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Homogeneous Alginate Gels: A Technical Approach

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ABSTRACT

Homogeneous alginate gels can be made by internal liberation of calcium ions. Non-toxic gels at neutral pH were achieved by mixing particles of $CaCO_3$ with the slowly hydrolysing proton donor D-glucono-δ-lactone into the alginate solutions. This paper summarizes the essential factors controlling the properties of the resulting gel, such as pH, optical clarity, gel homogeneity, gel strength and syneresis.

INTRODUCTION

Water-soluble polysaccharides able to form strong homogeneous gels show an increased demand world-wide. The use of gelling agents as food additives, such as the phycocolloids agar, alginates and carrageenans is becoming more widespread (Sandford 1987; Indergaard & Østgaard 1990). Within the rapidly growing field of biotechnology, alginate has found an application for immobilization of cell and enzyme systems (Brodelius, 1984; Draget *et al.*, 1988; Martinsen *et al.*, 1989). However, agar and agarose are the almost excusively used polymers for solidification of biological media both in traditional microbiology and in new biotechnological applications.

The main disadvantage of agar is the instability concerning supply and rising costs due to the limited resources of the species of red algae from which it is isolated (McLachlan, 1985). This factor has led to considerable investigation in an attempt to find polymers and gelling systems which can act as agar substitutes. In food, this has been important for the development of the carrageenan industry (Bjerre-Petersen *et al.*, 1973; Indergaard & Østgaard, 1990), but numerous attempts have shown difficulties in replacing microbiological-grade agar (McLachlan, 1985).

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Some substitutes have been developed based on polysaccharides of non-algal origin, such as Plantgar™ and gellan gum.

Compared to agar, there seems to be no global shortages of resources for alginates (Jensen, 1978; McLachlan, 1985) and they may thus provide a cheap and steady supply alternative to agar as a basis for homogeneous hydrogels. A prerequisite will be that there exists a simple and non-toxic method of an in-situ introduction of Ca^{2+} ions and that the gelling conditions result in stable, homogeneous gels. In-situ gelation is necessary because it is proven that alginate gels made by dialysis are basically non-uniform with respect to polymer distribution and often exhibit syneresis (Martinsen *et al.*, 1989; Skjåk-Bræk *et al.*, 1989).

In industry several methods are in use for in-situ gelation of alginate. Perhaps the most well-known is the mixing of sodium alginate with complexed calcium by the aid of ethylenediamine tetraacetic acid (EDTA) and using the slowly hydrolysing D-glucono-δ-lactone (GDL) to lower the pH and release the complexed calcium into the solution (Toft, 1982). The general problems with the use of complexed Ca^{2+} are the relatively low pH necessary to release calcium and the potent toxicity of the complexing agent itself. The method is therefore not suitable for biotechnological cultivation media.

The authors have already reported the use of highly dispersed $CaCO_3$ and GDL for in-situ gelation of sodium alginate solutions (Draget *et al.*, 1989). The main advantage of this method is that homogeneous alginate gels can be made over a wide pH-range just leaving the essentially non-toxic end products CO_2 and D-gluconic acid. For plant cell and tissue systems, such alginate gels are not inferior compared to traditional agar gels (Draget *et al.*, 1989).

The scope of this work was to develop the basic knowledge of how fundamental variables in this gelling system influenced gelation and the properties of the resulting gel, i.e. information necessary to obtain and optimize homogeneous alginate gels suitable for various types of cultivation media in microbiology. This also includes kinetic studies of the gelling system with a Bohlin VOR rheometer.

MATERIALS AND METHODS

Materials

Alginates isolated from *Laminaria hyperborea* stipe were commercial samples provided by Protan A/S denoted LF 10/60 and HF 200.

Another alginate sample, isolated from *Macrocystis pyrifera*, was obtained from Sigma (Sigma Chemical Company, St. Louis, USA). Chemical and physical properties of the alginate samples, such as fraction of guluronic acid residues (F_G), fraction of G-units located in blocks of more than one G-unit (F_{GG}), average length of G-blocks ($\bar{N}_{G>1}$) and intrinsic viscosity $[\eta]$ are summarized in Table 1. D-glucono-δ-lactone was obtained from Sigma and $CaCO_3$ used was p.a. grade from Merck (E. Merck, Darmstadt, FRG). Xanthan ('Keltrol'), $[\eta]=60$ (100 ml/g), was obtained from Kelco (Kelco Division of Merck & Co., San Diego, USA).

TABLE 1
Types and properties of alginates used

Type	*Source*	F^a_G	F^b_{GG}	$\bar{N}^c_{G>1}$	*Intrinsic viscosity [η] (100 ml/g)*
LF 10/60	*Laminaria hyperborea*	0·66	0·56	11·4	6·3
HF 200	*Laminaria hyperborea*	0·69	0·57	11·4	14·2
Sigma alginate	*Macrocystis pyrifera*	0·40	0·20	6·3	11·4

[a] F_G = Fraction of guluronic acid residues.
[b] F_{GG} = Fraction of G-units in blocks of more than one G-unit.
[c] $\bar{N}_{G>1}$ = Characteristic average length of G-blocks (Skjåk-Bræk, 1988).

Gel formation

Gelling of sodium alginate solutions by $CaCO_3$ and GDL were executed as described earlier (Draget *et al.*, 1989). Briefly, $CaCO_3$ was dispersed in the sodium alginate solution and a freshly made aqueous GDL solution was added. Unless otherwise stated, all gels were made with 15 mM $CaCO_3$ and 30 mM GDL, giving a final gel at pH 7.

Gelling under CO_2 atmosphere was carried out by placing a petri dish (diameter = 45 mm) with a solution of sodium alginate with dispersed $CaCO_3$ in an exicator with a volume of about 7 litres. The exicator was flushed with pure CO_2 to remove the air until 5 min after a solution with diluted phenol red had lost its colour. The exicator was closed, and the dishes incubated with CO_2 under 1 atm pressure.

As a third alternative, direct addition of acid (2·0 M HCl) was tested for dissolution of the $CaCO_3$.

