

Preparation of Gelatin and Chitosan Hydrogels Beads

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Abstract: In this study, a series of gelatin and chitosan hydrogel beads were prepared via covalently crosslinking of these polymers. At the first step, three types of gelatin beads were obtained by dropping polymer solution into Glutaraldehyde (GA)-oil emulsion. While the concentration of polymer solution was varied from 7% to 14%, the concentration of crosslinking solution kept constant. Secondly, two types of chitosan beads were prepared by ionic gelation interaction procedure using Sodium Tripolyphosphate (TPP) as crosslinker. 1% of polymer solution was dropped into TPP solutions at different concentrations. At the last step, the different series of gelatin hydrogels were prepared by cutting the crosslinked polymer roads to form beads. Swelling behaviors of all the hydrogel beads were studied by changing time at constant temperature and pH. Swelling percentages (S%) increased with time initially, and then remained constant at close to 24 h. In general, it was determined that the gelatin hydrogel beads were much more swellable than the chitosan beads.

Keywords: Gelatin, Chitosan, Hydrogel, Glutaraldehyde

Introduction

Hydrogels are three-dimensionally cross-linked hydrophilic polymers that can absorb and retain large amounts of water up to thousands of times of their own weight. The hydrophilic property is due to presence of chemical residues such as -OH, -COOH, -NH₂, -CONH₂, -SO₃H and others with in molecular structure. Hydrogels have been extensively studied and preferred for a large number of industrial fields [Pulat and Asil, 2009]. Because of their excellent characteristics, they can also be used for a large number of applications in the medical field such as controlled drug release matrices, enzyme and yeast cell immobilization, blood-contacting applications, and others. Hydrogels are also very useful in production of agricultural and horticultural materials [Pulat and Yoltay, 2016].

Most of the synthetic polymers used to prepare hydrogels causes some problems because of their long degradation times and degradation products. Natural polymers are a good choice to overcome this issue [Pulat and Akalın, 2013].

Gelatin is a biodegradable natural polymer with extensive industrial, pharmaceutical, and biomedical uses that has been employed for coatings and microencapsulating various drugs, and for preparing biodegradable hydrogels [Pulat and Akalın, 2013]. As gelatin is an animal protein, it is obtained by thermal denaturation or physical and chemical degradation of collagen; the most widespread protein in the body occurs in most connective tissues as skin, tendon, and bone [Kushibiki et al. 2003, Gilsenan and Ross-Murphy, 2001] (Figure 1). Since it is soluble in aqueous solutions, the materials for long-term applications must be submitted to crosslinking, which improves both thermal and mechanical stability of gelatin [Bigi et al, 2001].

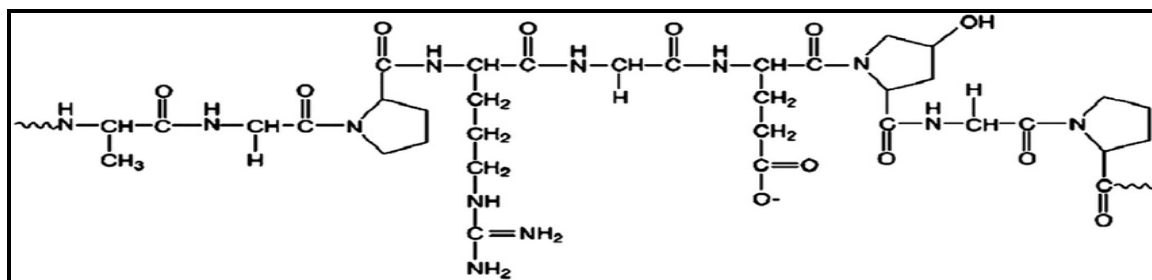


Figure 1. Molecular formula of gelatin

Chitosan (Cs) is a natural polymer obtained through the alkaline deacetylation of chitin (Figure 2). The hydrophilicity of Cs, due to the presence of amine and hydroxyl functional groups in its repeat unit, makes the polymer soluble in dilute acidic solutions and yields to a rubbery hydrogel in water [Pulat M. et al., 2011]. The propensity of CS to absorb water and swell into a soft rubbery material makes it a good matrix material for incorporating hydrophilic drugs.

