Eur Biophys J (2003) 32: 412–417
DOI 10.1007/s00249-003-0298-7

ARTICLE

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Phase separation in calcium alginate gels

Received: 11 June 2002 / Revised: 10 February 2003 / Accepted: 10 February 2003 /

Abstract Alginates are polysaccharides consisting of β-D-mannuronate and α-L-guluronate units. In the presence of bivalent cations like calcium the guluronate blocks form physically cross-linked gels. The gelation properties of alginates play an important role in the stability of extracellular polymer substances and in the food industry. When stock solutions of Ca^{2+} ions and alginate are mixed, the gelation starts before the Ca^{2+} ions are evenly distributed, which leads to non-uniform gels. In this contribution, Ca alginate gels were prepared by in situ gelation using glucono-δ-lactone and $CaCO_3$. In this way, uniform gels could be prepared directly in the measuring cell. Below a critical concentration, highly viscous solutions were obtained, which were below the critical point of gel formation. In these solutions at low rotational speeds a Schlieren peak arose, which became smaller and steeper with increasing time until a new meniscus could be detected. This behaviour is in contrast to the peak broadening due to diffusion after a synthetic boundary was formed. Evaluation of the data leads to negative diffusion coefficients. It has been shown by others that the mutual diffusion coefficient must be negative in the spinodal region. This phenomena is known as uphill diffusion and leads to phase separation of a binary system. The formation of the gel phase in this case is therefore discussed as uphill diffusion.

Keywords Alginate · In situ gelation · Uphill diffusion

Presented at the Conference for Advances in Analytical Ultracentrifugation and Hydrodynamics, 8-11 June 2002, Grenoble, France

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Introduction

Alginates are polysaccharides which are known to form gels in the presence of bivalent cations. They are composed of the two monomers β-D-mannuronate (M) and α-L-guluronate (G). The monomers are distributed as homogeneous blocks (GG or MM) or as alternating sequences (GM) (Braccini et al. 1999). The mannuronate monomers are equatorially linked and form a flat ribbon (Fig. 1a). The guluronate blocks are linked diaxially as a buckled chain (Fig. 1b). If two guluronate blocks are positioned opposite to each other, a cavity is formed in which bivalent cations can be enclosed. Ca^{2+} ions, especially, have the right size to fit in this cavity. The oxygen containing groups of the guluronate can chelate these ions and form stable links. For a block of at least 20 guluronate monomers, the structure of these linked chains is known as the egg box model, initially proposed by Rees and Welsh (1977) (Fig. 2). Owing to the flat shape of the mannuronate chains, they cannot chelate the Ca^{2+} ions. The solution and gelling properties of alginates depend therefore on the composition and the monomer sequences of the polysaccharide.

If a solution with Ca^{2+} ions is added to a solution of alginate, the chelation process is much faster than the diffusion of the Ca^{2+} ions. Therefore strongly inhomogeneous gels with the highest concentration of Ca^{2+} at the surface are formed in this case. A method of internal gelation to produce homogeneous gels was invented by Draget et al. (1989). A suspension of insoluble sonicated $CaCO_3$ in glucono-δ-lactone (GDL) solution is used as the source of Ca^{2+} ions. The glucono-δ-lactone hydrolyses slowly to δ-gluconic acid in water. Ca^{2+} ions are released in an acid-base reaction, and will be chelated to the guluronate residues. The carbon dioxide produced in the reaction escapes out of the gel. Draget et al. reported the highest gel strength for equimolar concentrations of Ca^{2+} ions and guloronic acid residues for different alginates with high

