Studies with microgels as matrices for molecular receptor and catalytic sites: stereoselectivity of copolymers of (−)-menthyl acrylate and *N*-methacryloyl-hydroxylamine *versus* chiral esters

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The reactivity of 4-nitrophenyl esters has been studied against an optically active microgel copolymer containing hydroxamic acid pendant groups. Saturation phenomena are observed in the kinetics consistent with formation of a complex followed by reaction of the ester in the complexed species. The polymer discriminates between R- and S-forms of a chiral substrate in both complexing and catalytic steps. The data are consistent with the existence of chiral spaces in the microgel bead which accept the chiral substrate. The selectivity of the polymer for the chiral substrate ($\alpha \sim 2$) compares favourably with that shown by conventional chiral chromatographic supports.

INTRODUCTION

Most of the naturally occurring enzymes exhibit a characteristic stereospecificity as well as a structural selectivity in their catalytic action towards substrates. Many synthetic co-polymers that contain reagent or catalytic groups have been investigated to provide simple enzyme-like catalysts which are chemically robust and selective[1-3]. Although synthetic polymers have simpler structural features on a connectivity basis than those of enzymes there are now many examples of synthetic polymers with much higher reactivity than their respective monomer analogues[4-6].

There are several reports of polymer catalysts which, like enzymes, exhibit specificity[6,7], saturation phenomena[8-10] and competitive inhibition[10,11]. Most studies of catalysis by polymers have involved homogeneous solutions of the linear species which lack significant rigidity and which are incapable of maintaining a time or pH-independent structure; these polymers may not be used as matrices for receptor sites with a fixed structure. Previous studies of stereoselective polymer catalyst have used linear systems and for this reason poor selectivity has been observed[12,13]. Incorporation of optically active monomer in the copolymer with a symmetrical reagent monomer is unlikely to yield significant enantiomeric selectivity as the chiral centre will be far removed from the reagent.

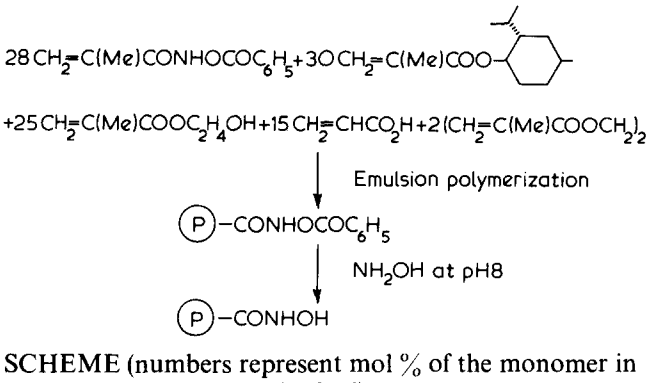
We have shown[14,15] how hydroxamic acid reactant groups may be incorporated into the framework of a microgel[16] and have recorded dramatically enhanced rate constants for reactivity against their reaction with 4-nitrophenyl esters. The advantage of the microgel is that it provides a time-table matrix for receptor site formation and yet is potentially soluble in solvents whereas massive cross-linked polymers are not. The present work is a study of the incorporation of optically active monomers into a microgel (Scheme) with a view to the induction of stereoselectivity into a symmetric nucleophile.

A microgel is defined as a spherical particle of diameter between 200 and 2000 Å composed of polymeric cross-linked chains[16]. These particles may be dispersed in water provided they possess sufficient hydrophilic groups and the resulting colloidal suspension is referred to in this paper as a polymer solution.

EXPERIMENTAL

Materials

(—)-Menthyl acrylate was prepared by adding triethylamine (21.3 g, 0.21 mol) in ether (100 ml) to an ice-cooled solution of acryloyl chloride (19 g, 0.21 mol) and (—)-menthyl alcohol (0.2 mol) in ether (100 ml). The solution was stirred and the addition regulated to maintain a mild reflux. After the addition was complete the



SCHEME (numbers represent mol % of the monomer in the feed).

