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ASSESSMENT OF CALCIUM ALGINATE GELS AS WALL MATERIALS FOR ENCAPSULATION SYSTEMS

Running title: CHARACTERIZATION OF CALCIUM ALGINATE GELS

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Abstract

Background. Calcium alginate gels are widely used to encapsulate active compounds. Some characteristic parameters of these gels are necessary to describe the release of active compounds through mechanistic mathematical models. In this work, transport and kinetics properties of calcium alginate gels were determined through simple experimental techniques.

Results. The weight-average molecular weight ($\overline{M}_w = 192 \times 10^3$ Da) and the fraction of residues of α-L-guluronic acid ($F_G = 0.356$) of sodium alginate were determined by capillary viscometry and ^{1}H NMR at 25 °C, respectively. Considering the *half egg-box* model, both values were used to estimate the molecular weight of calcium alginate as $M_g = 2.02 \times 10^5$ Da. An effective diffusion coefficient of water ($D_{eff,w} = 2.256 \times 10^{-9}$ m^2 s^{-1}) in calcium alginate was determined using a cell diffusion at 37 °C. Finally, a kinetics constant of depolymerization ($k_m = 9.72 \times 10^{-9}$ m^3 mol^{-1} s^{-1}) of calcium alginate was obtained considering dissolution of calcium to a medium under intestinal conditions.

Conclusions. The experimental techniques used are simple and easily reproducible. The obtained values may be useful in the design, production, and optimization of the alginate-based delivery systems that require specific release kinetics of the encapsulated active compounds.

Keywords: Calcium alginate gel, Molecular weight, Water effective diffusion coefficient, Kinetics constant of depolymerization.

1. Introduction

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jsfa.13131

In recent years, the encapsulation of food active compounds (vitamins, unsaturated fatty acids, etc.) has increased in response to the strong demand for healthy foods by consumers.[1] These active compounds tend to degrade under harsh environmental conditions.[2] Therefore, their encapsulation is a good strategy to preserve them and ensure their release with a desired kinetics in the intestinal tract.[3] In this regard, the selection of the appropriate wall material is very important for a successful encapsulation.[1] Moreover, some properties of the wall material are necessary when mathematical models are used to predict the release kinetics of an encapsulated food active compound.[4]

Polysaccharides are commonly used to encapsulate lipophilic active compounds.[1] In particular, alginate is a natural polysaccharide with many advantages over other biopolymers used to obtain hydrogel beads, especially due to its versatility and wide applications.[3,5] In general, calcium alginate gels are stable at room temperature, biocompatible, exhibit good encapsulation efficiency, and have adequate pH responsiveness. In addition, the alginate production is simple, low cost, and allows obtaining gels with a wide range of mechanical properties.[2,5]

Calcium alginate gels are three-dimensional networks formed by ionic crosslinking between alginate and Ca^{2+} ions. Alginate is a linear polysaccharide of the residues of β-D-mannuronic acid (M group) and α-L-guluronic acid (G group) linked by glycosidic 1-4 bonds.[5] The monomers can be linked forming homogeneous sequences (M blocks and G blocks) or heterogeneous sequences (MG blocks). Since alginate is a natural polymer, its characteristics can define the alginate-based delivery system[2] and must be known.

The objective of this work was to apply simple techniques to determine some characteristic parameters of calcium alginate gel. These parameters are necessary for a rational design of alginate-based delivery systems, as they are generally used in mathematical models to represent the release kinetics of active compounds (*i.e.*, the molecular weight of calcium alginate, the effective diffusion coefficient of water in a calcium alginate gel, and the kinetics constant of depolymerization of calcium alginate gel).[4] The molecular weight of calcium alginate and its kinetics constant of depolymerization are necessary to solve the mass balance of the polymeric matrix, while the effective diffusion coefficient of water in calcium alginate gel is necessary to solve the mass balance of water.

2. Materials and methods

2.1. Materials

Food grade low viscosity sodium alginate (Kelco, Atlanta, USA), dihydrate calcium chloride ($CaCl_2.2H_2O$, Anedra, Buenos Aires, Argentina), urea ($CO(NH_2)_2$, Biopack, Buenos Aires, Argentina), urea assay kit (Wiener lab., Santa Fe, Argentina), soy oil (density = 0.919 ± 0.001 g mL^{-1} at 20 °C, data provided by the manufacturer) (AGD, Córdoba, Argentina), sodium chloride (NaCl), polysorbate 80, monosodium phosphate (NaH_2PO_4), and disodium phosphate (Na_2HPO_4) (Cicarelli Reagents S.A., Santa Fe, Argentina) were used in the present work.

2.2. Determination of the molecular weight of a calcium alginate gel (M_g)

Braschler et al.[6] proposed that the G groups of alginate can bind a specific number N_c of Ca^{2+} resulting in the formation of calcium alginate gels. Accordingly, N_c is the stoichiometric coefficient of Ca^{2+} [-] that depends on the G groups of the alginate and on the model used for describing the gel structure. Therefore, the molecular weight of calcium alginate (M_g) can be estimated by,[6]

$$M_g = N_c M_c + M_a \quad \textbf{(1)}$$

where M_c and M_a are the molecular weight [Da] of calcium and alginate, respectively.

The *egg-box* model was proposed to describe the calcium alginate gel structure. According to this model, Ca^{2+} ions bind selectively to the G blocks of the alginate chain due to their compatible form and charge. Then, a stoichiometric ratio of G:Ca^{2+} of 2:1 is expected when G groups are in G blocks. However, G blocks can be more cross-linked when Ca^{2+} ions are in excess.[5] Then, taking into account that the initially formed dimers bind to Ca^{2+} ions forming aggregates (**Fig. 1**), an improved model called the *half egg-box* model was proposed.[7] Here, the stoichiometric ratio G:Ca^{2+} is 4:3. As a consequence, considering that all G groups in the alginate molecule are forming G blocks,[8] N_c can be estimated by,[6]

$$N_c = \frac{3}{4}\frac{M_a}{M_m}F_G \quad \textbf{(2)}$$

where M_m is the molecular weight of G groups [Da] and F_G is the G fraction in the alginate [-].

