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Investigation of the properties of novel acrylamido monomers by capillary zone electrophoresis

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ABSTRACT

A series of mono- and disubstituted acrylamide monomers [monomethylacrylamide, dimethylacrylamide, trisacryl (Tris-A), dideoxytrisacryl and acryloylmorpholine] were investigated as potential candidates of a novel class of polyacrylamide matrices, exhibiting high hydrophilicity, high resistance to hydrolysis and larger pore size than conventional polyacrylamide gels. However, the most promising monomer (Tris-A) exhibited first-order degradation kinetics in 0.1 *M* NaOH, suggesting that such a structure is electronically unstable. On the other hand, the fact that, once incorporated into a polymer chain, such a monomer exhibited a strong resistance to alkaline hydrolysis suggests that, perhaps, a poly(trisacryl) matrix could be stereoregular, perhaps via helix formation. Another unique finding is the inverse relationship between the partition coefficient of such monomers and the incorporation efficiency: the more hydrophobic members of the family exhibit a very poor conversion from monomer into polymer. The efficiency, however, can be dramatically increased by increasing the polymerization temperature from 25 to 60°C.

INTRODUCTION

Polyacrylamide, first reported by Raymond and Weintraub in 1959 [1] as a supporting medium for zone electrophoresis, has enjoyed enormous popularity in biochemical separations. While the chemistry of acrylamide monomers has been extensively developed (see refs. 2 and 3 for reviews), not much work has been done on the structure and properties of the polymer. Following earlier studies on the properties of some cross-linkers [4], Gelfi and coworkers [5–7] started a decade ago an extensive investigation on polymerization conditions as a function of different types and amounts of cross-linkers, temperature and amount and type of catalysts. Some general rules were derived: (a) the order of reactivity for copolymerization with acrylamide decreases for cross-linkers in the following order: N,N′-methylenebisacrylamide (Bis) ≈ N,N′-(1,2-dihydroxyethylene)bisacrylamide (DHEBA) > ethylene diacrylate ≈ N,N′-bisacrylylcystamine (BAC) ≫ N,N′-diallyltartardiamide (DATD) [5]; (b) high temperatures (ideally 50°C) greatly favour polymerization [6]; and (c) photopolymerization produces in general poor gels with poor conversion of the monomers into the polymer [7]. In particular, DATD was found to be an inhibitor of gel polymerization and its use was discouraged.

A decade later, having synthesized a novel series of monomers (mostly mono- and disubstituted acrylamide), we decided to investigate some physico-chemical properties of such monomers and of the matrix thus obtained by using capillary zone electrophoresis (CZE). CZE is a suitable technique for separating and determining small molecules, for which gel electrophoresis does not have much to offer. In particular, we have studied the following parameters: (a) resistance to hydrolysis of the different monomers in free solution; (b) resistance to

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hydrolysis of the monomers in the polymeric phase; (c) relative hydrophobicity of the different monomers; and (d) extent of incorporation of the various monomers into the polymer. For all these aspects, CZE was instrumental in producing quantitative and highly reliable data, except for point (b); in this last instance, we had to resort to frontal analysis, which gave a precise determination of the extent of production of acrylic acid in the polymer on hydrolysis of amido bonds. These investigations were prompted by a series of studies performed by our group in the last few years on the acrylamido weak acids and bases copolymerized into polyacrylamide gels for performing isoelectric focusing in immobilized pH gradients [8–11].

As we understand more of the physico-chemical properties of such monomers, we hope to arrive at novel gel formulations.

EXPERIMENTAL

Materials

The following six monomers were analysed: acrylamide (Acr), N-methylacrylamide (MMA), N,N-dimethylacrylamide (DMA), N-acryloyltris(hydroxymethyl)aminomethane (trisacryl, Tris-A), N-acryloyldimethylhydroxymethylaminomethane (dideoxytrisacryl, DD-Tris) and N-acryloylmorpholine (ACM). Their formulae are listed in Table I and their syntheses have been described elsewhere [3,12]. All gels were prepared by cross-linking with Bis. Acrylamide, Bis, TEMED and ammonium peroxodisulphate were obtained from Bio-Rad Labs. (Richmond, CA, USA). Mandelic acid and pK 9.3 Immobiline, used as internal standards in CZE runs, were purchased from Aldrich (Steinheim, Germany) and Pharmacia–LKB (Uppsala, Sweden), respectively.

