Polyacrylamide gels: monitoring of the crosslinking polymerization by means of fluorescent probes

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The crosslinking copolymerization of acrylamide (AA) and *N,N'*-methylenebisacrylamide (BA) has been monitored *in situ* by running the fluorescence spectra of a probe, pyrene, and a label, dansylacrylamide. This procedure has been applied to study the reaction at 25°C of different systems covering a broad range of AA and BA concentrations in the feed. In each case, the fluorescence intensity I_F of both dyes increases with an increase in the local viscosity during the course of the polymerization. Changes of I_F during the reaction allow one to determine the rate of polymerization. Three different reaction stages are clearly observed: pre-gelation, gelation and post-gel reactions. Evidence for post-gel reactions is found. It is at this stage of the reaction that some new domains of low polarity are formed and the morphological heterogeneity of the final gel is developed.

(Keywords: fluorescent probes; gelation; polyacrylamide; hydrogels)

INTRODUCTION

In the last few years we have been concerned[1–6] with the structural characterization of polyacrylamide (PAA) gels. It is well known that PAA gels are heterogeneous[1–12] on the scale of micrometres to nanometres. Such a conclusion has been reached by the analysis of various physical properties of the gels[1,2,6–12] and through direct determination of their microstructure[3–5].

Some authors[7–9] suppose that it can be explained by the existence of *N,N'*-methylenebisacrylamide (BA) aggregates in the feed mixture, which incorporates into the network, forming hydrophobic domains. The solubility of BA in water is 0.1 M (smaller than for acrylamide (AA)) but ^{1}H n.m.r. spectra reproduce[4] the gravimetric fraction of BA in aqueous solutions of AA and BA within a 1% error, and therefore BA aggregation must be considered negligible.

We think that the macroscopic heterogeneity of PAA gels is generated by some microheterogeneities[3], which are the consequence of the different reactivities of AA and BA[4,5]. For example, drifts in the feed cause[5] a certain microheterogeneity due to changes in the composition of the network portions formed at different instants of the reaction. On the other hand, BA is not randomly distributed but forms sequences of a number of monomeric units[4–6]; BA sequences give rise to multifunctional knots and decrease the crosslinking efficiency[1,2,6] by simple concatenation and through network defects like pendant vinyl groups. These microheterogeneities are very much dependent[1–6] on the crosslinker ratio (C in w/w)[13] and the total comonomer concentration (C_T in w/v)[13] in the feed.

Liquid–liquid phase separation is frequently operative in crosslinking copolymerizations of vinyl–divinyl type and it gives rise to heterogeneous structures[14]. It can be induced by an increase of crosslinking density or by a change in the solvent thermodynamic power. The critical values for phase separation may be reached at any instant of the reaction, before or after gelation. It has been suggested[12] that a hypothetical phase separation could play an important role in determining the morphology of PAA gels. Phase separation can be detected through turbidity or light scattering measurements during the reaction.

Here we intend to determine how microheterogeneities give rise to the final biphasic structure of the gel obtained at total conversion. To that end, we use fluorescent chromophores dissolved in an aqueous medium (pyrene) or attached to comonomeric unit (dansylacrylamide). The fluorescence of probes and labels has recently been found to be very useful in studying homopolymerizations or cure reactions of epoxy resins[15–19]. The fluorescence spectra also allow the measurement of the light scattering at the excitation wavelength, and therefore we also expect to detect the existence of phase separation at any instant of the reaction.

EXPERIMENTAL

AA and BA were purchased from Eastman Kodak and used without further purification. The dyes used were: pyrene (Py), purchased from Eastman Kodak; 1-pyrenebutanol (PyBuOH) and dansyl chloride (DNS), both from Polysciences. Py was purified by several recrystallizations in methanol. DNS was left overnight to react with AA (slightly in excess) in a concentrated aqueous solution. The end-point of the reaction was checked by spectrophotometry.

In all cases the initiator concentration (3×10^{-3} M potassium persulphate/6×10^{-3} M triethanolamine) was

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the same. Two pattern solutions were mixed in the quartz cell at time 0: one containing BA and potassium persulphate and the other containing AA and triethanolamine. This procedure ensures the observation of the real spectra at time 0 but forces one to use small volumes of the pattern solutions (1 and 3 ml respectively); as a consequence, an uncertainty of 5% in the BA concentration and 1% in the AA one, must be expected.

