Microgels as Matrices for Molecular Receptor and Reactive Sites: Synthesis and Reactivity of Cavities possessing Amino-functions

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Non-fluxional polymeric particles, microgels, have been prepared containing amino-functions situated in cavities in the polymer matrix. The microgels possess binding properties and exhibit greatly enhanced rates of reaction *versus* isoquinoline-*N*-sulphonate and 4-nitrophenyl ester substrates compared with simple amines. The polymer reagents possess selectivity against the molecular size of the substrate due to finite cavity size and this selectivity may be modified by variation of pH which causes a change in size of the cavities.

It has long been recognised that binding of substrate to enzyme is a major factor in rate accelerations relative to the reaction of the substrate with simple chemical reagents in a bimolecular reaction.[1] Considerable effort has been invested in the study of complexation of substrates in aqueous solution with a variety of acceptor molecules. Many small acceptors such as cyclodextrins,[2,3] cryptands,[4] and cyclophanes [5] have been intensively investigated and much work reported on the modification of these species as model enzymes.[2-5]

A completely different approach to enzyme modelling involves the use of linear [6] and branched [7] random polymers and structures related to the micellar state such as vesicles [8] and synthetic membranes.[9] These systems suffer from the absence of non-fluxional receptor sites which are an essential feature of the catalytic power of enzymes.

A major advance in enzyme modelling would be the provision of non-fluxional receptor sites in colloidal particles of dimensions similar to those of enzymes. A microgel is a colloidal particle formed in emulsion polymerisation with a feed possessing cross-linking monomer units.[10] The term microgel was the original nomenclature for these small particles [10b] and has been defined by Medalia.[10c] Microsphere [11] is another term but only conveys the size and shape of the particle and not its cross-linked structure. The polymerisation process yields high molecular weight essentially monodisperse polymer solutions which can be stabilised as colloidal suspensions in water by the introduction of acidic monomer units. The diameter of the microgel sphere can be regulated by varying the composition of the monomer feed and particles can be produced of a size approximating that of large enzymes (40—300 nm).[11] Little work has been reported on the reactivity of reagents attached to microgels but we have reported dramatic increases in the reactivity of microgel hydroxamates [12] and stereochemical selectivity of optically active microgel hydroxamates.[13] There is also evidence of binding of substrates to microgels [13] and of a molecular exclusion effect depending on the size of substrate species.[12]

Microgels are probably ideal as matrices into which reactive sites may be tailored to model an enzyme. An active site may be introduced easily by incorporating a former monomer in the polymerisation step which when released after polymerisation has been completed will reveal the active site cavity. The cavity produced will be non-fluxional owing to the cross-linked nature of the polymer.[14] This simple process has clear advantages of cheapness and generality over the stepwise synthetic approach required for the production of the small acceptor molecules mentioned above. The advantage over linear and branched polymers and micelles is the non-fluxional nature of the matrix supporting the cavity and over massive cross-linked resins (such as ion exchange resin beads) of surface area and accessibility of the substrate. Microgels can be obtained as colloidal suspensions as are enzymes. A corollary of the small particle size is that a higher loading of active site cavities is possible compared with that obtainable in massive cross-linked resins leading to a higher specific activity relative to the latter supports.

We were interested to investigate the amino-function as a reactive species in microgels because this group is less ambiguous as a reactant than the previously studied hydroxamates. The reactivity of the amino-function generated in cavities in the microgel is assayed against the well known aminolysis reactions (1) and (2).

$$R^1NH_2 + R^2CO\text{-}O\text{-}4NP \longrightarrow R^1NHCOR^2 + 4NPO^- \quad (1)$$

$$R^1NH_2 + R^2{}_3\overset{+}{N}\text{-}SO_3^- \longrightarrow R^1NHSO_3^- + R^2{}_3N \quad (2)$$

(4NP = 4-nitrophenyl)

Experimental

Methods.—Most monomers were obtained from Aldrich or Fluka and were used immediately after inhibitors had been removed by distillation at reduced pressure. 4-Nitrophenyl acetate and caproate were from Sigma and isoquinoline-*N*-sulphonate was prepared from sulphur trioxide and isoquinoline.[15] Tris (trishydroxymethylaminomethane) and t-butylamine buffers were prepared from analytical grade reagents and water was doubly distilled from glass. Acetonitrile was purified by the method of Lewis and Smyth.[16]

