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SWELLING AND CONTRACTION OF POLYACRYLAMIDE GEL SLABS IN AQUEOUS SOLUTIONS

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SUMMARY

Polyacrylamide gel slabs shrink in water at sufficiently low temperatures and swell at higher temperatures, slowly and reversibly, the extent and inversion temperature depending on gel composition. Mixed agarose–acrylamide gels show similar but less extensive changes. High concentrations of initiators during polymerization favour swelling, and conversely. High polymerization temperatures also favour swelling. Compression of gels leads to a slow loss of water. The presence of solutes affects swelling in various ways; with salts, it increases as the cation is changed in the order $(CH_3)_4N^+$, Li^+, NH_4^+, K^+, Cs^+, Na^+ (at 0.5 *M*) and, as the anion is changed, in the order CH_3COO^-, F^-, $HCOO^-$, Cl^-, Br^-, NO_3^-, I^- (at 0.5 *M*).

INTRODUCTION

Polyacrylamide gels usually swell in water or aqueous buffer solutions. One might expect this phenomenon to have considerable influence on the molecular sieve behaviour of such gels in electrophoresis or chromatography, but there has been remarkably little germane discussion or application. Of many possible influences on the extent of swelling, only the effect of gel composition has been well studied[1–4]. The starting point of the present investigation was an observation, alluded to earlier[5], that swelling of the carrier gel in an electrophoresis apparatus was temperature dependent, to the extent that it could be kept within bounds by operating at temperatures below 10°. This paper describes the phenomenon of swelling, and the factors influencing it, with the expectation that this will assist in improving the design of apparatus and procedures that depend upon the properties of polyacrylamide gels. The results may assist in resolving the apparent discrepancy between the behaviour of acrylamide gels in electrophoresis and their behaviour in chromatography[2,6].

MATERIALS AND METHODS

Early experiments were carried out at Makerere University, Kampala, Uganda, with acrylamide and N,N′-methylenebisacrylamide (BIS) obtained from Koch-Light (Colnbrook, Great Britain). The critical series was carried out in Hong Kong with

materials obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.), the batch numbers being N7B and A2C, respectively. The materials from the two sources gave reasonably comparable results. Recrystallisation of acrylamide[7] was effected with the Eastman-Kodak product as starting material. N,N,N′,N′-tetramethylethylenediamine (TEMED) was purchased from Eastman-Kodak.

Ammonium persulphate obtained from BDH (Poole, Great Britain) was stored in a loosely stoppered bottle in a desiccator over dry silica gel. Riboflavin was purchased from Merck (Darmstadt, G.F.R.) and agarose from BDH, "for electrophoresis". Other chemicals used were of analytical-reagent grade when available commercially, otherwise of reagent or laboratory-reagent grade, as supplied by BDH or Merck, except that lithium chloride and tetramethylammonium chloride were made by adding lithium carbonate and tetramethylammonium hydroxide (Sigma, St. Louis, Mo., U.S.A.), respectively, to standard hydrochloric acid, to give a pH of 7, and then diluting to volume.

Gel preparation

Gel preparation is best expressed in terms of a standard method and variations of it: thus, unless otherwise stated, gels were prepared as follows. Immediately before use, a solution was prepared containing a 5% (w/v) final concentration of total monomers, of which 5% (w/w) was BIS. This solution also contained 1 g/l final concentration of ammonium persulphate [added as a 10% (w/v) solution made up freshly at least once a week] and 1 ml/l final concentration of TEMED, giving a final pH close to 7. After dissolution of the other components was complete, the TEMED was added, the whole solution mixed quickly and thoroughly and then poured out into a mould 5.0 mm deep, which was covered, with exclusion of bubbles, and put aside on the bench (at 21–25.5°) to allow polymerization, signalled by the appearance of slight opacity or opalescence after about 4–5 min.