Two sets of experiments must be distinguished. In the first case, the solvent used was a saturated aqueous solution of Py or PyBuOH filtered with 0.2 μm pore size. In the other set, dansylacrylamide was added to the feed in a small proportion in such a way that its optical density was always less than 0.5 at the excitation wavelength. In this way, the concentration of labelled AA was less than 1.5×10^{-4} M in each case, and that makes a percentage of less than 0.02% with respect to the total AA in the feed. The very small fraction of DNS–AA ensures that it will be randomly distributed independently of its relative reactivity.

The total comonomer concentration and the crosslinker ratio of both sets of experiments are summarized in *Table 1*. All the samples were in the transparent gel zone of the phase diagram[13].

The fluorescence spectra were recorded in a Perkin–Elmer LS-3 fluorometer at 25°C. The excitation wavelength was 315 nm for Py (to minimize the overlap of light scattering and fluorescence spectra) and 320 nm for DNS. Broad slits with 5 and 10 nm bandpass were employed in excitation and emission, respectively. It was necessary to make the fluorescence intensity of the very dilute Py solutions measurable, but this made it impossible to achieve good vibrational resolution of the fluorescence spectra. The fluorescence intensity I_F of the dyes was always referred to the I_F of external standards purchased from Perkin–Elmer. We have found that this method is reproducible within less than the experimental error: 2% of I_F. Nitrogen bubbling increases I_F but disturbs the gelation process; therefore, aerated solutions were used.

The light scattering intensity I_{LS} can be measured on the fluorescence spectra through the height of the Raman light scattering peak (*Figure 1*).

Table 1 Composition of the feed mixtures and rate of polymerization during gelation M_g and post-gel M_{pg} reactions. The chromophore used was pyrene except when otherwise stated

Sample	C (%) (w/w)	C_T (%) (w/v)	$M_g \times 10^4$ (min^{-1})	$M_{pg} \times 10^4$ (min^{-1})
P3(1)	1.00	2.93	1.30	0.72
P4(1)	1.00	4.00	3.85	1.67
P5(1)	1.00	5.00	3.33	1.82
P6(1)	1.00	6.00	5.26	1.89
P7(1)	1.00	7.00	9.10	2.13
P5(1)	0.99	4.80	3.57	1.10
P5(2)	2.02	4.80	5.88	1.37
P5(2.5)[a]	2.57	4.74	–	–
P5(3)	2.79	4.80		
P5(4)	4.17	4.80	7.69	1.85
			20.00	–
D5(1)[b]	1.05	4.85		
D5(5)[b]	5.09	4.86		
D7(0.5)[b]	0.45	7.00		
D7(1)[b]	1.01	7.00		

[a] The probe was PyBuOH
[b] The label was DNS-AA

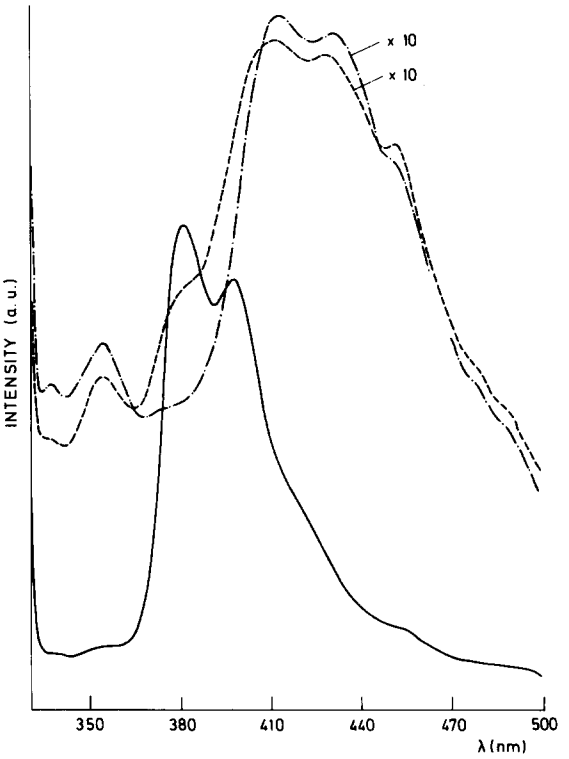

Figure 1 Fluorescence spectra of PyBuOH in aqueous saturated solution (——), in aqueous solution with BA (– – – –) and in aqueous solution with AA and BA (–·–·–)

RESULTS

Fluorescence spectra of dyes

The solubility of Py and PyBuOH in water is very low (10^{-6} M) but their fluorescence spectra are clearly observed (*Figure 1*), peaking at about 380 nm. In aqueous solutions of AA and BA, the fluorescence of both dyes is strongly quenched and a new band red-shifted to about 410 nm is observed under some conditions (*Figure 1*). The intensity ratio I_{410}/I_{380} increases with BA concentration and is larger for PyBuOH than for Py. The new band can thus be ascribed to exciplex emission[20].

