Asian Journal of Chemistry; Vol. 26, No. 19 (2014), 6567-6570

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16608

A Novel CCAG Hydrogel: The Mechanical Strength and Crosslinking Densities

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Received: 30 October 2013; *Accepted*: 21 March 2014; *Published online*: 16 September 2014; AJC-15962

In this study, swelling behaviour and mechanical properties of the CS-HLC-HA/β-GP (chitosan-human like collagen-hyaluronic acid/β-sodium glycerophosphate) hydrogels, were investigated. The pH/temperature sensitive CCAG (CS-HLC-HA/β-GP) hydrogels were successfully synthesized by a combination of CCA (CS-HLC-HA) and β-GP by self-assemble technique. Compression-strain measurements were used to analyze the mechanical properties of the hydrogels. It was found that increasing the amount of hyaluronic acid comonomer in the gel structure increases the compression modulus of the materials. The results of mechanical measurements were used to characterize the network structure of the hydrogels, namely the effective crosslinking density (ρ). It was found that ρ increasing calculated from the initial amount of hyaluronic acid used for hydrogel synthesis. These hydrogels demonstrated dual sensitivity to both pH and temperature. It showed that the pH-sensitive or temperature-sensitive phase transition behaviour of the gels can be changed by changing the temperature or pH of the swelling medium at constant hydrogel composition. Finally, the results of equilibrium swelling and compression-strain measurements were used to calculate the interaction parameters of these hydrogels using a gel strength instrument.

Keywords: Hydrogel, Mechanical strength, Crosslinking densities, Chitosan, Hyaluronic acid, β-Sodium glycerophosphate.

INTRODUCTION

Hydrogels are of interest in biomedical applications due to their tunable chemical and three-dimensional physical network structures, high water content in an aqueous solution without dissolution good mechanical property and biocompatibility[1-3]. "Smart" hydrogels are the ones that absorb or desorb large quantities of water in response to some environmental trigger such as changes in temperature, pH or concentration of some analyte[4]. In particular, great attention has been focused on the intelligent ones, which can change their volumes as a result of a slight variation of external stimuli, such as temperature[5], light[6], chemical environment[7], electric field[8], antigen[9], *etc*. Generally, stimuli responsive polymers play an important role in the development of intelligent hydrogels. pH- and/or temperature-sensitive hydrogels are perhaps the most extensively studied classes of smart hydrogels. As the representative one, poly(N-isopropylacrylamide) (PNIPAAm) is a well known temperature sensitive polymer, which exhibits phase separation at a lower critical solution temperature (LCST) of 32 °C in aqueous solution[10,11]. The unique characteristics make PNIPAAm hydrogel specially useful in biomedical applications, such as in the controlled release of drugs and in tissue engineering[12-14]. Chitosan/β-glycerophosphate hydrogel is a thermo-sensitive hydrogel that was first reported by Chenite *et al.*[15]. However, this hydrogel system has undesirable properties, such as a loose structure, a low compressive strength and poor control over gel formation *in situ*. Therefore, several chitosan derivatives with improved properties for enhanced applicability have been developed over recent years[16-20].

Therefore, in this study, we report on the mechanical properties and pH- and temperature-sensitive swelling behaviour of CCAG hydrogels. The effect of addition of hyaluronic acid to the gel chemical structure on the mechanical properties was investigated and the results were used to characterize the network parameters. The role of pH, temperature and comonomer content in the swelling behaviour of the resulting hydrogels was also investigated.

EXPERIMENTAL

HLC was expressed by *E. coli* with a cloning partial cDNA derived from human collagen Mrna. It is a macromolecular water-soluble protein with molecular weight of 97,000 (China patent number: ZL01106757.8). Hyaluronic acid (molecular weight, 1,000,000) was purchased by Shandong Frieda biological technology limited company (China). Chitosan (molecular weight, 550,000; 90-92 % deacetylation) was obtained by Qingdao Ocean Ltd. Co. (China). All other solvents and reagents were analytical grade.