It exhibits excellent biological properties such as biodegradation in the human body and immunological, antibacterial, and wound-healing activity. Cs has also been found to be a good candidate as a support material for gene delivery, cell culture, and tissue engineering [Macleod et al. 1999]. Moreover, Cs has antacid and anti-ulcer activity, and can prevent or weaken drug-induced irritations in the stomach. In addition, this cationic biopolymer has been reported to improve transport across biological barriers [Kotze' A F et al,1999]. From a biomedical point of view, Cs has demonstrated high activity as wound healing activators or accelerators, and now is used in human and veterinary medications. Several researchers have reported on the mechanism of the activation of wound healing considering the activation of polymorphonuclear cells, protective effects against microorganisms, or the promotion of granulocyte tissue formation with angiogenesis. Finally, Cs is very abundant and its production is both environmentally safe and of low cost. These interesting properties make Cs an ideal candidate for use in controlled drug release formulations.

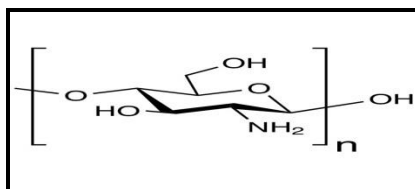


Figure 2. Molecular formula of Cs

The purpose of this study is to develop a series of gelatin and chitosan hydrogels in bead form. Hydrogels will be prepared by crosslinking of gelatin with glutaraldehyde (GA) and Cs with sodium *tripolyphosphate* (TPP). It is aimed that to obtain utilizable hydrogels at different swelling values.

Methods

Preparing of Hydrogel Beads

A series of Cs (Aldrich) beads were obtained by ionotropic gelation method (Nitsae M. et al, 2016). 1% of Cs solution was prepared in 5% of acetic acid (Merck) solution. This solution was added drop wise into TPP solutions of 1% and 3%. The mixture was gently stirred with a speed of 60 rpm for 1h. The formed beads were collected by filtration and immersed in distilled water in order to remove unreacted ingredients. After purification, the beads were dried in oven at 40 °C.

Aqueous gelatin (Fluka, Type B, 280-Bloom) solutions were prepared at 7%, 10% and 14% concentrations and added drop wise into emulsion bath including GA as crosslinker and oil. This bath was prepared using 20 mL of oil and 5 mL of GA solution. The mixture was gently stirred with a speed of 60 rpm for 1h. The formed beads were collected by filtration and immersed in distilled water in order to remove unparticipated ingredients. After purification, the beads were dried in oven at 40 °C. The schematic representation was given in Figure 3.

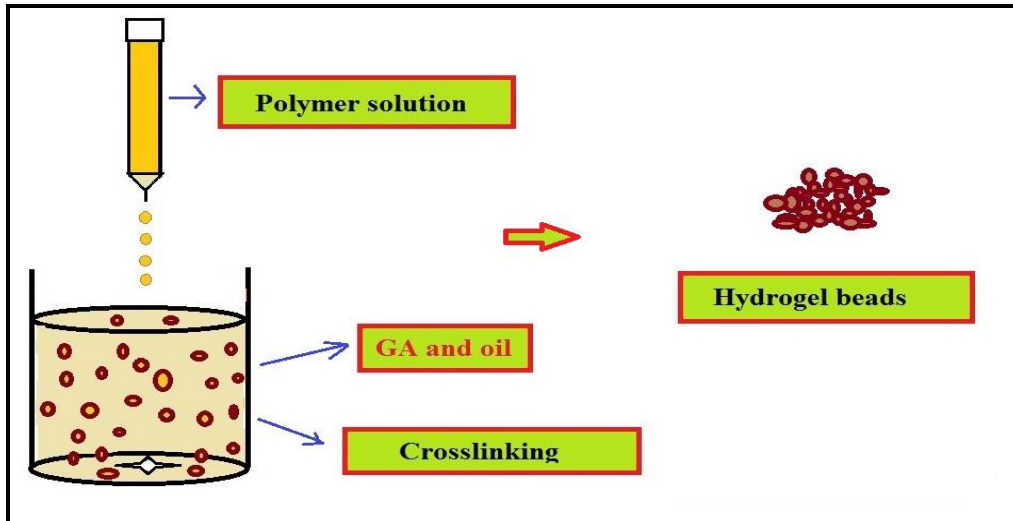


Figure 3. Preparation of hydrogel beads by dropping gelation

Another series of gelatin hydrogel beads were prepared by directly mixing of gelatin and GA solutions in a glass tube. The steps of procedure were presented in Figure 4. Four types of gelatin hydrogels were obtained by using the polymer and crosslinker solutions at different concentration as given in Table 1. Crosslinking reaction was preceded for 24 h at room temperature in a glass tube. The fresh hydrogel rods were taken from the tube and were cut into pieces 0.5 cm long. After the discs were left overnight at room conditions, they were washed several times with distilled water to remove unreacted chemicals [Blanco M.D. et al, 2003].

