Polymer(Korea), Vol. 40, No. 1, pp. 63-69 (2016)
http://dx.doi.org/10.7317/pk.2016.40.1.63

ISSN 0379-153X(Print)
ISSN 2234-8077(Online)

가교제가 Sodium Alginate와 Lignosulphonic Acid의 블렌드에 미치는 영향

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(2015년 8월 7일 접수, 2015년 10월 13일 수정, 2015년 10월 17일 채택)

Effects of Crosslink Agents on Sodium Alginate and Lignosulphonic Acid Blends

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(Received August 7, 2015; Revised October 13, 2015; Accepted October 17, 2015)

Abstract: Blends of sodium alginate (SA) and lignosulphonic acid (LS) has been prepared in the ratio of 80/20. The prepared blends were crosslinked using chlorides of calcium, barium, strontium and aluminum. The crosslinking of blends was done for different time intervals and then these blends were subjected for swelling studies in aqueous medium of pH 7.4. The observations indicate that the crosslinking is diffusion controlled and is affected by the size of metal ion and the type of alginate used. The improved swelling time for crosslinked blends in aqueous medium supports the fact that the stability under physiological conditions of the blends is improved due to crosslinking with the metal ions. Calcium chloride and barium chloride forms strong crosslink with the blend. Calcium ion crosslinked blends can be considered suitable for biomedical drug applications. The investigations on crosslinked blends using FTIR, SEM, XRD and EDAX are in close agreement with swelling results.

Keywords: sodium alginate, lignosulphonic acid, swelling, crosslinking.

Introduction

Natural polymers are used in many forms for the controlled delivery of drugs mainly because of their availability, stability and biocompatibility. Controlled drug release has other benefits like reduced frequency of drug dosing, limited fluctuation within the therapeutically effective level, reduced side effects, reduced toxicity, improved patient compliance etc.[1-5] Sodium alginate, a biopolymer from marine brown algae is widely used in pharmaceutical formulations. The polymer contains β-D-mannuronic acid (M) and its C-5 epimer α-*L*-guluronic acid (G) as monomers bonded together by 1-4 glycosidic linkages. The structure and biocompatibility of alginate depends on the G:M ratio. In general alginates are biodegradable, nontoxic and biocompatible and have the ability to form hydrogels under mild conditions.[6] The poor mechanical stability of alginate hydrogels under physiological conditions[7,8] is an unwanted attribute for the controlled drug release system. It can be improved by blending with a suitable polymer and then crosslinking the blend with crosslinking agents. Crosslinking forms strong networks and imparts desired mechanical property in a natural polymer.[9-12] Crosslinking with glutaraldehyde is achieved through chemical method where covalent bond formation takes place. Also glutaraldehyde is toxic so it is not a suitable candidate for biomedical applications. Crosslinking with the metal ion on the other hand is physical (due to ionic bond formation). Divalent metal ions such as Ca^{+2}, Ba^{+2}, Sr^{+2} form hydrogels with SA by crosslinking while no gel formation is observed with Mg^{+2}, Na^{+} and K^{+} ions. Crosslinking of SA with calcium chloride gives calcium alginate which is water insoluble.[13] In alginates the physical crosslinking is preferred to avoid the use of crosslinking agents which are often toxic compounds and have to be removed or extracted from gels before use. Crosslinking of sodium alginate (SA) is carried out at room temperature and physiological pH medium which is advantageous for encapsulating living cells, proteins, and drugs for control release.[14]

Lignosulphonic acid (LS), is natural biodegradable polymer and a plant by-product from paper industry. LS is well known as a super plasticizer. Biocompatibility and water solubility

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makes it suitable for blending with SA under mild conditions. Also the blends are safe for biomedical applications. Blends of SA/LS were prepared in various proportions and improvement in the properties of SA was reported in our previous works.[15] The SA/LS blends in the 80/20 ratio have shown suitable for the controlled release of drugs.[16]

In this article, SA/LS (80/20) blend was crosslinked using different crosslinking agents such as calcium chloride, barium chloride, strontium chloride and aluminum chloride at room temperature. The crosslinked blends were investigated for swelling in aqueous medium of pH 7.4. At pH 7.4 the carboxylate groups in alginates are ionized and the electrostatic repulsions between –COO- groups increases swelling efficiency. Whereas, in acidic or basic media, ionic strength of the medium is increased and the repulsions between groups is shielded by the ions present in the solution resulting to poor swelling.[17] The crosslinked blends were further investigated using FTIR, XRD, SEM and EDAX for chemical interactions, crystallinity and morphological changes.

