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

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2019

Volume 6, Pages 32-38

ICRES 2019: International Conference on Research in Education and Science

Preparation and Characterization of Na-Alginate Hydrogel Beads

Mehlika PULAT
Gazi University

Duygu OZUKAYA
Gazi University

Abstract: A series of Na Alginate (Na-Alg) hydrogel beads were prepared by using ionic cross linking method. The beads were obtained by dropping of Na-Alg solution in chilled oil bath at -18 ºC first and then leaving the hardened beads into the cross linker solution. CaCl2 solution was used for ionic cross linking of Na-Alg molecules. While the concentration of Na-Alg solution was kept constant, crosslinking time was changed from 5 to 30 minutes. In general, longer crosslinking times negatively affect hydrogel formation. Swelling behaviors of the hydrogels were studied by changing time, temperature and pH. The higher crosslinker / polymer rates caused lower swelling values. It was determined that the swelling percentages regularly decreased with increasing the crossliking time. While the time increased from 5 to 30 minute, swelling percentages decreased from 158 % to 33 %. Temperature changing negligibly affects the swelling percentages. The prepared Na-Alg hydrogels could not be commended as temperature sensitive.

Keywords: Hydrogel, Na-Alginate, CaCl2, Swelling

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 num¬ber of industrial fields [Pulat and Asıl, 2009, Akalin and Pulat, 2018]. Because of their excellent characteris¬tics, 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. Natu¬ral polymers are a good choice to overcome this issue [Pulat M. & Uğurlu N., 2016].

Sodium alginate (Na-Alg) is sodium salt of alginic acid, a naturally occurring polysaccharide obtained from marine brown algae [Sapana A et al., 2014]. Alginic acid is a linear copolymer composed of D-mannuronic acid (M) and L-guluronic acid (G). Alginates are linear unbranched copolymers of -d-mannuronic acid (M) and -l-guluronic acid (G) units. The M and G monomers are 1→4 linked by glycosidic bonds, forming homopolymeric M- or G-blocks and heteropolymeric MG blocks. In the presence of polyvalent cations such as Ca 2+ or Al 3+, cross-linking occurs to form gels. The cations act as bridges between the anionic polymer chains, constituting junction zones, forming a hydrogel network. Ca 2+, a commonly used cross-linker, preferentially interacts with G blocks due to structurally favorable chelation sites formed by the corrugated chains.

Alginate is commonly employed as cross-linked matrices or coats in drug delivery systems. It is also extensively used to encapsulate living cells, such as yeasts for fermentation and pancreatic islets for clinical applications. Na-Alg is a biopolymer that is widely used as an encapsulation matrix due to its ability to form hydrogels upon

---

cross-linking. Its ability to gel under mild conditions makes alginate the polymer-of-choice in food, pharmaceutical and biotechnological applications. Its unique property of forming water insoluble calcium alginate gel through ionotropic gelation with calcium ions is a simple, mild and eco-friendly condition has made possible to encapsulate macromolecular bio-active agents like cell, enzyme, protein and vaccine.

The aim of this study is to develop a series of hydrogel beads via ionic gelation method using Na-Alg and CaCl2 as crosslinker. By changing the polymer/crosslinker ratio and crosslinking time, it is planned to obtain 21 types of hydrogel beads having different swelling values.

Methods

Preparing of Hydrogel Beads

A series of Na-Alg hydrogel beads were prepared by using ionic cross linking method (Reddy S. Giridhar et al, 2011). Aqueous Na-Alg solutions at different concentrations were dropped into chilled oil bath at -18 ºC and waited for 15 minutes. Then the hardened beads were collected and left into 100 mL of crosslinker solution. CaCl2 (Sigma-Aldrich) solution was used for ionic cross linking of Na-Alg molecules. The mixture was gently stirred with a speed of 60 rpm. This procedure was carried out by changing the concentrations of Na-Alg and CaCl2 solutions and crosslinking time. Two of the hydrogels, A-6 and A-7 were obtained by intermittently crosslinking. At this step, the beads were exposed with crosslinker solution for 5+5 and 5+5+5 minutes. After first 5 minute, the beads were taken out from crosslinker medium, waited for 5 minutes and then left again into crosslinker solution. Preparation conditions were summarized into Table 1.

