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Morphology, Absorptivity and Viscoelastic Properties of Mineralized PVP-CMC Hydrogel

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Abstract. A simple liquid diffusion mineralization technique was applied for the incorporation of calcium carbonate (CaCO_3) in PVP-CMC hydrogel. The hydrogel was prepared 6.5 mm thick to achieve around 1 mm thick sample after mineralization of hydrogel matrix with calcite. The calcite crystals were round shaped and organized as building blocks inside the porous three dimensional cross linked structure of the PVP-CMC hydrogel. The present study was designed to evaluate the properties of mineralized (calcite) hydrogel with respect to freshly prepared hydrogel and those swelled in water (H_2O) after drying. The viscoelastic properties of swelled and mineralized samples were reported though the dry PVP-CMC hydrogel were swelled and mineralized with calcite until 150 min. It is observed that there is not much difference in elastic property of fresh and 60 min mineralized hydrogels but the values of elastic property are decreased in the case of swelled hydrogels. It is interesting that in case of swelled samples the values of complex viscosity (η^*) are increased with the increase of swelling time after 90 min but in case of calcite hydrogel the values (η^*) are gradually decreased with the increase of time.

Keywords: Mineralized hydrogel, Viscoelastic properties, Absorptivity, Calcite, PVP, CMC.

PACS: 83.60.Bc, 83.60.Df, 81.07.Pr, 81.05.Rm, 91.65.An

INTRODUCTION

Polymeric hydrogels are attracted by many researchers because of several significant properties like three dimensional cross-linked structures, high water holding capacity, flexibility, biodegradability, good biocompatibility and can be formulated into various forms. Several natural and synthetic hydrogels are reported already which comprises similar properties and applicable in medical, tissue engineering and drug delivery field. There are several drawbacks associated with these hydrogels like weak mechanical strength, immunogenic reactions, animal virus contamination in case of natural based hydrogels whereas synthetic hydrogels are bio-inert in nature [1]. To address this issue, nowadays, attention is given on biomimetic structure development within the hydrogel to improve the mechanical properties of it. Further, that mimics natural extracellular micro-environment to generate novel hybrid materials by incorporating both organic biopolymers and inorganic phase [2,3]. Hydrogel as an implant, itself lack the ability to mineralize into bone tissues, also preventing chemical bond formation with hard tissues like bones. But, the inorganic

substances of the hydrogel matrices play an important role in mineralization process into bone tissues [4]. Thus, the studies on bio-inspired mineralization approaches come up together with bio-polymers to create organic-inorganic hybrid structure [2]. The involvement of inorganic phase into the hydrogel matrix acts as nucleation site, as a result formation of crystal as well as mineralization occurs, and gradually improving the mechanical property of material (i.e. hydrogel matrix). Biomimetic mineralization strategies include soaking of hydrogel in inorganic solutions, incorporation of enzymes or synthetic analogues to matrix that are its initial sites [4]. Calcium carbonate (CaCO_3 /calcite) is one of the most abundant biomineral with decreasing solubility and increasing stability. It has wide application in environmental, pharmaceutical industries, medical field, as fillers in paints, plastics, rubber and many others [5]. Moreover, it is bioactive and biogenic in nature. Motivated by these fascinating features of bioinspired mineralization, a calcite based mineralized PVP-CMC hydrogel has been prepared following a simple liquid diffusion method [2] that can be utilized in biomedical applications like bone regeneration, dental plaque and tissue replacement etc. The research team of Tomas Bata University in Zlin has already been developed and patented the method of preparation of PVP-CMC hydrogel which has been recommended as wound dressing material especially for burn/cut wounds [1]. As per our knowledge, till now there is no report about mineralization or formation of calcite in PVP-CMC hydrogel. In this study, the characteristics of such bio-mineralized PVP-CMC hydrogel have been assessed in the form of morphology, interaction between hydrogel matrix and calcite, nature of absorption of CaCO_3 and its rheological properties. This paper is reporting mainly about calcite based mineralized hydrogel in comparison with non-mineralized PVP-CMC hydrogel.