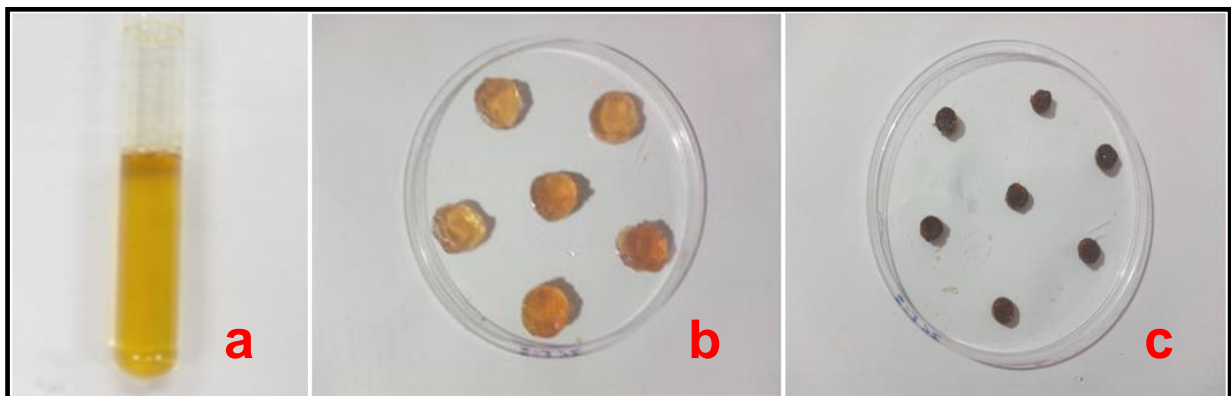


Figure 4. The preparation of gelatin hydrogel beads in tube (a), swollen (b) and dry (c) beads

The abbreviations and preparing conditions of all hydrogels were summarized in Table 1.

Table 1. Preparing conditions of the hydrogels

HYDROGEL	Gelatin (%)	GA (%)	Chitosan (%)	TPP (%)	C/P
C-1	-	-	1	1	1.0
C-2	-	-	1	3	3.0
G-1	7	25	-	-	3.6
G-2	10	25	-	-	2.5
G-3	14	25	-	-	1.8
	Gelatin (g)	GA (g)			
G-4	1.0	0.4	-	-	0.4
G-5	1.0	2.0	-	-	2.0
G-6	1.5	0.4	-	-	0.3
G-7	1.5	2.0	-	-	1.3

Swelling Tests

Swelling tests of hydrogel beads were gravimetrically carried out. The dried discs were left to swell in a Britton-Robinson buffer (BRB) solution (pH = 7.0) at 30°C. Swollen gels, removed from the swelling medium at regular intervals, were dried superficially with a filter paper, weighed, and placed into the same bath. The measurements were performed until a constant weight was reached for each sample. The swelling percentage (S% values) was calculated with the following equation [Pulat and Asil 2009, Chen et al, 2005]:

$$\text{Swelling (\%)} = \frac{M_w - M_d}{M_d} \times 100 \quad (1)$$

where M_w is the wet weight of the sample and M_d is the dry weight of the sample before swelling. Reproducible results for all swelling studies were obtained with triplicate measurements.

Results and Discussion

Cs beads were prepared by ionotropic gelation method based on the interaction of oppositely charged macromolecules. Ionotropic gelation involves simply the interaction of an ionic polymer with oppositely charged ion to initiate cross linking [Sapana A et al., 2014]. TPP has often been used to prepare chitosan particles because TPP is nontoxic, multivalent and able to form gels through ionic interactions. Cs gel beads were obtained by the fixation of its amine groups with TPP (ionic crosslinker). The negative charged TPP ions can react with positive charged chitosan via electrostatic attraction to form ionic crosslinked networks. A schematic mechanism about ionic crosslinking of Cs was presented in Figure 5.

