**The Eurasia Proceedings of Science, Technology,
Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199**

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2018

Volume 4, Pages 149-155

IConTES 2018: International Conference on Technology, Engineering and Science

The Preparatıon of Gelatin Coated Sodium Alginate Hydrogels

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Abstract: In this study, a series of gelatin coated sodium alginate (Na-Alg) hydrogels were prepared. Firstly, three types of hydrogels in bead form were obtained by using the classical ionic crosslinking method of Na-Alg with calcium chloride ($CaCl_2$). Second group was a series of Na-Alg hydrogel beads coated with gelatin. The immersing method was used to get the coated beads. The hydrogel samples were diversified by changing the concentrations of gelatin and $CaCl_2$ solutions. The percentages of Na-Alg solution was kept constant at 10%. Swelling tests of all hydrogel beads were gravimetrically carried out in buffer solution at pH=7.0 and swelling percentages were calculated. It is found that swelling value is decreased by increasing of $CaCl_2$ from 5% to 30%. In general, the hydrogels prepared using 5% of crosslinker were unstable. Coating conditions of Na-Alg hydrogel were varied by changing gelatin concentrations from 10% to 20%. The increasing amount of gelatin in coated part causes the decreasing in swelling values of hydrogel beads.

Keywords: Hydrogel, Sodium alginate, Gelatin, Swelling, Sequentially releasing

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, $-NH_2$, $-CONH_2$, $-SO_3H$ and others with in molecular structure. Hydrogels have been extensively studied and preferred for a large number of industrial fields (Pulat and Asıl, 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.

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].

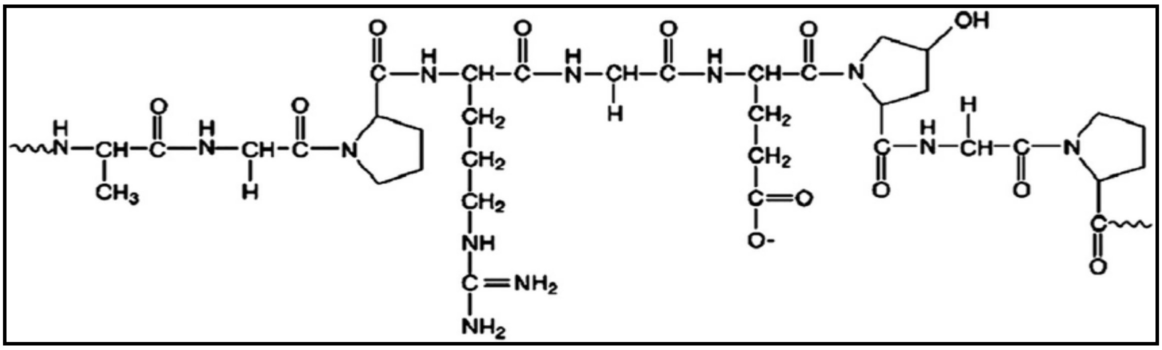

Figure 1. Molecular formula of gelatin

Alginates are isolated from brown seaweed using dilute alkaline extraction. Alginic acid is a linear polymer consisting of d-mannuronic acid and l-guluronic acid residues (Figure 2). It forms a high-viscosity acid gel in the presence of water, which is attributed to the hydration of the polymer chain and intermolecular hydrogen bonding. [Kunal Pal et al., 2013).