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

Hydrogel	Na-Alg (%)	CaCl$_2$ (%)	Crosslinking Time (min)
A-1	10	10	30
A-2	10	10	20
A-3	10	10	15
A-4	10	10	10
A-5	10	10	5
A-6	10	10	5+5
A-7	10	10	5+5+5
A-8	10	5	5
A-5	10	10	5
A-9	10	15	5
A-10	10	20	5
A-13	10	35	5
A-16	8	5	5
A-19	6	5	5

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 30 °C.

The schematic representation was given in Figure 1.

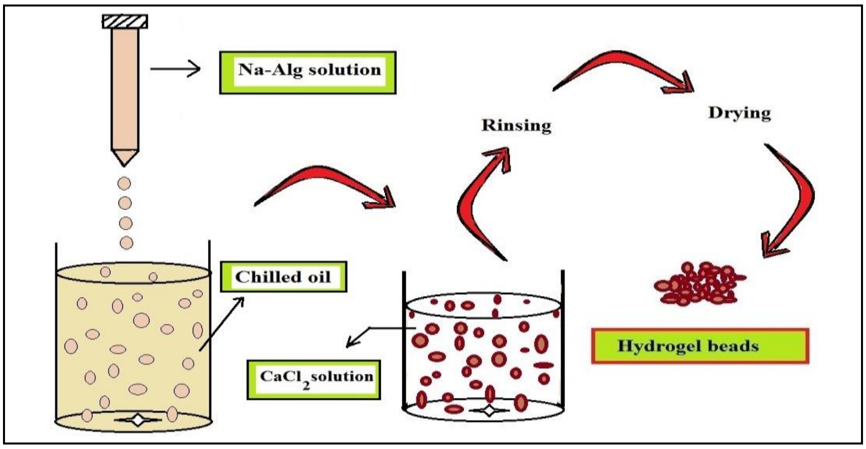

Figure 1 Preparation of hydrogel beads by dropping gelation method

Swelling Tests

Swelling tests of hydrogel beads were gravimetrically carried out in three steps. In the first step, the weighed dried hydrogels were immersed in 100 mL of distilled water at 30^{0}C. Swollen beads removed from the swelling medium at regular intervals. Then, they were dried superficially with a filter paper, weighed, and placed into the same bath. The tests were performed until constant weight was reached. The swelling percentages (%) of hydrogels were calculated from [Pulat and Asıl 2009, Hsiue et al. 2001]:

$$S (\%) = \frac{Mw - Md}{Md} \times 100 \qquad (1)$$

where Mw is the wet weight of the sample and Md is the dry weight of the sample before swelling.

In the second step, it is decided to work with only 5 types of hydrogel beads for determining the temperature effect on swelling behaviors. According to the swelling values A-5, A-8, A-9, A-10 and A-13 hydrogls were chosen. The dried hydrogels were swollen in distilled water (pH = 7.0) at different temperatures ranging from 10 to 60^{0}C to determine the effect of temperature on swelling behaviors. Equilibrium swelling time was kept as 12 hours. The swollen hydrogels were taken out from swelling medium, dried, and weighed. Swelling values were calculated from Equation 1.

In the third step, the tests were repeated for 2 hydrogels used in the second step. The dried hydrogels were immersed in different Britton–Robinson buffer (BRB) solutions at various pH values (from 2.0 to 12.0) to investigate the effect of pH on the swelling behaviors. At the end of 12 h incubation, the swollen hydrogels were taken out from swelling medium, dried, and weighed [Pulat M., Akalın G.O.,2013]. Swelling values were calculated using Equation 1.

