

Ester fission catalysed by microgels possessing hydroxamic acid groups: structure–reactivity studies

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Abstract

Second order kinetics of the reaction of polymer-attached hydroxamic acids with substituted phenyl acetates (k_{pol}) have been measured in aqueous solution at 25°C and shown to follow the following empirical rate law for degrees of ionisation (α) ranging from 0.1 to 1: $k_{\text{pol}} = a \cdot \alpha + b \cdot e^{-c/\alpha}$. The slopes of the plots of k_{pol} against α measures the reactivity of the polymer (k_{react}) at given values of α and these increase as α is increased.

The reactivity against substituted phenyl acetates at given α values increases with increasing cross-linking content of the microgel support. The reactivities at $\alpha = 0.1$ and 0.9 obey the Brønsted equations:

$$\log k_{\text{react}} (\alpha = 0.1) = -0.55 \pm 0.065 pK_a^{\text{ArOH}} + 3.26 \pm 0.44 \quad (n = 5; r = 0.9799)$$

$$\log k_{\text{react}} (\alpha = 0.9) = -0.37 \pm 0.06 pK_a^{\text{ArOH}} + 3.13 \pm 0.43 \quad (n = 5; r = 0.9594)$$

The similarity between the Brønsted β values for the microgel-supported hydroxamate nucleophile at low degree of ionisation (α) and monomer hydroxamate ion is consistent with the microscopic medium of the transition-state's being water-like. At a high α value the less negative β value indicates a smaller charge change in the transition state; this result and the effect of cross-linking on reactivity supports the hypothesis that the substantial reactivity increases are due to hydroxamate ions buried in the microgel particle and electrophilic assistance of nucleophilic attack.

De-cinnamoylation of *O*-cinnamoylated microgel-supported hydroxamic acid is only some ten-fold less reactive to hydroxide ion than is the model *O*-cinnamoyl-*N*-benzoylhydroxylamine. The polymer deacylation possesses a significant water term which means that at low pH it is faster than that of the model reaction; intramolecular catalysis by carboxylate ions is held responsible for this acceleration.

Keywords: Esters; Fission; Hydroxamic acid groups; Microgels; Reactivity; Structure

1. Introduction

The study of polymers as catalysts and reagents is of interest because it may lead to a better understanding of enzyme reactivity and to the preparation of inexpensive, stable catalysts able to work in a variety of conditions which would degrade

these biological catalysts. Water-soluble polymeric catalysts have been widely investigated as models of hydrolytic enzymes [1] and have been shown to act by complexation with substrate.

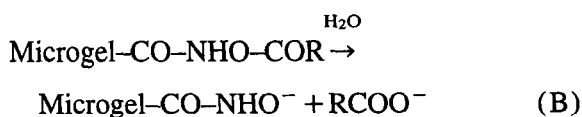
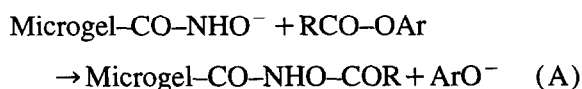
The reactivity towards esters of hydroxamate ions attached to microgel particles increases with increasing degree of ionisation (α) [2]; at high values of α the reactivity can dramatically exceed that of the monomeric hydroxamate anion with the same substrate. The remarkable nucleophilic

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reactivity is ascribed to penetration of substrate into the microgel particle followed by reaction with buried hydroxamate ions attached to the polymer [2a,b]. Increasing the size of the ester decreases the change in reactivity consistent with the reagent's not being able to burrow into the microgel. Negatively charged esters do not show enhanced reactivity with negatively charged microgels because they are electrostatically repelled and are prevented from penetration. The observation of stereoselectivity in the reaction of microgel–hydroxamate polymers prepared from optically active monomer is also consistent with reaction with buried nucleophiles [2c].

Because of the general interest in the reactivity of polymer groups it was decided to investigate the origins of the enhanced reactivity of microgel–hydroxamate ions further. We have carried out a structure–reactivity study where the reactivity is studied as a function of the cross-linking content of hydroxamate-containing microgels. The effect of polar substituents on reactivity is recognised as a sensitive probe of mechanism because it enables measurements of change in charge to be made between reactant and transition states [3]. In the present case comparison is made with polar substituent effects in reactions of well-defined mechanism.

