

## Triethoxysilane-substituted acrylate copolymers as reagents for the derivatization of porous silica beads

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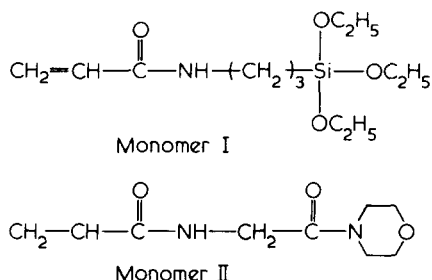
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Reaction schemes for the covalent derivatization of porous silica beads have, hitherto, involved coating of the internal surface with trialkoxysilane derivatives of small, functionally active molecules<sup>1,2</sup> or by radiation-induced graft polymerization of simple vinyl monomers<sup>3-5</sup>. In this Communication we describe an alternative, versatile derivatization procedure in which the internal silica surface is coated by reaction with purpose-synthesized triethoxysilane-substituted copolymers.

The triethoxysilane-substituted copolymers are prepared by free radical copolymerization of *N*-[(3-triethoxysilyl)propyl]acrylamide<sup>6</sup> (Monomer I) and 4-(*N*-acryloylglycyl)morpholine (Monomer II), either alone or in the presence of other functionally active acrylate monomers.

Choice of Monomer I was dictated by



the need to incorporate pendant groups for the subsequent covalent anchorage of the copolymers to the internal silica surface, Monomer II, which was prepared by established procedures of amino-acid and peptide chemistry, was selected in order to incorporate into the copolymer a substantial proportion of side-chains with terminal *N*-acrylmorpholine residues. The latter are known to guarantee good solvation of copolymers in water and in all-polar organic solvents<sup>7</sup>. Simple copolymers incorporating Monomers I and II only could be reacted with porous silica beads to give composites with a large, passive, internal surface which was wholly compatible with these solvents.

A triethoxysilane-substituted acrylate copolymer (Figure 1) which provides an internal coating of porous silica beads potentially active in the covalent bonding of enzymes was prepared as follows<sup>8</sup>. A solution of Monomer I (1.10 g, 0.004 mol), Monomer II (3.96 g, 0.02 mol) and *N*-acryloyl-*N'*-*t*-butoxy-carbonyl hydrazine (0.74 g, 0.004 mol) in dry methanol (30 cm<sup>3</sup>) was deoxygenated by bubbling nitrogen for 1 h. Initiator,  $\alpha, \alpha'$ -azobisisobutyronitrile (0.1 g), was added and the solution heated under reflux for 4 h. The solution was evaporated to dryness under reduced pressure. The residue was finely ground and any monomeric and oligomeric species removed by repeated trituration with dry acetone followed by ethyl acetate. The copolymer had a number-average molecular weight (membrane osmometry)  $\bar{M}_n = 101\,000$  and an intrinsic viscosity at 25°C in chloroform  $[\eta] = 0.42$  dl g<sup>-1</sup>.

Spherosil porous silica beads (Rhône-Poulenc Industries, Paris-Cédex) Type XOB 015 (Internal surface area  $S_A =$

25 m<sup>2</sup>g<sup>-1</sup> and average pore diameter  $\bar{d}_p = 125$  nm) and Type XOC 005 ( $S_A = 10$  m<sup>2</sup>g<sup>-1</sup> and  $\bar{d}_p = 300$  nm), each with pore volumes of 1 cm<sup>3</sup>g<sup>-1</sup>, were selected for derivatization. The beads were pretreated by heating in 0.2 M HNO<sub>3</sub> at 80°C for 4 h with continuous sonication after which they were washed repeatedly with distilled water and dried by heating at 640°C for 18 h. Coating of the internal surfaces of the porous silica beads was effected by heating the beads under gentle reflux in a 10% w/v chloroform solution of the copolymer for 18 h. Following exhaustive washing with hot chloroform, the resulting composite materials were dried under reduced pressure. Samples of the internally coated beads were subjected to controlled hydrolysis with 2M HCl in MeOH/H<sub>2</sub>O (2/1) at ambient temperature over 1 h in order to deprotect the acyl hydrazide groups pendant on the attached copolymer. The degree of acyl hydrazide derivatization was determined by conversion of the acyl hydrazide groups to their picrate salt followed by dissociation with 5% diisopropylethylamine in chloroform and spectrophotometric estimation of the washings at 358 nm<sup>9</sup>. The materials obtained from Spherosil Type XOB 015 and Type XOC 005 had acyl hydrazide contents respectively of  $2.84 \times 10^{-5}$  and  $1.23 \times 10^{-5}$  mol g<sup>-1</sup>. The corresponding polymer contents, calculated from elemental analysis, were 58.2 and 25.2 mg g<sup>-1</sup>. Thus the amount of copolymer bound to the silica surface is proportional to the internal surface area of the Spherosil porous silica beads.