The reproducible results for all swelling studies were obtained with triplicate measurements.

Results and Discussion

13 of Na-Alg 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 charge ion to initiate cross linking [Sapana A et al., 2014]. CaCl2 has often been used to prepare Na-Alg particles because of its nontoxic and ionic interactions properties. Na-Alg polymers and CaCl2 are a favorable pairs for crosslinking mechanism. Na-Alg molecules are ionized in water by losing of their Na+ ions and meanwhile Ca+2 ions cross-linked two different polymer rings. The positive charged Ca+2 ions can react with negative charged alginates molecules via electrostatic attraction to form ionic crosslinked networks. A schematic mechanism about ionic crosslinking of Na-Alg was presented in Figure 2 [Kühbeck D et al., 2015].

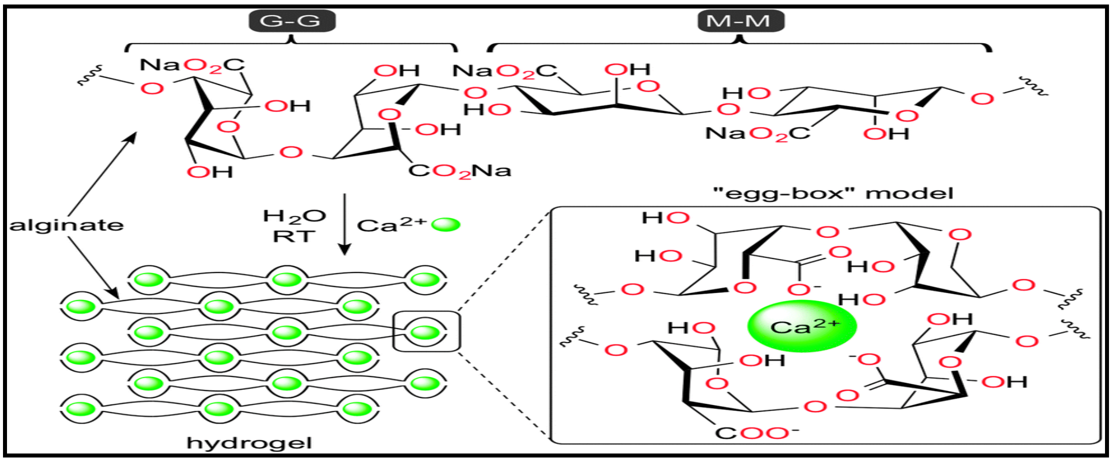

Figure 2. A schematic presentation for crosslinking mechanism of Na-Alg polymers with Ca+2

Swelling Results

All of the swelling percentages belong to hydrogel beads were calculated from Equation 1. As explained in Experimental Part, the beads could be divided into four groups.

In the first group, the effect of crosslinking time to S% was investigated. A-1, A-2, A-3, A-4, A-5, A-6 and A-7 beads were prepared by changing crosslinking time while polymer and crosslinker concentration were kept constant. As indicated in Table 2, crosslinker/polymer (C/P) rate was 1.0 for these samples. Swelling variations with time were given in Figure 3. The swelling increased with time initially and then remained constant at close to 8 h. As crosslinking time increased from 5 to 30 minutes, swelling values dramatically decreased. S% values were determined to be 158% for the most swollen hydrogel A-5, and 33% for the least swollen hydrogel A-1. In this step, A-6 and A-7 hydrogels were prepared by intermittent exposing of the beads with crosslinker. The results show that not any considerable amounts of variation on swelling values were obtained.