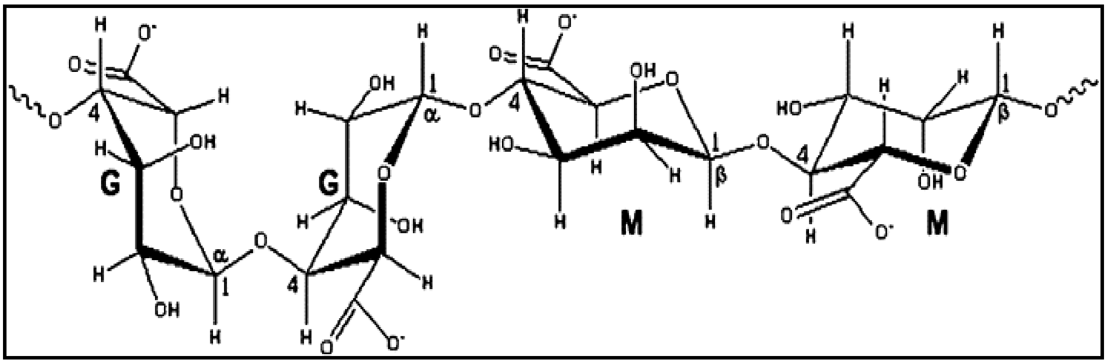

Figure 2. Chemical structure of alginic acid

Sodium alginate ($NaC_6H_7O_6$) is a linear polysaccharide derivative of alginic acid comprised of 1,4-β-d-mannuronic (M) and α-l-guluronic (G) acids. Sodium alginate is a cell wall component of marine brown algae, and contains approximately 30 to 60% alginic acid. The conversion of alginic acid to sodium alginate allows its solubility in water, which assists its extraction. Alginates could be also synthesized by using two bacterial genera, *Pseudomonas* and *Azotobacter.*

Sodium alginate polymers form gels in the presence of divalent and multivalent cations (except Mg^{2+}) by cross-linking of the carboxylate groups on the polymer backbone.

Alginates are used in various applications such as food manufacturing, pharmaceuticals and in textiles and cosmetics, particularly as an emulsifier, and are also used in dentistry to make molds. More recently, they have been studied extensively due to its tissue compatibility and use in tissue engineering, including the regeneration of skin tissue, cartilage, bone, pancreas, liver, muscles and nerves (Lee and Mooney, 2012), in addition to being used in the encapsulation of cells for the controlled release of drugs.

In the food industry, sodium alginate is used as thickener in sauces, syrups and toppings for ice cream. By thickening pie fillings with sodium alginate, softening of the pastry by liquid from the filling is reduced. Water-in-oil emulsions such as mayonnaise and salad dressings thickened with sodium alginate are less likely to separate into their original oil and water.

The aim of this study is to develop a sequential release material using two kind of natural polymers. The material is designed as a bead in two components. Inner part was prepared from Na-ALg hydrogel and this core was surrounded by gelatin shell. Both of the polymers are suitable for loading of various releasing substances. So it will be possible to reach the sequentially releasing of active ingredients depending on swelling/degradation behaviors of Na-Alg and gelatin polymers.

Method

Preparation of Hydrogel Beads

At the first step, a container was filled with Vaseline. A sequence of hollows was burrowed on its surface and the Vaseline bath was cooled at -18 ºC. After %10 of Na-Alg (Sigma-Aldrich) solutions was dropped into the cavities, this system was left at -18 ºC for 15 minutes. The solidified Na-Alg beads were placed in 100 mL of $CaCl_2$ (Merck) solution at four different percentages for crosslinking reaction for 1 h. The collected beads were washed several times with distilled water and dried first in air and then in a vacuum oven at 30°C (Pulat and Asıl 2009, Blanco et al. 2003). The average diameter was 0.8 ± 0.2 cm according to $CaCl_2$ percentages. The Na-Alg hydrogel beads were coated by immersing them into gelatin solution at different concentrations. The coated beads were dried at 30 ºC and they were stored for further use. The conditions were presented in Table 1.

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 Asıl 2009, Hsiue et al. 2001):

$$\text{Swelling (\%)} = \frac{M\text{w} - M\text{d}}{M\text{d}} \times 100 \qquad (1)$$

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.

Table 1. The preparation conditions of Na-Alg and gelatin coated Na-Alg hydrogel beads

Components / Hidrojel	Na-Alg (%)	$CaCl_2$ (%)	Gelatin (%)
A-1	10	5	-
A-2	10	10	-
A-3	10	20	-
A-4	10	30	-
AG-1	10	5	10
AG-2	10	5	15
AG-3	10	5	20
AG-4	10	10	10
AG-5	10	10	15
AG-6	10	10	20
AG-7	10	20	10
AG-8	10	20	15
AG-9	10	20	20
AG-10	10	30	10
AG-11	10	30	15
AG-12	10	30	20

Results and Discussion

The most common method to prepare hydrogels from an aqueous alginate solution is to combine the solution with ionic cross-linking agents, such as divalent cations (i.e., Ca^{2+}). The divalent cations are believed to bind solely to guluronate blocks of the alginate chains, as the structure of the guluronate blocks allows a high degree of coordination of the divalent ions. The guluronate blocks of one polymer then form junctions with the guluronate blocks of adjacent polymer chains in what is termed the egg-box model of cross-linking, resulting in a gel structure. Calcium chloride is one of the most frequently used agents to ionically cross-link alginate (Yong Lee and Mooney, 2012). A schematic mechanism about ionic crosslinking of Na-Alg was presented in Figure 3.

