

ALGINATE-BASED RAPID PROTOTYPING SYSTEM

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Abstract

Alginate hydrogels are an important class of ionic biopolymers for medical and biotechnological applications, produced by the combination of alginate with a proper cross-linking agent. During the gel formation, cross-links can be formed between the alginate chains and cationic species, changing the elastic behaviour of the material that controls the volume change phenomena of the gels.

This paper proposes a new rapid prototyping system to produce three-dimensional alginate structures by extruding, layer-by-layer, a previously prepared solution of sodium alginate in water mixed with a solution of calcium chloride, both of known concentration. The building process to obtain these gel structures is described, from a chemical point of view, and some obtained structures are shown. The alginate concentration over both the kinetics and accuracy effects of the process is investigated. The preliminary findings of this research work promise to open an exciting new area for medical applications.

Keywords: Alginate, Hydrogels, Biomimetics, Medical Applications, Rapid prototyping.

Introduction

The engineering activity, a *design by human* process, is usually portrayed as a unique human process, relying on the intuition, experience and judgment of engineers and designers, to create a representation of objects meeting a set of requirements. However, there is also a *design by nature* (Bartolo and Bartolo, 2003) process, the process through which all the organisms dynamically adapt to its environment, performing its different functions, such as respiration, reproduction, sensing, movement, etc. This *design by nature* is an evolutionary transformation process with a unique goal, the survival of each population of organisms through the correct adjustment to the environment. This kind of design process has been optimised throughout years and years of natural evolution.

Just as an ecosystem incorporates change and adapts towards self-sustaining processes, lessons from nature can be used to support and optimise the *design by human* activity. The philosophy under this research project is the belief that engineers can learn with nature. This process of borrowing the best from nature is called biomimetics (Jeronimidis and Atkins, 1995) and this research study focuses on this new concept, investigating a new route to produce three-dimensional patterns in alginate hydrogels for medical applications, through a biomimetic free form manufacturing system. This system intends to replicate natural procedures used by some marine brown algae, namely *Laminaria Hyperborea*, to produce alginate, a structural component of the algae.

Hydrogels have been receiving much attention due to their potential use in a wide variety of biomedical applications, including tissue engineering scaffolds, drug delivery, contact lenses, corneal implants and wound dressing (Griffith and Naughton, 2002; Garcia and Ghaly, 1996;

Kim et al., 1998). Most hydrogels are usually synthesized using reactive monomers and cross-linkers. However, they do consume non-renewable resources, employ hazardous manufacturing steps and are not biodegradable, apart from not always being biocompatible so their use in medical applications is limited. Consequently, research attention has been centred on generating hydrogels from natural and biocompatible polymeric materials, such as collagen, gelatin, chitosan and alginate. Biomaterials present properties, such as biocompatibility, biodegradability, sensitivity to pH, electrical or temperature stimuli, that enables its use in biotechnology and medical applications.

Biologic manufacturing system

The alginate-based rapid prototyping system, which is in a preliminary research stage, intends to produce alginate solid structures by extruding a previously prepared solution of sodium alginate in water mixed with a solution of calcium chloride, both of known concentration. Understanding the reaction kinetics of the gelation process of alginate-based systems is the first step for the development of an intelligent system leading to the design, optimisation and control of an alginate-based rapid prototyping system.

Alginate is a natural linear polysaccharide that contains 1,4-linked β -D-mannuronic (M) and α -L-guluronic (G) acid residues (Figure 1), arranged in a non-regular and block-wise fashion along the chain (Kang *et al*, 2002). The biological properties of this biopolymer have been exploited in numerous medical and surgical applications, such as drug delivery, cell encapsulation, etc (Gombotz and Wee, 1998; Bucke, 1987).

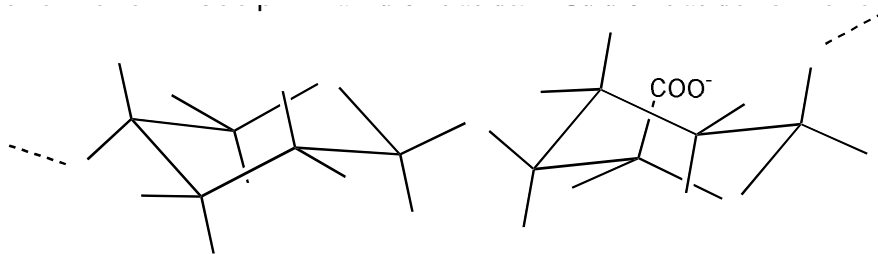


Figure 1: Structure of an alginate showing a linkage between the mannuronic and guluronic acid.

