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A new approach for estimating the crosslink density of covalently crosslinked ionic polysaccharide gels

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For an ideal polysaccharide gel with a known total polymer chain contour length, crosslinks all of the same functionality and elastic chains all with the same contour length and stiffness, the gel crosslink density can readily be determined from measurements of the maximum volume of the swollen gel (Moe *et al., (1991) Food Hydrocolloids*, **5**, (1/2), 119–23. In the case of randomly crosslinked polysaccharide gels, where the chain contour length between two adjacent crosslinks may vary greatly, it is often much more difficult to determine the crosslink density. This paper reports on an attempt to extend the use of maximum gel volume measurements to estimate crosslink density for the latter type of gel. This is done by calculating the maximum swelling volume for polymer networks with four-functional crosslinks, known elastic chain mean contour length and standard deviation. The numerical analysis involves the calculation of the equilibrium force at each crosslink as the network expands. This allows a detailed study of how the distribution of individual polymer chain contour lengths affects the maximum swelling volume. The computer simulation results are compared with the results from experimental measurements of the maximum volume of swollen covalently crosslinked sodium alginate gels.

INTRODUCTION

The chemistry of crosslinking polysaccharides with epichlorohydrin (ECH) is well known (Dreyfus, 1921) and has been utilised in the industry for more than 30 years, for example, for the production of ion-exchange and gel-permeation chromatography matrices (Pharmacia, 1961; Flodin, 1962). ECH has also been used for coupling of ligands to affinity chromatography matrices and for themanufacturing of affinity chromatography matrices (Rombouts *et al.*, 1979; Weber *et al.*, 1986). ECH crosslinking of alginate, the linear binary copolymer of α-L-guluronate and β-D-mannuronate, has been suggested in order to produce a matrix that has the characteristic ion-binding properties of alginates (Cereti Mazza & Ferrero, 1971; Ferrero & Piccinini, 1982; Ferrero *et al.*, 1982) or for the manufacture of a novel superswelling material (Moe *et al.*, 1991; Skjåk-Bræk & Moe, 1992). The molecular structure of this gel is fundamentally different from the ionically crosslinked alginate gel. The long junction zones in the ionically crosslinked alginate gel are substituted by discrete, covalent crosslinks between chains, as illustrated in Fig. 1.

Although the ECH crosslinking process itself is well known and many studies have been carried out on the crosslinking of polysaccharides with ECH, little is known about the crosslink density in such gels. The highly reactive ECH crosslinks polysaccharides by a nucleophilic attack from the hydroxyl groups on the polysaccharide (Flodin, 1962), thus giving rise to a stable gel, but also to a number of side reactions (Kuniak &

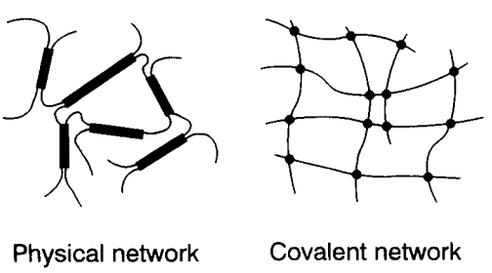


Fig. 1. Two-dimensional representation of the molecular structure of covalently crosslinked polysaccharide networks and physically crosslinked polysaccharide networks.

Marchessault, 1972; Holmberg, 1983; Yu, 1984; Kartha & Srivastava, 1985). The side reactions makes it impossible to use radiolabelled ECH or spectroscopic methods for quantitative determination of crosslink density. The experimental method which until now has been shown to give the most reliable estimates of crosslink density for epichlorohydrin crosslinked polysaccharide gels is therefore an acid degradation of the gel followed by gas chromatography–mass spectrometry (GC–MS) analysis (Holmberg, 1983). This method is both time- and labour-consuming and has not been attempted in this work.

If the network follows the theory of swelling developed by Flory (1953), the gel crosslink density may, in principle, be determined by measuring the degree of gel swelling for a given set of conditions. The swelling of ionic gels, according to this theory, is described by the equilibrium

$$\Pi_M + \Pi_D + \Pi_E = 0 \tag{1}$$

where Π_M is the osmotic pressure in the gel due to polymer solubility (Flory–Huggins term), Π_D the osmotic pressure due to concentration difference for mobile ions between the inside of the gel and the supernatant, and Π_E the elastic reaction of the network expressed as an osmotic pressure (Flory, 1953).