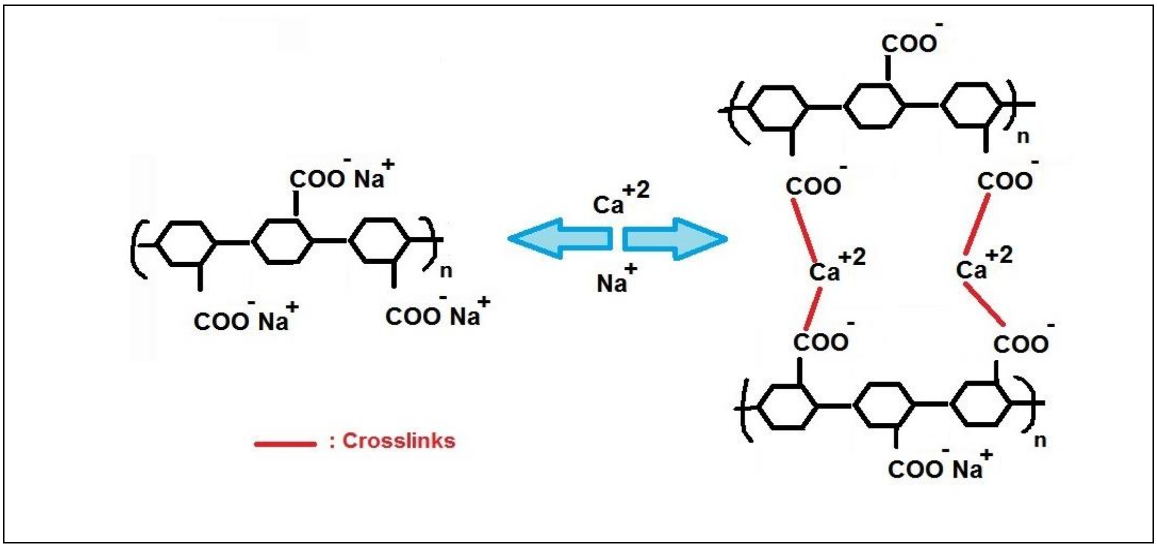

Figure 3. A schematic mechanism for ionic crosslinking of Na-Alg with Ca^{+2}

Swelling Behaviors

At the first step, swelling tests were carried for uncoated Na-Alg beads and the results were presented in Figure 4. In general, swelling behaviors of a hydrogel depend on composition, monomer ratio, ionic charge content, and polymerization route, type and density of crosslinker, and so on (El-Sherbiny *et al.*, 2005). In this study, Na-Alg hydrogels were prepared by changing crosslinker percentages. The swelling behaviors were inversely proportional with the amount of crosslinker. While $CaCl_2$ content increases, equilibrium swelling values of the hydrogels decreased. As the percentages of $CaCl_2$ are increased from 10% to 30%, swelling values decreased from 60% to 30%. The bead prepared using 5% of $CaCl_2$ solution dissolved in 3 hours.