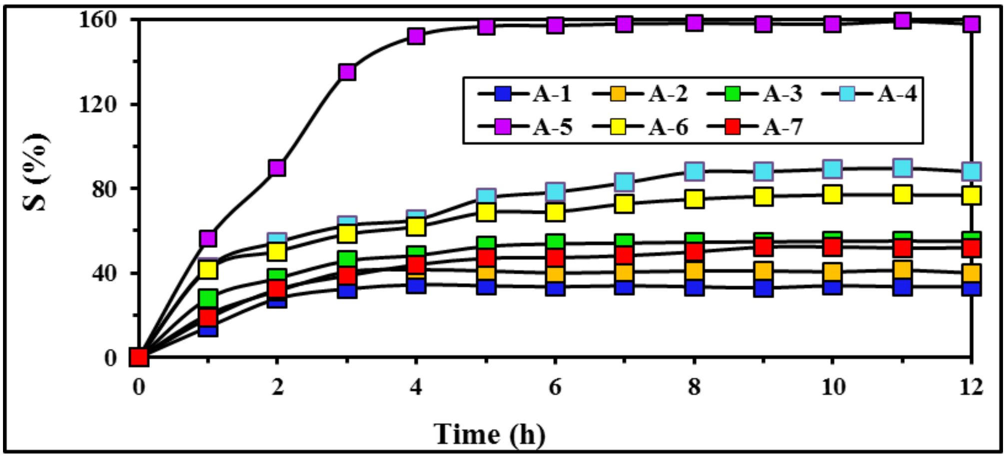

Figure 3. The variations of S% values of A-1 – A-7 beads with time (30°C; pH=7.0)

According to these results, it is decided to keep the crosslinking time as 5 minutes for other three steps.

Table 2. C/P and swelling values of Na-Alg hydrogel beads

Hydrogel	Na-Alg (%)	C/P	Swelling (%)
A-1	10	1.00	33.5
A-2	10	1.00	40.6
A-3	10	1.00	54.6
A-4	10	1.00	87.9
A-5	10	1.00	157.8
A-6	10	1.00	76.3
A-7	10	1.00	52.3
A-8	10	0.50	Dispersed
A-5	10	1.00	157.8
A-9	10	1.50	75.6
A-10	10	2.00	55.4
A-13	10	3.50	1.9
A-16	8	0.62	88.5
A-19	6	0.83	45.2

In the second group, the effect of crosslinking concentration to S% was investigated. A-5, A-8, A-9, A-10 and A-13 beads were prepared by changing crosslinker percentages while polymer concentration and crosslinking time were kept constant. C/P rates were changed from 0.5 to 3.5.

As seen from Figure 4, the swelling increased with time initially and then remained constant at close to 8 h. While crosslinker concentration increased from 5% to 35%, swelling values decreased. S% values were determined to be 158% for the most swollen hydrogel A-5, and 2% for the least swollen hydrogel A-13. 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]. As C/P ratio increased from A-

8 to A-13, 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. A-8 hydrogel which prepared at the minimum C/P rate dispersed in a short time.