Based on the above, the values of M_c, M_a, M_m and F_G are required to calculate M_g. The values of M_c = 40 g mol^{-1} and M_m = 198 g mol^{-1} [9] were obtained from the literature, while M_a and F_G were experimentally determined from capillary viscometry[10] and by proton nuclear magnetic resonance,[11] respectively.

Figure 1

2.2.1. Determination of the molecular weight of alginate (M_a)

The molecular weight of alginate (M_a) was estimated as the weight-average molecular weight ($\bar{M}_w$) from its intrinsic viscosity. The estimation was carried out using the Mark-Houwink-Sakurada (MHS) equation,[10]

$$[\eta] = k_{MHS}\bar{M}_w^a \quad \textbf{(3)}$$

where $[\eta]$ is the intrinsic viscosity [mL g^{-1}], and k_{MHS} [g mL^{-1}] and a [-] are empirical constants that depend on the polymer, solvent and temperature.

The value of $[\eta]$ was obtained from the reduced viscosity (η_{red}) values, which were determined by capillary viscometry. Eight sodium alginate solutions of concentrations ranging from 0.25 to 1 g L^{-1} were prepared in 0.1 M NaCl as solvent.[13] Each solution was filtered through a 0.45-µm nylon filter (D'Amico Sistemas, Buenos Aires, Argentina). An AVS300 Schott Geräte viscometer (Schott Geräte, Mainz, Germany), composed by a Cannon-Fenske size-50 capillary with a CT 1150/2 Schott Geräte thermostatic bath, was used. The η_{red} values were calculated from the flow time measured for each alginate solution at 25 °C, using **Eq. (4)**.[14]

$$\eta_{red} = \frac{t/t_0 - 1}{c} \quad \textbf{(4)}$$

where t and t_0 are the flow times of alginate solution and solvent [s], respectively, and c is the concentration of the alginate solution [g mL^{-1}]. The capillary viscometry experiments were carried out in duplicate. In each experiment, the measurements at each time t were obtained in quadruplicate.

The $[\eta]$ value was determined by fitting the η_{red} values as a function of c, using **Eq. (5)**,[14]

$$\eta_{red} = \frac{[\eta]}{1 - k_H[\eta]c} \quad \textbf{(5)}$$

where k_H is a constant [-].

The goodness of fit between the theoretical (**Eq. 5**) and experimental values of η_{red} was evaluated through a mean absolute percent error ($MAPE$) defined by **Eq. (6)**,

$$MAPE = \frac{100}{J}\sum_{j=1}^{J}\left|1 - \frac{\Gamma_{T,j}}{\Gamma_{E,j}}\right| \quad \textbf{(6)}$$

where J is the number of experimental data [-] and $\Gamma_{T,j}$ and $\Gamma_{E,j}$ are the theoretical and experimental j-th values, respectively.

2.2.2. Determination of the G fraction of alginate (F_G)

The composition of a 50 g L^{-1} sodium alginate solution in deuterium oxide (D_2O) were determined by proton nuclear magnetic resonance (^1H NMR) analysis on a Bruker Advanced II 300 MHz spectrometer (Bruker, Rheinstetten, Germany). Complete solubilization was achieved by sonication in warm water using a Teslab T302 TA ultrasonic bath (Teslab S.R.L., Buenos Aires, Argentina). Then, the alginate solution was stored overnight at 4 °C.

Pulse sequence for ^1H NMR analysis

^1H NMR experiments were performed with a pulse sequence denoted *ledbpgp2s1d* in the Bruker pulse sequence library **(Fig. 2)** at 25 °C. This sequence includes a longitudinal eddy current delay (LED) experiment using bipolar gradients.[15] In general, this pulse sequence consists of applying a gradient of pulsed field that it is added to the main magnetic field. This produces the diffusion of the sample molecules inside the tube at different velocities than the ones associated to their diffusion coefficients. Accordingly, the signals of the sample compounds are separated in the spectrum.[16] **Fig. 2** shows a schematic representation of the pulse sequence *ledbpgp2s1d*. In the ^1H channel, $d1$, $d21$, AQ, and δ_G are the recycle delay, the recovery time, the acquisition time, and the length of the applied gradient, respectively. Pulse gradients are represented in the G_z channel, where G1-G2 and G3-G4 are the gradients of bipolar pulses, and the Gp are purge gradients.[16] **Table 1** shows the parameter values of the pulse sequence used to obtain the best resolution of the spectrum for an alginate sodium solution at 25 °C.

Figure 2

Table 1 Parameters of the pulse sequence for the determination of sodium alginate composition by ^1H NMR at 25 °C

Parameter	Symbol	Unit	Value
Time domain size	TD	-	32768
Number of scans	NS	-	256
Number of dummy scans	DS	-	8
Spectral width	SW	ppm	26.7
	SWH	Hz	812.8
Acquisition time	AQ	s	2.0
Diffusion time	Δ	s	0.1
Gradient strength	G	%	98
Gradient length	δ_G	µs	2500

2.3. Determination of the effective diffusion coefficient of water in a calcium alginate gel ($D_{eff,w}$) at 37 °C

The transport of water molecules in an occluded solution within the pores of an alginate gel can be affected by various factors (*e.g.*, different types of diffusive transport). For this reason, an often used and widely accepted engineering simplification is to consider that the global transport of water in a porous gel can be described by an effective diffusion coefficient ($D_{eff,w}$). This value can be related to the molecular diffusion coefficient of water in the occluded solution ($D_{w,s}$) as,[17]

$$D_{eff,w} = KD_{w,s} \quad \textbf{(7)}$$

where K is a factor [-] that takes into account the structural effects of the gel on the water diffusion process.

