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

I. Evaluation of a Method for the Determination of Stiffness

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Solutions of sodium alginate contained in small cylinders covered with a cellophane membrane at both ends, were dialysed against aqueous calcium chloride until cylindrical pellets of gel of reproducible size and shape were formed. The stress-strain behaviour of the pellets was studied by compression at a constant rate in an Instron TTK Universal Testing Machine. After the gels had been aged for at least two days, the initial slope in the stress-strain diagram could be determined with good accuracy. It varied considerably with the rate of compression, but at a rate of 0.2 cm/min, it corresponded very closely to the modulus of stiffness G, of viscoelastic gels. Data for gels of different concentration and size could be compared, since the slope was proportional to the square of the concentration of alginate, and to the cross-sectional area of the cylinders of gel, and inversely proportional to the initial length of the gels.

The behaviour of gels in which polymeric chains are cross-linked by primary bonds, *e.g.* rubber-benzene gels, is quite well understood in the sense that theories have been developed which predict many of their known physical properties.[1] On the other hand, gels in which the "crosslinks" are due to secondary bonds are understood only to a very limited extent. There are many reasons for this. First, the chemical structure of many gel-forming polymers is not completely known; the gross chemical composition is often known, but the sequence of the monomers or substituents in heteropolymers is seldom known in detail. Second, the rather complex nature of the polymer, which is usually a polysaccharide or a protein, and of the solvent, which is usually water or an aqueous salt solution, makes the expected type of physical interaction more difficult to treat theoretically than those of the rubber-benzene systems. A considerable amount of experimental work has been carried out on gels of this kind (for recent review articles see Schachat *et al.*,[2] Solms,[3] Lipatov *et al.*[4] and Rees [5]), but still the intermacromolecular bonds in

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the gels are described only in vague terms such as "ordered zones"[4] or "micro-crystallites".[5]

Since "gel" is the term used for substances with properties intermediate between those of fluids and solids, these properties are so diverse that the mere problem of characterizing them is great. Typical is the fact that each laboratory and each industry develops measuring techniques that serve the purpose of characterizing the particular kind of gel in which it is interested, while the same method may be quite useless for studying other types of gel. Many gels are not "equilibrium gels"[4] and their nature will therefore often depend upon the method of preparation. This adds to the problem of charac-terizing and comparing different types of gel.

The gels studied here are those formed by introducing calcium ions into solutions of soluble salts of polyhexuronic acids. The main aim of this work has been to obtain reproducible data which could characterize the stiffness of the gels. To do this, it was necessary to test both the methods of preparation of the gel samples and the measuring techniques in some detail.

EXPERIMENTAL

The sample of sodium alginate was prepared [6] from *Laminaria digitata* harvested at Tarva August 29. The ratio [6] between D-mannuronic acid and L-guluronic acid residues was 1.6. The weight-average molecular weight, as determined from viscosity measure-ments, was 620 000.

The gels were prepared by dialysing for two days solutions of sodium alginate, contained in small cylinders [diameter $(D) = 14$ mm and length $(L) = 15$ mm unless otherwise stated] covered with sheets of cellophane dialysis casing at both ends, against large amounts $(4 \times 50$ ml) of 0.34 M aqueous solutions of $CaCl_2$. The gels formed had in most cases a smaller volume than the cylinder and did not have an exact cylindrical shape. The side walls of the gels had a concave curvature and the circular ends were not in all cases parallel. The diameter and the length to be used in the calculation of the modulus of stiffness were measured where these distances had their minimum value. The gels were weighed in order to calculate the amount of shrinkage and the concentration of alginate.

The mechanical properties of the gels were determined by compressing the gels at a constant rate of deformation in an Instron TTK Universal Testing Machine. The gels were kept in the dialysis medium until immediately before testing. The testing temperature was $20 \pm 1°C$. To avoid evaporation of water from the gels in the cases in which the testing time was long (at rates of compression lower than 0.01 cm/min) the gels were compressed while submerged in 0.34 M $CaCl_2$ kept at $20 \pm 0.1°C$ by a thermostat. Compression in air and aqueous $CaCl_2$ gave stress-strain diagrams which were not significantly different. The load and the distance of deformation were recorded with high accuracy down to loads of a few grams and deformations of less than one tenth of a millimeter.

RESULTS

Appearance of the gels. It is known from the work of Thiele *et al.*[7-9] and of Baardseth [10] that capillaries are formed in the gels when salts of divalent metal ions are allowed to diffuse into solutions of sodium alginate. The number and size of the capillaries depend both on the type of divalent metal ions [9] and on the type of alginate.[10] It therefore had to be decided whether gels prepared by dialysis were suitable for the determination of stiffness.