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mixture was refluxed for 0.5 h. The ether solution was filtered to remove the hydrochloride precipitate, washed with 10% aqueous sodium bicarbonate and dried with Na_2SO_4. The ether was removed *in vacuo* and the ester distilled b.p. 120–2°/15 torr (lit. b.p. 128/18 torr)[17]; $[\alpha]_D^{20} = -66.16°$ (neat, $l=0.5$); yield almost theoretical.

N-Methacryloylhydroxylamine and *O*-benzoyl-*N*-methacryoylhydroxylamine were prepared as previously described[15]. Other monomers were obtained commercially and distilled before use to remove radical traps used as preservative. The products were stored in bottles, sealed under nitrogen, in a freezer at −20°. 4-Nitrophenyl esters were purchased from Sigma Chemical Company or were from previous investigations[15]. The structures of the purchased and synthesized monomers were confirmed by their i.r. and n.m.r. spectra.

Buffer materials and reagents were either of analytical grade or were redistilled or recrystallized before use. Water was doubly distilled from glass.

METHODS

Polymerization

Water (100 ml) was deaerated and placed in a thick walled glass bottle fitted with a polythene-lined screw cap (8 oz tonic water bottles are ideal). (—)-Menthyl acrylate (0.88g, 30 mol %) 2-hydroxyethylmethacrylate (0.42g, 25 mol %), 1,2-ethylenedimethacrylate (0.05g, 2 mol %) acrylic acid (0.14 g, 15 mol %) and *O*-benzoyl-*N*-methacryloylhydroxylamine (0.75 g, 28 mol %) were added to the bottle with sodium lauryl sulphate (100 mg). The bottle was placed in a trigol bath set at 60–70° and the contents stirred with a magnetic stirrer. The emulsion was purged with nitrogen, potassium persulphate (50 mg) added and the bottle sealed. The polymerization mixture was kept stirred and heated for 3–4 h and the reaction stopped by the addition of quinol (100 mg), cooled to room temperature and filtered to remove any massive particles. The solution, which had a blue opalescent appearance, was passed through an ion exchange column to remove the emulsifying agent; the removal of sodium laurylsulphate was monitored with $BaCl_2$ solution. Hydroxylamine hydrochloride (1g) was then added and the pH adjusted to 8.0. The material was stirred for 24 h and then dialysed *versus* distilled water.

Analytical procedures

Carbon, hydrogen and nitrogen analyses were carried out on the dialysed polymer after it had been evaporated to dryness. We thank Mr. A. J. Fassam who made the determinations using a Carlo Erba Elemental Analyzer model 1106.

Hydroxamic acid concentration was determined from the change in absorbance at 300 nm from acid to alkaline pH. The extinction coefficient change is assumed to be identical with that of monomer hydroxamic acid; previous work from this laboratory[15] and that of Kunitake[18] indicates this assumption to be valid and enables us to use the more convenient spectrophotometric method (molarity of hydroxamic acid = Δ absorbance at 300 nm × 1230).

The pH-titration of the polymer was measured spectrophotometrically using the absorbance at 300 mm. Polymer solution (25λ) was added on the flattened end of a glass rod to a solution of buffer (0.1 M, 2.5 ml) in a silica cell in the thermostatted cell compartment of a Unicam SP 800 recording spectrometer. The absorbance was recorded and the trace extrapolated to zero time to allow for changes due to the Lossen rearrangement which occurs at high pH. The absorbance is treated according to the Henderson–Hasselbach equation (equation 1) where FB* is the fraction of base which is determined from the difference in absorbance

$$pK_a = pH + n \log [(1 - FB)/FB] \quad (1)$$

from that at pH 1 and the total change in absorbance from pH 1 to 12.

Optical rotations were carried out with Bellingham and Stanley PEPOL 60 polarimeter using a 0.5 dm path length cell and an Isotope Developments EHT unit and Pye Scalamp Microameter.