Gels were left covered for a minimum of 1 h before the mould was dismantled, and were then cut with a knife or spatula into pieces weighing 2.5–10 g, according to the particular experiment, and lifted out of the mould with a spatula. For riboflavin photopolymerization[2], concentrations of monomer were as above and the solution also contained the following (final concentrations per litre): TEMED, 1 ml; acetic acid, 6 mmoles; and riboflavin, 5 mg. Light entered the mould through its glass lid (about 3 mm thick), the mould otherwise being made of Perspex. The temperature in the immediate environment of the mould was recorded as 27–28°. Deoxygenation, when performed, was by degassing under a high vacuum. Combined agarose–polyacrylamide gels were made by dissolving monomers in a solution of agarose 1% (w/v), maintained at 45° in a water-bath, to give a volume of 49.5 ml, adding 0.5 ml of ammonium persulphate solution, 50 µl of TEMED, and immediately mixing and pouring into the mould.

Following a convention widely, but not universally, accepted, the concentration of total monomers in percent (w/v) is designated T and the proportion of BIS in percent (w/w) designated C. Thus in this paper the composition of a standard gel is abbreviated as T = 5, C = 5, and gels of variant composition correspondingly. In the literature, usage has varied somewhat. Morris and Morris[2] use the present convention, whereas Morris[8] writes T for grams of total monomers per 100 ml of solvent.

Extent of swelling

Gels were weighed on removal from the mould and again after being exposed for 7 days to several changes of the chosen solution. Adherent surface water was removed by blotting them until dry with Whatman No. 540 filter paper, which is preferred to the No. 50 filter paper used by Morris and Morris[2]. To minimise evaporation the gels were kept when possible in folded "envelopes" of polythene sheet. The standard deviation of repeat weighings (*i.e.*, the complete sequence: moistening in water, blotting dry and weighing) was about 0.1 % for 5 mm thick gels.

Extent of polymerization: dry weight of gel matrix

Portions of gel, freed from small molecules by dialysis, were dried by heating in an oven at 90° and then exposing to a vacuum over phosphorus pentoxide. But this treatment gives falsely high weights, and therefore falsely low results for water regain[2]. Further drying at 140° under high vacuum over phosphorus pentoxide for 3 days indicated that polymerization was 98 % complete, as would be expected[9].

Further details of all procedures will be provided on request.

RESULTS

Progress of swelling and contraction of gels

Fig. 1 shows the results of an experiment with slabs of gel 6 mm thick. The gels swelled rapidly and extensively, approaching a plateau after 3–6 days. The extent of swelling was temperature dependent and the gels shrank again on exposure to a temperature of 4°, whether immersed in fluid or in air in a moist chamber at the same temperature, but did not reach their initial weight. Gels exposed to a temperature, of 0° shrank below their initial weight.

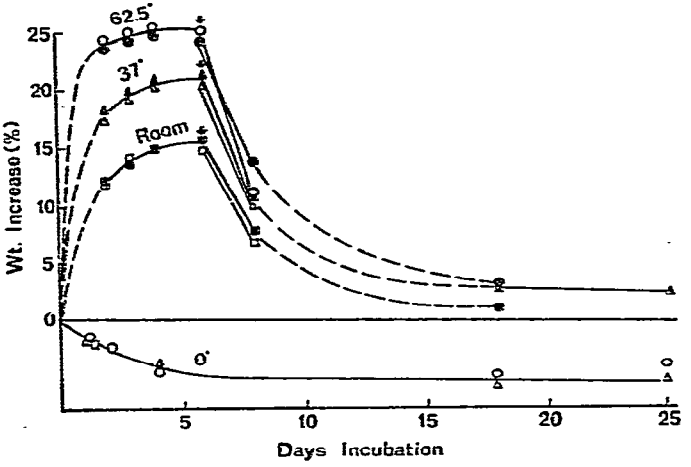

Fig. 1. Progress of swelling and shrinking of gels. Gels were made by the standard procedure but with Koch-Light materials and cast in a mould 6 mm deep. The weight history of individual pieces of gel is shown by individual symbols. In the experiments at higher temperatures, each piece was first incubated in water at the temperature shown and later transferred to 4°. Open symbols, pieces which, when incubated at 4°, were kept in a moist chamber; filled symbols, those in water.

Effect of temperature

Fig. 2 indicates the effect of temperature on swelling in gels of various composition. An additional result not shown in the figure is that at 90° a portion of standard gel showed a weight increase of 183% in 24 h and of 733% in 3 days, by which time the gel was extremely friable.

