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Hydrogels based carboxymethylpullulan: preparation and investigation on the rheological and swelling behavior

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ABSTRACT

Novel hydrogels based carboxymethylpullulan CMP were elaborated using Sodium trimethaphospahe STMP as the crosslinker agent. The phoshpating agent provides negative charges on the crosslinking nodes and leads to sensitive gels, their rheological and swelling properties were evaluated.Analysis of the swelling kinetics data has revealed that the swelling of hydrogels followed the second-order kinetic model. The results indicated that the three hydrogels had a great swelling capacity which was greatly affected by various physical and chemical parameters like ionic strength, cation valence and pH. The rheological characteristics in terms of storage modulus *G′*, loss modulus *G″*, the loss factor *tan (δ)* and the gel point were assessed. For all frequencies investigated, *G′* remained considerably higher than *G″* which is in favor of elastic behavior and solid material. *G′* increased with increasing the cross-linker concentration while gelation time of the systems decreased. These highly desirable results both in terms of swelling properties and rheological characteristics of the CMP hydrogels hold great promise for medical and industrial applications.

Keywords: *carboxymethylpullulan, hydrogel, swelling behavior, rheology.*

1. INTRODUCTION

Hydrogels are three-dimensional (3D) networks consisting of hydrophilic polymer chains which are crosslinked to form matrices capable to absorb high amounts of water or biological fluids [1]. Hydrogels have received considerable attention in the past 50 years, due to their exceptional promise in medicinal, pharmaceutical, biotechnological, cosmetics [2] and agricultural field. The main applications of hydrogels in biomedical areas are drug delivery systems [3-8], hygienic products [9], tissue engineering and regenerative medicines [10-13], wound dressings [14-16], contact lenses [17-19]. In agriculture, hydrogels are used as soil conditioners [20, 21] and systems for nutrient release [22-24]. The environmental applications of hydrogels were explored in water treatment for removal and separation of hazardous metal ions and organic contaminants [25-28].

The ability of hydrogels to absorb water arises from hydrophilic functional groups attached to the polymeric backbone, while their resistance to dissolution arises from cross-links between network chains. Many materials, both naturally occurring and synthetic, fit the definition of hydrogels. They have been successfully used as superabsorbent materials and in drug delivery, cell encapsulation and tissue repair due to their high water content and consequent biocompatibility. Considering the fact that water retention in the hydrogels provides a suitable drug diffusion pathway; many hydrogel-based networks have been designed and fabricated as intelligent carriers of drugs. The rate and degree of hydrogel swelling are the most important parameters which control diffusion and the release patterns of solvents and molecules of these polymeric networks. Therefore, the monitoring of hydrogel behaviour as well as mathematical description of equilibrium swelling, dimensional changes due to solvent uptake, desorption and drug release profiles were the main objectives in many investigations andare currently subject of considerable scientific research.

Hydrogels can be stimuli sensitive and respond to the changes of surrounding environment [29]. They may perform considerable volume transitions in response to a variety of physical stimuli like temperature, electric or magnetic field, light, mechanical stress , pressure, and sound, while the chemical stimuli include pH, solvent composition, ionic strength, and chemical agents [30-32].

Depending on their method of preparation, ionic charge, physical properties, origin (natural synthetic or hybrid), type of crosslinking, biodegradability (biodegradable and non-biodegradable hydrogels), hydrogels can be classified in several categories [33-34]. We distinguish homopolymeric or copolymeric hydrogels and multipolymer Interpenetrating polymeric hydrogel. On the basis of presence or absence of electrical charge, hydrogels can be cationic, anionic, neutral or ampholytic. Again, they can be physically or chemically crosslinked. Physical hydrogels are reversible where chemical hydrogels are permanent and irreversible. On the basis of the physical structure of the networks, hydrogels can be amorphous, crystalline and semicrystalline.