In this study, $D_{eff,w}$ was determined at 37 °C (intestinal temperature) and pH 7 because swelling and degradation of the alginate gel were not significant at these conditions (see **Supporting information**). Also, the occluded solution in the gel was regarded as an aqueous solution. Then, $D_{w,s}$ was considered to be equal to the water self-diffusion coefficient. The value of K can be estimated experimentally through **Eq. (7)** using an adequate tracer compound diffusing in the same medium to obtain its corresponding effective and occluded-solution diffusion coefficients.[17] The following method was used to obtain $D_{eff,w}$: (1) urea was selected as a tracer compound because of its low toxicity, high water solubility (1510 g L^{-1} at 37 °C), known diffusion coefficient in water ($D_{u,s}$), and a negligible interaction with calcium alginate gel in the experimental conditions used,[18–20] (2) the effective diffusion coefficient of urea in calcium alginate gel ($D_{eff,u}$) was determined using a diffusion cell (**Fig. 3**), (3) the K factor in **Eq. (7)** was determined as $K = D_{eff,u}/D_{u,s}$ and (4) $D_{eff,w}$ was calculated.

2.3.1. Determination of the effective diffusion coefficient of urea in a calcium alginate gel ($D_{eff,u}$) at 37 °C

The effective diffusion coefficient of urea in a calcium alginate gel ($D_{eff,u}$) was determined using a similar procedure and diffusion cell described by Zorrilla and Rubiolo[21] (**Fig. 3**). Briefly, the diffusion cell is composed of two compartments (A and B) separated by a wall containing a disk of the studied gel. The disk had a surface S available for the diffusion and a thickness L. The compartments A and B were filled with the same volume V of a urea solution and water, respectively. In consequence, a diffusive flux of urea was established through the disk between compartments A and B. Urea concentration in the compartment B (c_B) was monitored at different times (t) using a urea assay kit. The coefficient $D_{eff,u}$ was determined by fitting the c_B vs t values to the **Eq. (8)**. This equation was obtained solving a mass balance

for urea in the disk considering that (a) there was no chemical reaction, (b) the urea convective flux was negligible, (c) the disk diameter was larger than L, (d) the compartments A and B were well stirred (perfect mixing), and (e) L and $D_{eff,u}$ remained constant during the experiments.[22]

$$c_B(t) - c_{B,0} = \frac{SL}{V}\left[\frac{D_{eff,u}}{L^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-\frac{D_{eff,u}\pi^2 n^2 t}{L^2}\right)\right]k\left(c_{A,0} - c_{B,0}\right) \quad \textbf{(8)}$$

Here, k is the partition coefficient [-], and $c_{A,0}$ and $c_{B,0}$ are the initial concentration of urea in the compartments A and B [g L^{-1}], respectively. Five independent experiments were performed. In each experiment, the determination of c_B at each time t was carried out in duplicate. The goodness of fit of **Eq. (8)** to the experimental data was evaluated through the $MAPE$ defined by **Eq. (6)** and considering that the fitted variable (Γ) is c_B.

Calcium alginate disks were prepared from a 20 g kg^{-1} sodium alginate solution and a 250 mM $CaCl_2$ solution. Briefly, an absorbent cloth and a filter paper were placed in a disk-shaped mold of 0.05-m internal diameter and 0.007-m depth. The cloth and paper placed in the mold were soaked with the $CaCl_2$ solution. An aliquot of 5 to 7.5 mL of the sodium alginate solution was transferred into the mold and kept for 15 min. Then, the mold was immersed in the $CaCl_2$ solution for 60 min until a gel was obtained. Finally, the gel was removed from the mold and rinsed with ultrapure water. The gel disk was trimmed to get a final diameter of 0.032 m and placed in the diffusion cell.

The experimental values of $c_{A,0}$, $c_{B,0}$, S and V used in the experiments are shown in **Table 2**. The values of L were measured using a digital disc micrometer Schwyz (Schwyz, China) and k was assumed equal to 1 because the interaction between urea and calcium alginate is supposed negligible during the experiment time.[18,23]

Figure 3

Table 2 Parameters used in **Eq. (8)**

Parameter	Unit	Value
$c_{A,0}$	g L^{-1}	100
$c_{B,0}$	g L^{-1}	0
S	m^2	4.909×10^{-4}
V	m^3	3.500×10^{-4}

2.4. Determination of the kinetics constant of depolymerization of calcium alginate (k_m) in simulated intestinal conditions

The kinetics constant of depolymerization of calcium alginate gel was determined through the kinetics of calcium release from calcium alginate particles under simulated intestinal conditions.[24] In this case, the depolymerization process can be characterized with a second-order kinetics model.[25] Taking into account that the level of polymerization can be represented by the molecular weight of calcium alginate (M_g), the process can be described as,

$$\frac{\partial M_g}{\partial t} = -k_m c_w M_g \quad \textbf{(9)}$$

where k_m is a kinetics constant of depolymerization [m^3 mol^{-1} s^{-1}] and c_w is the water concentration [mol m^{-3}]. An experimentally useful form of **Eq. (9)** can be obtained assuming that: (a) the gel hydration is faster than the gel depolymerization, *i.e.*, c_w rapidly reaches the equilibrium water concentration ($c_{w,\infty}$), and (b) the release of calcium from the gel to the dissolution medium can be represented by a kinetics of equal magnitude that the depolymerization kinetics, but opposite in sign.[24]

Consequently, the depolymerization kinetics of calcium alginate gel under intestinal conditions can be studied through the release kinetics of calcium that is described by,

$$\frac{\partial \tilde{c}_{Ca}}{\partial t} = \kappa \tilde{c}_{Ca} \quad \textbf{(10)}$$

where $\tilde{c}_{Ca} = c_{Ca}/c_{Ca,0}$ is the dimensionless calcium concentration [-], c_{Ca} is the calcium concentration in the dissolution medium, $c_{Ca,0}$ is the initial calcium concentration in the particles [mg g_{ds}^{-1}], and κ is a constant [s^{-1}] defined as,

$$\kappa = k_m c_{w,\infty} \quad \textbf{(11)}$$

Equation (10) can be integrated and linearized to obtain,

$$ln(\tilde{c}_{Ca}) = \kappa t + ln(\tilde{c}_{Ca,0}) \quad \textbf{(12)}$$

Now, k_m can be found by fitting **Eq. (12)** to experimental data of the calcium concentration released from alginate calcium beads immersed in an aqueous medium under intestinal conditions.

