Polymeric Biomaterial Hydrogels: II. Behavior of Some Coordination Biopolymeric Metal-Alginate Ionotropic Hydrogels in Aqueous Solutions

Refat M. Hassan *1, Ishaq A. Zaafarany[2], Adil A. Gobouri[3] , Fahd A. Tirkistani[2], Ahmed Fawzy[1,2], Hideo Takagi [4]

[1] Chemistry Department, Faculty of Science, Assiut University, Assiut 71516, Egypt
[2] Chemistry Department, Faculty of Applied Sciences, Umm Al-Qura University, Makkah Al-Mukarramah 13401, Saudi Arabia Kingdom
[3] Chemistry Department, Faculty of Science, Taif University, Taif 21995, Saudi Arabia Kingdom
[4]Chemistry Department, School of Science, Research Center for Physical Inorganic Science, Nagoya 464-01, Japan.
[1*] rmhassan2002@yahoo.com ; [2] ishaq_zaafarany@yahoo.com; [2]drfahd999@gmail.com; afsaad06@yahoo.com;
[3]agobouri00@yahoo.com, [4] h.d.takagi@nagoya-u.jp

Abstract-**The behavior of some biopolymeric metal-alginate hydrogel spheres of ionotropic nature in aqueous solutions has been investigated. Cross-linked ionotropic biopolymeric hydrogels such as Mn^{II} , Co^{II} , Ni^{II} , Ca^{II}, Sr^{II} and Se^{IV}-alginate showed an appreciable tendency for swelling in water, whereas that of Cu^{II}-, Sn^{II}-, Ba^{II}, Pb^{II}, Al^{III}, Cr^{III} and Fe^{III}-alginates showed a remarkable tendency for shrinking. The swelling processes were accompanied by an increase in volume and transparency; while a decrease in the pore size with a simultaneous increase in hardness was followed the shrinking processes. Drying of these metal-alginate ionotropic hydrogel spheres indicated that the water content exceeds 90% of the gel weights. The kinetics of swelling and shrinking processes have been studied. The factors which affect that behavior were examined and discussed in terms of the mechanical stability and the changes in some rheological properties of these ionotropic hydrogel spheres.**

Keywords*- Biomedical Materials; Polysaccharides; Metal-alginate Complexes; Hydrogels; Swelling; Shrinking; Kinetics*

I. INTRODUCTION

Hydrogels that exhibit volume changes under the influence of environmental changes are promising materials in biotechnology owing to its wide applications in biomedical applications such as in sensors, drug delivery, tissues engineering, skin grafting, medical adhesive, dressing in the treatment of thickness wounds and encapsulation of live-cells [1, 2]. It also covers targeted drug delivery to the nervous system, gastrointestinal tract, and kidneys etc. Again, some cross-linked metal-alginate ionotropic hydrogels especially calcium-alginate spheres and beads [3-5] are used in immobilization systems as biocatalysts, separation processes in biotechnology and processing of agricultural products [6, 7].

Cross-linked metal-alginate ionotropic hydrogels are usually prepared by sol-gel transformation processes of addition of sodium-alginate sol to a polyvalent metal ion electrolyte [5-7]. The kinetics of such gelation processes have been studied for the first time by us elsewhere [8-11]. Furthermore, the diffusion controls which affected such sol-gel transformation, has been also examined in more details and discussed earlier [12].

Torres *et al.* [13] studied the behavior of Ca-alginate spheres in both pure solvents and other water-solvent mixtures. They reported that the gel particles were suffered different changes depending on the nature of the solvents. Tanaka et al [14] presented a theory on the kinetics of swelling of gels. They reported that the characteristic type of swelling was proportional to the square of linear dimension of the gel and the diffusion coefficient of the gel network. Hassan and coworkers [15] investigated the behavior of ionotropic alginate complexes in particularly Cu-alginate membrane hydrogel as a coordination biopolymeric materials in pure organic solvents and buffer solutions. They reported that the change in volume and other rheological properties were mainly dependent on the nature of solvent and the pHs of the buffer solution used.

Mongar and Wassermann [16] investigated the swelling of alginates in the form of thread fibers and reported that an axial concentration was followed the swelling process for calcium alginates. The possibility of application the rubber-like elasticity theories to the swollen alginate gels has been examined by Tempel et al [17]. They explained the observed Gaussian behavior in terms of strength- distribution of cross-links. Again, the shrinking kinetics of calcium-alginate beads in alcoholic soda has been investigated by Ferrero and coworkers [18].

In view of the above aspects and our interesting in physicochemical studies of cross-linked metal-alginate biomaterials [8-12,15], the present work aims to shed some highlights on the behavior of spherical cross-linked metal-alginate ionotropic hydrogels in aqueous solutions compared to that investigated in non-aqueous solutions [15].