2-Phthalimidoethanol was prepared by mixing phthalic anhydride (0.1 mol) and ethanolamine (0.1 mol) and heating (180°; 2 h). The product solidified on cooling and was recrystallised twice from ethanol to yield plates, m.p. 127—128° (lit.,[17a] 129.5°). 2-*Phthalimidoethyl methacrylate* was prepared by mixing 2-phthalimidoethanol (0.026 mol) and triethylamine (0.026 mol) in toluene (100 ml) and slowly adding methacryloyl chloride (0.026 mol) with stirring and reflux. After 4 h reflux the cold suspension was filtered, washed with dilute HCl, dried (Na_2SO_4), and evaporated. The crude material was recrystallised from ethanol–light petroleum and had m.p. 97—99° (Found: C, 64.7; H, 5.0; N, 5.1. $C_{14}H_{13}NO_4$ requires C, 64.9; H, 5.0; N, 5.4%). The n.m.r. and i.r. spectra were consistent with the proposed structures for both the above compounds. We are grateful to Dr. D. O. Smith for running the n.m.r. spectra on a JEOL 100 MHz instrument.

Preparation of Polymers.—The general polymerisation process was as follows. Water (100 ml) was degassed by evacuation and placed in a thick-walled glass bottle with a screw top fitted with a polythene liner. Sodium dodecyl sulphate (0.1 g) was added and the bottle flushed gently with

Table 1. Monomer feeds for emulsion polymerisation: polymers (I)—(X)

Polymer	MM [a]	EEM [b]	EDM [c]	CYC [d]	XLink [e]
(I)	2.23	2.5	0.27	0	3.18
(II)	2.23	2.5	0.27	5	3.18
(III)	2.23	2.63	0.14	5	1.8
(IV)	1.96	2.5	0.54	5	7.2
(V)	2.23	2.5	0.27	0	3.18
(VI)	2.23	2.5	0.27	10	3.18
(VII)	1.96	2.5	0.54	0	7.2
(VIII)	1.5	2.5	1.0	0	14.2
(IX)	1.5	2.5	1.0	5	14.2
(X)	1.75	2.5	0.75	0	10.2

[a] MM = methyl methacrylate in g per100 ml. [b] EEM = 2-ethoxyethyl methacrylate in g per 100 ml. [c] EDM = ethylene dimethacrylate in g per 100 ml. [d] CYC = cyclohexane in ml per 100 ml. [e] Percentage of monomer as the cross-linking agent.

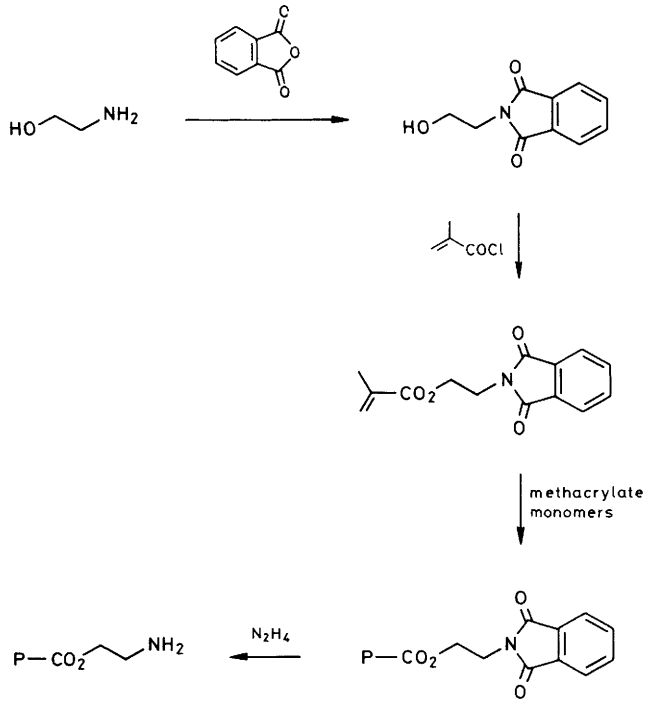
Table 2. Monomer feeds for emulsion polymerisation: polymers XI) and (XII) [a]