Preparation of CCAG hydrogels: The pH/temperature-sensitive CCAG hydrogels were prepared by mixing 5 % (w/v) chitosan dissolved in 0.1 mol/L aqueous solution of acetic acid with an isocratic volume of 3 % (w/v) human-like collagen aqueous solution. The mixture was stirred for 20 min in an ice-water bath until dissolution was complete. Then, the different concentration of hyaluronic acid (2, 1, 0.5 and 0.1 %) was added with an volume ratio 1:0.1 while stirred until fibrous form and mixture becoming crystalloid copolymer. 1 mL 40 % β-sodium glycerophosphate was added dropwise until the pH of the sol solution reached 7.4 (Table-1). The solutions formed were placed in a thermo cell for gelling at various temperatures.

TABLE-1
CCAG HYDROGEL COMPOSITION

Sample	CS (mL)[a]	HLC (%)	HA (%)	β-GP (mL)[b]
Gel1	2	3	0.1	1
Gel2	2	3	0.5	1
Gel3	2	3	1	1
Gel4	2	3	2	1

[a]CS is 5 wt. % aqueous solution, [b]β-GP is 40 wt. % aqueous solution

Swelling measurement: For temperature/pH dependent swelling studies, dried gels were placed in triplicate PBS buffer solutions with pH value of 5, 7.4 and 9 at various temperatures for at least 24 h to reach equilibrium, after which the gels were weighed. The classical gravimetric method was employed to measure the swelling ratio of the hydrogels. The equilibrium swelling ratio was given as $(W_s\text{-}W_d)/W_s$, where W_s and W_d represent the weight of swollen gel and the dried gel weight, respectively.

Compression experiment: The hydrogels were tested by a gel strength instrument (Electronic Universal Testing Machine) as follows: firstly, the height of the compression plate was adjusted to 10 mm and its final height was adjusted to 4 mm. The disc of instrument was circular with a diameter of 10 mm and the compression speed was adjusted to 2 mm/min. The gel was cut to a length of 10 mm and placed on the instrument disc. When the compression displacement reached 6 mm, the test was stopped. The compression modulus and the relationship between the compression stress and compression displacement were calculated. The effective crosslinking density (ρ) was determined by the compression modulus and polymer swelling ratio (Q), shown as follows[21]:

$$\rho = GQ^{1/3}/RT \qquad (1)$$

G was the compression modulus, R was the gas constant (8.314×10^3 KPa·cm^3 mol^{-1} K^{-1}) and T was 310 K.

Calculations and data analysis: Data were collected in a Microsoft Excel 2000 database and the results were presented as means and standard deviations using the Origins 7.0 software. A student's t-test was performed to determine the statistical significance between experimental groups. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Structure of hydrogels: The morphology of the lyophilized CCAG hydrogels was examined by SEM, which showed that the hydrogels pore structure changes from nonporous to microporous and the hole wall changes from thin to thick with different concentration of hyaluronic acid throughout the cross-section (Fig. 1). For higher concentration of hyaluronic acid, the CCAG hydrogels did not have a uniform microporous structure and had poor pore interconnectivity (Fig. 1, D). For lower concentration of hyaluronic acid, the CCAG hydrogels demonstrated a uniform microporous network, indicating good interaction between the components (Fig. 1, A and B). However, for Gel3, the morphology of cross-section showed a more rough and did not homogeneous and uniform structure. The smoother and their surface structures were compact with uniform pore distribution, which renders them favorable for water and small molecule movement within the network.

