cient with molecular weight, for which we used the results of Berry.[18]

Our results suggest that α decreases first rapidly and then gradually as concentration increases. Such behavior has been predicted by some authors.[1-3,5,6] However, further measurements are required for more precise comparison with these predictions.

Experiments are under way to determine the dimensions over a wider concentration range, including the bulk state, and to study the effects of solvent and molecular weight of polymer.

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Kinetic Studies on the Conformational Transition of the Copolymer of Styrene and Maleic Acid in Aqueous Salt Solution

The pH-induced conformational transition of the hydrophobic poly(carboxylic acids) in aqueous salt solutions has been studied by many authors,[1-5] and the transition has been found to take place from a compact form in the non-charged state to an expanded coil form in the fully charged state. Such polyacids may be useful to study the intramolecular hydrophobic interaction, which may contribute to the structural stability of the globular proteins. Recently, two research groups[6,7] found a similar pH-induced conformational transition of an alternating copolymer of styrene and maleic acid (St–MA). The thermodynamic analysis of the transition in aqueous NaCl solution was investigated with data for fractionated samples from pH titration, viscosity, and dilatometric measurements.[6] The compact form at small degrees of dissociation of the primary carboxyl group seems to be stabilized by the hydrophobic interaction between phenyl residues buried in the compact polymer, and the coil form appears to have exposed phenyl residues. Therefore ultraviolet absorption of the phenyl group may reflect the conformational transition.

Kinetic studies of the transition by means of measurements of change in ultraviolet absorption of the phenyl group are reported here. Although there have been many equilibrium studies of the conformational transition of the hydrophobic poly(carboxylic acids), no kinetic studies have been found.

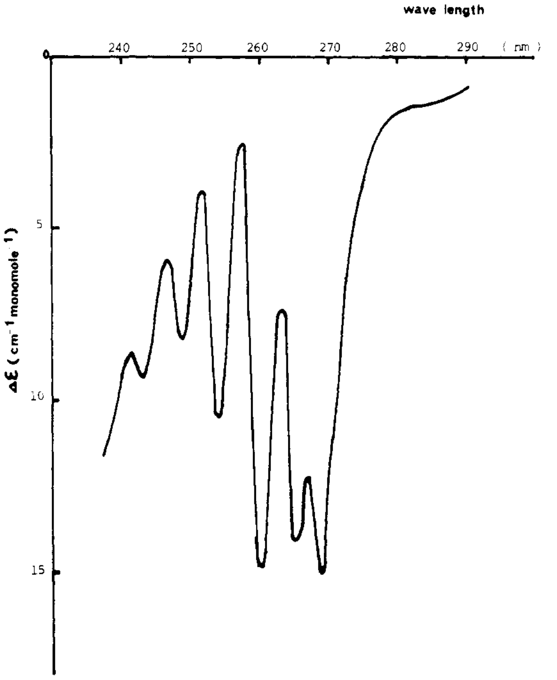

Figure 1. Difference spectrum of St–MA with $\alpha = 0.18$ in aqueous NaCl solution (polymer concentration = 0.0123 monomol/l. $I = 0.0091$, mol wt = 580,000).

St–MA was obtained and fractionated by the method reported previously.[6] The reagents used here and the method of preparation of the aqueous NaCl solution of St–MA were also identical.[6] Difference spectra were obtained with a Hitachi Perkin-Elmer spectrophotometer 139 or EPU-2A having a sample and reference temperature control at 25.0 ± 0.25°. As a reference the aqueous NaCl solution of St–MA completely in the coil form with the degree of dissociation of the primary carboxyl group $\alpha = 0.94$ was used. The kinetic measurements were done at 25.0 ± 0.25° with use of a rapid reaction analyzer (Union Giken, RA-1100)[8] whose dead time was less than 4 msec. The α jump of the polymer solution occurred during rapid mixing of equal volumes of polymer–NaCl and NaOH–NaCl. The change in transmittance at 269 nm of the polymer solution due to the α jump at constant NaCl concentration was recorded by use of a digital memory (Union Giken, RA-108S) and a pen recorder. The α value of the solution before mixing α_0 was determined by pH measurements with a Radiometer pH meter M-4 calibrated by use of the standard buffers, and its change $\Delta\alpha$ due to mixing was calculated from the quantities of NaOH introduced.

