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Properties of Poly(1,4-hexuronates) in the Gel State

II. Comparison of Gels of Different Chemical Composition

OLAV SMIDSRØD and ARNE HAUG

Norwegian Institute of Seaweed Research, N-7034 Trondheim-NTH, Norway

The modulus of stiffness, transparency, and the decrease in volume associated with formation were determined for some calcium-alginate gels. From the dependence upon molecular weight of the modulus of stiffness it is suggested that the distance between the junctions is similar to, or smaller, than the Kuhn segment-length, and that the parts of the alginate molecule between the junctions, therefore, are very restricted in their movements. Calcium polymannuronate does not form gels with a measureable stiffness, and the modulus of stiffness increases with increasing content of guluronic acid residues. The properties of calcium pectate resembled those of alginates rich in guluronic acid residues. The modulus of stiffness also depended upon the type of divalent metal ion used, and in all cases the stiffness increased with increasing affinity of the polyuronate for the metal ion. The transparency and the stiffness of the gels always varied in the same way.

The gelling of polyuronates with divalent metal ions has been studied quite extensively, and most of the relevant papers have been reviewed by Schachat *et al.*,[1] Solms,[2] Lipatov *et al.*,[3] Haug,[5] and Rees.[4] Because the gels formed by diffusion of divalent metal ions into solutions of polyuronates are birefringent [6–8] and give X-ray diffraction patterns,[8] it is quite clear that they have an ordered structure. Since the polyuronates in solution behave like flexible coils,[9,10] the divalent metal ions must have an ordering effect upon the macromolecules. It is thought that the metal ions are involved in the formation of secondary bonds between macromolecules. The magnitude and the origin of the energy associated with their formation is, however, not known. Mongar and Wassermann found that heat effects accompanied the absorption of sodium ions by swollen calcium-alginate fibres [11] and that the dependence upon temperature of the elasticity of calcium and sodium alginate fibres was different.[12] They were not able to calculate thermodynamic quantities for the formation of inter-chain linkages in calcium alginate, but they were able to explain most of their results [12] by assuming that "the main valency chains in stretched calcium alginate fibres are attached to each other, in

broadside-on positions, by salt bridges operating between the bivalent calcium and the carboxylate groups of the polyanion". Rees [4] rejected the possibility of "salt bridges" in polyuronate gels and described the inter-chain linkages as "microcrystallites".

We believe that at present too little is known about the correlation between the chemistry of the gels and their physical properties to allow any detailed discussion of the nature of the intermolecular forces. In this work we use simple measuring techniques [13] to compare some properties of gels formed by introducing different alkaline earth metal ions into solutions of some chemically well characterized polyuronates.

MATERIALS AND METHODS

The samples of sodium alginate were prepared and analyzed as previously described.[5] Molecular weight was obtained from viscosity measurements using previously determined [9] indices in the Mark-Houwink equation.

The sample of sodium pectate was kindly supplied by Dr. R. Kohn, Slovak Academy of Sciences, Bratislava, and has been characterized by him.[14]

The gels were prepared [13] by dialysing sodium polyuronate solutions against 0.34 M solutions of alkaline-earth metal chlorides. The volume of the gels and their moduli of stiffness were determined as described previously.[13] When "solvents" other than water in the gel were wanted, the gels were first prepared as usual, and then dialysed against a large excess (volume ratio 100:1) of the "solvent".

The gels for the measurements of light transmittance were prepared by performing the dialysis with the sodium alginate solutions contained in square cells ($0.2 \times 1.5 \times 4$ cm), covered with dialysis tubing at two ends (1.5×4 cm). The length of the light path was 0.2 cm. The light transmittance was determined at 436 mμ in a Brice-Phoenix light scattering photometer. The light transmittance was calculated as a percentage of the transmittance of the sodium alginate solutions before dialysis. No corrections were made for the small decrease in the length of the light path due to the shrinkage of the gels.