The DNS fluorescence spectrum depends on the polarity of the solvent surrounding the label. In pure water and in the aqueous solution of AA and BA, the maximum emission is observed at 500 nm, whereas in solvents of lower polarity, the spectrum is blue-shifted (e.g. $\lambda = 460$ nm in tetrahydrofuran).

Py has been used like a probe, that is to say, just dissolved in the feed mixture. DNS was anchored in AA (see the 'Experimental' section) and the labelled monomer was added to the feed in a very small ratio with respect to unlabelled AA (less than 0.02%).

Time course of the polymerization

Following the method described in the 'Experimental' section, samples with the initial conditions summarized in *Table 1* were prepared in the quartz cell and the polymerization was then monitored by running fluorescence spectra of the dye every 10 min over the course of 4 h. In each case, we have observed that I_F increases during polymerization, as shown in *Figures 2* and *3* for Py.

In accordance with previous results[3], three different

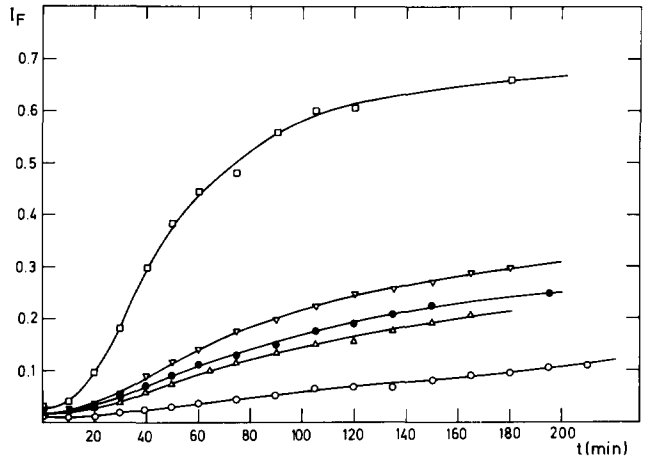

Figure 2 Fluorescence intensity I_F of Py (relative to an external standard) as a function of polymerization time in different systems, which differ in the total comonomer concentration in the feed C_T: (□) P7(1), (▽) P6(1), (△) P5(1), (●) P4(1) and (○) P3(1) (see *Table 1*)

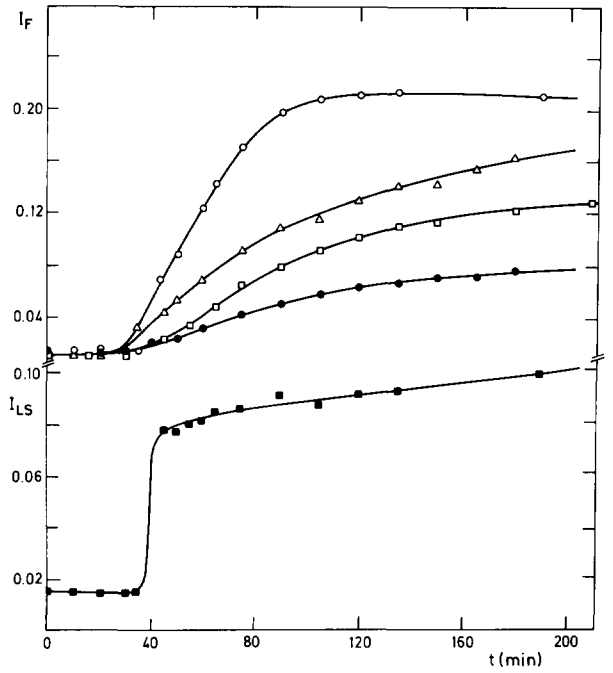

Figure 3 Fluorescence intensity I_F of Py (relative to an external standard) as a function of polymerization time in different systems, which differ in the initial crosslinker ratio C: (○) P5(4), (△) P5(3), (□) P5(2) and (●) P5(1) (see *Table 1*). The light scattering intensity I_{LS} of the system P5(4) has also been plotted (■)

stages of the reaction can clearly be observed in *Figures 2* and *3*. In the first stage (pre-gelation), I_F and I_{LS} remain constant. Nevertheless, on pouring the reaction mixture into methanol, a precipitate appears, which proves that soluble pre-network particles are being formed in the pre-gelation stage.