In Figure 1, an example of the difference spectra of the aqueous NaCl solution of St–MA is shown. The spectrum has some peaks at 269 nm and so on, and such a red-shift spectrum may be characteristic of the phenyl group buried in the hydrophobic domain.[9] The difference between the molar extinction coefficients of the sample and the reference at 269 nm, $\Delta\epsilon_{269}$, decreases in a sigmoidal fashion with increasing α in the case of low ionic strength I as shown in Figure 2. In the case of $I = 0.27$, molecular aggregation seems to affect the change in $\Delta\epsilon_{269}$ in the region of small α.

$\Delta\epsilon_{269}$ may be taken to be composed of two terms: perturbation of the phenyl group produced by change in the charge on the nearby carboxyl group in the same molecule, $\Delta\epsilon_{269}{}^{e}$, and that by change of environment near the phenyl group, $\Delta\epsilon_{269}{}^{t}$. The former should be proportional to this

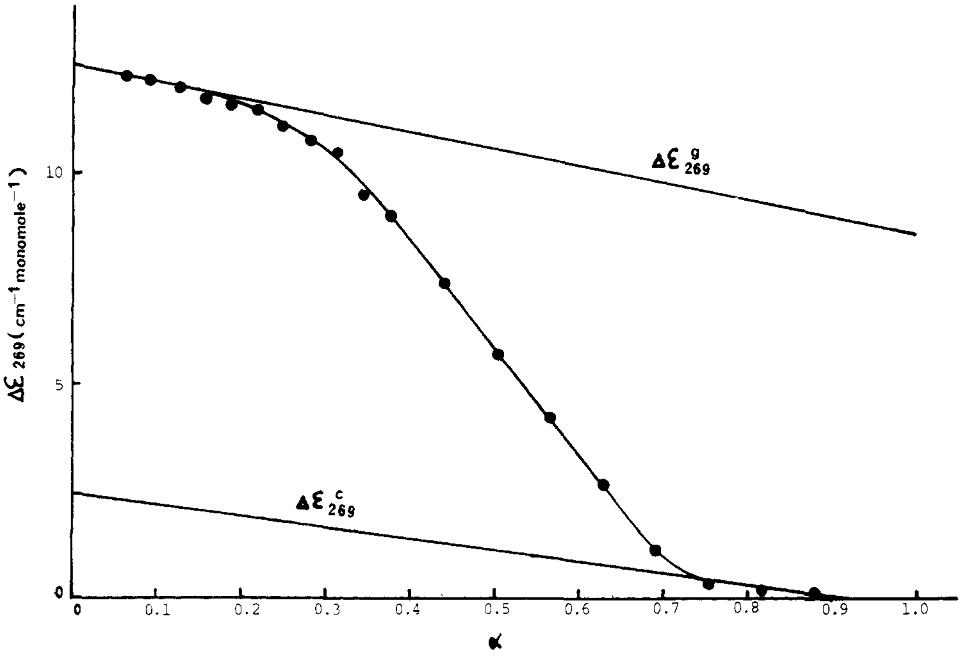

Figure 2. Dependence of $\Delta\epsilon_{269}$ on α in a case of $I = 0.091$ (polymer concentration = 0.0141 monomol/l., mol wt = 580,000).

