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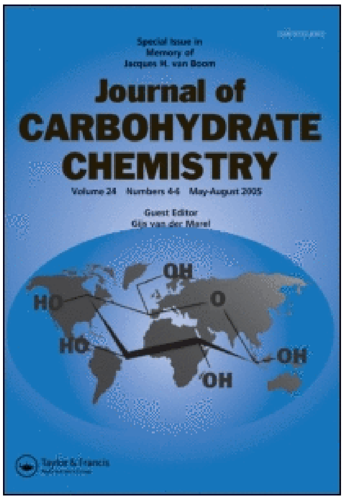

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Copper Ions Binding in Cu-Alginate Gelation

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Alginate polysaccharide forms viscous aqueous dispersions and has the ability to form gels in the presence of divalent cations such as calcium and copper. In this work, we have studied cooper ions binding during Cu-alginate gelation, obtaining quantitative information about the amount and kinetics of cation binding. Our results indicate that copper binding during gelation occurs until a Langmuir-type equilibrium is reached between bound and free ions in the gel-contacting solution. The kinetics of metal ions binding can be modeled using Ritchie equation–derived models, allowing the prediction of ionic binding and gel formation temporal evolution. The ratio between cationic and polysaccharide quantities in the gelation system determines the kinetics of gelation and the characteristics of the gel formed. The experimental results and models applied in the work give more insights on alginate gelation and contribute to a reliable design and control of production methods for alginate gel structures.

Keywords Alginate, Copper ions, Gel, Kinetics, Langmuir model, Biomaterials

INTRODUCTION

Alginate is a major component of the cell wall of brown algae (*Phaeophyceae*) and is produced by certain bacteria, including *Pseudomonas aeruginosa*.[1] It is a linear polysaccharide composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues, linked by $1 \rightarrow 4$ glycosidic bonds. Within the alginate chains, the M and G residues are sequentially distributed in repeating blocks of mannurate residues (M blocks) and guluronate residues (G blocks) interspersed by blocks of alternating M and G residues (MG blocks).[2]

Alginates are used in diverse fields, from food industry to environmental technology. In foods, alginates are extensively employed as thickening agents,

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emulsifiers, and stabilizers.[3] In biotechnology, alginate gels are exploited as immobilization matrix of cells and enzymes for use in bioreactors.[4,5]

Calcium alginate gel beads have also been tested with success for the removal of heavy metals, such as Pb^{2+}, Zn^{2+}, Hg^{2+}, Cd^{2+}, Cu^{2+}, Mn^{2+}, and Cr^{3+}, from industrial wastewaters by a technique called biosorption.[6–9] Alginate is one of the most indicated polymers for biomaterials and has been used in a variety of medical applications, including dental impressions, drug delivery systems, and engineered tissue replacements.[10] This carbohydrate polymer has a low toxicity and is readily available, but its wide range of applications arises from the ability to gel under gentle conditions. In the presence of di- and trivalent cations, alginate polymer establishes interchain associations, leading to formation of a gel.[1]

In the gelation mechanism, cations such as calcium, barium, and copper ions cooperatively interact with blocks of G residues, forming ionic crosslinks between different polymer chains. Sequences of polyguluronate pack together with the cations strongly coordinated in cavities between the chains. This is the commonly known "egg-box" model for alginate gelation.[11,12] Recent studies suggest refinements to this model and new hypotheses for the mechanism of cation binding and alginate gelation (Fig. 1).[13–16]

The vast research regarding the molecular details involved in alginate gelation contrasts with the insufficient work done on extending this knowledge to additional physical and chemical aspects of the gelation phenomena. For this reason, and in spite of its many applications, alginate use has been mostly based on empirical knowledge.

