



## Preparation and Characterization of Xanthan Gum-based Biodegradable Polysaccharide Hydrogels

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### Abstract

The hydrogels are tridimensional crosslinked polymeric network with the ability to hold large amount of water. The purpose of this study was to prepare xanthan gum-based polysaccharide hydrogels with polymeric networks and to evaluate their physical and chemical properties. Xanthan gum, sodium benzoate, potassium sorbate, HPLC graded water and glycerine was used in the preparation of hydrogels. The physical and chemical properties of hydrogels were determined to characterize moisture content, biodegradability, relative hydrogen bonding strength, viscosity and surface morphology of the hydrogel formulations. On the basis of this analysis, hydrogel 04 and 05 with relatively higher xanthan gum concentration were found to be more effective product in comparison with other variations of hydrogels. The reasons behind the effectiveness of these two hydrogels were biodegradability, lower moisture content, higher viscosity and better crosslinking polymeric network morphology.

**Keywords:** Hydrogel, Xanthan gum, Polysaccharide..

### Introduction

The hydrogel can be defined as a water-swollen crosslinked polymeric three-dimensional network formed by covalent cross-linking and via various non-covalent intramolecular and intermolecular bonds<sup>1</sup>. The water holding capacity of the hydrogels is attributed to the hydrophilic moieties such as hydroxyl, amide, amino, and carboxyl groups distributed along the polymeric chains' backbone<sup>2,3</sup>. Hydrogels have gained extensive interest in the last few decades due to their wide range of clinical and biomedical application such as in tissue engineering, drug delivery, regenerative medicine, wound dressing etc<sup>3</sup>.

In general, hydrogels can be prepared from either synthetic polymers or natural polymers. Natural hydrogel-forming polymers include proteins such as gelatine and collagen and polysaccharides such as alginate, starch, chitin, chitosan, agarose, dextran and chondroitin sulfate<sup>1,3</sup>. Chitin and chitosan, of these, have been demonstrated as potential tissue supporting materials<sup>4</sup>.

Xanthan gum is an exopolysaccharide secreted by the plant pathogenic bacterium *Xanthomonas campestris*<sup>5</sup>. It is an acidic polymer of pentasaccharide repeat units, comprising glucose,

mannose, and glucuronic acid in the molar ratio 2:2:1<sup>6</sup>. It is well known because of its use as a food additive and rheology modifier<sup>7</sup>, and also as a food thickening agent and an emulsifier in food industry as well as in oil recovery. Previously, xanthan gum in combination with konjac gum,  $\kappa$ -carrageenan and  $\iota$ -carrageenan was used in the preparation of a biodegradable hydrogels for skin scaffold<sup>8</sup>. In this study, we used different proportion of xanthan gum, sodium benzoate, potassium sorbate, HPLC graded water and glycerine for the preparation of novel hydrogels and evaluated their different physico-chemical and biological properties to fine tune the characteristics such as biodegradability, surface moisture content, relative hydrogen bonding strength, viscosity, surface morphology and antimicrobial activity.

### Materials and Methods

**Materials:** For the preparation of polysaccharide based hydrogel, we used xanthan gum, sodium benzoate ( $C_7H_5NaO_2$ ), potassium sorbate ( $C_6H_7KO_2$ ), HPLC grade water and glycerine ( $C_3H_8O_3$ ). Xanthan gum and potassium sorbate were obtained from Zhengzhou Sino Chemical Co. Ltd., while sodium benzoate and glycerine were purchased from Sigma Aldrich and P and G chemicals respectively. HPLC grade water was prepared in the laboratory.

**Preparation of Hydrogels:** For the preparation of hydrogel with different composition, all the chemicals (xanthan gum, sodium benzoate, potassium sorbate and glycerine) with different proportions were dispersed in HPLC grade water. Afterwards, the mixture was stirred and heated at 85°C and was then poured into petri dishes and allowed to cool at room temperature to obtain polymeric hydrogel. Then prepared hydrogels were preserved at 4°C.

Table-1 shows the composition of different hydrogels prepared in this study.