The use of natural polymers such as polysaccharides to design innovative hydrogels has been the subject of several studies [35-37] and has gained much attention over the years because of their nontoxicity, biocompatiblility, biodegradablilty and their cost effectiveness. The exploitation of physicochemical characteristics offered by polysaccharide-based hydrogels make them more attractive materials in different fields [38-40, 22]. Pullulan, a non-ionic polysaccharide with fascinating properties, is a promising material for various applications, including coating, packaging, cosmetic emulsions, biotechnological and biomedical uses [41-43].

The aim of this work was to study the elaboration of novel hydrogels based on Carboxymethylpullulan. Sodium trimetaphosphate (STMP) has been chosen as a crosslinker, STMP is a non-toxic cyclic triphosphate [44], already reported as an effective crosslinker for starches [45-46], guar gum [47], hyaluronic acid [48], pullulan [49] or pullulanderivates [50] in order to synthesize gels for pharmaceutical purposes. These new hydrogels were characterized by infrared spectroscopy and their rheological and swelling properties were determined.

2. EXPERIMENTAL

2.1. Materials. Pullulan PI-20 (Mw = 216500 g mol^{-1}, Mn = 101700 g mol^{-1} determined by SEC/MALLS) was purchased from Hayashibara Biochemical Laboratory (Okayama, Japan), sodium chloroacetatefrom Fluka , sodium chloride from Merck, sodium hydroxide, trisodiumtrimetaphosphate (STMP) and isopropanol from Sigma-Aldrich (France). Water was purified with the milli-Q water reagent system (Millipore). All compounds were used without further purification.

2.2. Methods

2.2.1. Synthesis of carboxymethylpullulan CMP. The carboxymethylpullulan (CMP) was synthesized in isopropanol/water medium (2/1 V/V) by reacting the hydroxyl groups of pullulan with sodium chloroacetate in the presence of sodium hydroxide, according to the procedure described in previous works [50]. The substitution degree (DS) of carboxylate groups per anhydroglucose unit (AGU) was determined by conductimetric titration [51].

2.2.2. Hydrogel synthesis. The synthesis of hydrogels based on CMP and crosslinked with STMP was realized using a previously procedure reported [49]**.** The CMP was dissolved in alkaline water (at fixed pH) and stirred under gentle agitation for two hours. Then, the phosphating agent STMP was added and the reaction mixture was stirred one minute [47].Different concentrations of STMP were tested (0.13, 0.20 and 0.26 mol/L) with r_2 = [STMP] / [AGU] and the concentration ratios r_1= [NaOH] / [AGU] was fixed where AGU is the anhydroglucose unit. The reaction was stopped after achieving neutrality by addition of HCl (0.1N), the product is subsequently immersed in an excess of water for at least two days, the water was renewed twice a day to reach conductivity less than 5 µS. The hydrogel particles were washed in acetone to remove water and dried at 45°C under vacuum.

The reaction scheme for crosslinking CMP hydrogels was depicted in Fig.1.

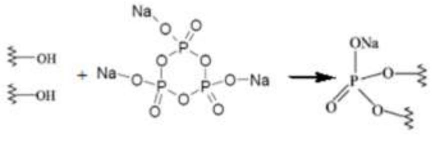

Figure 1. Crosslinking reaction of CMP with STMP [47].

In this work, three hydrogels G1, G2 and G3 were prepared at different concentrations of STMP whereas the other parameters remained constant.The synthesis conditions are reported in Table 1.

Table 1. The synthesis conditions of hydrogels.

CMP						
M_n(kDa)	**68.46**	**sample**	**[CMP] g/L**	**[NaCl] mol/L**	**r_1**	**r_2**
M_w(kDa)	148.2	G1	200	1	0.16	0.21
I_p	2.16	G2	200	1	0.16	0.16
DS	0.64	G3	200	1	0.16	0.10

2.2.3. Molecular weight. The determination of molecular weight of samples was performed by coupling on-line a size exclusion chromatography (SEC), a multi-angle laser light scattering photometer (MALLS) and a differential refractive index detector (RI). The eluent was $LiNO_3$ 0.1 mol/L.

