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The spatial distribution of calcium in alginate gel beads analysed by synchrotron-radiation induced X-ray emission (SRIXE)

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Abstract

The spatial distribution of calcium ions in alginate gel spheres was investigated by synchrotron radiation induced X-ray emission. X-ray emission spectra were obtained from 1 mm spherical calcium alginate beads gelled either in the presence or in the absence of sodium ions. Evidence was found for an inhomogeneous distribution of calcium ions with the highest concentration at the surface, and the lowest concentration in the core of the spheres, which reflected the spatial distribution of alginate in the gel spheres. The form and the steepness of the calcium concentration gradient was strongly affected by the presence of sodium ions.

Keywords: Alginate gel beads; X-ray fluorescence; Calcium distribution

1. Introduction

It was previously demonstrated that calcium alginate gels prepared using a dialysis method often exhibited a concentration inhomogeneity where the polymer concentration was considerably lower in the core of the gel than at the edges [1,2]. When divalent metal ions such as calcium diffused into an alginate solution, the rapid ion-binding and formation of the polymeric network produced an inwardly moving gelling zone. In fact, alginate moved from the core of the gel towards this gelling zone, leading to a depletion of alginate in the core. The polymer gradient is essentially governed by the relative diffusion rate between the soluble alginate molecules and the gel-forming ion [1]. The actual concentration profiles in the final calcium alginate gels were previously determined for gel slabs, simply by filling plastic cylinders with sodium alginate solution, closed at both ends with a semi-permeable membrane, and placed in a Ca(II) ion reservoir [1]. The alginate concentration profile depended on different parameters such as the calcium-to-alginate concentration ratio, the molecular

Abbreviations: SRIXE, synchrotron radiation induced X-ray emission; G, α-L-guluronic acid

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weight of the alginate, and partly also on the chemical composition of the alginate polymers. In general, low molecular weight alginate and a low concentration of gelling ions yielded inhomogeneous gels, while high molecular weight alginate, high concentration of gelling ions, and high concentration of non-gelling ions such as supporting salt, favoured a homogeneous distribution of polymers in the final gel. In addition, it can be assumed that there is a dependence of the chemical reaction rate on the overall ionic strength of the system.

In recent years, entrapment into spheres of calcium alginate gel has become the most widely used technique for immobilization of living cells [3] and one of the most exciting applications of alginate gels is the potential use of encapsulated pancreatic islets for the treatment of type I diabetes [4]. It was shown that inhomogeneity may be the most suitable structure in microcapsules due to lower porosity, higher charge density on the surface, and improved stability compared with homogeneous gels [5,6]. Therefore an assessment of the actual polymer gradient in small spherical gels is of significance in their application.

Recently, Mikkelsen and Elgsaeter [7] reported on a mathematical model of alginate gel formation. They were able to simulate the data obtained by Skjåk-Bræk et al. [1] on the cylindrical system described above. In addition, they put forward predictions of gel inhomogeneity for more complicated systems which exhibited different geometry such as gel fibres and gel beads. In their paper, the parameters which influence the gel inhomogeneity were also considered and discussed. In this respect, the alginate molecular weight was shown to influence the gel structure through the alginate diffusion constant; the calcium-to-alginate concentration ratio exhibited a similar effect, if a high reaction rate between divalent cation and polymer, with respect to the diffusion rate of calcium ions, is assumed. In addition, a high guluronic content in the alginate polymer was expected to enhance the inhomogeneity due to the concomitant high value of the chemical reaction constant. Finally, the addition of non-gelling ions, such as sodium or magnesium, lowered the gel inhomogeneity effectively, by increasing the total ionic strength and, therefore, affecting the ion-to-polymer affinity.

In order to compare theoretical expectations [7] with experimental results, we decided to investigate the calcium ion distribution in small alginate gel beads. Since the alginate gels, in spite of their inhomogeneity with respect to alginate concentration, were shown to be compositionally homogeneous [7], the spatial distribution of calcium ions should give a realistic picture of the of the spatial distribution of alginate in the gel spheres.