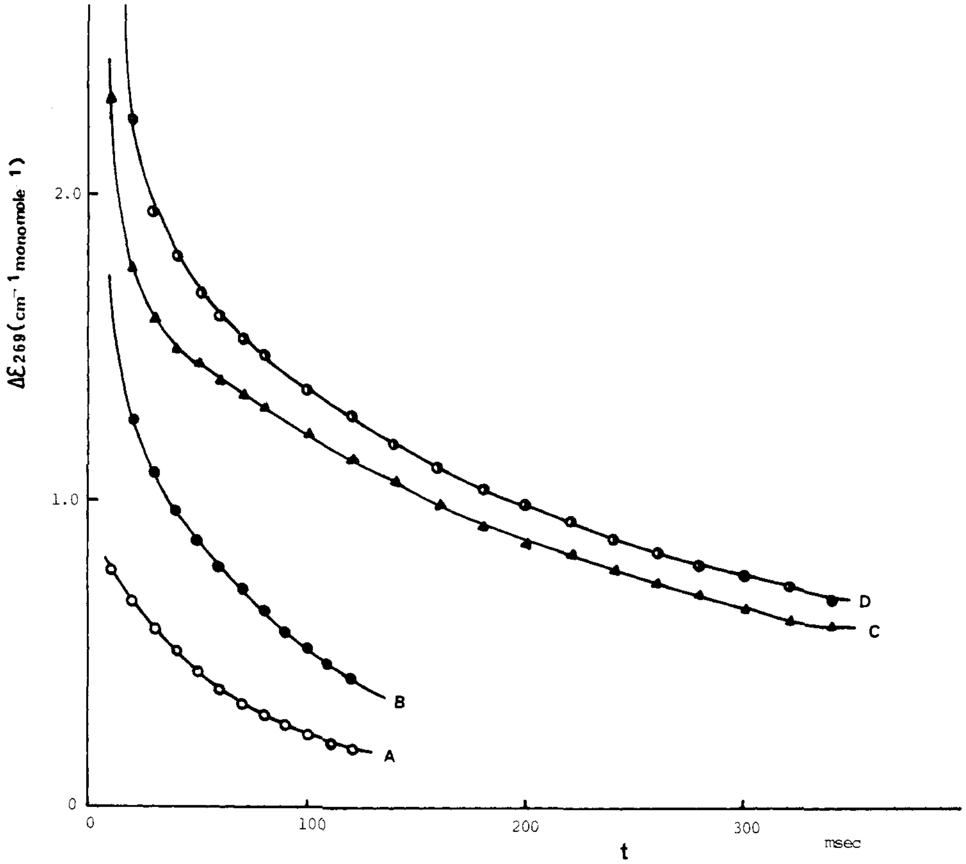

Figure 3. Kinetic trace of $\Delta\epsilon_{269}$ in a case of $I = 0.091$ and $\alpha_0 = 0.17$ (polymer concentration = 0.0135 monomol/l., mol wt = 450,000): α_∞ = 0.24 (A), 0.31 (B), 0.44 (C), and 0.64 (D).

difference between α's of the sample and the reference. Therefore, the sigmoidal changes in $\Delta\epsilon_{269}$ suggest that the difference spectra are mainly caused by the conformational transition accompanying the exposure of the phenyl group. The changes reflect very well the conformational transition estimated previously with pH titration.

In Figure 2 the linear relation of $\Delta\epsilon_{269}$ to α in the range of the completely compact or coil form is extrapolated to obtain $\Delta\epsilon_{269}$ for the hypothetical compact or coil form over the whole range of α studied. Such values of $\Delta\epsilon_{269}$ for the hypothetical forms are similar to that of $\Delta\epsilon_{269}{}^{e}$ of phenylalanine.[9] With the assumption used by Leyte and Mandel for the conformational transition of poly(methacrylic acid) (PMA)[1] that the transition of St-MA occurs between the compact and coil forms, dependence of the coil form fraction on α can be obtained from the difference spectra data.