Experimental

Chemicals. SA (seaweed product) and LS (plant byproduct) were purchased from Sigma Aldrich Bangalore, India. Calcium chloride, barium chloride, strontium chloride and aluminum chloride were obtained from Ranbaxy Chemicals, India. Aqueous solutions of chemicals were prepared using distilled water. All chemicals were of analytical grade and used without any further purification.

Preparation of Sodium Alginate (SA) and Lignosulphonic Acid (LS) Blends. 2% w/v aqueous solutions of SA and LS were prepared in 80/20 ratio and stirred using magnetic stirrer for 30 min. The solution was casted on to the glass plate and heated in hot air oven at 60 ºC for 3 days. Films of thicknesses 0.2±0.05 mm thus obtained were cut in circular shape of diameter 1.9±0.1 cm as reported in reference.[18]

Preparation of SA/LS Crosslinked Blends. The blends SA/LS (80/20) were crosslinked with calcium chloride, barium chloride, strontium chloride and aluminum chloride. Crosslinking was done by keeping the blend films in 2% aqueous solution of each of the crosslinking agents separately for different intervals of time. The crosslinked films are then wiped with tissue paper and dried in a dust free chamber at 30 ºC till constancy in weight was observed.

Swelling Behavior of the Blends. The crosslinked blends were subjected to swelling in pH 7.4 medium at room temperature. Blends were then removed from the aqueous medium at regular interval of time; their surface was wiped with tissue paper and then weighed. The weight change was calculated using the relation:

$$\% \text{ of Swelling} = (W_2 - W_1)/W_1 \times 100$$

Where, W_2 is final weight and W_1 is initial weight of the hydrogels.

Thermo Nicolet, Avatar 370. This FTIR instrument was used in the spectral range of 4000-400 cm^{-1} to detect the nature of crosslinking between SA/LS blends and the metal ions. The IR spectrum of uncrosslinked and crosslinked SA/LS blends were taken using this instrument.

Bruker D8 X-ray Diffractometer. This instrument was used to investigate the crystallinity of uncrosslinked and crosslinked SA/LS blends. The X-ray of wavelengths (1.548 Å) were generated by a CuKα source. The diffraction angle between 5º to 60º was used to identify the change in the crystallinity in blends due to crosslinking.

JEOL Model JSM - 6390LV, JEOL Model JED -2300 (SEM-EDS). SEM– energy dispersive spectrometer is used for qualitative elemental analysis of blend films. X-ray line scans and mapping was performed with SEM-EDS combination. The surface morphology and chemical analysis of uncrosslinked and crosslinked SA/LS blends for 30 min were investigated using this instrument.

Results and Discussion

Effect of Different Crosslinking on SA/LS (80/20) Blends. In order to study the effect of different crosslinking agents, the SA/LS (80/20) blends are crosslinked using different crosslinking agents for various intervals of time (10, 20, 30 and 60 min). The swelling due to water uptake of crosslinked blends was investigated by keeping the samples in an aqueous medium of pH 7.4.

Effect of Calcium Chloride Solution (2% $CaCl_2$): Calcium ion's ability to bind with the anionic part of some polymers and form hydrogels under mild conditions makes it a preferred choice to crosslink alginates for biomedical applications.[19-27] Also it is non-toxic, and its crosslinked films or beads obtained are compact in nature.[28-31] In case of SA the crosslinking is due to ionic interaction between Ca^{2+} ion and the carboxyl groups of the guluronic acid residues of two neighboring alginate chains as shown in Figure 1.

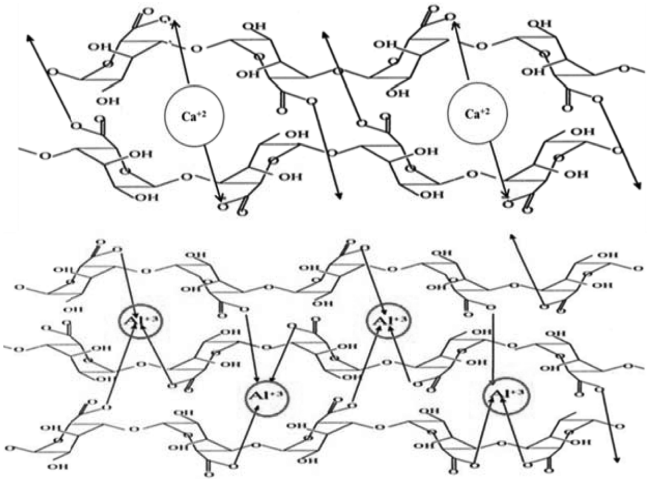


Figure 1. Expected mechanism of reaction between calcium and aluminum cations on sodium alginate matrices.[21]