By assuming four-functional crosslinks and inserting

$$\Pi_M = \frac{RT}{V_1^0}(\ln(1-\phi) + \phi + \chi\phi^2)$$

$$\Pi_D = -\mathrm{RT}\Delta C_{\text{mobile ions}}$$

and

$$\Pi_E = \mathrm{RT}\frac{\nu}{V^0}\left(\phi^{\frac{1}{3}} - \frac{1}{2}\phi\right),$$

this relation may be rearranged to yield the crosslink density in the gel:

$$\nu = \frac{V^0}{V_1^0} \times \frac{-(\ln(1-\phi) + \phi + \chi\phi^2) + V_1^0\Delta C_{\text{mobile ions}}}{\phi^{\frac{1}{3}} - \frac{1}{2}\phi} \tag{2}$$

where ν is the molar number of crosslinked chains in the gel, V^0 the volume of the gel when the crosslinks were introduced, V_1^0 the molar volume of water, ϕ the volume fraction of polymer in the gel, χ the Flory–Huggins interaction parameter, and $\Delta C_{\text{mobile ions}}$ the difference in molar concentration of mobile ions between the gel and the liquid surrounding the gel. The concentration difference of mobile ions, $\Delta C_{\text{mobile ions}}$, may be calculated using the well-known relationships valid for the Donnan equilibrium (Tanford, 1961).

A similar expression can be obtained for polymer networks crosslinked in the gel state (Tanaka, 1979, 1981, 1987; Ohmine & Tanaka, 1982):

$$\nu = \frac{V^0}{V_1^0} \times \frac{-(\ln(1-\phi) + \phi + \chi\phi^2) + V_1^0\Delta C_{\text{mobile ions}}}{\left(\frac{\phi}{\phi_0}\right)^{\frac{1}{3}} - \frac{1}{2}\frac{\phi}{\phi_0}} \tag{3}$$

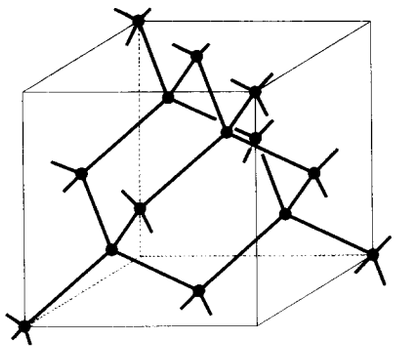
where ϕ_0 is the volume fraction of polymer in the gel when the crosslinks were introduced.

However, eqns (2) and (3) both assume, that the network consists of Gaussian chains. This assumption is generally not valid for polysaccharide networks, and in particular not for alginate networks (Bailey *et al.*, 1977; Moe *et al.*, 1992). The elastic modulus of some biopolymer networks is three to 10 times that predicted using rubber elasticity theory (Clark *et al.*, 1983; Higgs & Ball, 1989). By introducing a front factor of this magnitude into the expression for the elastic term, one may obtain estimates for crosslink density which are more in accordance with reality. The magnitude of this front factor has, however, not been determined for these gels. Also, the magnitude of this front factor would most probably be dependent on the average elastic chain length as the network chains approach Gaussian behaviour when the elastic chain length increases (Bailey *et al.*, 1977). It has been observed (Moe, unpublished data) that storing covalently crosslinked sodium alginate beads for long periods in Milli-Q quality water (ionic strength $\approx 10^{-7}$ mol/litre) results in some disintegration of the gels. In this case, it is evident that some polymer chain breakage must take place, which indicates that the maximum attainable volume of the gels is probably determined simply by the topological restrictions of the polymer network. A better approach to estimating crosslink density may therefore be to analyse the topological restrictions on maximum gel swelling rather than using the equations describing the intermediate degree of equilibrium swelling. An important added benefit is that this analysis also eliminates the necessity of using a front factor of undocumented magnitude. This approach has been suggested by Moe *et al.* (1991) using a simple model based on the molecular structure of the covalent gel pictured in Figs 1 and 2(a).