In order to measure the calcium ion concentration, we used X-ray fluorescence by means of synchrotron radiation. In fact, the availability of an X-ray microbeam collimated to 10×10 microns made the scanning of bead sections, with concomitant high lateral resolution, feasible.

2. Experimental

Calcium alginate beads (0.7–1.0 mm diameter) were obtained by means of the experimental set-up already described by Skjåk-Bræk et al. [2] and Martinsen et al. [8]. Commercial samples of Na-alginate, an alginate extracted from the stipe of *Laminaria hyperborea* containing 63% α-L-guluronic acid (G) with an average length of consecutive G units, $\langle N \rangle = 12.6$ and with an average molecular weight ($\langle M \rangle_W$) of 277 kD (Pronova Biopolymers, Drammen, Norway), and an alginate from *Macrocystis pyrifera* with 43% G, $\langle N \rangle = 6.3$ and $\langle M \rangle_W = 210$ kD (Kelco, Division of Merck), were used for these experiments. Ca-alginate gel beads (0.7–1.0 mm) were made by letting droplets of aqueous solution of Na-alginate (1.8% w/v) fall into a solution containing Ca^{2+} ions (0.02–0.1 M). Beads were made with and without 0.15 M NaCl and 0.075 M NaCl in the alginate solution and calcium solution, respectively. The size of the beads was controlled by a coaxial airstream, and the diameter were measured in an Nicon inverted microscope (Diaphot-TMD). After hardening, the alginate beads were dehydrated directly with successive changes of ethanol (25, 30, 40, 50, 60, 70, 80, 90, 96 and 100%), 20 min in each solution. Propylene oxide were then introduced with further changes with 1:2 and 1:1 mixtures of propylene oxide and 100% ethanol, and finally pure propylene oxide. This procedure ensures that the perfect spherical form of the gel beads is preserved with just a minor (12%) reduction in volume. The beads were embedded in a polymeric material, LX 112 (Ladd Research Industries Inc.) according to the following schedule: propylene oxide:LX 112 1:1 in a sealed container for 6 h, propylene oxide:LX 112 in a sealed container for 12 h and propylene oxide:LX 112 1:1 in an open container for 24 h. The infiltrated beads were then placed in plastic capsules with pure LX 112 and polymerized at 60 °C for 48 h. Five micron semi-thin sections were obtained from the resin blocks by

means of an ultra microtome and attention was paid to obtain equatorial section of the included beads. Finally the section was laid on a polypropylene film exhibiting the purity suitable for X-ray spectroscopy (3520 polypropylene 0.2 MI, Spex Industries Inc., USA).

In order to have a high number of photons in a highly collimated X-ray beam, calcium X-ray fluorescence experiments were carried out by means of synchrotron radiation at the National Synchrotron Light Source (NSLS, X26A beam line) hosted at the Brookhaven National Laboratory (BNL, USA). NSLS is characterised by the following parameters: electron energy: 2.53 GeV, critical energy: 5.0 keV, maximum ring current approximately 250 mA. The X26A beamline uses the continuous X-ray spectrum produced by a bending magnet. A set of four stepper-motors-driven tantalum slits can be used to produce collimated beams down to 40×40 micron on the specimen. In our experiments, a second tantalum collimator, placed at 3.5 cm from the sample position was used to reduce the X-ray beam size to about 10×10 micron.

X-ray fluorescence can be used either in point analysis or in the scanning mode. The former allows bulk analysis of very small specimens, whilst multi-elemental 2-dimensional maps of elements can be obtained in the scanning mode. The position of the sample, with respect to the X-ray beam, was changed by means of a computer-controlled stepper motor allowing micrometric movements. X-ray fluorescence was detected by means of a Si(Li) X-ray detector (30 mm^2 sensitive area), provided with an Ag collimator, positioned at 90° with respect to the incident beam. All experiments were performed in air. Scans of the bead sections, throughout section diameters, were carried out using steps of either 50 or 20 micron, with a residence-time per point of 120 s. Two-dimensional scans of overall sections were performed using 50 micron steps in both x- and y-directions and a residence-time per point of 30 s.