However, the development of new applications and time- and cost-effective manufacturing processes require a tight control of the gelation reaction and the knowledge of how different factors affect the reaction time-course and the final product features. For example, the processes widely used in biotechnology for gel bead production are performed without a rationale to

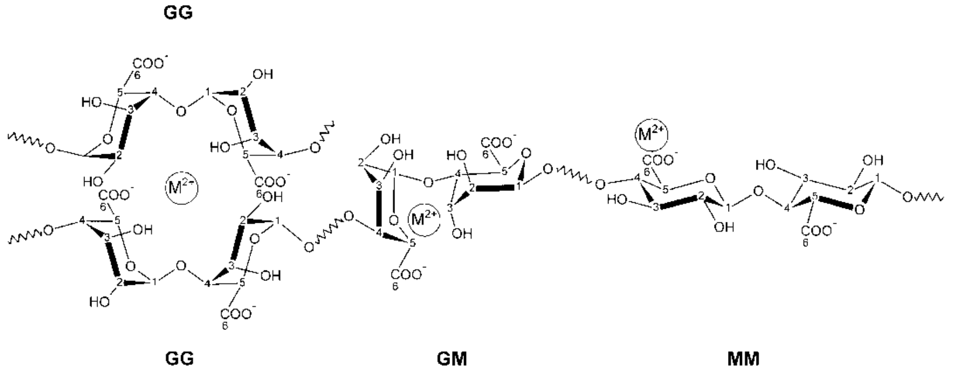

Figure 1: Alginate molecular structure showing some binding sites for divalent cations.(14,15)

predict the time needed for gelation or the influence of the cation's concentration on the beads' size. In tissue-engineering applications, which requires the manufacturing of relatively large and morphologically complex structures, new gelation processes must be developed and optimized in terms of gelation kinetics, limits to morphology, shrinkage control, and mechanical properties.[10]

In this work, we focus on the ionic binding during gelation because it is an essential basis for the design of gel production processes. The aim of the work was to study cations binding during alginate gelation, to obtain quantitative information on the amount of binding, and to find modeling tools to predict the amount and kinetics of binding. Copper ions were chosen for this work because of the high selectivity of alginate for Cu^{2+} and the easy analytical determination of these ions.

EXPERIMENTAL

Chemicals and Alginate Solution

Calcium chloride and copper sulphate were supplied by Merck and Carlo Erba, respectively. All solutions were prepared with Milli-Q water, with conductivity of 0,054 μS/cm or less.

Sodium alginate was purchased from Panreac and used without any further purification. Alginate solutions 2% (w/w) were prepared by addition of a weighted amount of sodium alginate to a portion of water, followed by agitation in an orbital shaker for 3 h at 50°C. Alginate solutions were prepared daily.

Gelation Essays

Copper alginate gelation essays were carried out by adding volumes of alginate solution dropwise to 50 mL of Cu^{2+} solutions with concentrations ranging from 3 to 32 mmol/L. Gelation was allowed to occur for 4 h, at 25°C, keeping the solution and gelling beads under stirring.

After gelation, gel beads were filtered and weighed. The dry weight of the gel was measured after drying the gel beads for 2 days at 50°C.

Copper ions' concentration in solution, before and after gelation, was determined by molecular absorption spectroscopy at $\lambda = 750\,\text{nm}$.

For the kinetic studies, aliquots were withdrawn from the solution at time intervals during the gelation reaction and Cu^{2+} concentration determined.

Data Treatment

The bound quantity of copper ions in the gel beads (Cu_{gel}), produced under different conditions, was determined from the initial Cu^{2+} concentration in the gelation solution ($[Cu^{2+}]_0$) and the Cu^{2+} concentration ($[Cu^{2+}]$) at a given instant during gelation, or after equilibrium had been reached ($[Cu^{2+}]_e$), taking in account the Cu^{2+} solution volume ($0.0500\,dm^3$):

$$Cu_{gel} = ([Cu^{2+}]_0 - [Cu^{2+}]) \times 0.0500 \tag{1}$$

For equilibrium modeling of ionic binding, the bounded quantity at equilibrium was calculated according to equation (2):

$$q_e = \frac{[Cu^{2+}]_0 - [Cu^{2+}]_e}{m} \times 0.0500 \tag{2}$$

where q_e is the bound quantity of copper ions per unit weight of alginate at equilibrium, $[Cu^{2+}]_0$ is the initial Cu^{2+} concentration in the gelation solution, $[Cu^{2+}]_e$ is the residual Cu^{2+} concentration at equilibrium, and m is the gel weight or the dry weight.