The application of the composite materials to the immobilization of two enzymes, bovine erythrocyte carbonic anhydrase (BDH Ltd, Poole) and horseradish peroxidase (BDH Ltd., Poole) was demonstrated. Following deprotection of the acyl hydrazide groups, these were activated for enzyme coupling by conversion to acyl azide residues by treatment with HNO<sub>2</sub>. Immobilization was effected by contacting samples of the activated composites with solutions of the enzymes in aqueous buffer. Control experiments were performed in which polymer-coated beads, not activated by HNO<sub>2</sub> treatment, were contacted with the enzymes. Both

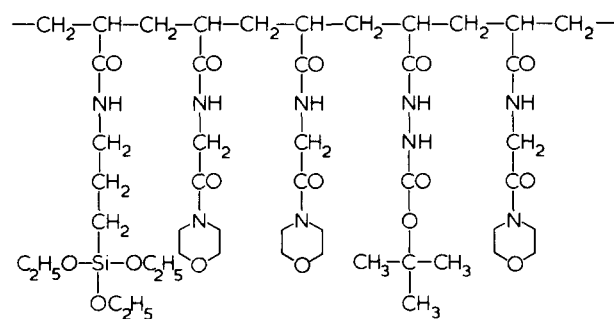


Figure 1 Schematic representation of copolymer of *N*-[(3-triethoxysilyl)propyl]-acrylamide, 4-(*N*-acryloylglycyl)-morpholine and *N*-acryloyl-*N'*-*t*-butoxycarbonylhydrazine (molar ratio 1:5:1)

the conjugates and the controls were subjected, in parallel, to exhaustive washing with buffered salt solution until both the enzyme activity of the conjugates became constant and the enzyme activity of the controls was zero. This dual check ensured that the possibility of non-specific adsorption of enzyme was eliminated. Spherosil Type XOB 015- and Type XOC 005-based carbonic anhydrase conjugates were found to have respective protein contents of 7.2 and 4.2 mg g<sup>-1</sup>. Carbonic anhydrase activity was determined by spectrophotometric estimation at 348 nm of 4-nitrophenol produced on enzyme hydrolysis of 4-nitrophenylacetate<sup>10</sup>. The activities of the bound carbonic anhydrase relative to equivalent amounts of the free enzyme in solution were 10.6 and 6.4%, respectively. Spherosil Type XOB 015- and Type XOC 005-based horseradish peroxidase conjugates had respective protein contents of 2.9 and 0.8 mg g<sup>-1</sup>. Horseradish peroxidase activity was determined by spectrophotometric estimation at 420 nm of purpurogallin produced on enzyme oxidation of pyrogallol in the presence of hydrogen peroxide<sup>11</sup>. The activities of the bound horseradish peroxidase relative to equivalent amounts of the free enzyme in solution were 0.5 and 1.24%, respectively.

Currently we are investigating copolymers incorporating

Monomer I, Monomer II and other functionally active acrylate monomers. Our aim is to use these to prepare other porous silica-based composites suitable for application as heterogeneous catalysts and as solid phase reagents.

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## Photoelasticity of glassy polymers

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The birefringence of plastically deformed glassy polymers is known to be related to molecular orientation induced by the deformation process<sup>1,2,3</sup>. However, the birefringence (often comparable in amount) that arises while a glassy polymer is elastically deformed is much less understood, despite the use of such materials in photoelastic stress analysis.

In this Communication we discuss the effect of elastic deformation on the local electric field, showing that this can produce a negative component of birefringence of approximately the correct magnitude for poly(methyl methacrylate) (PMMA).

Figure 1 shows the birefringence of PMMA (ICI Perspex) for tensile specimens held at constant length at approximately 20°C, birefringence being measured with a six-order Berek compensator. By using a wide-angle X-ray diffraction method of orientation measurement<sup>4</sup>, we have shown that the chain orientation in the elastically deformed specimens is negligible in comparison with that in plastically deformed specimens having the same birefringence. However, diffraction measurements did show that, as might be expected, the average interchain spacing parallel to the extension direction increased during elastic straining whereas that perpendicular to the extension direction decreased. These changes, shown in Figure 2, are discussed elsewhere<sup>5</sup>. They imply that the material surrounding any particular chain segment can no longer be considered to be isotropic and the usual Lorentz treatment of the local field<sup>6</sup> cannot be applied.

In anisotropic crystals, the local field has been evaluated

by summing over all the lattice points using special methods to achieve convergence<sup>7</sup>. Such methods cannot be applied to anisotropic non-crystalline solids and so we have used a modification of the Lorentz approach originally proposed by Havelock<sup>8</sup> in 1908 for inorganic glasses. This models the local anisotropy of the strained solid by allowing the imaginary spherical cavity of the Lorentz model to deform into an ellipsoid. This effectively compensates for the local anisotropy and allows the continued use of the assumption that the molecules inside the cavity make no net contribution to the field. We have used the measured changes in interchain spacings to give the axial ratio of the ellipsoid, whereas Havelock assumed that the cavity deformed by the same amount as the solid.

For an ellipsoidal cavity with semi-axes (*a*, *b*, *c*), the local field, *E'*, due to an applied field, *E*, is given (in SI units) by<sup>8</sup>:

$$E'_x = E_x + \frac{1}{\epsilon_0} A_x P_x \text{ etc.} \quad (1)$$

where *P* is the polarization of the solid and the correction factors are

$$A_x = \frac{abc}{2} \int_0^\infty \frac{du}{(a^2 + u)^{3/2}(b^2 + u)^{1/2}(c^2 + u)^{1/2}} \quad (2)$$

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