1028. *Counter-ion Fixation in Alginates.*

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Donnan equilibria between alginate sols or highly swollen gels and uni- or bi-valent counter-ions were measured and from the results the fractions, $\phi_{p'}$ of the free counter-ions and the mean activity coefficients of the free ions in the sols or gels were calculated. For comparison counter-ion fixation was also studied by an osmotic method. Both series of measurements showed that the ϕ_p values of the bivalent or complexing counter-ions are relatively small. The relevance for previously described mechanochemical effects is discussed.

It is known that some negatively charged macromolecules are capable of binding simple cations (see refs. 1—3 and earlier literature cited there). In order to increase the

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[1] Mock and Marshall, *J. Polymer Sci.*, 1954, **13**, 263; Kagawa and Katsuura, *ibid.*, 1955, **17**, 365; Nagasawa and Kagawa, *ibid.*, 1957, **25**, 61; Nagasawa, Izumi, and Kagawa, *ibid.*, 1959, **37**, 375; **38**, 213.

[2] Alexandrowicz, *J. Polymer Sci.*, 1960, **43**, 325, 337.

[3] Katchalsky, Alexandrowicz, and Kedem, " Polyelectrolyte Solutions," Interscience Publ. Inc., New York, in preparation.

knowledge of these effects the cation fixation by alginates * has now been investigated. This work is also of interest for an understanding of the mechanochemistry of swollen cylindrical alginate gels,[5] and of molecular size and shape changes accompanying the conversion of sodium or potassium into magnesium alginate.[6]

This paper deals with alginic acid gels and with alginates fully neutralised by one type of counter-ions, no attempt being made to deal with systems containing different cations. The binding of sodium, potassium, ammonium, hydrogen, silver, ethylenediammonium, magnesium, calcium, and cupric ions has been deduced from a study of Donnan and osmotic equilibria. The former technique is applicable to sols and gels and enables, moreover, a computation to be made of the activity coefficients of the permeant electrolyte in presence of the alginates. Attempts to deduce counterion fixation from Donnan equilibria have been made before (see, *e.g.*, ref. 7), but the present method of calculation has not been used hitherto.

Theoretical.—Recent investigations [2] have shown that the osmotic pressure, π, of a solution containing a uni-univalent salt and a polyelectrolyte can be expressed by $\pi = \pi_p + \pi_s$, where π_p is the osmotic pressure of the polyelectrolyte solution without the salt and π_s is the osmotic pressure of the salt solution free from macromolecules; π_p is given by $(\boldsymbol{R}T/1000)(m_p + m_p\nu\phi_p)$, where m_p is the molarity of the polyelectrolyte, ν is the number of charges per macromolecule, and ϕ_p is the osmotically active fraction of the counter-ions. In most systems here considered ν equals P, the total number of carboxylate groups in the fully neutralised alginic acid, so that $m_p\nu$ equals m_pP or m_m, the last symbol indicating the concentrations of repeating unit in moles per litre. The product $\nu\phi_p$ can be taken as the effective charge on the macromolecule and $m_p\nu\phi_p$, which equals $m_m\phi_p$, is the concentration of " free " counter-ions. Experiment [1,2] and theory [3] show that ϕ_p is determined by the charge density on the polyelectrolyte rather than by its concentration or the ionic strength of the solution, and it appears that each macromolecule contributes ϕ_p free counter-ions whatever the contribution of permeant electrolytes in the solution.

In this situation one is justified [2,3] in expressing the activity of a uni-univalent salt in a polyelectrolyte solution by

$$a_s = (m_p\nu\phi_p + m_s)m_sf^2, \qquad (1)$$

where m_s is the concentration of the uni-univalent salt and f is the mean activity coefficient of the " free " ions. As usual the activity is the product of the activities of counter- and co-ions, each activity being obtained by multiplying the concentration of the " free " ions with an activity coefficient, probably similar to that of the salt without the polyelectrolyte. If a Donnan equilibrium is set up, the equality of activities is represented by

$$(m_p\nu\phi_p + m_s)m_sf^2 = (m_s')^2f'^2. \qquad (2)$$

Here and below primed symbols relate to permeant electrolytes free from macromolecules. In equations (2), (3), (4), and (8) a factor depending on solvent activities [8] has been omitted because it is close to unity in the systems here considered.