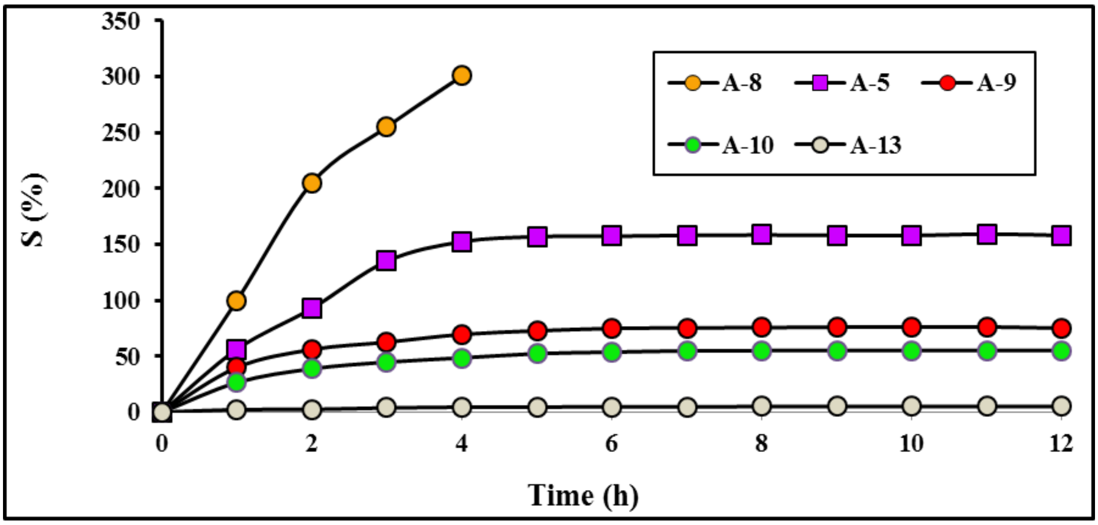

Figure 4. The variations of S% values of A-8 – A-15 beads with time (30°C; pH=7.0)

In the last group, 2 types of hydrogel beads were prepared by minimizing of the polymer amount to obtain various C/P rates. In general, higher crosslinker amounts per Na-Alg causes less S% values. It is observed that, as C/P ratio increased from A-16 to A-19, S% values sharply decreased. The results were presented in Figure 5.

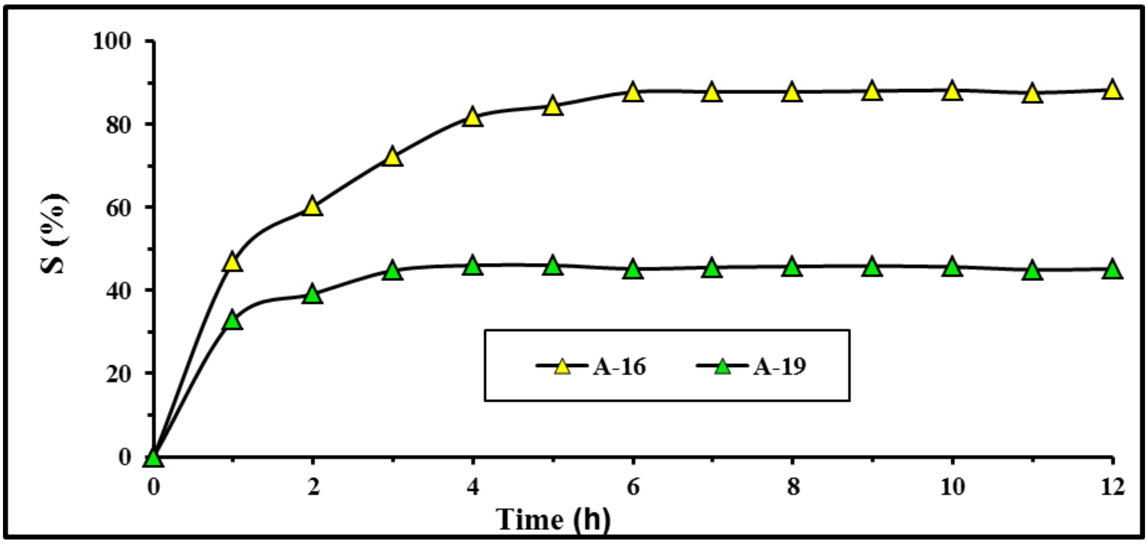

Figure 5. The variations of S% values of A-16 – A-19 beads with time (30°C; pH=7.0)

The variations of swelling values with temperature at pH 7.0 and 12 h are presented in Figure 6. Swelling percentages slightly increased with temperature and then remained constant. The most swollen bead is A-5. As the temperature increases, thermal mobility of the polymer chains increases and H-bonds were broken, and hydrogels can easily swell [Esposito, E et all, 1996]. A-8 rapidly degraded over 20^{0}C. It is commended that, Na-Alg hydrogels are suitable for using in wide temperature ranges.