Feed component	Weight (g per 100 ml suspension) (XI)	(XII)
Methacrylic acid	10.26	10.26
2-Hydroxyethyl methacrylate	25.24	25.24
Methyl methacrylate	49.18	61.33
Ethylene dimethacrylate	3.17	3.17
2-Phthalimidoethyl methacrylate	12.15	0

[a] Ethyl acetate (5 ml) was added to the feed in both polymers. This solvent is necessary to solubilise the 2-phthalimidoethyl methacrylate.

nitrogen. The monomer feed was then added with (or without) a suitable diluent such as cyclohexane. The bottle was then stirred in an oil-bath set at 60—70°, potassium persulphate (50 mg) was added, purged gently with nitrogen, sealed, and kept for 3.5 h with stirring. Polymerisation was stopped by addition of hydroquinone (50 mg) and the polymer precipitated by adding a saturated salt solution (10 ml), filtered at the pump, and washed with cold water. The polymer was air dried and redispersed in 1,2-dichloroethane and surfactant removed by stirring with ion exchange resin. This process is repeated if sulphur (determined by evaporation of a small portion and microanalysis) was found in the product. Unchanged monomer and all small molecular weight impurities were removed by chromatography on a Sephadex LH 20 support in a pumped system. The pure polymer solution was then adjusted to a total volume of 100 ml. Yields of polymer based on the monomer feed were between 60 and 70%. Details of the monomer feeds are given in Tables 1 and 2 for polymers (I)—(XII).

Polymer (XI) and the control polymer (XII) were prepared in a similar manner to the above except that the dried polymer product was redispersed in methanol. The colloidal solution in methanol was refluxed with hydrazine hydrate (0.24 g) for 24 h to remove the phthalimide protecting groups (Scheme), acidified (pH 2.25), filtered, and the polymer precipitated by the addition of water (10 ml). The polymer was dried, dispersed in methanol, treated with ion exchange resin to remove surfactant, and the resulting dispersion passed through a Sephadex LH 20 column to exclude all small molecular weight impurities. No u.v. peaks due to phthalic acid or phthalimide were present in the product suspension which was made up to 100 ml. The weight per ml (21.1 mg ml^{-1}), yield (42%), and incorporation of the phthalimide monomer unit (42%) were



Scheme. Preparation of the amino-polymer

Table 3. Assay of the amino-function in polymer (XI)

Method	10^2 Concentration in stock (merlar) [b]
Combustion analysis	2.0 ± 0.1
U.v. assay	1.35 ± 0.3
pH-titration	1.60 ± 0.4 [a]

[a] In 40% methanol pK = 7.68, n = 1.0 [see equation (3)]; in 30% methanol pK = 8.16 and n = 0.97. [b] Merlarity in this case represents the concentration of total amine sites per litre.

recorded. A similar procedure was carried out on the control polymer (XII) to yield stock at 23.2 mg ml^{-1}. Data were collected from samples of polymer from a single batch.

Methods

Amino-group Analysis.—Polymer (XI) was titrated with standard HCl (0.01M; 40% MeOH) using a Radiometer pH-titration set comprising recording titration assembly (REC 61/REA 60), pH-meter (PHM 62), and autoburette (ABU 11). Titration of the sample [stock polymer (0.05 ml) in 40% methanol (5 ml) at pH 10.0] was corrected by a background titration to assay the amino-groups and determine their pK. The resulting data were fitted to equation (3) and the experiment was repeated using 30% methanol solutions.