If an alginate anion with P carboxylate groups is fully neutralised by a bivalent counter-ion X^{2+}, the resulting species, $Alg^{P-}X_{P/2}{}^{2+}$ may be in equilibrium with a permeant

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* The repeating units of alginic acid are D-mannuronic and L-guluronic acid.[4] This polyelectrolyte is either a mixture of poly-D-mannuronic and poly-L-guluronic acid or a copolymer; in the latter case the copolymer composition may depend on the degree of polymerisation.

[4] Fischer and Doerfel, *Z. physiol. Chem.*, 1955, **302**, 186.

[5] Mongar and Wassermann, *J.*, 1952, 492, 500, 510; Harkness and Wassermann, *ibid.*, p. 497; MacArthur, Mongar, and Wassermann, *Nature*, 1949, **164**, 110.

[6] Buchner, Cooper, and Wassermann, *J.*, 1961, 3974.

[7] Strauss and Anders, *J. Amer. Chem. Soc.*, 1958, **80**, 6494.

[8] Donnan and Guggenheim, *Z. phys. Chem.*, 1932, *A*, **162**, 346.

bi-univalent salt, $X^{2+}Y_2^-$ or with a bi-bivalent salt, $X^{2+}Y^{2-}$. Considerations similar to those leading to equation (2) show that the Donnan equilibrium conditions are

$$(m_s + m_m\phi_p/2)4m_s^2f^3 = 4(m_s')^3f'^3, \tag{3}$$

$$(m_s + m_m\phi_p/2)m_sf^2 = (m_s')^2f'^2. \tag{4}$$

On rearrangement of expressions (2), (3), and (4) and replacement of νm_p by m_m one obtains (5), (6), and (7) which apply to uni-univalent, bi-univalent, and bi-bivalent salts, respectively.

$$m_s = [(m_s')^2/m_s][(f')^2/f^2] - \phi_p m_m, \tag{5}$$

$$m_s = [(m_s')^3/m_s^2][(f')^3/f^3] - \phi_s m_m/2, \tag{6}$$

$$m_s = [(m')^2/m_s][(f')^2/f^2] - \phi_p m_m/2. \tag{7}$$

So far it has been assumed that covalent-bond formation plays no significant rôle in the counter-ion fixation. In systems where this assumption is not justified, an equilibrium constant, K, has to be introduced. To show this we consider the equilibrium between alginic and hydrochloric acid and designate chloride-ion concentrations by m_{Cl} and m_{Cl}'. Equality of activities of hydrochloric acid solutions with and without alginic acid requires that

$$(\alpha\phi_p m_m + m_{Cl})m_{Cl}f^2 = (m_{Cl}')^2(f')^2, \tag{8}$$

where α, the degree of ionisation of alginic acid, equals $\nu m_p/m_m$. The mass-action law requires that

$$\alpha \times a_{H^+}/(1 - \alpha) = K, \tag{9}$$

a_{H^+} being the activity of the hydrogen ions, which may be identified with the first terms, $\alpha\phi_p m_m$ and m_{Cl}, in expression (8). In presence of hydrochloric acid, $\alpha \ll 1$, so that

$$\alpha(\alpha\phi_p m_m + m_{Cl}) = K. \tag{10}$$

Dividing (10) by (8) and introducing the resulting expression for α in (10) lead to

$$(m_{Cl}')^2 = [(m_{Cl}')^4/m_{Cl}^2](f'^2/f^2) - K\phi_p m_m(f^2/f'^2). \tag{11}$$

If, therefore, the ratios of the activity coefficients, the ϕ_p values, and the product $K\phi_p$ in expressions (5), (6), (7), and (11) remain effectively constant, their numerical values can be computed from the slopes and intercepts of the straight line obtained by plotting m_s against $(m_s')^2/m_s$ and $(m_s')^3/m_s^2$, or $m_{Cl}'^2$ against $(m_{Cl}')^4/m_{Cl}^2$. If it is possible to determine K by an independent method, ϕ_p in expression (11) can also be estimated. Alternatively one can make the reasonable assumption that ϕ_p approaches unity if $\alpha \ll 1$; if this is accepted, equation (11) enables one to estimate K from a study of Donnan equilibria.