Gel homogeneity

Gel cylinders were made by placing aqueous solutions of sodium alginate, containing dispersed $CaCO_3$ and freshly made GDL, in plastic cylinders (diameter = 14 mm, length = 15 mm) and covering the cylinders with glass lids. After 48 h the gels were taken out of the cylinders and sliced perpendicular to the cylinder axes into *c.*2 mm discs in a self-built gel slicer as described earlier (Skjåk-Bræk *et al.*, 1989). The wet weight of the slices was immediately determined, and polymer concentration in each slice was determined after drying at 40°C for 24 h. A correction was made for the extra contribution to the dry weight by GDL. A simplified indicator of gel homogeneity was determined by calculating the mean values of alginate content in the upper half relative to the lower half of the cylinder. Thus, a percentage approximation ($P_{1/2}$) of the vertical alginate distribution was obtained.

$CaCO_3$ particle size and sedimentation

To obtain smaller $CaCO_3$ particles with a narrower size distribution, a sample of dispersed batch $CaCO_3$ was sonicated with a B. Braun 'Labsonic 1510' (B. Braun Melsungen AG, Melsungen, FRG) for 5 min at 400 W. The stability of the smaller particles towards aggregation was tested by freeze-drying a sonicated sample followed by an aqueous re-dispersion.

The particle size distribution of aqueous dispersed $CaCO_3$ was monitored in a Nikon (Nippon Kogaku K.K., Tokyo, Japan) inverted light microscope at 200 × magnification.

The sedimentation velocities in polymer solutions of the $CaCO_3$ particles were tested by a simple turbidity method. A spectrophotometrical cell (volume 3 ml) was filled with a fixed volume (2·5 ml) of alginate solution containing dispersed $CaCO_3$. After mixing to get a uniform particle distribution, the cell was placed in a Shimadzu UV-260 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Sedimentation was monitored by measuring the decrease in the observed absorbance at 500 nm, and a half-time ($t_{1/2}$) was determined as the time necessary for the particles sedimenting to give a decrease in recorded turbidity corresponding to 50% of the initial value.

Rheology

Gelling kinetics of the $CaCO_3$/GDL system was monitored by using an oscillation test on a Bohlin VOR rheometer (Bohlin Reologi AB, Lund,

Sweden) at 20°C. The amplitude, initially 100%, was reduced subsequently with 40–50% each time the torque range exceeded 10%. Frequency was 1·0 Hz, the torque was 12.19 *g* cm and the gap between the serrated plates was 0·76 mm. The time lap between each measurement was 2·0 min. Liberation of Ca^{2+} was measured by an Orion Research Ca^{2+} selective electrode (Orion Research Inc., Boston, USA).

Syneresis and gel strength

Syneresis was measured as final gel weight relative to the initial weight determined by the total volume of the gelling solution made as described above. This was simply done by removing the gel plug from the plastic cylinder followed by a gentle drying on water absorbent paper and weighing. Gel strength was determined as the force necessary to compress the 15 mm alginate gel cylinders 3 mm by a Stevens LFRA Texture Analyzer (C. Stevens & Son, St Albans, UK).

RESULTS AND DISCUSSION

Gel formation

As presented earlier, the pH in the $CaCO_3$/GDL system varies according to a standard acid-base relationship; i.e. equivalent amounts of GDL and $CaCO_3$ give neutral gels (Draget *et al.*, 1989). In order to avoid formation of acid gels, it was decided to use equivalent molar amounts of GDL and carbonate (30 and 15 mM respectively for 1% alginate).

Alginate gels with $CaCO_3$ could also be made by gassing with CO_2 to form the necessary acid. However, under 1 atm CO_2 pressure this gelling system proved to be extremely dependent on gel depth. A 3 mm-thick gel slice of 1% LF 10/60 was formed during 4–6 h, whereas an 8 mm slice had not gelled completely within 48 h of incubation. It is reasonable to believe that an increase in pressure would enhance the gelling process. The main disadvantage of using CO_2 as acidifier in this system is the creation of additional HCO^{3-}. CO_2 may thus lower the speed of Ca^{2+} liberation by influencing the kinetics of carbonate hydrolysis.

Attempts to make alginate gels based on $CaCO_3$ with addition of diluted (2.0 M) HCl, led to instant gelling and gave irregular gel lumps where the acid entered the alginate/$CaCO_3$ dispersion before the components could be properly mixed.

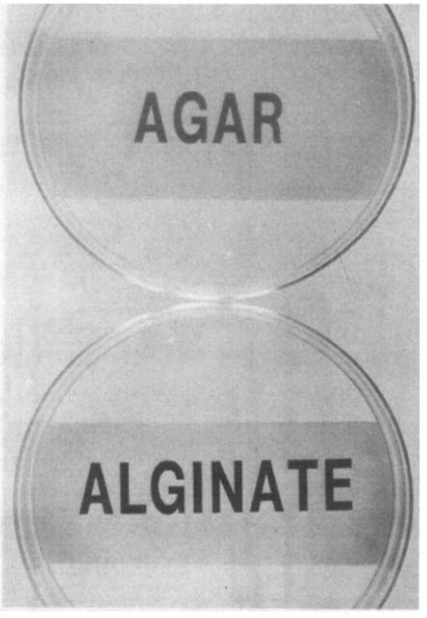


Fig. 1. Optical clarity of 10 mm thick alginate and agar based gels at 1·0% concentration: Alginate, HF 200; agar, Difco Bactoagar.

Capture of gas bubbles inside the final gel may occur when making large gels with a high volume/surface ratio. This capture is reduced by degassing the alginate solution prior to gelling. Gels in petri dishes did not show observable gas capture due to the low volume/surface ratio of these systems. Figure 1 shows 10 mm deep gels of alginate and agar made in 90 mm petri dishes. The observed optical clarity was better in a technical grade high-G alginate than in bacterial grade agar based gels.