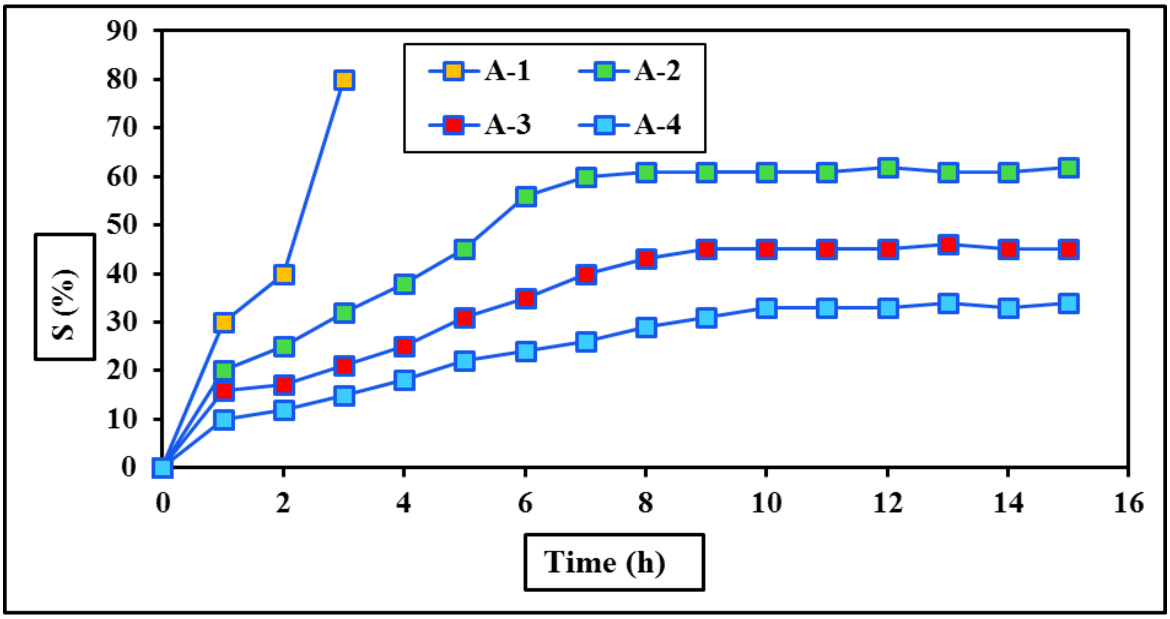

Figure 4. The swelling percentages of Na-Alg hydrogel beads with variation of time at 30°C, pH=7.0.

As indicated in Table 1, a series of coated beads were obtained using 10%, 20% and 30% of gelatin solutions. These hydrogel beads have a monolayer gelatin shells at different thicknesses.

The first groups of gelatin coated beads prepared using 5% of crosslinker are named as AG-1, AG-2 and AG-3. The variations of their swelling values with time were presented in Figure 5. Although the crosslinking of the beads prepared using 5% of $CaCl_2$ solution was relatively weak then other AG hydrogel series, gelatin shell onto their surfaces could preserve them from decomposing for a while. AG-1 bead prepared using 10% gelatin solution swelled rapidly by changing of time and then completely disappeared near 7 hours. Its conjugative bead, A-1, was dissolved in 3 hours. So, the decomposition time was extended two times for these weakest crosslinked beads. As the gelatin concentration was increased from 15% to 20%, AG-2 and AG-3, swelling values were slightly decreased. It was not observed any degradation for these beads.

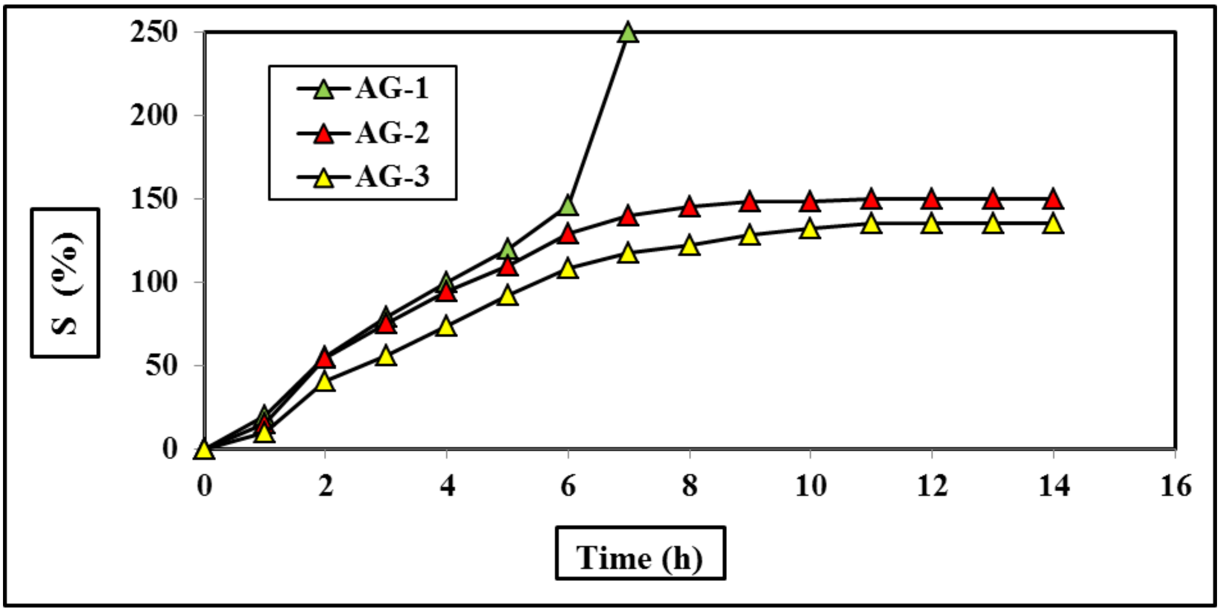

Figure 5. The swelling percentages of gelatin coated Na-Alg hydrogel beads prepared using 5% of crosslinker with variation of time at 30°C, pH=7.0.