Experimental

The present work extends the use of model for the preparation of PVP-CMC hydrogel [6]. For the preparation of mineralized hydrogel, at first PVP-CMC hydrogel were prepared using PVP (0.2 % w/v), CMC (0.8 % w/v), PEG (1% w/v), agar (2 % w/v), and glycerin (1% v/v) to achieve the final thickness approximately 1 mm in mineralized state of PVP-CMC hydrogel (as to investigate viscoelastic properties, the sample thickness must be between 1-3 mm). The obtained fresh hydrogel is soft, smooth and off-white round (thickness: 6.5 mm, diameter: 80 mm) which were kept for drying until 48 hrs at room temperature (22-25°C). The dry hydrogel became off-white to ivory, transparent, reasonably flexible and size (thickness: 0.8 mm, diameter: 75 mm). To achieve the mineralized hydrogel, experiment was carried out following liquid-diffusion method [2]. For the formation of calcite crystals into the PVP-CMC hydrogel matrix, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (14.7%, 1M, pH = 8.4) solution and Na_2CO_3 (10.5%, 1M, pH = 7.4) solution were used as the source of Ca^{+2} and CO_3^{-2} respectively. The dry hydrogel films were initially immersed in the 50 ml solution of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ for 15 min and then transferred into 50 ml Na_2CO_3 solution for another 15 min. The first round mineralization of PVP-CMC hydrogel was carried out for 30 (15+15) min and then continued upto 150 min. Thereafter, the mineralized membranes were washed with distilled water and used for further investigations. Finally, the obtained hydrogel

is termed as mineralized/calcite PVP-CMC hydrogel. The scheme for the mineralization of PVP-CMC hydrogel and the formation of calcite crystals inside the pores of hydrogel matrix is depicted in Figure 1.