2.3. Physico-chemical characterization

2.3.1. FTIR analysis.FTIR spectra of CMP and crosslinked CMP were recorded between 250 and 4500 cm^{-1} with a AVATAR 360 FT-IR spectrophotometer following the classic procedure.

2.3.2. Scanning electron microscope (SEM) analysis. Micro structure characterization of CMP and crosslinked CMP was conducted byscanning electron microscopes. The samples were freeze dried and kept in vacuum for silver sputtering treatment before scanning.

2.3.3. Swelling Measurements. A known amount of dried hydrogel (0.03g) was immersed in an excess amount of distilled water (250 ml) at 25°C during 24h to obtain equilibrium. The swollen gel was filtered at different intervals and accurately weighed, then the swelling degree (the water absorption amount)Q was calculated according to equation 1:$Q = \frac{m-m_0}{m_0}$ (1)

Where m is the weight of the swollen hydrogel and m_o, the weight of the dry hydrogel, Q was calculated as grams of water per gram of sample. We checked that there was no loss during the swelling and the drying steps by weighting the dried gels at the end of the measurements. The swelling value was averaged using at least three samples.

2.4. Rheological characterization. The rheological properties of the hydrogels were evaluated on a AR2000 rheometer TA Instruments (New Castle, Delaware, USA) using cone/plate (2°, 4 cm radius cone) geometry. Oscillation frequencies varied from 0.1 to 10 Hz at the controlled temperature of 25 °C and suitable stresses were applied to be in the linear viscoelastic region. The dynamic viscoelastic functions, such as the dynamic shear storage modulus (G') and loss modulus (*G"*), were measured.

3. RESULTS AND DISCUSSION

3.1. FTIR analysis. Infrared analysis of the CMP and crosslinked CMP was performed to verify the introduction of new functional groups along the chains. The spectra obtained for CMP and CMP hydrogels are displayed in Fig. 2(a) and (b).

Table 2. The infrared results.

sample	P=O	P-O-C
G1	1240	945
G2	1226	941
G3	1130	920

We noted three new shoulders in spectra of hydrogel G3. An intense band was centered at 1130 cm^{-1} which was related to free P=O stretching and two stretching bands at 1015–930 cm^{-1} attributed to P–O–C [52]. The infrared results for the three samples were summarized in table 2.

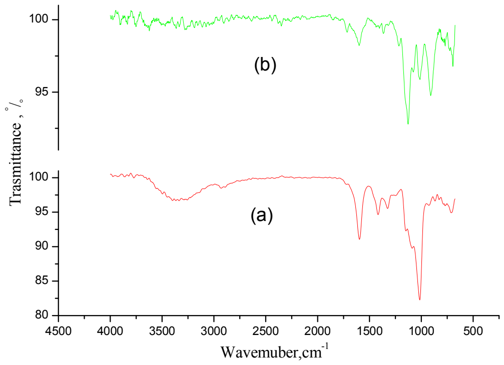

Figure 2. FT-IR spectra of (a) CMP and (b) CMP hydrogel G3.

3.2. SEM analysis. Fig.3shows the SEM image of CMP hydrogel. As it is clear from this figure, the hydrogel possessed a great number of interconnected pores indicating that hydrogel has a porous structure. We assume that these pores will permit the water to penetrate into the hydrogel networks or drug molecules to diffuse out of them or to adsorb environmental contaminants.

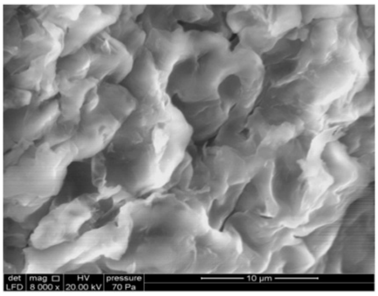

Figure 3. SEM image of surface morphology of CMP hydrogel.

Swelling behavior of CMP hydrogels. The dynamic swelling behavior of the CMP hydrogel over 24 h of immersion in water is represented in Fig.4 (a). The results showed that for the high concentration of STMP (0.26 mol/L), the swelling equilibrium was achieved in 90 min approximately where a total adsorption was noticed 230 g/g. This high water uptake capacity was due to the presence of several charges (COO^- and POO^-) and to the porous structure of the hydrogel which would benefit the diffusion of water molecules into the network.