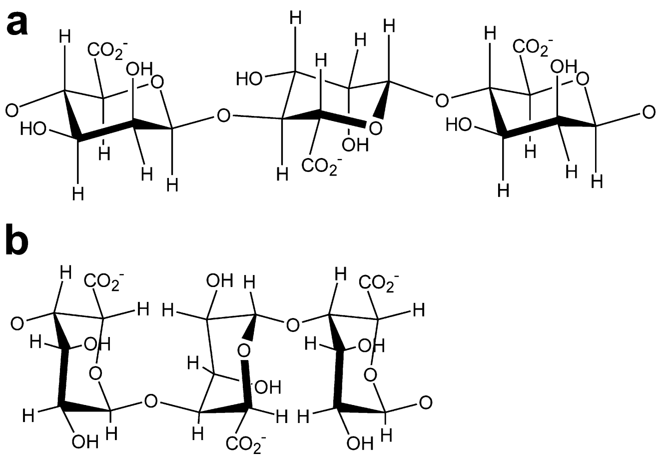

Fig. 1 a Block of β-D-mannuronate. **b** Block of α-L-guluronate

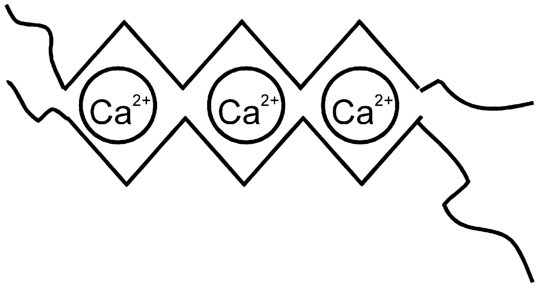

Fig. 2 Egg box model (Rees and Welsh 1977)

guluronate fractions. Syneresis was observed at higher Ca^{2+} concentrations (Draget et al. 1991), indicating that these gels are close to a stability limit, where phase separation occurs. Kramarenko and Khokhlov (1998) showed that polyelectrolyte gels may undergo intra-network phase separation or syneresis due to their charged residues.

Although phase separation has been widely discussed theoretically, only a few investigations on phase separation under a centrifugal field have been reported. Borchard and Holtus (1989) studied the phase separation of the system polystyrene/cyclohexane, showing that the concentration gradient near the stability curve must be high and at the stability limit be infinite. At the phase boundary a discontinuity of the concentration is predicted. A similar behaviour of Ca alginate is described in this paper.

Materials and methods

Sample preparation

Sodium alginate (Manucol DM, Monsanto) with a molar mass of 130,000 g/mol and an M/G ratio of 2.1:1, glucono-δ-lactone (99%, Aldrich) and calcium carbonate (99.5%, Alfa Aesar) were used.

The sodium alginate was dialysed against water for 24 h (cut-off of the dialysing tubing, 15,000 g/mol) and freeze dried. The alginate was dissolved in water over night and the stock solution was diluted afterwards. To prevent bacterial attack, 0.15 mg of a 5% solution of 4-chloro-3-methylphenol was added per gram dry alginate. In a freshly made glucono-δ-lactone solution, $CaCO_3$ was dispersed, leading to a ratio of 2 mmol GDL per 1 mmol $CaCO_3$. To 8 mL alginate solution, 2 mL of the dispersion were added. The residues of the dispersions were allowed to hydrolyse completely. The total alginate concentrations were in the range from 0.2 wt% to 2 wt% (Table 1). After stirring for 2 min, 0.3 mL of the mixture was injected into the measuring cells. The cells were set aside for 24 h. After this time the $CaCO_3$ had fully reacted and the gelation was completed. The ultracentrifugal runs were started afterwards.

Refractive index increment

The refractive indices of the gels and the solutions of $CaCO_3$ in glucono-δ-lactone were measured with an Abbé refractometer after the hydrolysis was completed. From the difference of the refractive indices of the gel and the solution without alginate, the refractive index increment could be calculated. A refractive index increment of 0.14 was obtained.

Sedimentation

The measurements were carried out in an analytical ultracentrifuge (Beckman model E) with modified Schlieren optics, which has a higher resolution than the original optics (Coelfen and Borchard 1994). A six-hole An-G titanium rotor with titanium cells of 3 mm path length and a sector angle of 4° was used. In order to prevent adhesion of the gels to the surfaces, the walls of the centrepieces and the surfaces of the cell windows were coated with a solution of Teflon AF 1600 (DuPont Polymers, Wilmington, USA) in Fluorinert 75 (from 3M). The solvent was removed by heating the pieces at 40 °C for 2 h.

The pictures were taken with a charge-coupled device camera (Digital Visions Technologies, Micam VHR 2000) transmitted to a TV card (Win TV GO, Hauppauge) and saved as bitmaps. The Schlieren curves were redrawn with a graphics digitizing tablet (Wacom).

The speed of the ultracentrifuge was accelerated to 9000 rpm and pictures were taken twice a day. After 25 days, the run was stopped.

The sedimentation coefficient was calculated from the second moment of the gradient curve after the method of Goldberg (1953). From the height area ratio the diffusion coefficients were calculated (Fujita 1962).