Kinetics

The reaction of the esters (see Table) with polymer solution was followed by spectroscopic determination of the release of 4-nitrophenol using a Unicam SP 500 instrument coupled with a linear-logarithmic converter and servoscribe recorder. The method was essentially that described for the pH-titration. Stock polymer solution (25μl) was added on the flattened tip of a glass rod to a solution of buffer (0.1 M, 2.5 ml; see *Tables 1* and *2* for the nature of the buffers) in a silica cell in the thermostatted cell compartment of a Unicam SP 800 spectrophotometer. Ester, dissolved as a stock solution in ethanol, was then added (25 μl) and the potentiometric recorder initiated to record the change in absorbance with time. The pH was measured before and after the kinetic run using a Radiometer digital pH-meter PHM 62.

RESULTS

The technique of emulsion polymerization is well established; previous electron micrograph studies from our laboratory have shown that the diameter of beads from the polymerization conditions as shown in the experimental are in the region of 400 Å (dry). The diameter of the microbeads depends mainly on the feed concentration[16c]. Inclusion of solubilizing agents (hydroxyethyl methacrylate and acrylic acid) in the monomer feed provides a stable emulsion even in the presence of high salt concentration or in the absence of sodium laurylsulphate. Removal of the *O*-benzoyl group subsequent to polymerization by hydroxylamine provides hydroxamic acid pendant groups irrespective of whether the site of attack is at the benzoyl or acryloyl carbonyl group[15].

Stoichiometry

The yield of polymer in the emulsion polymerization obtained by weighing the dried dialysed solid varied from ~20 to 60%. The combustion analysis (C, 59.9%; H, 7.0%; and N, 3.1%) agrees reasonably well with the nitrogen analysis as measured from the hydroxamic acid assay (3.7%). The monomer feed ratio would give a nitrogen content of 2.3% and the excess may be due to reaction of the hydroxylamine with normal ester links; this is not

* FB = [B]/([B] + [BH]) where BH and B are respectively hydroxamic acid and its conjugate base

Table 1 Rate constants for release of 4-nitrophenol from esters as a function of polymer concentration[a,g]

pH	[HA] x 10^4 (M_i)[b]	k_{obs} x 10^3 (s^{-1})	k x 10^3 (s^{-1})[c]
4-Nitrophenyl acetate (1.2 x 10^{-5} M)[d]			
10.41	2.92	3.2	0.85
10.42	5.84	3.9	1.6
10.42	8.76	4.7	2.4
10.40	11.7	5.3	2.9
10.39	14.6	6.4	4.0
10.38	17.5	7.4	5.0[h]
10.40	20.4	8.3	6.0
10.38	23.4	8.5	6.2
10.38	26.3	9.6	7.3
10.44	0	2.35	7.3
4-Nitrophenyl caproate (1.81 x 10^{-5} M)[d]			
11.70	2.78	10.7	0.78
11.67	5.56	12.0	2.02
11.66	8.34	12.8	2.82
11.66	16.7	16.0	6.08
11.64	19.5	16.9	6.98
11.73	0	9.92	
4-Nitrophenyl *N*-Z-glycine ester (2.16 x 10^{-5} M)[e]			
9.95	2.74	17.4	6.4
9.93	4.11	20.9	9.9
9.92	5.48	23.0	12.0
9.92	6.85	25.2	14.2
9.92	8.22	29.1	18.1
9.92	11.0	33.8	22.8
9.90	12.3	38.3	27.3
9.91	13.7	39.7	28.7
9.91	16.4	41.1	30.1
9.95	0	11.0	
4-Nitrophenyl *N*-Z-S-phenylalanine ester (1.70 x 10^{-5} M)[d,f]			
10.23	25.6	17.9	9.9
10.18	20.5	17.7	9.7
10.27	17.9	17.4	9.4
10.22	15.4	15.7	7.7
10.21	10.2	13.6	5.6
10.24	5.1	11.3	3.3
10.23	2.56	9.59	1.6
10.21	0	7.99	
4-Nitrophenyl *N*-Z-R-phenylalanine ester (5.81 x 10^{-5} M)[d,f]			
10.24	25.6	14.4	6.4
10.20	20.5	14.4	6.4
10.20	15.4	13.7	5.7
10.08	10.2	12.3	4.3
10.21	5.1	11.0	3.0
10.21	0	7.99	
4-Nitrophenyl *N*-Z-R-alanine ester (1.00 x 10^{-6} M)[d]			
10.30	0.6	8.16	0.48
10.32	1.2	9.59	1.9
10.34	1.8	10.6	2.7
10.30	2.4	11.6	3.9
10.30	3	12.3	4.6
10.35	4.2	14.4	6.7
10.32	0	7.68	