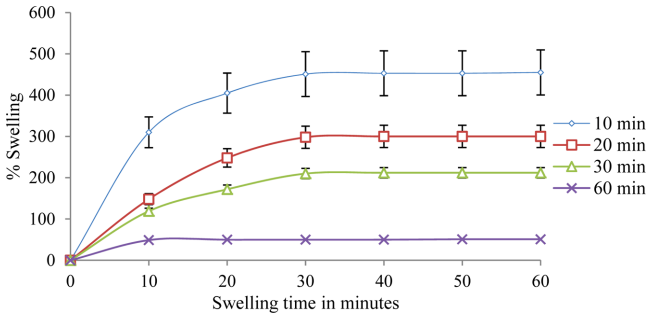


Figure 2. Effect of 2% $CaCl_2$ solution on swelling behavior of SA/LS (80/20) blends crosslinked for 10, 20, 30 and 60 min. The error bar represents the ±SD (n=3).

The blends crosslinked for 10, 20, 30 and 60 min show swelling up to 450%, 280%, 220% and 55%, respectively (Figure 2). It is clear that the swelling tendency decreases as the time of crosslinking increased. The swelling experiment is conducted till 1 h; later the blend film start degrading and turns sticky.

Effect of Barium Chloride Solution (2% $BaCl_2$): It is noticed that blends appear to be physically stable in aqueous medium even after 1 h and no sign of degradation is observed till 2.5 h. The percentage swelling observed for blends crosslinked for 10, 20, 30 and 60 min is 201%, 68%, 50%, and 40%, respectively (Figure 3). A drastic reduction in swelling for barium ion crosslinked blends is observed as compared to the calcium ion crosslinked blends which is in consistence with the earlier reports that crosslinking with Ba^{+2} forms stronger films.[6,14,32]

Effect of Strontium Chloride Solution (2% $SrCl_2$): The

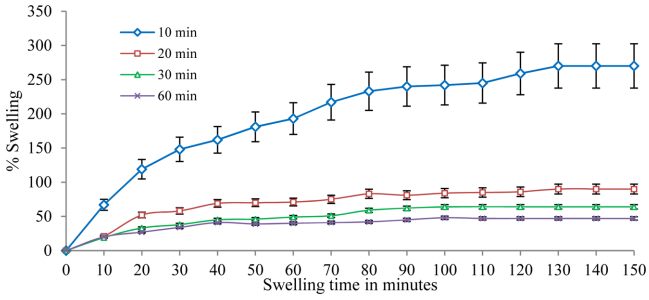


Figure 3. Effect of 2% $BaCl_2$ solution on swelling behavior of SA/LS (80/20) blends crosslinked for 10, 20, 30 and 60 min. The error bar represents the ±SD (n=3).

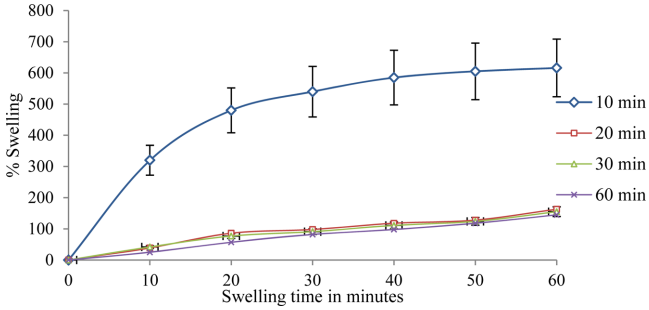


Figure 4. Effect of 2% $SrCl_2$ solution on swelling behavior of SA/LS (80/20) blends crosslinked for 10, 20, 30 and 60 min. The error bar represents the ±SD (n=3).

study shows that 10 min crosslinked blend swells up to 616±20%, while the 20 and 30 min crosslinked blends swell about 145±8% (Figure 4) which is an indication of poor crosslinking. Further increase in crosslinking time has no effect on swellings.

Effect of Aluminum Chloride Solution (2% $AlCl_3$): The blends crosslinked with Al^{+3} ions show poor swelling as degradation of blends start after 10 min of being kept in the swelling medium. Figure 5 shows that trivalent aluminum chloride crosslinked blends swell little as compared to bivalent cations (Ca^{2+}, Ba^{2+} and Sr^{2+}).