In some introductory experiments, the gels were cross-sectioned and studied under the microscope. Capillaries running parallel to the direction of diffusion were found in the centre of the cross-section. The rest of the cross-section, which comprised approximately 90 % of the total area, appeared to be homogeneous. Since no conclusions were drawn from differences in measured properties of the order of 10 % or less, the gels were assumed to be homogeneous in all measurements and calculations. It should also be pointed out that the satisfactory reproducibility of the determinations of gel strength, and the fact that variation of the length and cross-sectional area of the cylinders of gel does not influence their modulus of stiffness (see below), strongly supports the assumption that the presence of the capillaries does not significantly influence the measurements.

Determination of the elastic properties of the gels. Several cylindrical gels were prepared by dialysing 2 % aqueous sodium alginate for two days against aqueous calcium chloride. Their stress-strain diagrams were determined in the testing machine at different, constant rates of compression. A typical stress-strain diagram for a rate of compression of 0.2 cm/min is shown in Fig.1, Curve 2.
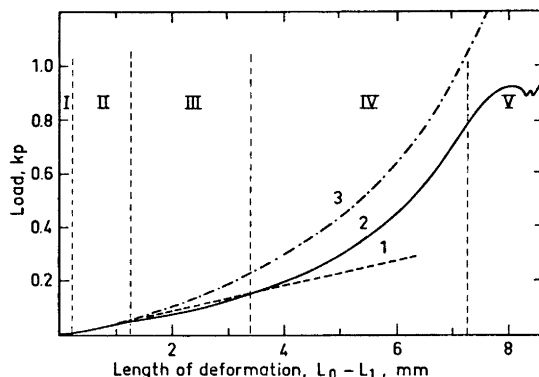

Fig. 1. Compression of a cylindrical alginate gel. Initial lenght, $L_0 = 1.3$ cm. Cross-sectional area, $A = 0.622$ cm². Rate of deformation 0.2 cm/min. Curve 1. Initial tangent. Curve 2. Experimental curve. Curve 3. Theoretical curve based upon the initial tangent and eqn. (1) in the text.

Curve 1 in the figure is the initial tangent and represents the behaviour of an elastic spring obeying Hooke's law. Curve 3 is calculated from the kinetic theory of rubber elasticity.[11-14] This theory considers the entropic work to be overcome by affine deformation of the chains, held together in a network by randomly spaced permanent "crosslinks", when the configuration of the part of the chains in between the crosslinks is given by the Gaussian distribution function.

The relevant equation for compression of such a network filled with an incompressible fluid is [15]

$$S = J\ T[(1/\gamma^2) - \gamma] \tag{1}$$

where S is the applied force per unit area, J is a constant, proportional to the concentration of crosslinks in the network, T is the absolute temperature, and γ is the relative length of the gel, equal to L/L_0 where L is the length at a certain applied force and L_0 is the initial length.

The experimental curve is not well represented by either of the two theoretical curves. The deviation is such that the diagram may be divided into 5 different zones as indicated in Fig. 1. Some comments on the physical significance of the zones will be given below.

The experimental curve in zone I has a slope which is lower than that in zone II. This "lag" in the curve was present when the circular ends of the gels were not parallel. In some badly-shaped gels the lag period existed up to 1 mm of deformation. It could be removed either by trimming the ends of the gel with a razor-blade or by using a device that allowed compression between two plates that were not parallel to each other, but were parallel to each end of the gel.

The curve in zone II is a straight line. Its slope can be determined with a good accuracy and seems well suited for characterizing the strength of the gels.

In zone III the curve is lying below the initial tangent. This negative deviation from Hooke's law was found to depend very much upon the rate of compression. At high rates of compression (0.5 cm/min) the effect was not observed. At rates of compression lower than 0.05 cm/min, the curve was lying below the initial tangent for most of the compression period. This effect is most probably associated with the observed release of water from the cylindrical wall of the gels during compression. Since the water has to travel some distance before reaching the surface of the cylinders of gel, the effect could be expected to be kinetically controlled, and should depend very much upon the rate of compression, as observed. It should be very small in the first part of the compression period, and the absence of the effect in zone 1 may therefore be understood. The effect should lead to a release of pressure if the gels were kept for a period of time at a constant length of deformation. Such behaviour was indeed observed in several experiments.

In zone IV, the experimental curve is lying between the initial tangent and the curve calculated from eqn. (1), and has a curvature very similar to that of the latter. This curvature was found to depend upon the rate of compression; the lower the rate of compression, the less pronounced was the increase in slope with the length of compression.