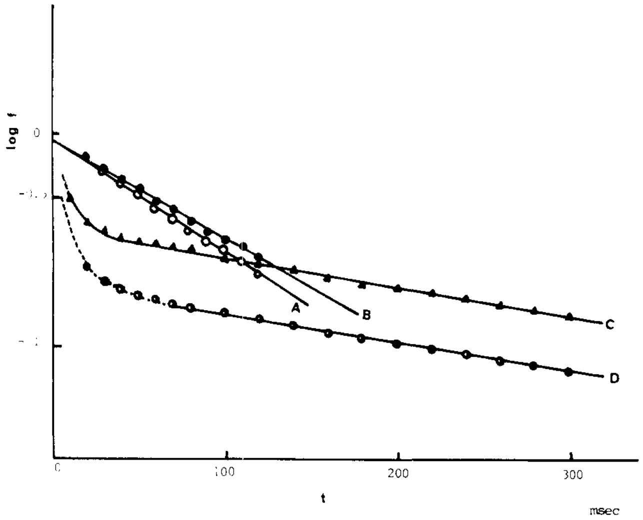

Figure 4. Semilogarithmic plots of the kinetic data in Figure 3. $f = \Delta\epsilon_{269}$/total change in $\Delta\epsilon_{269}$ expected from Figure 2.

Table I
Mean Relaxation Times (msec)

Polymer concn, monomol/l.	Ionic strength	α_0	α_∞	τ^a	$\tau_s{}^b$
0.0135	0.0091	0.07	0.20	100	
			0.27	150	
			0.41		290
			0.54		370
	0.091	0.17	0.24	80	
			0.31	100	
			0.44		300
			0.64		350
	0.27	0.44	0.58		290
			0.64		300
			0.78		400

[a] τ: mean relaxation time of the change in $\Delta\epsilon_{269}$ due to the α jump with small $\Delta\alpha$. [b] τ_s: mean relaxation time of the slow process with large $\Delta\alpha$.

The fraction from the present optical data coincides very well with that from the pH-titration results.

Figure 3 shows the kinetic trace at 269 nm after the α jump from the compact form of St–MA in solution with $I = 0.091$. The changes in $\Delta\epsilon_{269}$ are apparently due to first-order processes in the case of small $\Delta\alpha$ as shown in Figure 4, in which semilogarithmic plots of the normalized kinetic data are shown. However, in cases of large $\Delta\alpha$, they are not due to first-order processes, but occur in two distinct time stages. Change in $\Delta\epsilon_{269}{}^e$ may be too fast to measure with the present apparatus, in which case the changes in $\Delta\epsilon_{269}$ in Figures 3 and 4 should be defined in terms of the conformational transition. In the cases of $I = 0.0091$ and 0.27, the changes in $\Delta\epsilon_{269}$ after the α jump with small $\Delta\alpha$ are apparently first-order processes. However, the α jump with large $\Delta\alpha$ to near the midpoint of the transition or beyond produces a very rapid process accounting for most of the optical change accompanied by another slow process of the first order.

In Table I the mean relaxation time of the change in $\Delta\epsilon_{269}$ due to the α jump with small $\Delta\alpha$ is shown. It increases with increase of α_∞, the degree of dissociation of the primary carboxyl group after mixing, or decrease of ionic strength. A possible cause of such a process is reorientation of the molecule in the nearly compact form. Wada, *et al.*, have pointed out the slow relaxation process of PMA due to its reorientation.[10]

The mean relaxation time of the slow process with large $\Delta\alpha$ is also shown in the same table; the fast process related to the conformational transition has a very short relaxation time which could not be determined exactly with the present apparatus. The slow process may also be expressed in terms of molecular reorientation in the transition region or in the nearly coil form.

In any case a conformational transition of St–MA in NaCl solution was observed which was faster than the denaturation process of a typical globular protein, such as lysozyme; however, the change was considerably slower than the helix–coil transition of poly(α-amino acids) in solutions. The relaxation time of the transition of St–MA may be near the dead time of the present apparatus. A more detailed study of the kinetic trace of the hydrophobic poly(carboxylic acid) should be made with other methods, such as the T-jump method.

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