasing. It is well known that LAOS behavior is sensitive to the interactions and to the shear-induced formation of microstructures.[21] Among the different behaviors reported in literature, the weak strain overshoot (G' decreasing and G'' increasing followed by decreasing) has been observed in different systems (xanthan gum solution, silica suspension, dough, etc.), but its molecular origin and physical mechanism is not yet clearly understood. As explained by Hyun *et al.*,[21] when weak structures like Carbopol systems are subjected to an external strain, the structure resists against deformation up to a certain strain (G'' increases) before being destroyed by large deformation over the critical strain (G'' decreases). Here, it is hypothesized that even if the degree of percolation of the Carbopol at 0.25% was not modified in the presence of sorbitol or mannitol as shown by similar values of G' versus frequency, the polyol modifies the interaction between microgel particles, possibly through hydrogen bonding and, consequently, affects the resistance of the material against deformation.[21]

Fluorescence Confocal Microscopy

The carbopol was labeled with a very low amount of Alexa Fluor 488 dye, namely 0.1% relative to the number of carboxyl groups, so that it should not affect the gel structure and properties. Images in Figure 3 reveal the carbopol structure at pH 7.4 and $c = 0.25$ wt % with and without addition of polyols. Conversely to the confocal images of the gel at pH 3 reported by Gutowski *et al.*,[11] where individual Carbopol particles can be clearly seen, here, the higher degree of swelling of the Carbopol particles at pH 7.4 favors their jamming into an almost continuous network structure. However, one can

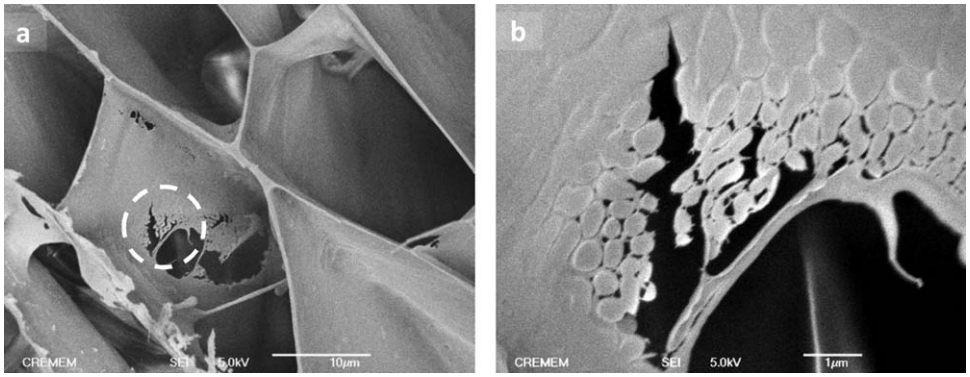

Figure 4. Cryo-SEM pictures of Carbopol 974P NF at 0.25% (w/v) with sorbitol 3.5% (w/v) at pH 4.0. The sample was frozen by direct immersion of a drop of hydrogel in liquid nitrogen at −180°C. The honeycomb structure results from the formation of ice crystals. Magnification a. ×2,500, b. ×15,000.