Gelling occurs when divalent ions (Ca^{2+} , Ba^{2+} , Fe^{2+} , Sr^{2+} , etc.) or trivalent ions (Al, etc.) take part in the interchain ionic binding between G-blocks in the polymer chain giving rise to a three dimensional network. Such binding zones between the G-blocks are often referred to as “egg boxes” (Figure 2). This ions act as cross-linkers that stabilise alginate chains forming a gel structure, which contains cross-linked chains interspersed with more freely movable chains that binds and entraps large quantities of water. The gelling process is characterised by a re-organisation of the gel network accompanied by the expulsion of water (Serp *et al.*, 2002).

Its reactivity with calcium and the subsequent gel formation capacity is a direct function of the average chain length of the G blocks, showing a great affinity for Ca^{2+} ions (Gombotz and Wee, 1998). Gels made of M-rich alginate are softer and more fragile, and may also have lower porosity. This is due to the lower binding strength between the polymer chains and to

the higher flexibilities of the molecules. The gelling process is highly dependent upon diffusion of gelling ions into the polymer network.

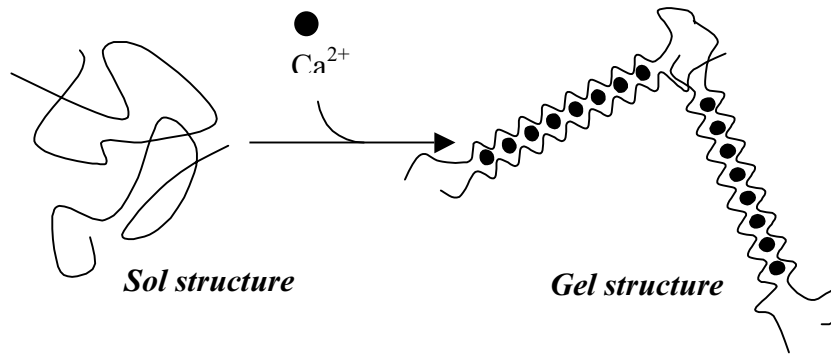


Figure 2: Alginate gelling results from reticulation of the chains by calcium ions.

Material

Sodium alginate was purchased at Panreac (Barcelona, Spain). Calcium chloride was supplied by Carlo Erba (Milano, Italy). All solutions were prepared with pure water, with conductivity of $0.054 \mu\text{S}/\text{cm}$. Alginate solutions were prepared by addition of weighted portions of sodium alginate to measured volumes of water. Due to their high viscosity, these solutions were agitated by orbital shaking for three hours at 50°C to ensure good homogeneity. Calcium chloride solution 5% (w/v) was obtained dissolving the salt in water. This solution was diluted to obtain solutions containing different concentrations of calcium chloride.

Results

To evaluate the kinetics of the gelling process solutions, containing different concentrations of alginate and different concentrations of calcium chloride (CaCl_2), they were prepared and mixed at room temperature. The effect of the alginate concentration is shown in Figure 3, which describes the weight loss as a function of time for solutions containing different amounts of alginate (1% and 2%) mixed with a solution of 5% CaCl_2 . Figure 4 illustrates the variation of weight loss as a function of time, for a solution containing 2% of alginate mixed with solutions containing different amounts of CaCl_2 . The experimental data (discrete values), was fitted using a sigmoidal equation. Fitting the experimental data enables us to have a continuous range of values to better evaluate the gelation process. To correlate the experimental data and the values obtained from the sigmoidal equation, a numerical routine using the Marquardt-Levenberg (Press *et al.*, 1986) multivariable non-linear regression method was employed. A good correlation was achieved by controlling the number of steps, the increment and a small tolerance parameter, corresponding to the difference of values at the step $n+1$ and values at the step n , which is used for convergence purposes.