Kinetic and equilibrium experimental data were fitted to models by minimizing the sum of the squared deviations between experimental and predicted values, using a nonlinear regression data analysis program. Model effectiveness was evaluated based on the correlation coefficients (R^2).

RESULTS AND DISCUSSION

Cu^{2+} Binding in Cu-Alginate Gelation

To study Cu-alginate gelation, several essays were done by the addition, drop by drop, of a fixed volume of sodium alginate solution to copper sulphate solutions. When alginate solution contacted the copper ion solution, gelation began immediately and was accompanied by Cu^{2+} removal from solution, as can be seen in Figure 2. Copper binding by the gelling beads was very fast in the first 10 mins of gelation and, afterward, the binding rate decreased until equilibrium was reached.

Copper binding and the concomitant gelation rate is higher in the initial instants because alginate solution drops exhibit a large superficial area contacting directly with the surrounding copper solution. After this initial phase, a gel layer covers the beads and cooper ions must diffuse through this layer to reach the inner alginate solution, making the gelation evolution and copper ion removal from the solution gradually slower throughout the process.

The experimental data in Figure 2 show that the time needed for equilibrium to be reached depends on the ionic concentration in the gelation solution. With an initial Cu^{2+} concentration of 32 mmol/L, ionic binding was ended after 30 min of contact, while with an initial Cu^{2+} concentration of 3 mmol/L, it was prolonged over 3 h. The results in Figure 2 also indicate

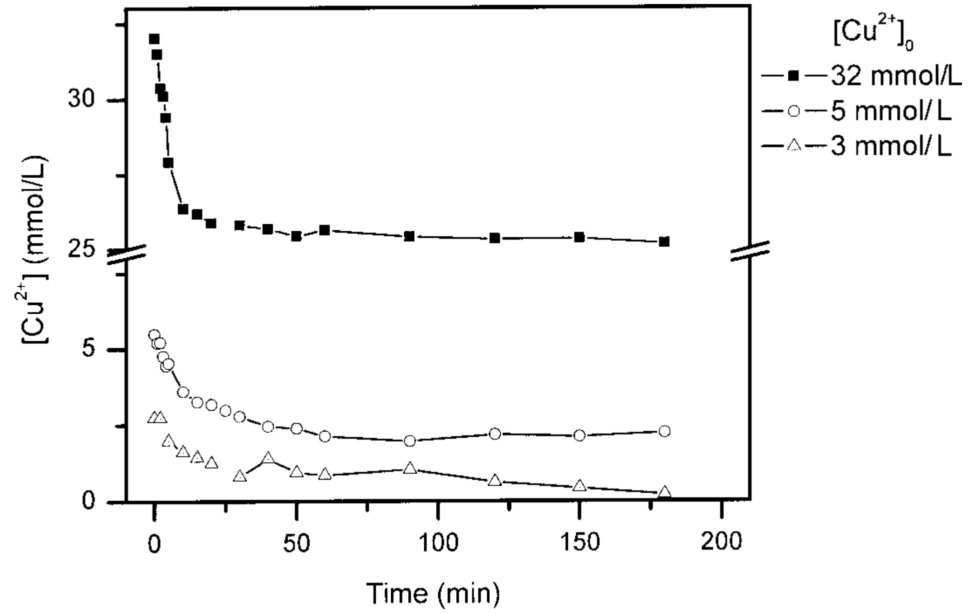

Figure 2: Time course evolution of copper ions' concentration during Cu-alginate gelation in copper solutions with different initial concentrations.

that the amount of ion binding by the alginate gel depends on the Cu^{2+} concentration in the gelation solution, as will be confirmed in a following section.