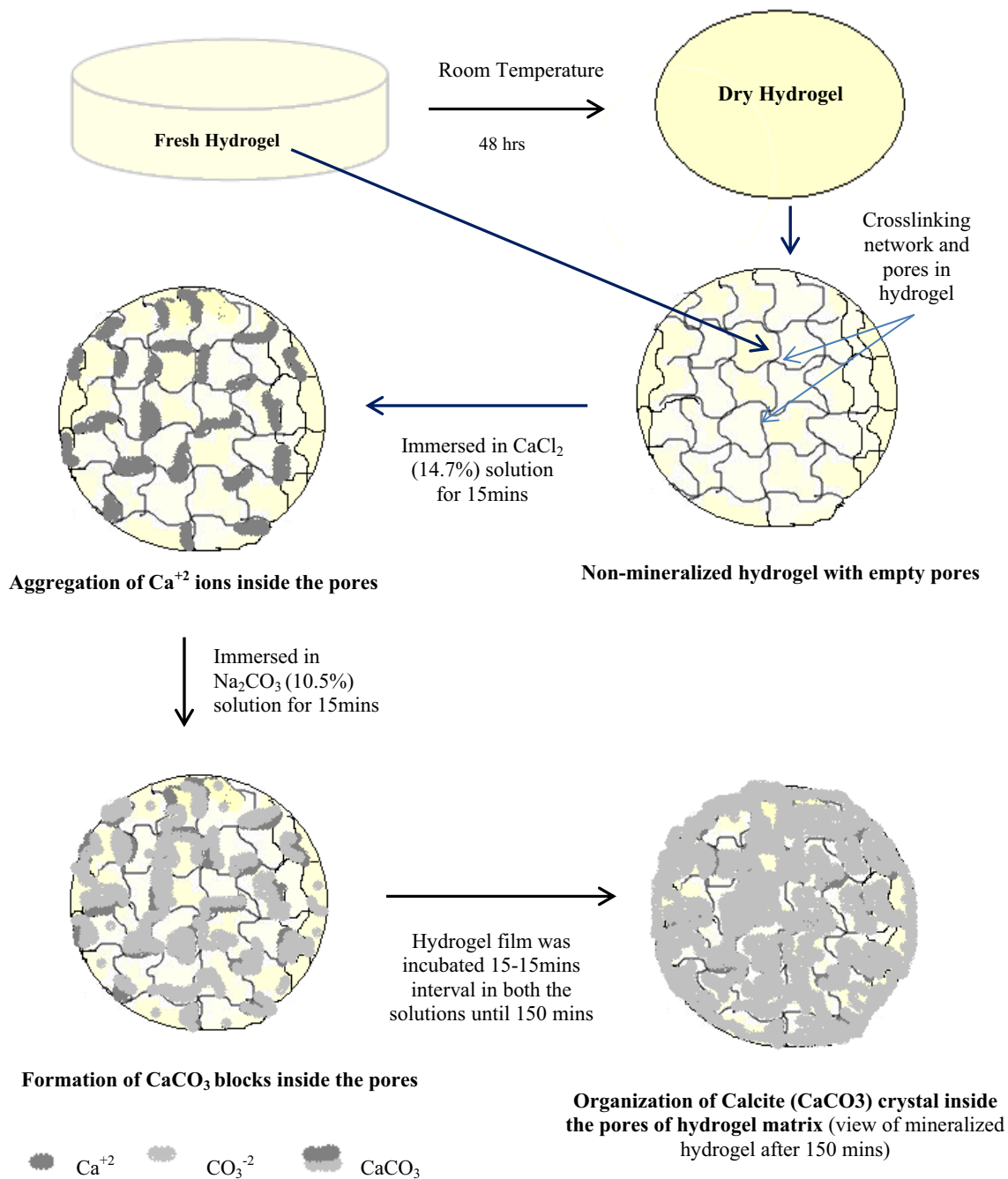


FIGURE 1. Scheme for the mineralization of PVP-CMC hydrogel/Preparation of mineralized (calcite) PVP-CMC hydrogel.

The non-mineralized and mineralized (calcite) PVP-CMC hydrogels are characterized based on its morphology, water/calcite absorptivity, confirmation of calcite/mineralization, and viscoelastic behavior. The assay methods are described below.

The morphology of the non-mineralized and mineralized (calcite) PVP-CMC hydrogels was determined by scanning electron microscopy (SEM) [6,7]. The SEM analysis was carried out on VEGA II LMU (TESCAN) operating in the high-vacuum/secondary electron imaging mode at an accelerating voltage of 5-20 kV. The samples were sputter coated with a thin layer of palladium/gold alloy to improve the surface conductivity and tilted 30° for better observation. The images were taken at magnification of 100x-10kx.

The absorptivity of water and formation of minerals (i.e. CaCO₃) in PVP-CMC hydrogel matrix were studied using dry hydrogel. The samples were immersed in distilled water and in mineral solutions (as shown in Figure 1) separately. The absorptivity was investigated at room temperature and after specified time intervals, the weight of the mineralized and non-mineralized hydrogels were recorded. The absorptivity of minerals (CaCO₃) as well as water (H₂O) by the hydrogel was determined using the equation mentioned below [6]; where, W_s is for swollen gel or CaCO₃ formation within the hydrogel and W_d is the weight of dried PVP-CMC hydrogel.

$$Absorptivity\% = \left[\frac{(W_s - W_d)}{W_d} \right] \cdot 100 \quad (1)$$

The formation of calcite crystals in PVP-CMC hydrogel was confirmed by using fourier transform infrared spectroscopy (FTIR) [6]. The ATR-FTIR spectroscopic analysis was conducted by using NICOLET 320 FTIR Spectrophotometer with “Omnic” software package over 4000-600 cm⁻¹ at room temperature. A uniform resolution of 2 cm⁻¹ was maintained throughout in all cases.