2.4.1. Preparation and characterization of the calcium alginate beads

Calcium alginate beads are generally used to encapsulate hydrophobic active compounds.[2] In this work, soy oil was encapsulated as a model hydrophobic active compound. Then, calcium alginate beads were obtained following the procedure used by Niizawa et al.[26]. Briefly, an emulsion of a 20 g kg^{-1} sodium alginate solution, soy oil and polysorbate 80 (emulsifier) was prepared with a homogenizer UltraTurrax T25 Basic (IKA, Staufen, Germany) at 20000 rpm for 3 min. The ratio alginate/oil was 3

(v:v) and the polysorbate concentration was 20 g kg^{-1}. The dispersion was dripped into a stirred 250 mM $CaCl_2$ solution, using a 22-gauge needle to form the calcium alginate beads. Then, the particles were maintained in suspension in the $CaCl_2$ solution for 30 min, filtered, washed and dried at 25 °C for 2 h.

The initial mean value of the radius of the particles radius (R) was determined by image analysis using the ImageJ software (National Institute of Health, Maryland, USA).[26] Also, the water content (H) of the particles was measured in triplicate by a gravimetric technique.[27]

2.4.2. Depolymerization of calcium alginate beads in simulated intestinal conditions

The depolymerization experiments were carried out in simulated intestinal conditions in duplicate using a 0.05 M phosphate buffer solution at pH 7.4, 37 °C, and 100 rpm of agitation. The experiments consisted of immersing the calcium alginate beads into the dissolution medium at different intervals of time (300, 600, 900, 1500, 2100, 3000, 4800 and 7200 s). At the end of each interval, samples were centrifuged at 2292 ×g for 10 min with a Capp CRC-658 centrifuge (Capp, Odense, Denmark). The supernatant was collected, filtered and used for determining the calcium concentration.

2.4.3. Calcium determination

The initial calcium content of the particles ($c_{Ca,0}$) and the released calcium from particles to the dissolution medium (c_{Ca}) were determined by flame atomic absorption spectroscopy (FAAS) (Perkin Elmer AAnalyst 200, Perkin Elmer, Massachusetts, USA) in duplicate. A flame of air-acetylene was used. Lanthanum oxide (1 g L^{-1}) was added to all samples to minimize phosphate interferences. The measurements were carried out at 422.67 nm.[28]

2.5. Statistical analysis

A statistical analysis to detect outliers or influential data was performed using RStudio 2021.09.2 (RStudio, PBC, Boston, USA). The linear model and the functions *outlierstest* e *influenceIndexPlot* were used. A significance level of 0.05 was considered. The variance homogeneity was checked with the functions *plot* and *Q-Q plot*.

3. Results and discussion

3.1. Molecular weight of a calcium alginate gel (M_g)

The molecular weight of calcium alginate gel is essential to shed light on the matrix behavior during the release process (e.g., its swelling and erosion), and then mathematically model the release kinetics of active compounds from an encapsulating matrix (e.g., calcium alginate gel).[4]

Fig. 4 shows a typical curve of the experimental data and the theoretical curve obtained using **Eq. (5)**. The values of k_H and $[\eta]$ obtained fitting the experimental data to **Eq. (5)** were 0.55 ± 0.06 and 406.94 ± 3.77 mL g^{-1}, respectively. The $MAPE$ values for all the independent experiments were less than 2 %, representing a highly accurate fitting.[29]

Figure 4

The values of k_H and $[\eta]$ were similar (same order of magnitude) to those reported for different alginates in NaCl solutions. Hermansson et al.[30] informed k_H values from 0.50 to 0.55 for sodium alginate in NaCl solutions (0.005 – 0.2 M). Benabbas et al.[31] obtained k_H values of 0.80 and 1.11, and $[\eta]$ values of 77.692 and 40.587 mL g^{-1} for two types of alginic acid. Fertah et al.[32] reported a $[\eta]$ of 243.1 mL g^{-1} for a sodium alginate extracted from Moroccan *Laminaria digitata* brown seaweed. da Costa et al.[33] reported values of 146 mL g^{-1} and 0.33 for $[\eta]$ and k_H, respectively.

Weight-average molecular weight ($\bar{M}_w$) was estimated through the MHS equation (**Eq. 3**) with the coefficients k_{MHS} and a equal to 2.3 g mL^{-1} and 0.987, respectively, obtained for sodium alginate in NaCl 0.1 M at 25 °C.[34] A $\bar{M}_w$ value of 192 (± 2) × 10^3 Da was obtained. This value was in the range of those reported the literature for a low viscosity sodium alginate.[35] Martinsen et al.[36] characterized a sodium alginate, isolated from *Macrocystis pyrifera* of Kelco Division of Merck, and dissolved in a 0.1 M NaCl solution at 25 °C. These authors reported values of 548 ± 3 mL g^{-1}, 0.421 ± 0.001 and 205.3 ± 5.3 kDa for $[\eta]$, k_H and $\bar{M}_w$, respectively.

Fig. 5 shows the spectrum obtained with the pulse sequence *ledbpgp2s1d*. A D_2O peak (δ = 4.709 ppm) and characteristics peaks of alginate (A, B, and C) can be observed. These data were used for determining F_G,[32]

$$F_G = \frac{I_A}{I_B + I_C} \qquad \textbf{(13)}$$

where I_A, I_B and I_C are the integrated intensities of the A, B, and C peaks, respectively, and their values are shown below their respective peak in **Fig. 5**. The values of F_M, F_G and F_M/F_G are showed in the **Table 3**. These values are similar to those reported in the literature for a sodium alginate isolated from

Macrocystis pyrifera obtained from Kelco Division of Merck (viscosity-average molecular weight $\bar{M}_v$ = 198.1 kDa, F_G = 0.43, F_M = 0.57, and F_M/F_G = 1.38).[37] Our results indicate that the studied low viscosity sodium alginate is rich in M monomer (F_M/F_G> 1), suggesting that this alginate would be more suitable for the formulation of softer edible coatings.[38]

Figure 5

Table 3 Composition of the studied low viscosity sodium alginate

Parameter	Value
F_G	0.356
F_M	0.644
F_M/F_G	1.809

Then, the estimated stoichiometric coefficient was $N_c \approx 259$ (**Eq. 2**), which was obtained using the values of $\bar{M}_w$ and F_G previously determined and a value of M_m= 198 Da obtained from the literature.[9] Finally, using **Eq. (1)** and considering M_c = 40.08 Da, the estimated molecular weight of calcium alginate was M_g = 2.02 × 10^5 Da.