Alkaline hydrolysis

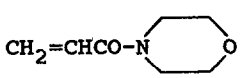
All monomers were dissolved (20 mM each) in 0.1 M NaOH and incubated at 70°C for up to 6 h. At given time intervals (see the relevant figures) aliquots were collected and diluted in 0.1 M sodium borate buffer (pH 9.0) to 2.5 mM. After adding mandelic acid (2.50 mM) as an internal standard, the samples were analysed by CZE.

Incorporation efficiency

In order to check for the extent of conversion of the various monomers into the polymer, 5 ml of gel (made with the different monomers in Table I) were

TABLE I

MONOFUNCTIONAL ACRYLAMIDO DERIVATIVES

Formula	Name	M_r
$CH_2=CHCONH_2$	Acrylamide (Acr)	71
$CH_2=CHCONHCH_3$	N-Methylacrylamide (MMA)	85
$CH_2=CHCON(CH_3)_2$	N,N-Dimethylacrylamide (DMA)	99
	N-Acryloylmorpholine (ACM)	141
$CH_2=CHCONHC(CH_3)(CH_2OH)_2$	N-Acryloyldimethylhydroxymethylaminomethane (DD-Tris)	143
$CH_2=CHCONHC(CH_2OH)_3$	N-Acryloyltris(hydroxymethyl)aminomethane (Tris-A)	175

polymerized in a test-tube under anaerobic conditions (protected by a thin film of water-saturated *n*-butanol). After polymerization (1 h, temperatures ranging from 25 to 60°C), the gel was extruded and homogenized and an equal amount of water was added (extraction was also performed in methanol). After stirring for 30 min, the gel phase was filtered through a Buchner funnel, the supernatant was diluted, internal standard (2.5 m*M* p*K* 9.3 Immobiline) was added and the mixture was filtered through an Ultrafree-MC Millipore filter (0.22 μm porosity) and analysed by CZE.

Capillary zone electrophoresis

CZE was performed in a Beckman (Palo Alto, CA, USA) P/ACE System 2000 instrument equipped with a 50 cm × 75 μm I.D. capillary. Runs were performed at 25°C in a thermostated environment in 0.1 *M* borate buffer (pH 9.0). In all instances the migration direction was toward the negative electrode, which means that the acidic species (mandelic acid) are transported there by electroosmosis as they migrate electrophoretically toward the positive electrode. The samples were injected into the capillary by pressure (55 kPa), usually for 10 s. The calibration graph for each acrylamido derivative analysed was constructed with the Beckman integration system Gold, with concentration points of 0.25, 0.50, 1.00, 1.25, 200, 250 and 3.50 m*M*. In each run the p*K* 9.3 Immobiline (2.50 m*M*) was used as an internal standard (except for the hydrolysis experiments, where mandelic acid was utilized). Runs were usually performed at 15 kV and 50 μA with the detector set at 254 nm.

Partition coefficient

In order to establish a hydrophobicity scale, the various acrylamido monomers were subjected to partitioning in water–1-octanol as described by Purcell *et al.* [13]. The partition coefficient *P* is defined as the ratio between the molarity of a given compound in the organic phase and that in the aqueous phase. Partitioning was performed as follows: each monomer was dissolved (2 m*M*) in water saturated with 1-octanol; 3.5 ml of this solution and 3.5 ml of 1-octanol were transferred to a separating funnel and shaken for 2 min. After decanting for 1 h, the aqueous phase was collected and centrifuged for 75 min at 1800 *g*. All operations were performed at 25°C. The clarified solution was diluted, internal standard (2.5 m*M* p*K* 9.3 Immobiline) was added and the mixture was analysed by CZE as described above.

Titration of acrylate groups in polyacrylamides by frontal analysis

In order to assess the amount of protolytic groups (acrylic acid) produced on extensive hydrolysis of the different types of polyacrylamides, the gels were cast not as continuous layers but as beads (with a concentration of 10%T and 8%C)[a] by emulsion polymerization [14]. The beads were extensively washed, dehydrated in methanol and dried *in vacuo*. A known amount of dry beads (from 0.25 to 1 g) was then reswollen in water and subjected to hydrolysis in 0.1 *M* NaOH at 70°C for up to 6 h. After extensive washing in water (to negligible conductivity), the beads were loaded on a 1.6-cm diameter column to a bed height of *ca.* 10 cm. Titration was performed with 0.1 *M* HCl and the eluate was pumped at constant speed (1.5 ml/min, LKB peristaltic pump) through a micro-conductimetric cell (4-μl volume, Orion conductimeter, 10 $m\Omega^{-1}$ full-scale). The signal was registered on a Kipp and Zonen recorder (20 mV full-scale, 10 mm/min chart speed). Once the conductivity curve had reached a plateau (corresponding to the conductivity of the titrant, diluted by the dead volume of the resin) the titration was stopped and a solution, with half the molarity of the titrant, was injected directly into the conductimeter cell; this was necessary in order to measure the inflection point of the curve. For calculating the total amount of protolytic groups (acrylic acid) generated on the resin by alkaline hydrolysis (C_{tot}), the following equation applies:

$$C_{tot} = [V/L(L_1 - L_0) - G_v]M_{tit}$$

where V is the volume of titrant utilized (in ml, as measured with a burette), L is the total length of the recorder tracing (in cm) for this V value, L_0 is the length of the recorder tracing (in cm) corresponding to the dead volume of all connecting tubings, L_1 is the length of the recorder tracing (in cm) up to the inflection point (as measured when pumping in the

[a] %C = g Bis/%T; T = g acrylamide + g Bis per 100 ml of solution.

titrant at half the molarity), G_v is the total gel volume (in ml) and M_{tit} is the molarity of the titrant. In our case, the value of C_{tot} obtained was divided by the resin dry weight so that our data were expressed in μequiv./g of dry beads. These data were finally converted into percentage hydrolysis with time (see Fig. 4).

RESULTS

It is well known that mono- and disubstituted amides are substantially more resistant to alkaline hydrolysis than unsubstituted species. As most zone electrophoretic runs in polyacrylamides are performed at alkaline pH values, it seemed worthwhile to explore new types of matrices formed with N-substituted monomers. At first glance, Fig. 1 seems to confirm this hypothesis: when a regular polyacrylamide matrix (5%T, 4%C) is briefly exposed to 0.1 *M* NaOH at 70°C, washed, dried and then reswollen in carrier ampholytes, during the focusing step a strong electrosmotic flow ensues, resulting in a marked acidification of the pH gradient, which suggests the formation of polyacrylate [15]. Conversely, when a similar matrix, but made with N,N-dimethylacrylamide, is subjected to the same protocol, the pH gradient generated is just as good as in a control, non-hydrolysed matrix, suggesting a strong resistance to hydrolysis of such a polymer.

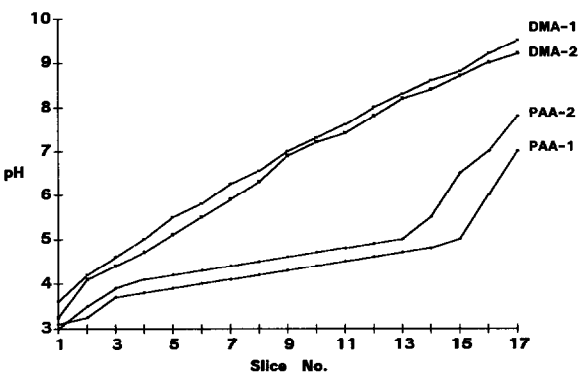


Fig. 1. Check for hydrolysis of polyacrylamide matrices. A polyacrylamide (PAA) and a polydimethylacrylamide (DMA) gel were cast on to a glass coated with Bind-Silane and then subjected to hydrolysis in 0.1 *M* NaOH for 20 min at 70°C. After extensive washing and drying, the gels were reswollen in 2% pH 3–10 carrier ampholytes and subjected to isoelectric focusing (2 h at 1500 V, 4°C). The gels were sliced along the electrode distance and the pH measured after equilibration in 300 μl of 10 m*M* NaCl. Note the flattening and marked acidification of the pH gradient in the PAA gels.

Given these findings, we decided to screen the series of monomers listed in Table I in order to check their resistance to alkaline hydrolysis. Fig. 2 shows a representative CZE run of DMA before and after hydrolysis; mandelic acid is always added as internal standard for quantitation purposes. A summary of

