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Modeling capillary formation in calcium and copper alginate gels

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ABSTRACT

Alginate solutions in the presence of bivalent ions can form ionic cross-linked gels. In particular gelation conditions the gel structure can be characterized by great anisotropy with the presence of straight capillaries along a preferential direction. These materials can find applications mainly in high-tech sectors, like tissue engineering, where the gel characteristics play a crucial role. Despite the need of mastering the capillary formation and properties, the process remains a poorly known problem, and its development is left to trial and error procedures.

In this work a quantitative approach to the description of the capillary formation process has been developed. The theory proposed by Treml et al. (2003) has been implemented and extended to an alginate different from the one used in that study and two different ions (calcium and copper). Some of the model parameters have been derived through simple measurements; others have been scaled using proper scaling equations.

Experiments have been performed in different gelation conditions, varying alginate and ionic solution concentrations, to highlight the effects of these parameters on the anisotropic structure and to validate the model. In all the analyses done, the model has performed nicely showing a good reliability in the prediction of gel characteristics like capillary formation, capillary length and process time.

1. Introduction

Alginate is a renewable polysaccharide commonly extracted from algal plants (brown algae or Phaeophyceae). It is a linear polymer consisting of (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) [1]. The polymer has been extensively used as thickener and gelling agent in the food and pharmaceutical industries. It is increasingly studied for use in biomedical applications partly related to its ability to form gels under mild conditions e.g. encapsulation of cells or microorganisms. Alginate form gels in the presence of di- and tri-valent ions, at room temperature and over a wide range of pH.

The ionically crosslinked alginate gel is formed by bi and trivalent ions and G-unit complexes in a structure described by the "egg-box" model [2]. It is predominantly G units that are involved in the junction zones, the gel properties are thus dependent on the G/M ratio of the polymer [1,3,4]. An alginate high in G units give a stronger and more brittle gel compared to alginates rich in M units [1,3,4]. The ratio and arrangement of the M and G units depend on the botanical source and the state of maturation of the plant [1].

As the gelation kinetics is fast, slow introduction of di- and tri-valent cations, e.g. calcium ions is needed to create an isotropic gel. This can be achieved by dispersing an insoluble calcium source in the alginate solution, followed by the addition of a slowly hydrolyzed acid. Such calcium alginate gels are of isotropic character and the gelation methodology is commonly referred to as the internal method [1,5].

Another gelation route involves the anchoring of alginate to the container surface and the creation of a top gelled alginate layer, over which an ion solution composed of ions enabling crosslinks is added. The ions diffuse in this way across the gelled membrane into the alginate solution, creating a highly anisotropic structure where macroscopic channels (Fig. 1) run from the top to the bottom of the gel [6].

Various routes aiming at preparing hydrogels with macroscopic pore structures are explored for the use in biomedicine and tissue engineering where successful growth of cells require gels containing pores with 100 μm in diameter or larger [7,8]. The anisotropic structure of ordered capillaries of defined diameters obtained using external gelation of alginate has therefore received an interest as potential scaffold material [9–11] but also as use as membranes [12] or to simply study the structure for better understanding of biological processes [6,13].

Studies have been devoted to the control of capillary diameter, height and number as well as mechanical and microstructural properties of the gel [14]. The mechanism behind the formation of ordered and repeated capillaries within the alginate gel is however still not fully understood.

The phenomena of obtaining ordered capillaries within alginate gels was first reported by Thiele who proposed that the capillaries originate from a phase separation mechanism of droplet segregation [6]:

$$\text{Sol} + \text{Electrolyte(A)} \leftrightarrow \text{Gel} + \text{Electrolyte(B)} + \text{Water}.$$

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Nomenclature

C_A^0	initial alginate sol concentration [mol/m^3]
$\tilde{C}_A^g$	alginate concentration of the gel [mol/m^3]
C_{ion}^0	initial ionic sol concentration [mol/m^3]
$\mathfrak{D}$	general definition of diffusivity [m^2/s]
$\mathfrak{D}_A$	alginate diffusivity close to the gelation front [m^2/s]
$\mathfrak{D}_A^{eff}$	effective alginate diffusivity [m^2/s]
$\mathfrak{D}_{ion}^{eff}$	effective ion diffusivity [m^2/s]
erf	error function [−]
k_B	Boltzmann constant [J/K]
k_r	reaction rate constant [m^3/(mol s)]
$\tilde{k}_r$	dimensionless reaction rate constant [−]
k_r^{diff}	reaction rate constant in the case of diffusion limitation [m^3/(mol s)]
l	length of a Kuhn segment of alginate [m]
l_m	diameter of an alginate monomer [m]
M_w	molar mass [g/mol]
N_A	alginate degree of polymerization [−]
N_{ag}	numbers of monomers forming a Kuhn segment [−]
N_{AV}	Avogadro constant [1/mol]
t	process time [s]
T	temperature [K]
v_c	contraction velocity [m/s]
v_c^{crit}	critical contraction velocity [m/s]
v_r	gel formation front velocity [m/s]
$\tilde{v}_r$	dimensionless gel formation front velocity [−]
z_0	time dependent thickness of the contraction layer [m]
$\tilde{z}_0$	dimensionless thickness of the contraction layer [−]
z_r	reaction front [m]
α	friction constant between contracting chains and fluid [kg/(mol s)]
γ	displacement parameter [m^2/s]
ε	degree of contraction [−]
η^*	viscosity of the fluid surrounding the alginate chains [Pa s]
θ	structure formation coefficient [−]
κ	coefficient of convective transport [mol/m^4]