Swelling kinetics. To investigate the swelling mechanism of the prepared hydrogels, the experimental swellingdata were analyzed by considering the first and the second order kinetics.

For the first-order kinetics model, as described by Pourjavadi and Mahdavinia[53], the rate of swelling at any time is proportional to the water content that the hydrogel must take up before attaining the equilibrium. The first-order kinetics of swelling is expressed as follows: $\mathrm{Ln}\frac{m_e}{m_e-m_t} = kt \quad (2)$

Where m_t and m_e are water contents of the hydrogel at time t and at equilibrium time and k is the rate constant. The model has been tested and the plot of the variation of Ln (m_e/ m_e-m_t) as a fonction of time is given in Fig.4 (b). None of the swelling studies in water yielded a straight line, consequently, the swelling process did not follow first-order kinetics.

Considering the second order kinetics, the swelling rate at any time may be expressed as Eq. 3

$$\frac{t}{m_t} = \frac{1}{km_e{}^2} + \frac{1}{m_e}t \quad (3)$$

According to this equation, the swelling data must fit a straight line with a slope of $1/m_e$and an ordinate of $1/km_e^2$. The results demonstrated that the swelling of synthesized hydrogelsis well fitted by the second-order kinetic model where the coefficient of determination is equal to 0.9986 (Fig.4 (c)). An experimental me value of 230 g/g was obtained for G1, the theoretical value was determined to be 233 g /g. For G2 and G3, the swelling rate was evaluated at 240 and 260 g/g respectively.

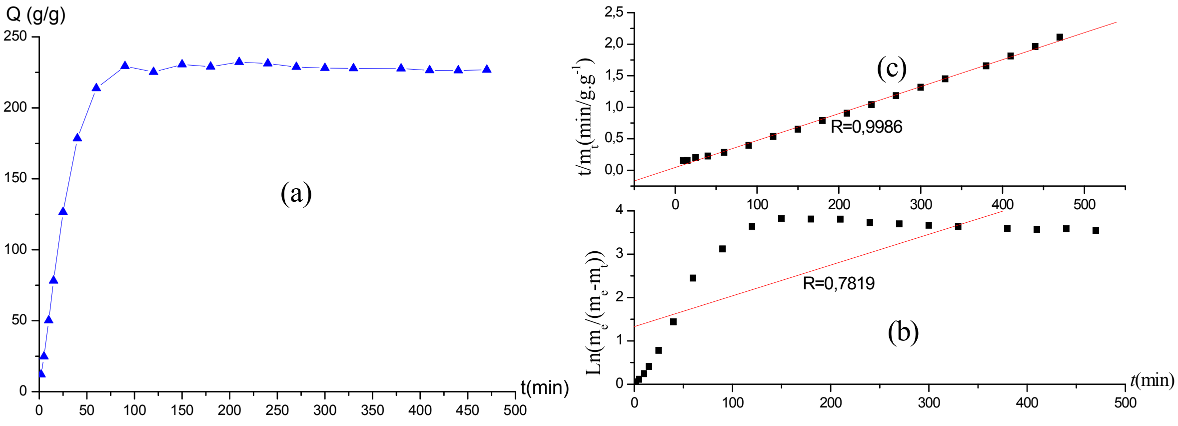

Figure 4. Swelling behavior of the hydrogel G1 versus time (a), Swelling kinetics of the hydrogel G1 according to the pseudo-first order (b), Swelling kinetics of the hydrogel G1 according to the pseudo-second order (c).

Effect of physical and chemical parameters on the swelling. The swelling behavior of hydrogels is an important parameter governing their applications and depends on several factors, including composition and concentration of chemical elements, crosslinking density and other environmental factors such as pH and ionic strength.