The function of the microgel–hydroxamate ion as a catalyst (Eqs. A and B)



is also of interest and it was decided to study the deacylation step which regenerates active catalyst. The hydrolysis of the cinnamoylated microgel is studied kinetically as a model of the deacylation of the acylated microgel; the cinnamoyl group was chosen because of the ease with which the reaction could be followed spectrophotometrically.

2. Experimental

2.1. Materials

Methyl methacrylate, methacrylic acid, ethylene dimethacrylate and 2-hydroxyethyl methacrylate were obtained from BDH Ltd; the stabilising inhibitors in each of the monomers were removed by shaking with dilute NaOH or by distillation in vacuo. 4-Nitrophenyl acetate was purchased from Aldrich and purified by recrystallisation from benzene. The other acetates were prepared as described in previous work [4].

O-Benzoyl-*N*-methacryloylhydroxylamine [5] was prepared from *N*-methacryloylhydroxylamine (prepared by the method of Smith [[5]]) by reaction with benzoyl chloride in the presence of base catalyst. Cinnamoyl chloride and *N*-benzoylhydroxylamine were purchased from Aldrich.

O-Cinnamoyl-*N*-benzoylhydroxylamine was prepared by slow addition of cinnamoyl chloride (1.83 g) to a solution of *N*-benzoylhydroxylamine (1.37 g) and triethylamine (1.01 g) in tetrahydrofuran (25 ml) at 0–5°C. The mixture was stirred for 1 h after which the reaction mixture was allowed to warm to room temperature. The solution was extracted with dilute HCl, with a saturated solution of NaCl and with water. The solution was then dried and evaporated to give a crude solid which on recrystallisation with ethyl acetate gave needles, m.p. 174–176°C. Found: C, 72.2; H, 4.9; N, 5.4% C₁₅H₁₃NO₃ requires: C, 71.9; H, 4.9; N, 5.2%.

O-Cinnamoyl-*N*-methacryloylhydroxylamine was prepared in a similar manner to that above. *N*-Methacryloylhydroxylamine (2.04 g) and triethylamine (2.02 g) were dissolved in benzene (100 ml) and the solution cooled to 5–8°C. Cinnamoyl chloride (3.09 g) was then added slowly with stirring. After the addition the solution was extracted with dilute HCl and water, dried with MgSO₄ and evaporated. The crude product was recrystallised from toluene to yield white needles, m.p. 95–96°C. Found: C, 66.9; H, 5.6; N, 6.2% C₁₃H₁₄NO₃: C, 67.5; H, 5.7; N, 6.1%.

Table 1
Physical constants of the polymers

	Polymer-1	Polymer-2	Polymer-3	Polymer-4	Cinnamoylated polymer
	Components of monomer feed/mole percentage				
methyl methacrylate	30.5	15	5	30	30.5
methacrylic acid	8.5	10	10	10	8.5
2-hydroxyethyl methacrylate	27.4	30	30	30	27.4
ethylene dimethacrylate	3.6	15	25	25	3.6
<i>O</i> -benzoylmethacryloylhydroxamic acid	30.0	30.0	30.0	5.0	
<i>O</i> -cinnamoyl- <i>N</i> -methacryloylhydroxylamine					30
cross-linking/mole percent ^a	3.6	15	25	25	3.6
pK_a ^b	8.2	8.8	9.2	8.5	–
n ^b	1.4	1.9	1.3	1.9	–
Diameter/nm ^c	110	105	120	115	123

^a Taken as the mole percentage of ethylene dimethacrylate in the monomer feed.

^b The pK_a and n refer to the equation in the text.

^c Measured in aqueous solution at 25°C, pH 9.0.

The materials were checked for purity by TLC on silica plates and the structures confirmed by NMR and IR spectroscopy.

Other materials were of analytical reagent grade or were recrystallised or redistilled from bench grade reagents.