These observations can be related to the mechanism of bonding of calcium, barium, strontium and aluminum cations with the alginate anions. The metal ions combine to the carboxylate ion of the alginate through ionic bond. Bivalent calcium, barium and strontium ions bond in a planar two dimensional manner as represented in egg box model[33] (Figure 6) while the trivalent aluminum cation forms a three dimensional structure with SA. The crosslinking occurs in two different planes of the film and at the same time it makes the alginate framework more compact.[34] The small size of alu-

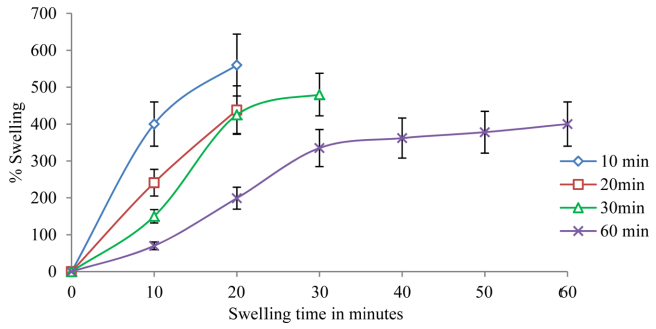


Figure 5. Effect of 2% $AlCl_3$ solution on swelling behavior of SA/LS (80/20) blends crosslinked for 10, 20, 30 and 60 min. The error bar represents the ±SD (n=3).

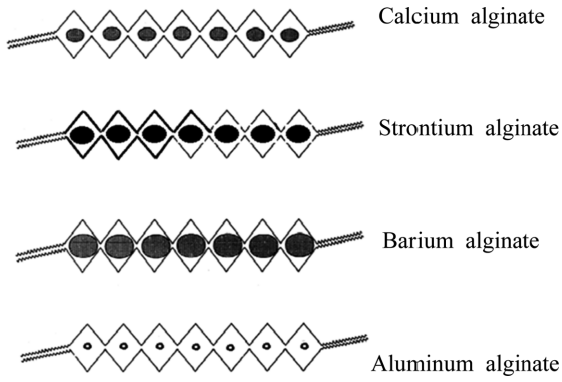


Figure 6. Egg box model representing calcium, barium, strontium and aluminum ions reacting with alginates.

minum cation (0.58 Å)[35] facilitates its diffusion into the body of the film without crosslinking on the surface and thus results in a poor crosslinking.

X-ray Diffraction Studies of Crosslinked SA/LS (80/20) Blends. The XRD spectra of uncrosslinked and crosslinked SA/LS (80/20) blends are shown in Figure 7. The diffractograms of uncrosslinked and crosslinked (Ca^{+2}, Ba^{+2} and Sr^{+2}) SA /LS blends indicate their semi crystalline nature. The shift in peaks confirms that Na^+ ions in SA/LS (80/20) blends are replaced by other ions such as Ca^{2+}, Ba^{2+} and Sr^{2+} while crosslinking. The XRD patterns of Al^{3+} ion crosslinked blends are almost same as uncrosslinked one, which confirms a poor crosslinking.

Fourier Transform Infra-Red Studies of Crosslinked SA/LS (80/20) Blends. The FTIR spectrum of the uncrosslinked and the crosslinked SA/LS blends are shown in Figure 8. The spectrum of uncrosslinked blends show the C=O stretching peak of the carboxylic group at 1651 cm^{-1} and the characteristic peak of free hydroxyl group the range of 3188-

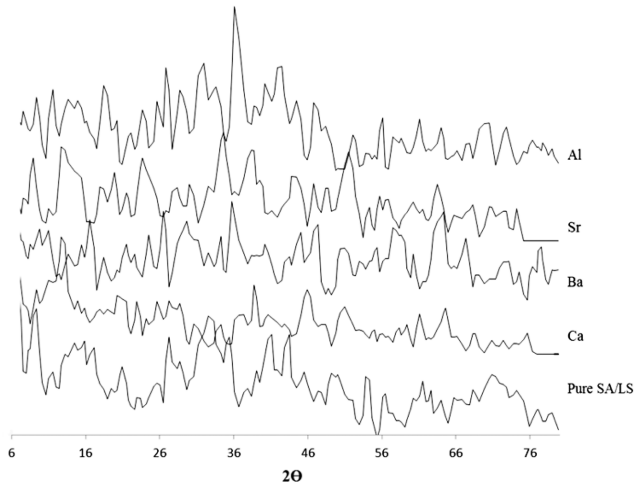


Figure 7. X-Ray diffractograms of uncrosslinked and crosslinked SA/LS (80/20) blends (Ca^{2+}, Ba^{2+}, Sr^{2+} and Al^{3+} ion).