Gel homogeneity

Figure 2 shows final gel homogeneity as a function of alginates with different intrinsic viscosities. The two highly viscous alginates gave homogeneous gels, whereas the low viscous sample shows a considerable degree of inhomogeneity. As predicted earlier, the observed inhomogeneity in the final alginate gel by using $CaCO_3$ and GDL was due to carbonate sedimentation during gelation (Draget *et al.*, 1989). Alginate concentrations in upper half relative to lower half ($P_{1/2}$) were 76, 97 and 99% for LF 10/60, *Macrocystis* and HF 200 respectively. This result was expected since decreased sedimentation by increased solvent viscosity is predicted by the Stokes' equation, in which the friction coefficient (f) of a

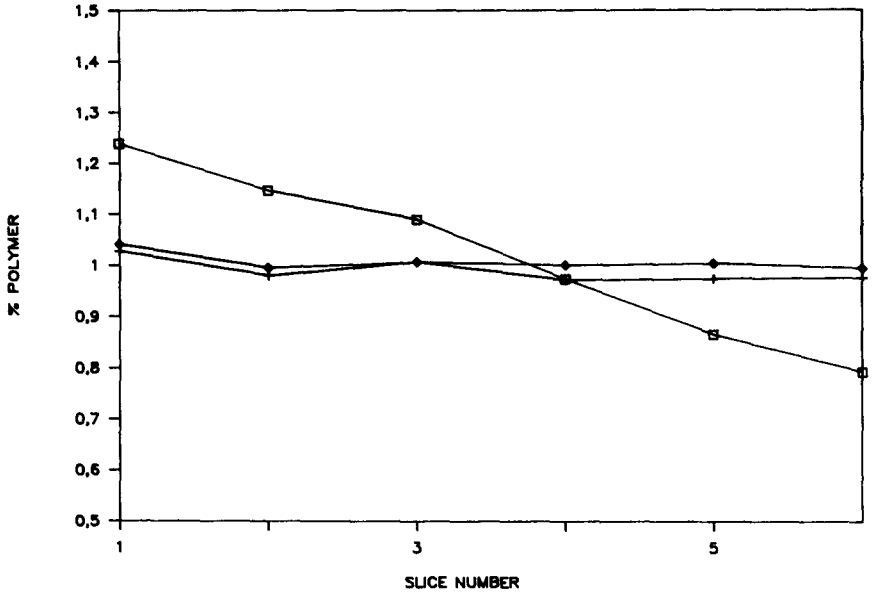


Fig. 2. Alginate concentration throughout gel cylinders made with different alginates at 1·0% (w/v), 6 = top of cylinder: (□) = LF 10/60; (+) = *Macrocystis*; (◇) = HF 200 alginate.

sphere in a solution is proportional to the solution's viscosity; $f = 6\pi\eta . R$, where R = radius of the sphere.

The results presented in Fig. 2 also show that there was no difference in homogeneity of the final gel of alginates with different guluronic acid (G)-content. Even though the *Macrocystis* alginate has far lower F_G and a somewhat lower intrinsic viscosity, the final gel homogeneity was essentially the same as for HF 200. Hence, the most important alginate property concerning final gel homogeneity is viscosity.

Sterile gels are necessary for biological applications. It has been observed (results not included) that autoclaved buffered alginate solutions at neutral pH retain their viscosity quite well. Autoclaving an alginate solution in the presence of dispersed $CaCO_3$ decreased the homogeneity of gels made by such autoclaved solutions (120°C, 15 min) as illustrated in Fig. 3. Reduced homogeneity was observed with the two initially highly viscous alginates, indicating chain breakages, loss in viscosity and increased carbonate sedimentation. This is most probably due to a base catalysed hydrolysis of the alginate chains because of the high pKa value of $CaCO_3$ (approx. 9·5) combined with high temperature. The $P_{1/2}$ values dropped to 80% for both *Macrocystis* and HF 200 alginate when autoclaved in the presence of $CaCO_3$.

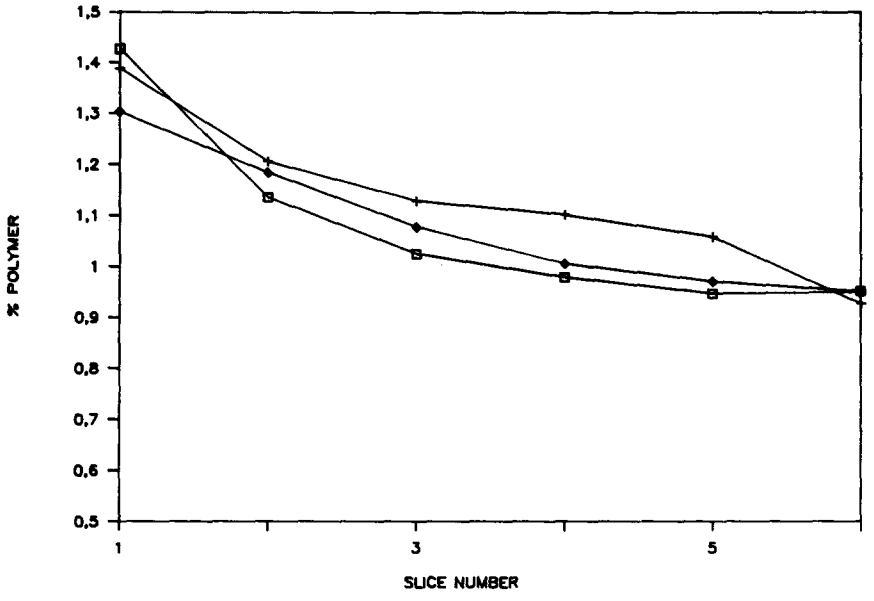
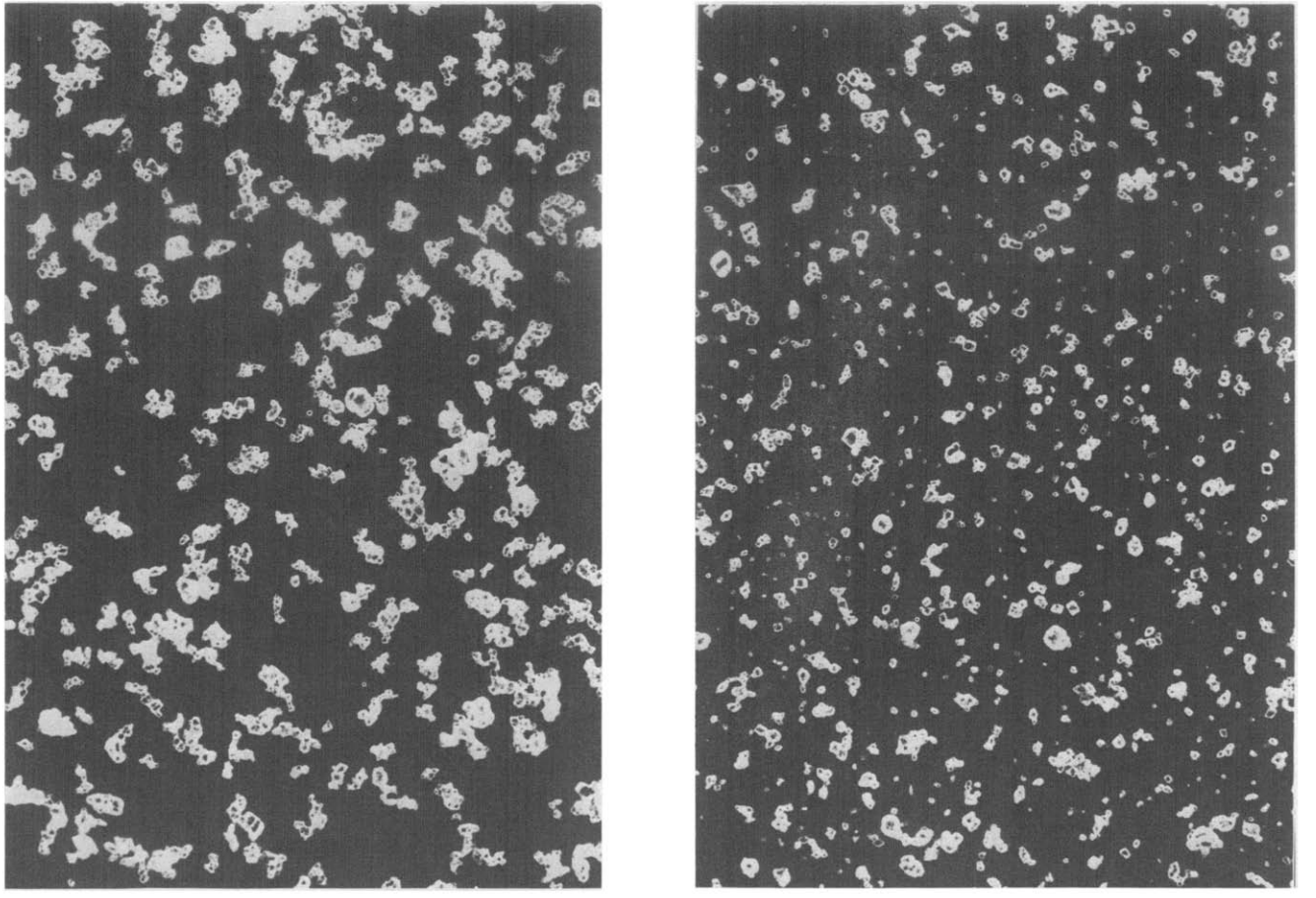