II. MATERIALS AND METHODS

A. Material

Sodium alginate (Cica-Reagent Chem. Co) was used. The degree of substitution was found to be 4.34 mmol g^{-1} (0.95 $molmol^{-1}$). The measured viscosities using an Ubbelhode viscometer for a 4% alginate sol in water (w/w) were found to be 2.78 and 9.87 dl g^{-1} at 25 °C for the inherent and reduced viscosities, respectively.

All other chemicals used were of Analar (BHD) grade. Doubly distilled conductivity water was used in all preparations.

B. Preparation of Alginate Sols

Sodium alginate sols of different concentrations (2%, 3% and 4%) in water (w/w) were prepared as described earlier [8-11]. This process was performed by stepwise addition of the alginate powder reagent to bidistilled solution whilst rapidly stirring the water to avoid the formation of lumps, which swell with difficulty. The prepared alginate sols were left at room temperature for about 24 h (25 °C) until became air-bubbles free before used.

C. Preparation of Ionotropic Metal-Alginate Spheres

Gels can be prepared by dropping sodium-alginate sols into electrolyte solutions of polyvalent metal ions using either a dropper or a syringe.

In our case, we used a special glass syringe for preparing cross-linked Mn^{II}, Co^{II}, Ni^{II}, Ca^{II}, Sr^{II}, Cu^{II}, Sn^{II}, Ba^{II}, Pb^{II}, Al^{III}, Cr^{III}, Fe^{III} and Se^{IV}-alginates of sphere nature as described earlier [12,15,19,20]. After completion of the sol-gel transformation, the formed spheres were carefully removed from the containers of metal ion electrolytes (conical flasks or beakers) and washed with deionized water several times until the resultant washings became free from non-chelated metal ions that surrounding the gel spheres. Then, these spheres were kept in doubly distilled conductivity water for use.

For each alginate sol and metal ion electrolyte, the following precautions were taken into consideration to maintain the size and shape of all alginate sol droplets constants as far as possible (a) the volume of the alginate sol inside the syringe and the distance of the syringe tip from the surface of the electrolyte were kept constant; (b) the force exerted upon the piston of the syringe was maintained fixed; and (c) the same syringe was used as a dropper in all preparations.

D. Swelling and Shrinking Measurements

This procedure comprised with the measurements of either the absorbed or released water by some metal-alginate hydrogel pellets immersed into containers which contained fixed volumes of bidistilled water maintained at the desired temperatures within ±0.1 °C.

After intervals of time, the swollen or shrunk spheres were removed from the water containers and blotted quickly by means of filter paper strips to remove the excess water adhered on the surfaces. Then the weights or volumes were determined. The degree of water uptake was calculated from the relationship

$$\text{Swelling (\%)} = ((W - W_0)/W_0) \times 100 \qquad (1)$$

where W_0 and W represent the weight of pellets before and after immersing, respectively. On the other hand, the rate of water released can be determined from the equation

$$\text{Shrinking (\%)} = ((W_0 - W)/W_0) \times 100 \qquad (2)$$

In a similar manner, the swelling or shrinking volumes can be evaluated. The results are shown in Figs. (1-4).

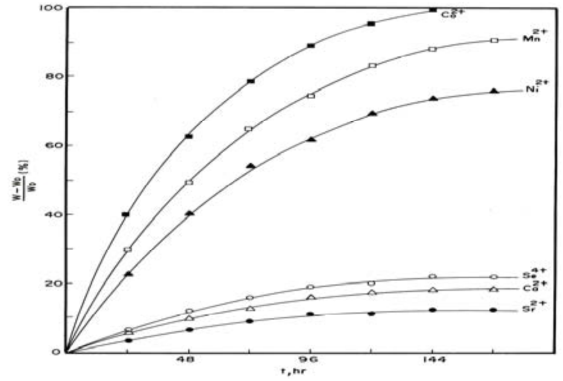


Fig.1 Change in weight-swelling with aging time at 22 ºC.