C, H, and N analyses (carried out by Mr. A. Fassam using a Carlo Erba analyser) on the dried polymer were employed to obtain a further value for the amine content. A third assay

$$pK = pH + n\log(1 - FB)/FB \qquad (3)$$

for amine utilised u.v. analysis of the phthalimide-containing polymer assuming a molar extinction coefficient identical with that of the monomer ($\Delta\varepsilon_{265}$ 3 330) and 100% conversion to the amine. There is considerable divergence in the results from

Table 4. Particle diameters of polymers (I)—(X) in 1,2-dichloroethane solution

Polymer	Mol. percentage cross-linking monomer	Cyclohexane [b]	Diameter (nm)
(I) [a]	3.18	0	123
(II)	3.18	5	141
(III)	1.8	5	772
(IV)	7.2	5	134
(V) [a]	3.18	0	120
(VI)	3.18	10	167
(VII)	7.2	0	109
(VIII)	14.2	0	69
(IX)	14.2	5	130
(X)	10.2	0	91

[a] Repeated polymerisation under the same conditions to demonstrate repeatability of the measurements. [b] Volume added as co-solvent in ml per 100 ml.

these assay methods as previous experience [12c] indicates that closer correspondence (Table 3) for the assays is probably not possible. We use in the following kinetic calculations an average value of 1.65×10^{-2} merlar for the concentration of amine in the stock solution of polymer (XI).

Kinetics.—Rates of reaction were measured using a Pye–Unicam SP 800 u.v.–visible spectrophotometer following the release of 4-nitrophenolate ion at 400 nm and the disappearance of isoquinoline-*N*-sulphonate at 339 nm. The techniques were the same for ester and sulphonate except that the latter was studied using 40% methanol (ionic strength 0.06M) and the former using 30% methanol at ionic strength 0.06M; the methanol was required to prevent precipitation of polymer or reaction products. Reactions were initiated by adding a portion (0.05 ml) of the stock solution of substrate on the tip of a glass rod to buffer solution (2.5 ml) containing the polymer and equilibrated in the thermostatted cell compartment of the spectrophotometer. The absorbance was then recorded at the appropriate wavelength. Pseudo-first-order rate constants were then estimated from the progress curves of the absorbance *versus* time by plotting $A_t - A_\infty$ against time on two-cycle semi-logarithmic graph paper. The pH of the buffer solution was recorded after the reaction and the run was discarded if the pH differed from the pH of the stock buffer by >0.1 units.

Particle Size Determination.—A Coulter Nanosizer was employed to measure particle size in the colloidal dispersions and we are grateful to Mr. B. V. Miller of the Coulter Company for the loan of an instrument for this purpose.

Results

Particle Size.—The particle diameters of the polymers (I)—(X) in 1,2-dichloroethane are recorded in Table 4 and vary between 60 and 770 nm. Although reproducibility in polymerisation has been effectively demonstrated elsewhere [11] we were able to confirm that different polymer batches of the same composition of feed had similar diameters. The particle diameter of polymer (XI) was measured as a function of pH and this variation is recorded in Figure 3.

*Isoquinoline-*N*-sulphonate.*—Reaction of isoquinoline-*N*-sulphonate with solutions of polymer (XI) followed excellent pseudo-first-order kinetics; in order to allow comparisons to be made accurately we used polymer (XI) from a single batch preparation. The pseudo-first-order rate constants (Table 5) exhibited a non-linear rate–polymer concentration plot and the kinetics were consistent with a Michaelis–Menten equation (4). Treatment of the polymer with further ion exchange resin

Table 5. Dependence of reactivity on the polymer concentration [c]

10^3[Polymer]/merlar [c]	$10^3(k_{obs.} - k_0)/s^{-1}$ Isoquinoline-*N*-sulphonate [a] pH 8.10 (FB = 0.72)	$10^3(k_{obs.} - k_0)/s^{-1}$ 4-Nitrophenyl acetate [b] pH 8.75 (FB = 0.80)
0	0	0
0.66	3.6	0.28
0.99	6.7	
1.65	11.4	0.53
1.98		0.63
2.64	15.9	0.88
3.30		0.98
3.96	20.9	
4.12		0.97
4.95	23.7	1.1

[a] 40% Methanol at 25°, $K = 5.5 \times 10^{-3}$ merlar and $k_m = 4.9 \times 10^{-2}$ s^{-1}. [b] 30% Methanol at 25°, $K = 1.25 \times 10^{-2}$ merlar and $k_m = 4.0 \times 10^{-3}$ s^{-1}. [c] Concentration of the macromolecule is expressed in the merlarity of the pendant amino-groups.

