Microgels as Matrices for Reagents and Catalysts: the Enhanced Reactivity of Hydroxamic Acid Groups Attached to a Water-soluble Emulsion Polymer with a High Level of Cross-linking

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Summary Water-soluble globular macromolecules (mol. wt. 18 000 000) possessing considerable internal cross-linking exhibit molecular exclusion phenomena; pendant hydroxamic acid groups possess enhanced reactivity (in excess of 1 000-fold enhancement) compared with the corresponding monomer species towards reaction with 4-nitrophenyl esters of a series of acids.

THERE is considerable interest in reactions of groups attached to water-soluble polymers where an important aim is the synthesis of selective catalysts with stability and economy of preparation not possessed by enzymes.[1] So far, work has centred on linear[2] or branched[3] polymers and on micelles;[4] these systems have flexible structures and are therefore poor candidates for a matrix for the construction of active sites similar to enzymes where the structures are essentially rigid. Considerable work has been carried out on the reactivity of groups attached to massive systems where the surface:bulk ratio is unfavourable.[5] Work on the tailoring of active sites in massive systems has commenced.[5b]

We report here the measurement of the reactivity (*vs.* the fission of 4-nitrophenyl esters) of hydroxamic acid groups attached to microgels[6] prepared by emulsion polymerisation. These macromolecules are of great significance in the search for enzyme-like polymer catalysts because their structure can be rigid, they may be modified for example to be water-soluble and, unlike suspension or bulk polymers their surface area:volume ratio is very large; the present microgels have a diameter of *ca.* 400 Å in the dry state.† Potentially the rigid structure of the microgel provides a matrix for the synthesis of active-sites.

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† Measured using an AEI model 801A electron microscope at 60 kV without shadowing.

Although microgels have been studied previously as reagents[7] investigations of the reactivity of functional groups have not been reported; these studies are most important in any assessment of the accessibility of pendant groups to solutes.

This communication is the first report of the reactivity of a group attached to a microgel and, moreover, describes a dramatic enhancement in the nucleophilicity of pendant hydroxamic acid groups over that of simple models; a molecular exclusion phenomenon is also observed.
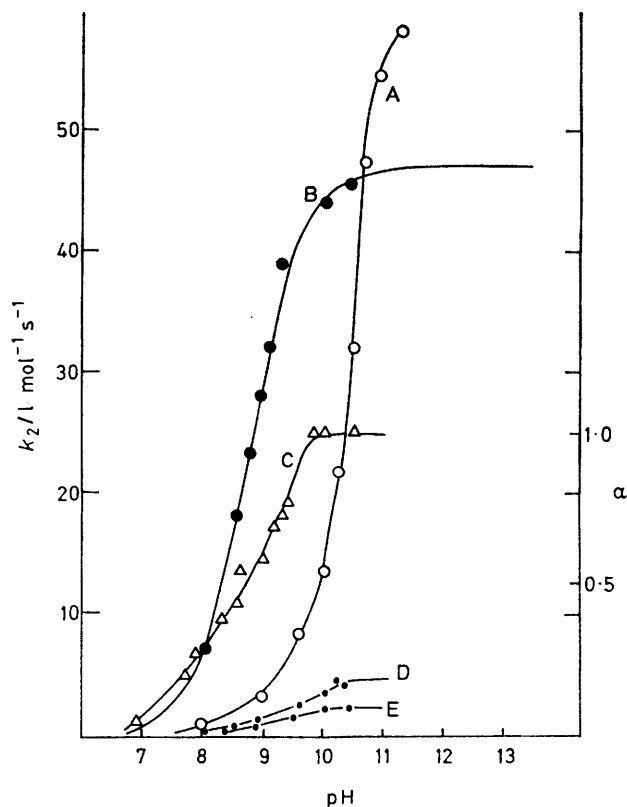

FIGURE. Second order rate constant (k_2) for the reaction of hydroxamic acids with esters as a function of pH. Conditions: 25 °C, ionic strength maintained at 0·1 mol l^{-1} with KCl; buffers are: borate, phosphate, carbonate, and hydroxide; [polymer] $= 2·74 \times 10^{-4}$ mol l^{-1} as the molarity of total hydroxamic acid groups; [ester] $= 5 \times 10^{-5}$ mol l^{-1} a concentration less than the critical micelle limit for most 4-nitrophenyl esters of the type employed here as substrates. Rate constants are corrected for background hydrolysis by buffer. Curves A, D, and E refer to the reaction of microgel polymer with 4-nitrophenyl acetate, caproate, and adipate, respectively. Curve B refers to the reaction of methacryloylhydroxamic acid with 4-nitrophenyl acetate and curve C is the pH-dependence of α for the microgel polymer. The line corresponding to the reaction of the control linear polymer with 4-nitrophenyl acetate lies between curves D and E. Kinetics were measured spectrophotometrically at 400 nm using conventional apparatus. Lines for curves B, D, and E are theoretical assuming reactivity is independent of α. The value of k_2 is derived by dividing the first order rate constant for nitrophenol release (corrected for background hydrolysis) by the total concentration of hydroxamic acid groups.