Effects of Alginate Quantity

In the preparation of alginate gels, the most convenient variables to control the process are the cationic concentration in the gelation solution and the amount of biopolymer applied.

Figure 3 shows results from gelation of different volumes of a sodium alginate solution in a Cu^{2+} solution with 6 mmol/L initial concentration. The residual copper ion concentration after gelation dropped from 5.3 to 0.7 mmol/L when the volume of alginate solution was increased from 1 to 10 mL. The copper uptake by the gel increased linearly in this range of alginate quantity, showing proportionality between the amount of polysaccharide and ionic binding. However, for volumes higher than 10 mL, the polymer was unable to bind more copper ions, although a low concentration remained in the solution, suggesting equilibrium between the solution and the alginate-bound ions.

The mass of gel, and respective dry weight, obtained using different volumes of polysaccharide solution were measured. As can be seen in Figure 3b, the gel weight increased nonlinearly with the amount of alginate

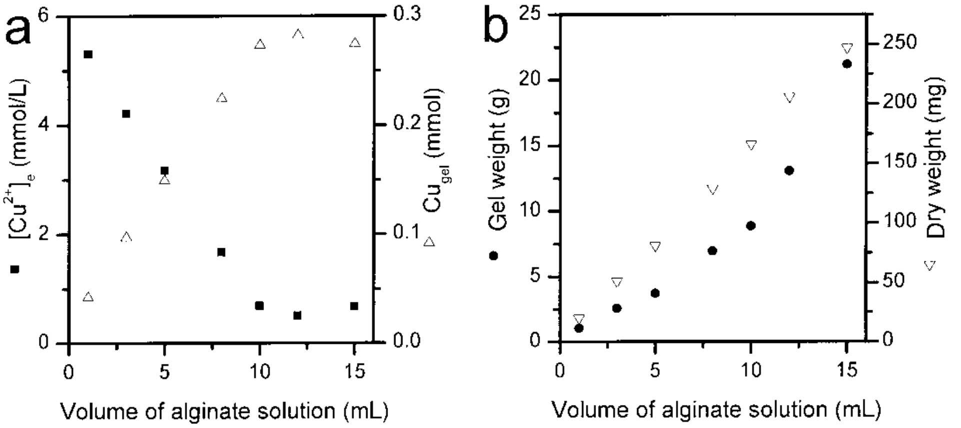

Figure 3: Influence of the amount of alginate applied on (a) copper binding and (b) weight of gel formed. Different volumes of sodium alginate 2% (w/w) were added to 6 mmol/L copper ion solution.

gellified. However, the dry weight of the gel was linearly related to the volume of alginate applied. Thus, it can be concluded that the gel from higher polysaccharide/cation ratios retains a greater percentage of water. The gel beads formed in these cases had a larger diameter and a lower rigidity (results not shown).

In the gelation of higher volumes of alginate, the ionic concentration in the solution is strongly reduced, affecting the degree of reticulation of the polysaccharide chains. In this case, the gel contracts less, retaining more water and giving rise to larger beads. The effects of the solution Cu^{2+} concentration on gelation are studied more thoughtfully in the next section.

Effects of Cu^{2+} Concentration

The affinity of alginates for different metal ions is well characterized,[17,18] but the influence of cationic concentration in the gelation process and in the properties of the resulting gel is an aspect poorly studied. However, as mentioned earlier, the ionic concentration is a convenient variable to control in the process of alginate gel formation.

The gelation of a fixed amount of sodium alginate in different concentrations of copper ion solutions resulted in distinct gelation kinetics and final gel weights (Fig. 4). With high concentrations of copper ions available for alginate gelation, the gel beads formed faster, becoming smaller and stiffer, because of a higher degree of reticulation and a lower retention of water in the gel (Fig. 4). Gelation in low concentration of copper ions was slower and led to brittle gels, and leaking of polymer chains from the gelling beads was visually observed, which explains the lower dry weight of the gel produced.