[a] 25°, carbonate buffers, ionic strength 0.04
[b] Molarity of the polymer as a function of the hydroxamic acid groups (determined spectrophotometrically)
[c] This value is corrected from the background rate constant at the pH in question
[d] 20% (v/v) EtOH/H_2O
[e] 8% (v/v) EtOH/H_2O
[f] Parameters k_{max} and K_m (equation 2) for S ester are 2.0 x 10^{-2} s^{-1} and 2.5 x 10^{-3} M_i and for R ester are 9.4 x 10^{-3} s^{-1} and 1.1 x 10^{-3} M_i respectively
[g] The polymer stock solution throughout this table is from the same batch of polymer
[h] Reactivity to 4-nitrophenyl acetate is $k_2 = k/$[total hydroxamic acid] = 2.9 M^{-1} s^{-1}

expected to contribute much as hydroxylamine has been shown not to react efficiently with microgels composed of acrylic ester monomer. It is possible that the *O*-benzoyl-*N*-methacryloylhydroxylamine monomer was incorporated to a larger extent than its monomer feed composition. The specific rotation of the polymer stock solution was found to be $[\alpha]_D^{25} = -66.16°$ (neat, $l = 0.5$).

Varying yields of polymer are obtained, therefore the detailed polymer structure is likely to be variable. Comparisons between ester reactivities must be made using the same stock polymer solution. Physical properties such as light scattering, water content or turbidity will not be affected by small differences in the internal architecture of the polymer. Comparison of reactivities using different batches of polymer is dangerous in view of the small differences expected in reactivity between enantiomeric esters. Kinetic reactivity toward ester hydrolysis is probably the best index of the structure of the polymer. Different batches of polymer have similar reactivities but the differences are larger than those observed between enantiomeric esters. Reference to *Tables 1* and *2* indicate that for this series of experiments two polymer batches have reactivities toward 4-nitrophenyl acetate varying two-fold at pH 10.38.

Kinetics

The liberation of 4-nitrophenol from the esters obeyed first order kinetics over at least 90% of the total reaction. The pseudo first order rate constants were proportional to polymer concentration at low concentrations; those for the phenylalanine substrates showed marked deviation at high polymer concentration and obeyed a hyperbolic rate law (equation 2).

$$k_{obs} = k^{max}[P]/(K + [P]) \quad (2)$$

The results for the R and S forms of 4-nitrophenyl N_α-benzyloxycarbonylphenylalaninate are illustrated in *Figure 1*. The other substrates exhibited a linear rate dependence on polymer concentrations (*Table 1*). The concentration of polymer [P] is expressed as the molarity of the hydroxamic acid monomer.

The dependence of the rate constant on pH follows the well established pattern for the microgel polymer hydroxamic acids[14] and involves a marked increase as the fraction of hydroxamic conjugate base (FB) increases. These results are illustrated in *Figures 2* and *3* for

Table 2 Reaction of 4-nitrophenyl acetate with polymer over a pH-range[a,f]

pH[e]	F.B.[b]	k_{obs} x 10^5 (s^{-1})	k_{buff} x 10^5 (s^{-1})[c]	k x 10^5 (s^{-1})	k_2^d (s^{-1} M^{-1})
7.60	0.13	4.57	2.99	1.58	0.062
9.04	0.60	36.5	21.9	14.6	0.57
9.50	0.75	64.0	48.6	15.4	0.60
9.84	0.84	132	123	90	3.52
10.16	0.91	349	213	136	5.31
10.54	0.94	640	492	148	5.78
10.76	0.94	1820	1250	570	22.3