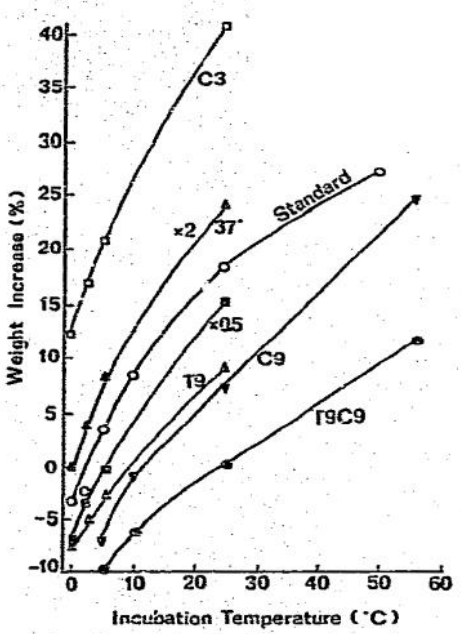

Fig. 2. Effect on swelling of gel composition, incubation temperature and conditions during polymerization. Gels made by the standard technique (5 mm thick), and with variations as stated, were incubated in water for 7 days: □, (C3), 3%C(5%T); ▲ (×2), double the normal amounts of initiators, *i.e.*, 2 ml/l TEMED and 2 g/l persulphate; ○, standard (5%T, 5%C); ■ (×0.5), half the normal amounts of initiators, *i.e.*, 0.5 ml/l TEMED and 0.5 g/l persulphate (otherwise standard); △, (T9) 9%T (5%C); ▼, (C9), 9%C (5%T); ●, (T9C9), 9%C, 9%T. Results for gels polymerized at an initial temperature of 37° were too close to those for double the normal amounts of initiators (×2) for the points to be shown separately, *i.e.*, this one curve represents the results for two different sets of observations. Results for gels polymerized at 5° (otherwise standard) were slightly higher than standard, and again cannot be separately shown.

Effect of conditions during polymerization

Fig. 2 also shows that gels set more rapidly than the standard (with higher initiator concentrations), swell more. Such gels were also softer, less opaque and more friable. Conversely, gels set with lower than standard initiator concentrations swelled less and were more opaque than usual. Additional results, not shown in the figure, are that at 25° gels made with five times the normal amounts of initiators (persulphate 5 g/l, TEMED 5 ml/l) increased in weight by 29.5%, and gels made with one fifth of the normal amounts of initiators (persulphate 0.2 g/l, TEMED 0.2 ml/l) swelled by 13.4%. Setting times in these experiments were: (initiator concentrations, time of appearance of opalescence) five times normal, 40 sec; twice normal, 2 min; normal, 4 min; half normal, 10 min; one fifth normal, more than 2 h (gel left overnight to set).

Gels set at an initial temperature of 37° swelled more than normal (Fig. 2). Gels made at 5° were much more opaque than normal, although they swelled slightly more than standard (omitted from Fig. 2, for clarity), showing that opacity is not necessarily correlated with resistance to swelling.

Deoxygenation made little difference to the extent of swelling, although polymerization was accelerated considerably, as judged by the time taken for opacity to appear.

Riboflavin photopolymerization results were too variable to allow definite conclusions to be drawn on the effects of setting temperature, abbreviated setting period or deoxygenation. The degree of swelling was rather similar to that found in contemporary experiments with standard gels, *i.e.*, 16.7% (mean of 16 results) compared with 17.5–18.1% (standard). The greater variability of results with these gels was unmistakable. This can possibly be explained by the softer surface leading to difficulties in removing adherent water, on the one hand, and more frequent damage to the surface, with loss of material, on the other. There seems to be a difference in the nature of the surface according to the mould material in contact with it during casting, the upper surface, that in contact with glass, being stronger. No such difference was seen with standard gels.

Effect of pH during incubation

Table I shows that in solutions of approximately equal osmolarity, there was little or no effect of pH over the range 4–8, but that at pH 9 and above there was progressively increasing extra swelling: in 0.05 *M* sodium hydroxide this effect was gross and the gel had become friable. Acetate and glycine solutions of the pH indicated were made up by titrating 0.1 *M* acetic acid or 0.1 *M* glycine with 0.1 *M* sodium hydroxide. In separate experiments, 0.05 *M* hydrochloric acid gave little more swelling than water alone.

TABLE I

EFFECT OF INCUBATION pH

Pieces of gel from a single casting were incubated in the solutions shown (see text).