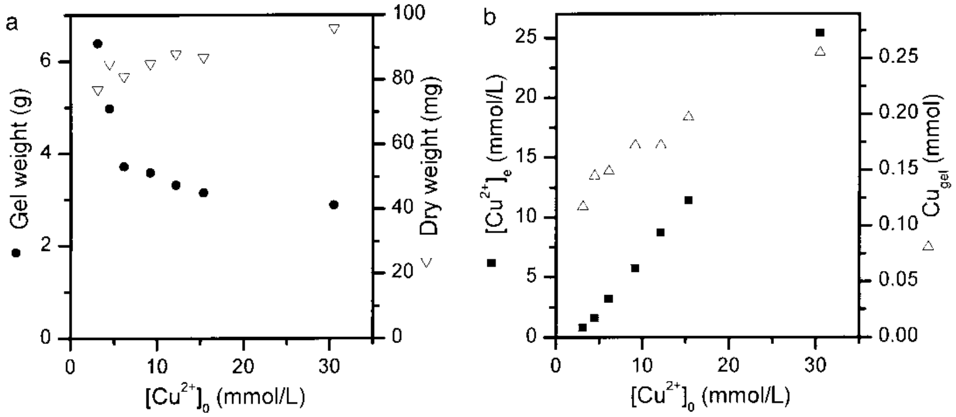

Figure 4: Influence of the initial copper ions concentration on (a) gel weight and (b) copper binding. A volume of 5 mL of sodium alginate solution was added to copper ion solutions with concentrations ranging from 3.1 to 31 mmol/L.

As can be seen in Figure 4, copper ions binding by the gel depended also on the Cu^{2+} concentration in the gelation solution, in addition to the quantity of alginate available, as discussed in the previous section. With higher Cu^{2+} concentrations, a larger amount of ions are bound and a higher residual copper concentration remained in the solution.

Siew and Williams found a binding curve for Mn^{2+} by alginate with a steep rise at low metal concentrations and a plateau region at higher concentrations.[15] This plateau region corresponds to the saturation of alginate chains, which are unable to bind more ions. For the range of copper ions-to-alginate ratios in Figure 4, saturation was not attained. For Pb^{2+} and Cd^{2+} complexation by alginate, similar curves were obtained.[19] According to Ouwerex et al., the elasticity of a gel is directly related to the number of reticulation points of the network.[18] These authors studied the influence of the gelation cation (Pb^{2+} and Cd^{2+}) concentration on the elasticity of the gels beads formed and found a relationship between the Young modulus of the beads and the cationic concentration used similar to our results for Cu^{2+}.

Equilibrium Modeling of Cu^{2+} Binding

The preceding results suggest that during Cu-alginate gelation, copper ions in solution are bound by the polysaccharide chains, lowering the Cu^{2+} concentration to a value resultant of equilibrium between Cu^{2+} in solution and alginate-bound. In order to predict the amount of ionic binding during alginate gelation, it is necessary to model this equilibrium.

The Langmuir model is an adsorption model widely employed in the study of sorption systems, including ionic binding by polysaccharide materials.[7,9,20,21]

The Langmuir model is represented by equation (3):

$$q_e = \frac{q_m b[\mathrm{Cu}^{2+}]_\mathrm{e}}{1 + b[\mathrm{Cu}^{2+}]_\mathrm{e}} \tag{3}$$

where q_e and $[\mathrm{Cu}^{2+}]_\mathrm{e}$ are the amount of metal attached to the adsorbent and the residual metal concentration in solution after equilibrium is reached, respectively; q_m is the maximum binding capacity of the adsorbent; and b is the Langmuir constant related to the affinity of the metal ions for the binding groups on the adsorbent.

