



Proceeding Paper The Influence of Calcium–Sodium Ion Exchange in the Rheometry of Sodium Alginate-Based Hydrogel ⁺

Marius Ghiurea 🖻, Naomi Tritean 🖻, Ştefan-Ovidiu Dima 🖻, Bogdan Trică, Ioana Hosu 🖻 and Florin Oancea *🖻

National Institute for Research & Development in Chemistry and Petrochemistry—ICECHIM, 202 Splaiul Independentei, 060021 Bucharest, Romania; marius.ghiurea@icechim.ro (M.G.); naomi.tritean@icechim.ro (N.T.); ovidiu.dima@icechim.ro (Ş.-O.D.); bogdan.trica@icechim.ro (B.T.); ioana.hosu@icechim.ro (I.H.)

* Correspondence: florin.oancea@icechim.ro

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Abstract: This work proposes a process for a bio-based hydrogel suitable for 3D-printing formulation. The components of the hydrogel are sodium alginate, tetrasodium pyrophosphate, and calcium sulfate. Changes in the rheology of hydrogels based on alginate cross-linked with calcium released from calcium sulfate are reported for further use in 3D printing. The effect of the total or partial reaction of the compounds on the microscopic morphology and, consequently, on the rheometric behavior is studied.

Keywords: biodegradable materials; microstructure; printing formulation; bioinspired hydrogels

1. Introduction

A high water-holding capacity, biodegradability, and a lack of toxicity recommend alginate as a biocompatible biopolymer that is widely employed in health sector research to develop new materials for drug delivery, tissue regeneration, wound healing systems, and biomedical implants [1–3]. In addition to biomedical applications, sodium alginate (NaAlg) has applications in various other fields, such as food packaging [4], electrolytes in chemical devices [5], wastewater treatment [6], emulsifiers [7], anticorrosive dyes [8], stabilizers [9], and textiles [10]. Alginate-based composites can be used in powder form [11], film form [12], and liquid phase in spin coating or 3D printing items [13].

Using alginate-based hydrogel for 3D printing is an emerging industrial application [14]. Alginate-based ink must have rheometric properties that allow it to flow through deposition nozzles of different diameters. Therefore, alginate-based inks must have a shear thinning capability. Also, alginate-based ink must have a zero shear viscosity high enough to support the structural stability of 3D-printed constructions made with additive layers [3].

Sodium alginate forms hydrogels in the presence of free Ca^{2+} ions. Gelling can be carried out in two ways: external setting or internal setting. External cross-linking occurs rapidly at the alginate solution/CaCl₂ solution interface and then increasingly slowly as the thickness of the alginate gel layer increases [15]. In this case, the gelling rate is diffusion-controlled and depends mainly on the contact area between the gel and the CaCl₂ solution relative to the total alginate mass. The reaction ends when the gel is saturated with Ca²⁺ ions. Another variant that is of interest in terms of controlling the gelation process is the internal setting, in which the source of Ca^{2+} ions is a relatively insoluble salt (CaSO₄) added to the alginate solution in the presence of a complexing agent (sodium pyrophosphate) that controls the reaction kinetics. This work investigates this approach for the formulation of a hydrogel suitable for 3D printing.



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2. Materials and Methods

Analytically pure substances were used to form the hydrogels: sodium alginate BioChemica (PanReac AppliChem ITW Reagents, Monza, Italy, molecular weight 10,000–300,000 dal), tetrasodium diphosphate decahydrate (Scharlau, Barcelona, Spain), calcium sulfate (Chimreactiv, Bucharest, Romania). For the formation of hydrogels, the amounts in Table 1 were used. Sodium alginate was dissolved in half the quantity of distilled water. After forming a uniform gel, the amount of tetrasodium pyrophosphate as a reaction retardant was added and mixed with a mechanical stirrer until homogenization. Calcium sulfate was used as an ionic cross-linker for the alginate. The quantity of calcium sulfate from Table 1 was dissolved in half the amount of water, and the solution was added over the alginate solution previously obtained under continuous stirring.

Table 1. The recipes used in the present study and the calculated zero-rate viscosity of each sample.

Sample	H ₂ O	Sodium Alginate	Tetrasodium Pyrophosphate	Calcium Sulfate	Zero-Rate Viscosity
S0	100 g	3 g	1 g	0 g	5.06309 Pa·s
S1	100 g	3 g	1 g	0.1689 g	7.07305 Pa·s
S2	100 g	3 g	1 g	0.3378 g	224.198 Pa·s
S4	100 g	3 g	1 g	0.6756 g	666.185 Pa·s

X-ray diffraction measurements were performed with a Smartlab diffractometer (Rigaku, Tokyo, Japan), using Cu K_{α} radiation ($\lambda = 1.54184$ Å). The accelerating voltage of the X-ray generator was set to 45 kV, and the emission current to 200 mA. The diffractograms were recorded in parallel beam geometry, in transmittance mode, in the range 20 5–40°, with a continuous scan rate of 6°/min. The crystalline phases were identified using the International Centre for Diffraction Data (ICDD) files (PDF).