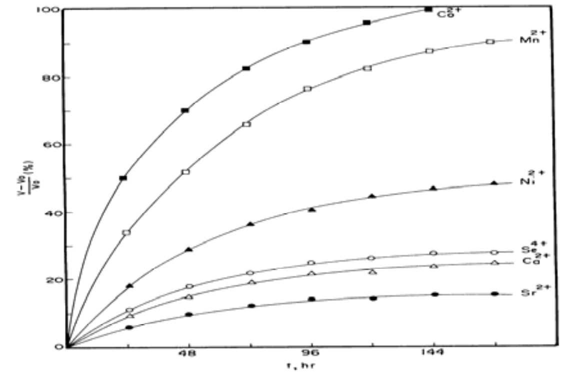


Fig.2 Change in volume-swelling with aging time at 22 ºC

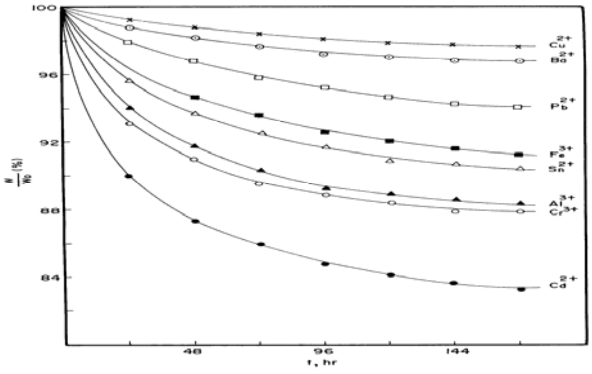


Fig. 3 Degree of shrinking as a function of time at 22 ºC

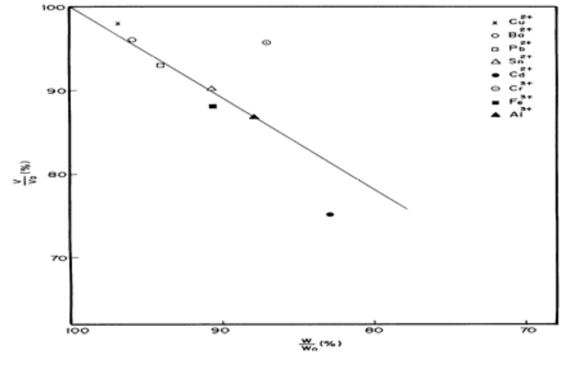


Fig.4 Volume shrinkage-weight loss plot after one week at 22 ºC

E. *Water-Content Determination*

The water content in these gels was determined by drying the gel pellets in an electric oven at 105 °C for about 72 hrs. The water content was calculated as follows,

$$\text{Water content (\%)} = 100-[(W`/W_0) \times 100] \qquad (3)$$

where W` denotes the weight of the dryness gel spheres. The water content and the degree of swelling or shrinking in metal-alginate hydrogel spheres are summarized in Table I. Again, some other rheological properties such as the mass and volume of the droplets as well as the mechanical properties such as the elasticity and transparency are summarized in Table II. All cited data are an average of five experimental runs.

TABLE I THE DEGREE OF SWELLING AND SHRINKING ([ALG] = 2%) AND THE WATER CONTENTS OF SOME CROSS-LINKED METAL-ALGINATE HYDROGEL SPHERES (2% AND 4%)

Metal ion	**Degree of Swelling^1 (%)**	**Water content^2 (%)**		**Metal ion**	**Degree of shrinking ^{1} (%)**	**Water content^2 (%)**	
		2%	**4%**			**2%**	**4%**
Ca^{II}	19.1	97.78	95.12	**Cu^{II}**	2.4	97.46	94.56
Mn^{II}	96.4	97.83	94.33	**Cd^{II}**	16.7	97.48	91.33
Co^{II}	106.5	97.85	93.75	**Sn^{II}**	9.6	97.71	94.31
Ni^{II}	70.9	97.54	95.25	**Ba^{II}**	3.2	96.56	93.04
Sr^{II}	12.2	96.94	92.47	**Pb^{II}**	6.0	96.33	93.76
Se^{IV}	21.8	98.74	95.89	**Al^{III}**	11.9	97.98	92.15
				Cr^{III}	12.2	98.43	93.62
				Fe^{III}	8.8	98.23	93.40

^{1} [Alg] = 2% ; ^{2} [Alg] = 2 and 4 %; Heating temp. = 105 °C for about72 h.

TABLE II SOME PHYSICAL PROPERTIES, RATE OF GELATION (RG, DM3MOL-1S-1) AND RELAXATION TIME (ΔT, MIN) OF SOME CROSS-LINKED METAL-ALGINATE IONOTROPIC HYDROGEL SPHERES. [ALG] = 2% AT 25 °C

Metal-alginate	**Δτ, min**	**Shape**	**Elasticity**	**Transparency**	**$^a10^4$Rg, M^{-1} s^{-1}**	**$^b10^2m_e$ g**	**$^b10^2m_i$, g**	**$^c10^2v_e$, cm^3**	**$^c10^2v_i$, cm^3**	**N**	**K**
Mn(II)	27.05	spherical	elastic	transp.	-	5.72	4.10	5.41	3.45	0.581	0.076
Co(II)	30.03	spherical	elastic	transp.	8.30	8.01	3.72	8.05	3.87	1.254	0.004
Ni(II)	31.52	spherical	elastic	transp.	4.12	5.50	3.07	5.13	3.55	1.004	0.012
Ca(II)	33.48	spherical	elastic	transp.	1.33	3.67	3.26	3.65	2.97	0.23	0.362
Sr(II)	34.01	spherical	elastic	transp.	2.51	3.20	2.87	2.97	2.70	0.210	0.391
Se(IV)	28.67	spherical	elastic	transp.	-	6.09	5.50	5.92	5.27	-	-
Cu(II)	33.11	spherical	elastic	transp.	3.33	3.33	3.44	3.29	3.55	0.085	0.68
Sn(II)	38.35	spherical	brittle	not	7.5	5.79	5.90	5.41	5.68	0.096	0.733
Ba(II)	30.48	spherical	elastic	transp.	3.78	2.45	2.65	3.38	3.46	0.160	0.480
Pb(II)	18.08	spherical	elastic	transp.	3.35	2.92	3.07	2.70	2.87	0.104	0.627
Al(III)	46.50	flat	brittle	semi	0.7	4.99	5.46	4.73	5.34	-	-
Cr(III)	>80	flat	brittle	not	-	4.82	5.44	4.54	4.80	-	-
Fe(III)	58.40	flat	brittle	not	-	5.41	4.99	4.80	5.47	-	-

a. R_g is the rate of sol-gel transformation.