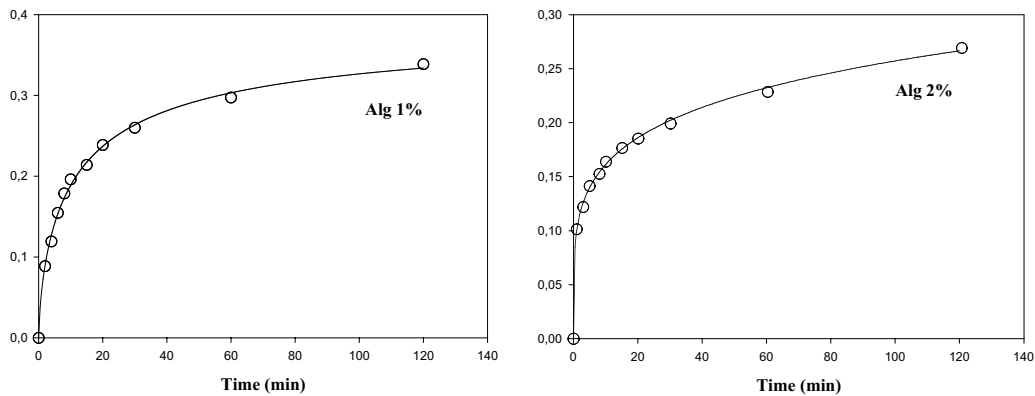


Figure 3: Weight loss vs gelation time for two solutions containing different concentrations of alginate mixed with a solution of of 5% CaCl₂

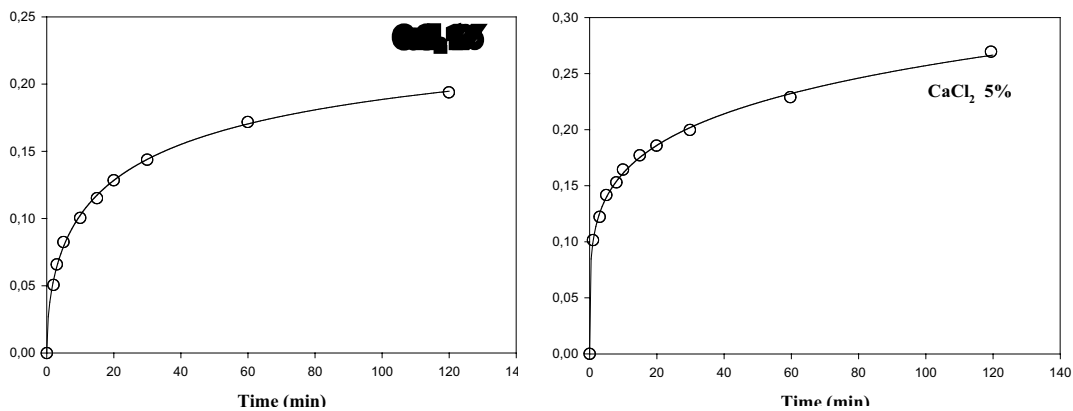


Figure 4: Weight loss vs gelation time for a solution containing 2% of alginate mixed with solution containing different concentrations CaCl₂.

The weight loss increases with time and is more significant for samples produced by solutions containing low contents of alginate and high contents of calcium chloride is shown in Figures 3 and 4. This is due to two main reasons:

- the amount of water present in the initial alginate solution, which is higher in more dilute solutions
- the kinetics of the gelation process, which is higher whenever solutions containing higher concentrations of CaCl₂ are used.

The calcium divalent ions act as cross-linkers that stabilise alginate chains forming a gel structure. Therefore, increasing the concentration of calcium chloride present in the solution increases the cross-linking of polymeric chains and the expulsion of water. During the gel formation, calcium ions diffuse from the liquid solution to the forming gel and its complexation by the carboxylic groups of G blocks increases the gel stiffness. This phenomenon is accompanied by a significant loss in water at counter-current of the flux of calcium ions (Figure 5). Ahmad and Huglin (1994) and Hu and Lin (1994) claim that the water contained in alginate gels can be found in two states: free-water and non-free-water.