3.2. Effective diffusion coefficient of water in calcium alginate gel ($D_{eff,w}$) at 37 °C

The diffusion coefficient of water is required to mathematically model and solve the mass balance of water and to evaluate the hydration characteristics of the encapsulating matrix during the release process.[4]

Fig. 6 shows a typical curve of the experimental data and the theoretical curve obtained using **Eq. (8)**. The goodness of fit for all the independent experiments was ranged good to highly accurate (*MAPE* = 15.1 – 6.6 %).[29] The average effective diffusion coefficient of urea in the calcium alginate gel obtained at 37 °C was $D_{eff,u}$= 1.490 (± 0.254) × 10^{-9} m^2 s^{-1}. This value is in the same order of magnitude as to those reported in the literature for calcium alginate gels (20 g kg^{-1}) at 25 °C (8.97 × 10^{-10} m^2 s^{-1}),[39] and alginate gels (10 g kg^{-1}) with different monomeric compositions, $CaCl_2$ concentration and at 30 °C (7 × 10^{-10} m^2 s^{-1} to 5 × 10^{-9} m^2 s^{-1}).[40]

Figure 6

The diffusion coefficient of urea in water ($D_{u,s}$) at 37 °C was estimated as 2.00 (± 0.20) × 10^{-9} m^2 s^{-1} using the data at 5 °C and 25 °C obtained from Cussler[41] and Geankoplis,[42] respectively. Then, a *K*

value equal to 0.745 was obtained using $D_{eff,u}$ and $D_{u,s}$ in **Eq. (7)**. That is, the effective diffusion coefficient is 25.5 % lower than the diffusion coefficient of urea in the occluded solution. This value is in agreement with the literature. Geankoplis[42] reported a reduction of 27 % when urea is diffusing through gelatin (29 g kg^{-1}). Estapé et al.[43] reported a reduction of 25 % for glucose in 2 g kg^{-1} calcium alginate. Chai et al.[44] found that this difference was 44 % in a 10 g kg^{-1} calcium alginate gel.

Finally, using $D_{w,s}(37\ °C) = 3.028 \times 10^{-9}$ m^2 s^{-1},[45] the calculated value of K, and **Eq. (7)**, the estimated effective diffusion coefficient of water in a calcium alginate gel was $D_{eff,w}(37\ °C) = 2.256 \times 10^{-9}$ m^2 s^{-1}.

3.3. Kinetics constant of depolymerization of calcium alginate (k_m) in simulated intestinal conditions

The depolymerization kinetics constant is necessary to complete the mathematical description of the mass balance of the polymeric matrix and to describe the effect of the erosion on diffusive and mechanical characteristics of the polymeric matrix during the release process.[4]

The initial mean values of the radius (R) and water content (H) of the particles were 1.17 (± 0.07) $\times 10^{-3}$ m and 611.83 ± 50.65 mg g_{ds}^{-1}, respectively. The average initial calcium concentration ($c_{Ca,0}$) in particles was 5.67 ± 0.45 mg g_{ds}^{-1}, which is in the magnitude order of the theoretical value of 3.06 mg g_{ds}^{-1}.[9]

Fig. 7 shows a typical evolution (in t and $t^{1/2}$) of dimensionless calcium concentration in the dissolution medium ($\tilde{c}_{Ca}$) as a result of the depolymerization of calcium alginate beads in simulated intestinal conditions. Initially, for 0 s < t < 2.4×10^3 s, $\tilde{c}_{Ca}$ values show an approximately linear increase with $t^{1/2}$ (0 s < $t^{1/2}$ < 49 $s^{1/2}$, **Fig. 7.b**). Then, a plateau is observed at $\tilde{c}_{Ca} \approx 0.3$. Finally, an increase is observed again starting from $t \approx 5.4 \times 10^3$ s ($t^{1/2} \approx 73$ $s^{1/2}$). These results were similar to the ones reported by Kikuchi et al.[8] for a similar system. The described shape of the profile could be related to the molecular phenomena involved. That is, initially, Ca^{2+} is strongly bound to the G blocks within the calcium alginate structure and interacts ionically (*i.e.*, weak interactions) with the M and MG blocks. Then, Na^+ ions (present in the phosphate buffer) replace Ca^{2+} ions linked to the M blocks, resulting in a diffusive mechanism that governs the first stage of the calcium release (*i.e.*, linear relationship between $\tilde{c}_{Ca}$ and $t^{1/2}$). In the second stage, Na^+ ions are interchanged with Ca^{2+} ions bound to the G blocks. These blocks form auto-cooperative binding of Ca^{2+} ions. Therefore, Ca^{2+} release is slower than the release

observed on the first stage. In the third stage, Ca^{2+} bound to the G blocks diffuse into the dissolution medium, disintegrating the gel.[8]

Figure 7

Fig. 8 shows the fitted **Eq. (12)** and the experimental values of $\tilde{c}_{Ca}$ used to estimate κ. The average fitted value of κ was 5.407 (± 1.003) × 10^{-4} s^{-1}. This result is in the order of magnitude of the reported values in the literature for similar systems (1.383 × 10^{-4} s^{-1} and 1.218 × 10^{-4} s^{-1}).[8,24] The difference could be attributed to a lower F_M/F_G ratio (same molecular weight) observed for the alginate samples reported in the literature. That is, lower F_M/F_G ratios allow the formation of a more rigid and less porous particles due to fewer interaction zones between G blocks and Ca^{2+}. Consequently, those gels are less permeable and the calcium release is slower (lower κ).[38]

Finally, the kinetics constant of depolymerization k_m = 9.72 (± 1.80) × 10^{-9} m^3 mol^{-1} s^{-1} was calculated using **Eq. (11)** and assuming that the equilibrium water concentration was $c_{w,\infty}$ = 5.56 × 10^4 mol m^{-3} (*i.e.*, pure water).