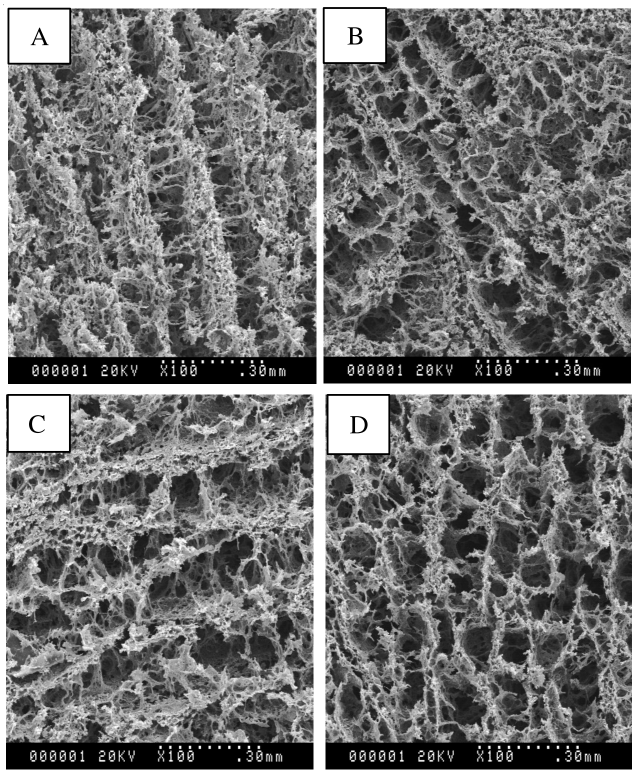

Fig. 1. SEM micrographs (× 100) of Gel1 (A), Gel2 (B), Gel3 (C) and Gel4 (D) at 37 °C after lyophilization

Swelling ratio analysis: From Fig. 2, the different pH value of PBS (5, 7.4 and 9) associated with hydrogels swelling ratio in contact with acidic (pH = 5) and basic solution (pH = 9) is usually higher than neutral solution (pH = 7.4) after immersing as some functional group of hydrogel can be protonated or ionized. Tjong and Bei[22] suggested that the strong dependence of acrylamide hydrogels swelling behaviour on initial monomer concentrations was a reflection of increasing network-chain interpenetration with rising monomer concentration. The swelling behaviours of natural synthetic hydrogels also similar to it. With increasing hyaluronic acid content, the absorbed of hydrogels gradient increased. Therefore, Gel4 result in higher equilibrium water content or swelling ratio no matter what condition in acidic, neutral or basic medium. For example, the swelling ratio of Gel4 was higher than other hydrogels (Gel1, Gel2 and Gel3) at different temperature and media (Fig. 2). The swelling ratio of the CCAG hydrogel in