Therefore, it is expected that the water leaving the gel during the gelation process is the one which interacts less with the polymer (free-water).

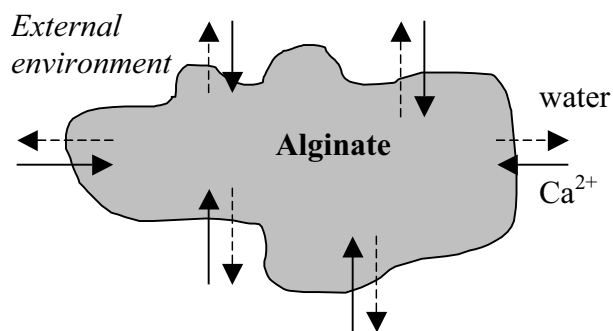


Figure 5: The diffusion of chemical elements during the gelation process of alginate structures.

Figure 6 compares the variation of the rate of weight loss with time as a function of both alginate and CaCl₂ concentration. This Figure shows that maximum value of the rate of weight loss is almost independent of the concentration of alginate or calcium chloride and occurs very fast, in less than one minute. However, the rate of weight loss is higher for samples produced with solutions containing higher concentrations of alginate or solutions containing higher concentrations of CaCl₂.

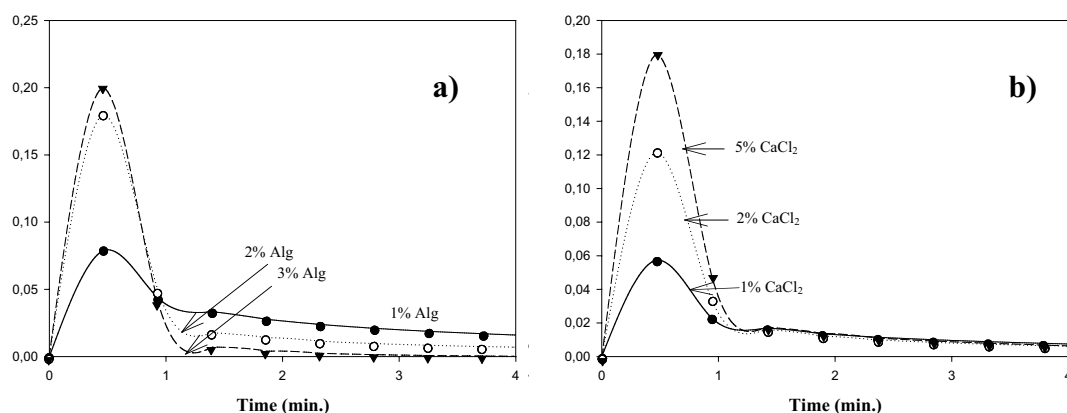


Figure 6: Variation of the rate of weight loss vs time as a function of a) alginate concentration for samples produced through a solution containing 5% of CaCl₂; and b) calcium chloride concentration for samples produced through a solution containing 2% of alginate.

The variation of the maximum value of the rate of weight loss with the concentration of calcium chloride for samples produced using a solution of 2% of alginate was fitted using a sigmoidal equation ($r^2=1$) as shown in Figure 7. This variation reveals two main regions. The first region corresponds to the gelation processes performed with solutions containing concentrations of CaCl₂ lower than 3%. In this region, the concentration of CaCl₂ has great impact on the rate of weight loss and consequently on the gelation kinetics. The second region appears at high values of CaCl₂. This can be attributed to diffusion limitations of the divalent ions of Ca²⁺ as the gel structure is formed. Analogous behaviour is observed in Figure 8 for

the variation of the maximum value of the rate of weight loss with the concentration of alginate produced through a solution of 5% of calcium chloride.