The second groups of gelatin coated beads prepared using 10% of crosslinker are named as AG-4, AG-5 and AG-6. The variations of their swelling values with time were presented in Figure 6. Fast swelling was occurred at first hours and then equilibrium was reached near 10 h. All of the beads were stable and no any decomposition was observed. As gelatin concentration was increased from 10% to 20%, swelling value decreased from 150% to 130%.

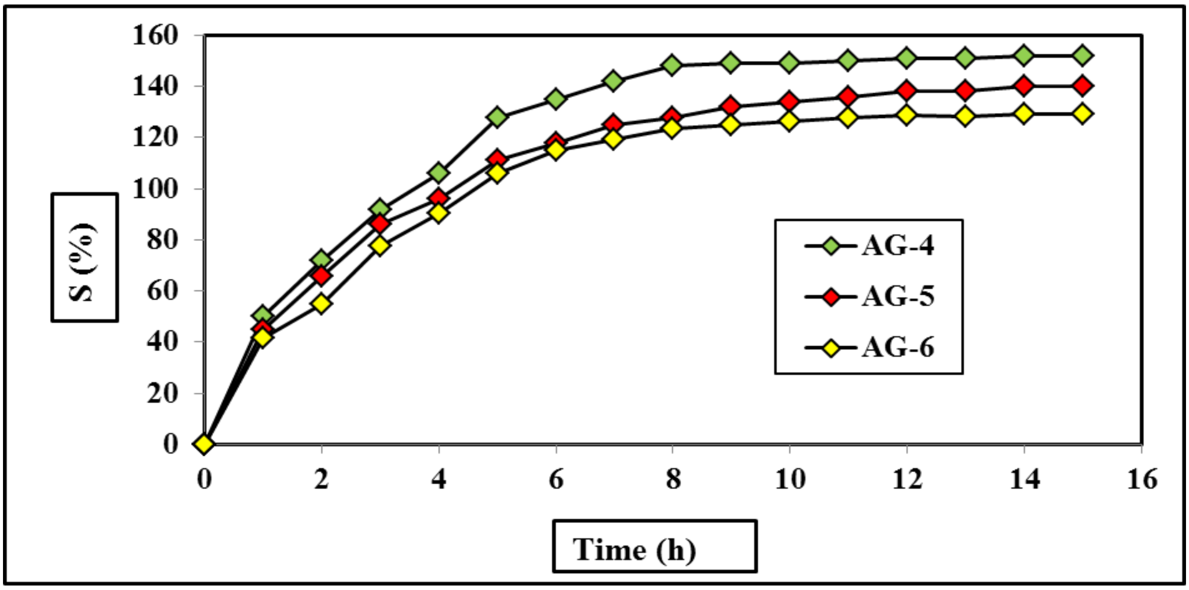

Figure 6. The swelling percentages of gelatin coated Na-Alg hydrogel beads prepared using 10% of crosslinker with variation of time at 30°C, pH=7.0.

The third groups of gelatin coated beads prepared using 20% of crosslinker were named as AG-7, AG-8 and AG-9. The variations of their swelling values with time were presented in Figure 7. The equilibrium swelling was reached near 10 h. The swelling percentages were slightly decreased for this group of beads depending on crosslinker concentration. As gelatin concentration was increased from 10% to 20%, swelling value decreased from 135% to 120%.