Selectivity coefficients were determined by an equilibrium dialysis technique as described previously.[15]

RESULTS

The influence of the molecular weight on the stiffness of calcium-alginate gels was investigated by performing experiments with samples of alginate ranging in weight-average degree of polymerization from 70 to 5100. The results, given as the modulus of stiffness of a 3 % alginate gel, $G_{C=3}$ (see Part I in this series [13]), are given in Fig. 1. It is seen that the stiffness is, within experimental error, independent of the degree of polymerization down to $\overline{DP}_w \approx 400$. Below this DP, a sharp decrease in stiffness occurs and it extrapolates to zero at $\overline{DP}_w = 65$. Experiments with samples of even lower molecular weights gave milky precipitates that could not be transferred to the testing machine without loss of their cylindrical shape.

In the previous paper, it was shown that a sample of alginate of $\overline{DP}_w = 3100$ gave a linear relationship between the stiffness and the square of the concentration. To see if this was the case for all degrees of polymerisation above $\overline{DP}_w = 400$, two more samples, one with $\overline{DP}_w = 440$ and one with $\overline{DP}_w = 5100$, were tested at different concentrations. The results, given in Fig. 2, show a linear relationship between the stiffness and the square of the concentration for all the three samples.

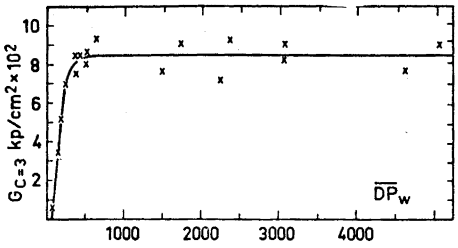

Fig. 1. Modulus of stiffness, $G_{C=3}$, as a function of the weight-average degree of polymerisation of calcium alginate. Alginate: *Laminaria digitata*, Tarva, August 29, 38.5 % guluronic-acid residues.

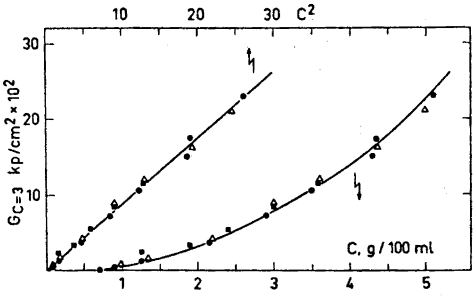

Fig. 2. Modulus of stiffness as a function of the concentration of calcium alginate in the gels. Alginate: As in Fig. 1. ■: $\overline{DP}_w = 440$; ●: $\overline{DP}_w = 3100$; △: $\overline{DP}_w = 5100$.

It should now be possible to compare alginate samples of different chemical composition by keeping the degree of polymerization of all samples well above $\overline{DP}_w = 400$ and by correcting for differences in concentration due to shrinkage of the gels as before.[13] Calcium gels from a number of different alginates with different proportion between the two monomers, all having $\overline{DP}_w$ between 1000 and 3000, were prepared, and the stiffness, light transmittance and

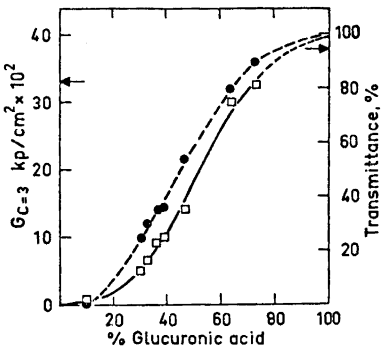

Fig. 3. Stiffness and light transmittance as a function of the chemical composition of calcium alginates. The arrows indicate values for calcium pectate. Alginates: *Fucus vesiculosus* receptacles (10 % guluronic-acid residues (G.A.)), *Ascophyllum nodosum* (37 % G.A.), *Laminaria digitata* (30–46 % G.A.), *Laminaria hyperborea* stipe (64 and 72 % G.A.). ●: Transmittance; □: Modulus of stiffness.