Zone V is separated from zone IV by a point of inflection in the experimental curve. In this zone, the load reaches its maximum value (~ 0.9 kp) after which the shape of the curve is irregular. This behaviour is indicative of breaking of the gel. The point of inflection and the maximum were not very reproducible. In some cases, especially at low rates of deformation, the gels could be compressed to less than one tenth of the original length without breaking. The point of inflection was observed only in the cases in which breaking of the gels occurred. Owing to this lack of reproducibility it seems that the load at the breaking point is not a good characteristic of the strength of calcium alginate gels prepared as here described.

Initial slope as a measure of the modulus of stiffness. A series of gels, prepared in the same way as before, were tested at several different rates of deformation between 0.001 and 0.5 cm/min. The signals from the testing machine were expanded in order to study only the first 0.5 mm of deformation. The initial slopes at different rates of deformation are given in Fig. 2. At rates of deformation higher than 0.1 cm/min the curve is linear and may be represented by the equation for the viscoelastic element of Voight:[15]

$$S/\gamma = G + \eta/\gamma \ (d\gamma/dt) \tag{2}$$

where G is the stiffness (modulus), η is the viscosity and $d\gamma/dt$ is the rate of deformation of the element. The straight line resulting from this equation is represented by the broken line in Fig. 2. For deformation of the stiffness of such an element, a straight-lined extrapolation to zero rate of compression is necessary. However, this procedure is very time-consuming, and it was decided to use a rate of deformation of 0.2 cm/min as standard in testing. The initial slope at this condition exceeded the extrapolated value by only some 6 % and may therefore be used with negligible error for calculating the moduli.

In Fig. 2 it is seen that the deviation from eqn. (2) becomes very marked at low rates of deformation. This is probably due to the reduction in volume caused by the loss of water from the gel during compression. The curve seems
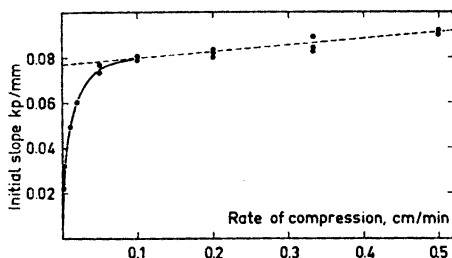

Fig. 2. Initial slope of the stress-strain diagrams as a function of the rate of compression of the gels. The size of the gels was approximately (± 5 %) the same as the gel used in Fig. 1.
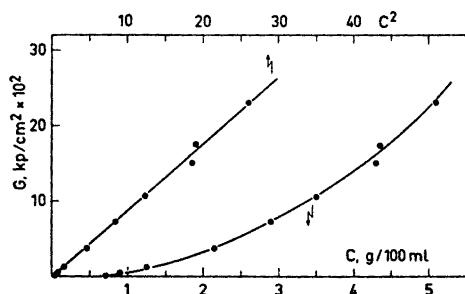
Fig. 3. Modulus of stiffness as a function of the alginate concentration to the first and the second power. Size of the gels as in Figs. 1 and 2.

to extrapolate to the origin. If a gel exhibited a tendency for syneresis, one would expect zero slope in the stress-strain diagram when the rate of compression equals the natural rate of contraction of the gels. It will be shown later that the calcium alginate gels showed syneresis.

Effect of the alginate concentration on the stiffness. Nine calcium alginate gels were prepared by dialysing alginate solutions ranging in concentration from 0.5 to 4 %. The concentration of alginate in the gels ranged from 0.7 to 5.1 %. From the initial slopes in the stress-strain diagrams the moduli were calculated. They are given, as a function of the alginate concentration, in

Fig. 3. A linear relation between the stiffness and the square of the concentration is seen in the figure. One may therefore compare the stiffness of gels of different concentrations by multiplying the modulus by the square of the ratio between a reference concentration and the concentration in the gel. In all the experiments reported below this method is used to compare the results, using 3 % (w/w) of alginate as the reference concentration.

Effect of the size of the gels on the stiffness. It may readily be shown that the slope in the stress-strain diagram, dp/dL, where p is the load and A cross-sectional area, is given by eqn. (3).

$$\frac{dp}{dL} = \frac{dS}{d\gamma}\frac{A}{L_0} = G\frac{A}{L_0} \tag{3}$$

In the calculation of the moduli in the preceding paragraph, the implicit assumption was made that this equation holds regardless the size of the gels. To test this point, a series of gels was prepared by performing the dialysis with cylinders of different size. The results are shown in Table 1.