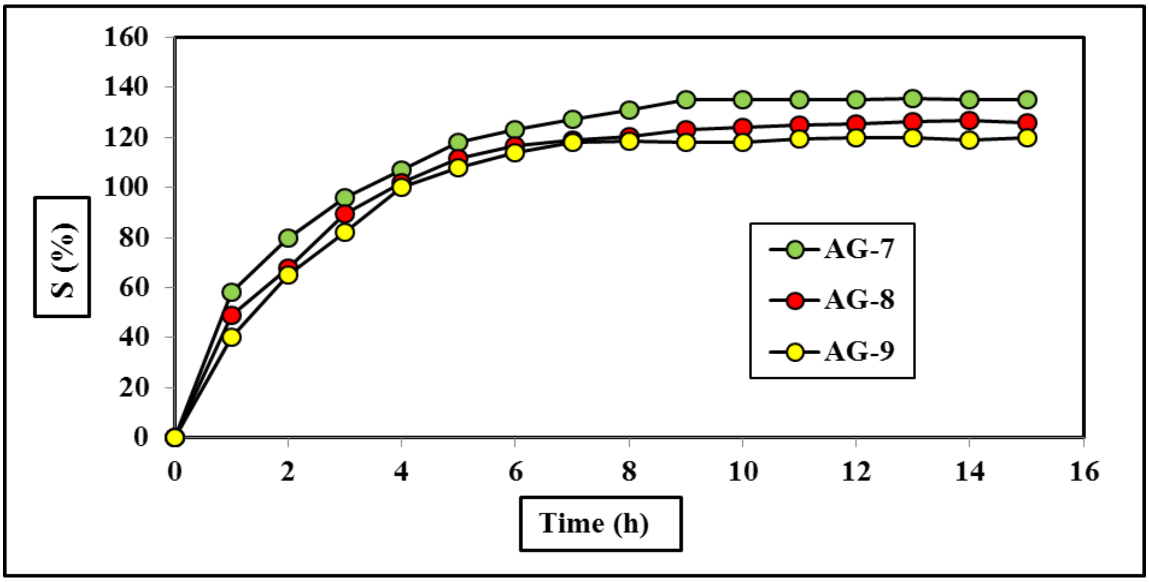

Figure 7. The swelling percentages of gelatin coated Na-Alg hydrogel beads prepared using 20% of crosslinker with variation of time at 30°C, pH=7.0.

The last groups of gelatin coated beads prepared using 30% of crosslinker were named as AG-10, AG-11 and AG-12. The variations of their swelling values with time were presented in Figure 8. The equilibrium swelling values were reached near 10 h. The swelling percentages were slightly decreased for this group of beads depending on crosslinker concentration. As gelatin concentration was increased from 10% to 20%, swelling value decreased from 130% to 105%.

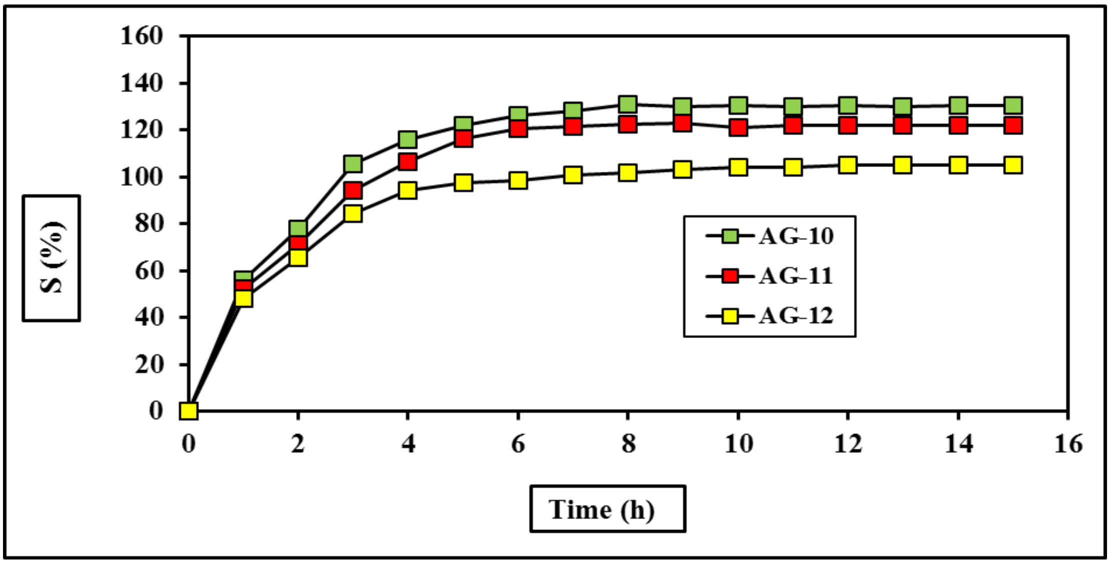

Figure 8. The swelling percentages of gelatin coated Na-Alg hydrogel beads prepared using 30% of crosslinker with variation of time at 30°C, pH=7.0.

Conclusion

The results of the present work indicate that the preparation of gelatin coated NaAlg hydrogels is possible. Various swelling percentages were obtained by changing of the crosslinking degree of Na-Alg and gelatin thicknesses. Both of the components, Na-Alg and gelatin could be used as separate loading medium for many different releasing substances. So it could be concluded that this prepared bead systems are satisfactory as sequentially releasing material.

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