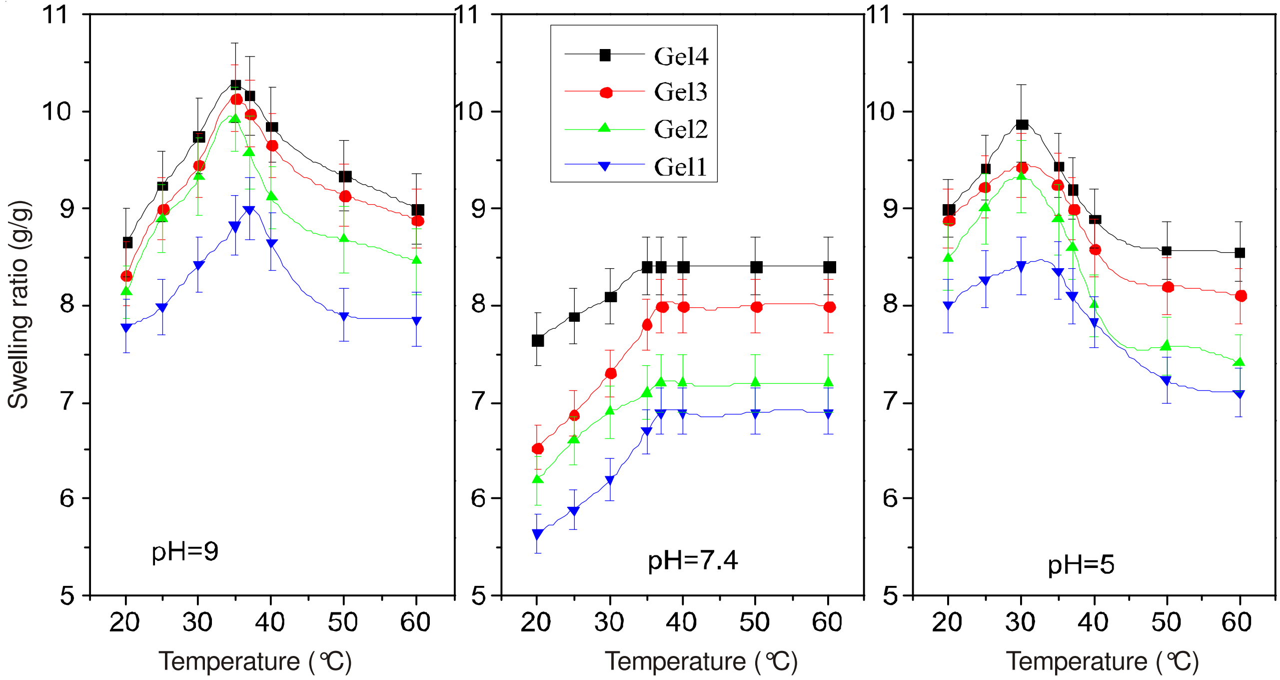

Fig. 2. Swelling ratios of gels dependence on pH (pH = 9, 7.4 and 5) and temperature

PBS (pH = 9) is higher than in PBS (pH = 7.4) and (pH = 5). The maximum swelling ratio of Gel1, Gel2, Gel3 and Gel4 was found at 35, 37 and 40 °C in PBS (pH = 9), PBS (pH = 7.4) and PBS (pH = 5), thus showing a "thermal-expansion" characteristic[23]. At temperatures either lower than 35 or 37 °C or higher than 35 or 37 °C in PBS (pH = 9 or 5), the swelling ratios of Gel1, Gel2, Gel3 and Gel4 were decreased, showing a "thermo-shrink" feature[23]. While at temperature higher than 37 °C, showing a swelling equilibrium in PBS (pH = 7.4). Therefore, the CCAG hydrogels is pH/temperature sensitive hydrogels.

Strength and crosslinking densities of the hydrogels: The compression modulus of the hydrogels is listed in Table-2. As shown in Fig. 3, the compression stress of Gel4 (2 KPa) was higher than that of Gel1 (1.5 KPa), Gel2 (1.7 KPa) and Gel3 (1.9 KPa) with the same compression displacement at 37 °C. This can probably be ascribed to the influence of both structure and porosity on the flexibility and plasticity of the gel. The compression modulus was directly proportional to compression stress; the better the flexibility of gel, the higher the compression modulus value. Table-2 shows that the compression modulus of the hydrogels increased with their cross-