[a] 25°, ionic strength at 0.1, 20% EtOH/H_2O (v/v)
[b] Calculated from pH and the Henderson–Hasselbach equation 1
[c] Rate constant for reaction with buffer alone
[d] $k_2 = k/$[total hydroxamic acid]
[e] Carbonate buffer from pH 9–11 and trishydroxy methyl amino methane at pH 7.60
[f] Polymer (2.56 10^{-4} M) was from a different stock solution from that used in *Table 1*

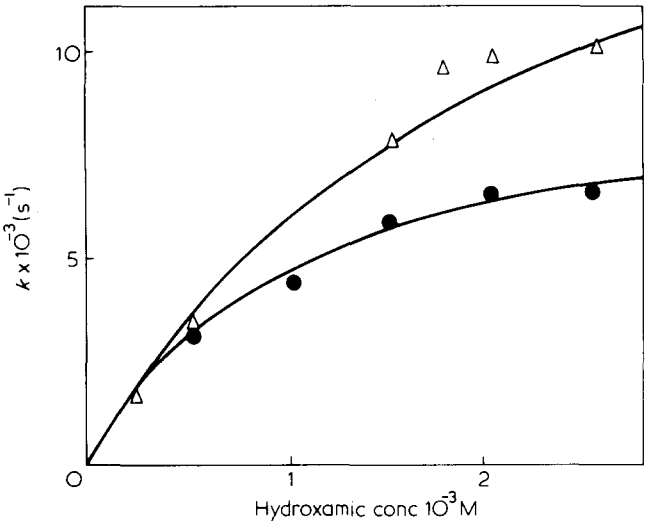

Figure 1 Dependence of the rate constant for 4-nitrophenyl release from 4-nitrophenyl *N*-Z-(S)-phenylalaninate (△), and *N*-Z-(R)-phenylalaninate (●), on the concentration of optically active polymer catalyst. The lines are theoretical from equation (2) using parameters from *Table 1*. The abscissa represents the molarity of hydroxamic acid groups and the ordinate is the rate constant corrected for background hydrolysis. Conditions are given in *Table 1*

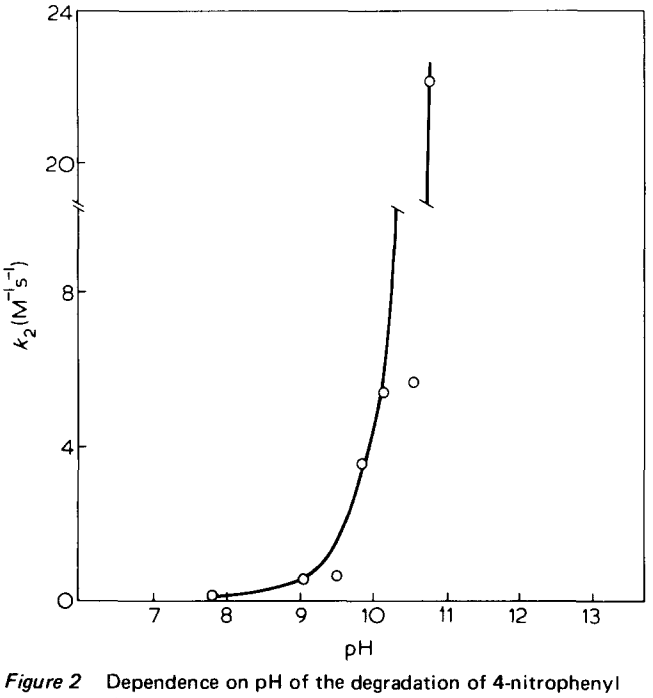

Figure 2 Dependence on pH of the degradation of 4-nitrophenyl acetate by the optically active, hydroxamic acid containing, polymer; conditions and data are given in *Table 2*

variation with pH and FB respectively. In all experiments control reactions were run in the absence of polymer to correct for background rate constants (*Table 2*).