EXPERIMENTAL

Two commercial sodium alginate samples (A) and (B) were used. The weight-average molecular weights (in 0·100M-sodium chloride at 22—24°) were respectively $(1{\cdot}5 \pm 0{\cdot}3) \times 10^5$ and $(5{\cdot}3 \pm 1{\cdot}3) \times 10^4$. Alginic acid and potassium, magnesium, calcium, copper, and silver alginate were prepared from sample (A) by techniques described previously.[5,6] Ammonium and ethylenediammonium alginate were made from sample (B), which was first converted into alginic acid by treatment with N-hydrochloric acid; after being washed with water, the fully swollen alginic acid gel was neutralised with ammonia or ethylenediamine, the pH being controlled with the help of a glass electrode and a Cambridge Instrument Co. pH meter. All alginates were freed from permeant electrolytes by dialysis against water or 1 : 1 water–acetone, or by washing of the fully swollen gels with water. The complete removal of permeant electrolyte from sols used for osmotic tests was checked by electrical-conductance measurements. All alginates were analysed by methods already described [5,6] and it was found that the ratio, counter-ion : carboxylate (equiv.), was 1·00 ± 0·03. No attempt was made to estimate the relative quantities of D-mannuronic and L-guluronic acid residues; a few measurements of optical rotation and rotary dispersion indicated that these ion-exchange reactions did not influence the stereochemical composition of the polyanion. (Earlier work [9] had shown that the specific optical rotation of sodium alginate is not significantly dependent on the molecular weight.) For a determination of the concentration m_m in the fully swollen gels, their specific gravity had to be determined.

Donnan Equilibria.—Alginate sols were filled in Cellophane bags, which were placed in glass-stoppered tubes containing solutions of the permeant electrolytes. The tubes were shaken in a thermostat bath until equilibrium was reached. Tests were carried out in which the initial solution in the Cellophane bag contained the alginate together with the permeant electrolyte, while the initial liquid outside was water. It was thus established that the same equilibrium was reached from both sides. Control experiments were carried out in which the bags, without the sols, were shaken with water or with a solution of the permeant electrolytes. In some of the tests the bags had been washed with a solution of EDTA sodium salt. The fully swollen fibrous gels (fibre diameter 0·1—0·4 mm.) were stirred or shaken with the solutions of the permeant electrolyte until equilibrium was reached. The equilibrated solution was separated from the gel by low-speed centrifugation.[5] The centrifuged gels were extracted with water until all permeant electrolytes had been removed. Control experiments showed that the composition of the gels was unaffected by centrifugation. The concentrations of the permeant electrolytes were determined by standard analytical methods, with an accuracy of 0·2—0·3%. Alginate concentrations in sols or gels were measured [5,6] with an accuracy of ±5%; in most cases there was no significant change during establishment of the Donnan equilibrium. The experiments with sols were done at 25·0° and those with gels at 22—24°; separate tests showed that between 20° and 25° the equilibrium positions were not detectably altered. The results in the last four lines of Table 1 were calculated from data obtained by Mongar.[10]

Osmotic-concentration Method.—This has been described by Alexandrowicz,[11] whose technique was used without essential modifications. Two samples of polyethylene glycols (supplied severally by Petrochemicals and by Croda Ltd.) of weight-average molecular weight, M_w, 4800 ± 200 (sample I) and 7400 ± 300 (sample II) were used to afford the solutions of known activity. The light-scattering equipment used for the standardisation has been described.[12] Purification was by high-speed centrifugation followed by filtration through sintered glass (porosity No. 5). The concentration dependence of the osmotic coefficient, ϕ, of the two polyethylene glycol samples was similar to that found by Alexandrowicz,[11] the ϕ values of sample (II) being larger. If, for instance, the glycol concentration was 1·00 g. per 100 ml. of solution, ϕ values of, respectively, 1·12 and 1·33 were observed. Tests were carried out in which the sodium ion fixation was determined with both standards: there was no significant difference. "Viskin" regenerated-cellulose membranes, which had been treated at 100° with a dilute solution of EDTA sodium salt, were used. To hasten attainment of equilibrium, the osmometers were shaken. In a typical experiment, a solution of sodium alginate (sample A) (0·133 g. per 100 ml.) was equilibrated against a solution of polyethylene glycol [sample (II)]; after 23, 30, and 46 hr. the interpolated glycol concentrations, corresponding to no solvent transport, were 1·00, 1·01, and 1·00 g. per 100 ml. of solution.