$$k_{obs.} - k_0 = k_m[\text{amine}]/(K + [\text{amine}]) \qquad (4)$$

did not alter the kinetics excluding catalysis by residues of the surfactant. The expression [amine] refers to the merlarity of the amino-functions in the solution rather than to a molarity; more than one amino-group is attached to a single polymer particle. The values of k_m and K derived from the concentration dependence are recorded in Table 5 for the pH in question. At pH 8.10 the ratio $k'_2 = k_m/K$ is the apparent bimolecular rate constant for reaction of sulphonate with polymer. This value, 8.9 l mol^{-1} s^{-1}, divided by the fraction of base (FB) of the amino-function (0.72) yields a value 12.4 l mol^{-1} s^{-1}, *ca.* 1 000-fold larger than the true bimolecular rate constant for reaction of the sulphonate with Tris (8.9×10^{-3} l mol^{-1} s^{-1}); [15] Tris was used as a comparison as it is arbitrarily assumed to have a similar steric requirement to the amine in the polymer.

The reactivity of polymer (XI) at a low constant merlarity of amine was measured against isoquinoline-*N*-sulphonate as a function of the pH. Division of $k_{obs.} - k_0$ by the amine merlarity gives k_2, an apparent bimolecular rate constant, provided the amine concentration does not exceed the value of K. The latter value is not known over the pH range but at pH 8.1 it is well in excess of the concentrations employed here (1.32×10^{-3} merlar). Figure 1 illustrates a plot of k_2 *versus* FB for reaction of isoquinoline-*N*-sulphonate with polymer (XI). The points are fitted to a polynomial [equation (5)]

$$k_2 = 19.9\text{FB} - 11.6\text{FB}^2 \qquad (5)$$

calculated with a program (G-PLOT) provided by the University of Kent Computing Centre.

4-Nitrophenyl Acetate and 4-Nitrophenyl Caproate.—Good pseudo-first-order kinetics were obtained in the reaction of the nitrophenyl esters with polymer (XI). Reaction of 4-nitrophenyl acetate with the polymer at pH 8.76 obeys a relationship non-linear in polymer concentration; the data (Table 5) are consistent with the Michaelis–Menten equation (4) and the parameters k_m and K are recorded in Table 5. The value of k'_2 for the reaction of 4-nitrophenyl acetate with

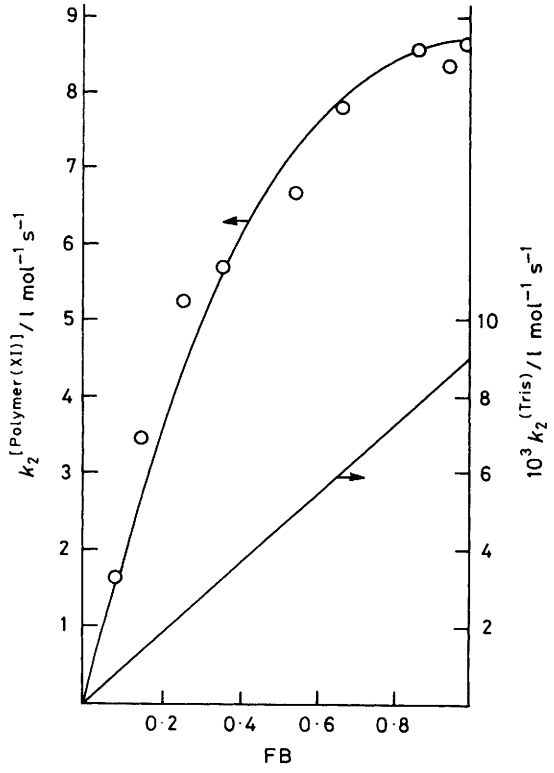


Figure 1. Dependence of k_2 on FB (fraction of total amine present as base) for reaction of polymer (XI) with isoquinoline-*N*-sulphonate (○) at 40% methanol–water (v/v), ionic strength 0.06M, and 25°. The line is theoretical for equation (5); the linear dependence is for the analogous reaction of isoquinoline-*N*-sulphonate with Tris buffer under the same conditions.[15] Polymer concentration 1.32×10^{-3} merlar