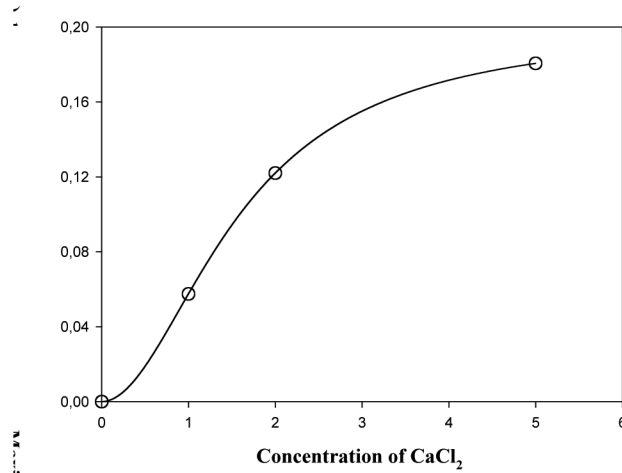


Figure 7: Variation of the maximum value of the rate weight loss vs. concentration of CaCl₂ for samples produced through a solution containing 2% of alginate.

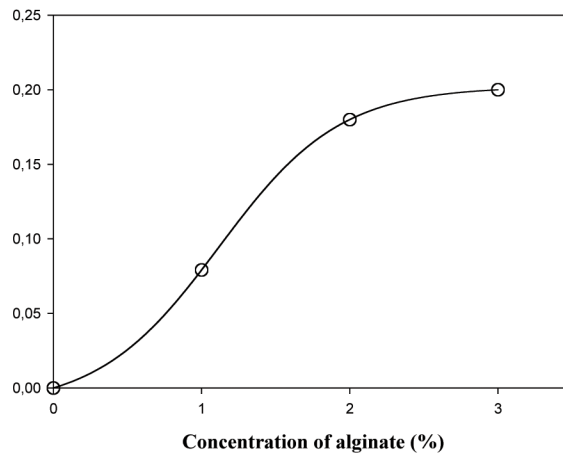


Figure 8: Variation of the maximum value of the rate weight loss vs. concentration of alginate for samples produced through a solution containing 5% of CaCl₂.

Due to the loss of water during the gelation process, high shrinkage values are observed compromising the accuracy of the process in terms of the dimensional generation of three-dimensional structures. The shrinkage is a function of the kinetics of the gelation process and, consequently, is a function of the concentration of alginate and calcium chloride. Figure 9 shows the variation of the linear shrinkage as a function of time and weight loss of a disc produced through a solution with 2 wt% of alginate combined with a solution containing 5% of CaCl₂.

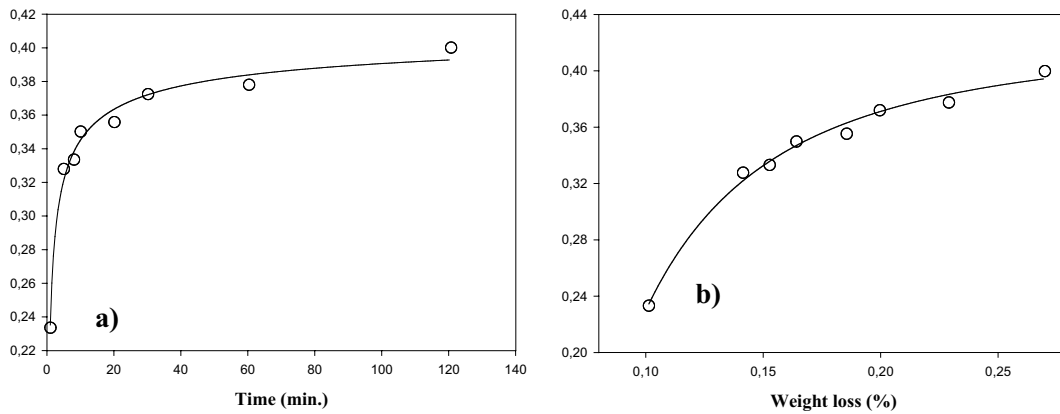


Figure 9: Variation of the shrinkage as a function of a) time and b) weight loss of a disc produced through a solution with 2% of alginate mixed with a solution containing 5% of CaCl_2 .

The accuracy in the definition of the geometric shape of alginate structures is also strongly dependent on both the concentrations of alginate and calcium chloride. Figure 10 displays the shapes of different circular structures produced by mixing a solution with 2% of alginate with solutions containing different concentrations of CaCl_2 . A better definition is obtained using high contents of alginate and CaCl_2 . The gelation process of alginate structures will enable to retain the shape of structures during a large period of time (see Figure 11), owing to the fast process of the gelation rate during the first 5 minutes, that slows down afterwards slowing down the rate of weight loss.