Effect of NaCl (Different ionic strength).To study the effect of NaCl on the swelling of prepared hydrogels, five solutions with different concentrations were prepared (10^{-3} - 10^{-1}mo/L), then0.03 g of G1 was immersed in the saline solutionsat pH=6.07. The swelling curve of hydrogel versus NaCl concentration is given in Fig.5 (a). As seen in this figure,swelling capacity at equilibrium and swelling rate were decreased with increasing salt concentration.

This phenomenon can be explained that with the increasing of NaCl concentration, the ionic strength and osmotic pressure of the external solution were reinforced. So the osmotic pressure difference between the internal environment of the polymer andthe external medium loaded with salts was reduced. This renders difficult the penetration of water into the inner of the hydrogel,which led to the decreasing of swelling capacity and swelling rate. This result agrees with the expected behavior.On the other hand, increasing ionic strength minimize the repulsion effect by screening the negative charges -COO$^-$ and hence the swelling will begin to decrease (Na$^+$ ions becomes so high that they condense around the fixed –COO$^-$ charges and causes a decrease in repulsive forces among –COO$^-$ groups).

Influence of metal cations on the swelling. To better understand the effect of various cations on the swelling of hydrogels, the chloride salts of Cu^{2+} and Ag$^+$ 1000 ppm were used as swelling media, the experiments were carried out with 0.03g of gelat pH = 4.3

The results (Fig.5 (b)) appreciably demonstrated that the presence of metal cations causes a drop in the rate of swelling of the gel. This effect is more pronounced in the presence of divalent cations. Indeed, the water absorbency of CMP hydrogel decreased with an increase in cation valence at the same salt solution concentrations. This fact mainly caused by complexing ability arising from the coordination of the bivalent metal with -COO$^-$ groups which could lead to the formation of intramolecular and intermolecular complexes thereby decreasing the space in the network chain andwater penetration [54, 55]. The complexation constant between Ag+ and -COO$^-$ is small and, therefore, the water absorbency of CMP hydrogelin Ag+ solutions is relatively high. Considering there were more Cl$^-$ ions in CuCl$_2$ solutions than that in AgCl, this led to a decrease onosmotic pressure difference, so water absorbency of hydrogels in CuCl$_2$ was lower than that of AgCl.

Effect of pH on the swelling. In order to investigate the influence of pH values on water absorption, swelling behaviors of prepared hydrogels were studied at various pH ranged from 2 to 8.

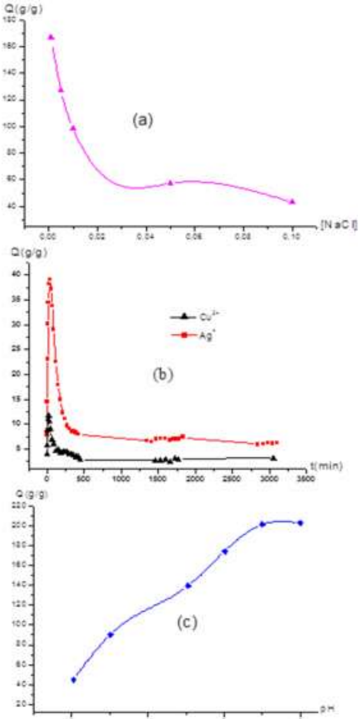

Figure 5. Effect of physical and chemical parameters on the swelling of G1: NaCl concentrations effect (a), Metal valence effect (b), effect of pH (c).

As shown in Fig.5 (c),the rate of swellingof the hydrogel increases with an increase in solution pH, signifying pH sensitivity of the hydrogels. At lower pH values,the low swelling degree was attributed to the higher protonation degree of carboxylate function according to the reaction:

$$\text{-COONa} + H^+ \rightleftharpoons \text{-COOH} + Na^+$$

Thus, a strong hydrogen bonding was easily producedby the -COOH groups in the hydrogel network structure which leads to a small swelling ratio.In addition, the main anion-anion repulsive forces are eliminated which leads to a considerable decrease in the swelling capacity.

In the other case, the increase in pH increases the number of charged sites in the gel causingimportant electrostatic repulsions among similarly –COO$^-$ groups,thus, the macromolecular chains become more flexible, which facilitatesthe expansion of the polymeric network.