The viscoelastic properties of non-mineralized (fresh and swelled) and mineralized (calcite) hydrogel were investigated by using a parallel plate rheometer (ARES; Rheometrics Scientifics, USA) testing with a “TA Orchestrator” software package. A 25 mm diameter parallel plate measuring geometry with a gap of about 2-3 mm was used, employed at strain amplitude (1%) to maintain the measurement range within the linear viscoelastic region. Dynamic frequency sweep test were carried out at 28°C temperature to observe the storage (G') and loss (G'') moduli, and complex viscosity (η^*) as a function of a wide range of angular frequency (ω : 0.1-100 rad.s⁻¹) [6,8].

Results and Discussion

Morphology of Non-mineralized and Mineralized (Calcite) PVP-CMC Hydrogels

The surface views of SEM micrograph of hydrogels are depicted in Figure 2 where it is clearly visible that the pores of PVP-CMC hydrogel (non-mineralized hydrogel) are filled with CaCO_3 . It can be seen from Figure 2 (a) about three dimensional cross-linking net work of PVP-CMC hydrogel and development of pores which have already been reported [9]. The pore sizes and shapes are not uniform. The size of the pores varies from 30 to 60 μm . During mineralization of PVP-CMC hydrogel, the pores are filled with CaCO_3 (see Figure 1). The surface image of SEM micrograph Figure 2 (b) confirmed the mineralization or formation of calcite within the matrix. The observed coarse surface and granular structure is due to the deposition or aggregation of crystal structure of CaCO_3 .

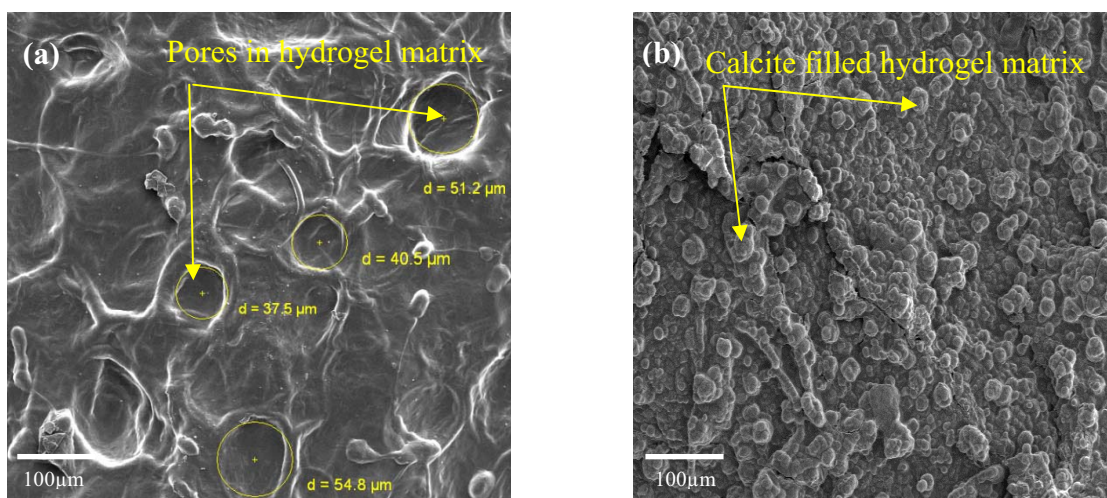


FIGURE 2: SEM Micrographs: (a) Non-mineralized PVP-CMC hydrogel, (b) Mineralized PVP-CMC hydrogel (surface view, 150 min mineralised).