Ionization

Plotting log FB/(1 − FB) *versus* pH yields a linear relationship (equation 1) with $pK_a = 8.55$. The relatively high value of n (1.5) indicates that there is considerable influence of the negatively charged groups on the polymer molecule as expected from an acrylic acid feed composition of 15 mol %.

DISCUSSION

The reaction being followed in the scission of the 4-nitrophenyl esters is the acylation of the polymer hydroxamic acid (equation 3).

$$\text{Ⓟ}-\text{CONHOH} + 4\text{-}NO_2C_6H_4OCOR \rightarrow \text{Ⓟ}-\text{CONHOCOR} + 4\text{-}NO_2C_6H_4OH \quad (3)$$

Comparison of the present polymer with that utilizing a methyl methacrylate backbone[14] reveals that at low values of FB the reactivity *versus* 4-nitrophenyl acetate is lower but becomes comparable as the pH increases. The behaviour is similar to that of the polymer with methyl methacrylate backbone cross-linked to the extent of 14% as there is a dramatic increase in reactivity at FB ~0.8 after a steady increase from FB = 0. With lower values of cross-linking comonomer feed (~2%) the increase in reactivity is not usually so marked[15]. Possibly the marked reactivity increase with the present polymers is related to the bulk of the menthyl group compared with the methyl group it replaces in polymers with similar cross-linking percentages. We have previously discussed the rate enhancements which the polymer hydroxamates bring about.

The phenomenon of saturation (*Figure 1*) observed with the bulky phenylalaninate substrates is probably a result of the filling of cavities in the polymer. We have indicated[14,15] that the most reactive hydroxamate ions (in non-chiral microgels) are in the bulk of the microsphere; the least active nucleophiles are those of the surface and their lower reactivity is presumably due to their solvation. Entry of ester to the reactive nucleophiles requires passage through channels or cavities and it is the occupation of these voids which we believe causes the saturation effect. There may be other, residual, complexing forces but we do not regard these as important as the permeation phenomenon. The saturation result is of course further evidence for the existence of cavities advanced previously[14,15].

Figure 1 shows a marked difference between polymer

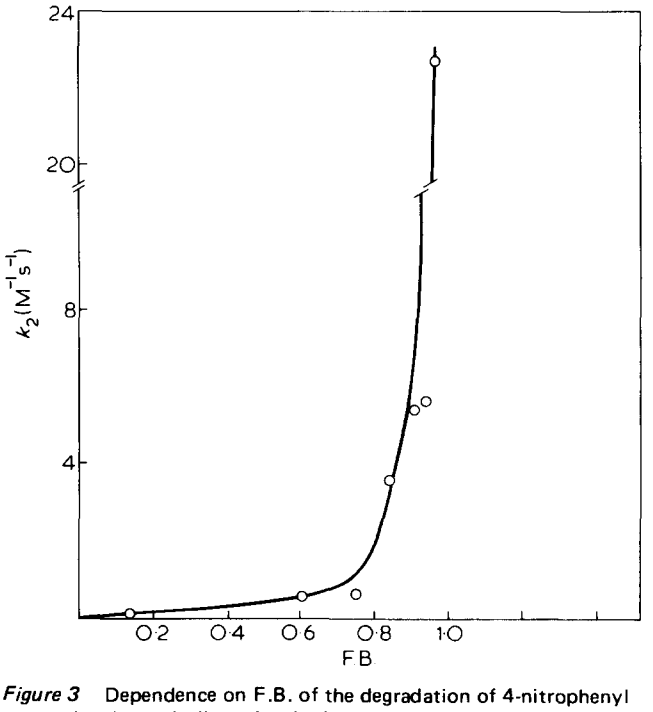

Figure 3 Dependence on F.B. of the degradation of 4-nitrophenyl acetate by the optically active, hydroxamic acid containing, polymer. F.B. is the fraction of total hydroxamic acid present as the free base form. Conditions and data are from *Table 2*

catalysis of scission of R and S forms of the phenylalanine ester. The kinetic rate law for both R and S forms of the substrate (equation 2) has $K^S \approx 2K^R$ consistent with weaker 'binding' for the S enantiomer. The complexed S-enantiomer decomposes (k^{max}) nearly twice as fast as the R-form.