Emulsion polymerisation was carried out with monomer feed (5 ml) comprising methyl methacrylate, 2-hydroxyethyl methacrylate, 1,2-ethylene dimethacrylate, methacrylic acid and *O*-benzoyl-*N*-methacryloylhydroxylamine (in the molar ratios given in Table 1). Double distilled water (100 ml) was deaerated, placed in a thick-walled glass bottle with a screw-cap fitted with a polythene liner and placed in an oil bath set at 60–70°C. Sodium lauryl sulphate (100 mg) was added and when dissolved the monomer feed (5 ml) with ethyl acetate (5 ml) was added. The mixture was stirred and purged with nitrogen, potassium persulphate (50 mg) added and the bottle sealed. After about 2 h the characteristic opalescence had appeared. The polymerisation was stopped by the addition of hydroquinone (50 mg), the product cooled and the suspension centrifuged with a small bench centrifuge to remove a small amount of coagulated material. Fission of the *N*-benzoyl group from the polymer was effected by adding hydroxylamine hydrochloride

(0.5 g) to the sol and the pH adjusted to 8 with KOH solution. The contents of the bottle were stirred for 24 h to ensure complete fission. The emulsifier was removed by stirring with an anion exchange resin (e.g., Dowex 2-X8, 20–50 US mesh) in its chloride form. The complete removal of the lauryl sulphate was checked by a precipitation test with BaCl₂. The sol was ultrafiltered with Amicon PM30 membranes repeatedly (volume was made up with distilled water) to remove any residual low molecular weight material. A sample of the stock solution of the microgel sol was evaporated to dryness in an oven and the weight employed to compute the microgel concentration. An approximately theoretical yield of polymer was obtained in the polymerisation process.

Microgel possessing *O*-cinnamoyl side chains was prepared essentially as for the above polymers except that the cinnamoyl group was not removed by reaction with hydroxylamine.

3. Methods

3.1. Assay of hydroxamic acid groups in microgel

Determination of the concentration of hydroxamic acid groups was carried out by elemental

analysis for nitrogen and by UV-spectral determination. The spectrophotometric determinations were carried out by adding alkali (0.1 M) from a Radiometer Autoburette (AB411) to a solution of the polymer (50 μl in 2.5 ml water) and observing the absorbance change at 300 nm. The technique enables both $\text{p}K_a$ and molaricity to be determined simultaneously. The results are analysed according to the modified Henderson–Hasselbalch equation (Eq. 1) where α is the degree of ionisation of the hydroxamic acid. The degree of ionisation is given by the Eq. 2 where A_0 , A_1 and A_2 are the absorptions (300 nm) at low pH (where hydroxamic acid is present in unionised form), the pH in question and high pH (where all hydroxamic acid is ionised).

$$\text{pH} = \text{p}K_a + n \log \frac{\alpha}{(1 - \alpha)} \quad (1)$$

$$\alpha = \frac{(A_1 - A_0)}{(A_2 - A_0)} \quad (2)$$

The extinction coefficient of the polymeric hydroxamic acid was assumed to be the same as that of the monomeric species at the same wavelength (1230 molar/cm at 300 nm).

The concentration of the *O*-cinnamoylated polymeric hydroxamic acid was determined from the absorbance at 315 nm assuming that the extinction coefficient is the same as that for the monomer *O*-cinnamoyl-*N*-methacryloylhydroxylamine. The data for the polymers are collected in Table 1.

4. Kinetics

Rates were measured by adding an aliquot (20 μl) of an acetonitrile stock solution of the substituted phenyl acetate to a microgel solution (2.5 ml) buffered to a specified pH in a silica cell in the thermostatted cell compartment of a Hitachi 124 double beam spectrometer. The wavelength for kinetic study was determined by carrying out a separate experiment under similar conditions involving repetitive scanning of the UV–Visible

spectrum. The hydrolysis of *O*-cinnamoyl-*N*-methacryloylhydroxylamine was followed spectrophotometrically from the decrease in absorbance at 315 nm. The de-cinnamoylation of the cinnamoylated polymer was followed in a similar fashion.

Pseudo first order rate constants were calculated from the change in absorbance with time by use of a curve-fitting programme with a microcomputer. A Radiometer PHM26 digital pH-meter was employed to determine the pH of the reactant solution in the silica cell after the reaction. The pH-meter was fitted with a Russell CMAWL CL057 combined pH probe which was calibrated with EIL standard buffer powders to ± 0.01 pH units.