Table 1 Composition of the alginate gels[a]

$w_2 \times 100$	c_{G+M} (mmol L^{-1})	c_G (mmol L^{-1})	c_{CaCO3} (mmol L^{-1})	$c_{lactone}$ (mmol L^{-1})	c_{G+M}/c_{CaCO3}	c_G/c_{CaCO3}
0.190	10	4	3	6	3.5	1.1
0.500	29	9	8	15	3.8	1.1
0.997	57	19	15	31	3.7	1.2
1.488	86	28	23	47	3.7	1.2
1.886	108	35	30	59	3.7	1.2

[a] w_2 = weight fraction of the alginate; c_i = amount of matter of *i* per volume

Theory

Superposition of phase separation and gelation

Homogeneous solutions may undergo phase separation into two different phases if the thermodynamic parameters are changed (Haase 1956). Within traditional theory the condition for the stability of a phase is:

$$\left(\frac{\partial^2 G}{\partial w_i^2}\right)_{T,P} > 0 \text{ or } \left(\frac{\partial \mu_i}{\partial w_i}\right)_{T,P} > 0 \qquad (1)$$

where μ_i is the chemical potential of compound i, w_i the weight fraction of compound i, T the temperature, P the pressure and G the free enthalpy of the system (Haase 1956). The stability curve or spinodal curve is defined by $(\partial \mu_i/\partial w_i)_{T,P} = 0$. Inside this curve, where the derivative of the chemical potential with respect to the concentration is negative, lies the unstable region. In the spinodal region, every fluctuation leads to a decrease in the free enthalpy and therefore results in spontaneous demixing.

From Fick's first law it is known that the flow J_2 is proportional to the negative concentration gradient, $J_2 = -D\left(\frac{\partial \rho_2}{\partial r}\right)$, where D is the mutual diffusion coefficient and r the radial distance from the axis of rotation. For normal diffusion the diffusion coefficient is defined as positive. When the system demixes the diffusion induces a concentration gradient (Voigt-Martin et al.1986), resulting in a negative diffusion coefficient. This is known as "uphill diffusion". Cahn (1965, 1971) showed that the diffusion coefficient inside the spinodal must be negative, using the thermodynamic relation (Flory 1953; Haase 1963):

$$D = u_2 \rho_2 (\partial \mu_2/\partial \rho_2)_{T,P} \qquad (2)$$

The diffusion coefficient D is composed of two factors. One is the mobility u_2, which describes the mean velocity of compound 2 and is always positive. The other is the thermodynamic factor $\rho_2(\partial \mu_2/\partial \rho_2)_{T,P}$, which is the product of the partial density and the derivative of the chemical potential. Since this derivative is negative inside the spinodal and the partial density cannot become negative, the diffusion coefficient must be negative.

Results and discussion

After the hydrolysis was completed, strong continuous gels were obtained at concentrations above 0.5 wt% alginate. All gels except the one with a weight fraction of 2 wt% were clear. After 3 days there was a second phase on top of these gels, which was an extremely viscous solution that had a lower concentration than the initial concentration.

The polymer concentrations of the two phases after phase separation of the gel can be seen in Table 2. Inside the gel the concentration of the alginate was increased. Draget et al. (1991) reported syneresis if the Ca^{2+} concentration was higher than the equivalent concentration of guluronic acid. Syneresis is a special case of phase separation, where a gel coexists with its solvent. If not enough solvent is available for syneresis, a separation into two gels will occur (Kramarenko and Khokhlov 1998).

Table 2 Concentration in the gel phase and in the solution phase after phase separation has occurred under standard pressure[a]

$w_2 \times 100$	$w_2 \times 100$ (gel phase)	$w_2 \times 100$ (solution phase)
0.997	1.03	0.82
1.488	1.48	1.05
1.886	2.07	1.36

[a] w_2 = weight fraction of alginate

If the Ca alginate gels were exposed to a centrifugal field before the phase separation had started, a dark zone close to the meniscus could be observed. The dark zone cleared within 4 days for the gels below 1.5 wt% alginate. This is in agreement with the results of Borchard and Holtus (1989), who showed that the meniscus in the system polystyrene/cyclohexane was dark when the phase separation started. A dark zone was also reported by Kisters et al. (2002), who observed dark zones when gelatine gels were exposed to high centrifugal fields.

After the second phase was formed, both the gel phase and the solution phase contained polymer and two separate Schlieren curves could be detected. When the solution of Ca gluconate without polymer was exposed to a centrifugal field, no gradient could be detected in the Schlieren pictures. Therefore it was concluded that the gradient in both phases could be attributed to the alginate. The integration of the concentration gradient is not possible if a discontinuity in the Schlieren curve occurs and for this reason no further evaluation of the data were done. The gel with the 2 wt% remained dark during the whole run. This might be explained by the higher turbidity due to inhomogeneities inside the gel.