Absorption of Water and Calcite in PVP- CMC Hydrogel

The absorptivity of the PVP-CMC hydrogel was studied using dry hydrogel in presence of distilled water and mineral solutions up to 150 minutes. The absorption capacity and nature of absorption (water and minerals) are shown in Figure 3. It can be seen from the figure that dry hydrogel absorbed much water at faster rate than calcite formation/deposition when immersed in water and mineral solutions respectively. High water retention capacity is one of the main features of the hydrogel. In the fresh hydrogel with increase in time the polymeric interactions increases and cause a very high sorption rate thus the uptake capacity of hydrogel increases [6]. To achieve mineralized/calcite hydrogel matrix, the dry hydrogel film is soaked in mineral containing solutions where, the Ca^{+2} ions on the top layer acts as nucleation sites and

slowly are subjected to diffusion inside the membrane. This leads to impregnation of calcite crystals in the cross linking network and pores inside the hydrogel structure [2]. It can be seen from Figure 3 that the absorptivity of CaCO_3 by PVP-CMC hydrogel film is slowly increasing with time, but the variation in values is not much high.

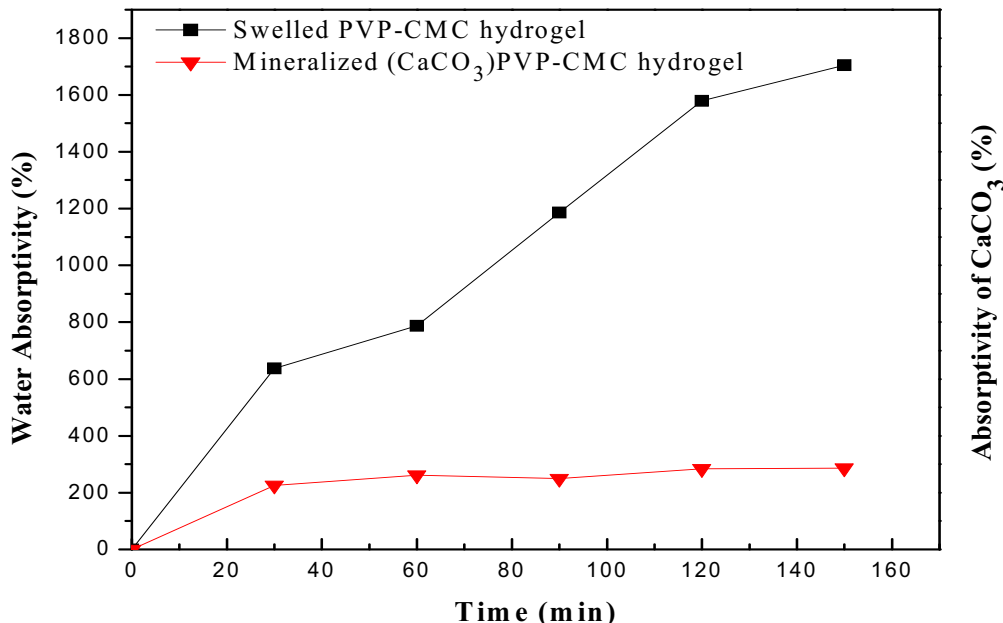


FIGURE 3. Absorptivity behavior of PVP-CMC hydrogel in presence of water and mineral solutions (see Figure 1).

Interaction between PVP-CMC Hydrogel and CaCO_3 to Form Calcite Based Mineralized Hydrogel

The FTIR spectra of pure CaCO_3 , calcite based PVP-CMC hydrogel and non-mineralized PVP-CMC hydrogels are shown in Figure 4. It can be seen from the figure that in the case of non-mineralized and mineralized PVP-CMC hydrogels (curve c and b in Figure 4) the broad absorption band between ($3000\text{-}3500\text{ cm}^{-1}$) is detected which represent the hydrogen bonding in the polymers. Further, when considering the mineralized PVP-CMC hydrogel and pure CaCO_3 (curve b and a in Figure 4) strong absorption bands related to CO_3^{2-} appear between $1320\text{-}1530\text{ cm}^{-1}$ and sharp peaks are noticed at 871 cm^{-1} and $672\text{-}745\text{ cm}^{-1}$ region [10]. The presence of such common features validated that the PVP-CMC hydrogel has been mineralized with CaCO_3 .