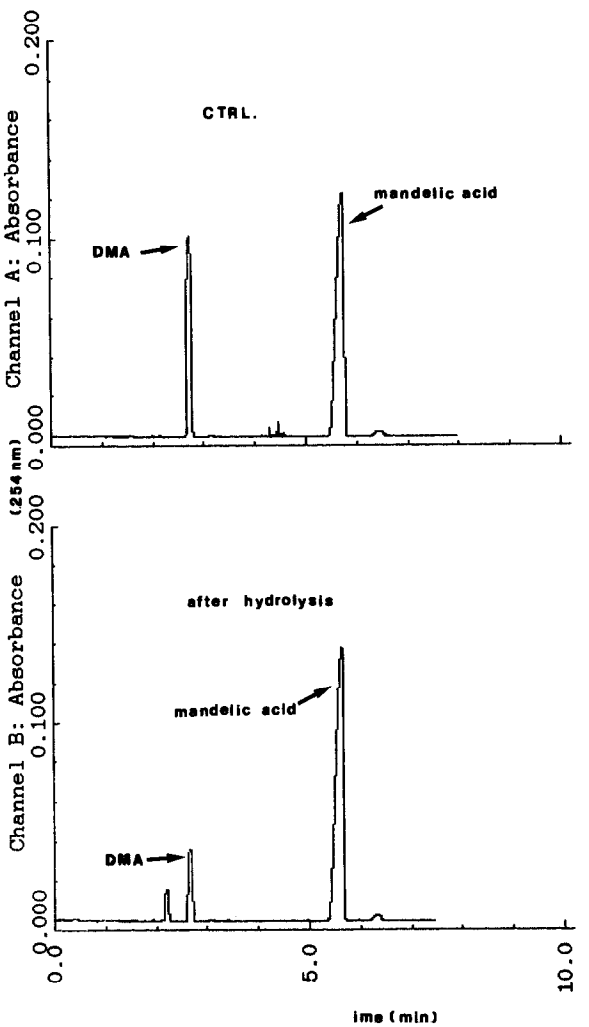


Fig. 2. Representative CZE runs for monitoring alkaline hydrolysis of acrylamido monomers. Conditions: 100 m*M* borate–NaOH buffer (pH 9.0), 15 kV, 86 μA at 25°C. Uncoated fused-silica capillary (50 cm × 75 μm I.D.). Beckman P/ACE 2000 instrument, monitoring at 254 nm. DMA = N,N-dimethylacrylamide; CTRL = control, before hydrolysis. In this and all subsequents runs the migration is towards the cathode.

all the hydrolysis data is shown in Fig. 3; surprisingly, not all substituted acrylamides exhibit a stronger resistance to alkaline hydrolysis than control acrylamide. In addition, whereas almost all acrylamido derivatives exhibit first-order degradation kinetics, one of them (trisacryl) shows zero-order kinetics, suggesting that such a monomer is intrinsically unstable. Based on the data obtained by CZE, Table II summarizes the relevant parameters of such hydrolytic processes, *viz.*, the half-lives ($t_{\frac{1}{2}}$) and the first-order rate coefficients.

The findings in Fig. 3 were disturbing, as we had hoped to locate new monomers which would be not only more resistant to hydrolysis, but also bulkier than acrylamide, so that they would form a more porous gel, having a larger fibre diameter. However, a completely different picture emerges if one looks at the relative resistance to hydrolysis not of the free monomers, but of the monomers after incorporation into the polymer chain. In order to measure such kinetics, the gels had to be polymerized into beads, subjected to hydrolysis and then titrated by frontal analysis. The data are summarized in Fig. 4; it can now be seen that all substituted acrylamides, independent of their degradation kinetics as free monomers, exhibit a much higher stability in the polymer matrix as compared with control acrylamide. Most striking is the behaviour of trisacryl, which, from zero-order degradation kinetics as the free monomer, exhibits a very slow degradation process in the polymer. Such findings could provide unique information about the three-dimensional structure of polyacrylamides, still believed to be a random network of fibres (see Discussion).

We next measured the relative hydrophobicities of

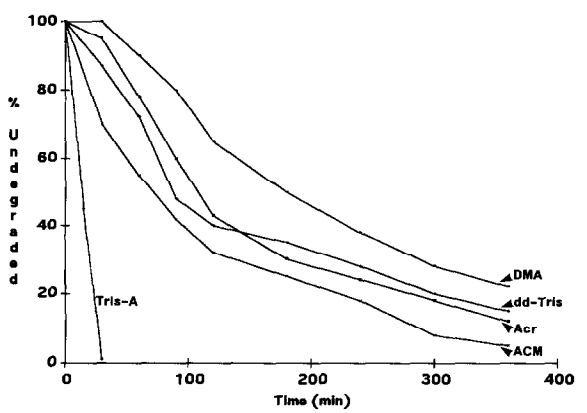


Fig. 3. Kinetics of hydrolysis of different acrylamide monomers. Hydrolysis was performed in 0.1 *M* NaOH at 70°C for the times indicated. The amounts were assessed by collecting in triplicate at each point, neutralizing and injecting into the CZE instrument (Beckman P/ACE 2000). Conditions: 100 m*M* borate–NaOH buffer (pH 9.0), 15 kV, 86 μA at 25°C. Uncoated fused-silica capillary (50 cm × 75 μm I.D.). Peak integration with the Beckman system Gold (mandelic acid was used as an internal standard). Abbreviations as in Table I. Note that whereas all the other monomers exhibit first-order kinetics, Tris-A follows zero-order degradation kinetics.