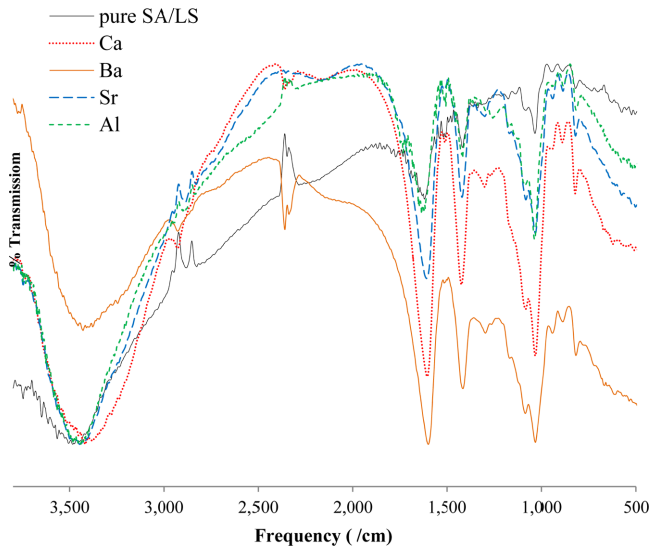


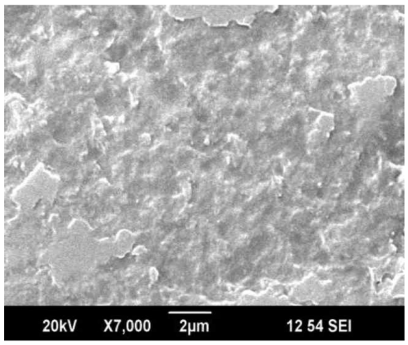
Figure 8. FTIR spectra of uncrosslinked (SA/LS:80/20) and Ca^{2+}, Ba^{2+}, Sr^{2+} and Al^{3+} ion crosslinked SA/LS (80/20) blends.

3583 cm^{-1}. The FTIR spectra indicates no change in the position of the peak for the C=O group in the blends. The change intensity of bands at C=O group indicates the extent of crosslinking of ions with SA in the order of $Ba^{2+} > Ca^{2+} > Sr^{2+} > Al^{3+}$.

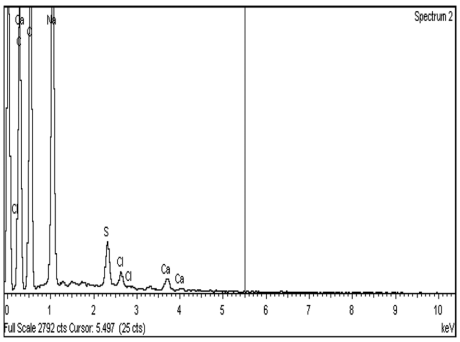
Scanning Electron Microscope Studies of Crosslinked SA/LS Blends. The surface morphological studies were made, using SEM. Images of uncrosslinked and crosslinked SA/LS (80/20) blends are shown in Figure 9. The surface appears to be plane. Observed cracks on the surface are due to fracturing of the films before scanning. A large number of Ba^{2+} and Sr^{2+} ions are just attached to outer surface of the film and are not able to penetrate in to film (Figure 9.3 and 9.4). Ca^{2+}

ions are able to diffuse inside the blend and partial crosslinking with SA is observed on the surface (Figure 9.2). Smaller Al^{3+} ions are able to diffuse into the film without crosslinking on the surface (Figure 9.5).

The extent of crosslinking for SA/LS (80/20) blends, with the various metal ions is also supported by EDAX spectrum using SEM. The atomic percentages are estimated in each case (Figure 9.1 to Figure 9.5). The atomic percentage of Ca^{2+}, Ba^{2+} and Sr^{2+} ions in the crosslinked SA/LS blends for 30 min is 8.88%, 2.85%, and 1.56%, respectively. The Al^{3+} ions uptake



(a)

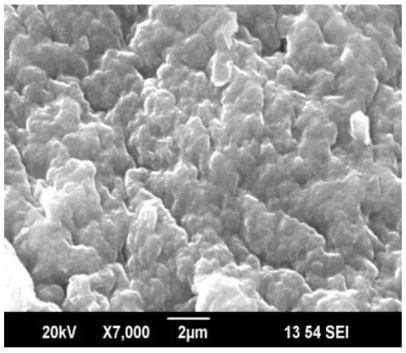


(b)

Element	At %
C	49.67
O	40.44
Na	8.9
S	0.59
Cl	0.21
Ca	0.19
Total	100

(c)

Figure 9.1. Scanning electron micrograph: (a) Uncrosslinked SA/LS (80/20) blend; (b) EDS spectrum of uncrosslinked SA/LS (80/20) blend; (c) atomic percentage from EDS.