Rheological characterization. Rheology is an experimental technique that allows us to probe the physical characteristics ofmaterials such as viscosity or elasticity in dynamic mode. These properties (visco-elastic) can be efficiently assessed using oscillatory tests to provide a more explicit and detailed characterization of materials structure. In this paper, the following rheological characteristics were determined:

- The storage modulus G′(also known as elastic modulus): represents the elastic property of material, it isa measure ofthe stored energy by the systemduringthe shearing process.
- The loss modulus G″(also known as viscous modulus): which is a measure of the irreversible energy loss during the shearing process and characterizes the viscous behavior of material.
- The loss factor tan(δ) = G"/G' is a measurement of the ratio of energy lost against energy stored

If G″ > G′ (tan δ > 1), the sample behaves more like a viscous liquid while, conversely, when G′ > G″, and, thus, tan δ < 1, the sample behaves more like an elastic solid.

- The critical gel point (the point where tan δ=1)

The effect of the STMP concentrations on the elastic and viscous properties of the hydrogels and on the gel point was investigated

Gelation process of CMP hydrogels:crosslinking Kinetics. The storage modulus (G′) and loss modulus G" as a function of time during the crosslinking of CMP hydrogels are shown in Fig. 6.

Initially, it is observed an increase of both moduli, with loss modulus G" greater than storage modulus G ', for example at t = 1359s and frequency=0.1Hz, G"= 2.228 Pa and G'= 0.2777 Pa. In this short period the system behaved as a viscous liquid until thegelation point was attained.

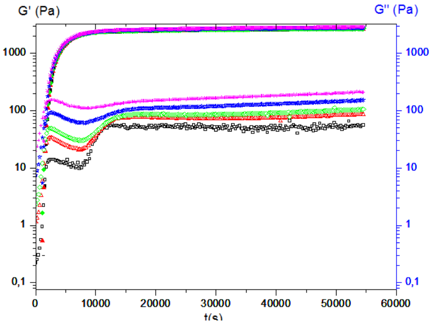

Figure 6. Variations of storage modulus G' and loss modulus G’’ as a function of crosslinking time for G1, square 0.1 Hz, up triangle 0.5Hz, diamond 1Hz, star 3Hz, pentagon 8Hz, solide G', open G"

After the onset of gelation, G′ increased rapidly as the crosslinking progresses for all frequencies investigated (at t= 53870 s and frequency = 0.1 Hz, G'=2596 Pa and G"= 56.61 Pa) , which means that the hydrogel developed a predominantly elastic character. If both moduli reached a plateau, thestorage modulus G' remained considerably higher than G", these results indicated that the elastic response of the hydrogelis stronger than the viscous response. The values of the storage modulus G' and the differences with those of the loss modulus G" show that the obtainedhydrogelcan be considered as stable crosslinked network with high elasticity. We also noted from fig.6that the value of G ' in the plateau region became clearly independent of the frequency.

Determination of the critical gel point. The rheological investigations, (G′) and (G") have been employed to monitor the visco-elastic changes that occurred during crosslinking reaction and consequently to study gelation. Thus, to accurately determine the gel point, the crosslinking was followed dynamically at several stress frequencies of samples. The critical gel point is the time when the curves of tanδ at various frequencies coincide. An example is given in Fig. 7 (a).

Effect of cross-linker concentration on the percolation time (gel point) t_{gel}. In order to examine the influence of cross-linker concentration [STMP] on the gel point, a series of experiments were performed with [STMP] equal to 0.13, 0.20 and 0.26 mol/L. The results are represented in fig.7 (b).