It was stated that the gelation process is accompanied by dehydration so that the finely distributed drops of water are trapped within the zone of sol–gel-transition. Further delivered water molecules will accumulate and be pushed by the gelation front towards the sol creating alginate free pore channels [11]. The research group of Kohler proposed later the theory of chemically fixed dissipative structure formation [15]. This idea was based on the observation [16] of convective movement, similar to Benard cells, close to the already gelled alginate. The phenomenon was explained considering the contraction of the alginate chains during the complexation by the bivalent cations. This induces a movement of solvent, close to the gelation front, that gives rise to periodic variations of the alginate gel density in the gelation plane (horizontal plane). This theory was implemented in a mathematical model and semi-qualitatively validated to a system of Cu-induced alginate gel [17].

The molecular dynamics measured using magnetic resonance (MR) upon the diffusion of Cu ions into an alginate solution suggest however that capillary formation is related to spinodal decomposition mechanism rather than the hydrodynamic convection roll model described by Kohler and co-workers [13]. This is concluded due to the lack of coherent transverse convective velocities within the Cu-alginate sample. Transverse convection would be necessary for the presence of “roll-like” convective movements. Capillary type structures have been predicted in two dimension model based on total free energy containing an elastic stress contribution and a dynamic coupling between this stress and diffusion [18]. The molecular dynamics of the calcium containing alginate gel was less conclusive than the copper containing gel potentially related to the lack of consistent capillary formation in the case of calcium as diffusive ions. Nevertheless, Maneval and co-workers propose that a model based on spinodal decomposition approach may be more fruitful owing to the measured anisotropic dynamics observed in their study.

Independent of the exact mechanism (hydrodynamic convection or spinodal decomposition) being responsible for the capillary formation in ion induced alginate gel, we show in this work that, through the generalization of the model suggested by Treml and co-workers (chemically fixed dissipative structure) it is possible to predict the minimum ionic strength required of the counterions for the formation of capillaries in alginate solution of different concentrations. The predictions give further the time required to obtain specific capillary lengths. The counterions chosen in this work are Ca^{2+} and Cu^{2+}, both extensively used in biomedicine and tissue engineering, however the present approach could be easily extended to other (multivalent) ions.

We present a quantitative analysis of prediction and experimental data obtained for the Cu-alginate system (previously only a semi-qualitative analysis was done) and the Ca-alginate system (which as far as we are aware has not been previously done). Such analysis will aid and fine tune the experimental preparation of capillary alginate gels and the work presented here highlights, by combined experimental and modeling approach, the conditions for the capillary formation process and their effect on the resulting structures.

2. Materials and methods

2.1. Materials

Alginate Protanal RF6650 was kindly provided by FMC Biopolymers (UK). It contains 70% guluronate according to the supplier. Calcium chloride dihydrate was purchased from Sigma–Aldrich (Germany) and copper chloride dihydrate was purchased from Apotek Produktion & Laboratorier (Sweden).

2.2. Methods

Alginate solutions were prepared by careful addition of alginate powder to deionized water at room temperature under vigorous stirring. The dispersion was thereafter heated to 353 K in a water bath and kept at this temperature for 30 min or until dissolution was obtained. The pH of the polymeric solution was adjusted from pH 7.3 to pH 7 using 0.1 M HCl. The solution was thereafter deaerated, to remove the trapped air bubbles, by leaving the solution in an open flask for 5–10 min inside a vacuum chamber.

2.2.1. Gel preparation

Externally set alginate gels were prepared by coating a glass beaker ($V = 50$ ml and $d = 40$ mm) brushing the internal wall with alginate solution, which then was allowed to dry in an oven set at 383 K for 30 min. The proceeding was repeated three times and the beaker was preheated in the oven at 393 K prior the first application. A known amount of alginate solution at 293 K was poured into the glass beaker. The surface of the polymeric solution was sprayed with 2 ml of bivalent ionic solution until a gel membrane was formed on top of the alginate solution. The gel membrane was left to set for 30 min after which other ionic solution at 293 K was carefully poured on top of the membrane. The ionic solution was left to diffuse through the membrane, and the beaker was covered with parafilm and allowed to rest for the desired gelation time. The coating procedure is necessary to assure capillary formation. Since the amount and concentration of both alginate and ionic solutions are experiment dependent, their values will be specified later on in the text.