Fig. 3. Alginate concentration throughout gel cylinders made with different alginate samples (1·0%) autoclaved in the presence of $CaCO_3$, 6 = top of cylinder: (□) = LF 10/60; (+) = *Macrocystis*; (◇) = HF 200 alginate.

A microscopical examination of carbonate revealed a polydisperse particle size distribution consisting primarily of relatively large 'microaggregates' (Fig. 4(a)). With the use of a powerful sonicator, these aggregates proved to be easily detachable. Figure 4(b) shows a micrograph of sonicated $CaCO_3$, with considerably smaller particles and a more uniform size distribution than batch carbonate. Sonicated and freeze-dried carbonate resuspended in water was similar to the sonicated particles presented in Fig. 4(b), showing preserved particle size after drying.

In Fig. 5 the sedimentation velocities of batch (non-sonicated) and sonicated carbonate samples are compared. In both cases, an approximate linear relationship was observed when the sedimentation periods ($t_{1/2}$) were plotted as a function of c^2. From the slopes in the linear area, it can be estimated that sonicated $CaCO_3$ sedimented 3·5 times slower than the batch sample. This should greatly increase the homogeneity of gels made with low viscous alginate due to the prolonged period of time in which GDL is allowed to react with carbonate before sedimentation. This is visualized in Fig. 6 in which $P_{1/2}$ is estimated on gel cylinders made with LF 10/60 (low viscosity) at different concentrations and with



(a) (b)

Fig. 4. Light micrograph of (a) non-sonicated (batch) carbonate particles and (b) carbonate particles sonicated dispersed in water for 5 min at 400 W.lcm = 36 μm.

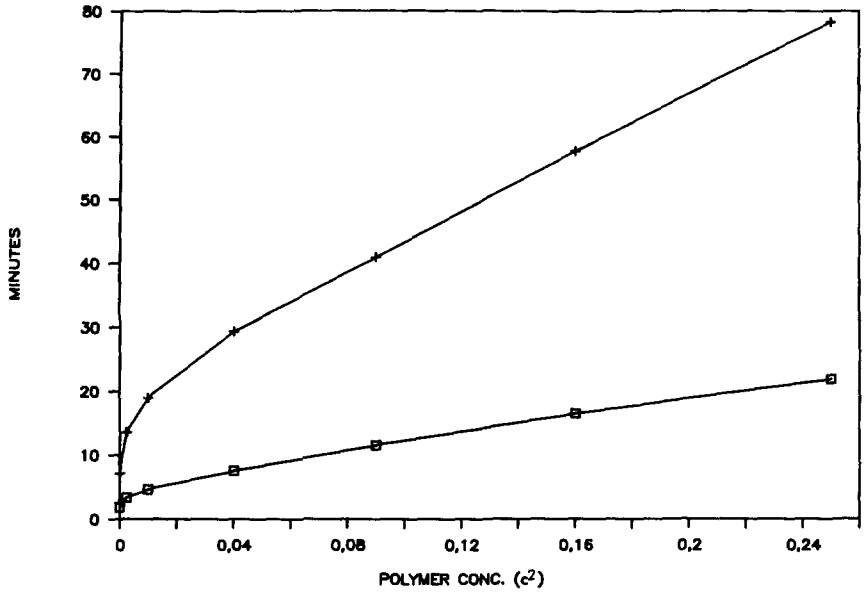


Fig. 5. Halftime ($t_{1/2}$) for sedimentation of sonicated (+) and batch (□) carbonate particles in solutions of LF 10/60 at different alginate concentrations.