**Determination of Moisture Content of Hydrogels:** Standard IUPAC method was followed for the determination of moisture content. 2 gm of sample was taken in a porcelain crucible and heated in a temperature controlled water bath for about six hours at 105°C. It was then cooled in desiccators and weighed again. The moisture percentage in hydrogels was calculated from the formula:

$$\text{Moisture} = 100(W_1 - W_2) \%$$

Here,  $W_1$  = original weight of the sample before drying and  $W_2$  = weight of the sample after drying.

**Biodegradability of Hydrogels:** This biodegradation process was carried out according to American Standard for Testing of Materials (ASTM) F 1635-95. In brief, samples were dried to a constant weight before initial weighing. Hydrogels were fully immersed in the physiological solution for a specified period of time (for example 1 week, 2 weeks, 3 weeks etc.). Upon completion of the specified time period, each sample was removed and dried to a constant weight. After weighing, the samples were not returned to the physiological solution and were retired from the study.

**FT-IR Studies:** The hydrogel preparations were dried and converted in powdered form for FT-IR study. The powdered samples were mixed with KBr and FT-IR spectrum was recorded over the range 600-4000 cm<sup>-1</sup> using FTIR- 8400S Spectrometer (Shimadzu, Japan).

**Scanning Electron Microscopy (SEM) Studies:** For ensuring whether hydrogels retain its structure, SEM (HITACHI S3400N operated at 15 kV accelerating voltage) was used to analyze its surface morphology. The desiccated hydrogels were sliced to expose their inner structure and each cross section was used for SEM studies.

**Determination of Rheological Characteristics:** Rheological properties of hydrogel preparations were monitored using rotating rheometer (RheolabQC, SN1648597) equipped with temperature controller and spindle (C 75-1). Viscosity of samples was measured at different shear rate. The shear rate was increased from 0–5 s<sup>-1</sup> in 1 min. The viscosity was determined from the flow curve obtained at different values of shear rate. All measurements were made in triplicate.

**Antimicrobial Activity Testing:** The antimicrobial activity of prepared hydrogel was investigated with modified Kirby-Bauer disc diffusion method<sup>9</sup> using *E.coli* and *Klebsiella spp.* as reference strain, whereas antibiotics, Gentamycin (10µg) and Ampicillin (120µg) were used as control.

**Table-1**  
**Composition of different hydrogels**

Hydrogel	Xanthan gum (g)	Pottasium sorbate (g)	Sodium benzoate (g)	Glycerine (g)	HPLC water
Hydrogel 01	0.5	0.02	0.02	1.0	92.3%
Hydrogel 02	1.0	0.02	0.02	1.0	89.8%
Hydrogel 03	1.5	0.02	0.02	1.0	87.3%
Hydrogel 04	3.0	0.02	0.02	1.0	79.8%
Hydrogel 05	2.0	0.02	0.02	1.0	84.96%

## Results and Discussion

**Moister Content of Hydrogels:** In a physiological environment, the interfacial free energy is reduced by the presence of water at the surface of a hydrogel, which improves their biological properties<sup>10</sup>. In this study, we investigated the moisture content of the prepared hydrogels. As shown in Table-2, the highest moisture content (%) was found in hydrogel 01, while lower moisture content (%) was observed in hydrogel 04. Notably, hydrogel 01 had lower xanthan gum concentration while hydrogel 04 contained higher concentration of xanthan gum (Table-1). Thus, this finding suggests that, the hydrogels with less amount of xanthan gum is inversely related to the moisture content and vice versa, which is compatible with the previous finding<sup>11</sup>.

**Table-2**  
**Moisture content of the hydrogels**

Hydrogel samples	Moisture content = 100 ( $W_1 - W_2$ ) %
Hydrogel 01	9.75
Hydrogel 02	9.05
Hydrogel 03	7.33
Hydrogel 04	3.83
Hydrogel 05	5.60

**Biodegradability of Hydrogels:** Biodegradability is often considered as an essential factor since hydrogels when used as tissue scaffolds should preferably be absorbed by the

surrounding tissues<sup>12</sup>. In this study, we analyzed the biodegradability of the hydrogel preparations as shown in Figure-1. Among all the hydrogel preparations, hydrogel 01, 02 and 03 had higher degradation rate than hydrogels 04 and 05. Hydrogel 05 had moderate while hydrogel 04 showed the lowest degradation rate (Figure-1). The degradation rate of hydrogels might be attributed to the concentration of xanthan gum since the concentration of xanthan gum was higher in hydrogel 04 and lower in hydrogel 01 (Table-1). Thus this observation suggests that, lower the concentration of xanthan gum in hydrogels, higher the degradation rate.