Measurements of particle size were carried out by use of a Malvern Automeasure instrument (4700V4).

5. Results

Fission of the aryl acetates in buffer solutions containing the microgel and monomeric hydroxamic acid obeyed excellent pseudo first order kinetics to over 90% of the total reaction. The observed pseudo first order rate constants depend on the concentration of polymer bound hydroxamic acid groups and on the degree of ionisation

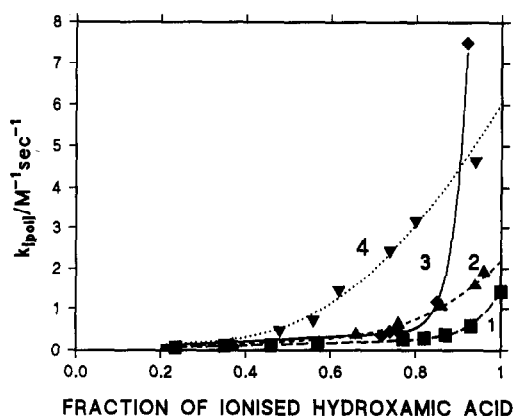


Fig. 1. Dependence on α of k_{poi} for the reaction of 4-nitrophenyl acetate with polymers 1 to 4; data from Table 2.

Table 2
Reaction of 4-nitrophenyl acetate with polymers-1 to -4 over a pH-range ^a

Polymer-1			Polymer-2			Polymer-3			Polymer-4		
pH	α^b	k_{pol}	pH	α^b	k_{pol}	pH	α^b	k_{pol}	pH	α^b	k_{pol}
7.48	0.235	0.0717									
7.82	0.35	0.107									
8.09	0.46	0.10	8.33	0.37	0.13	8.97	0.48	0.49	9.29	0.72	0.36
8.33	0.57	0.14	9.29	0.66	0.38	9.25	0.56	0.73	9.39	0.74	0.45
8.94	0.77	0.27	9.65	0.76	0.64	9.46	0.62	1.46	9.44	0.76	0.45
9.12	0.82	0.30	9.96	0.86	1.07	9.83	0.74	2.44	9.84	0.85	1.16
9.30	0.87	0.38	10.38	0.94	1.58	10.03	0.80	3.17	10.12	0.92	7.50
9.62	0.93	0.59	10.66	0.96	1.92	10.58	0.94	4.64			
10.43	1.00	1.42									
<i>a</i>	$0.305 \pm .028$		0.44 ± 0.13			0.66 ± 0.08			0.55 ± 0.03		
<i>b</i>	$1.39 \pm 0.23 \times 10^7$		709 ± 7.9			107 ± 6.3			$1.14 \pm 0.09 \times 10^{13}$		
<i>c</i>	16.3 ± 1.7		6.0 ± 1.1			2.99 ± 0.069			25.9 ± 0.8		
<i>r</i>	0.9919		0.9972			0.9937			0.9999		
$k_{\text{react}}(0.1)$	0.305		0.44			0.66			0.55		
$k_{\text{react}}(0.9)$	3.39		5.85			12.1			94.3		
[CONHOH]/mMer	2.6		1.7			0.41			0.112		

^a Conditions: 25°C, 1 M ionic strength made up with KCl. Buffers were tris(hydroxymethyl)aminomethane (0.05 M, pH 7.48–9.49) and carbonate (0.02 M, pH 9.46–10.66).

^b Degree of ionisation of hydroxamic acid calculated from the UV absorption at 300 nm as described in the text.

^c Estimated from the UV absorption data at 300 nm and an assumed extinction coefficient (see text).