b. m_i and m_e are the masses of the droplet before and after equilibrium

c. v_i and v_e are the volumes of the droplet before and after equilibrium

F. *Gravimetric Analysis*

Thermal decomposition technique was used in order to determine the stoichiometric molar ratio of the (metal: alginate) in these cross-linked metal-alginate ionotropic hydrogels. This technique was based on the results obtained from TGA data that reported earlier [21-24]. Therefore, these metal-alginate ionotropic hydrogel spheres were heated at about 105 for about 5 h until constant weight in order to evaluate the water contents. Then, the dried spheres were heated over its corresponding decomposition temperature (from TGA data) for about 24 h in order to give rise to their corresponding metal oxides as final products. The stoichiometric molar ratio can be evaluated from the weights of dried metal-alginate spheres and the weights of the final metal oxides.

III. RESULTS AND DISCUSSION

A. *Stoichiometry*

Ion exchange is inherently a stoichiometric process [25]. This means that, the Na^+ counter ions which leave the macromolecular chains of alginate during the gelation process must be replaced by an equivalent amount of other metal ions from the electrolyte solutions, i.e. the fluxes of the two exchanging counter ions should be equal in magnitude even the mobilities of the two counter ions may be different.

The experimental results obtained from the gravimetric method analysis indicated that one mole of each divalent-, trivalent and tetravalent metal ions chelates two, three and four moles of alginate macromolecule monomers, (Alg^-), respectively. This result conforms to the following stoichiometric overall reactions,

$$Z(Na\text{-}Alg)_n + nM^{Z+} = (M-Alg_z)_n + n(ZNa^+) \qquad (4)$$

sol electrolyte gel electrolyte

where Na-Alg and M-Alg_z denotes sodium alginate sol and its corresponding cross-linked metal-alginate gel, M is the metal ion and Z stands for its valency. The molar stoichiometry obtained here was found to be in good agreement with that obtained in other kinetic studies [8-11]. When the metal-alginate ionotropic gels immersed into aqueous solution, it may to expect an occurrence of either shrinking or swelling processes. The results are summarized in Table 1.

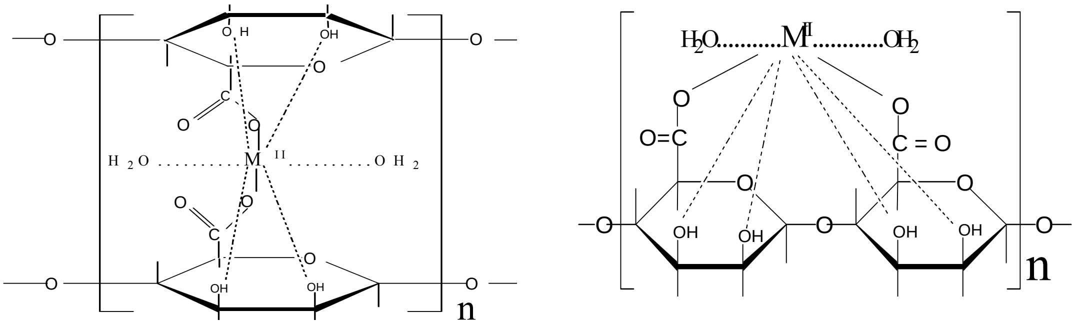
B. Gelation Mechanism

As the droplet of alginate sol comes in contact with the metal ion electrolyte; a primary membrane is rapidly formed around the alginate sol droplet. This formed membrane will keep the alginate sol droplet in a spherical shape as well as prevents the alginate sol inside the droplet from deterioration or scattering onto the external metal ion electrolyte. At the beginning, the alginate sol droplet will float as just below the surface of the electrolyte because its density is less than that of the metal ion electrolyte. Then, an exchange process will take place between the Na^+ counter ions of the alginate sol macromolecule and the metal cations of the electrolyte through that formed primary membrane. When the density of the droplet exceeds that of the electrolyte, it begins to sink into the electrolyte by a certain velocity is termed by velocity of acceleration [12]. The time consumed between the floating and sinking of the droplet may be called the relaxation time (ΔT). It noticed that both relaxation time and velocity of acceleration are mainly dependent on the nature of the metal ions (such as its mass, density, radius, concentration and pH's of both alginate sol and electrolyte solutions) as well as the temperature [12, 13]. The relaxation times and gelation rates along with some other reological properties are summarized in Table 2. The mechanism of such sol-gel transformation with respect to these cross-linked metal alginate ionotropic spheres was explained in more details earlier [8-11].