The homogeneity of the formed hydrogels and their morphology were studied using a TM4000plus II scanning electron microscope—SEM (Hitachi, Tokyo, Japan). For visualization, samples were deposited on a copper band and dried at room temperature. The electron accelerating voltage was 15 kV, and a standard vacuum was used in the sample chamber as specified by the manufacturer.

For each experiment, 5 g of each sample was dried in Petri dishes in an oven at 50 °C. The film formed was used for Fourier transform infrared (FTIR) analysis. The spectra were recorded with the attenuated total reflectance (ATR) technique on an IRTracer-100 FTIR (Shimadzu, Kyoto, Japan) in a wavenumber range from 8000 to 400 cm⁻¹ via integration of 45 spectra at a resolution of 4 cm⁻¹ for chemical structure analysis of the alginate-based samples.

The dynamic viscoelastic measurements were conducted with Discovery HR20 rheometer (TA Instruments, New Castle, DE, USA), with a parallel plate. The rheometer was used to determine the storage and loss moduli $G'(\omega)$ and $G''(\omega)$. The determinations were conducted at various angular frequencies ω . The non-linearity of the viscoelastic response was assayed in dynamic flow experiments.

Reaction steps in an aqueous environment for hydrogel formation can be summarized with the following chemical reaction equations [16]:

$$NaAlg \rightarrow Na^+ + Alg^-$$
 (1)

$$Na_4P_2O_7 \to 4Na^+ + P_2O_7^{4-}$$
 (2)

$$CaSO_4 \to Ca^{2+} + SO_4^{2-} \tag{3}$$

$$2Ca^{2+} + P_2O_7^{4-} \to Ca_2P_2O_7 \tag{4}$$

$$Ca^{2+} + Alg^{-} \rightarrow CaAlg^{+}$$
 (5)

$$2Na^{+} + SO_4^{2-} \rightarrow Na_2SO_4 \tag{6}$$

The formation of crystalline phases due to the reactions in the mixtures was followed by X-ray diffraction (Figure 1).



Figure 1. XRD patterns collected on obtained gels and the identification of used calcium sulfate and sodium alginate.

The diffractogram obtained for the calcium sulfate shows diffraction maxima specific to anhydrite (ICDD PDF: 01-076-6906). The diffractograms of samples S0 and S1 present weakly intense diffraction maxima in the noise domain. This result is an indication of the homogeneity of the obtained solutions, as well as of the disordered state in which they are. In the case of sample S2, it is observed that by adding a double amount of calcium sulfate, sodium ions from sodium pyrophosphate are replaced by calcium ions, and the formation of dicalcium pyrophosphate tetrahydrate crystals ($Ca_2P_2O_7 \cdot 4H_2O$) occurs, according to Equation (4).

When the Ca/Na ionic molar ratio is ~4/10, crystallite formation is observed in the case of sample S4. Diffraction peaks can be attributed to sodium sulfate (according to reaction (6)), intermediate compounds sodium hydrogen phosphate (Na₂H₂P₂O₇ ICDD PDF: 00-010-0192), calcium hydrogen phosphate (CaH₂P₂O₇ ICDD PDF: 00-009-0354), and calcium sulfate in the form of unreacted gypsum (ICDD PDF: 00-033-0311) but not in hydrated or anhydrite forms (ICDD 01-076-6906).

Using Fourier-transform infrared spectroscopy analysis, working in ATR reflectance mode, absorption bands characteristic of composite samples were revealed after ionotropic cross-linking of Na⁺ alginate solution dislocated by Ca²⁺ ions, as shown in Figure 2. The most intense and broad absorption band in the diagnostic range 4000–1800 cm⁻¹ is that at 3400–3200 cm⁻¹, characteristic of stretching and deformation vibrations of the functional hydroxyl-OH groups of the polysaccharide chain of alginate, groups involved in hydrogen bonding with the interstitial water adsorbed by the hydrogel. Also, the band of -CH groups in the structure of the alginate aromatic chain is visible in the diagnostic region at 2893 cm⁻¹, with relatively low intensity compared with the broad band of -OH groups.

In the region of double bonds, $2200-2000 \text{ cm}^{-1}$, the absorption band of C=O bonds at 2158 cm^{-1} is observed, and the pair of asymmetric and symmetric vibration bands of C=O groups linked to the aromatic structures of the alginate biopolymer chain are observed.