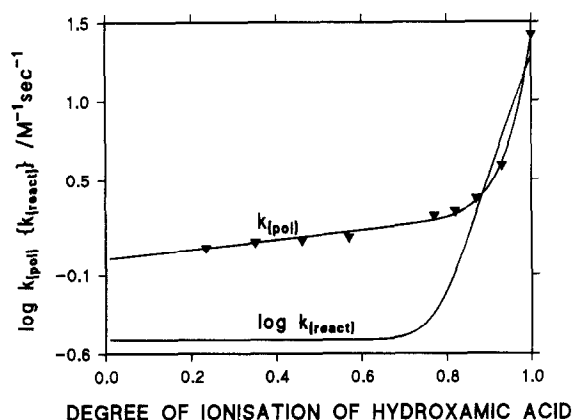


Fig. 2. Dependence on α of k_{pol} and $\log k_{\text{react}}$ for the reaction of 4-nitrophenyl acetate with polymer-1; data are from Table 2 and lines are theoretical from equations given in the text and parameters in Table 2.

of the hydroxamic acid. The concentrations of polymer were kept less than 10^{-3} M Mer in hydroxamic acid as under these conditions the rate constants are proportional to concentration; complexation effects are observed at higher concentrations [1c]. The pseudo first order rate constants

were corrected for background hydrolysis and division by hydroxamic acid molarity gave the pseudo second order rate constants (k_{pol}). The values of k_{pol} for the polymer dependent reactions obey a non-linear function of the degree of ionisation (α) and illustrated in Fig. 1 for the reaction of 4-nitrophenyl acetate with polymers-1 to -4.

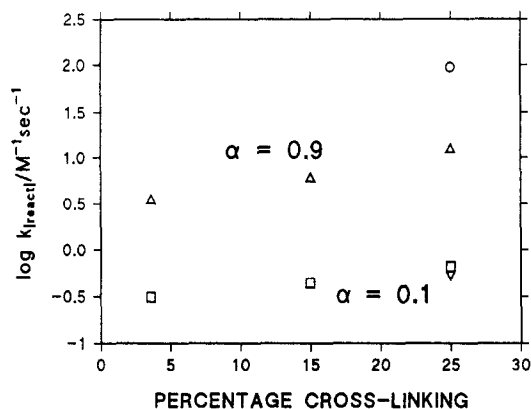


Fig. 3. Values of k_{react} at $\alpha=0.1$ and 0.9 for 4-nitrophenyl acetate plotted as a function of cross-linking percentage for polymers-1 to -4; data from Table 2. Polymer-4 = \circ and ∇ .

Table 3
Reaction of substituted phenyl acetates with polymer-1 over a pH-range^a

2,4-DNPA			2-Cl-4-NPA			4-APA			3-NPA		
pH	α	k_{pol}	pH	α	k_{pol}	pH	α	k_{pol}	pH	α	k_{pol}
7.47	0.23	3.30									
7.71	0.31	4.44									
8.07	0.46	6.4	8.17	0.50	0.42	8.39	0.59	3.1	8.31	0.56	1.9
8.27	0.54	8.8	8.60	0.67	0.72	8.57	0.69	3.9	8.70	0.70	5.0
8.68	0.69	12.1	8.81	0.72	0.88	8.83	0.74	5.8	8.89	0.78	7.7
8.88	0.75	14.8	9.21	0.84	1.37	9.01	0.78	6.9	9.28	0.86	13.5
9.10	0.81	17.2	9.60	0.92	2.02	9.34	0.88	12.3	9.62	0.93	23.1
9.32	0.87	23.4	9.94	0.97	2.41	9.60	0.92	22.3			
9.63	0.93	26.5									
9.98	0.98	30.9									
<i>a</i>	14.3 ± 1.4		0.84 ± 0.07			0.068 ± 0.008			0.040 ± 0.010		
<i>b</i>	1680 ± 11		324 ± 15			2.79 ± 0.12 · 10 ⁷			350 ± 30		
<i>c</i>	4.5 ± 0.08		5.1 ± 0.5			17.5 ± 3.8			6.99 ± 0.81		
<i>r</i>	0.9966		0.9933			0.9940			0.9987		
$k_{\text{react}} (\alpha=0.1)$	14.3		0.84			0.068			0.040		
$k_{\text{react}} (\alpha=0.9)$	65.2		6.33			1.82			1.08		

^a Concentration of polymer = 2.6 mMer in hydroxamic acid. Conditions as given in Table 2.