The present polymer has a separation factor K^S/K^R of approximately 2 for the selectivity for the two enantiomers. The massive polymers prepared by Wulff and coworkers[19] using 'templates' to provide chiral cavities had separation factors α (α is the ratio of the distribution coefficients of enantiomers between solution and polymer) in the region 1.15–1.24. In the latter case the massive polymer was fabricated into a column and the selectivity measured by passage of the enantiomers. Reference to a recent successful chromatographic separation of the enantiomers of mandelamide on chiral acrylamide polymers[20] indicates that a separation factor of $\alpha = 1.4$ gives an excellent division of enantiomers. The recent work of Cram[21] on the chiral recognition of amino acids by chiral cyclic polyethers indicates values 2.3–52 for the separation factor between enantiomers. The better selectivity observed for the small molecule receptors is probably due to the existence of one relatively good site; in the polymer the selectivity is due to a statistical combination of good and poor sites and if no effort is made to increase the former then it is not surprising that seemingly low values of α are obtained.

The success of the present method and that of Wulff[19] is probably due to the synthesis of chiral voids within the bulk of the polymer molecule. Blaschke and Donow[22] showed that post-polymerization modification of a non-chiral acrylamide with chiral reagent produced a chiral polymer with little resolving power; preparation from chiral monomers produced a polymer with excellent resolving power for R,S-mandelamide[20]. In the former case the chirality presumably resides on the surface of the polymer whereas in the latter it is homogeneously distributed and may therefore participate in forming chiral voids within the bulk.

Stereoselectivity has been observed in reactions of chiral micelles with chiral substrates (usually esters); the system may involve a nonchiral surfactant with chiral nucleophile and ester[23] to achieve modest selectivity or the chiral nucleophile may be covalently linked to the surfactant[24]. A further variation has been the use of optically active surfactant, non chiral nucleophile and substrate exemplified by the sodium cholate mediated asymmetric reduction of aromatic ketones[25]. Both Brown and Bunton[24] and Ihara[26] observe saturation of the chiral micelles with the ester substrate and note that the dissociation constant for the complex is not stereoselective (in contrast to our results). These authors[24,26] conclude that stereoselectivity resides in the transition state of the reaction rather than the ground-state. Extremely small selectivities were observed when the surfactant but not the nucleophile was chiral[25]. Even placing the centre of chirality on an atom α or β to the nucleophilic species did not increase selectivity greatly when either non-chiral surfactants were used or those chiral nucleophiles with covalently attached non-chiral surfactant chains[27]. There is no possibility that chiral cavities are formed in micelles with lifetimes greater than a diffusion process[28]; this is borne out by the non-selective dissociation constants. The stereoselectivity arises for the micelle catalysis through a difference in energy between diastereoisomeric transition states. The present case of microgel selectivity arises purely from chiral voids recognizing a complementary structure. We note that the chiral centre (the menthyl group) can never be closer than 7 atoms from the nucleophilic oxygen atom of the hydroxamic acid monomer.

This paper, together with our previous work[14,15] establishes that microgels form excellent vehicles for the attachment of reagent groups. The dimensions of microgels are such that these macromolecules are large enough to possess the rigidity necessary if they are to act as matrices for molecular recognition sites; they are small enough to be easily solubilized without surfactant in water or organic solvents to enable kinetic studies to be carried out. Our future work in the field of microgel reactivity is a study of the possibilities of tailoring molecular recognition sites already pioneered with massive polymers[19,29–31].

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