TABLE II

HALF-LIVES ($t_{\frac{1}{2}}$)[a] AND FIRST-ORDER RATE COEFFICIENTS (K)[b] FOR DEGRADATION OF ACRYLAMIDES IN 0.1 *M* NaOH, 70°C

Monomer	$t_{\frac{1}{2}}$ (min)	10^3 K (min^{-1})
N,N-Dimethylacrylamide	185	3.7
Dideoxytrisacryl	130	5.3
Acrylamide	111	6.2
Acryloylmorpholine	80	8.6
Trisacryl	15	–

[a] The half-life of the process was calculated from the equation $t_{\frac{1}{2}} = 0.693/K$.

[b] The first-order rate coefficient (K) was calculated from the slope of the line obtained when the logarithm of the residual molarity (undegraded monomers) was plotted against time.

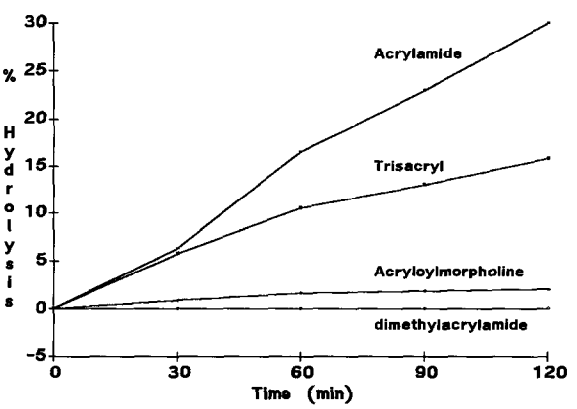


Fig. 4. Degradation kinetics of the monomers into the polymeric gel. The different monomers were polymerized (by emulsion polymerization) into beads, subjected to hydrolysis in 0.1 *M* NaOH at 70°C for the times indicated and then analysed for hydrolytic products. Hydrolysis was assessed in the beads by titrating free acrylic acid residues, produced by hydrolysis of the amide bond, by frontal analysis. Note tne much increased stability of trisacryl in the polymer as compared to its behaviour as free monomer (Fig. 3).

the six monomers in Table I by partitioning in water–1-octanol phases. It is well known that, for protein separation by electrokinetic processes, the supporting matrix should be highly hydrophilic so as to avoid any hydrophobic interaction. The quantitative data after the partitioning process, as measured by CZE (see Fig. 5 as a representative CZE run), are summarized in Fig. 6. It is seen that, in