Figure 2. Fourier-transform infrared spectroscopy of sodium alginate and the obtained samples.

In the 1800–400 cm⁻¹ molecular fingerprint region, the most intense absorption band is that at 1636 \pm 100 cm⁻¹, characteristic of the -C-O-C (1 \rightarrow 4) glycosidic bond between the β -D-mannuronate (M) and the α -L-guluronate (G) monomer of the alginate copolymer. The following two bands, although of low intensities at 1414 and 1327 cm⁻¹, specific to the vibrations of the C=O and C-O bonds in the carboxy -COO- functional groups of the M-mannuronate and G-guluronate monomers to which Na⁺ ions are attached, further lose intensity in samples S2 and S4, also suggesting their involvement in stronger ionic bonds with Ca²⁺ ions, confirming the success of the cross-linking process. This finding is further strengthened by the decrease in intensity of the absorption band at 1092 cm⁻¹ specific to the in-plane deformation vibrations (scissoring and rocking) of the C=O and C-O bonds in the 2COO-Ca²⁺ carboxy groups, confirming the stabilization of the groups via ionotropic cross-linking.

SEM images of the sodium alginate–sodium pyrophosphate mixture show good homogeneity (Figure 3). The surface of the dry film shows surface ripples due to the formation of associations between alginate chains and sodium pyrophosphate. In sample P2, agglomeration of submicron particles in the alginate matrix can be observed. The brightest acicular formations in the SEM image, which means an increased electron density, are mainly due to the presence of calcium ions. By increasing the amount of calcium sulfate in the reaction medium, agglomerates (S2 and S4) are formed mainly in areas rich in calcium ions. The non-uniformity of the formations may be due to the fact that calcium sulfate is a poorly water-soluble compound. Most probably, in the case of mild dilution of anhydrite (S2), it hydrates, and the crystal structure changes from orthorhombic to monoclinic. This crystal structure change is demonstrated by corroborating the SEM results with X-ray diffraction. The compound gels quickly when more calcium sulfate is added (5 min). The SEM image shows multiple formations due to the non-uniform distribution of reaction centers. Due to the higher concentration of calcium sulfate, it does not solubilize entirely, and the exchange between calcium and sodium ions is hindered due to ion mobility.



Figure 3. Backscattered electron SEM images of the samples: (**a**) S0, (**b**) S1, (**c**) S2, (**d**) S4. Same magnification for all images; 50 μm scale bar.



The rheological behavior of the studied sample is presented in Figure 4.

Figure 4. Rheological curves for studied samples: (a) inflow sweep and (b) frequency sweep, where the straight line represents the storage modulus (G') and the dotted line the loss modulus (G'').

From the point of view of rheology, fluids are divided into Newtonian fluids, in which the viscosity is constant with applied shear rate, and non-Newtonian fluids. The time dependence, temperature dependence, and physicochemical properties of the incorporated particles generally drive the rheological properties of non-Newtonian fluids. Dozens of models have been proposed to study non-Newtonian liquids, each explaining the specific behavior of a class of fluids [3].

The Carreau–Yasuda model is the most suitable for analyzing the data of the obtained composites. Due to the multicomponent character and the turbulent flow of the obtained fluids, the modeling can be performed according to the following equation [17]:

$$\eta = \eta_{\infty} + (\eta_0 - \eta_{\infty}) \left[1 + \left(\lambda \dot{\gamma} \right)^a \right]^{\frac{n-1}{a}},\tag{7}$$

where η is the dynamic viscosity; η_0 , η_∞ is the viscosity at 0 and infinite shear rate; $\dot{\gamma}$ is the shear rate; and λ , n, a are model constants.

After modeling, it can be seen that the sample without calcium sulfate and the one in which a small amount of calcium sulfate was introduced have a shear-thinning non-Newtonian liquid behavior. A small amount of calcium sulfate forms submicron agglomerates, and the sodium or calcium alginate formed allows a sufficient distance so that the particles do not interact with each other from a rheometric point of view. Samples with a high content of initial calcium sulfate (S2, S4) exhibit a pseudoplastic behavior. Their zero-rate viscosity is two orders of magnitude larger than that of the previous samples (Table 1). The pseudoplastic behavior can be more clearly explained by the morphology of the particles formed in the alginate matrix. It can be understood that there is a complex ionic interaction between partial or total reaction compounds and a change in the hydrodynamic index due to the formation of agglomerates of different sizes and shapes.

By analyzing the storage modulus (G') and loss modulus (G'') curves from Figure 4b, it can be concluded that the addition of calcium sulfate in the initial solution (S0) changes the character of the viscoelastic liquid into a viscoelastic solid.

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