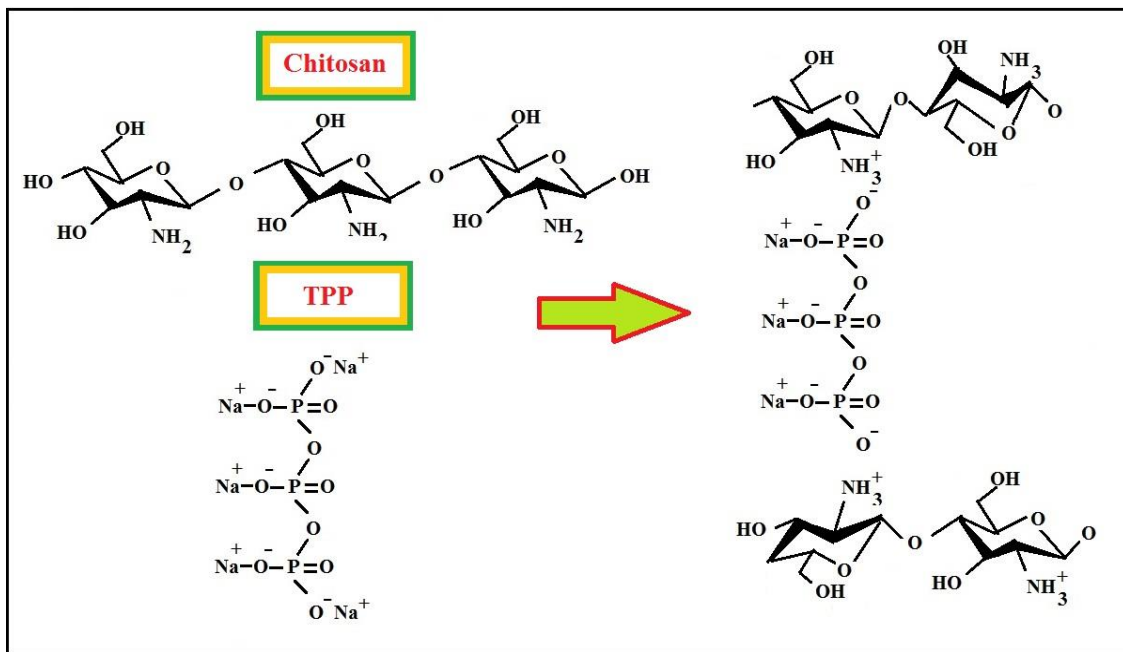


Figure 5. Ionic crosslinking mechanism of Cs with TPP

Gelatin beads were prepared by covalently crosslinking of polymer with GA. A schematic mechanism about ionic crosslinking of Na-Alg was presented in Figure 6.

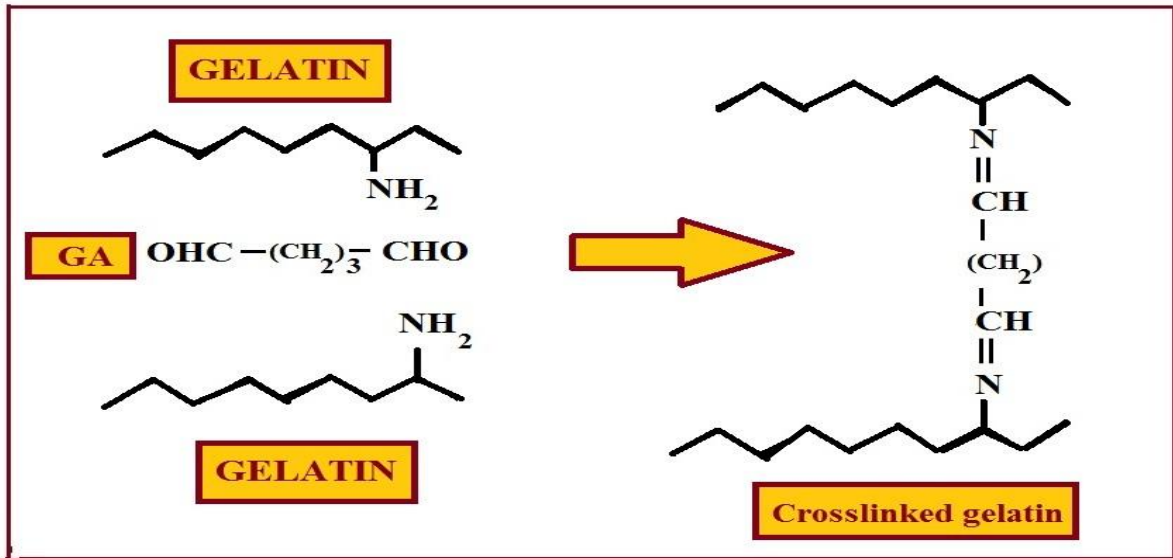


Figure 6. Crosslinking mechanism of gelatin with GA

Swelling Results

All of the swelling percentages belong to hydrogel beads were calculated from Equation 1. The photographs of dry and swollen hydrogels were presented in Figure 4.