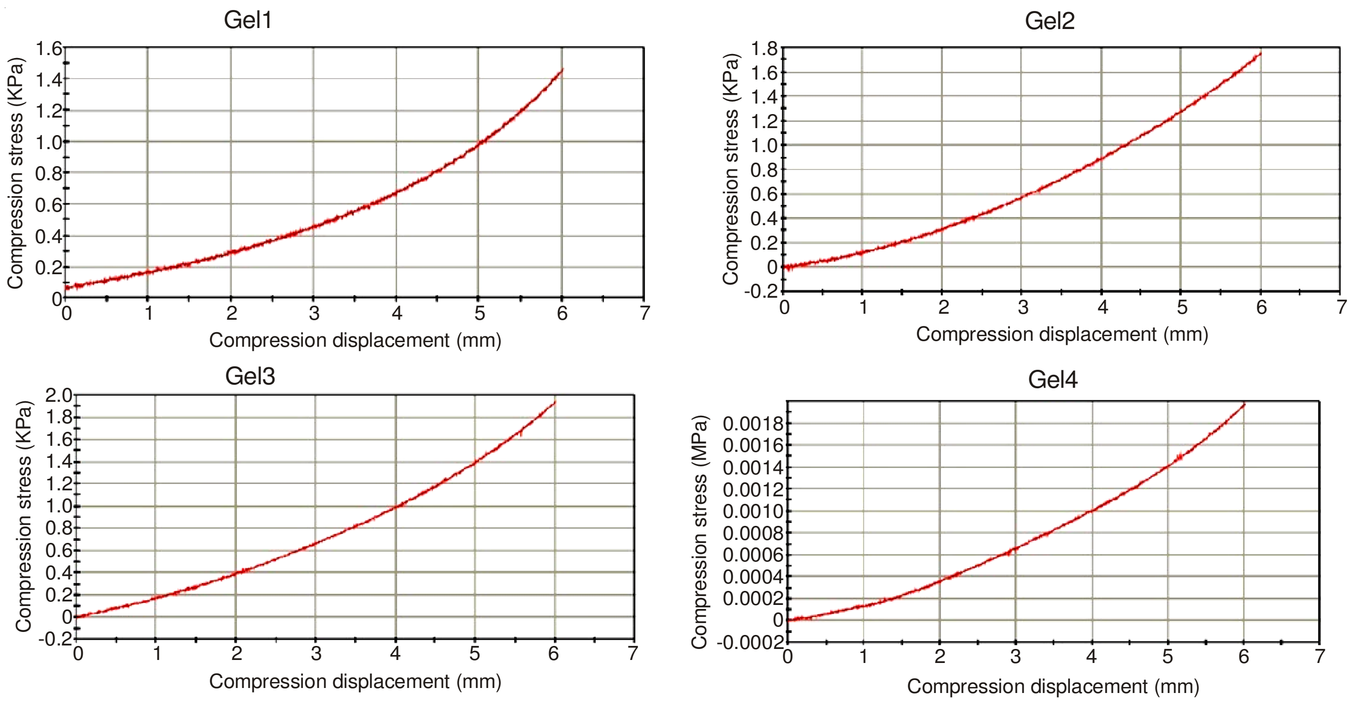

Fig. 3. Relationship between compression stress and compression displacement: Gel1, Gel2, Gel3 and Gel4

linking densities at the same temperature. This could be explained in eqn. 1, where the compression modulus depended on the crosslinking density and the swelling ratio at the same temperature. As the values of $Q^{1/3}$ were nearly equal to 2 for Gel1, Gel2, Gel3 and Gel4 (Table-2), the compression moduli of these gels were directly proportional to their crosslinking densities. Therefore, crosslinking density played an important role in the formation of the pH/temperature-sensitive hydrogels. The CCAG hydrogels exhibited more adequate mechanical strength (1.5-2 MPa) and crosslinking densities (2.5-4.5 × 10^{-3} moL/cm^3). Therefore the CCAG hydroels are suitable for biomaterials as medical application such as soft tissue defect filling.

TABLE-2
COMPRESSION MODULE AND CROSSLINKING DENSITY OF GELS

Gel	G (KPa)	Q	$Q^{1/3}$	$\beta \times 10^{-6}$ mol (cm^3)
Gel1	4.37	6.9	1.5	2.54
Gel2	4.95	7.2	1.81	3.48
Gel3	5.79	8	1.87	4.2
Gel4	6.0	8.4	1.89	4.4

Conclusion

The pH/temperature sensitive CCAG hydrogels were successfully synthesized by a combination of hyaluronic acid, chitosan, human like collagen and β-sodium glycerophosphate. In this study swelling and mechanical properties of the CCAG hydrogels were investigated. Mechanical measurements showed that addition of a hydrophobic comonomer (hyaluronic acid and HLC) increases the mechanical strength of the network. Swelling studies showed that these hydrogels are both pH- and temperature-sensitive. It is possible to control the pH- or temperature-sensitive phase transition characteristics of these hydrogels without changing the chemical structure of the hydrogel by a change in the temperature or pH of the surrounding environment, respectively. Crosslinking density played an important role in the formation of the pH/temperature-sensitive hydrogels. With excellent pH and temperature sensitive property, the CCAG hydrogels have potential biomedical applications, such as release of drugs and tissue engineering.

ACKNOWLEDGEMENTS

This study was financially supported by the National Natural Science Foundation of China (21276210, 21106112, 21206135, 31000019 and 21106111) and the NWU Graduate Innovation and Creativity Funds (YZZ12037) and the Shaanxi Key Subject Program, China.

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