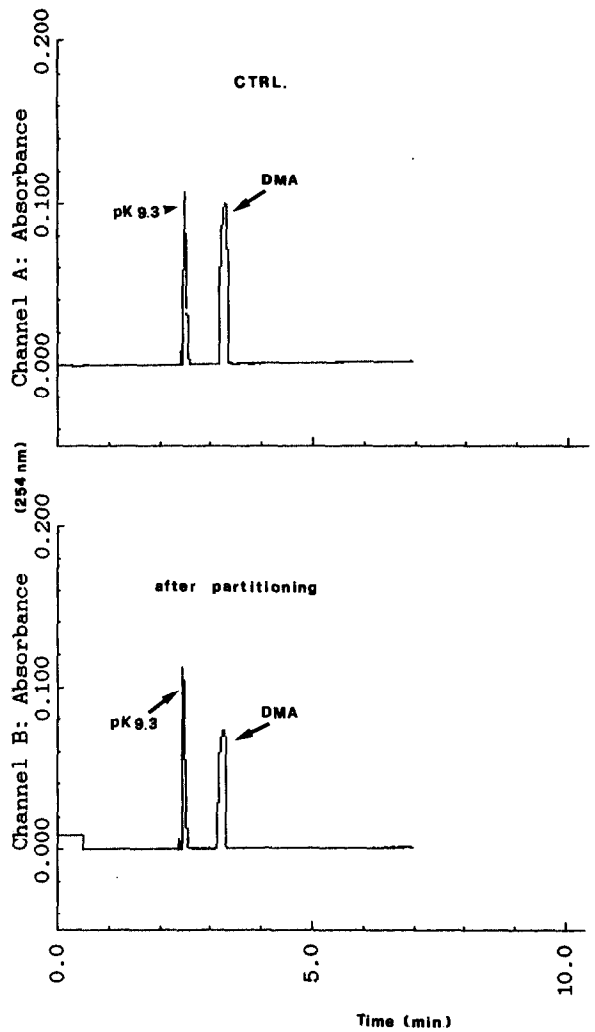


Fig. 5. Representative CZE runs for measuring partition coefficients (in the aqueous phase) of acrylamido monomers. Conditions: 100 mM borate–NaOH buffer (pH 9.0), 15 kV, 86 μA at 25°C. Uncoated fused-silica capillary (50 cm × 75 μm I.D.). Beckman P/ACE 2000 instrument, monitoring at 254 nm. DMA = N,N-dimethylacrylamide; pK 9.3 = Immobiline of pK 9.3 used as internal standard for peak integration.

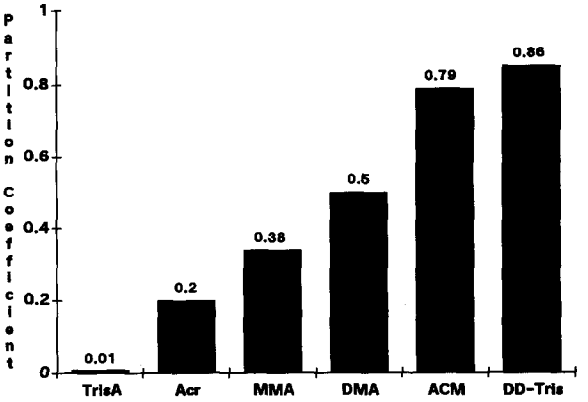


Fig. 6. Hydrophobicity scale for six acrylamide monomers. It was obtained by partitioning in water–1-octanol at room temperature and determining the concentrations in the two phases by CZE. Conditions: 100 mM borate–NaOH buffer (pH 9.0), 15 kV, 86 μA at 25°C. Fused-silica capillary (50 cm × 75 μm I.D.). Peak integration with the Beckman system Gold (pK 9.3 Immobiline was used as an internal standard). Abbreviations as in Table I.

general, trisacryl (as expected from its formula) is the most hydrophilic monomer: its P value is almost two orders of magnitude lower than that of DD-Tris. Acrylamide also exhibits good hydrophilicity whereas all other substituted acrylamides show progressively increasing hydrophobicity. It might be asked if there is a limiting value above which, owing to cooperative hydrophobic increments into the polymer, the gel matrix might be unable to re-swell in water. Empirically, we find this value to be located around P = 0.8 (the partition coefficient of dideoxytrisacryl), as poly(DD-Tris) becomes a white plastic, which collapses in the gravitation field and exudes all the water solvent.

There seems to be another undesirable feature associated with increasing hydrophobicity of the monomers: as the P value increases, the efficiency of incorporation into the growing polymer chain diminishes. This unique finding, previously unreported, is illustrated in Fig. 7; it is seen that in fact there is an inverse correlation between P and the extent of monomer incorporation. Under exactly the same polymerization conditions, ACM (P = 0.79) exhibits only 55% incorporation, while Tris-A (P = 0.01) is 95% incorporated. Fig. 7 also shows another unique finding: even the poorly reacting, more hydrophobic monomers can be incorporated as efficiently as Tris-A by increasing the polymerization temperature from 25 to 60°C. The three curves