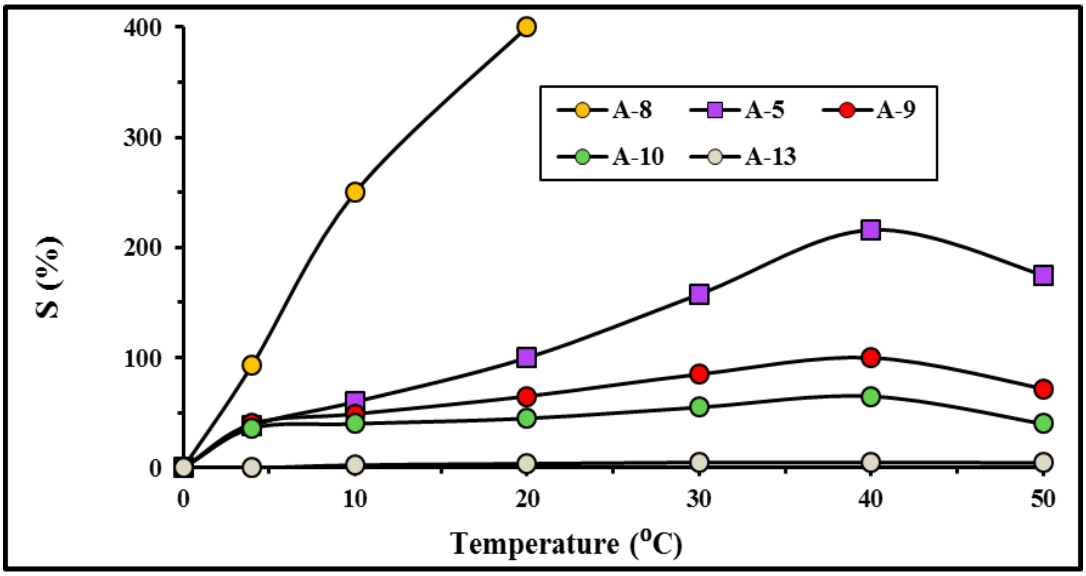

Figure 6. The variation of S% values with temperature at pH = 7.0 and 12 h.

Figure 7 presents the variation of S% values of hydrogels with pH at 30°C and 12 h. As the pH is increased from 2 to 7, a sharp increment was observed. At low pH's, ionization of Na^+ is not easy and it is thought that the polymer molecules are in neutral position. While pH values rise, ionization could be active and swelling values increases. The ionized negatively charged pendant groups on the polymer chains caused repulsion leading to swelling. As swelling pressure increased, hydrogel expanded and thereby maximizes the repulsion between the ionized groups. After a summit was observed near pH= 7, the swelling sharply decreased. A-5 hydrogel degraded near pH=8.