The replacement of Na^+ counter ions by the metal ions leads to the well-known sol-gel transformation phenomenon with formation of their corresponding biomaterial hydrogels ionotropic cross-linked metal alginates. Here, a sort of bridges is formed between the interdiffused metal ion and the carboxylate and hydroxyl functional groups present on alginate monomer chain through formation of partially ionic and partially coordinate bonds, respectively. In reality, these bonds are not just simple, but a kind of chelates involves one pair or more of carboxylate groups and one or more pairs of hydroxyl groups in an egg-carton like structure is formed [26, 27] depending on the valence of interdiffused metal ion and its coordination number.

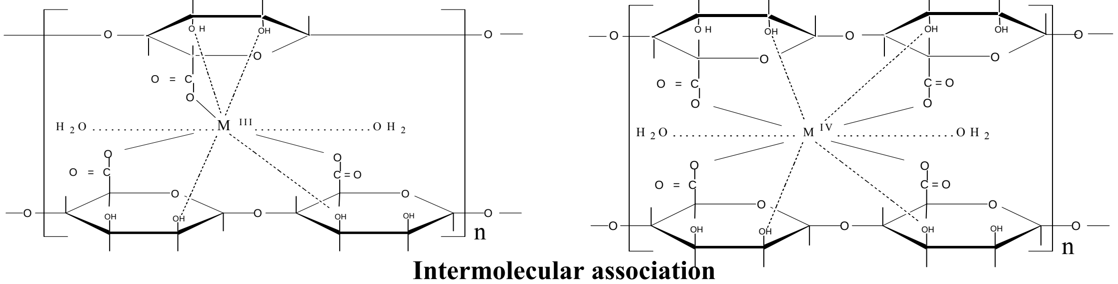
The coordination geometry in these ionotropic gels are shown in Scheme I.

Cross linked divalent metal alginate complexes



Intermolecular association **Intramolecular association**

Cross linked trivalent & tetravalent metal alginate complexes



Intermolecular association

Scheme I. Types of cross- linking

The kinetics of swelling or shrinking of divalent metal-alginate hydrogels were examined according to Peppes kinetic formula [28-30]

$$m_t / m_e = k\, t^n$$

Linearized form of this equation can be rewritten as follows,

$$\ln\ (m_t / m_e) = \ln k + n\ \ln t$$

where m_i and m_e are the weights of the hydrogels at time t and at equilibrium, respectively, whereas k and n are constants. The values of the constants k and n are dependent on the degrees of swelling or shrinking (in term of expansion or construction of the matrix network). Plots of the left-hand side ln (m_t / m_e) against ln t gave good straight lines from whose slopes and intercept the values of n and k can be evaluated. These values were calculated by the least-squares method and are summarized in Table 2. It noticed that n value is a function of the swelling or shrinking magnitudes. The n value was found to increase with increasing either the degree of swelling or shrinking which in turn relates to the degrees of expansion or construction in the matrix network, respectively. Hence, the values of n may be considered as an indirect evidence for the ability of those hydrogels for swelling or shrinking. On the other hand, the constant k depends on the nature of metal ion such as its ionic radius, polarizability and the orientation of the solvent molecules and the macromolecular chains toward the chelated metal ion.

C. Gel Morphology

These cross-linked metal-alginate hydrogels were characterized by a distinct morphological structure depending on the direction of diffusion of the interdiffused metal cations towards the alginate sol during the replacement of the leaving Na^+ counter ions. When the metal ions are allowed to diffuse upward such as in case of columns(where one side of the column was previously closed with a cellophane paper and then filled with the alginate sol) immersed in electrolyte solutions, non-capillary structural gels are formed. This method of gel preparation is called “ascending technique” [31-33]. On the other hand, as the metal ions are diffused downward such as in case of spheres and membranes, the direction of diffusion becomes trapping-capillaries that are parallel and symmetrically identical, and this method is termed by “descending technique” [34, 35]. When a traverse section is made in the capillary zone, a porous structure image is obtained. The pores seem to be identical and symmetrical in size [11, 34]. This kind of gels has anisotropic properties owing to the orientation of the solvent molecules and the macromolecular chains toward the chelated metal ions and such properties are mainly dependent on the nature of metal ion used [36-38].