Table 1. Elastic properties of cylindrical gels of different dimensions.

Length of cylinder	30	25	20	15	10	5	20	15
Diameter of cylinder	14	14	14	14	14	14	10	62
Initial length, L_0, mm	28.3	23	18.5	13.9	7.9	3.9	18.2	12.2
Initial diameter, D_0, mm	10.1	10.3	10.2	10.2	10.2	10.4	7.6	51
Cross-sectional area, A, cm²	0.800	0.832	0.818	0.818	0.818	0.850	0.578	20.5
Alginate concentration, c %	3.23	3.35	3.21	3.12	4.00	4.81	3.46	3.50
Initial slope, dp/dL g/mm	24.5	33	40	49	167	375	27	1900
Modulus, $G_{c=3} \times 10^2$, kp/cm²	8.7	8.2	9.16	8.5	8.2	8.1	7.8	8.2

It is seen that the stiffness at an alginate concentration of 3 % ($G_{c=3}$) does not vary systematically with either the length or the diameter of the gels. The stiffness is therefore an apparatus-independent property, and one may choose any size of gel as a standard condition for testing. After weighing two conflicting requirements against each other — that the size should be as small as possible to minimize the diffusion time and the amount of alginate required, and that the size should be as large as possible to minimize errors in the measuring of length and diameter — a cylinder with length 15 mm and diameter of 14 mm was chosen as the standard size in all measurements.

Effect of aging. So far all the results have been obtained on gels that have been dialysed for two days before measurements. It was considered important to find out if this time was long enough to give a good characteristic of calcium alginate gels. A series of gels was prepared from 2 % sodium alginate by dialysing for different periods of time between one and fourteen days. The initial slopes in the stress-strain diagrams, the volume of the gels (in per cent of the volume of the sodium alginate before dialysis) and $G_{c=3}$ are given in Fig. 4. It is seen that most of the decrease in volume (30 %) occurs as a result of the introduction of calcium ions during the first day of dialysis. In the next 13 days only a 10 % reduction in volume occurs. The rigidity of the gels, as
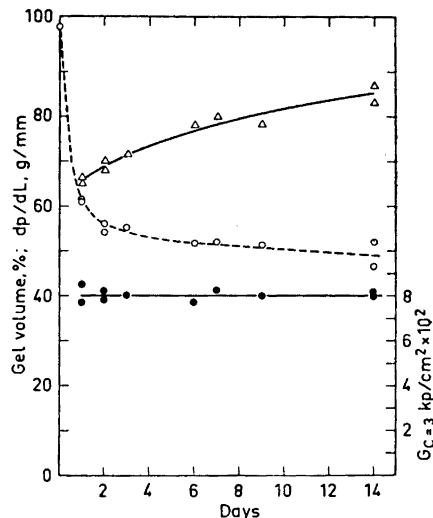
Fig. 4. Effect of aging on the volume and the rigidity of the gels. O: Gel volume; △: Initial tangent, dp/dL; ●: Modulus of a 3 % gel, $G_{C=3}$.

measured by the initial slopes, increases slightly in the whole time period. However, when correction for the increase in concentration is made, the modulus was found to be independent of time. It seems, therefore, that a period of dialysis of two days gives gels that may be regarded as in a state of "practical equilibrium", *i.e.* that, after this time, some variation in the period of dialysis (which will occur in practice) will not affect the reproducibility of the results.

Accuracy of the measurements of stiffness. Twenty-five gels were prepared and measured under standard conditions. The calculation of $G_{C=3}$ yielded the result 8.02×10^{-2} kp/cm^2 with a standard deviation, σ, of 0.565×10^{-2}, or 7.%

CONCLUSIONS

The method developed for measuring stiffness (modulus) of calcium alginate gels is well suited for routine measurements. The accuracy is only fairly good, but this is compensated by the speed with which the measurements may be carried out. One may test 10 – 15 gels per hour, and it is easy, therefore, to run several parallel experiments if high accuracy is required. None of the experimental parameters is critically affected by changes in conditions. This makes it possible to choose the detailed experimental conditions rather freely and facilitates simple experimentation. The calcium alginate gels are visco-elastic, and the determination of the modulus does not constitute a full description of their mechanical properties. Certainly, from a practical point of view, the determination of the modulus by no means gives a satisfactory description of all the properties of the gels that are of interest. However, the modulus is a quantity that is well established both practically and theoretically, and it is well suited for correlating the mechanical properties to the chemistry of different gel systems. The method will be used in following papers.

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