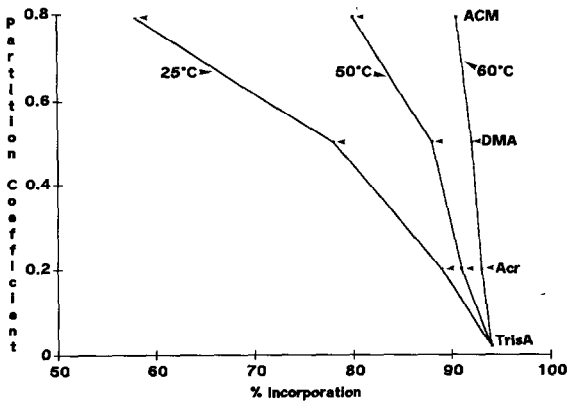


Fig. 7. Incorporation efficiency of different monomers *vs.* partition coefficient (*P*). There seems to be a unique inverse correlation between *P* and incorporation efficiency. The latter was assessed by polymerizing the gel into beads, extracting ungrafted monomers by extensive washing and determining them by CZE. Conditions: 100 m*M* borate–NaOH buffer (pH 9.0), 15 kV, 86 μA at 25°C. Fused-silica capillary (50 cm × 75 μm I.D.). Peak integration with the Beckman system Gold (p*K* 9.3 Immobiline as internal standard). Abbreviations: as in Table I. Note how, even for hydrophobic monomers, much better incorporation efficiencies can be obtained simply by increasing the polymerization temperature from 25 to 60°C.

seem to have as a pivotal point the Tris-A incorporation efficiency, the upper, slower reacting members rotating from a diagonal curve to a vertical position above the Tris-A point. These findings seem to be paradoxical, as the acrylamide polymerization reaction is known to be strongly exothermic. By using the Van 't Hoff equation, and plotting $-R\ln K$ *vs.* $1/T$ (Fig. 8), it is possible to calculate ΔH° for such reactions. It is seen that ΔH° for Tris-A and acrylamide conversion is essentially constant, whereas for the more hydrophobic members of the series it increases progressively with increasing temperature.

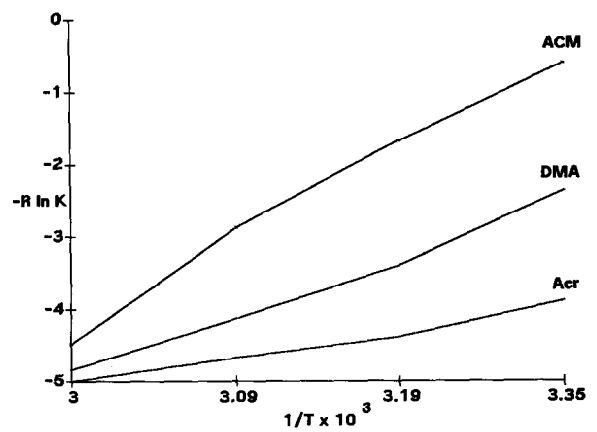


Fig. 8. Van 't Hoff plot of the data in Fig. 7. The equilibrium constant (*K*) represents the molar ratio between the incorporated and free monomer at the different temperatures. The slope of the different curves represents ΔH°. Note that ΔH° is essentially constant for Tris-A and acrylamide, whereas it increases progressively with increasing monomer hydrophobicity.

DISCUSSION

Our aim is to arrive at novel polyacrylamide gel formulations that satisfy the following requirements: high hydrophilicity, high resistance to alkaline hydrolysis and higher porosity. The reason is that in modern separation science there exist a dichotomy between the two most popular gel matrices: agaroses have been confined mostly to DNA separations [16], whereas polyacrylamides are utilized only for protein analysis [2,3]. However, as recently demonstrated with the Hydrolink matrices [17], there is a need for modified polyacrylamides that could properly sieve nucleic acids starting from oligonucleotides up to a few thousand base pairs (BP). This is a sort of "dark" region, where regular polyacrylamides sieve too much (in the Ogston model) [18] and agaroses are too porous. As demonstrated by Smith *et al.* [19], in a plot of log (BP) *vs.* %T, Hydrolink matrices are able to link diagonally the behaviour of polyacrylamides and agaroses, whose plots run parallel to each other at a distance of *ca.* three orders of magnitude (in sieving ability; see Fig. 1 in ref. 19). Hence novel monomers able to fulfil the above requirements would be welcome in electrokinetic separations. One such monomer appeared to be trisacryl; this molecule has been described by Boschetti's group [3] and represents the backbone of a number of highly hydrophilic ion exchangers for chromatography [20]. The amino-2-hydroxymethyl-2-propanediol residues create a micro-environment that favours the approach of hydrophilic solutes (proteins) to the polymer surface. As a result of grafting the "tris" moiety on to the acrylamido residue, the polyethylene backbone remains buried underneath a layer of hydroxymethyl groups. Such a matrix therefore has a pronounced advantage over polyacrylamide- or hydroxymethyl methacrylate-based supports, which have a hydrophobic character. Our data on such substituted acrylamides bring a unique insight into

the chemistry of polyacrylamides, which we shall discuss below.