Solution	*pH*	*Weight increase (%)*
0.1 *M* Acetic acid	3.4	19.7
Acetate	4	17.6
	6.3	18.0
Glycine	7	17.1
	8	17.9
	9	22.6
	10	48.3
0.05 *M* NaOH		552

Effect of solutes during incubation

Table II demonstrates that many solutes markedly increase swelling and that the effect increases with concentration. But ethanol, sodium acetate and sodium

TABLE II

EFFECT OF SOLUTES AND THEIR CONCENTRATIONS ON GEL SWELLING

All results are quoted as weight increase (%). Values given in parentheses have been calculated as volume increase (%), correcting for final density of the gel. The lower four rows show experiments, each carried out with portions of gel from a single casting.

Water	Tris–HCl*	0.2 *M* NaCl	0.5 *M* NaCl	1.0 *M* NaCl	2.0 *M* NaCl	0.1 *M* Sucrose	0.25 *M* Sucrose	0.5 *M* Sucrose	1.0 *M* Sucrose	2 *M* Sucrose
18.4	22.4	23.3	29.2 (27.0)	35.3 (30.2)	45.9 (35.4)	21.8	25.1 (21.1)	38.5 (30.0)	39.9 (24.1)	49.7 (20.2)
0.2 *M* Urea	0.5 *M* Urea	1.0 *M* Urea	2.0 *M* Urea	0.5 *M* Glucose	1.0 *M* Ethanol	0.5 *M* *n*-Butanol	1.0 *M* Na acetate	0.67 *M* $CaCl_2$	0.67 *M* Na_2SO_4	TEMED–$S_2O_8^{2-}$**
20.5	23.8	29.2	38.4 (34.1)	31	9.5	17.8	12.1	44.5	25.3	17.8
0.5 *M* LiCl	0.25 *M* $MgCl_2$	0.25 *M* $CaCl_2$	0.5 *M* KCl	0.25 *M* $MnCl_2$	0.5 *M* NaCl					
24.4	26.2	26.8	27.0	27.4	28.0					
0.5 *M* NaF	0.5 *M* NaCl	0.5 *M* NaBr	0.5 *M* KI	0.5 *M* KNO_3	0.5 *M* $NaNO_3$	0.5 *M* Na formate	0.5 *M* $(CH_3)_4NCl$	0.5 *M* NH_4Cl	0.25 *M* $CoCl_2$	0.5 *M* CsCl
15.0	28.0 (25.7)	30.4 (26.8)	40.2 (32.8)	33.8 (29.9)	34.2 (30.6)	19.4 (17.1)	18.0 (17.8)	25.4 (24.6)	28.3 (24.7)	33.4 (25.4)
Water	0.5 *M* LiCl	0.5 *M* NH_4Cl	0.5 *M* KCl	0.5 *M* CsCl	0.5 *M* NaCl					
17.4	25.3 (23.7)	25.2 (24.5)	28.0 (25.5)	33.0 (25.3)	28.0 (25.8)					
Water	0.1 *M* $(CH_3)_4NCl$	0.2 *M* $(CH_3)_4NCl$	0.5 *M* $(CH_3)_4NCl$	1.0 *M* $(CH_3)_4NCl$						
18.0	19.5	19.7	19.0 (18.9)	13.0 (12.7)						

* 0.25 *M* Tris, 0.05 *M* chloride (pH 8.76)[2].

** $(NH_4)_2S_2O_8$, 0.44 m*M*; TEMED, 0.86 m*M*.

fluoride cause less swelling than water alone, the swelling-potentiation effect of sucrose diminishes above 0.5 *M* and the effect of tetramethylammonium chloride, always slight, also passes through a maximum.

Among salts at moderate concentrations, the following relationships can be seen. (1) At constant chloride concentration (0.5 *M*), swelling increases in the order $(CH_3)_4N^+$, Li^+, NH_4^+, K^+, Cs^+, Na^+, and results for the bivalent ions tested all lie in the vicinity of results for NH_4^+ and K^+. (2) At constant sodium concentration (0.5 *M*), and allowing for some interpolation, swelling appears to increase in the order (CH_3COO^-, F^-, $HCOO^-$), Cl^-, Br^-, NO_3^-, I^-, the bracketed ions giving swelling equal to or less than water alone.