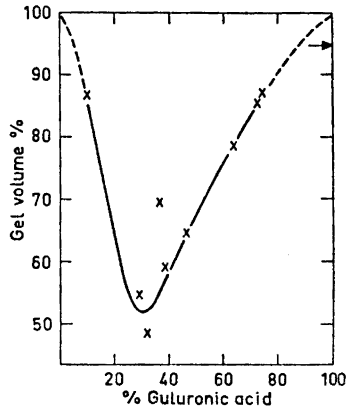

Fig. 4. Volume of gel relative to sodium alginate for different calcium polyuronides The polyuronates are the same as in Fig. 3.

volume of each were determined. The last two quantities were calculated as a percentage of those of the corresponding sample of sodium alginate. In Figs. 3 and 4, the results are given as a function of the fraction of L-guluronate residues (G.A.) in the alginates. Both the stiffness and the light transmittance is seen to correlate closely with the G.A. content. Very high turbidity and virtually no stiffness in the gel is characteristic for the poly-(D-mannuronate) whereas the extrapolated values for poly-(L-guluronate) indicate that this gel has a transparency close to that of sodium alginate, and a very high modulus of stiffness. The volume of the gel seems to extrapolate to 100 % for both the homopolymers and has a marked minimum for samples of intermediate composition (Fig. 4). One of the samples, containing 37 % G.A., is seen to give a higher volume than that corresponding to the drawn curve. This is the only sample in the series that was prepared from *Ascophyllum nodosum*, and it has been shown to contain a higher proportion of sequences of the alternating type [16] than samples from *Laminaria digitata* of the same G.A.-content.[17] It may be, therefore, that some properties of the gels are not solely dependent upon the average composition of the alginate, but also on the sequence of the two uronic acid residues in the chains. This point has not been further studied.

The most significant result in Figs. 3 and 4 is the profound difference between poly(D-mannuronate) and poly(L-guluronate) gels; the first is a voluminous slurry of aggregates, while the other is a very rigid, transparent gel. It must, therefore, be the L-guluronate residue that are responsible for gel-formation in alginates.

From ion-exchange experiments,[15,18] it is known that poly(L-guluronate) binds calcium more strongly than does poly(D-mannuronate). The affinity for calcium of the D-galacturonate residues in pectate has been shown [19] to be very similar to that of L-guluronate residues in alginates, and a sample of calcium pectate was included in the investigation of gel properties. The results are indicated by arrows in Figs. 3 and 4. The calcium pectate is seen to resemble poly(L-guluronate) in its gel-forming properties. The results suggest, therefore, that a correlation exists between both the light transmittance and the stiffness of the gels, and the strength of the bonds between the calcium ions and the polyuronates.

To test the general validity of this correlation, a comparison of the strengths of magnesium, calcium, strontium, and barium polyuronate gels was made. From potentiometric titration and ion-exchange experiments, it is known [18] that alginates containing both D-mannuronate and L-guluronate residues, have a sequence of affinity for these metal ions decreasing in the order $Ba > Sr > Ca \gg Mg$. For poly(D-mannuronate) the sequence is Ba > Sr, Ca, Mg. For pectate it is found [18] that in the exchange-reaction between calcium and magnesium, the affinity for calcium depends very much upon the ionic composition of the pectate gel. When ion-exchange experiments between pairs of magnesium and the other alkaline earth metal ions were performed to give equivalent amounts of the two ions in the gel at equilibrium, the sequence of affinity was found to be Ba, Sr, $Ca \gg Mg$.