Structure–stability relationship for N-substituted acrylamides

This is an argument that we have elucidated in the case of the Immobiline chemicals, which are mono-N-substituted acrylamides bearing a weak protolytic group. Although we agreed with the general knowledge on the greater stability of such compounds, as compared with unsubstituted amides, it was clear from our results [8–10] that there were other, more subtle mechanisms governing such stability. On the basis of our data, and of the known structures of these acrylamido derivatives, we derived the following rules:

(a) to afford protection of the amido bond, the most important parameter is not the degree of substitution in the nitrogen engaged in the amido plane (mono- or disubstituted) but the type of substituent;

(b) in particular, rigid ring structures are inefficient in protecting the adjacent amido bond, as their rigidity prevents them from oscillating in the surrounding space and thus shielding the amido plane;

(c) flexible chains bound to the nitrogen of the amido bond are capable of protecting the amido plane, as they can fluctuate in the nearby space and shroud the amido group;

(d) if rigid structures are present in the nitrogen substituents, they should be some distance from the plane of the amido bond;

(e) if a simple, flexible chain is present as a substituent on the nitrogen of the amido bond, greater protection of the latter is afforded by a longer chain.

None of these general rules applies to the inborn instability of the trisacryl monomer; this is due to the fact that the 2-hydroxymethyl group neighbouring the amido bond can form a hydrogen-bonded ring with the latter and, for electronic reasons, favour the hydrolytic attack (by a mechanism of N,O-acyl transfer) [21]. Thus, paradoxically, such a mono-substituted acrylamide degrades with a zero-order kinetics. However, more surprising, once this monomer is inserted into a polymer chain, the poly(trisacryl) becomes much more resistant to hydrolytic attack than a regular polyacrylamide. This novel finding could have interesting implications with regard to the three-dimensional structure of such a polymer. The added stability in the polymer could be due now to steric factors: it could be possible that the polymer matrix is stereoregular, perhaps due to helix formation of the poly(trisacryl) coil. We are currently trying to decode such a structure (if any) by small-angle soft neutron scattering. If we could demonstrate such spatial organization, it could be an interesting advance in decoding the structure of polyacrylamide matrices, still believed to be "a random meshwork of fibres".

Polymerization efficiency

Because the monomers are neurotoxins, one should always try to drive the reaction to completion, an almost impossible task, in fact, with radical polymerization. However, over the years, we have described conditions that ensure incorporation of the monomers into the growing chain to the extent of >95% [4–6]. An efficient removal of ungrafted monomers is also necessary in zone electrophoresis of proteins, as at alkaline pH values the double bond of unreacted acrylamide could easily add to free SH groups, forming a cysteine adduct [22]. An alternative to this could be the use of "washed" matrices, a technique we have introduced since 1980 when developing immobilized pH gradients (IPG). It is a routine, in IPGs, to wash the matrix extensively, dry it and re-swell it in any desired additive [11].

An unexpected finding was the strong dependence of the efficiency of conversion of the more hydrophobic monomers on the polymerization temperature. We had always advocated polymerizing standard polyacrylamide gels at 50°C (1 h reaction time), but the increase in conversion was from *ca.* 88% to 95% on going from 25 to 60°C (see Fig. 7). Considering that the heat of polymerization from aqueous monomer to aqueous polymer solutions is *ca.* 82.8 kJ/mol (a large amount of heat indeed), and that the overall activation energy for polymerizing in water–peroxodisulphate initiator is 70.7 kJ/mol, it is surprising that one could drive the reaction to even better yields by increasing the temperature, as we routinely do. Even more surprising is the great temperature dependence of the more hydrophobic monomers in the series; as shown in Fig. 7, for ACM, increasing the polymerization temperature from 25 to 60°C increases the efficiency from barely 55% to 94%. There might be several possible

explanations for this phenomenon: (a) the decrease in viscosity with higher temperature (nascent polymer chains would greatly augment the viscosity of bulk solution); (b) the possibility that the more hydrophobic elements of the series are not fully dissolved in water, but secluded into aggregates [6]; (c) the possibility that, as the hydrophobicity of the series increases, this is paralleled by an increase in overall activation energy (as also indicated by the increment in ΔH°, see Fig. 8). The present findings, hopefully, should terminate a 20-year-long disagreement among two main groups of polyacrylamide users: those who regularly polymerize at high temperatures and those who have been pestering us by suggesting polymerization in a thermostated ice-bath!

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