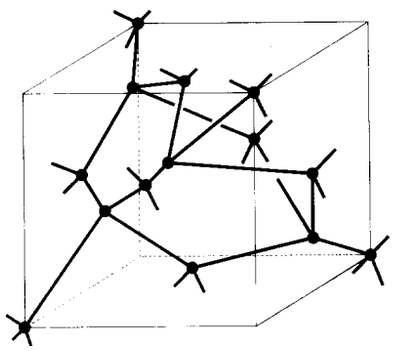
The original network model consisted of a network of elastic chains of equal length bound together in four-functional crosslinks (analogous to the well-known 'phantom network' treated by Flory (1953)), as shown in Fig. 2(a). This model included some assumptions which clearly were unrealistic:

(1) all network chains have the same contour length;
(2) the distribution of crosslinks in the unstrained network is completely uniform; and
(3) there are no 'loose ends' in the network.

In the original network model (Moe *et al.*, 1991) the maximum swelling ratio of the gel corresponds to all network chains being fully stretched. In a model where assumptions (1) and (2) are no longer satisfied, the maximum gel swelling may be expected to a large extent



(a)



(b)

Fig. 2. Network structure in (a) the regular network, and (b) the stress-free network.

to be determined by the topology close to the shorter network chains. This paper reports on a numerical analysis of such a gel network where assumptions (1) and (2) have no longer been incorporated. The numerical analysis involves calculation of the force equilibrium at each crosslink in the network as the network is being expanded. This allows a detailed study of how the distribution of individual polymer chain contour lengths affects the maximum swelling volume. The computer simulation results are compared with experimental measurements of the maximum volume of swollen covalently crosslinked sodium alginate gels.

MATERIALS AND METHODS

Materials

Alginate was supplied by Pronova Biopolymer A/S (Drammen, Norway). The alginate had been extracted from *Laminaria hyperborea* stipes, and had a guluronate content (F_G) of 67% and an average number of guluronate units in *G*-blocks ($\overline{N}_{G>1}$) of 14·0, as determined by ^{1}H-NMR (Grasdalen, 1983). ECH was Merck p.s. grade. Sodium chloride, calcium chloride, sodium hydroxide and EDTA was Merck p.a. grade and ethanol was 96% by volume.

Estimation of crosslink density from swelling experiments

Sodium alginate gel bead samples crosslinked for 1, 2, 3, and 4 h were prepared according to Moe *et al.* (1991). The diameter of the gel beads in various concentrations of sodium chloride was measured by microscopy, and the volume was calculated. From the swelling experiments, the crosslink density was calculated using eqns (2) and (3). The volume giving a polymer concentration of 20 g/litre, which equalled the volume of the beads at the onset of crosslinking, was taken as V^0. The difference in ion concentration, $\Delta C_{\text{mobile ions}}$, was calculated using the relations for the ideal Donnan equilibrium for monovalent electrolytes (Tanford, 1961):

$$\begin{aligned}(C'_+)^2 &= (\gamma_\pm/\gamma'_\pm)^2 C_+(C_+ + zC_p)\\ (C'_-)^2 &= (\gamma_\pm/\gamma'_\pm)^2 C_-(C_- + zC_p)\end{aligned} \tag{4}$$

where C_+ and C_- are the molar concentrations of ions inside the gel, C'_+ and C'_- the molar concentrations of ions outside the gel, $\gamma_\pm$ and $\gamma'_\pm$ the activity coefficients for the salt inside and outside the gel, respectively, z the fraction of non-condensed ions per monomer residue and C_p the molar concentration of monomer residues. Assuming that $\gamma_\pm = \gamma'_\pm$, one obtains two quadratic equations for the concentration of ions inside the gel which are easily solved. It was further assumed that the alginate had a fraction on non-condensed ions (z) of 0·6 (Büchner *et al.*, 1961).

Estimating crosslink density using numerical simulations

One numerical analysis of the network swelling consists of the following main steps.

(1) Establish the crosslink coordinates of a stress-free three-dimensional polymer network with four-functional crosslinks and selected polymer chain stress–strain relation, mean contour length and standard deviation.
(2) Increase the overall dimensions of the network by a small amount; recalculate the equilibrium coordinates of all crosslinks; save the value of the relative extension of each network chain.
(3) Repeat step (2) until the relative extension of at least one of the network chains equals 98% of the contour length of the chain in question; this is used as the criterion for when the network maximum swelling volume has been reached.