Gels were prepared from one sample of pectate, and from two samples of alginate, one of an intermediate composition and one containing mainly D-

mannuronate residues, by dialysis against 0.34 M solutions of the four alkaline earth chlorides. The results of the measurements of stiffness are given in Fig. 5. The light transmittance was not measured, but it could be visually observed that the transparency and the rigidity of the gels in all cases varied in the same way. By comparing the result in Fig. 5 with the given affinity series, it

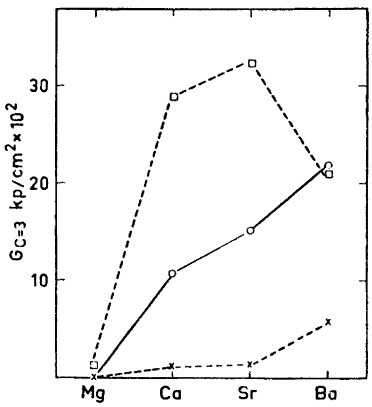

Fig. 5. Modulus of stiffness, $G_{c=3}$, for different alkaline earth polyuronide gels. □: Pectate; ○: Alginate from *L. digitata*, 38.5 % G.A.; ×: Alginate from *Fucus vesiculosus* receptacles (10 % G.A.).

is seen that the two samples of alginate give the same sequence of affinity and rigidity. The sample of pectate apparently does not follow the affinity series in that the barium pectate has a markedly lower stiffness than the calcium and strontium pectate gels. However, it may be that the affinity series, which is based on selectivity coefficients determined with 50 % of each metal ion in the gel is not representative of the differences in affinity when the pectate gel is fully converted into the barium form. Equilibrium dialysis experiments were therefore performed with a ratio between barium and strontium chloride of 100:1 in the solution. The resulting gel was found to have an equivalent ratio of Ba^{2+} to Sr^{2+} of 28:1, showing that a pectate gel almost fully in the barium form has a lower affinity for barium than for strontium ($K_{Sr}^{Ba} = 0.28$). The correlation between rigidity of the polyuronate gels and their affinity for alkaline-earth metal ions exists, therefore, in all cases.

Experiments were also performed in which the strength of the bonds between the divalent metal ions and the polyuronate was changed by changing the temperature and the "solvent" in the gel. Ion-exchange experiments have indicated that the affinity of polyguluronate for calcium ions decreases with increasing temperature.[18] Measurements on calcium alginate gels at increasing temperatures gave a decrease in stiffness, as shown in Table 1.

Table 1. Modulus of stiffness of a 3 % calcium alginate gel at different temperatures. Alginate: *Laminaria digitata*, $\overline{DP}_w = 3100$, 38.5 % guluronic acid residues.

Temperature, °C	7.5	20.0	29.0	72.0	79.0
$G_{c=3}$,[a] kp/cm^2 × 10^2	11.0	8.0	7.5	5.9	5.7

[a] All numbers are averages from at least five measurements.

The affinity of alginate for calcium ions in solvents other than water has not been studied. It would be expected, however, that a solvent like ethanol, which has a lower dielectric constant than water, should lead to an increase of the strength of the bonds between a polyanion and cations. Results from measurements of the stiffness and volume of calcium alginate gels that had been dialysed against large volumes of ethanol-water mixtures, are given in Table 2. The observed independence of the volume upon the composition of

Table 2. Modulus of stiffness and volume of calcium alginate gels dialyzed against ethanol-water mixtures. Alginate: As in Table 1.

% (v/v) ethanol	0	20	40	60	80	96
Volume, %	42.2	38.5	39.6	42.0	42.2	43.8
$G_{C=3}$, kp/cm^2 × 10^2	7.0	11.3	16.2	21.7	25.7	34

the solvent indicates that the average distance between the alginate segments is not dependent upon the concentration of ethanol in the gel. This suggests that the number of segments involved in molecular interaction has not increased. The observed increase in stiffness by the introduction of ethanol in the gel is therefore probably again connected with a corresponding increase in the strength of the calcium-polyuronide interaction.

The exchange of ethanol in the gels for other organic solvents gave gels of widely different modulus of stiffness. For instance, the "inert" solvent cyclohexane gave a markedly higher modulus than ethanol. These effects are not well understood and will be the subject of a special investigation in this laboratory.