In general, these hydrogel spheres tend to equilibrate with the external solvent through swelling or shrinking processes. In case of swelling, the gels are swollen by uptake some water molecules from the swelling media. As shown in Fig. 1, the rate of swelling is gradually decreased with time. When an equilibrium condition is attained, no change in the swelling rate of the gel is observed. The equilibrium is established when the chemical potential of the solvent in the swelling media reaches that of the swollen gel. It has been found that Mn(II), Co(II), Ni(II), Ca(II), Sr(II), and Se(IV)- alginates having a greet tendency for swelling in water. The degree of swelling was increased in the order Sr(II)- < Ca(II)- < Se(IV)- < Ni(II)- < Mn(II)- < Co (II)-alginates, where the cross-links did not affect by the solvent chemical potential in the gel. This property may be attributed to many factors such as the mixing property of the free-energies of alginate sol as an anionic polyelectrolyte and solvent, the extension of the macromolecule network, and the mixing properties of both solvent and the mobile ions. Again, the experimental observations indicated that the volume-swelling is closely related to the weight-swelling during the swelling process as shown in Fig. 2.

On the other hand, the property of shrinking occurs by the removal of the liquid from the gel network to the external media. As shown in Fig. 3, the weight of the gel is gradually decreased by time. This behavior may be explained by an alternation of swelling-heat which is caused by the presence of a large number of segments of adjacent chains in juxtapositions. These juxtapositions were prevented from taking up some positions of a relatively high statistical probability for streochemical reasons. The degree of shrinking was found to decrease in the order: Cd(II)- > Cr(III)- > Al(III)- > Sn(II)- > Fe(III- > Pb(II)- ≈ Ba(II)- > Cu(II)-alginates. A plot of volume-shrinking vs. weight-loss was fairly linear as shown in Fig. 4. This linearity may indicate that the volume-reduction is closely related to the weight loss, i.e. to the density of the gel by about 0.95 g/cm^3 in a very close value to the density of water as a swelling media.

It is well known that the metal-alginate ionotropic gels must possess acceptable properties for their handling in practical use. The stress strain [39] and percent elongation [39, 40] for alginate gels have been examined in details previously. Therefore, it may be concluded that these ionotropic hydrogels may change some of their physicochemical properties by the shrinking or swelling. The experimental observations indicated the occurrence of a remarkable alternation in the macroscopical shape and optical properties of these gels. The volume and pore size are increased by swelling of the gel which may be explained by the increase in the wideness of the network-capillaries. Again, the increase in the gel-transparency on swelling may be attributed to the birefringence loss of the gel. As the gel shrinks, its stiffness increases because the network becomes more tightly packed. Also, a decrease in the volume, pore size, chemical potential and the birefringence of the gel should follow the shrinking process. The latter can be interpreted by the expulsion of the liquid from the hydrogel pores to the external media and, hence, a reduction in the gel/liquid interfacial area is occurred. Again, the decrease in the birefringence was attributed to the removal of water molecules from the gel which leads to an increase in the molecular orientation.

The water content in these gels was found to be more than 90% (Table 1). The pore size distribution showed a large tendency to vary with simultaneous contraction of the gel during drying. This can be interpreted by the huge capillary stresses and resistance of the fluid-flow in the narrow and small pores of the hydrogel network.

The factors which affect the behavior of metal-alginate gels in aqueous solutions such as temperature, concentration of alginate sol, width of capillaries and the radii of the metal ions have been examined. These factors may be explained by some variations which occur in the chemical potential between the swelling media and the gel-water content, the free-energy of the gel stretching of the gel network (the free-energy of the gel is apart from that of standard free- energy of components which results from mixing the gel with water) and the electrostatic free energy [41]. This variation leads to a contraction in the network of the gel. The degree of swelling or shrinking was found to be largely affected by the sol concentration. As the alginate sol increases the swelling or shrinking rate is increased. This fact can be interpreted by the increase in the bridging bonds which causes swelling or contraction of the gel network, respectively. The magnitude of swelling or shrinking was found to agree with the order of capillary width in these metal alginates [7]. As the width of capillaries increases, the swelling process is also increased whereas that of shrinking is decreased.

The slope of the curves obtained from swelling (or shrinking)-time plots may be considered as a determining factor for the mechanical stability of these metal-alginate ionotropic hydrogels. The experimental observations indicated that the rate of mechanical stability in divalent metal-alginate hydrogels is decreased in the following order Cu > Ba > Sr > Ca > Pb > Mn > Co-alginate gels in good consistent with the stability of these ionotropic gels as was reported elsewhere [6, 42]. The high mechanical stability of copper-alginate gel may be attributed to its high degree of orientation [43] as well as to its distorted octahedral structure. The geometrical configuration for chelation in these metal alginate hydrogel complexes was described in more details by Hassan elsewhere [27].

ACKNOWLEDGMENT

This work is dedicated to the memory of Professor Ibrahim Awad, Professor of Organic Chemistry & Professor Said Abu-Bakr, Professor of Analytical Chemistry, Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt.

REFERENCES

[1] T. Espevik , and G. Skjak-Braek “Application of alginate gels in biotechnology and biomedicine”, Carbohydrate European, vol. 14, pp. 19-25, 1996.