I_F and I_{LS} increase abruptly in the second stage or gelation process (*Figure 2*). The gel point depends on C_T more than on C (*Figures 2* and *3*). I_F is related to the rate constants for radiative K_F and non-radiative decay K_{NR} through the following expression:

$$I_F = K_F/(K_F + K_{NR}) \quad (1)$$

K_F does not depend on temperature or on the environmental characteristics of the chromophore. The increase of I_F during polymerization must be ascribed to the decrease of K_{NR} as a consequence of the increasing rigidity (or microviscosity) of the domains in which the chromophore is placed. The slope of I_F *versus* t plots (M_g or $M_{pg} = dI_F/dt$) represents a measurement of the rate of polymerization. The value of dI_F/dt depends on the size of the probe, and thus only the experiments made with Py have been considered in determining the rate of polymerization. During gelation M_g is the largest and increases with C and C_T (*Table 1*). Double logarithmic plots of M_g *versus* C and C_T result in straight lines with slopes equal to 1.0 and 2.0, respectively.

I_{LS} increases abruptly at the gel point simultaneously with I_F. This means that during gelation large structures are being formed by bonding of pre-network particles. It suggests, too, that there is no phase separation, which would yield an additional increment of I_{LS} independently of changes in I_F. But some more research is needed on that point.

Post-gel reactions

After gelation the rate of polymerization decreases (*Table 1*) (with respect to gelation) and I_{LS} increases slowly (*Figure 3*). It is known[3,4] that, in this last stage, changes in conversion are very small, that is to say, free monomers are incorporated into the network in very small amounts that depend on the feed mixture. Nevertheless, changes of I_F and I_{LS} show that some reactions or physical processes are taking place in the system.

The rate of polymerization in this stage (M_{pg}, *Table 1*) is less than half of M_g. The value of M_{pg} increases with C and C_T but with orders of reaction (0.5 and 1.0, respectively) smaller than during gelation. Diffusion-controlled processes are probably unimportant in that respect since post-gel reactions do not involve free monomeric units but pendant vinyl groups. We believe that the decrease of the rate of polymerization and the order of reaction is due to the smaller reactivity of pendant vinyl groups with respect to the divinylic BA unit involved in gelation. Pendant vinyl group reactions were previously observed in post-gel reactions of styrene–divinylbenzene crosslinking copolymerizations[21].

I_F of dansylacrylamide changes during polymerization like I_F of Py or PyBuOH except in the post-gel stage. The fluorescence spectrum of dansylacrylamide during pre-gelation or gelation shows only one band, peaking at 500 nm, the intensity of which remains constant in the pre-gel stage and increases abruptly during gelation. Just after gelation, a new band appears, peaking at 420 nm (*Figure 4*). The emission at 500 nm corresponds to dansyl groups surrounded by water, whereas the emission at 420 nm corresponds to domains of much lower polarity. The apolar domains grow at the expense of the domains previously swollen by water, and as a consequence I_F measured at 500 nm decreases slightly during post-gelation whereas the total emission intensity increases like for Py.

It is interesting to remark that (i) the new apolar domains are formed mostly during post-gel reactions (*Figure 5*) and (ii) their composition is a definite one. There is not a continuous shift of the 500 nm band to the new one (*Figure 4*). This means that the apolar domains nucleate in unswollen regions and remain hydrophobic when growing.

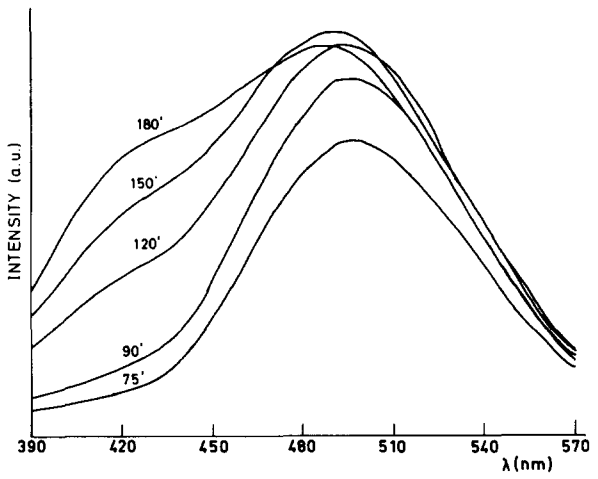

Figure 4 Fluorescence spectra of dansylacrylamide at different instants of the polymerization of the system D7(1) (see *Table 1*)