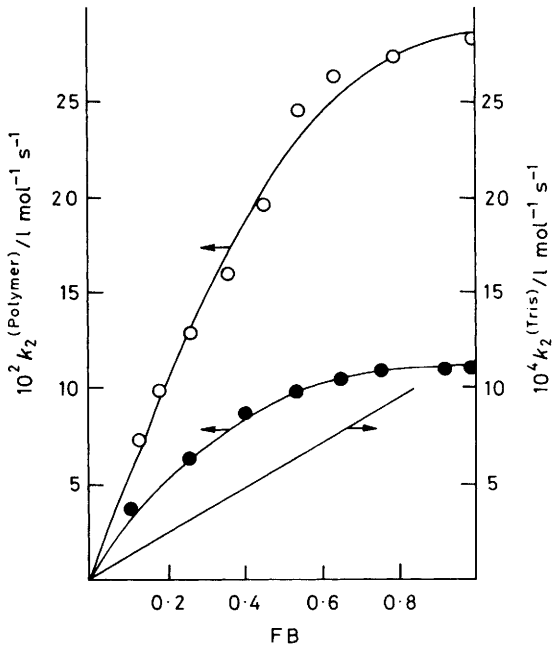


Figure 2. Dependence on FB of k_2 for the reaction of polymer (XI) with 4-nitrophenyl acetate (○) and caproate (●) at 30% methanol–water (v/v), ionic strength 0.06M, and 25°. The lines are theoretical from equations (6) and (7). The linear dependence is for the reaction of Tris with 4-nitrophenyl acetate under similar conditions.[17b] Polymer concentration 1.63×10^{-3} merlar

the polymer at pH 8.76 (FB = 0.80) is 0.4 l mol^{-1} s^{-1} which is *ca.* 340-fold larger than the bimolecular rate constant for the reaction of Tris with the ester (1.17×10^{-3} l mol^{-1} s^{-1}).[17b] Reactivity of the acetate ester against the control polymer (XII) gave a small decrease relative to the background rate constant (k_0).

The pH-dependences of the reaction of 4-nitrophenyl acetate and caproate against polymer (XI) were determined at 1.65×10^{-3} merlar in amine (concentration lower than the value of K at pH 8.76). The dependence of k_2 is illustrated in Figure 2 and the points fit polynomials (6) and (7) for acetate and caproate, respectively.

$$k_2^{acetate} = 0.57FB - 0.29FB^2 \quad (6)$$

$$k_2^{caproate} = 0.33FB - 0.33FB^2 + 0.11FB^3 \quad (7)$$

Discussion

Polymer Structures.—The closeness of the results from three assay procedures confirms the presence of free primary amino-groups produced by the removal of the phthalimido protecting group. The control polymer possesses no reactivity against 4-nitrophenyl acetate excluding a possible acylhydrazide nucleophile formed by reaction of hydrazine with the methyl ester pendant group on the polymer. Each amino-function must be associated with a cavity corresponding to the phthalimido-group in addition to any other space available in the cross-linked matrix of the microgel. The swollen spherical microgel particles of polymer (XI) have a diameter of 129 nm at pH 8.74. Calculations show that there are 5.3×10^5 amine groups per microgel sphere of molecular weight 6.8×10^8 (based on the volume of the sphere and assuming a unit density). There is thus one amine group on average per 1.29 nm cube of matrix. The closeness of the fit of the pH-titration to the theoretical equation with $n = 1$ (Table 3) is indicative of only weak or non-existent interaction between the individual amino-functions; thus we can regard the reactant amines as being mutually exclusive. This result is in accord with a large average distance between the amino-functions of 1.29 nm.

There is a clear correlation between microgel diameter in solution and the percentage of cross-linking monomer in the feed. Polymers formed from feeds containing no inert solvent such as cyclohexane have a smaller diameter than those formed with the co-solvent. This observation is expected from the proposed mechanisms for emulsion polymerisation;[17c,d] if co-solvent immiscible with water is added in the monomer feed it should partition between the droplets in the water phase and the micelles where the polymerisation is occurring. Thus the co-solvent will dilute the monomer in the micelle and produce polymer looser in network and greater in size than polymers from feed without co-solvent. Cyclohexane appears to exert a limiting effect on the size of the microgel; the diameter rapidly decreases as the percentage cross-linking increases to 3% and becomes independent of cross-linking at higher values. There is an apparent linear dependence of diameter on cross-linking for polymerisation effected without co-solvent.