The crosslink coordinates of the stress-free three-dimensional network (step (1)) is obtained by first making a cubic lattice of n^3 points with selectable lattice constant (distance between lattice points). The location of the crosslinks and network topolgy is as illustrated in Fig. 2(a). This initial network has a regular tetrahedral crosslink structure analogous to the crystal structure, of for example, diamond. New crosslink coordinates were

then generated for each crosslink by adding a vector chosen from a three-dimensional Gaussian distribution with mean equal to zero and with selectable standard deviation. The contour length assigned to a chain connecting two crosslinks was calculated using a worm-like chain model (Kratky & Porod,. 1949) with a persistence length, a, of one-half of the value for the Kuhn segment length in 0·1 M NaCl reported by Smidsrød (1970) (39 nm, giving $a = 19{\cdot}5$ nm). The final stress-free network was finally obtained by using an interative algorithm making certain that

$$\sum_{i=1}^{N} \vec{F}_{ji} \simeq 0$$

for all j, where $\vec{F}_{ji}$ is the force on crosslink j by chain i connected to crosslink j. The magnitude of the force vector, $|\vec{F}_{ji}|$, was calculated using the Warner force law equation for a finitely extensible non-linear spring (Warner, 1972):

$$F(r) = \frac{3kT}{A_m} \times \frac{r/L_C}{1-(r/L_C)^2} \qquad (5)$$

where k is Boltzmann's constant, T is absolute temperature, A_m is the Kuhn segment length, r the end-to-end distance of the elastic chain and L_C the contour length of the elastic chain. Here, it is important to note that the calculated maximum swelling is independent of the front factor chosen in eqn (5).

The algorithm used to simulate gel swelling implicitly neglects any chain entanglements as the network chains are allowed to stretch without taking into account loops and entanglements (phantom network).

This model network was then allowed to swell (step (2)) by increasing the network dimensions by a small amount (affine deformation). The increment was limited by the condition that no chain end-to-end distance could exceed its contour length. The new coordinates for each crosslink corresponding to local equilibrium is then determined using the same algorithm as in step (2). This process was continued until the end-to-end distance of at least one chain was longer than 98% of its contour length (Odell *et al.*, 1988) (step (3)). The volume of the network was then calculated. Since the amount of polymer in the network was known from the contour lengths of all chains and the number of crosslinks was predetermined by the size of the three-dimensional lattice (n), this enabled the calculation of polymer concentration and average crosslink density at maximum swelling.

RESULTS AND DISCUSSION

The experimentally observed equilibrium swelling of the sodium alginate gel beads as a function of ionic strength is given in Fig. 3. The crosslink density as a function of ionic strength, calculated from these data using eqns (2) and (3), is given in Figs 4(a) and (b). The volume corresponding to random-walk behaviour of the elastic chains (V^0) has been chosen equal to the volume at which the polymer concentration is 20 g/litre, the concentration of polymer at the onset of crosslinking. This also supports the use of eqn (3) rather than eqn (2)

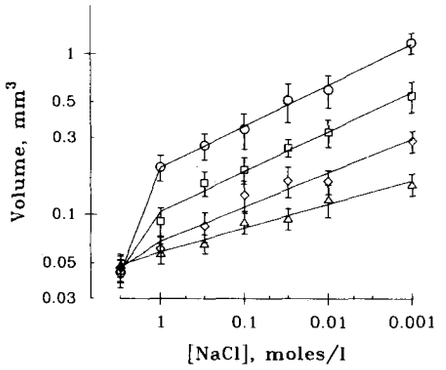


Fig. 3. Swelling of sodium alginate gel beads in sodium chloride solutions. Gel crosslinked for (○) 1 h; (□) 2 h; (◇) 3 h and (△) 4 h.