DISCUSSION

Although the details of most biopolymeric gel structures are unknown, it is clear that the gel state requires the existence of strong forces between the individual molecular chains. The simplest possible model for a gel-network would therefore be a network of chains linked together with chemical bonds, "crosslinks". Vulcanized rubber swollen in, for example, benzene, is such a gel. Both the elasticity and the swelling behaviour of such gels are well explained by theoretical models.[20] It has been shown that the dominating factor in determining their elastic properties is the entropic change suffered by the segments of the chains lying in between the crosslinks that is associated with positional changes of the crosslinks.

In polyuronate gels there is no possibility for covalent cross-linking, but at the outset we assume that the structure of these gels is such that it can be divided, as above, into regions with intermolecular contacts and regions of free chains between them. In harmony with recent theoretical work of Ziabicki and Takserman-Krozer [21] we use the term "junction" for points or zones of contact between the chains. Ziabicki and Takserman-Krozer's definition of a junction "as a contact between two (or more) macromolecules, with the mobility of one macromolecule with respect to another decreased due to inter-

action" is so wide that it covers a number of possibilities from chemical crosslinks (permanent junctions) to contacts caused by a rapid macromolecular association-dissociation equilibrium (temporary junctions).

One important question in the discussion of the properties of gels is whether they represent a state in thermodynamic equilibrium. Both Lipatov [3] and Rees [4] emphasise that many biological gels are not "equilibrium gels", in contrast to the gels of the rubber-benzene-type. It seems clear that this also holds for the present polyuronate gels. All the present gels have been prepared by extensive dialysis of the sodium polyuronate solutions against 0.34 M alkaline-earth chloride solutions. If the same amount of salt is added under stirring, a precipitate is formed.[22] In the preceding paper [13] it was shown that the gels shrank when left in calcium chloride solutions for long periods of time. Both these results indicate that the equilibrium position is nearer to a swollen precipitate than to a gel. However, the rate of syneresis was very low [13] compared to the standard rate of compression. For this reason most of the results may be discussed as if the state of the gels when tested represented an equilibrium condition. The question as to how the gels are able to stay for long periods of time in a metastable state is considered very important, but will not be discussed in much detail in this paper.

Takserman-Krozer and Ziabici [23] have shown theoretically that the dynamic behaviour of macromolecular networks is different, depending upon the type of junction. No general equations relating the mechanical properties of a gel to the number and properties of different types of junction have yet been presented. Here we shall assume that the modulus of stiffness is proportional to the number of junctions in the gel. The proportionality between the stiffness and the square of the concentration (Fig. 2) suggest, then, that the junctions are formed by a bimolecular reaction. The same behaviour has been found for gelatin gels.[24]

Experiments of the type shown in Fig. 1 have been used in the study of vulcanized rubber as a way of relating the stiffness to the number of permanent junctions per macromolecule. Linear macromolecules may form an infinite network when the number of chemical crosslinks exceeds the number of macromolecules in the network by one (every macromolecule is then linked to two other macromolecules). It has been shown,[25] however, that measurable stiffness does not occur in rubber before the number of crosslinks is about 3 times higher than this minimum value. A very steep increase in stiffness was found when this number was increased from about 3 to 10 – 15. A further increase in the number of crosslinks per molecule had only a small effect upon the stiffness.

When experiments were performed to give a constant concentration of crosslinks, regardless of the molecular weight of the macromolecule (as was the case in the experiment cited above), a molecular-weight dependence similar to that found in Fig. 1 was obtained. If the number of junctions in alginate gels is dependent upon the segment concentration rather than on the number of macromolecules (as has already been suggested) the steep increase in stiffness between $\overline{DP}_w = 65$ and 400 may be explained as an effect of an increasing number of junctions per molecule. The lack of further increase in stiffness

above $\overline{DP}_w = 400$ suggests that the number of junctions per molecule now becomes so high that each macromolecule looses its individuality in the network.