Figure 5 shows that the mathematical equation from the Langmuir model describes quite well Cu^{2+} binding by alginate during Cu-alginate gelation. The lower correlation coefficient for the results based on gel weight can be a consequence of the greater uncertainty of weighting the humid mass of the gel. Keeping in mind that copper ions' concentration changes the solution pH, thus affecting alginate chains ionic properties, only results corresponding to $[\mathrm{Cu}^{2+}]_\mathrm{e}$ lower than 10 mmol/L were taken in account. The estimated maximum sorption capacities were 57.4 μmol/g gel or 2.1 μmol/g dry weight

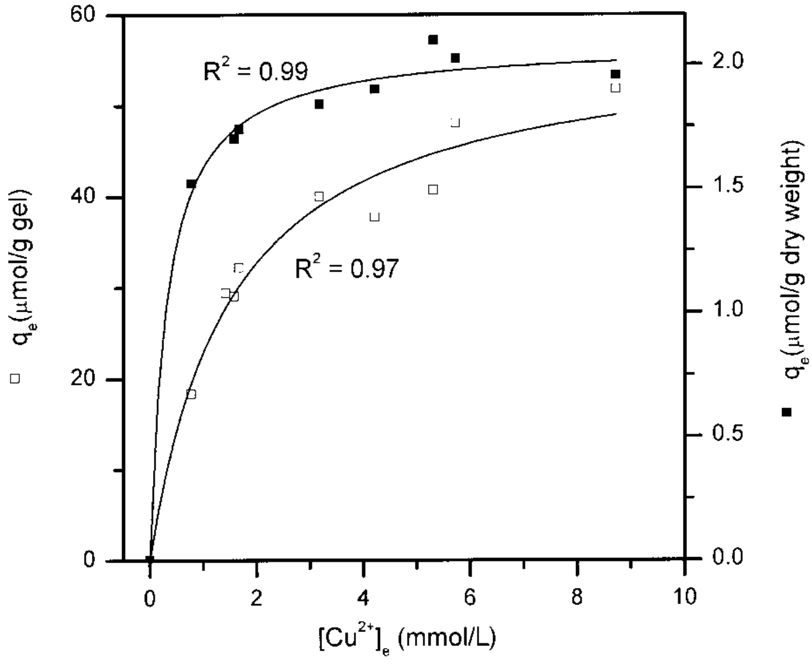

Figure 5: Langmuir adsorption isotherms of Cu^{2+} binding by alginate during Cu-alginate gelation. The two sets of results correspond to binding capacities, calculated for several gelation essays according to equation (2), based on the gel weight and the dry weight. The lines are the best fit of Langmuir model (equation (3)) to the experimental results.

and the Langmuir constant value was 0.665 L/mmol on a gel weight basis or 3.19 L/mmol on a dry weight basis.

The maximum binding capacity and Langmuir constant values will depend on the alginate sample in use, namely on the length and frequency of G blocks in the chains where the predominant binding sites are found. Additionally, the binding capacities will be affected by physicochemical conditions like pH and ionic strength. Nevertheless, these results show a way to predict ionic binding during alginate gelation. Using the model, it is possible to choose an initial cation concentration so that a specified final concentration and reticulation degree, critical for gel properties, are attained.

Kinetic Modeling of Cu^{2+} Binding

For an efficient control of shaped gel manufacturing, the kinetic behavior of the gelation process is an important feature to envisage. Given that the gelation is dependent on the ionic crosslinking of the polysaccharide chains, the monitoring of the ions' removal from solution can give an indication of the kinetics of gel formation. In accordance with this idea, Siew and Williams found a correspondence between the maximum Mn^{2+} binding and the maximum in G' (storage modulus) of alginate gels, for two kinds of alginate with different M:G ratios.[15] Kinetic modeling of cation binding during alginate gelation provides an instrument for monitoring gel production and can be used to examine the rate-limiting step of ionic sorption by the biopolymer.