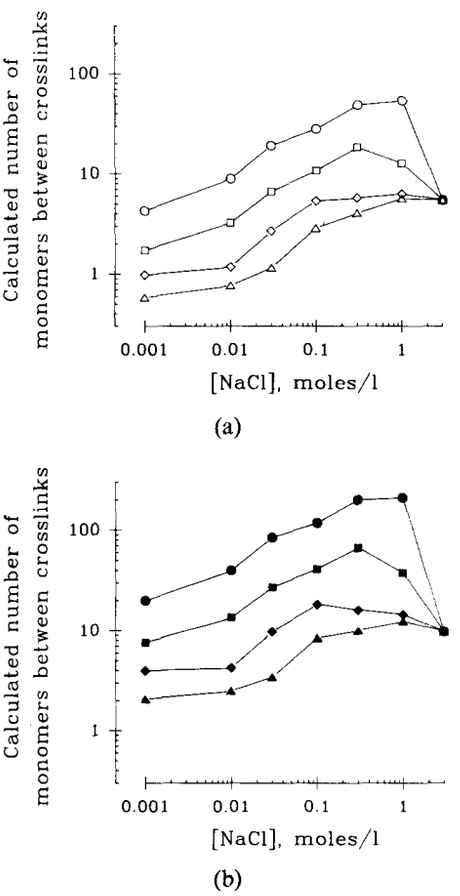


Fig. 4. Number of monomers between crosslinks, calculated using (a) eqn (2); dependence on ionic strength. Gel crosslinked for (○) 1 h; (□) 2 h; (◇) 3 h and (△) 4 h. (b) Calculated using eqn (3); dependence on ionic strength. Gel crosslinked for (●) 1 h; (■) 2 h; (◆) 3 h and (▲) 4 h.

for the determination of crosslink density from equilibrium swelling measurements. The average elastic chain length as obtained by this method, vary from approximately 0·6 to 200 monomer units depending on the ionic strength and crosslink density. An elastic chain length of 0·6 monomer units, corresponding to 1·7 crosslinks per monomer unit, is clearly without any physical meaning and indicates that the gel is in the finite extension regime. One can of course introduce a Gaussian front factor in the range of 3–10 in eqns (2) and (3), but, as already mentioned, the magnitude of this front factor is not determined and would probably also be dependent on the elastic chain length (i.e. the crosslink density). As one can see from Figs 4(a) and (b), the results are also strongly dependent on ionic strength. This is attributed to the onset of non-Gaussian behaviour of the chains (finite extension). These results therefore show that such methods for estimating the crosslink density of these alginate gel networks are questionable. They also suggest that an alginate gel network is a non-Gaussian network, as discussed by Moe *et al.* (1992).

The correlation between polymer concentration at maximum volume and number of monomers between crosslinks as obtained by numerical simulation is given in Fig. 5. Table 1 shows the maximum and minimum number of monomers (n_v) between crosslinks calculated from the experimentally observed minimum polymer concentration for the four gel samples. Maximum n_v is obtained with standard deviation of chain lengths 300%; minimum n_v is obtained with standard deviation of chain lengths 0, i.e. a regular network containing chains all of equal length.

The numerical simulations suggest that the elastic chain length of sodium alginate beads crosslinked for 2 h is between 45 and 173 monomers, corresponding to a contour length of between 22 and 83 nm. The results may be correlated to values for the temperature coefficient of the storage modulus reported for gels swollen in 0·4 M NaCl (Moe *et al.*, 1992). According to Smidsrød (1970), the Kuhn segment length for alginate varies between 15·5 and 85 nm, depending on ionic strength and is proportional to the inverse of the square root of the ionic strength. This yields a Kuhn segment

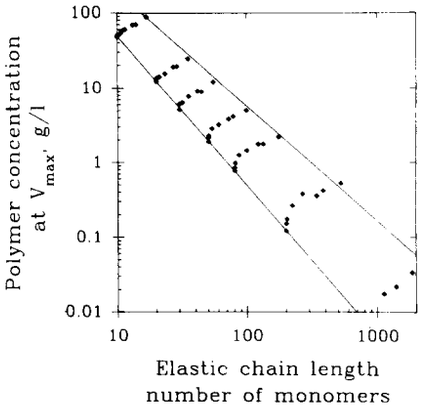


Fig. 5. Correlation between the maximum swelling polymer concentration and number of monomers between crosslinks from a computer simulation. Standard deviation of crosslink coordinates varied from 0 to 300%.