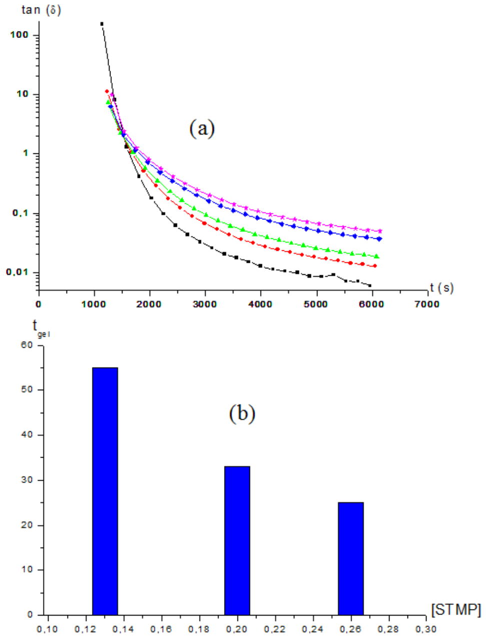

Figure7. (a) Plots of tangent δ versus time, each curve refers to a given frequency ω of the oscillary shear applied (0.1 to 5 Hz), square 0.1 Hz, circle 0.5Hz, up triangle 1Hz, diamond 3Hz, star 5H,(b) Effect of the STMP concentration on the gel point

As can be observed, t_{gel} decreases with increasing concentration of STMP, this means thatwith an excess of STMP new linkages are formed among the CMP molecules and more sites forcrosslinking are created leading to the largest phosphate incorporation,the crosslinking kinetics increases andthegelation time gets shorter.

Effect of cross-linker concentration on the structure of the gel. The concentration of crosslinking agent is the parameter generally used to control the final state of crosslinking and therefore the characteristics of thehydrogel.

Fig. 8 (a) indicates the variation of elastic modulus G' as a function of time for different cross-linker concentrations. G' increased with increasing the cross-linker concentration, this is probably due to the formation of additional crosslinking nodes leading to an increase in the network junction density and causes gel hardness.

Fig. 8 (b) represents the evolution of G 'and G" of CMP hydrogel samples as a function of frequency. The result confirmed that the plateau modules were independent throughout the entire frequency range, this is typical of gel behavior and indicative of a homogeneous cross-linked gel network in agreement with previous papers [56]

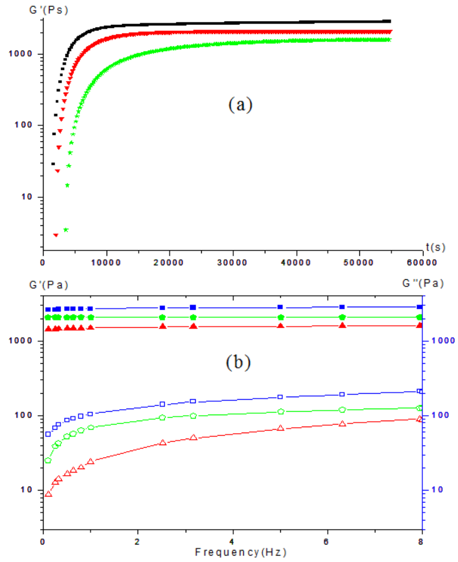

Figure 8. (a) Variation of G’ as a function of time for different cross-linker concentrations, square 0.26M, triangle 0.20M, star 0.13M, (b)Plots of G' and G" as a function of frequency, open G", solid G'.

4.CONCLUSION

The purpose of this study was the preparation of a smart macrogels based on carboxymethylpullulan for encapsulation and release of active agent. Taking the STMP as crosslinking agent, we have prepared an anionic matrice which absorbs important amounts of water. In particular, this work focused onthe effect of physicochemical parameters on the swelling and rheological properties of the materials.

The results of FTIR and SEM confirmed that CMP chains had been crosslinked and new functional groups were interpenetrated throughout the polymer net-work. The CMP-STMP hydrogels with porous and interconnected structure displayed good swelling and were highly sensitive to pH environment. The swelling was affected by the ionic strength, presence of metal cation and its valence. Second order kinetics appeared the best suited on swelling data acquired in water.

Based on the results from oscillatory rheology, the sol–gel transition kinetics of CMP hydrogels were accelerated by increased cross-linker concentration and the elastic properties prevailed over viscous.

In conclusion, hydrogels based on CMP-STMP which show specific characteristics can be considered as materials of choice for potential applications in medical and industrial fields.

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