The kinetic binding models used in this study are derived from the Ritchie equation for adsorption kinetics, which assumes that the binding rate depends on the fraction of binding sites unoccupied at each instant[22]:

$$\frac{d\theta}{dt} = k(1-\theta)^n \tag{4}$$

In equation (4), θ is the fraction of binding sites on the polymer occupied by bound ions at time t; n is the number of binding sites occupied by each ion; and k is the rate constant. From this general equation, different models can be deduced assuming different values for n. Considering $\theta = 0$ for $t = 0$, Equation (4) integrates to:

$$\theta = 1 - e^{-kt}, \quad \text{for } n = 1 \tag{5}$$

or

$$\frac{1}{(1-\theta)^{n-1}} = (n-1)kt + 1, \quad \text{for } n \neq 1 \tag{6}$$

Introducing the amount of copper bound, Cu_{gel}, at time t, equation (5) becomes:

$$Cu_{gel} = Cu_{gel,e}(1 - e^{-k_1 t}) \tag{7}$$

and equation (6) becomes:

$$Cu_{gel} = \frac{Cu_{gel,e}k_2t}{1 + k_2t}, \quad \text{for } n = 2 \tag{8}$$

or

$$Cu_{gel} = Cu_{gel,e}\left(1 - \frac{1}{\sqrt[3]{1 + 3k_4t}}\right), \quad \text{for } n = 4 \tag{9}$$

The application of the Ritchie equation to model Cu^{2+} binding during alginate gelation was tested for three hypotheses: $n = 1$, $n = 2$, and $n = 4$ (equations (7–9)). The first- and second-order equations have been used in modeling biosorption processes.[7] The equation with $n = 4$ was also tested here because, according to the "egg-box" model of calcium chelation by alginate chains, each cation is coordinated by four ligands from the chains.[13]

Figure 6 shows kinetic data for Cu^{2+} binding during alginate gelation with different copper ion concentrations fitted with the three models derived from the Ritchie equation. The rate constants and correlation coefficients for the fits are presented in Table 1. The comparison of the amount of copper bound at experimental equilibrium and the one predicted by the models, taken together with the examination of the correlation coefficients, endorses the models with $n = 1$ and $n = 2$ as the best suited for the prediction of copper ion binding.

The model derived for $n = 4$, corresponding to the "egg-box" coordination of copper ions by crosslinked chains of alginate, showed some applicability only for low Cu^{2+} concentrations. That is probably because this binding mechanism

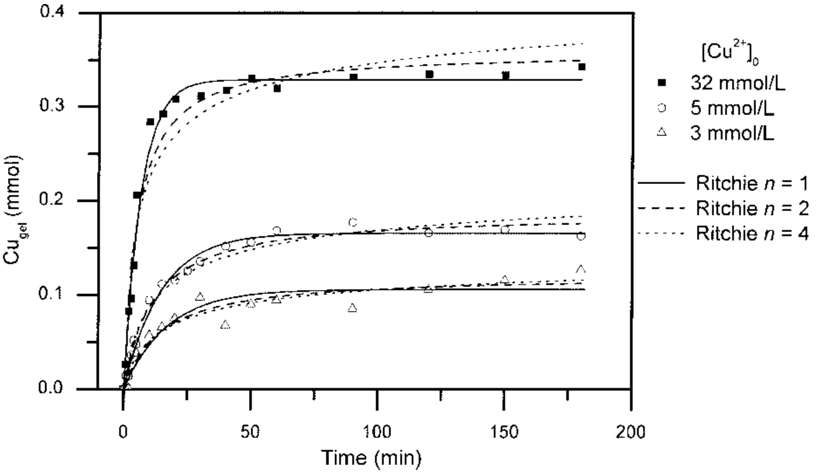

Figure 6: Fit of experimental results of Cu^{2+} binding by alginate during Cu-alginate gelation with the models derived from the Ritchie equation (equations (4) and (7–9)).