The polymerization initiator ions are without effect at plausible concentrations. The Morris and Morris[2] Tris buffer produces less swelling than 0.5 *M* sodium chloride, but more than water alone.

Effect of mechanical compression

Fig. 3 indicates that gels subjected to compression (with distortion) gradually lost water by comparison with an uncompressed gel, apparently approaching a limit. At ordinary room temperatures, the weight of standard gels can certainly fall below its initial value.

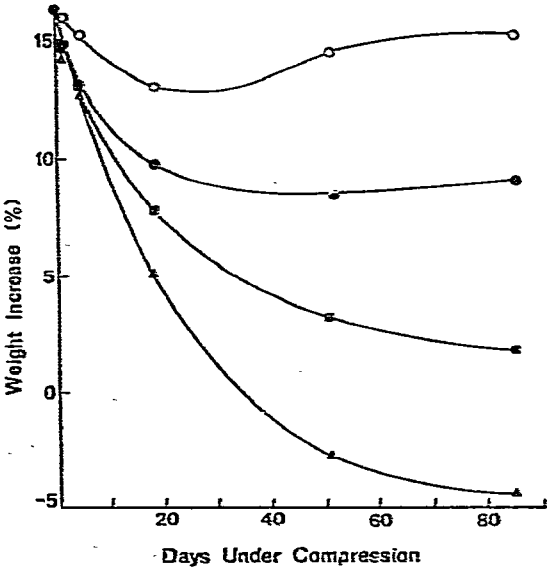

Fig. 3. Contraction of gels under compression. Four portions of a single gel were allowed to swell in water for 1 week, three were then trapped under perspex plates larger than themselves, upon which were placed weights of 200, 500 and 1000 g, the whole remaining in a tray of water, so that the gels were continuously immersed. Temperature control was not possible, and extreme limits of water temperature observed were 18.0–25°, such fluctuations affecting all portions equally. The gels were deformed (squashed out) by the imposed weights, so that the stated pressures are nominal, referred to the original, unswollen cross-sectional area of the portion concerned. ○, Free uncompressed; ●, 200 g, 10.4 g/cm²; ■, 500 g, 23.4 g/cm²; ▲, 1000 g, 50.0 g/cm².

Gels prepared according to ref. 2

Fig. 4 shows the weight history of gels exposed to the sequence of conditions recommended by Morris and Morris[2]. It was not possible to reproduce these conditions exactly, as they are not completely specified, and in the present work each stage was prolonged somewhat in order to show the phases more distinctly. Two separate experiments are shown. In one, the final incubation (corresponding to the 24-h electrophoresis procedure of ref. 2) was carried out in contact with solution; in the other, the gel was instead wrapped in a plastic sheet and subjected to slight compression in order to mimic a possible compression effect of the upper cooling plate employed by Morris and Morris[2].

These authors do not specify the temperature at which washing procedures were carried out, but it is clear from their description that gels were first exposed to the working electrophoresis temperature (10–11°) when placed in the apparatus shortly before beginning the run. The results in Fig. 4 make it seem unlikely that such gels will have shrunk to their final equilibrium position, even at the end of a 24-h "run".

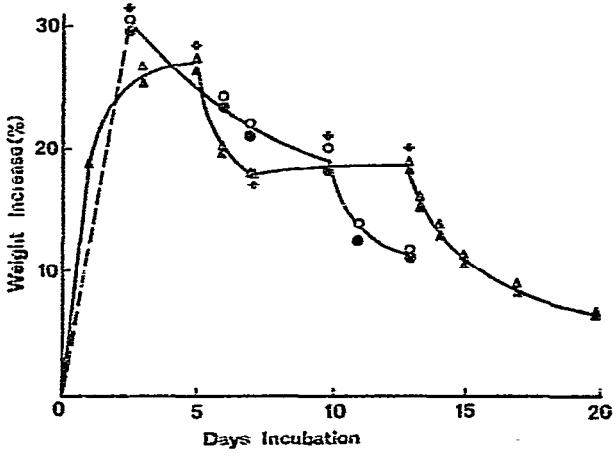

Fig. 4. Weight history of gels treated as in ref. 2. Gels were prepared by photopolymerization. Each symbol shows the weight history of an individual portion of gel. (1) ●, ○; the mould in this instance was 3.2 mm deep. Portions of gel were exposed to the following sequence of conditions. 0.5 *M* NaCl: 25°, 2.5 days; Tris–HCl buffer[2]: 25°, 7 days; Tris–HCl buffer: 11.5°, 2 days. (2) ▲, △; the standard mould was used and the gels exposed to the following sequence of conditions. 0.5 *M* NaCl: 25°, 5 days; water (3 changes): 25°, 48 h; Tris–HCl buffer: 25°, 6 days; wrapped in thin polythene film and compressed at 6.3 g/cm², 10.5°, 7 days.