RESULTS

In studying the Donnan equilibria, experiments were carried out in which the concentration of the polyelectrolytes or of the permeant electrolyte was kept constant, the variables being respectively m_s, or m_{Cl} and m_m. Plots of m_s or $(m_{Cl}')^2$ against $(m_s')^2/m_s$, $(m_s')^3/m_s^2$, or $(m_{Cl}')^4/m_{Cl}^2$ were straight lines, within the limits of the experimental errors, as shown for typical cases in the Figures. Numerical values of the ratios * of the activity coefficients, ϕ_p, and ϕ_pK (see equations 5, 6, 7, and 11) were then calculated and are given in Table 1. The K value

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* Those in columns 7 and 8 of Table 1 relate respectively to equations 5—7 and equation 11.

[9] Rose, Ph.D. Thesis, London, 1937, Table H; Vincent, Goring, and Young, *J. Appl. Chem.*, 1955, **5**, 374.

[10] Mongar, Ph.D. Thesis, London, 1951, Table 14 and unpublished results.

[11] Alexandrowicz, *J. Polymer Sci.*, 1959, **40**, 107, 113.

[12] French, Roubinek, and Wassermann, *J.*, 1961, 1953.

in the last line is of the same order of magnitude as that of alginic acid deduced from potentiometric titration [13] in aqueous suspension. In the case of silver alginate ϕ_p and K were calculated from equations (5) and (11) which fit the observation equally well. It is not possible, therefore, to use these results in order to decide whether the binding of the silver ion is governed by a mass-action equilibrium. The activity coefficients, f, in the last column of Table 1 were computed from the slopes in columns 7 and 8, by using the activity coefficients f' of column 11, taken from the literature [14] and relating to concentrations near the arithmetic mean of the values given in column 5. The difference between the concentrations of the "free" ions inside a sol or gel and in the external solution is not large in any of the systems here considered.

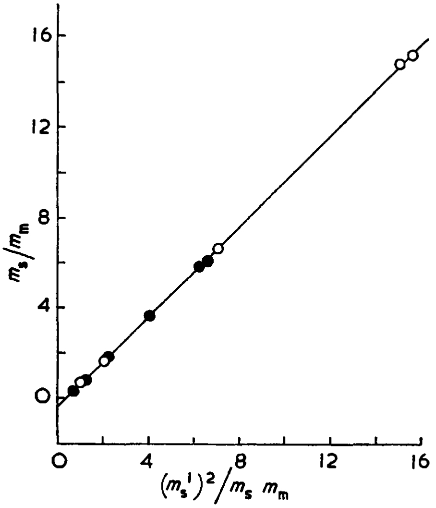

FIG. 1. Donnan equilibrium between sodium alginate sol and sodium chloride solution at 25·0°. Plot of m_s/m_m against $(m_s')^2/(m_s m_m)$. The concentration m_s' and m_s are in moles l.$^{-1}$; m_m in monomoles of alginate per l. of sol.

● m_m = 0·0165. ○ m_m = 0·00650.

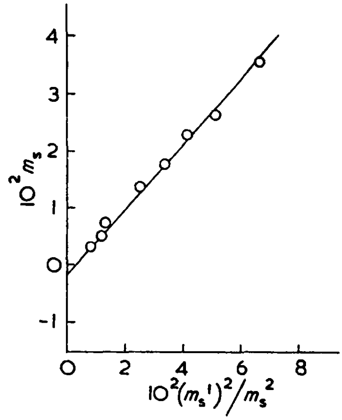

FIG. 2. Donnan equilibrium between fully swollen fibrous calcium alginate gel (fibre diameter 0·4 mm.) and calcium chloride solution at 23·5°. Plot of m_s against $(m_s')^3/m_s^2$, concentration being in mole l.$^{-1}$; m_m was 0·308 monomole of alginate per l. of fully swollen gel.