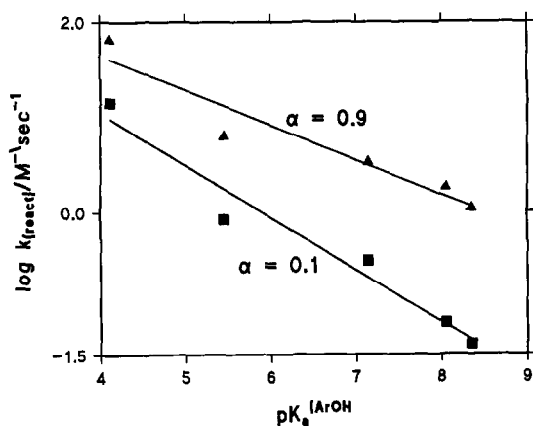


Fig. 4. Brønsted-dependence of the reactivity of polymer-1 with substituted phenyl acetates at $\alpha=0.1$ and $\alpha=0.9$; data from Table 3.

The data fit Eq. 3 and values of k_{pol} are obtained for given degrees of ionisation (α). The values of k_{pol} are fit to the empirical Eq. 4 which enables the reactivity (the slope of the plot of k_{pol} versus α { $k_{\text{react}} = dk_{\text{pol}}/d\alpha = a + b \cdot c \cdot e^{-c/\alpha/\alpha^2}$ }) to be estimated at different values of α . The values of the parameters *a*, *b* and *c* are recorded in Table 2 for the reaction

Table 4
Dependence on pH of the de-cinnamoylation of *O*-cinnamoyl-*N*-benzoylhydroxylamine and cinnamoyl-polymer^a

pH	$k_{\text{obs}}/s^{-1} \times 10^5$
<i>O</i> -cinnamoyl- <i>N</i> -benzoylhydroxylamine	
12.3	6.76
12.73	20.0
13.01	28.0
13.23	54.0
13.4	75.0
13.5	114
13.6	138
Cinnamoyl-polymer	
12.23	3.16
12.75	4.17
13.09	6.7
13.38	6.9
13.48	7.1
13.53	9.0
13.62	10.0
13.93	18.3

^a Conditions as given in Tables 2 and 3; concentration of polymer: [pol-CONHOCOCH=CHPh] = 5.6×10^{-5} M; [Ph-CONHOCOOCOCH=CHPh] = 8.0×10^{-5} M.

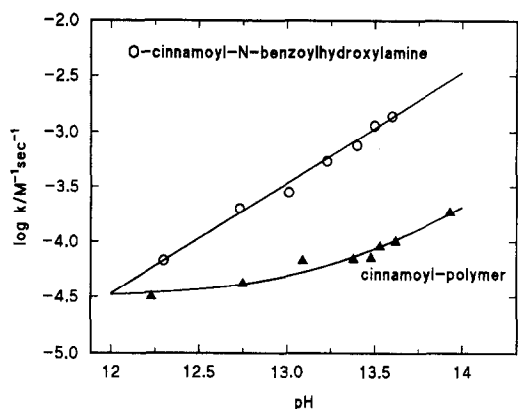


Fig. 5. Dependence on pH of the deacylation of cinnamate esters of *N*-benzoylhydroxylamine and microgel-hydroxamic acid polymer; data from Table 4. Lines are theoretical from Eqs. 8 and 9 and parameters given in the text.

$$k_{\text{obs}} = k_{\text{background}} + k_{\text{pol}} \quad (3)$$

$$k_{\text{pol}} = a \cdot \alpha + b \cdot e^{-c/\alpha} \quad (4)$$

of 4-nitrophenyl acetate with polymers (1–4) together with the values for k_{react} at $\alpha = 0.1$ and 0.9. The goodness of fit of the data to the empirical Eq. 4 is illustrated in Figs. 1 and 2. The values of k_{react} are illustrated graphically in Fig. 3 as a function of the percentage of cross-linking monomer.