Figure 8

4. Conclusions

This study provides simple and easily reproducible experimental techniques to characterize the structure and behavior (transport and kinetics properties) of calcium alginate gels. The molecular weight of calcium alginate was estimated through the determination of the weight-average molecular weight ($\bar{M}_w$) and the G fraction (F_G) of alginate by viscometry and ^{1}H MNR, respectively. Both techniques allowed obtaining good quality results at low temperature, in short time and without modifying the samples. An effective diffusion coefficient of water in calcium alginate gel was estimated using a diffusion cell and urea as tracer compound. The proposed technique is low-cost, versatile, and non-destructive. Finally, a kinetics constant of depolymerization of calcium alginate was estimated through the release of calcium from calcium alginate beads, measured by flame atomic absorption spectroscopy. It should also be noted that the data obtained were not available in the literature. These data are essential to complete and/or improve mechanistic mathematical models used to represent the release kinetics of active compounds.

The data obtained to characterize the structure and the behavior of calcium alginate gels may be useful during the design, production, and optimization of the alginate-based delivery systems that require specific behavior and release kinetics of the encapsulated active compounds.

Acknowledgments. The authors would like to thank the Universidad Nacional del Litoral (UNL) (Santa Fe, Argentina) [project CAI+D: 506 201901 00005 LI], Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (Agencia I+D+i) (Argentina) [project: PICT 2020-544], and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (Argentina) [project CONICET: 11220200100440CO].

Conflict of Interest Statement. The authors declare no conflicts of interest.

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Figure legends

Figure 1. Schematic representation of the formation of calcium alginate gels (adapted from Djabourov et al.[8] and Silva et al.[12]).

Figure 2. Schematic representation of the pulse sequence *ledbpgp2s1d* used in ^{1}H NMR (adapted from Parella[15]).

Figure 3. Cross-section view the diffusion cell.

Figure 4. Theoretical and experimental values of the reduced viscosity (η_{red}) as a function of the alginate concentration (c). Error bars correspond to the standard deviation of four replicates.

Figure 5. ^{1}H NMR spectrum obtained with pulse sequence *ledbpgp2s1d* at 25 °C for the studied low viscosity sodium alginate.

Figure 6. Experimental and theoretical profiles of c_B as a function of time (t). Error bars correspond to the standard deviation of two replicates.

Figure 7. Dimensionless calcium concentration in the dissolution medium ($\tilde{c}_{Ca}$) as function of **(a)** t and **(b)** $t^{1/2}$. Error bars correspond to the standard deviation of two replicates.

Figure 8. Experimental and theoretical profiles of $\ln(\tilde{c}_{Ca})$ as a function of time (t). Filled symbols represent data used to fit **Eq. (12)**.

