A Novel Polymeric Phosphine Oxide-derived Support for Enzyme Immobilisation

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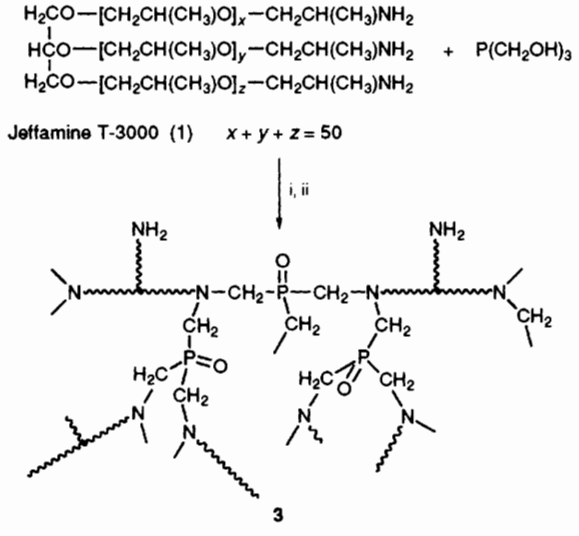
Synthesis of a novel polymeric support by Mannich condensation of $P(CH_2OH)_3$ with a polyetheramine provides flexible side chains for enzyme immobilisation without loss of catalytic activity.

The immobilisation of enzymes on polymeric supports is a highly attractive method for recovering and re-using the catalysts and a wide range of supports have been used. However, there is a continued need for durable supports which are resistant to mechanical, chemical and microbial degradation, and are capable of maintaining high enzymatic activities.[1] Here we report a novel, readily synthesised polymeric phosphine oxide support for the immobilisation of enzymes, together with some preliminary results on the immobilisation and activity of immobilised urease. Related polymeric phosphine oxides are currently finding commercial application as flame retardants for cellulosic materials.[2]

Mannich condensation polymerisation of tris(hydroxymethyl)phosphine [generated *in situ* from tetrakis(hydroxymethyl)phosphonium chloride (THPC) and sodium hydroxide][3] with 1 equiv. (*i.e.* NH:OH = 2.0) of glyceryl poly(oxypropylene)triamine (Jeffamine T-3000) **1** proceeded in ethanol over several hours to yield the rubbery polymeric phosphine **2**. Oxidation of **2** proceeded readily on treatment with dilute aqueous hydrogen peroxide, followed by washing and drying to give the polymeric phosphine oxide **3** in 86% overall yield as a white, semi-rubbery granular solid. Scheme 1 shows the synthetic procedure employed, together with a segment of the polymer indicating the types of functional groups present. The polymer has been characterised† by elemental analyses, and by ^{31}P-{^1H}, ^{13}C-{^1H}, and ^1H NMR spectroscopy as a solvent-swollen gel. Thus, the ^{31}P-{^1H} NMR spectrum of **3** showed a broad resonance ($\omega_{1/2}$ 260 Hz) at *ca.* δ 35. SEM shows the polymer to have smooth but cavitated surfaces, suggesting that the material will have significant surface area for enzyme immobilisation while minimising nonspecific adsorption.

We reasoned that the presence of the terminal long chain polyether-NH_2 groups in **3** would provide highly flexible 'anchors' for the immobilisation of enzymes, thereby minimising the reduction in activity often observed on immobilisation. Previous studies have shown that enzymes and rhodium(I) hydroformylation catalysts bound *via* short chains to the support are less active than those bound *via* longer links.[4]



Scheme 1 *Reagents and conditions*: i, polymerise, EtOH; ii, H_2O_2

Accordingly, we have investigated the immobilisation of the enzyme urease onto **3** by coupling the amine groups with glutaraldehyde.

In a typical immobilisation experiment, the polymer was stirred in 5% glutaraldehyde for 1 h at 30 °C and was subsequently washed until the washings were free of glutaraldehyde. Reaction with urease (10 mg enzyme and 200 mg polymer in 5 ml phosphate buffer at pH 7.4) then proceeded for 2 h at 30 °C followed by 24 h at 4 °C. The urease-modified polymer was washed thoroughly with water and 0.1 mol dm^{-3} phosphate buffer to remove unbound urease.

Activity determinations of the immobilised urease were carried out in 0.1 mol dm^{-3} phosphate buffer at pH 7.4, 30 °C with 100 mmol dm^{-3} urea to be certain that the reaction was zero order and not dependent upon substrate concentration. Samples were removed from the reaction mixture at intervals for estimation of NH_3 by the phenol-hypochlorite method.[5]

The kinetic parameter, K_m, was determined from linear Lineweaver–Burk plots in which the reaction was first order in urea concentration. The polymer-immobilised urease exhibited a K_m of *ca.* 8 mmol dm^{-3} at pH 7.4 and 30 °C, very similar to that of the free urease (K_m of *ca.* 5 mmol dm^{-3}). This similarity in K_m indicates that the environment around the immobilised enzyme is maintaining the flexibility necessary for enzyme activity and substrate accessibility to the active site. This result is in contrast to more rigid immobilisation supports which often result in a much higher K_m for urease, presumably due to the lack of mobility of the enzyme and inaccessibility of substrate to the active site.[6] We believe that these results can be extended to enzymes with larger substrates (*e.g.* proteases) since penetration/diffusion limitations should be minimised owing to the long flexible anchors with which the enzymes are bound, and the smooth surface of the polymer particles (from SEM).

These poly-phosphine oxide supports are remarkably robust to changes in pH, temperature, and chemical environment. Furthermore, the described synthetic protocol can be generalised to make the synthesis of a large variety of enzyme-to-polymer 'tethers' possible. We are continuing to explore a variety of polyphosphine oxide derivatives as enzyme supports capable of stabilising catalytic activity.

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Footnote

† Polymer **3**: NMR: ^{31}P-{^1H} (24 MHz, EtOH, external D_2O lock), δ 35 (br, $\omega_{1/2}$ *ca.* 260 Hz, LB = 1.7 Hz); ^{13}C-{^1H} ($CDCl_3$, 75 MHz), δ 77–72 (m, CH_2O and CHO), and 17.5 (s, CH_3). Resonances due to the PCH_2N carbons could not be observed, though these are expected to occur at *ca.* δ 40.[7] ^1H ($CDCl_3$, 300 MHz), δ 3.6 (vbr) and 1.1 (vbr).

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