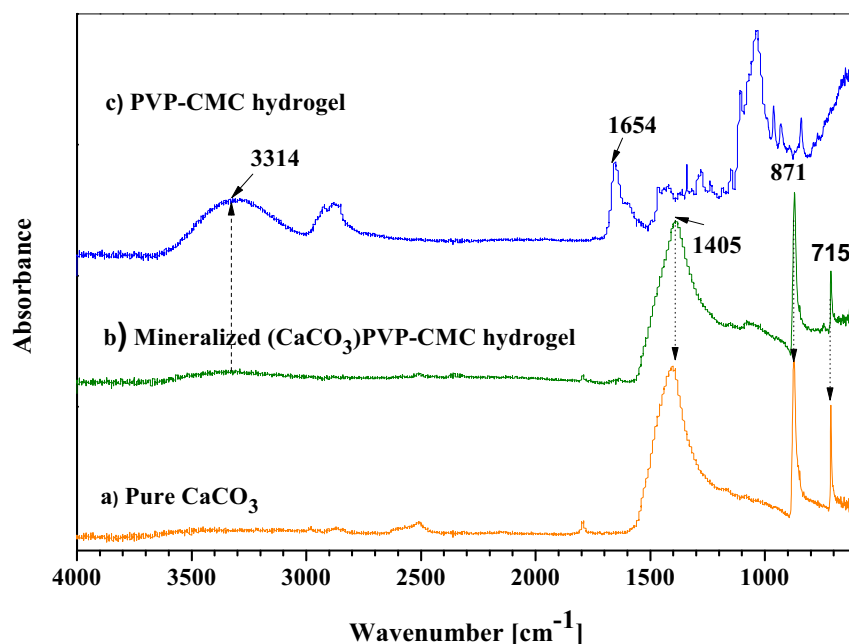


FIGURE 4. FTIR Spectra of a) Pure CaCO₃, b) Mineralized (CaCO₃) PVP-CMC hydrogel and c) PVP-CMC hydrogel.

Viscoelastic Properties of Fresh, Swelled and Calcite PVP-CMC Hydrogel

The viscoelastic properties of PVP-CMC hydrogel of fresh (before dry sample), water swollen (dry hydrogel swelled for 60 min) and mineralized (dry hydrogel mineralized for 60 min as shown in Figure 1) hydrogel samples were measured. Figure 5 reveals the effect of angular frequency (ω) on rheological properties of hydrogels: swelled and mineralized hydrogels in comparison to fresh form of PVP-CMC hydrogel on storage modulus (G') and loss modulus (G''). It can be seen from the Figure 5 that in each case G' are much more higher than G'' in a wide range of angular frequency (ω : 0.1-100 rad.s⁻¹). This indicates that in every cases (fresh, swollen and mineralized) the elastic behavior of hydrogel shows its influences compared to the viscous nature, moreover all the hydrogels exhibited the linear curve which is also confirmed about typical characteristics for cross linked gels or solid materials [6,8]. The observed results (Figure 5) also proved that there is not much difference in elastic property of fresh and 60 min mineralized hydrogels but the values of elastic property is decreased in the case of swelled hydrogel due to uptake of water (as shown in Figure 3) within the hydrogel matrix. Further, it can be seen from Figure 6 that even though the swelled hydrogel decreases its elastic property due to incorporation of water into the pores of three dimensional cross linked PVP-CMC hydrogel (Figure 2a) but the mineralized/calcite hydrogel (Figure 2b) keeps higher G' and G'' and thus typically shows higher complex viscosity (η^*) compared to that of water swollen hydrogel, and these values (η^*) are more or less similar to the fresh hydrogel.