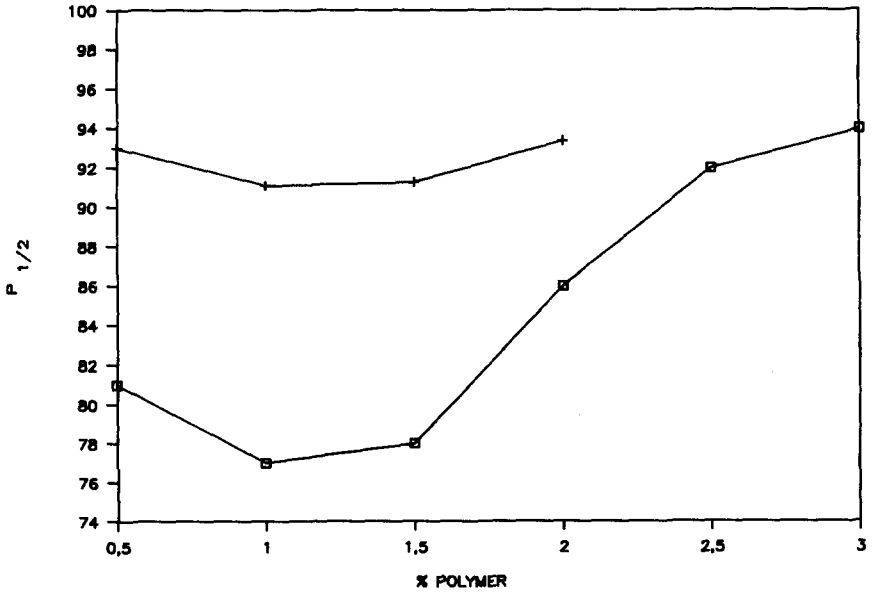


Fig. 6. Alginate concentrations in upper relative to lower half ($P_{1/2}$) of gel cylinders made with sonicated (+) and batch (□) $CaCO_3$ and LF 10/60 at different concentrations.

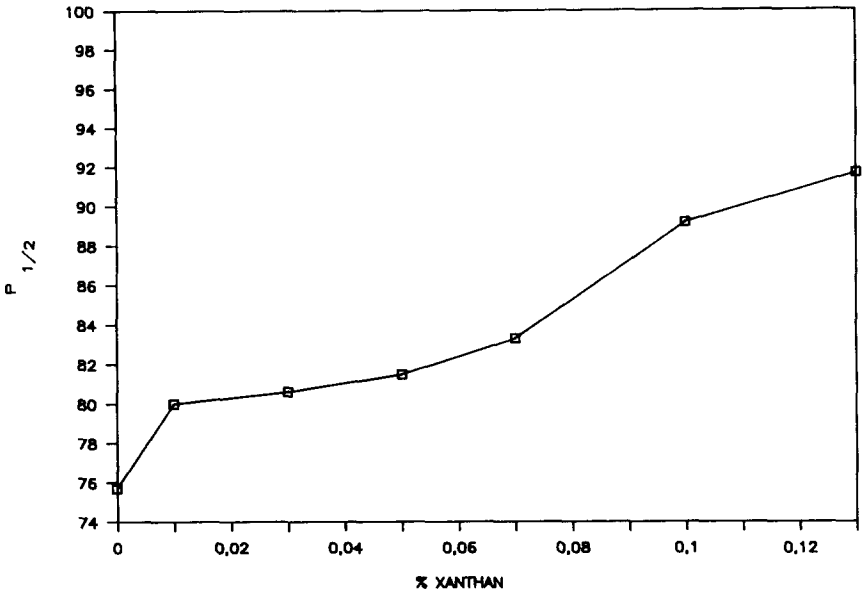


Fig. 7. Alginate concentrations in upper relative to lower half ($P_{1/2}$) of gel cylinders made with 1·0% LF 10/60 as a function of xanthan added as artificial viscosifier.

sonicated and batch carbonate. Sonicated $CaCO_3$ gave homogeneous gels even at low concentrations of alginate. Fig. 6 also shows that gel homogeneity increased with increasing concentrations of the alginate with lowest intrinsic viscosity. The results presented show that homogeneity in a gel made by a low viscous alginate can be increased by simply adding more polymer and thereby increasing relative viscosity of the solution. The same result was obtained by addition of an independent, non-gelling polymer as viscosifier. As presented in Fig. 7, addition of xanthan to a solution initially giving inhomogeneous gels improved homogeneity with increasing xanthan concentration.

None of the final gel cylinders at the given height (15 mm) made by this method exhibited extreme degrees of inhomogeneity. The degree of inhomogeneity is expected to increase with increasing gel height, and it was observed that the most extreme inhomogeneous gels made by low-viscosity alginate and non-sonicated $CaCO_3$ demanded up to 24 h to gel all the way up if made at heights of about 5 cm. By optimizing gel homogeneity with high-viscosity alginates, 2·5 dm^3 gels with a height of 15 cm have been made at 1% alginate concentration showing no problem in scaling-up the system.

Gelation kinetics

At an early stage a considerable difference in gelling velocities between gels made with sonicated and non-sonicated carbonate was visually observed. It was therefore necessary to describe the gelling system more precisely with rheological measurements, and an oscillation test on a Bohlin VOR rheometer was chosen for this purpose.

Figure 8 presents the rheological recordings of gelling with 1·0% HF 200 and batch $CaCO_3$. It is seen that dynamic complex modulus (G*) and dynamic storage modulus (elastic modulus, G′) exhibited a relative steady slope showing a continuous sol–gel transition. In Fig. 9, G′ and δ (phase angle) of the two different carbonate samples are compared directly. The sonicated carbonate system showed a sharp primary gelling phase culminating at about 1 h, followed by a plateau phase and a less pronounced secondary gelling phase. It can also be seen that δ dropped after a lag period of 17 min for systems with sonicated $CaCO_3$, while batch carbonate showed a slower and more steady decrease. This difference is also reflected in the dynamic storage modulus of the two systems. Sonicated $CaCO_3$ gave a considerably faster sol–gel transition and the slope of the initial gelling phase was much steeper.

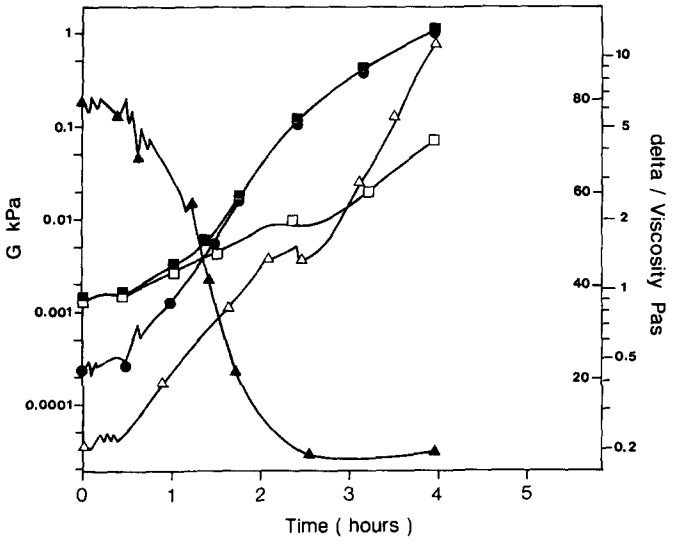


Fig. 8. Bohlin VOR rheometer recordings of the initial gelling process of a 1·0% HF 200 solution and batch $CaCO_3$: (■) = dynamic complex modulus (G*); (●) = dynamic storage modulus (G′); (□) = dynamic loss modulus (G″); (▲) = phase angle (δ); (△) = system dynamic viscosity.