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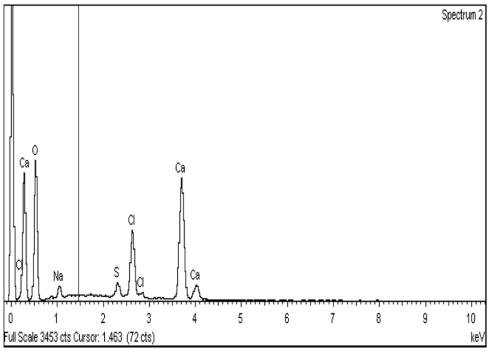
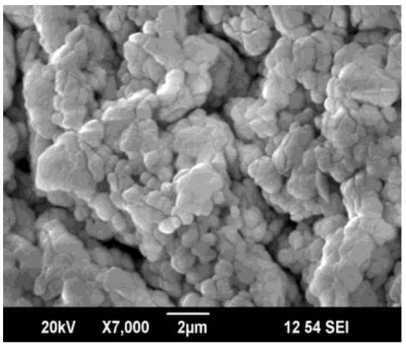


(b)

Element	At %
C	44.6
O	38.51
Na	2.03
S	0.88
Cl	4.58
Ca	8.88
Total	100

(c)

Figure 9.2. Scanning electron micrograph: (a) Ca^{2+} ion crosslinked SA/LS (80/20) blend; (b) EDS spectrum of Ca^{2+} ion crosslinked SA/LS (80/20) blend; (c) atomic percentage from EDS.



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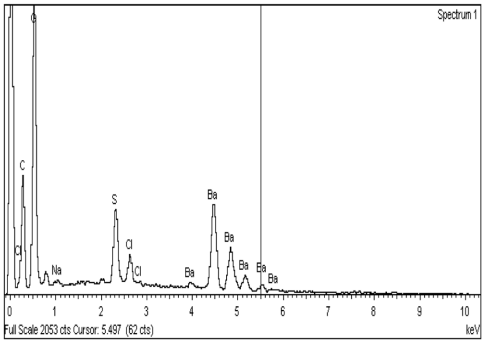
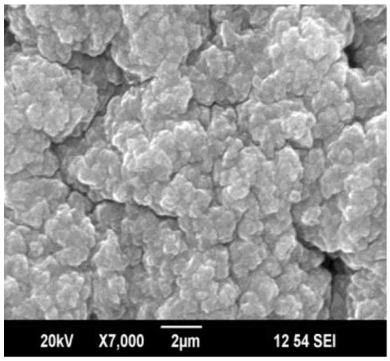


(b)

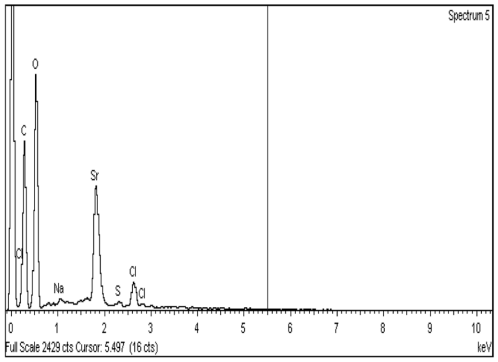
Element	At %
C	42.15
O	51.95
Na	0.17
S	2.05
Cl	0.82
Ba	2.85
Total	100

(c)

Figure 9.3. Scanning electron micrograph: (a) Ba^{2+} ion crosslinked SA/LS (80/20) blend; (b) EDS spectrum of Ba^{2+} ion crosslinked SA/LS (80/20) blend; (c) atomic percentage from EDS.