Below 0.5 wt% alginate the mixtures were still fluent after the hydrolysis of the glucono-δ-lactone was completed and highly viscous solutions were attained instead of a gel. It is presumed that the polymer concentration was not high enough to form a continuous network. Instead, large but finite clusters are formed which increased the viscosity of the primary solution. These solutions remain stable with regard to phase separation, if they are not exposed to a centrifugal field. During the ultracentrifugal run a Schlieren peak is observed inside the solutions, which appears after a few hours. Within a few days these gradients become smaller and steeper until they are infinitely high in the case of gels. Simultaneously, the peak is sedimenting in the direction of the bottom of the cell with decreasing velocity until the sedimentation stops before reaching the bottom (Fig. 3).

The expected behaviour for a sedimenting species is a peak broadening due to polydispersity and diffusion. In some systems, self-sharpening has been reported. This may occur if the sedimentation coefficient is concentration dependent. In this case the macromolecules on the leading side of the moving boundary are slower because they are in the region with higher concentration, while the solutes on the trailing side become faster because of the dilution (Schachmann 1959). In this case it would be expected that the Schlieren peak migrates to the bottom of the cell without stopping. For sodium alginate solutions, peak broadening during velocity ultracentrifugatin was observed (Straatmann and Borchard 2002).

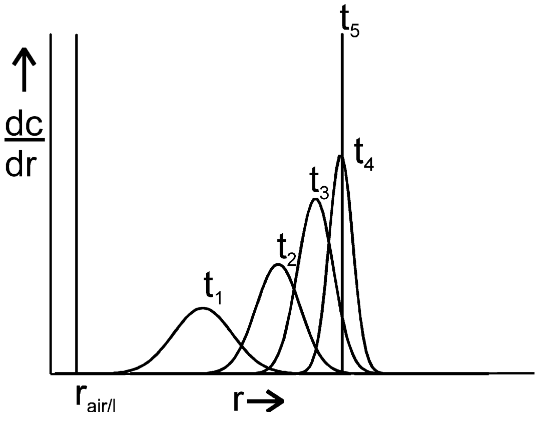

Fig. 3 Scheme of the Schlieren peak sharpening of a polymer solution during sedimentation

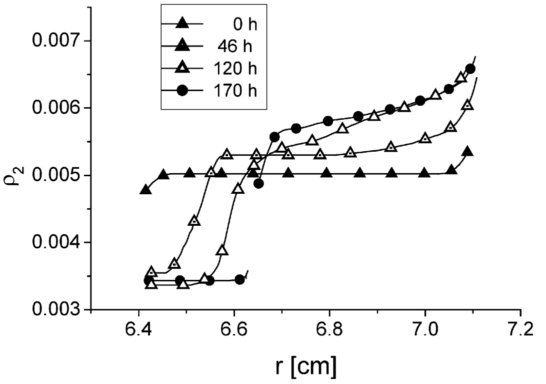

Fig. 4 Concentration profile of a Ca alginate gel ($w_2 = 0.5$ wt%) at 9000 rpm at different times

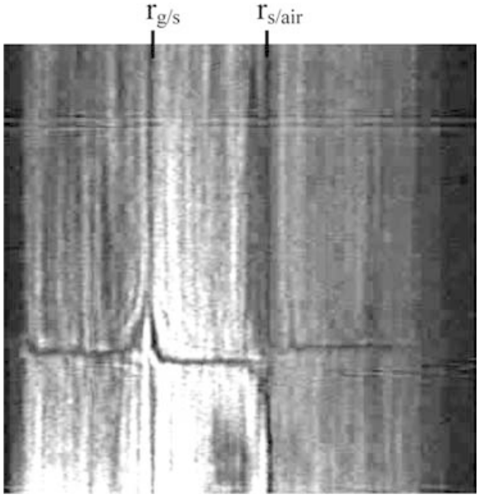

Fig. 5 Schlieren picture of a Ca alginate gel ($w_2 = 0.5$ wt%) at 9000 rpm after 25 days; $r_{g/s}$ = gel/solution phase boundary; $r_{s/air}$ = solution/air phase boundary (see text)

A continuous gradient is expected when equilibrium is established, but if phase separation occurs in an centrifugal field, an infinite Schlieren gradient is predicted at the stability limit. Because the infinite gradient remains within three weeks, it is therefore concluded that a new phase is formed, resulting in a phase boundary at this position. After the ultracentrifugal runs had been stopped, the cells were removed from the rotor. It could be seen that all the cells contained two phases, one gel phase and one solution phase. The position of the phase boundary was approximately the same as detected with the Schlieren optics. The change of the position was due to the absence of the ultracentrifugal field. The solution phase decreased, reaching a final position within a week. Within three weeks, no homogeneous solution could be detected.