Reaction of Polymer (XI) *with Substrates.—Stoicheiometry.* The reaction of isoquinoline-*N*-sulphonate with polymer (XI) is essentially the displacement of the isoquinoline moiety by the amine function [equation (2)]. This reaction has been demonstrated for simple amines[15] and the u.v. spectral

Table 6. Comparison of the dissociation constants for substrate–polymer complexes with other polymer systems

Polymer	Substrate	10^4K/merlar
PIM [a]	Charged esters	2—4 [h]
PVP [b]	4-NPBenz [c]	2.34 [i]
PVP [b]	NABA [d]	37.4 [i]
PIM [a]	NABA [d]	80.0 [18]
		90.0 [21]
PIM [a]	NABS [e]	3.8 [23]
PHA [f]	4-NPPhe [g]	20.0 [13]

[a] PIM = Poly(vinylimidazole). [b] PVP = Poly(vinylpyridine). [c] 4-NPBenz = 4-Nitrophenyl benzoate. [d] NABA = 3-Nitro-4-acetoxybenzoic acid. [e] NABS = 3-Nitro-4-acetoxybenzenesulphonic acid. [f] A microgel containing hydroxamic acid pendant groups. [g] 4-NPPhe = 4-Nitrophenyl benzyloxycarbonylphenylalaninate. [h] C. G. Overberger and T. J. Pacansky, *J. Polymer Sci.*, 1975, **13**, 391. [i] T. Kunitake, S. Shinkai, and S. Hirotso, *Biopolymers*, 1976, **15**, 1143.

changes are similar in the polymer case. Release of 4-nitrophenol (4-NPOH) is measured for the reaction of the polymer with the nitrophenyl esters where the product will be the amide [equation (1)].

Binding. The pseudo-first-order rate constants for reaction of sulphonate with polymer (XI) fit a non-linear rate equation in amine merlarity [equation (4)] consistent with substrate binding by the polymer and similar to that observed with enzymatic catalysis. Binding phenomena have been observed with linear polymers and comparison with the dissociation constants obtained kinetically for the substrate–polymer complex (Table 6) indicates similar orders of magnitude. Probably the main contributors to binding of organic species to polymers and enzymes not requiring metals are electrostatic and hydrophobic bonding. The phenomenon of binding of substrates to branched and linear polymers has been investigated by several groups, notably Kunitake's [18,19] and Overberger's.[20] The binding ability of poly(vinylpyrrolidone) and poly(methacrylic acid) is explained on a hydrophobic basis; the polymers in aqueous solution 'ball up' forming hydrophobic domains into which any substrate present may partition. Catalysis may be inhibited by the addition of molecules which bind without reacting.[21]

Overberger [22] showed that a polymer with carboxylate subunits exhibits marked selectivity towards a positively charged substrate as does partially protonated poly(vinylimidazole) towards negatively charged substrates in solution.[23] Reversible association of positively charged substrates was demonstrated for poly(acrylic acids) by Morawetz and Shafer.[24]

The minimum catalytic effect of poly(vinylimidazole) on the hydrolysis of 4-nitrophenyl acetate was shown to occur at a solvent composition where viscosity was at its maximum due to 'opening up' of the polymer to give long chains. The highest rate of reaction was at low ethanol content where the polymer chains had 'balled up' to form hydrophobic domains. The acceleration in rate is moderate compared to some that have been reported [25] although difficulty has been experienced in repeating these high catalytic rates.[26]

The association of the polymer (XI) with a neutral substrate demonstrates that hydrophobic domains [27,28] exist in the polymer and explains the increase in catalytic reactivity over Tris a primary amine which is probably not as sterically hindered as those residing in the polymer. Different considerations need to be made for the present microgel as against the linear polymers studied by previous workers because it is cross-linked and therefore non-fluxional. Cleavage of the phthalimido-group to form the amine function must leave