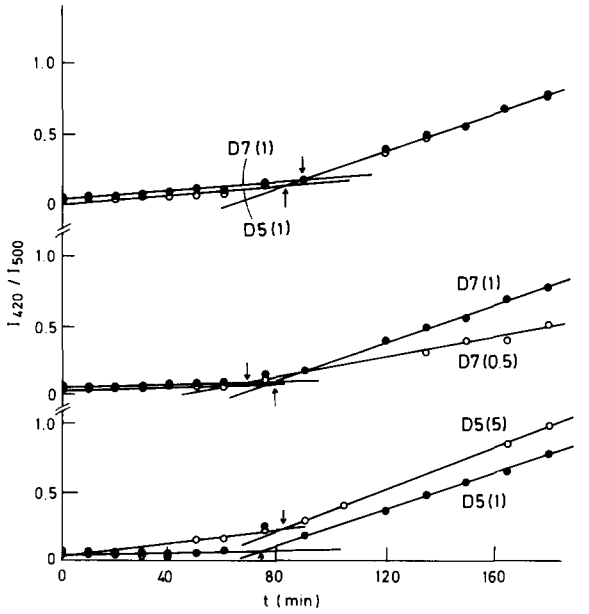

Figure 5 Fluorescence intensity ratio of dansylacrylamide as a function of polymerization time for the different systems indicated in the figure. The arrows mark the beginning of post-gel reactions and the slopes represent the rate of appearance of apolar domains

The rate of formation of apolar domains is very sensitive to changes of C in the feed but it does not change much with C_T (*Figure 5*). All these results suggest that BA sequences (which are larger as C becomes large but do not depend much on C_T[4,5]) nucleate the formation of hydrophobic domains through the reaction of pendant vinyl groups. Those post-gel reactions make the gel more compact (I_F increasing) and more heterogeneous (I_{LS} increasing too). Therefore, attention must be paid to the curing time of PAA gels, since it may modify their structure and draining properties.

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REFERENCES

1 Baselga, J., Piérola, I. F., Hernández-Fuentes, I. and Llorente, M. A. *Makromol. Chem., Macromol. Symp.* 1981, **20/21**, 369
2 Baselga, J., Hernández-Fuentes, I., Piérola, I. F. and Llorente, M. A. *Macromolecules* 1987, **20**, 3060
3 Nieto, J. L., Baselga, J., Hernández-Fuentes, I., Llorente, M. A. and Piérola, I. F. *Eur. Polym. J.* 1987, **23**, 551
4 Baselga, J., Llorente, M. A., Nieto, J. L., Hernández-Fuentes, I. and Piérola, I. F. *Eur. Polym. J.* 1988, **24**, 161
5 Baselga, J., Llorente, M. A., Hernández-Fuentes, I. and Piérola, I. F. *Eur. Polym. J.* 1989, **25**, 471
6 Baselga, J., Llorente, M. A., Hernández-Fuentes, I. and Piérola, I. F. *Eur. Polym. J.* 1989, **25**, 477
7 Hsu, T. P. and Cohen, C. *Polymer* 1980, **25**, 1419
8 Gupta, M. K. and Bansil, R. *J. Polym. Sci., Polym. Lett.* 1983, **21**, 969
9 Richards, E. G. and Temple, C. J. *Nature (Phys. Sci.)* 1971, **22**, 92
10 Nossal, R. *Macromolecules* 1985, **18**, 49
11 Weiss, N., Van Vliet, T. and Silberberg, A. *J. Polym. Sci. (A-2)* 1979, **17**, 2229
12 Janas, V. F., Rodriguez, F. and Cohen, C. *Macromolecules* 1980, **13**, 977
13 Bansil, R. and Gupta, M. K. *Ferroelectrics* 1980, **30**, 63
14 Dusek, K. *Chem. Zvesti* 1971, **25**, 184
15 Sung, C. S. P., Pyun, E. and Sun, H. L. *Macromolecules* 1986, **19**, 2922
16 Mathisen, R. J., Yoo, J. K. and Sung, C. S. P. *Macromolecules* 1987, **20**, 1414
17 Wang, F. W., Lowry, R. E., Pummer, W. J., Fanconi, B. M. and Wu, E. S. in 'Photophysics of Polymers' (Eds Ch. E. Hoyle and J. M. Torkelson), ACS Symp. Ser., American Chemical Society, Washington, DC, 1987
18 Wang, F. W., Lowry, R. W. and Grant, W. H. *Polymer* 1984, **25**, 690
19 Levy, R. L. and Ames, D. P. *Proc. Org. Coat. Appl. Polym. Sci.* 1983, **48**, 116
20 Gordon, M. and Ware, W. (Eds). 'The Exciplex', Academic Press, New York, 1975
21 Hild, G., Okasha, R. and Rempp, P. *Makromol. Chem.* 1985, **183**, 407