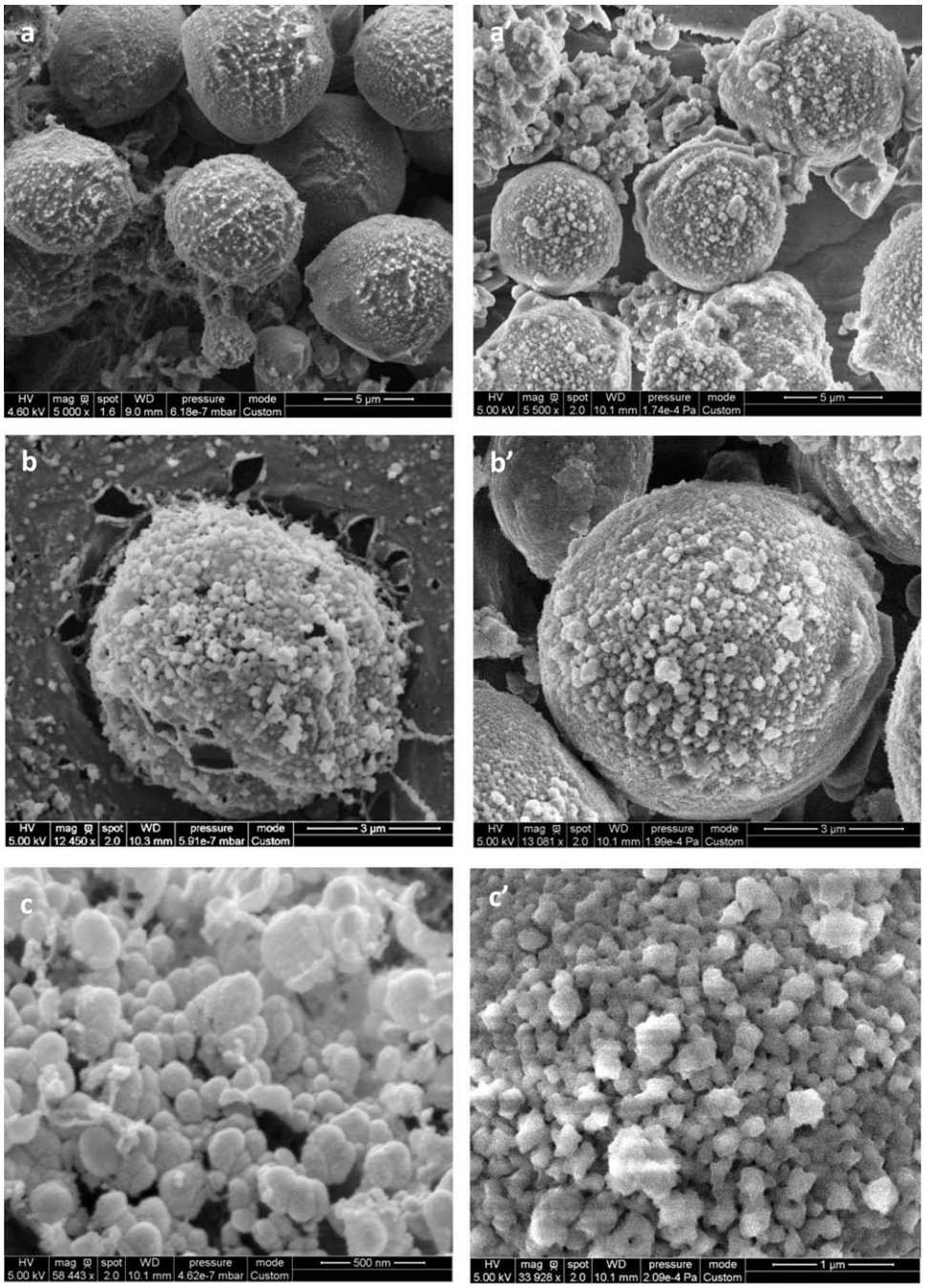

Figure 5. Cryo-SEM pictures of Carbopol 974P NF at 0.25% (w/v) adjusted to pH 7.4 without sorbitol (a–c) and with 3.5 wt % added sorbitol (a′–c′). A high-pressure freezing technique was used for the sample preparation to avoid the formation of ice crystals. Magnification: (a) ×5000, (a′) ×5500, (b) ×12,450, (b′) ×13,081, (c) ×58,443, and (c′) ×33,928.

notice the presence of small pools of liquid, highlighting the fact that the Carbopol concentration is not high enough to obtain a complete space-filling structure. In the presence of polyol, and especially mannitol, the network structure looks more heterogeneous with also larger pools of liquid [Figure 3(c)]. Again, this may be interpreted as the consequence of hydrogen bonding between the polyol molecules and the Carbopol microgel particles, even though no direct experimental evidence of this interaction could have been obtained in this work.