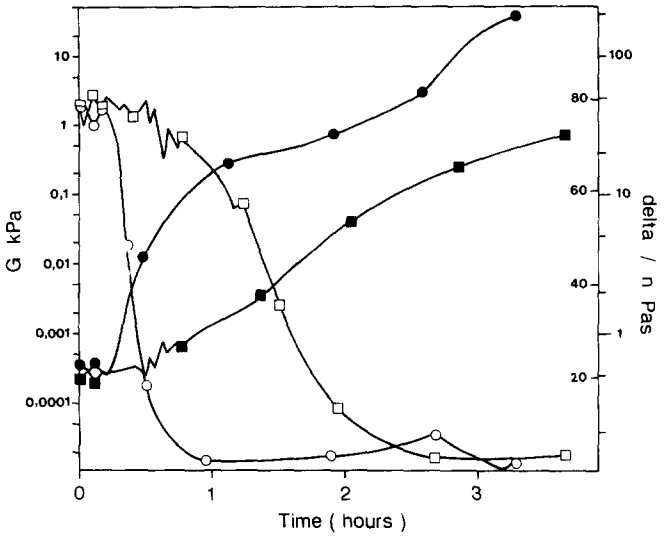


Fig. 9. Bohlin VOR recordings of phase angle (δ) and elastic modulus (G′) of gelation with 1·0% HF 200. Batch $CaCO_3$: (■) = G′ and (□) = δ. Sonicated $CaCO_3$: (●) = G′ and (○) = δ.

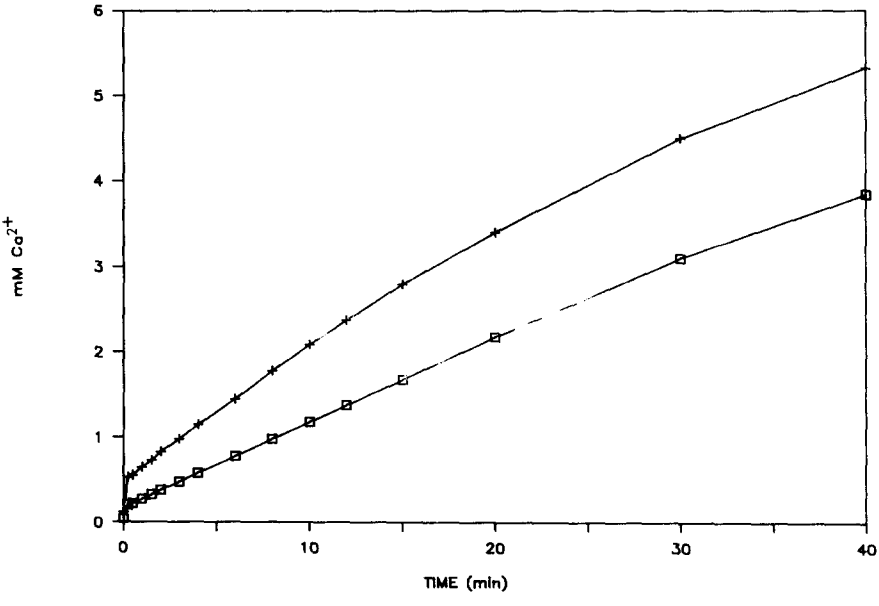


Fig. 10. Initial Ca^{2+} release from dispersed 15 mM batch (□) and sonicated (+) carbonate after addition 30 mM GDL.

This difference can be related to liberation of free Ca^{2+} as measured by a calcium selective electrode. The results of mixing $CaCO_3$ and GDL in the absence of alginate are presented in Fig. 10. It can be seen that the Ca^{2+} concentration corresponding to approx. 3 mM fits well with the observed initial lag periods before sol–gel transitions obtained with the Bohlin measurements. This means that systems containing sonicated carbonate responded almost twice as fast as those with batch $CaCO_3$. The concentration measured with the Ca selective electrode may, however, be different from the actual gelling system since the latter contains alginate. Alginate may increase the Ca^{2+} liberation by rapidly binding the continuous supply of free ions.

Since sonicated carbonate gave faster gelation, the dissolution and protonization of $CaCO_3$ rather than the hydrolysis of GDL is likely to be the rate limiting step. This view was also supported by making gels of LF 10/60 (inhomogeneous) with batch carbonate at different temperatures up to 52°C (results not included). The hydrolysis of GDL increases with an elevation of temperature, but no improvement of gel homogeneity was observed. Sonicated $CaCO_3$ will at the same concentrations as batch carbonate have a greater particle surface and is therefore more liable to acid attack and dissolution. Even though gelling velocities are not directly dependent upon the speed of GDL hydrolysis, some kind of

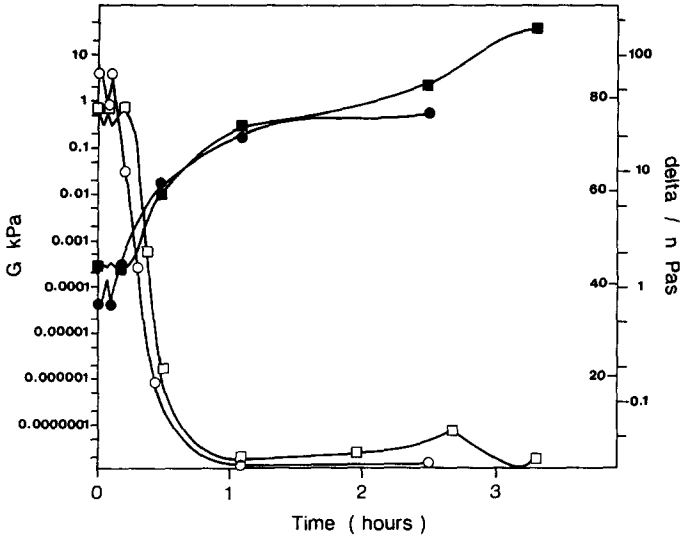


Fig. 11. Phase angle (δ) and elastic modulus (G′) of gelation with sonicated $CaCO_3$ in 1·0% alginate solutions of HF 200 (□ and ■ respectively) and *Macrocystis* (○ and ● respectively.)

slow proton release is basically necessary. As already presented, HCl initiated gelation before proper mixing could be achieved, leading to irregular gel lumps.