**Rheological Characteristics of Hydrogels:** The viscosity of hydrogel scaffold formulations at different shear rates ( $0-5 \text{ s}^{-1}$ ) was obtained as shown graphically in Figure-2. Though all the formulations showed pseudoplastic flow which is in line with the previous observation<sup>13</sup>, hydrogel 04 and 05 showed better pseudoplastic behavior with optimum steep in graph. This might be due to higher concentration of xanthan gum that results better cross-linking and showed saturated viscosity.

**Determination of Bonding Strength of Hydrogels:** In this study, various raw materials were used in the preparation of hydrogels. Polysaccharide xanthan gum was mainly used in this composition which contains -OH, -COOH, -CH<sub>2</sub>OH etc. bonds in its chain. Besides this, Sodium benzoate ( $\text{C}_7\text{H}_5\text{NaO}_2$ ),

glycerine ( $\text{C}_3\text{H}_8\text{O}_3$ ), potassium sorbate ( $\text{C}_6\text{H}_7\text{KO}_2$ ) and HPLC grade water were used which also contain several bonds like -OH, -COOH, -C=O, =C-C= etc.

In FTIR spectroscopy, free -OH bond usually shows a peak of band at  $3500 \text{ cm}^{-1}$  to  $3700 \text{ cm}^{-1}$ . But FTIR spectra of hydrogels showed no such peak at that region (Figure-3). The peak of the band was found to shift towards  $3200 \text{ cm}^{-1}$  to  $3400 \text{ cm}^{-1}$ . This region generally shows a sharp peak for hydrogen bond formation. Thus, this observation suggests that the free -OH group in the preparations was involved in hydrogen bond during the formation of hydrogels. Free -COOH group shows a peak at  $2400 \text{ cm}^{-1}$  to  $3000 \text{ cm}^{-1}$ , but the spectra of hydrogels showed no peak at this region. This implies that free -COOH took part in bond formation which caused the peak to be shifted to other region. In acidic substances, free -C=O group shows a peak at  $1600 \text{ cm}^{-1}$  to  $1610 \text{ cm}^{-1}$  region, but we did not observe any peak at this region. Besides, the spectra showed a clear peak in the region of  $1633 \text{ cm}^{-1}$  to  $1643 \text{ cm}^{-1}$  which confirmed the presence of conjugation bond (=C-C=). Free H<sub>2</sub>O usually shows a broad peak at  $3500 \text{ cm}^{-1}$  region in the IR spectroscopy. But the spectra of hydrogels showed no such peak at this region which confirmed that there was no free water in this hydrogel substances. All free water participates in the reaction of various bond formations.