The swelling variations of Cs beads with time at pH 7.0 and 30°C were shown in Figure 7. The swelling increased with time initially and then remained constant at close to 12 h. S% values were determined to be 135% for the most swollen hydrogel C-1, and 115 % for the least swollen hydrogel C-2. Swelling values were directly connected with composition, monomer ratio, ionic charge content, polymerization route, type and density of cross-linker, and so forth [El-Sherbiny *et al.*, 2005]. At this part of the work, hydrogels were prepared by changing crosslinker (C) amount while the polymer (P) amount was kept constant. As C/P ratio increased from C-1 to C-2, S% values decreased. This behavior is attributed to the fact that the network chains became inflexible at higher crosslinker density and thus, fewer amounts of water molecules penetrated the hydrogel structure.

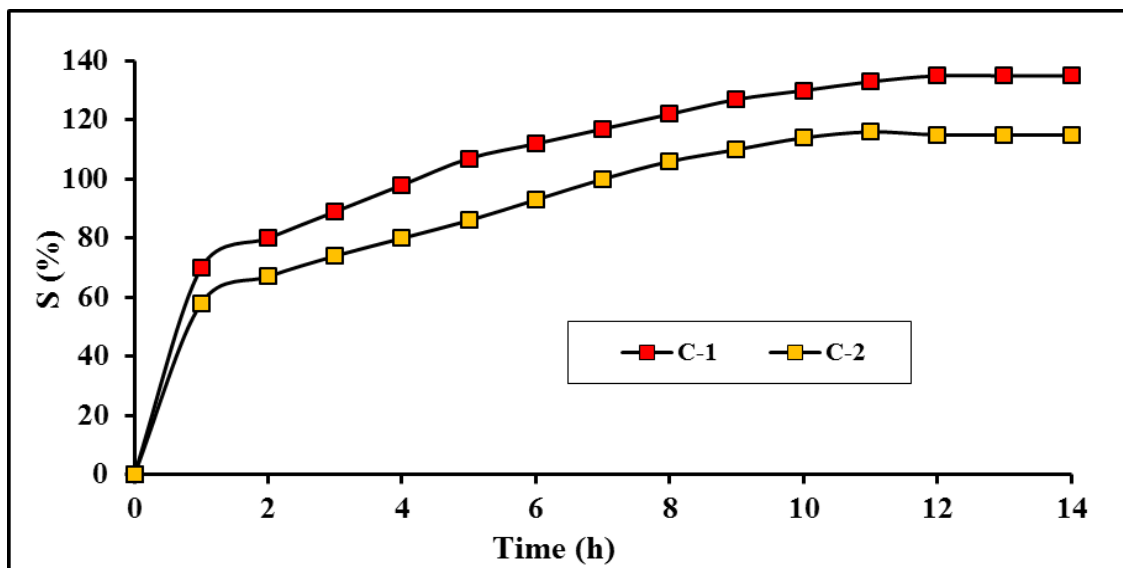


Figure 7. The variations of S% values belongs to chitosan beads with time (30°C; pH=7.0)

Swelling behaviors of the gelatin beads obtained by dropping were followed explained at experimental part. The variation of S% values with time was presented in Figure 8. The swelling increased with time initially and then remained constant at close to 24 h. S% values were determined to be 433% for the most swollen hydrogel G-1,

and 290 % for the least swollen hydrogel G-3. At this part of work, gelatin hydrogel beads were prepared by changing crosslinker amount while the polymer amount was kept constant. G-1 beads include the least amount of gelatin than G-2 and G-3. So, having relatively more crosslinker causes the less swelling value. G-3 beads were the most swollen hydrogel prepared from 14% of gelatin solution.