Rheological parameters (G′ and δ) of high- and low-G alginates are compared in Fig. 11. There was virtually no difference between the two samples during the first 1·5 h; low-G and high-G alginates gelled initially with the same speed. After 1·5 h the high-G alginate entered the secondary gelling phase with increasing gel strength, whereas the low-G sample seemed to have entered a very pronounced plateau phase. The observed final difference in gel strength between the two samples started to develop 1·5 h after initialization of gelling, which may be called the consolidation phase of high-G alginate gels.

As might be expected from the similarity between freshly sonicated and sonicated/freeze-dried carbonate in the microscopical inspections, the Bohlin rheometer measurements of freeze-dried and redispersed sonicated gave the same results as freshly sonicated $CaCO_3$ (results not included). The practical consequence is the improved handibility achieved by using dried sonicated carbonate instead of water dispersed. It should be pointed out that sonication is just one way of reducing carbonate particle size in a laboratory scale; any method resulting in reduced size particles will increase gel homogeneity with low viscous alginates and speed up the initial gelling.

Gel strength and syneresis

Figure 12 shows the gel strength of homogeneous alginate gel cylinders at different polymer concentrations at a fixed $CaCO_3$/GDL concentration (15/30 mM). It can be seen that maximum gel strength within the described system may be correlated to G-content in the alginate samples. Maximum gel strength was reached at 1% alginate concentration for HF 200 and around 1·5–2% for *Macrocystis*. Calculating the ratio between the actual Ca^{2+} concentrations (15 mM) and equivalent amount of uronic acids ($Mr \simeq 200$ D) at these alginate concentrations it gives approx. 0·6 and 0·3–0·4 for HF 200 and *Macrocystis* respectively. These values fit well with the F_G ratios presented in Table 1. In Fig. 12, the polymer concentration in the shrunken cylinders was corrected to final values as indicated.

Figure 12 also shows that an addition of polymer beyond optimum at this fixed carbonate level gave a weaker gel. Polymer levels below optimum caused syneresis. Both these effects were most pronounced for the high-G alginate, and both effects were reduced by addition of 0·1 M NaCl. The effects of ionic strength on polyionic molecules in solution are

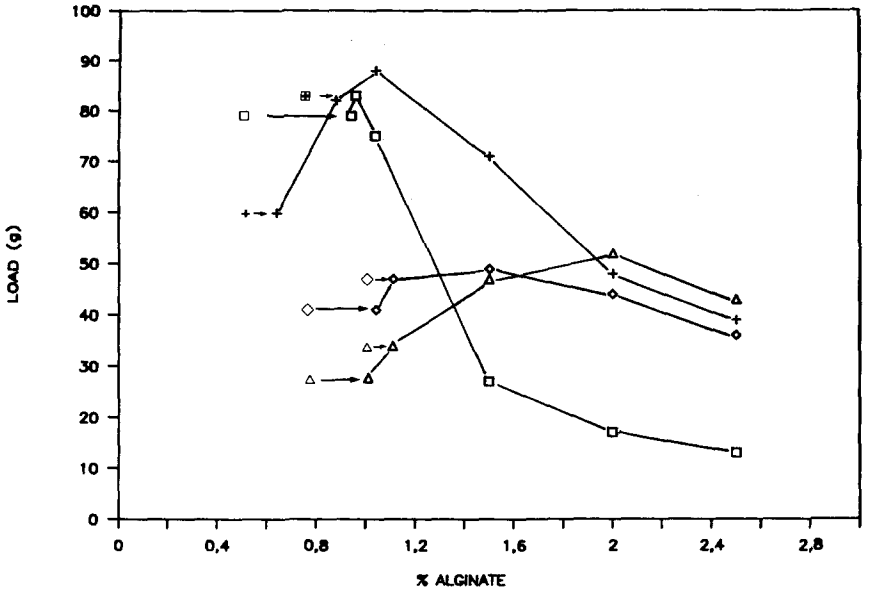


Fig. 12. Gel strength in HF 200 and *Macrocystis* gel cylinders at different alginate concentrations: (□) = HF 200 alginate, non-salted; (+) = HF 200 with 0·1 M NaCl; (◇) = *Macrocystis* alginate, non-salted; (△) = *Macrocystis* with 0·1 M NaCl. $CaCO_3$ and GDL concentrations were fixed at 15 and 30 mM respectively. Arrows show syneresis at suboptimal alginate concentrations by indicating transfer from initial to final concentration.

well documented (Smidsrød & Haug, 1971). In this particular experiment, Na^+ may compete with Ca^{2+} on binding to uronic acid residues, thereby decreasing sub-optimal binding of Ca^{2+} not involved in junction zones.

Figure 13 shows final gel strength of cylinders made of one low-G (*Macrocystis*) and one high-G (HF 200) alginate at four different polymer concentrations (0·5, 1·0, 1·5 and 2·0%) and optimized concentrations of $CaCO_3$/GDL for HF 200 (at 7·5/15, 15/30, 22·5/45 and 30/60 mM respectively). Both sonicated and batch carbonate were tested. It can be seen that the high-G alginate gels increased considerably more in gel strength with polymer content than the low-G alginate gels. This is consistent with earlier results showing that alginate gel strength is correlated to the polymer's content of guluronic acid residues (Smidsrød, 1974) and the length of the G-blocks within the polymer (Skjåk-Bræk *et al.*, 1986). Although the measured gel strength values might have been somewhat higher for the *Macrocystis* cylinders if optimized according to Fig. 12, the difference between HF 200 and *Macrocystis* gels at a given