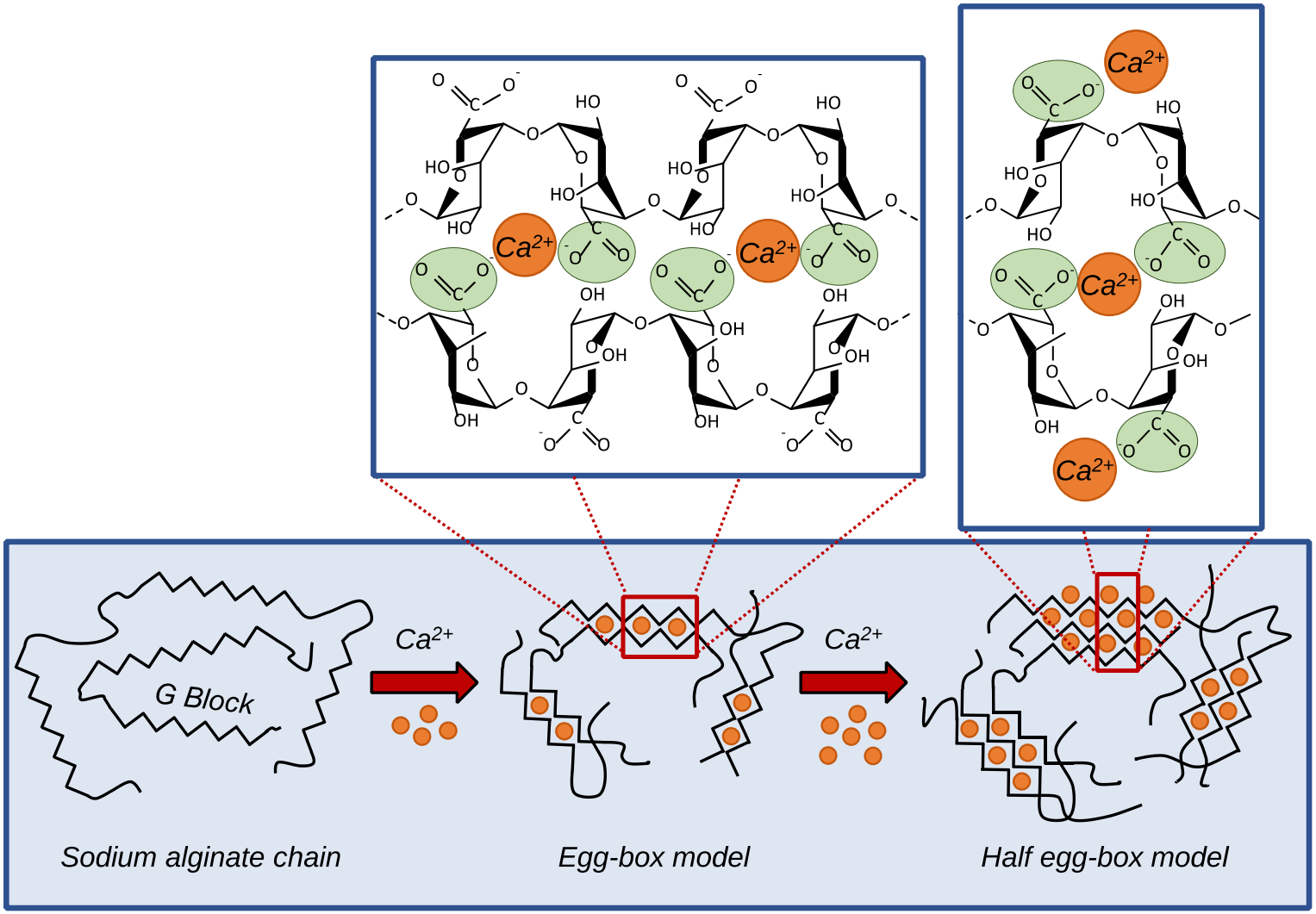
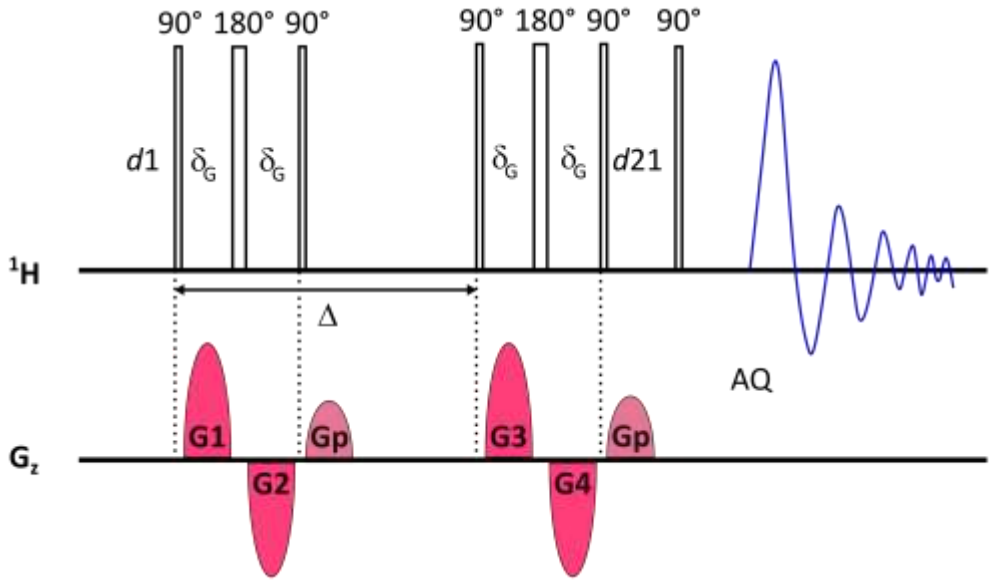


Figure 1.



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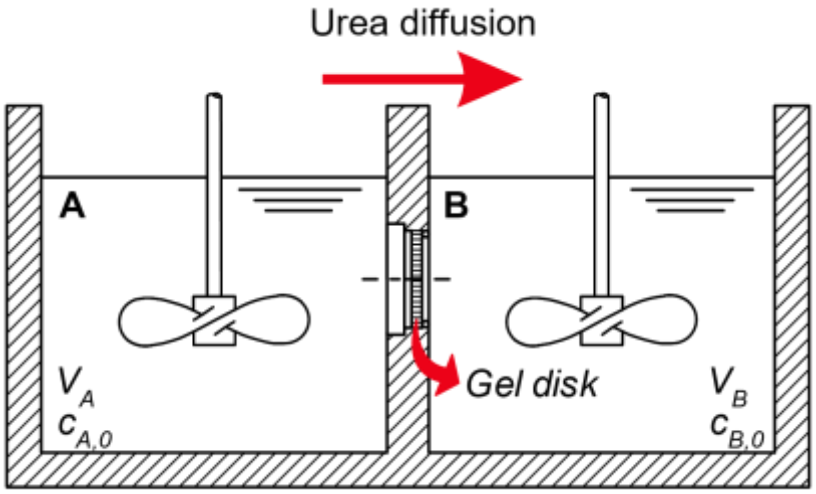

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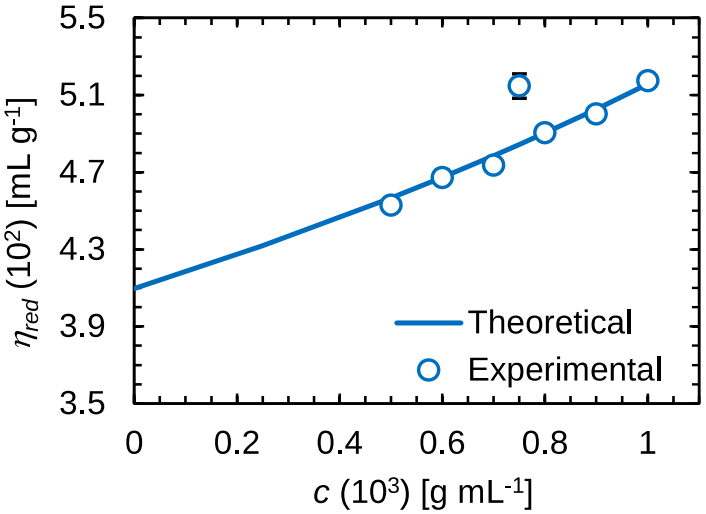