Table 1: Copper bound at equilibrium from the experimental data (experimental $Cu_{gel,e}$) and estimated parameters ($Cu_{gel,e}$, k_1, k_2, and k_4) and correlation coefficients (R^2) for the kinetic models.

		n = 1			***n = 2***			***n = 4***		
(Cu^{2+})$_0$ (mmol/L)	**Experimental $Cu_{gel;e}$ (mmol)**	**$Cu_{gel,e}$ (mmol)**	**k_1 (min^{-1})**	**R^2**	**$Cu_{gel,e}$ (mmol)**	**k_2 (min^{-1})**	**R^2**	**$Cu_{\ gel,e}$ (mmol)**	**k_4 (min^{-1})**	**R^2**
3 mmol/L	0.119	0.106	0.059	0.878	0.121	0.070	0.919	0.165	0.069	0.929
5 mmol/L	0.168	0.166	0.069	0.986	0.187	0.088	0.986	0.254	0.085	0.971
32 mmol/L	0.336	0.329	0.153	0.984	0.359	0.196	0.965	0.461	0.218	0.933

is valid only for the fraction of cations tightly bound by the G blocks of alginate chains, while more ions may be bound in other binding sites. Emmerichs et al., using ^{13}C NMR spectroscopy, found that in addition to G blocks, C6 and, in lesser extent, C5 positions in pure mannuronate blocks, as well as adjacent MG residues, also bind Mn^{2+}.[14] Siew and Williams attributed alginate and pectin gelation to the formation of polymer-metal complexes involving one or two carboxylate groups.[15]

Taking into account these recently proposed ionic binding and gelation mechanisms, it can be accepted that most of the bound metal ions in alginate have one or two binding sites, at least in the case of high availability of cations in the gel-contacting solution, justifying the better fit for the Ritchie equation with $n = 1$. The high-affinity binding sites in the G blocks complexes low-concentration copper ions in the "egg-box" fashion, making the Ritchie equation with $n = 1$ less suitable to predict the process in these conditions.

The Ritchie equation was originally developed for the adsorption of gases on an adsorbent solid. The ionic binding during alginate bead gelation is a more complex phenomenon, where factors like chain mobility and ionic diffusion in the gel particles are expected to influence the kinetics of the overall process. However, the results show that Cu^{2+} binding can be reasonably predicted with simple physicochemical models.

CONCLUSIONS

During Cu-alginate gelation, copper ions in solution binds to the alginate polymer rapidly in the first minutes of gelation, but the process prolongs for some hours, depending on the Cu^{2+} concentration in the gel-contacting solution.

The alginate quantity in the gelation system and the Cu^{2+} concentration in solution affect markedly the cation binding and the formation of the gel. If a high quantity of polysaccharide is applied or if the initial Cu^{2+} concentration is low, the availability of crosslinking ions will limit the gelation, leading to gels with low metal bound and higher percentage of water retained. In gelation systems with high concentrations of cations available, gelation occurs faster and with more volume contraction, resulting in stiffer gels with higher dry weights.

Copper ion binding occurs until equilibrium between alginate-bound and metal ions in solution is attained. The modeling of this equilibrium with the Langmuir equation yields a maximum binding capacity of 57.4 μmol Cu^{2+}/g of gel. Importantly, the amount of metal binding and the residual copper ions' concentration can be predicted according to the initial composition of the gelation system using the equilibrium model.

The kinetics of metal binding during alginate gelation can be examined with Ritchie equation-derived models, assuming a number of binding sites depending on the cation availability in the solution. The physicochemical meanings of these models are in accordance with recent studies on the mechanism of metal binding and alginate gelation. In typical alginate gel applications, like cell entrapment and tissue-engineering fabrication, in which high ion concentrations are used, process kinetics can be predicted using the Ritchie equation, assuming the existence of one binding site for each bound cation.

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