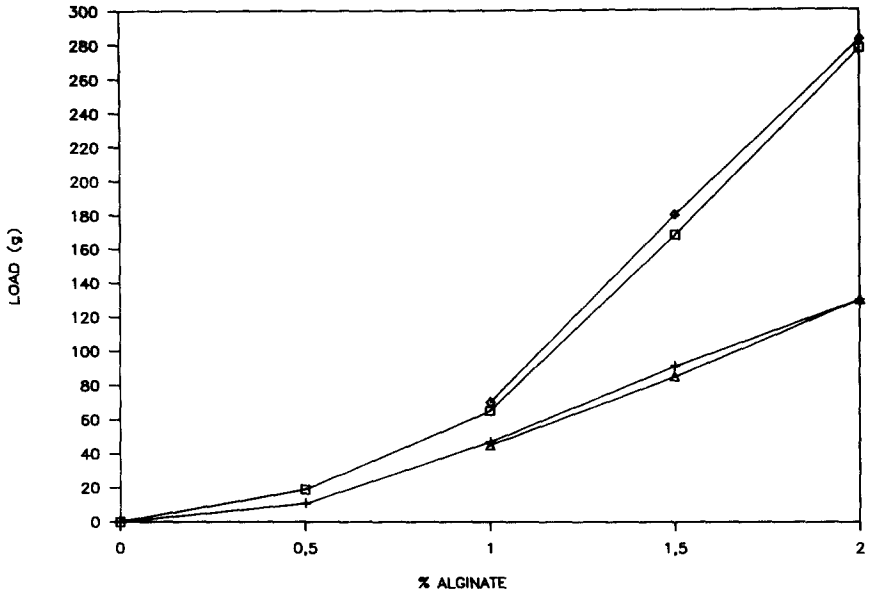


Fig. 13. Final gel strength in gel cylinders made of 0·5, 1·0, 1·5 and 2·0% solutions of HF 200 and *Macrocystis* alginate. The $CaCO_3$/GDL molarities were 7·5/15, 15/30, 22·5/45 and 30/60 mM respectively. HF 200: Batch carbonate (◇) and sonicated (□). *Macrocystis*: Batch carbonate (△) and sonicated (+).

polymer concentration fits well with the $\bar{N}_{G>1}$ values presented in Table 1 (11·4 versus 6·3).

The gel strength measurements are in accordance with the Bohlin rheological results of gelling with low-G alginate. The primary gelling phase culminating in a very pronounced plateau phase should lead to a lower final gel strength than the high-G alginate exhibiting a significant secondary gelling phase (Fig. 11). The results presented in Fig. 13 showed no significant differences between cylinders made of batch and sonicated $CaCO_3$. The difference in elastic modulus present in Fig. 9 is therefore probably lost later in the gelling process, i.e. within 48 h.

As mentioned earlier, syneresis was observed when the potential amount of free Ca^{2+} exceeded the optimal alginate concentration correlated to their content of guluronic acid residues. This phenomenon is quantified in Fig. 14. It can be seen that for gels made at standard 15 mM $CaCO_3$ and 30 mM GDL, syneresis became prominent at polymer concentration below 1·0% and showed extreme values at 0·5% alginate. However, when dialysed against 100 mM $CaCl_2$, gels at all polymer concentrations showed high degrees of syneresis after being exposed to these superoptimal Ca^{2+} concentrations. This syneresis was

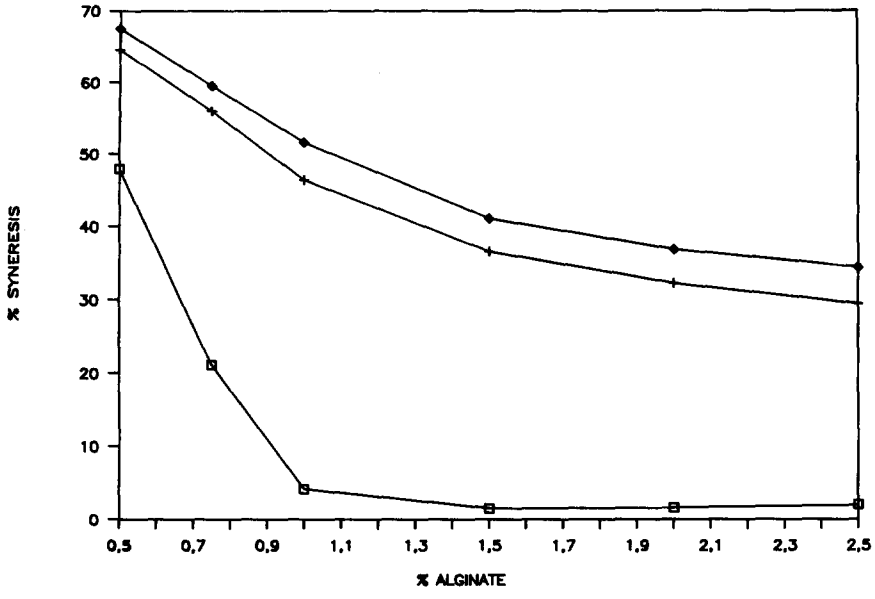


Fig. 14. Syneresis of gel cylinders made with HF 200 alginate at different concentrations. Initial gel cylinders were made with 15 and 30 mM $CaCO_3$ and GDL respectively (□). The final gel cylinders were later dialysed against 100 mM $CaCl_2$ (+) and finally redialysed against 10 mM $CaCl_2$(△).

not reversible, as illustrated by the upper curve where the shrunken gels were redialysed against 10 mM $CaCl_2$. Actually, syneresis showed a small additional increase with this treatment. This may, however, be due to a slow ageing of the gels.

By reducing carbonate levels in accordance with polymer content in order to avoid syneresis, stable homogeneous gels have been made at as low alginate concentrations as 0.5% (w/v, see also Fig. 12). With high-G alginates, stable gels can probably be made at even lower polymer concentrations.

CONCLUSIONS

Homogeneous alginate gels can be made at neutral pH by balancing $CaCO_3$ and GDL at the molar ratio 1:2.

Gas capture within the gel may occur at large volumes compared to the gel surface. When high optical clarity is wanted in such cases, degassing of the alginate solution will improve the turbidity of the gel. This is not a problem at the petri dish level.

Homogeneity is achieved by avoiding sedimentation of $CaCO_3$ before gelation. This is influenced and controlled by alginate viscosity and concentration, addition of artificial viscosifier and carbonate particle size. The last variable also influences the initiation and speed of gelling because particle size determines the release of Ca^{2+}.

Low-G and high-G alginates gelled with the same speed, and gel strength was found to be independent of G-content during the first 1·5 h. Later there was a consolidation phase with high-G gels which gave higher final gel strength. This final gel strength is quantitatively correlated to the average length of the G-blocks in the alginate samples.

Maximum gel strength was reached when the Ca^{2+} concentration was equivalent to the amount of guluronic acid residues. Syneresis became prominent when the calcium content exceeded this value.

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