The fact that a degree of polymerization as low as 65 does give a gel suggests that the distance between the crosslinks must be small. A calculation of the exact distance is not possible because of the lack of a theoretical model for the gelation process. It seems safe to conclude, however, that the average distance must be well below that corresponding to a degree of polymerization of 65. It is known that the Kuhn statistical segment-length of the alginate sample used in this experiment is as high as 155 Å at infinite ionic strength, corresponding to approximately 30 monomer units.[10] The part of the alginate chain in between the junctions must, therefore, be very stiff and restricted in its movement.

The sequence of the two monomers in the alginate molecule should also be taken into account. The molecule consists of three types of blocks, homopolymeric blocks of guluronic acid, homopolymeric blocks of mannuronic acid, and blocks with a predominantly alternating structure.[16,17] Assuming the homopolymeric blocks to behave as the polyuronates, it would be expected that the blocks of mannuronic-acid residues are not involved in junctions contributing to the strength of the gels. To what extent the blocks of alternating sequence contribute to the gel-strength cannot be decided, as polymers containing mainly this type of sequence have not been investigated. However, the close correlation between the modulus of stiffness and the guluronic-acid content (Fig. 3) obtained with samples containing varying proportions of guluronic-acid residues in alternating sequences, might suggest that both types of guluronic acid-containing blocks take part in the formation of junctions.

The good correlation found in the preceding paragraph between the modulus of stiffness and the affinity of the polyuronide for the divalent metal ion indicate that these ions contribute to the energy-interactions in the junctions. The correlation might suggest that bonds between the ions and the polyuronides are broken when the moduli of stiffness are determined, that is, in the initial phase of the compressing period.[13] This would lead to a correlation between the work needed for compressing the gels and the strength of the bonds between the divalent metal ions and the polyuronates. A similar correlation would, however, also be observed if the *number* of junctions in the gel increased with increasing affinity between the divalent metal ions and the polyuronates. A deeper understanding of the polyuronide gels requires, therefore, more knowledge of the mechanism with which the polyuronides bind divalent ions. The type of forces involved in the junctions and the number of metal ions in each junction are critically important factors to be known.

Even if the results do not allow us to conclude whether bonds between metal ions and polyuronate molecules are broken when the moduli are determined, it can be concluded that a rapid formation and rupture of junctions when no forces are acting upon the gels, does not take place. If this were the case, one would expect phase separation, and formation of the thermodynamically more favourable precipitate. In particular, the exchange of water in the gel with a "non-solvent"-like ethanol (Table 2) would be expected to

result in a contraction in volume, or precipitation. The rupture of junctions must therefore be a slow process, probably with high activation energy. Important in this connection is the fact that, in the cases in which the ion-polyuronide linkages are weak (in Ca-polymannuronate, Fig. 3, and in the Mg-polyuronates, Fig. 4) the systems do not form transparent gels, but voluminous milky precipitates. It seems, therefore, that strong ion-polyuronide bonds are a fundamental condition for gelation, not only because it causes phase separation to be energetically favourable, but because it gives the junctions a high activation energy for rupture, and thereby prevents the rearrangements of bonds which would lead to precipitation. A study of the kinetics of the exchange of ions in the network with the solution may yield a better understanding of this effect.

The measurements of optical transparency in the gels do not give any quantitative information about the gels structure, but such experiments have been considered to give a relative measure of inhomogeneity in gelatin gels.[25] The larger the inhomogeneity, the more light is scattered. The most rigid polyuronate gels are almost as transparent as the sodium alginate solutions, while the soft gels are very turbid. This should indicate that long sequences of several chains linked together is a characteristic feature of precipitates and soft gels, and that the formation of rigid gels is associated with a hindrance of the formation of large, crystalline regions.

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