Effect of gel composition

Where comparison is possible, the results on swelling at 25° confirm those of Richards and Lecanidou[4], and are therefore not reported in detail: there is a minimum of swelling at 9–15% T (varying with C). As polymerization is exothermic, high temperatures may occur and thus affect gel resistance to swelling (*cf.* Fig. 2). Some experiments were therefore performed at low initial temperatures, and the results suggest that this high-temperature effect is present but is not sufficient to account for all the excess of swelling observed at high T values (Table III).

TABLE III

MONOMER CONCENTRATION AND POLYMERIZATION TEMPERATURE: EFFECTS ON SWELLING

Gel preparation was as in text, but C = 0.5% and T as indicated.

T (%)	*Initial temperature (°C)*	*Maximum observed temperature during polymerization (°C)*	*Weight increase (%)*
5*	21		270
10	21		179
15	21	40	176
30	22	80	227
30	0	40	201

* Weight of dried gel matrix indicates that extent of polymerization was only 90%.

Gels containing only agarose (1% w/v) appear not to swell in water at 25° or in 100 m*M* sodium chloride, and may even decrease in weight. These gels lose water very quickly to filter paper, certainly quickly enough to disturb the results, and it is difficult to be sure that the observed loss is not an artifact. However, gels containing both agarose (1%) and acrylamide (T = 5, C = 5) behave rather like gels of acrylamide alone, although within much narrower limits. The swelling observed was (incubation temperature, weight increase): 5.0°, −0.2%; 25°, +7.4%; 40°, +12.0%.

The initial swelling of gels made with recrystallised acrylamide was indistinguishable from that of the standard gels.

Effect of gel thickness

As might be expected, gels thinner than the standard gel achieved quasi-equilibrium more quickly, and conversely: 2 mm thick gels had almost completed swelling within 24 h (at 25°).

DISCUSSION

The dependence of swelling on gel composition has been known for many years[1], and a theory exists that links this effect with gel opacity[2]. The present paper describes a variety of other phenomena connected with gel swelling, which have either not been reported or are mentioned only indirectly in previous publications. Theoretical explanations are not yet available for most of these phenomena, but such are the implications for the theory and practice of gel electrophoresis and its correlation with gel chromatography, that to wait indefinitely for the formulation and testing of such explanations appears to be unjustified.

Gels distort and shrink under a compressive load, behaving like slow-acting sponges. The loads concerned are modest and it seems unlikely that a gel cast and kept confined within a rigid container would burst its bounds, but that is not to say that such a gel would be unaffected by the phenomenon of swelling. Uneven distribution of imbibition seems likely and where the ends of a gel slab or cylinder are unconfined, as is usual, there is no doubt that swelling does occur, with local and perhaps more general distortion of the gel structure, spreading a considerable distance

into the main body of the gel. Mechanical distortion of gel structure, without an apparent change in volume, has been used to control the activity of enclosed enzymes[10], a remarkable instance of the possible effects of such stresses.

While the usual analytical applications of gel electrophoresis may be little affected by swelling, because cautious practitioners avoid using the ends of the gels, this effect is much more serious in preparative electrophoresis. Further, shrinkage cannot be controlled by this type of mechanical restriction: there is no doubt that it occurs, and that its effects may be serious in some applications.

One might predict that the extent of swelling would be temperature dependent, but perhaps not that the common types of gel could easily be made to shrink by cooling sufficiently and that some would do so even at normal ambient temperatures. This shrinkage is quite independent of, and much more extensive than, that which occurs by reason of gel formation[4] or simply by thermal contraction. These temperature effects are bound to give rise to problems in prolonged preparative procedures (because not all parts of the gel can be at the same temperature) even if they also offer some hope of controlling swelling.