3. Results and discussion

The X-ray fluorescence spectrum of the polypropylene film used to support bead sections is shown in Fig. 1a. Besides a minor peak on the low energy edge of the spectrum, only the fluorescence of Argon (2.96–3.19 keV), present in the air surrounding the experimental set-up, was detected. In particular, no calcium fluorescence (3.69–4.01 keV) was present in

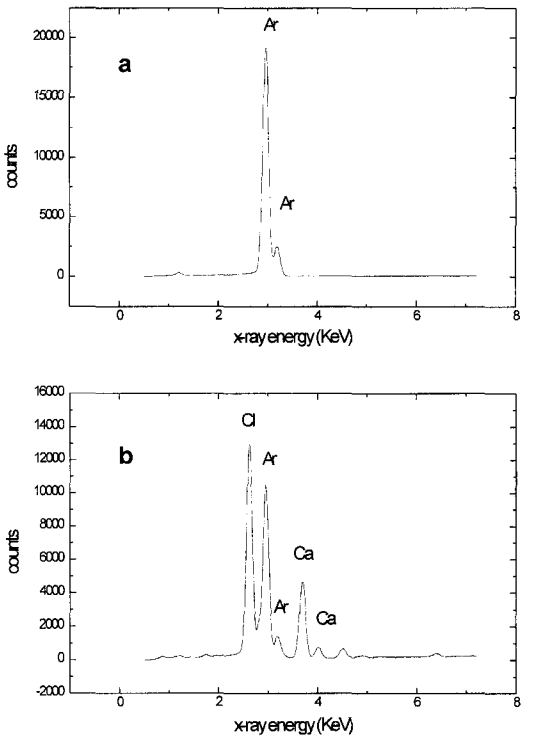

Fig. 1. (a) X-ray fluorescence spectrum of the poplypropylene film used as the supporting material. (b) X-ray fluorescence spectrum of a sample point on a gel bead section.

the spectrum. The fluorescence spectrum relative to sample points randomly analysed on bead sections is shown in Fig. 2b. Besides the peaks due to Argon, the spectrum exhibited both chlorine and calcium fluorescence (2.62–2.81 keV and 3.69–4.01 keV, respectively), which was obviously produced by the addition of the gelling salt.

Fig. 2a shows the profile of the calcium distribution along the diameter of an equatorial section of a bead made with an alginate fraction which exhibited high guluronic content (alginate extracted form *Laminaria hyperborea*), and Fig. 2b shows the 3-d analysis relative to half section of the bead. The bead was obtained in the absence of NaCl and in the presence of 100 mM Ca^{2+} ions. The gel bead exhibited some degree of inhomogeneity since the calcium concentration was much higher near the bead surface than in the core of the bead. Fig. 3 shows the calcium concentration profile obtained for a bead formed in 20 mM $CaCl_2$ using the same high-guluronic alginate fraction. In this case, the difference of calcium concentration between the surface and the core of the bead was clearly larger than that shown in Fig. 2a. When the gel formation occurred in the presence of a mixture of sodium and calcium ions (20 mM $CaCl_2$, 75 mM NaCl) and using a mannuronic rich alginate