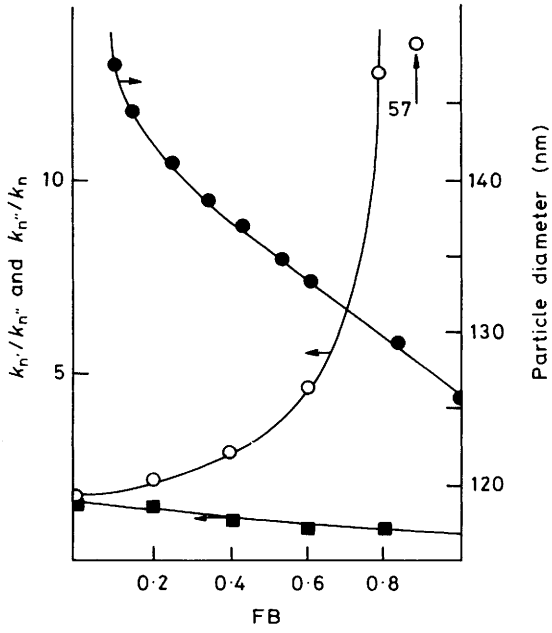


Figure 3. Comparison of the variation of selectivities as a function of pH with the particle diameter of the polymer (XI) (●). The diameter increases very rapidly at low FB values and this results in reversible coagulation if the pH is lowered too much. Reactivity is not recorded at pH values where FB is essentially zero. The values of k_n'/k_n'' (○) (4-nitrophenyl acetate–4-nitrophenyl caproate) and k_n''/k_n (■) (4-nitrophenyl caproate–isoquinoline-*N*-sulphonate) are calculated from equations (5)—(7)

cavities adjacent to the amino-groups. These cavities will be able to accept the substrate and retain it adjacent to the reactive nucleophile in a hydrophobic environment thus enhancing the nucleophilicity of the amino-function by a microscopic medium and propinquity effect.

Effect of pH. Figure 3 reveals that there is a progressive decrease in particle diameter as the pH of the solution increases for polymer (XI). Neutralisation of the ammonium groups by increasing pH decreases the electrostatic repulsion effect. The decrease in particle size is reflected in the plot of k_2 values *versus* the FB of the polymer amine (Figures 1 and 2) and which is not linear. Values of k_n for each of the substrates may be obtained from the differential of the polynomial (dk_2/dFB) at different FB values. Kunitake and Okahata [29] suggested that the upward curvature (increasing k_n) in plots of k_2 *versus* FB for *linear* polymers with hydroxamic acid units is due to the polyelectrolyte effect where charge on the polymer swells the macromolecule and reduces steric hindrance. The polyelectrolyte phenomenon as described by Kunitake cannot be the complete explanation for the increased reactivity of *microgel* hydroxamates towards esters. As the FB of the hydroxamate is increased the k_2 value *exceeds* that of monomer hydroxamate at the same FB.[12] The factor causing the increase in k_n is thus sufficient to overcome any steric hindrance inevitably present in the microgel reagent. We believe that in the present case access to the reactive sites is decreased by the decrease in diameter of the microgel and hence k_n decreases.

Selectivity may be defined as the ratio of k_n for different substrates and we consider the selectivity of 4-nitrophenyl acetate relative to caproate (k_n'/k_n'') and 4-nitrophenyl caproate relative to isoquinoline-*N*-sulphonate (k_n''/k_n). Figure 3 illustrates the effect of FB on selectivity and it can be seen that the acetate becomes a much better substrate com-

pared with caproate by a factor of six-fold as the fraction of base increases from zero to 0.7. The caproate becomes a poorer substrate relative to the sulphonate by a factor of only two-fold as the fraction of base rises to 0.8 from zero. The accomodation of the 4-nitrophenyl acetate becomes easier in the cavity relative to the caproate owing to the shrinkage of the cavity with increase in FB. Isoquinoline-*N*-sulphonate and the caproate are commensurate and are thus affected only to a small extent in relative selectivity as the cavity shrinks. Selectivity is not likely to be as strict as that obtained in enzymatic or small molecule systems such as cyclodextrin binding because the cavities produced in the polymer are of a random nature but have a statistically average shape and size caused by the mode of their formation.

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