As in the case of the gels described above, both phases seen in the Schlieren pictures contained polymer. The concentration profiles before the start of phase separation were determined by integration over the whole Schlieren curve. To obtain the concentration profile after the phase separation had started, the amount of alginate before the maximum of the Schlieren peak was determined. It was assumed that this was the amount of alginate in the solution phase in the first picture afterwards. Both phases could be integrated separately to derive the concentration profile. Since the menisci were moving further to the bottom at later times, it was not possible to estimate the concentration in each phase and therefore further evaluation was impossible.

In Fig. 4 there is first a continuous curve of the concentration with a moving boundary. After 170 h (approx. 7 days), when the phase separation started, there appears a concentration jump as expected at the phase boundary. Looking at the concentration gradient within the gel phase, the typical form with the highest gradients at the boundary and the bottom of the cell can be seen.

When the equilibrium is established the gradient inside the gel should become smaller at the meniscus. After the phase boundary reaches the final position, the Schlieren peak remains constant (Fig. 5). The equilibrium concentration gradient is therefore not established and the system is probably hindered from reaching demixing equilibrium. This has been qualitatively treated recently (Stauffer et al. 1982; Tanaka 1999)

Figure 6 shows the phase diagram of a polymer-solvent mixture with a miscibility gap, which is intersected by a gel curve. The white region describes the composition where the system is stable with regard to phase transitions. The solid line is the binodal; the dotted line is the spinodal curve. If the concentration of a solution with the initial concentration $w_{1,0}$ at point *a* is increased to a composition which corresponds to point *b*, the metastable region is reached. The stability limit is arrived at point *c*. Any further increase leads into the unstable region (point *d*). Therefore phase separation starts spontaneously. In Fig. 6, a gel curve reaches into the spinodal region (Tanaka 1999). Therefore the liquid-liquid phase separation will not be completed and the equilibrium concentrations *b* and *f* will not be

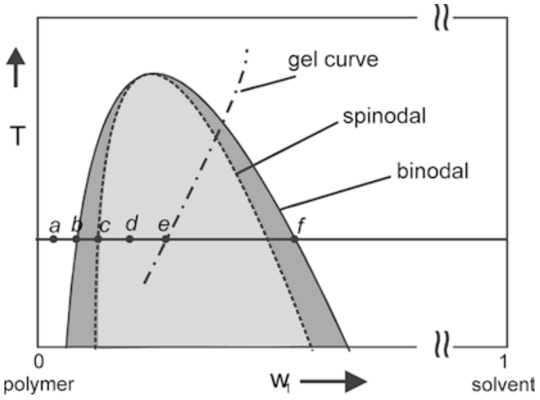

Fig. 6 Phase diagram of a binary mixture with a miscibility gap and a gel curve (see text)

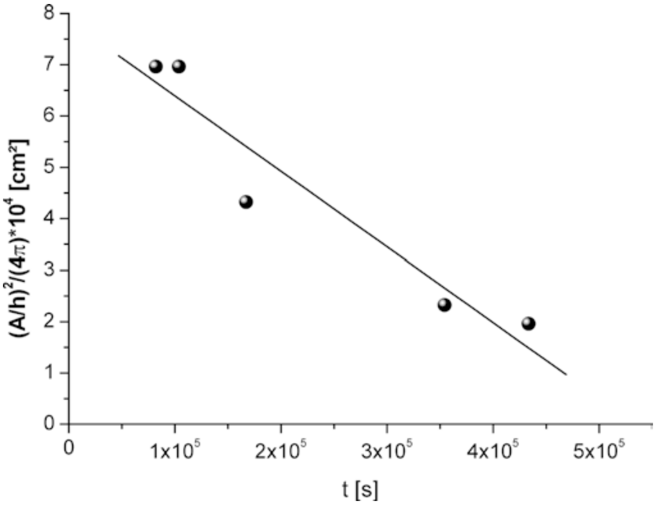

Fig. 7 Plot of the height area ratio against the time *t*

established. The concentrated phase will start gelling at point *e* instead. This gelation inhibits the liquid-liquid phase separation. The higher concentrated phase has a concentration between the gel curve and the equilibrium curve. The lower concentrated phase will have a higher concentration then the equilibrium concentration due to the conservation of mass.