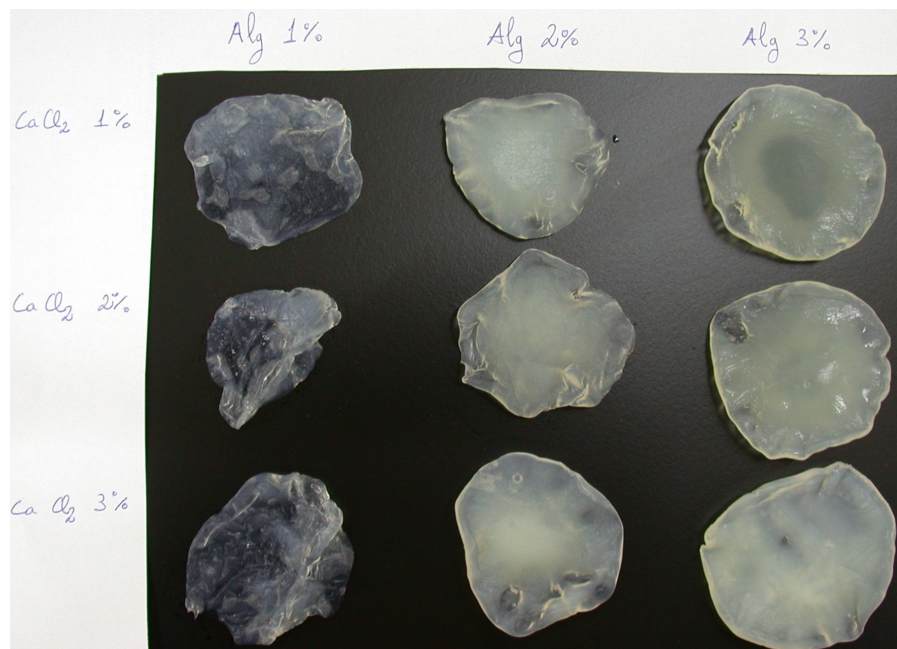


Figure 10: Circular structures produced by mixing solutions containing different concentrations of alginate with solutions containing different concentrations of CaCl_2 .



Figure 11: An alginate structure three weeks after its generation.

A phenomenological model previously developed for stereo-thermo-lithographic processes (Bartolo and Mitchell, 2003; Bartolo and Mitchell, 2001) was adopted to evaluate the kinetics of the gelation process of alginate systems. According to this model, the rate of weigh loss, dW/dt , is given by the following equation:

$$\frac{dW}{dt} = \Gamma \cdot W^m (1 - W)^n$$

where Γ is the gelation rate constant, W is the fractional value of weight loss, and m and n are the exponential coefficients of the kinetic model. All the kinetic parameters have been found to be dependent on both the alginate and calcium chloride concentration.

The rate constant is given by the following equation:

$$\Gamma = \xi \cdot \exp\left(\frac{\gamma}{[CaCl_2]}\right) \cdot \exp\left(\frac{\varphi}{[Alg]}\right)$$

where ξ , γ and φ are constants, $[CaCl_2]$ is the concentration of calcium chloride and $[Alg]$ is the concentration of alginate. The rate constant was found to be dependent on the alginate and calcium chloride concentrations in an Arrhenius way as shown in Figure 12.