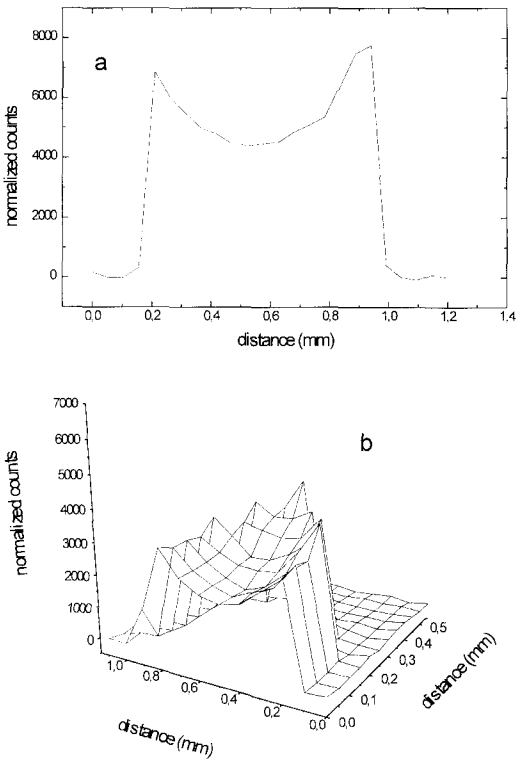

Fig. 2. (a) Profile of the calcium concentration along the diameter of an equatorial section of a bead made in 100 mM Ca(II) with an alginate fraction exhibiting high guluronic content. (b) 3-Dimensional image of the calcium distribution (half-bead analysis).

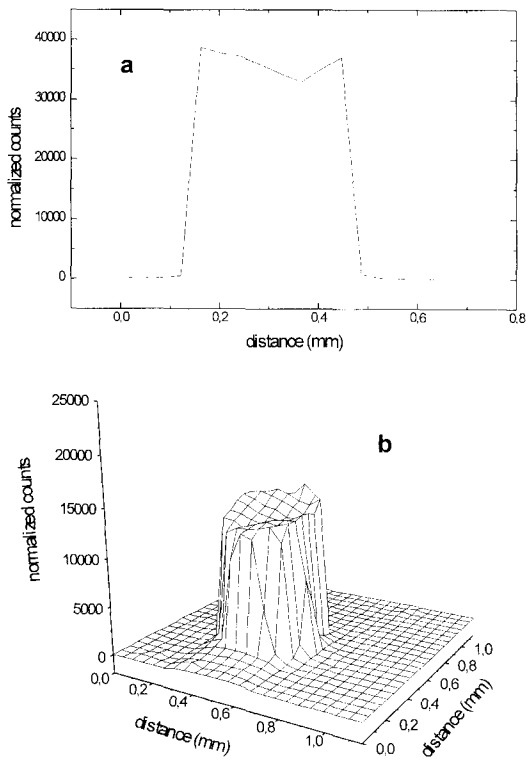

Fig. 4. (a) Profile of the calcium concentration along the diameter of an equatorial section of a bead made in the presence of a mixture of sodium (75 mM) and calcium (20 mM) ions with an alginate fraction exhibiting high mannuronic content. (b) 3-Dimensional image of the calcium distribution.

fraction, gels became significantly more homogeneous as shown for two and three dimensions in Fig. 4a and b.

The data obtained on the calcium distribution in alginate gel beads agree very well both with theoretical expectations and other experiments carried out on similar systems, and also demonstrated the power of the microscopic X-ray fluorescence analysis performed by means of synchrotron radiation for both successful investigation of microscopic volumes of samples and for trace-element detection.

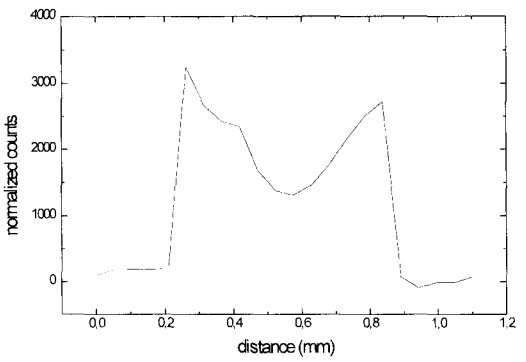

Fig. 3. Profile of the calcium concentration along the diameter of an equatorial section of a bead made in 20 mM Ca(II) with an alginate fraction exhibiting high guluronic content.

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