[2] N. Saha, A. Sarrai, N. Ray, T. Kilano, and P. Shasaha “ Polymeric biomaterial based hydrogels for biomedical application”, Journal of biomaterial nanotechnology, vol. 2, pp. 85-90, 2011.

[3] L. G. Torres, A. Sanchez-de-la-Vega, N.A. Beltran, and B.E. Jimenez “Production and characterization of a Ca-alginate biocatalyst for removal of phenol and chlorophenols from wastewaters”, Process Biochemistry, vol. 33, pp. 625-634, 1998.

[4] A. Martinsen, Skjak-Braek, and O. Smidrsrod “Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads”, Biotechnol. Bioeng., vol. 33, pp. 79-89, 1989.

[5] A. Haugh, and O. Simdrsrod “Selectivity of some anionic polymers for divalent metal ions”, Acta Chem. Scand., vol. 24,pp. 843-854, 1970.

[6] R. G. Schweiger “Complexing of alginic acid with metal ions”, Koll. Z., vol. 196, pp. 47-53, 1964.

[7]H. Thiele, and K. H. Hallich “Capilar structure in ionotropic gels”, Koll. Z., vol. 151, pp. 1-12, 1957.

[8] R. M. Hassan, M.H. Wahdan, and A. Hassan “Kinetics and mechanism of sol-gel transformation on polyelectrolytes of nickel alginate ionotropic membranes”, European Polymer Journal, vol. 24, pp. 281-283,1988.

[9] R. M. Hassan, S. A. El-Shatoury, M. A. Mousa, and A. Hassan “Kinetics and mechanism of sol-gel transformation for polyelectrolytes of capillary copper alginate ionotropic membranes”,European Polymer Journal, vol. 24, pp. 1173-1175, 1988.

[10] R. M. Hassan, A. M. Summan, M. K. Hassan, and S.A. El-Shatoury “Kinetics and mechanism of sol-gel transformation on polyelectrolytes of some transition metal ions especially cobalt alginate ionotropic membranes”, European Polymer Journal, vol. 25, pp. 1209-1212, 1989.

[11] K. S. Khairou, W. M. El-Gethami, and R. M. Hassan “Kinetics and mechanism of sol-gel transformation between sodium alginatepolyelectrolyte and some heavy divalent metal ions with formation of capillary polymembranes ionotropic gels”, Journal of Membrane Science, vol. 209, pp. 445-465, 2002.

[12] R. M. Hassan, M. Th. Makhlouf, and S. A. El-Shatoury “Alginate polyelectrolyte ionotropic gels. IX. Diffusion control effects on the relaxation time of sol-gel transformation of divalent metal alginate ionotropic gel complexes”,Colloid Polymer Science, vol. 12, pp. 1237-1242, 1992.

[13] L. G. Torres, A. Velasquez, and M. A. Brito-Arias “Ca-alginate spheres behavior in presence of some solvents and water-solvent mixtures”, Advances in Bioscience and Biotechnology, vol. 2, pp. 8-12, 2011.

[14] T. Tanaka, and D.J. Fillmore “Kinetics of swelling of gels”, J. Chem. Phys., vol. 70, pp. 1214- 1218, 1979.

[15] R. M. Hassan, F. A. Tirkistani, I. A. Zaafarany, A. Fawzy, M. Khairy, and S. Iqbal “Polymeric biomaterial hydrogels. I. Behavior of some ionotropic cross-Linked metal-alginate hydrogels especially copper-alginate membranes in some organic solvents and buffer solutions”. Adances in BioScience and Biotechnology, vol. 3, pp. 845-854, 2012.

[16] J. L. Mongar, and A. Wasseemann **“**Influence of ion exchange on optical properties, shape, and elasticity of fully-swollen alginate. Fibres”, Journal of the Chemical Society, pp. 500-510, 1952.

[17] A. J. M. Segeren, J. V. Boskamp, and V. D. Tempel “Rheological and swelling properties of alginate gels”, Farad. Dics. Chem. Soc., vol. 57, pp. 255-262, 1974.

[18] F. Ferrero, P. Compagna, and N. Piccinini “Shrinking kinetics of calcium alginate beads in alcoholic soda”, Chem. Eng. Commun., vol. 15, pp. 197-206, 1982.

[19] R. M. Hassan “Alginate polyelectrolyte ionotropic gels. III. Kinetics of exchange of chelated divalent transition metal ions especially cobalt(II) and copper(II) by hydrogen

ions in capillary ionotropic metal alginate polymembrane gels", Journal of Material Science, vol. 26, pp. 5806-5810, 1991.

[20] R. M. Hassan "Part II: Kinetics and mechanism of exchange of chelated nickel(II) by hydrogen ions in capillary ionotropic nickel-alginate polymembrane gel complex", Journal of Material Science, vol. 28, pp. 384-388, 1991.

[21] A. A. Said, and R. M. Hassan "Thermal decomposition of some divalent metal alginate gel compounds", Journal of Polymer Degradation Stability, vol. 39, pp. 392-397, 1993.