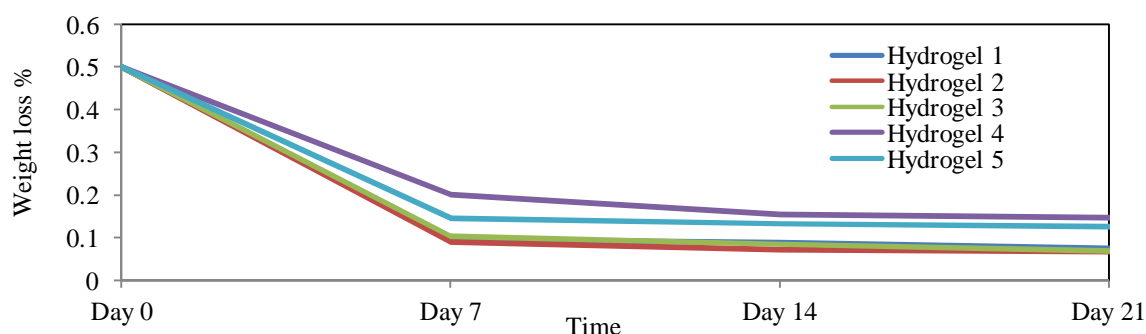


Figure-1  
Biodegradation Rate of Hydrogels

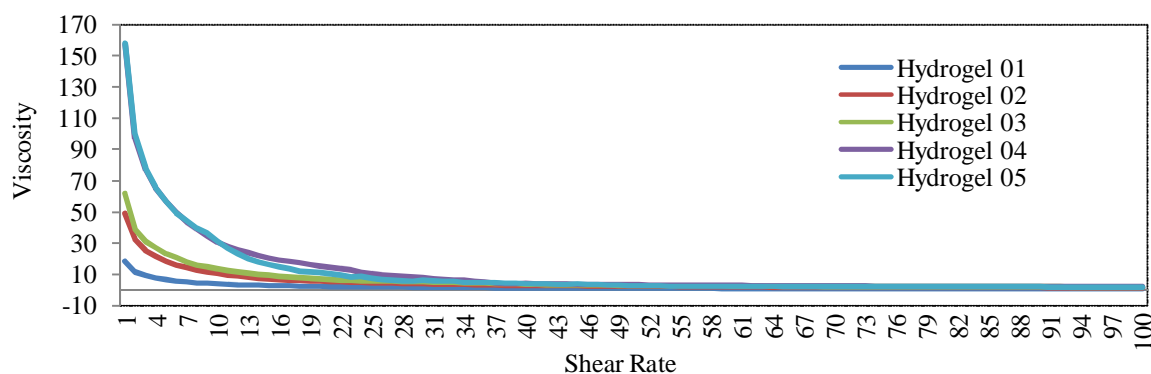
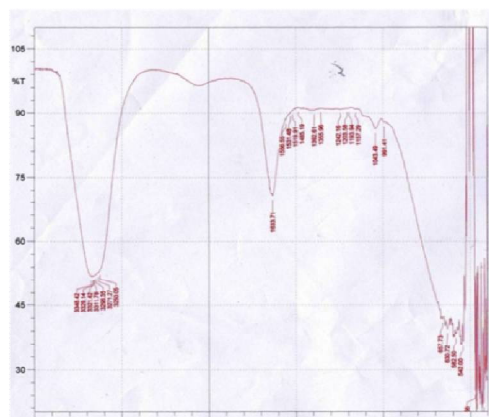
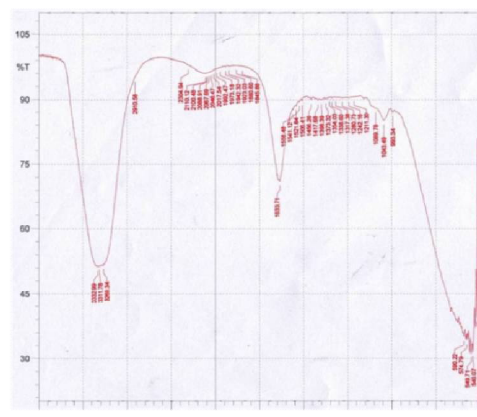


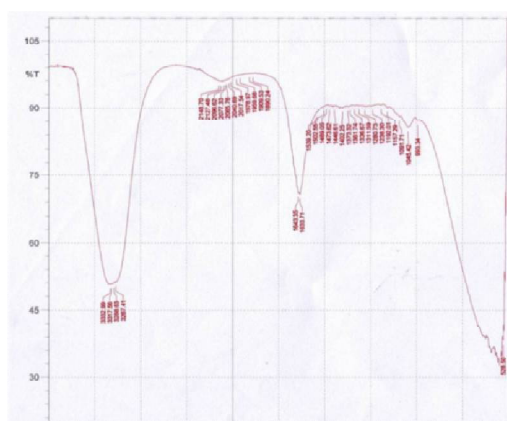
Figure-2  
Rheological Behavior of Hydrogels