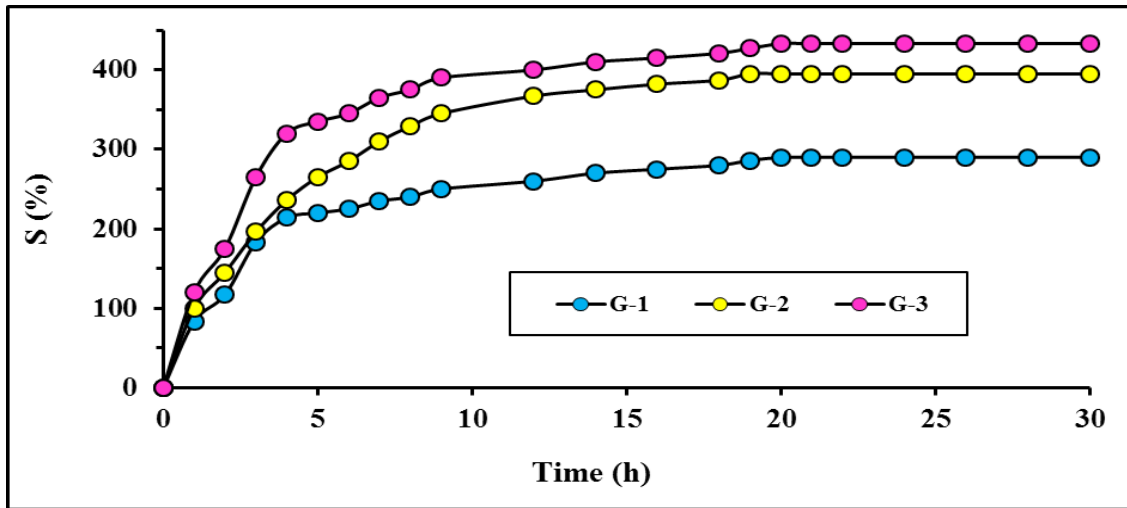


Figure 8. The variations of S% values belongs to gelatin beads with time (30°C; pH=7.0)

The last part of swelling experiment was carried out for the gelatin beads prepared in the glass tube. The variations of S% were given in Figure 9. The swelling increased with time initially and then remained constant at close to 24 h. S% values were determined to be 419% for the most swollen hydrogel G-4, and 304% for the least swollen hydrogel G-5. At this part of work, gelatin hydrogel beads were prepared by changing both crosslinker and polymer amounts. G-4 and G-5 hydrogels were obtained by using same amount of polymer despite varying amounts of crosslinker. C/P rates changed from 0.4 to 2.0 caused decreasing of S% values from 419 to 304. G-6 and G-7 hydrogels were prepared by changing C/P rates from 0.3 to 1.3. As the rates increases, S% values decreased from 385 to 347. All of the swelling values are compatible with their polymer/crosslinker contents.

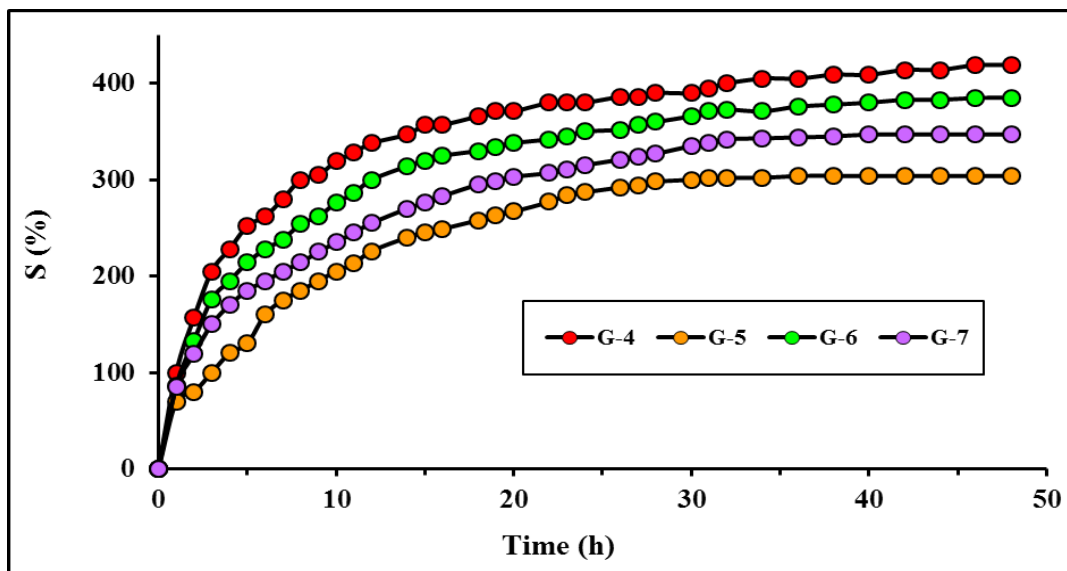


Figure 9. The variations of S% values belongs to gelatin beads with time (prepared in glass tube, 30°C; pH=7.0)

Conclusion

The results of the present work indicate that the beads type of hydrogels could be prepared by using gelatin and chitosan natural polymers. GA and TPP are good choices to obtain various hydrogels having different swelling values.

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