Kinetics for the reactions of the substituted phenyl acetates with polymer-1 exhibit behaviour similar to that for the reaction of 4-nitrophenyl acetate and the values of a , b and c together with k_{react} for degrees of ionisation at 0.1 and 0.9 are recorded in Table 3. The Brønsted dependences of k_{react} for $\alpha = 0.1$ and 0.9 are illustrated in Fig. 4. The values of k_{react} at a value of α of 0.1 and 0.9 respectively obey Eqs. 5 and 6.

$$\log k_{\text{react}} (\alpha = 0.1) = -0.55 \pm 0.065 pK_a + 3.26 \pm 0.44 \quad (n = 5; r = 0.9799) \quad (5)$$

$$\log k_{\text{react}} (\alpha = 0.9) = -0.37 \pm 0.06 pK_a + 3.13 \pm 0.43 \quad (n = 5; r = 0.9594) \quad (6)$$

The deacylation of both *O*-cinnamoyl-*N*-benzoylhydroxylamine and *O*-cinnamoylated polymeric hydroxamic acid obeys pseudo first order kinetics in the pH-range from 12 to 14. The rate constants for the monomer reaction obey a simple

law (Eq. 7) and the polymer rate constants obey a law involving both water and hydroxide catalysis (Eq. 8).

$$k_{\text{obs}} = 3.38 \pm 0.14 \times 10^{-3} [\text{OH}] \text{ M}^{-1} \text{ s}^{-1} \quad (7)$$

$$k_{\text{obs}} = 3.17 \pm 0.38 \times 10^{-5} \text{ s}^{-1} + 1.69 \pm 0.18 \times 10^{-4} [\text{OH}] \text{ M}^{-1} \text{ s}^{-1} \quad (8)$$

The experimental data are recorded in Table 4 and the pH-dependences illustrated in Fig. 5.

6. Discussion

6.1. Polymer structure

The values of n in the Henderson–Hasselbalch type Eq. 1 indicate that there is considerable interaction between the charged groups in the microgels. This result is consistent with a relatively small average distance between the hydroxamic acid functions and is furthermore affected by the degree of ionisation of the carboxylic ion groups in the region of pH where the hydroxamic acid is being titrated. The particle size does not change significantly as a function of the percentage of cross-linking monomer.

6.2. Effect of cross-linking on reactivity

The increase in reactivity of polymer-bound hydroxamic acid groups as a function of their increasing degree of ionisation has been observed in linear polymers [6] and is thought to be due to destruction of side-chain aggregation in the linear polymer due to repulsive interactions caused by the ionisation. Microgels are unable to disaggregate due to cross-linking and there is no evidence for a dramatic dependence of particle size on pH [7]. The data in Table 2 and Fig. 3 indicate that increasing the percentage of cross-linking monomer in the monomer feed results in microgels with increased reactivity and, moreover, the percentage change of reactivity between polymer at degrees of ionisation at 0.1 and 0.9 increases with increase in cross-linking content of the monomer feed. The

Table 5
Substituent effects on the reaction of nucleophiles with substituted phenyl acetates

Reaction	Value of β_{lg}	Reference
Hydroxide ion with aryl acetates	-0.45 ^a	[8]
Imidazole with aryl acetates	-0.79 ^a	[9]
Acylation of α -chymotrypsin with aryl acetates	-0.8 ^a	[10]
Acylation of alcalase with aryl acetates	-0.51 ^a	[11]
Acylation of microgel-hydroxamate with aryl acetates	-0.55 ($\alpha=0.1$)	This work
	-0.37 ($\alpha=0.9$)	This work
Acylation of N-phenylhydroxamate with phenyl acetates	-0.69	[12]

^a These data were originally quoted as Hammett ρ values [8–11] and the β_{lg} was obtained from the equation $\beta_{lg} = -\rho/2.23$ [21].

phenomenon is reasonably explained by the requirement of complexation of ester with microgel and penetration of substrate within the particle at the high values of α . Complexation is most certainly involved because there is precedent for it from other studies [7] with substituted phenyl acetates and microgels. The majority of the reaction must occur within the bulk of the microgel because the number of 'surface' hydroxamic acid groups (available in base form at low values of α) is too small to sustain the reaction flux. Although the limit of increase in reactivity with cross-linking content may not have been reached at 25% cross-linking it is expected that a value of the cross-linking percentage will be reached where pore size will be so reduced as to prevent entry of substrate into the microgel and thus reactivity should start to fall.