(a)

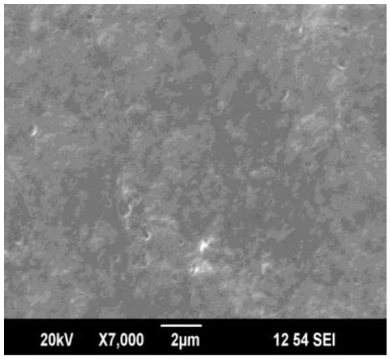


(b)

Element	At %
C	51.86
O	45.78
Na	0.18
S	0.1
Cl	0.52
Sr	1.56
Total	100

(c)

Figure 9.4. Scanning electron micrograph: (a) Sr^{2+} ion crosslinked SA/LS (80/20) blend; (b) EDS spectrum of Sr^{2+} ion crosslinked SA/LS (80/20) blend; (c) atomic percentage from EDS.



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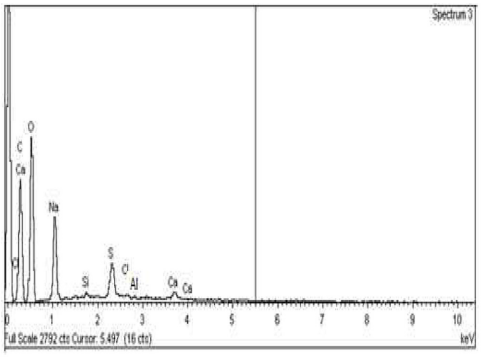

(b)

Element	At %
C	49.67
O	40.44
Na	8.9
S	0.59
Cl	0.21
Al	0.19
Total	100

(c)

Figure 9.5. Scanning electron micrograph: (a) Al^{3+} ion crosslinked SA/LS (80/20) blend; (b) EDS spectrum of Al^{3+} ion crosslinked SA/LS (80/20) blend; (c) atomic percentage from EDS.

is only 0.19% which is very low. The above observations substantiate the fact that crosslinking of the SA/LS film with Ba^{2+}, Ca^{2+}, Sr^{2+} and Al^{3+} ions is a diffusion controlled process.[35] That is the ions diffuse through the film. The diffusion ability is a function of the ionic size. Since the Ba^{2+} has a radius of 1.35 Å compared to 0.97 Å for Ca^{2+} ion,[35,36] the Ba^{2+} ions are expected to fill a larger space between the alginate molecules producing a tight arrangement at the surface with smaller voids as shown in Figure 9.3. A reduced swelling rate is thus expected which matches with our observations (Figure 3). Al^{+3} ions having very small size diffuse through the body without crosslinking. A poor crosslinking for Al^{3+} ion crosslinked blend supports the swelling results. Whereas strontium ion is expected to form stronger film because of its bigger size but this is contradictory to our observations. Studies have shown that binding of ions also depends on type of alginates used. The alginates consist of different ratios of G and M blocks based on the origin. Different block structures of M and G in the alginates bind the ions to a different extent. Ca^{2+} ion bind to G and MG blocks, Ba^{2+} to G and M blocks, and Sr^{2+} to G blocks solely.[37]

Conclusions

The crosslinking plays an important role in the stability of films under physiological conditions. The crosslinking metal ion and the time of crosslinking has influence on the swelling behavior. The nature of binding depends on the size and valency of the cations and also on the type of SA used. Calcium chloride solution forms stable hydrogels under mild conditions and its swelling can be controlled by crosslinking for various intervals of time. Ba^{+2} ions show strong crosslinking between polymer chains. Water uptake is considerably reduced in SA/LS blends prepared for higher crosslinking time. Al^{+3} ions show poor crosslinking and hence do not influence water uptake behavior of the blends. The FTIR studies confirm phys-

ical (ionic) interaction between the polymer and the metal ion. EDAX and SEM studies confirm the amount of crosslinking ions with SA.