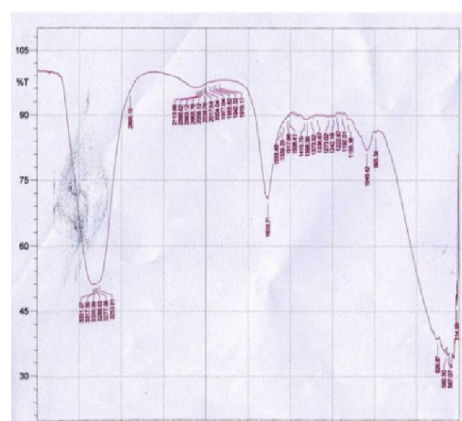
a



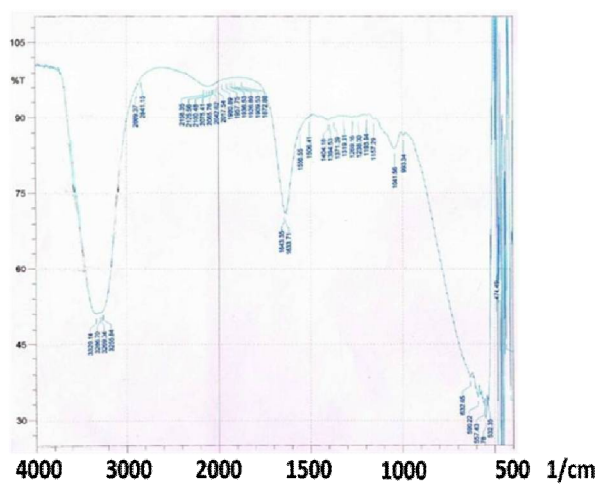
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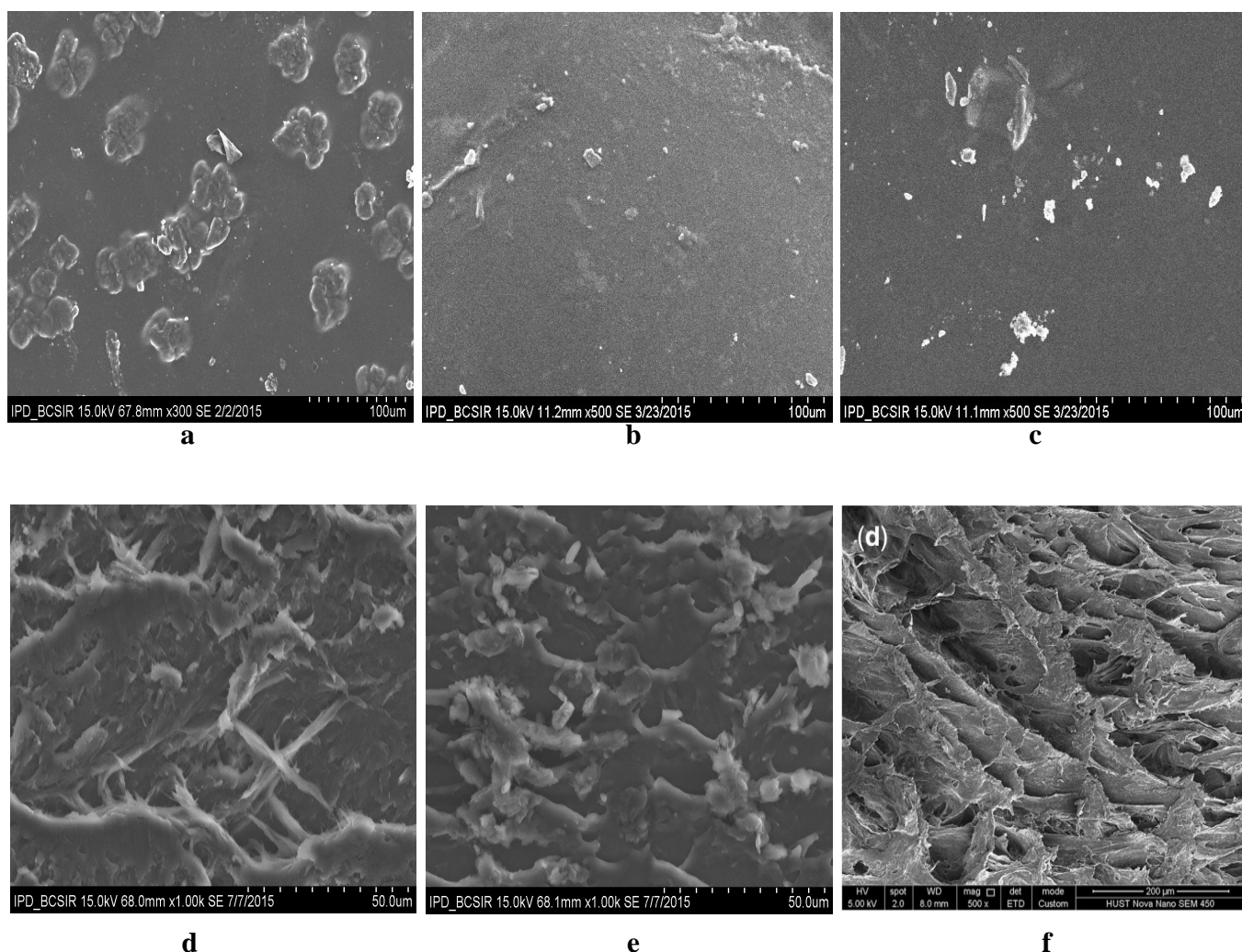
e