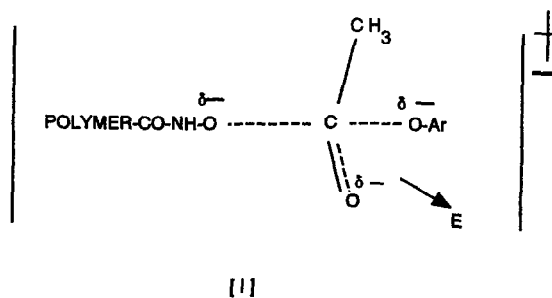
6.3. Polar effects

Penetration of the ester into the bulk of the microgel group should give rise to altered polar effects caused by a change in the electronic structure of the reactant and transition state. In the case under investigation the rate law measures the energy from uncomplexed ester to the transition state for the acylation reaction in the ester complex with microgel. Table 5 collects data for β_{lg} for a series of reactions of nucleophiles with substituted

phenyl acetates. The values of β_{lg} in the table indicate relative charges on the transition state because the reactant states all have the ester in its uncomplexed form.

The hydroxamate ion belongs to a class of nucleophiles whose reactivity is very much larger than nucleophiles of similar pK_a which do not possess an adjacent electronegative atom; these are called ' α -nucleophiles' [13,14]. The polymeric hydroxamate ion acting at low degree of dissociation ($\alpha=0.1$) is within experimental error the same as that for a monomeric hydroxamate ion acting in water [12]. This is consistent with the reaction of nucleophiles on the 'surface' of the microgel; nucleophiles at the surface of 'oil-in-water' micelles also behave as if they were in an aqueous bulk medium [15].

Reactivity at $\alpha=0.9$ indicates less development of negative charge in the transition state of acylation compared with the models. The acylations of many hydrolytic enzymes by specific aryl ester substrates also exhibit less negative charge development in the transition states and this has been ascribed to charge neutralisation by electrophilic groups at the active site [16]. We propose that charge neutralisation is occurring in acylation of the polymeric hydroxamate ion at high degree of dissociation. This could involve the side chains of the polymer as illustrated (I) and is consistent with the hypothesis that reaction is occurring within the bulk of the microgel.



6.4. Deacylation

The hydrolysis of *O*-acetyl-*N*-benzoylhydroxylamine has been investigated by Ashfaq, Pytela, Socha and Vecera [17] who showed that the Los-

sen rearrangement did not occur in the region of pH studied by us. The rate constant for alkaline hydrolysis of ethyl cinnamate ($4.97 \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$ [18]) is about the same as that for a cinnamate with a leaving group (hydroxamate ion) which is some 7 powers of 10 more acidic than ethanol. A possible explanation of the reduced reactivity of the ester with hydroxamate leaving group is that the reaction is at an already ionised ester which electrostatically repels the attack of the negatively charged hydroxide ion. The deacylation step in the catalytic action of linear polymers containing hydroxamic acid has been addressed by Kunitake and his co-workers [1d][19]. Addition of extra catalytic functions such as the imidazolyl group accelerates deacylation and hence improves the potential of the polymers as catalysts rather than as simply reagents. The pH-dependence of the de-cinnamoylation of the polymer (Table 4 and Fig. 5) indicates that the hydroxide term is only some 30-fold less than that of the monomeric species. This result is interesting in that the reactivity would be expected to be much less on account of the steric hindrance caused by the polymer architecture. The de-cinnamoylation of the polymer also possesses a pH-independent term (Eq. 8) due to spontaneous hydrolysis and this is probably caused by intramolecular catalysis by the ionised carboxyl groups present in the polymer. This behaviour is expected from previous results of Morawetz and his co-workers who demonstrated enhanced rates of hydrolysis of esters attached to polymers and small molecules possessing carboxylate functions [20].

7. Conclusions

The reactivity of microgel–hydroxamic acid polymers towards esters increases with the percentage of cross-linking in the monomer feed. The relatively small change in charge from reactant to transition state for the reaction in the bulk of the polymer is consistent with an electrophilic interaction between polymer and ester. Deacylation is

assisted by intramolecular attack by the pendant carboxyl functions in the polymer backbone.

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