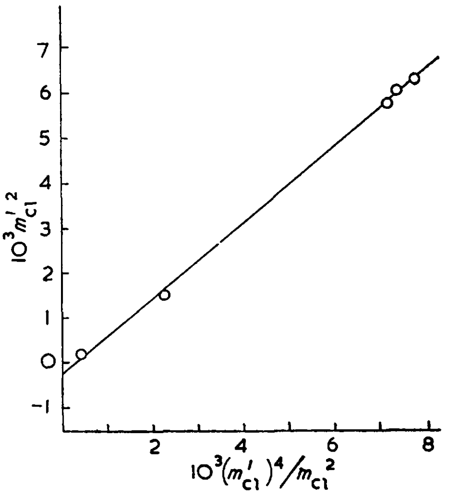

FIG. 3. Donnan and mass-action equilibrium between fully swollen fibrous alginic acid gels (fibre diameter 0·4 mm.) and hydrochloric acid solution at 22°. Plot of $(m_{Cl}')^2$ against $(m_{Cl}')^4/m_{Cl}^2$, concentrations being expressed in moles l.$^{-1}$; m_m was 0·341 monomole of alginic acid per l. of fully swollen gel.

[13] McGee, Fowler, and Kenyon, *J. Amer. Chem. Soc.*, 1947, **69**, 353.

[14] Harned and Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd ed., Reinhold Publ. Corp., New York, 1950.

It is understandable, therefore, why, for a given concentration, the f and f' values are similar. This is not incompatible with the slopes, deviating significantly from unity, if the ratios of the activity coefficients appear in the second or third power.

The ϕ_p values obtained with the help of the osmotic concentration method are in Table 2.

TABLE 1.

Counter-ion fixation in alginate sols and gels at 22—25°. Results of Donnan equilibrium measurements.

Alginate			Concentration range								
sol.	gel.	P.E.*	Alginates (equiv./l.)	P.E.* (mole/l.)	No.†	$(f'/f)^{2 \text{ or } 3}$	$(f'/f)^2$	ϕ_p	10^4K (mole/l.)	f^1	
Na		NaCl	0·00658—0·0165	0·00518—0·103	11	1·0 ± 0·03		0·40 ± 0·05		0·86 ± 0·07	0·86 ± 0·10
K		KCl	0·00600—0·0164	0·00641—0·100	8	1·0 ± 0·03		0·35 ± 0·05		0·92 ± 0·03	0·92 ± 0·06
NH_4		NH_4Cl	0·0104	0·00200—0·0138	8	1·0 ± 0·05		0·27 ± 0·05		0·90 ± 0·03	0·90 ± 0·06
Mg		$MgCl_2$	0·00610—0·0167	0·00358—0·0303	17	1·00 ± 0·05		0·15 ± 0·02		0·60 ± 0·08	0·60 ± 0·1
ED ‡		ED ‡ chloride	0·00567—0·00602	0·00251—0·0219	13	0·97 ± 0·04		<0·02			
	Ca	$CaCl_2$	0·308	0·00440—0·0432	8	0·55 ± 0·05		0·01 ± 0·005		0·70 ± 0·1	0·85 ± 0·1
	Cu	$CuSO_4$	0·291	0·00430—0·0618	17	1·6 ± 0·2		0·03 ± 0·02		0·27 ± 0·05	0·21 ± 0·05
	Ag	$AgNO_3$	0·218	0·00830—0·0874	8	0·80 ± 0·08	0·76 ± 0·08	0·03 ± 0·015	2 ± 1 §	>0·73	>0·81
	H	HCl	0·341	0·00830—0·768 ¶	9		0·86 ± 0·1		8 ± 4 §	0·91 ± 0·07	0·95 ± 0·05

* P.E. = Permeant electrolyte. ‡ ED = Ethylenediammonium.

† No. = Number of tests. § Calc. from equation 11, with $\phi_p = 1$.

¶ Fig. 3 represents results obtained with the lowest five concentrations in this range; the results obtained from tests with the highest four concentrations fall on a continuation of the straight line shown in the Figure.

TABLE 2.

Counter-ion fixation in alginate sols at 22—25°; results of osmotic measurements.

Alginate	Conc. range (equiv. $l.^{-1}$)	No. of tests	ϕ_p
Na *	0·00500—0·0136	7	0·29 ± 0·05
Na †	0·0115	1	0·33 ± 0·05
K	0·00460—0·0190	3	0·31 ± 0·05
NH_4	0·00300—0·0100	3	0·30 ± 0·05
Ethylenediammonium	0·00474—0·0116	4	0·07 ± 0·01
Mg	0·00398—0·0136	5	0·16 ± 0·02

* Weight-average molecular weight, $M_w = 1·5 \times 10^5$. † $M_w = 5·3 \times 10^4$.