The swelling of polymer gels has been treated as an osmotic phenomenon[11,12]. The equilibrium of swelling is then "a balance between the internal osmotic pressure of the gel and the negative entropy of distension", or, put crudely, between a swelling pressure and the elastic retraction of the gel mesh. This theory provides at least a qualitative fit with the observed dependence of swelling on temperature and mechanical restriction, including the behaviour of agarose–acrylamide gels, in which the agarose can be regarded as providing a less extensible network than acrylamide alone.

In vinyl polymerization, polymer chain length is inversely related to initiator concentration[11]. This suggests an explanation for the influence of initiator concentration on swelling: longer polymer chains might imply larger and less numerous osmotic units (even if these are not co-extensive with covalently linked "molecules") and might also result in an inherently stronger gel matrix. The greater swelling tendency found for high polymerization temperatures may be similarly explicable.

It is not so easy to explain the occurrence of increased opacity in gels set at low initiator concentrations or at 5°, nor the rather large swelling tendency of the latter. Opacity is known to depend upon gel composition and is correlated with swelling resistance[4]. However, it has been shown above that gels of standard composition ($T = 5$, $C = 5$) are more opaque than is normal and more resistant to swelling if set with low initiator concentrations; here opacity is related to swelling resistance but not to degree of cross-linking. Further, if gels of standard composition are polymerized at 5°, they are much more opaque than is normal, but show a standard or greater swelling tendency. In this instance, not only is the opacity unrelated to degree of cross-linking, but a breakdown in the predicted relationship to swelling resistance occurs. The same theory[4] was used to predict the observed increase in swelling tendency at high T values, on grounds simply of the structure of the gel matrix, but it has been shown above that this increase is due, at least in part, to self-induced high temperatures during polymerization. Clearly, the theory is incomplete.

The above observations on the effect of polymerization temperature are not necessarily valid for photopolymerized gels, in which the generation of free radicals probably depends chiefly on the intensity of illumination.

There is not yet any evidence to indicate the mechanism of the effect of various

solutes of low molecular weight, which must be presumed to be capable of penetrating almost all interstices of the gel meshwork. Factors that may be considered to have potential effect are: the affinity of a component of the gel for molecules that are hydrated to a greater or lesser extent; a Donnan-type effect; and competition for water between the gel and the solution. Evidence in respect of these possibilities should be obtainable from experiments on the distribution of water and solute molecules between the gel and the solution. The competition theory is attractive; it could account for the behaviour of ethanol and, at least in part, the relative effects of metal ions, but it is difficult on this basis to account for phenomena such as the widely varying effects of halide and other anions.

Attributing effects to one ion or the other in a salt solution is fraught with difficulties and may be impossible without recourse to theory or extraneous experimental evidence. The present results could be fitted by postulating a swelling-potentiating effect of anions, which can be outweighed by a sufficient concentration of appropriate cations. With gels carrying charged groups, the effects of ionic solutions are readily understood and have been well studied[11], but simple polyacrylamide gels have no ionisable groups deliberately included in their make-up. There may well be some adventitiously present, but no information is available on this aspect and in any event coulombic interactions would not provide an explanation for the effects of non-ionic solutes.

Evidence provided here shows that it may be possible to eliminate or control the swelling of polyacrylamide gels used for electrophoresis by any or all of the following means: (i), selecting polymerization conditions, including avoidance of large masses of monomer solution; (ii), allowing pre-swelling to the desired dimensions; (iii), controlling actual run temperature and temperature differentials; (iv), mechanical restriction, including incorporating of agarose, etc.; (v), choice of buffer, selection of ions, concentration and pH; and (vi), pre-shrinking at a selected temperature and then operating at a higher temperature. Of the above, perhaps only (ii) and (iii) have so far deliberately been made use of for this purpose. Swelling or distortion of gels caused by the process of electrophoresis itself may modify some of these conclusions.

Very probably, the phenomena recorded here will account for the difficulties of Ogston and Preston[13], who found that their cellulose–polyacrylamide bilayer strip osmometer was sensitive to both salt concentration and temperature. It was not, of course, their purpose to explain these findings, and the nature of their experimental material would prevent a conclusion as to whether the original effect was upon cellulose or polyacrylamide, or both.

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