The sedimentation constant could be determined by evaluation of the Schlieren pictures before phase separation. By plotting the height area ratio against the time, a negative slope was detected (Fig. 7). Since the slope gives the diffusion constant *D*, this means that a negative diffusion coefficient results. This is coincident with the fact that peak sharpening occurred. In the case of normal diffusion, peak broadening would be observed. Negative diffusion coefficients can only occur if the system is in the spinodal or unstable region where $(\partial\mu_2/\partial w_2)_{T,P}$ or $(\partial\mu_2/\partial\rho_2)_{T,P}$ is negative. This is in agreement with the fact that phase separation occurs at the same time. Negative diffusion coefficients in the case of phase separation were found by Voigt-Martin et al. (1986) during phase separation of the system polystyrene/poly(vinyl methyl ether).

Table 3 Sedimentation coefficients and diffusion coefficients of the Ca alginate before gelation[a]

$w_2\times100$	s_2(S)	D(cm^2/s)
0.190	2.0	-5×10^{-10}
0.500	2.8	-17×10^{-10}

[a] w_2 = weight fraction of alginate

The values of the sedimentation and diffusion constants of the Ca alginate in the solutions with concentrations below the gel point are summarized in Table 3. Since the alginate is polydisperse, it is expected that the shape of the Schlieren peak is not only influenced by diffusion but also by the polydispersity. In general, diffusion coefficients are overestimated, if calculated from the height area ratio. In the case of peak sharpening, the effect of peak broadening due to polydispersity produces the opposite of this effect. Both effects cannot be separated and the peak sharpening might become slower than it would be for a monodisperse system.

The sedimentation coefficient of the more concentrated solution is higher than of the other one. For sodium alginate solutions the sedimentation coefficients decrease with increasing alginate solutions (Wedlock et al. 1986; Straatmann and Borchard 2002). This is the expected behaviour for polymer solutions (Schachmann 1959). The opposite effect in the case of the Ca alginate can be explained by the aggregation of the alginate. If more polymer is available in the solution, larger clusters can be formed, which will migrate faster than smaller ones.

The sedimentation coefficient (1.9 S) of Manucol DM in sodium chloride solution ($c = 0.15$ mol/L) is for a solution with a weight fraction of 0.19 wt%, which is comparable to the sedimentation coefficient of the Ca alginate solution with the same weight fraction. In contrast, the Schlieren peaks of sodium alginate solution became broader with time and showed no peak sharpening.

The sedimentation coefficients of chemically fully cross-linked polymers have been found to be lower than the sedimentation coefficients of polymers in solution (Kisters et al. 2002). After complete gelation the sedimentation coefficients of the alginate are therefore expected to slow down. It was observed that the velocity of the movement of the phase boundary of the alginate gels after cross-linking tended to zero, which is similar to a slower sedimentation.

The occurrence of the real phase transition observed in this paper is completely different from the appearance of a phase boundary anomaly known as the Johnston-Ogston effect (Johnston and Ogston 1946; Fujita 1962). The latter has been observed in concentrated solutions of two polymers in a solvent where one of the polymer components sediments faster than the other (Pedersen

1936). In this case the Schlieren picture reveals finally two separated peaks combined with two plateau zones in the concentration-distance curve.

In this paper the transformation of a homogeneous solution into a two-phase system is stated where one phase is a gel and the other is a solution. During the time allowed (27 days) the gel seems to be a stable system, allowing the formation of a concentration gradient like a chemically cross-linked gel, because no collapsing properties which are expected for transient gels (Tanaka 1999) can be found.

Conclusion

It could be shown that Ca alginate gels are unstable with respect to phase separation into a gel phase and a solution phase. Below a critical polymer concentration, instead of a continuous gel, only clusters are formed and a viscous fluid is obtained. After a concentration gradient is formed due to a centrifugal field inside this solution, spinodal demixing via uphill diffusion is initiated. This could be evidenced by calculating the diffusion coefficients, which were negative. The phase separation is superimposed by gelling, which prevents the system from establishing a liquid-liquid phase equilibrium.

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