length in 0·4 M NaCl of 27 nm. The average elastic chain length of sodium alginate gels crosslinked for 2 h is then equivalent to 0·8–3 Kuhn segments lengths. The elastic chain length obtained of the order of approximately one Kuhn segment length also is indicative evidence of the alginate gel network being a network of stiff chains. A comparison of obtained elastic chain lengths with data for elastic modulus and temperature coefficient of the elastic modulus obtained for the sodium alginate gels (Moe *et al.*, 1992) is given in Table 1. For sodium alginate gels crosslinked for 2 h and equilibrated in 0·4 M NaCl the modulus increases with increased temperature, indicating that the elastic chains in this case are approaching Gaussian behaviour. In view of this, one may argue that the length of the average elastic chain corresponds to the maximum elastic chain length obtained by simulation (for 2 h: 173 monomers, corresponding to a contour length of 80 nm or 3 Kuhn segments). This also supports criterion used for maximum swelling (minimum one chain where $r > 0{\cdot}98L_C$). Albeit a 'local' criterion, it was observed that a large amount of the chains had an extension approaching $0{\cdot}98L_C$, especially for the lower values of chain length distribution (Fig. 6).

Table 1. Comparison of elastic chain length data with physical properties for sodium alginate gel cylinders[a]

Crosslink time (h)	Polymer concentration at maximum swelling (g/litre)	Typical storage modulus (Pa)	Typical temperature coefficient (Pa/K)	Numer of monomers between crosslinks (n_v) obtained by simulation		Number of Kuhn segments between crosslinks obtained by simulation	
				Minimum	Maximum	Minimum	Maximum
1	0·91	ND	ND	73	326	1·3	5·8
2	2·4	150	0·4	45	173	0·80	3·0
3	4·6	550	−0·2	33	114	0·59	2·0
4	8·9	1100	−0·7	23	74	0·41	1·3
5	ND	1800	−1·1	ND	ND	ND	ND

[a]Data taken from Moe *et al.* (1992).
[b]ND — not determined.

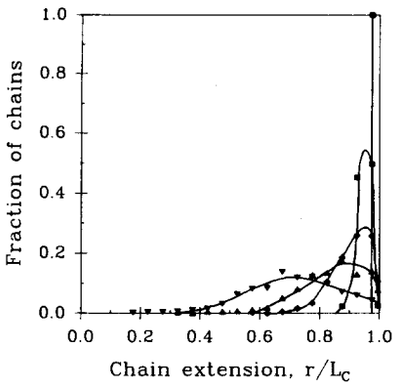


Fig. 6. Distribution of chain extensions in fully stretched networks determined from numerical simulations. Average chain length: 20 monomer residues. Chain length distributions: (●) SD = 0; (■) SD = 10%; (◆) SD = 30%; (▲) SD = 50% and (▼) SD = 100%.

From the results obtained by swelling theory and those obtained by numerical simulation, one can calculate the Gaussian front factor which has to be used to obtain reasonable estimates by equilibrium swelling experiments. As can be seen from Table 2, this front factor varies from 1·6 for gels with low crosslink density to approximately 8 for the gels with high crosslink density using eqn (3) from swelling data in the low-volume region. Alginate chains containing approximately 300–500 monomers have been shown to approach Gaussian behaviour (Bailey *et al.*, 1977), thus a Gaussian front factor in the order of unity is to be expected for the low-crosslink density gels. On the other hand, the large value for the front factor in the order of ten can also be expected for the non-Gaussian chains of about 75 monomer units. These values for the front factor as a function of crosslink density can then be used as an indication that the estimates obtained by numerical simulation are reasonable.

CONCLUSION

This paper reports on an attempt to extend the use of maximum gel volume measurements for estimation of crosslink density of covalently crosslinked polysaccharide networks. This is done by calculating numerically the maximum swelling volume for polymer networks with four-functional crosslinks, known elastic chain mean contour length and standard deviation. The estimates obtained by this method are consistent with the physical properties of these gels.

Table 2. Estimates for elastic chain lengths and Gaussian front factor for sodium alginate gels

Swelling experiments, eqn (3)	Number of monomers in the average elastic chain				Gaussian front factor			
	1h	2h	3h	4h	1h	2h	3h	4h
I = 0·001 M	20	7·6	4·0	2·1	16	23	29	36
I = 0·01 M	40	13	4·2	2·5	8·2	13	27	30
I = 0·03 M	84	27	9·8	3·4	3·9	6·4	12	21
I = 0·1 M	118	41	18	8·4	2·8	4·2	6·3	8·8
I = 0·3 M	198	67	16	10	1·6	2·6	7·1	7·4
I = 1 M	208	37	14	12	1·6	4·6	7·9	6·0
I = 3 M	10	10	10	10	34	18	12	7·6
Simulation	326	173	114	74				

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