DISCUSSION

Ion fixation by the three non-complex-forming univalent counter-ions, sodium, potassium, and ammonium is similar, as shown by the ϕ_p values in the first three lines of Table 1 and in the first four lines of Table 2. These values show that 60—70% of the counter-ions are sequestered by the polyanion. Similar binding of univalent counter-ions was found for other polycarboxylic acids.[1-3] Bivalent or complex-forming univalent counter-ions, on the other hand, are characterised by smaller ϕ_p values, as in other systems (see, for instance, ref. 15), the figures in the last lines of Tables 1 and 2 indicating that between 85% and >98% of these species can be inactivated. Comparison of the binding of

[15] Kotliar and Morawetz, *J. Amer. Chem. Soc.*, 1955, **77**, 3692; Wall and Eitel, *ibid.*, 1957, **79**, 1556, 1556; Inagaki, Hotta, and Hirami, *Makromol. Chem.*, 1957, **21**, 1.

magnesium, ethylenediammonium, calcium, and copper makes it obvious that unknown factors play a rôle. Copper possibly forms a complex with the alginate, and the same may happen with silver, the ϕ_p value of which is much smaller than that of the other univalent counter-ions.

The intrinsic viscosities, $[\eta]$, of sodium and magnesium alginate solutions [6] show that on addition of permeant salt the polyanion contracts substantially, the ratios ($[\eta]$ salt-free solution) : ($[\eta]$ 0·1M-salt solution) being >10. Thus, in the conditions of the Donnan equilibrium measurements, these polyelectrolytes must be characterised by a much more compact configuration than in the conditions of the osmotic tests, where no permeant salts are present. These marked changes in shape do not, however, influence the ϕ_p values to any significant extent, as follows from a comparison of the values in Tables 1 and 2. The values in the first two lines of Table 2 show, moreover, that the molecular weight of the alginate has no substantial influence on ϕ_p. The fraction of "free" sodium or magnesium ions in sodium or magnesium alginate solutions has been found by transference measurements [6] to be larger than the ϕ_p values deduced from osmosis. Thus, some of the counter-ions fixed to the alginate under static conditions are removed if migration in an electric field occurs.

It has been suggested [5,6] that bivalent or complex-forming univalent counter-ions are capable of joining together carboxyl groups attached to different alginate chains, and that such intermolecular salt bridges are sufficiently stable to withstand the thermal impact of the solvent. This hypothesis is supported by the observed small ϕ_p values. In dilute solutions of polyelectrolytes the probability of intermolecular cross-links is low, but most bivalent and complex-forming univalent counter-ions nevertheless give rise to gel formation which indicates intermolecular bonds. In the case, previously [5] discussed, of alginate fibres the aggregation occurred while the main valency chains were oriented by an external force, so that the cross-linking points were amplified and increased in extension through local crystallisation. It appears that each cross-linked segment undergoes a decrease of solubility, a suggestion which is in accordance with unpublished results of Michaeli who studied solubility changes accompanying the interaction of polyelectrolytes with bivalent counter-ions. It is suggested that stretched, fully swollen alginate fibres, cross-linked, for instance, by calcium ions, contains regions with a high degree of swelling and high mobility of chain segments, alternating with regions in which local precipitation and crystallisation have occurred. The existence of the latter segments was demonstrated by measurements of birefringence and low-angle *X*-ray scattering.[5] If the bivalent counter-ions are replaced by sodium or potassium ions, the ϕ_p values of which are larger, a "chemical melting" of the ordered segments in the gel takes place which alters the optical properties,[5] the elasticity,[5] and the overall length.[5] This type of counter-ion replacement may give rise to local solubility increases in those regions of the gel which become less ordered. If, however, the counter-ion exchange is carried out in a poor solvent, *e.g.*, 5N-sodium chloride or 10N-potassium carbonate, over-all dissolution of the gel does not take place.

We are indebted to Dr. Z. Alexandrowicz for the gift of one of his concentration osmometers.

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