Figure-3

FTIR Spectroscopy of the Hydrogels; a) Hydrogel 01, b) Hydrogel 02, c) Hydrogel 03, d) Hydrogel 04 and e) Hydrogel 05

**Scanning Electron Microscopy (SEM) Studies:** In tissue engineering, structural morphology of the hydrogel scaffold is important for the distribution and interconnection of cells in a homogeneous manner throughout engineered tissues and thus, plays crucial role to direct tissue formation and function<sup>14</sup>. Hydrogels with increased porosity, in the case of functional insufficiency in vascular system, is highly advantageous for effective diffusion of nutrients and oxygen in addition to local angiogenesis to occur<sup>14</sup>. Thus, porosity and pore interconnectivity are important factors to consider for constructing functional hydrogels scaffold in tissue engineering for the survival, proliferation and migration of cells. In this study, the differences in surface morphology of prepared hydrogels were characterized by using scanning electron microscopy (SEM). Hydrogels 04 and 05 were found to form polymeric network in its surface area (Figure-4, d-e), however, the polymeric network was not formed in hydrogels 01, 02 and

03 (Figure-4, a-c). SEM analysis of hydrogels were also compared with the SEM analysis of a reference article<sup>15</sup> shown in Figure-4, f, which revealed that surface morphology of prepared hydrogels was almost similar to the reference. It is evident from the figure that, surface morphology of hydrogel swollen beads was spherical in shape, while minute pores were found on the surface of those beads. It is interesting to note that hydrogel 04 and 05 with higher concentration of xanthan gum had much better polymeric network than hydrogel 01, 02 and 03 having lower xanthan gum content. Thus this analysis suggests that, surface morphology of hydrogels might be related to the concentration of xanthan gum. Further, the water content of hydrogels makes preparation for electron microscopy challenging<sup>16</sup>. Thus, it is assumed that hydrogels 04 and 05 with lower moisture content had much better surface morphology than hydrogels 01, 02 and 03 with higher moisture content.



**Figure-4**

**SEM analysis of the Hydrogels; a) Hydrogel 01, b) Hydrogel 02, c) Hydrogel 03, d) Hydrogel 04, e) Hydrogel 05 and f) Reference Hydrogel**



**Antimicrobial Activity Testing:** Previously, xanthane gum/tapioca starch edible matrices were reported to have antimicrobial activity<sup>17</sup>. However in this study, none of the hydrogels showed antimicrobial activity (data not shown).

## Conclusion

Xanthan gum was used in different concentration with a view to preparing different variations of biodegradable polymeric cross-linked hydrogels. After analysing all the data, it can be concluded that, hydrogel 04 and 05 with relatively higher xanthan gum concentration are more effective product in comparison with other variations of hydrogels. The reasons behind the effectiveness of these two hydrogels are biodegradability, lower moisture content, higher viscosity and better crosslinking polymeric network morphology.

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