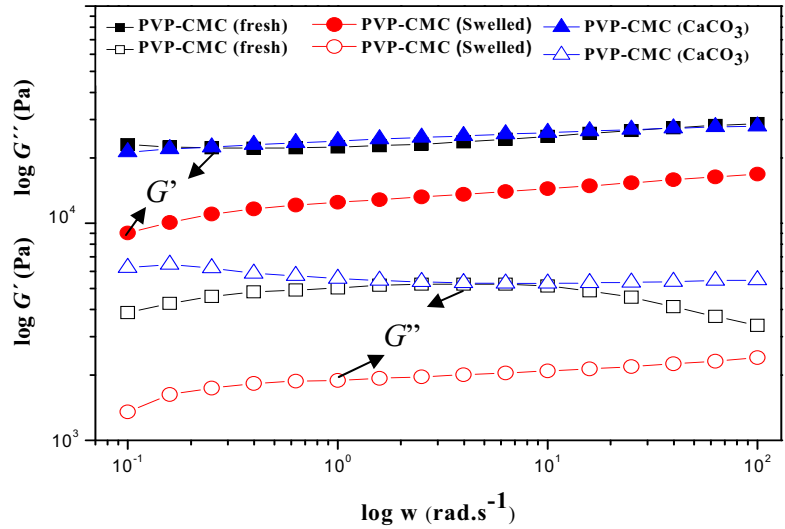


FIGURE 5. Effect of angular frequency (ω) at 1% strain on storage modulus (G' , filled symbol) and loss modulus (G'' , non filled symbol) for fresh, swelled (60 min) and mineralized (CaCO_3) PVP-CMC hydrogel.

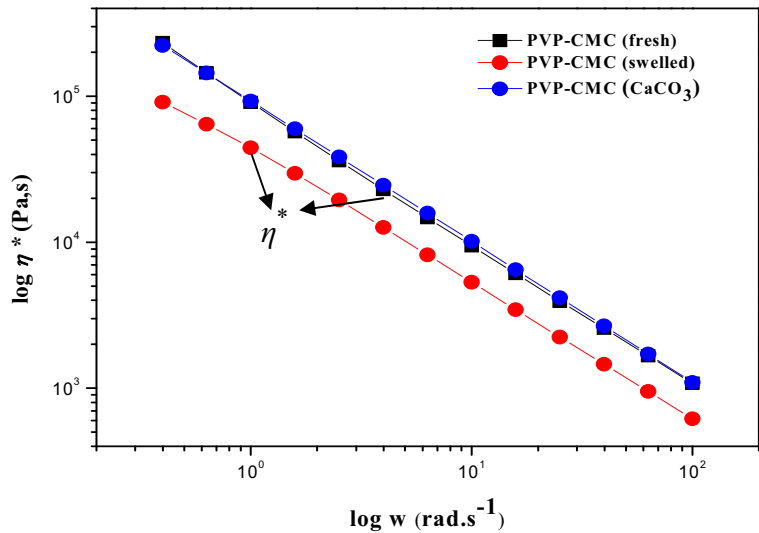


FIGURE 6. Effect of angular frequency (ω) at 1% strain on complex viscosity (η^*) for fresh, swelled (60 min) and mineralized (CaCO_3) PVP-CMC hydrogel.

Although, in this article it is reported about the viscoelastic properties of only 60 min swelled and calcite hydrogels, it is interesting to mention that the values (G' , G'' and η^*) are increased with the swelling time (after 90 min) in the case of swelled hydrogel, and decreased with the increase of mineralization time for the case of calcite hydrogel.

CONCLUSION

A simple liquid diffusion method was implemented to mineralize the PVP-CMC hydrogel matrix with CaCO₃ for biomedical applications (especially for bone tissue regeneration). The study of absorptivity proves that in time the formations of calcite crystals are increased within the PVP-CMC hydrogel and the formation of crystals inside the hydrogel could be explained by mesocrystal theory [2]. The FTIR and SEM images confirmed the presence of calcite mineral in PVP-CMC hydrogel. Further, from application point of view, the study of viscoelastic properties of biomineralized hydrogels is important because through this study it is possible to understand the elastic nature of mineralized/calcite hydrogel.

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