Water-soluble polymer was prepared by hydroxylaminolysis‡ of an emulsion polymer from the feed composition: methyl methacrylate (57 mol %), ethylene dimethacrylate (14 mol %), and 4-nitrophenyl acrylate (29 mol %). The reaction of polymer (in excess) with nitrophenyl esters was first order in total pendant hydroxamic acid and in substrate and second order rate constants (k_2) were plotted against pH (Figure). The reaction followed is the acylation of the hydroxamic acid [equation (1)].

$$R^1CONHOH + R^2CO\text{-}Onp \longrightarrow R^1CONHOCOR^2 + npOH \quad (1)$$

(np = 4-nitrophenyl)
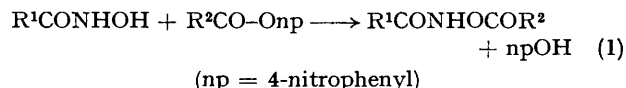
The degree of dissociation (α) and concentration of the hydroxamic acid were determined from the u.v. absorbance at 300 nm;[8] values for the total hydroxamic acid concentration were checked using other analytical methods. Whereas the pH-profile (Figure) for the reaction of methacryloylhydroxamic acid with 4-nitrophenyl acetate follows the ionisation of the acid (α) the value of k_2 for the polymer increases with pH almost six-fold after ionisation is substantially complete $(\alpha = ca.$ 1 after pH 9·8). The value of k_2 for those hydroxamic acid groups on the polymer ionising after pH 9·8 $(< 0·1\%$ of the total) is greater than that due to the totality of the groups on the monomer. A plot of k_2 vs. α indicates that the reactivity[8b] of the polymer hydroxamates vary from 1·76 to $> 50\,000$ l mol^{-1} s^{-1} at zero and full ionisation respectively. Reactivity of the monomer is independent of α and has the value 47 l mol^{-1} s^{-1} for 4-nitrophenyl acetate.

A linear copolymer was prepared by hydroxylaminolysis of a copolymer from solution polymerisation [MeCN solvent using 2,2'-azobis(2-methylpropionitrile) initiator] of a feed composition methyl methacrylate: acrylamide in the molar ratio 1 : 5·7; the increase in reactivity of this polymer against fission of 4-nitrophenyl acetate was from 0·54 to 9·03 l mol^{-1} $(ca.$ 17-fold) from zero to full ionisation of the hydroxamic acid groups.

We ascribe the enormous increase of the reactivity to the ionisation of hydroxamic acid groups in the interior of the microgel 'bead'; the last groups to ionise will, according to Faraday's rule be those of the interior. The enhanced nucleophilicity of the inner hydroxamates is probably due to the reaction of the anionic species with the substrate in a non-polar microscopic medium provided by the polymer backbone. This explanation is similar to that for enhancements in reactions catalysed by micelles and polysoaps.[9] This work and an earlier study with linear polymeric hydroxamic acids as reagents for nitrophenyl ester cleavage do not indicate such dramatic enhancements[8] and the upward curvature in plots of k_2 vs. α for these reagents is attributed to the expansion of the polymer due to increased electrostatic repulsion;[8] the expansion is not sufficient to overcome entirely the steric crowding of the polymer reagent relative to that of the monomer and even at full ionisation the control polymer is still only 1/5 as active as the monomer reagent.

We note that the microgel reagent must be excessively crowded so that the true enhancement of reactivity must be relative to the crowded reagent; thus a value of $> 28\,000$

‡ The pendant 4-nitrophenyl ester was converted by hydroxylaminolysis at alkaline pH into a mixture of carboxylic and hydroxamic acid groups in the ratio 5·5 : 1. The molecular weight calculated from an assumed density of 1 and a contracted diameter of 400 Å is 18 000 000. Passage of the polymer through a mixed bed ion exchange resin removed surfactant.

is obtained. With respect to the monomer an enhancement of $> 1\,000$ is observed.

Increasing the size of the substrate markedly reduces the reactivity of the polymer at complete ionisation while not altering that at low values of α. A slight increase in reactivity in the case of the 4-nitrophenyl caproate is taken to mean that there is partial entry of the substrate to the interior of the microgel 'bead'.§ 4-Nitrophenyl adipate behaves as a normal substrate with a reduced rate and little curvature in the plot of k_2 vs. α; in this case both electrostatic and steric forces allow the substrate to react only at the surface of the polymer.

§ Since we are suggesting an apolar micro-environment a type of non-productive binding between the n-alkyl 4-nitrophenyl esters might be envisioned (see, for example, T. Kunitake and S. Shinkai, *Polymer J.*, 1973, **4**, 253). This is probably not the case with our system since the negatively charged adipate also shows no enhancement; this behaviour is consistent with molecular exclusion but not with a non-productive binding explanation.

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[7] S. P. S. Yen, A. Rembaum, R. W. Molday, and W. Dreyer in 'Emulsion Polymerisation,' eds. I. Piirma and J. L. Gardon, The American Chemical Society Symposium Series 24, 1976, p. 236.

[8] (a) T. Kunitake, Y. Okahata, and R. Ando, *Bull. Chem. Soc. Japan*, 1974, **47**, 1509; (b) Reactivity is defined as the slope of the plot of k_2 vs. α and represents the second order rate constant of those anions ionising at a given α.

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