References

1. S. Haznedar and B. Dortunc, *Int. J. Pharmaceut.*, **269**, 131 (2004).
2. M. N. Prabhakar, U. S. Rao, P. K. Babu, M. C. S. Subha, and K. C. Rao, *Indian J. Advances Chem. Sci.*, **1**, 240 (2013).
3. B. Mallikarjuna, K. M. Rao, P. Sudhakar, K. C. Rao, and M. C. S. Subha, *Indian J. Advances Chem. Sci.*, **1**, 144 (2013).
4. S. K. Sindhu, D. V. Gowda, V. Datta, and Siddaramaiah, *Indian J. Advances Chem. Sci.*, **2**, 89 (2014).
5. P. Sudhakar, K. M. Rao, S. Siraj, A. C. Babu, K. C. Rao, and M. C. S. Subha, *Indian J. Advances Chem. Sci.,* **2**, 50 (2013).
6. A. Shilpa, S. S. Agrawal, and A. R. Ray, *Polym. Rev.*, **43**, 187 (2003).
7. S. Kalyania, B. Smithab, S. Sridhar, and A. Krishnaiah, *Desalination*, **229**, 68 (2008).
8. S. A. Riyajan and P. Tangboriboonrat, *Polym. Korea*, **39**, 550 (2015).
9. E. J. Lee and Y. H. Kim, *Polym. Korea*, **37**, 539 (2013).
10. J. Ku, M. S. Kim, B. Lee, G. Khang, and H. B. Lee, *Polym. Korea*, **32**, 103 (2008).
11. J. H. Kim, S. G. Kim, and J. Jegal, *Polym. Korea*, **28**, 352 (2004).
12. H. G. Kim, *Polym. Korea*, **39**, 714 (2015).
13. Y. Senuma, C. Lowe, Y. Zweifel, J. G. Hilborn, and Marison, *Biotech. Bioeng.*, **67**, 616 (2000).
14. S. Takka and F. Acarturk, *Pharmazie*, **54**, 137 (1999).
15. R. S. Giridhar and A. S. Pandit, *Polímeros*, **23**, 13 (2013).
16. R. S. Giridhar and A. S. Pandit, *Int. J. Polym. Mater. Polym. Biomater.*, **62**, 743 (2013).
17. G. R. Bardajee, Z. Hooshyar, and H. Rezanezhad, *J. Inorg. Biochem.*, **117**, 367 (2012).
18. A. Sujith, G. Unnikrishnan, and S. Thomas, *J. Appl. Polym. Sci.*, **90**, 2691 (2003).
19. S. Takka and F. Acarturk, *J. Microencapsulation*, **16**, 275 (1999).
20. L. Whitehead, J. H. Collett, and J. T. Fell, *Int. J. Pharm.*, **210**, 45 (2000).
21. T. Ostberg and C. Graffner, *Int. J. Pharm*., **111**, 271 (1994).
22. Y. Murata, N. Katayama, T. Kajita, E. Miyamoto, and S. Kawashima, *Drug Del. Syst.,* **12**, 49 (1997).
23. T. Yotsuyanagi, I. Yoshioka, N. Segi, and K. Ikeda, *Chem. Pharm. Bull.*, **38**, 3124 (1990).
24. M. R. Rubio and E. S. Ghaly, *Indian Pharm.*, **20**, 1239 (1994).
25. O.-J. Kwon, S.-T. Oh, S.-D. Lee, N.-R. Lee, C.-H. Shin, and J.-S. Park, *Fibers and Polymers*, **8**, 347 (2007).
26. S. H. Yuk, B. C. Shin, S. H. Cho, and H. B. Lee, *Polym. Korea*, **14**, 675 (1990).
27. Y. S. Kim, K. Y. Kim, Y. K. Sung, and C. S. Cho, *Polym. Korea*, **12**, 10 (1988).
28. T. Yotsuyanagi, T. Ohkubo, T. Ohhashi, and K. Ikeda, *Chem. Pharm. Bull.*, **35**, 1555 (1987).
29. G. Fundueanu, C. Nastruzzi, A. Carpov, J. Desbrieres, and M. Rinaudo, *Biomaterials*, **20**, 1427 (1999).
30. R. Bodmeier and O. Paeratakul, *J. Pharm. Sci.*, **78**, 964 (1989).
31. A. Kikuchi, M. Kawabuchi, A. Watanabe, M. Sugihara, Y. Sakurai, and T. Okano, *J. Controlled Release,* **58**, 21 (1999).
32. A. Gaumann, M. Laudes, B. Jacob, R. Pommersheim, C. Laue, W. Vogt, and J. Schrezenmeir, *Biomaterials*, **21**, 1911 (2000).
33. G. T. Grant, E. R. Morris, D. A. Rees, P. J. Smith, and D. Thom, *FEBS Lett.*, **32**, 195 (1973).
34. S. Al-Musa, D. A. Fara, and A. A. Badwan, *J. Controlled Release*, **57**, 223 (1999).
35. J. Burgess, *Metal Ions in Solution*, 1st Ed., Ellis Horwood, New York, pp. 137 (1978).
36. F. A. Johnson, D. Q. M. Craig, and A. D. Mercer, *Pharm. Pharmacol.*, **49**, 639 (1997).
37. Y. A. Mørch, I. Donati, B. L. Strand, and G. Skjàk-Bræk, *Biomacromolecule*, **7**, 1471 (2006).