[22] A. A. Said, M. Abd El-Wahab, and R. M. Hassan "Thermal and electrical studies on some metal alginate compounds", Thermal Chemica Acta, vol. 233, pp. 13-24, 1994.

[23] M. A. El-Gahami, K. S. Khairou, and R. M. Hassan "Thermal decomposition of Sn(II), Pb(II), Cd(II) and Hg(II) cross-linked metal-alginate complexes", Bulletin of Polish Academic Science, vol. 51, pp. 105-113, 2003.

[24] I. A. Zaafarany, K. S. Khairou, F.A. Tirkistani, I. Sayed, M. Khairy, R.M. Hassan "Kinetics and mechanism of non-isothermal decomposition of Ca(II)-, Sr(II)- and Ba(II)-cross-linked- divalent metal alginate complexes", Inter. J. Chem., vol. 4, pp. 7-14, 2012.

[25] F. Hellferich, "Ion exchange", McGraw-Hill, New York, 1962.

[26] D. A. Rees "Polysaccharide gels, a molecular view", Chemical Industry, pp. 630-636, 1972.

[27] R. M. Hassan "Alginate polyelectrolyte ionotropic gels. XIII. Geometrical aspects for chelation in metal alginate complexes related to their physicochemical properties", Polymer International, vol. 31, pp. 81- 86, 1993.

[28] N. M. Franson, and N. A. Peppas "Influence of polymer composition on non-Fickian water transport through glassy-polymer", J. Appl. Polym. Sci., vol. 28, pp. 1299-1310, 1983.

[29] P. L. Rigter, N. A. Peppas "Simple equation for description of solute-release II. Fickian and anolalous release from swellable devices", J. Contr. Rel., vol. 5, pp. 37- 42, 1987.

[30]C. Ozeroglu, and A. Birdal "Swelling properties of acrylamide-N,N`-methylene bis(acrylamide) hydrogels synthesized by using meso-2,3-dimercaptosuccinic acid0cerium(IV) redox couple", Express Polym. Lett., vol. 3, pp. 168-176, 2009.

[31] R. M. Hassan "Influence of frequency on electrical properties of acid and trivalent metal alginate ionotropic gels. A correlation between strength of chelation and stability of polye1ectrolyre gels", High Perform. Polymer, vol. 1, pp. 275-284, 1989.

[32]R. M. Hassan, M.Th. Makhlouf, and A. M. Summan "Influence of frequency on specific conductance of polyelectrolyte gels with special correlation between strength of chelation and stability of divalent metal alginate ionotropic gels", European Polymer Journal, vol. 25, pp. 993-996, 1989.

[33] R. M. Hassan, S. A. El-Shatoury, and M.Th. Makhlouf "Alginate polyelectrolyte ionotropic gels. XII. Chromatographic separation of metal ions in mixture solutions". High Performance Polymer, vol. 4, pp. 49-54, 1992.

[34] R. M. Hassan, A. Awad, and A. Hassan "Separation of metal alginate ionotropic gels to polymembranes with especial evidence on the position of chelation in copper alginate complex", Journal of Polymer Science A, vol. 29, pp. 1645-1648, 1991.

[35] H. Thiele, and K. H. Hallich "Capillary structure in ionotropic gels", Koll. Z., vol. 151, pp. 1-12, 1957.

[36] H. Thiele, and A. Awad "Ions and their biological effects. Investigation on ionotropic gels", Biotechnology, vol. 3, pp. 63-75, 1966.

[37] E. M. Obolonkova, E. M. Belavtseva, E. E. Braudo, and V. B. Tolstoguzov "Formation and structure of ionotropic gels. II. Electron microscopic investigation of anisotropic calcium-alginate gels", Colloid Polymer Science, vol. 252, pp. 526-529, 1974.

[38] V. B. Tolstoguzov " I. Ionotropic gels of a laminated structure", Colloid Polymer Science, vol. 253, pp. 109-113, 1974.

[39] O. Simdrsrod "Some physical properties of alginates in solution and in the gel state"; Thesis, Norwegian, Institute of Technology, Trondheim, 1973.

[40] P. Gemeiner, L. Kurillova, A. Malovikova, D. Toth, and D. Tomasovicova "Properties of spherical calcium pectate and alginate gels and their use in diffusion chromatography, solids separations and immobilization of enzymes and cells", Folia Microbiology, vol. 34, pp. 214-227, 1989.

[41] A. Katchalsky, and I. Michael "Polyelectrolyte gels in salt solutions", Journal of polymer Science, vol. 15, pp. 69-86, 1955.

[42] O. Simdrsrod, and A. Haugh "The effect of divalent metals on the properties of alginate solutions. I. Calcium ions", Acta Chem. Scand, vol. 19, pp. 329-340, 1965.

[43] H. Thiele, and C. Anderson "Ionotropic gels of polyuronic gels. Part II and Part III. Degree of order", Koll. Z., vol. 76, pp. 140-148,1955.