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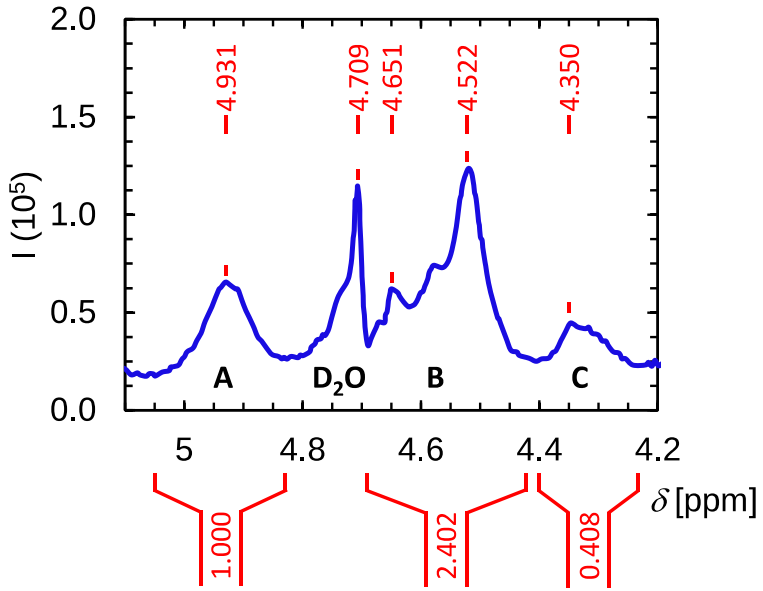

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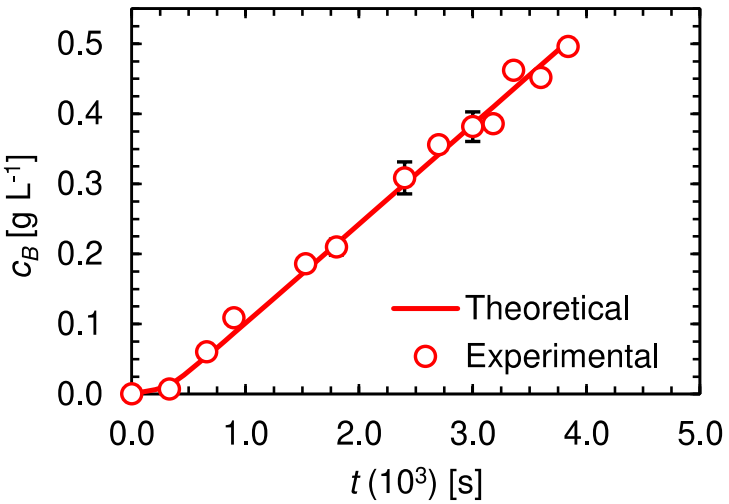

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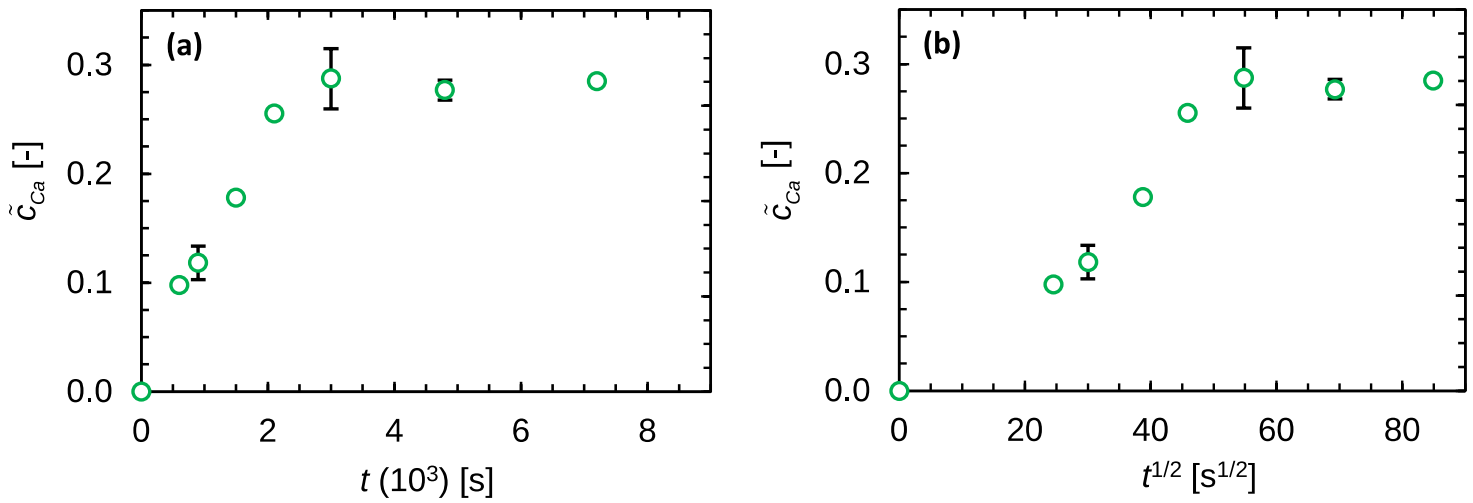

Figure 7.

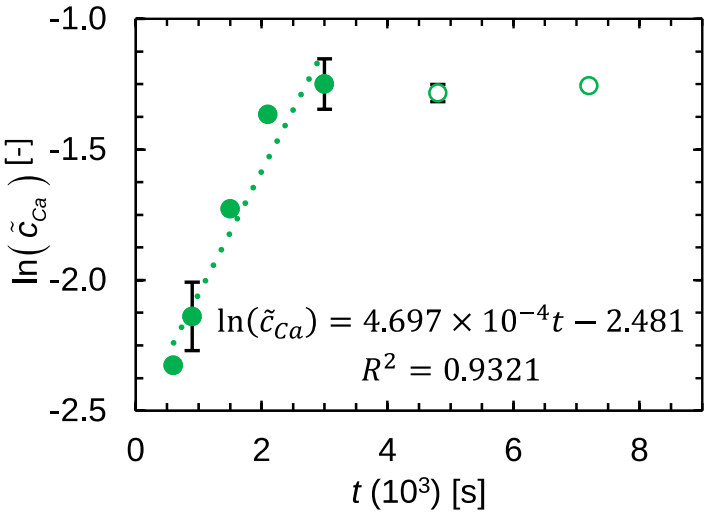

Figure 8.