Cryo-SEM Imaging

It is known that cryo-SEM analysis can be perturbed by the presence of artifacts related to the cryogenic process used. Especially, typical honeycomb structures are obtained with polymer and colloid materials when the samples are frozen with liquid nitrogen at ambient pressure.[22,23] Such structures result from the formation of ice crystals, which expell the solute to the boundaries between adjacent ice crystals. Subsequent freeze drying gives rise to a cryogel that are characterized by walls of

matter enclosing empty areas where ice crystals originally resided.[22] Although the formation of cryogel from Carbopol material was not the purpose of this study, it has been found incidently that microgel particles can be clearly evidenced in the walls of the cryogel when the sample was frozen in liquid nitrogen (Figure 4). The size in the 500 nm range is in good agreeement with previous results of capillary viscosimetry.

To avoid the formation of ice crystals, an extremely rapid freezing of aqueous samples is needed. For instance, the use of a nitrogen slush, which is able to absorb heat without increasing its own temperature, can minimize ice crystal formation.[24] An alternative consists in using a high-pressure freezing technique, which drastically reduces the nucleation and thus the crystal growth rate.[25] By applying this protocol, the honeycomb structure was not observed by SEM anymore after freeze drying. Instead, large spherical structures of a few micrometers in size which are themselves assembled from microgel particles of 100–300 nm in size can be clearly seen in Figure 5. Such hierarchical gel structure is in excellent agreement with previous results obtained by Lee *et al.*,[10] who pointed out the existence of two characteritics length scales, $\xi_1 > 6$ μm and $\xi_2 \sim 400$ nm, in Carbopol hydrogels through static light scattering and small-angle neutron scattering. The information about the microstructure gained by cryo-SEM are complementary to these obtained with confocal microscopy at larger scale. Therefore, one can state that the Carbopol gel at pH 7.4 and $c = 0.25$ wt % is an almost space-filling structure, as revealed by confocal microscopy, composed of compressed or "jammed" spherical structures made themselves of agglomerated microgels of a few hundreds of nanometers as seen by cryo-SEM. The same kind of microstructure and characteristic length scales were found in the presence of 3.5 wt % sorbitol, the only difference being that microgel particles have a more angular shape. By examining closely the Figure 5(c'), it seems that microgel particles have fused together to some extent in the presence of sorbitol, which confirms the nonspectator role of the polyol in the Carbopol formulation.

CONCLUSIONS

In this work, we have studied the solution behavior of Carbopol 974P NF with and without polyols, commonly used in pharmaceutical formulations. For the first time, well-resolved and artifacts-free cryo-SEM pictures of the Carbopol hydrogel structure have been obtained through the help of high-pressure freezing process preventing ice crystallization. It has been shown that the hydrogel is made of spherical structures of few microns, which are closed packed in the hydrogel. Each of these aggregates is composed of several microgel particles of few hundred nanometers that have been characterized by capillary viscometry in the dilute state. These observations are in excellent agreement with previous results obtained by scattering and rheology techniques that have pointed out the formation of a hierarchical gel structure.[10,11] Especially, it has been shown that both polyols slightly modified the hydrogel structure and properties of Carbopol 974P NF as seen by fluorescence confocal microscopy where a more heterogeneous network was obtained, especially with mannitol. Although the rheological behavior of the Carbopol in the linear domain was almost unchanged in the presence of polyol, a significant change was put in evidence using LAOS, where a sharper transition from the linear to nonlinear domain was observed when polyol was added to the Carbopol formulation. This constitutes a nonclassical modification of a rheological signature of a hydrogel resulting from the jamming of microgel particles.[26] The molecular origin of such subtle modification remains to be determined. It is hypothesized that the polyol may modify intramolecular interactions within microgel particles through hydrogen bonding and could, therefore, influence the behavior of the micron-size spherical structures under shear flow. Scattering technique under shear flow as well as rheo-optical techniques could bring useful information about the modification of the hydrogel microstructure in the presence of polyols.

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