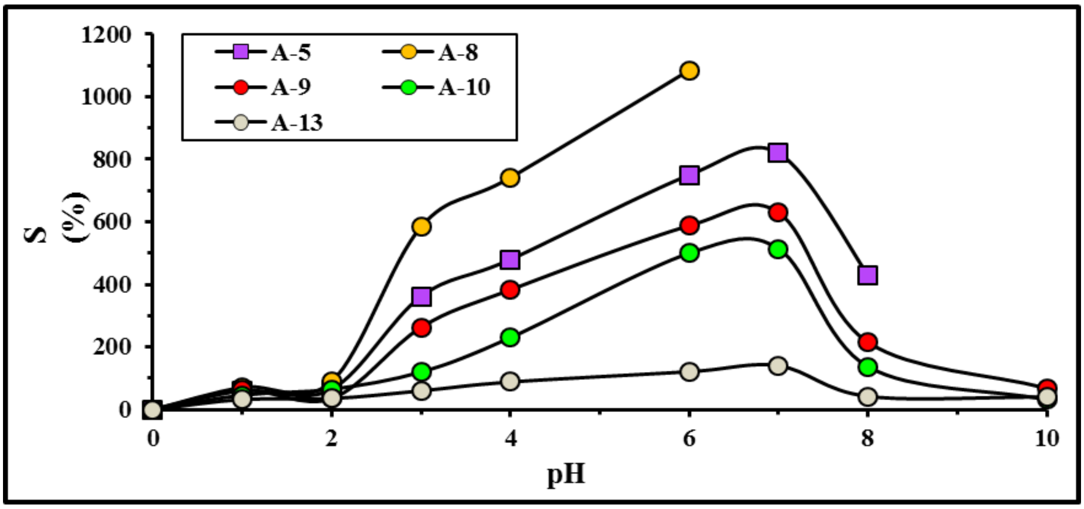

Figure 7. The variation of S% values with pH at 30°C, 12 h

Conclusion

The results of the present work indicate that the Na-Alg hydrogels in a wide range of swelling behaviors could be prepared by dropping method. It can be concluded that the hydrogel beads obtained in this study is much promising in utilizing a natural resource like Na-Alg in the production of matrix material which could significantly reduce the production costs and offering a quite environmental friendly alternative technique.

References

El-Sherbiny I.M., Lins R.J., Abdel-Bary E.M., Harding D.R.K.(2005). Preparation, characterization, swelling and in vitro drug release behaviour of poly(*N*-AG-chitosan) interpolymeric pH and therm.-responsive hydrogels. *Europ. Polym. J. 41*, 2584-2591.

Esposito, E., Cortesi, R., Nastruzzi, C., (1996). Gelatin microspheres: Influence of preparation parameters and thermal treatment on chemico-physical and biopharmaceutical properties", ***Biomaterials,*** 17: 2009–2020.

Akalin G.O and Pulat M. (2018). Preparation and Characterization of nanoporous Sodium Carboxymethyl Cellulose Hydrogel Beads, *Journal of Nanomaterials*, Volume 2018, 1-12.

Hsiue, G.H., Guu, J.A., and Cheng, C.C. (2001) Poly (2-hydroxyethyl methacrylate) film as a drug delivery system for pilocarpine. *Biomaterials*, 22:1763–1769.

Kühbeck, D., Mayr, J., Häring, M., Hofmann, M., Quignard, F., Díaz, D. D. (2015). Evaluation of the nitroaldol reaction in the presence of metal ion-crosslinked alginates'' *New Journal of Chemistry*, *39*(3), 2306-2315.

Pulat M., Akalın G.O. (2013). Preparation and characterization of gelatin hydrogel support for immobilization of *C. Rugosa* lipase. *Artif. Cells Nanomed. Biotechnol. 41*, 145-151.

Pulat M., Asıl D. (2009). Fluconazole release through semi-ipn hydrogels based on chitosan, AA, and citraconic acid. *J. Appl. Polym. Sci. 113*, 2613-2619.

Pulat M. & Uğurlu N. (2016). Preparation and characterization of biodegradable gelatin-PAAm-based IPN hydrogels for controlled release of maleic acid to improve the solubility of phosphate fertilizers, *Soft Materıals*, , 14:4, 217–227.

Pulat M., Yoltay N. (2016). Smart fertilizers: preparation and characterization of gelatin-based hydrogels for controlled release of MAP and AN fertilizers**,** *Agrochimica*, 60:4, 249-261.

Reddy S. Giridhar and Saxena Pandit Akanksha (2011). Swelling Behavior of Calcium–Ions Crosslinked Bipolymeric Sodium Alginate–Lignosulphonic Acid Blends, International Journal of Polymeric Materials, 60:1123–1129.

Sapana P. A., Paraag S.G., Shrivastav B and Sharma P. (2014). Ionotropic gelation: a promising cross linking technique for Hydrogels, *Research and reviews: journal of pharmaceutics and nanotechnology*, Volume 2, Issue 1, 1-6.

Author Information

Mehlika Pulat
Gazi Üniversitesi, Fen Fakültesi,
Kimya Bölümü–Teknikokullar, Ankara/Türkiye
Contact E-mail: *mpulat@gazi.edu.tr*

Duygu Ozukaya
Gazi Üniversitesi, Fen Fakültesi,
Kimya Bölümü–Teknikokullar, Ankara/Türkiye