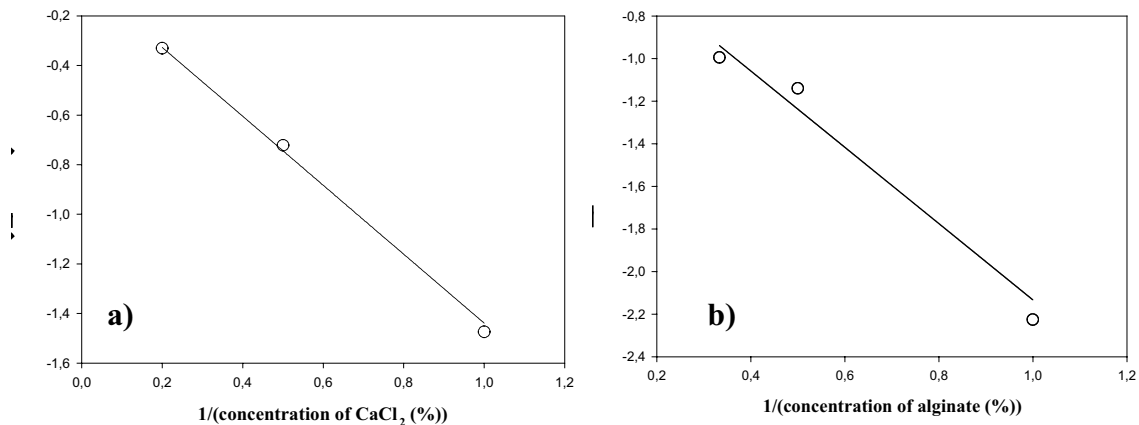


Figure 12: a) Variation of $\ln(k)$ vs. $(1/\text{concentration of } CaCl_2)$ for samples produced using a solution containing 2% of alginate. b) Variation of $\ln(k)$ vs. $(1/\text{concentration of alginate})$ for samples produced using a solution containing 5% of $CaCl_2$.

The exponential coefficients' dependencies with the CaCl_2 concentration are determined through an exponential regression ($r^2=1$ for the exponential coefficient n and $r^2=0.98$ for the coefficient m), and are shown in Figure 13. This Figure reveals that the exponential coefficient m increases with the concentration of calcium chloride, while the coefficient n decreases. Similar behaviour is observed for exponential coefficients' dependencies with the alginate concentration.

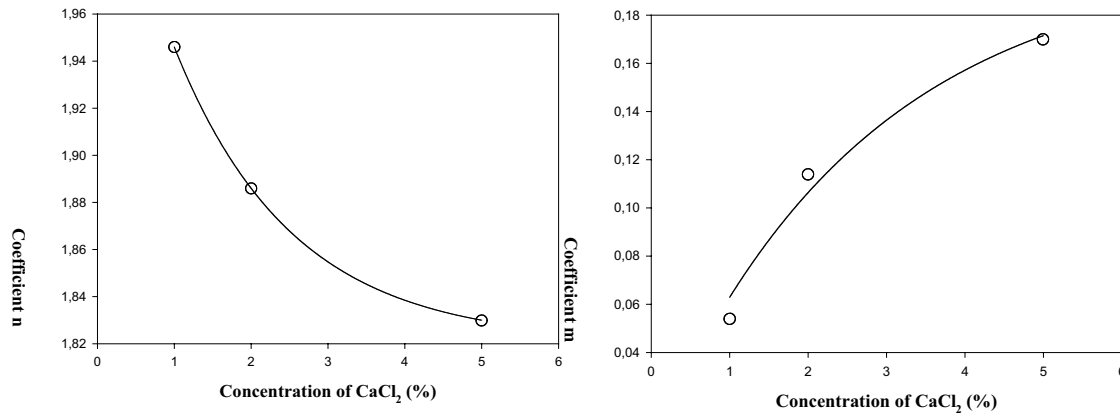


Figure 13: Variation of the exponential coefficients vs. concentration of CaCl_2 for samples produced using a solution containing 2% of alginate.

The model intends to provide:

- a better understanding of the gelation process;
- a more accurate estimation of build times and weight loss profiles;
- a platform for testing new alginate structures or other bio-polymeric systems and process improvements;
- the development of a standard set of parameters and gels for some pre-determined three-dimensional model characteristics.

Conclusions

A new rapid prototyping system to produce three-dimensional alginate structures has been described from a chemical point of view. The gelation process is strongly dependent on both the alginate and calcium chloride concentrations. During the gelation process a significant water loss is observed influencing the shrinkage of the produced structures. Stable alginate structures are obtained after 20 minutes and the shape of these structures is retained for a long period of time.

A kinetic model, describing the gelation process, is also proposed. This computational model will support the development and optimisation of this alginate-based rapid prototyping system, avoiding a great amount of experimental work.

Future work will focus on the relationship between morphology/mechanical properties and gelation kinetics in order to better understand the generation process of gel structures. This will help to produce appropriated porous structures for tissue engineering.

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