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Adsorption and Expression of Penicillin G Acylase Immobilized onto Methacrylate Polymers Generated with Varying Pore Generating Solvent Volume†

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ABSTRACT

Adsorption and expression of penicillin G acylase was studied on macroporous methacrylate polymer beads of differing pore volume, generated with kerosene. The absorption and expression of the penicillin G acylase was dependent on pore volume. Maximum expression of 57% of adsorbed enzyme was obtained on beads synthesized with 40 mL of kerosene, indicating minimum pore-diffusion limitations.

Index Entries: Penicillin G acylase; immobilization; methacrylate polymer beads.

INTRODUCTION

Penicillin G acylase (EC 3.5.1.11) catalyze cleavage of linear amide bond in the penicillin G molecule thereby generating 6-aminopenicillanic acid (6-APA) and phenyl acetic acid (*1*). This group of enzymes has gained

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commercial importance since 6-APA is processed for the production of semisynthetic penicillins such as ampicillin and amoxycillin. Immobilized preparations of penicillin G acylase are used commercially in pharmaceutical industries for the production of 6-APA. A wide range of matrices have been used for the immobilization of penicillin G acylases (*1*). In recent commercial practices, synthetic polymers have acquired greater importance over natural polymers as supports for enzyme immobilization because these offer a number of advantages (*2*). Though the enzymatic process for the production of 6-APA is economically viable, scientific innovations and efforts to immobilize penicillin G acylase to newer type of matrices are continued with the objective of producing more 6-APA per unit of enzyme therby reducing the process costs further.

Recently, we have reported the relation of crosslinking agents, crosslink density and comonomers on the performance of penicillin G acylase immobilized onto macroporous methacrylate beads (*2*). In the present study, the effect of pore generating solvent concentration used in synthesis of macroporous methacrylate polymer beads on the adsorption and expression of penicillin G acylase is investigated.

MATERIALS AND METHODS

Materials

Penicillin G K and purified penicillin G acylase (sp. activity 7.5 IU/mg) from *Escherichia coli* were obtained from production unit of M/S Hindustan Antibiotics Ltd., Pune, India. The following chemicals were obtained from the suppliers indicated: ethylene glycol dimethacrylate and acrylic acid (Fluka AG); methacrylic acid (Gujarat State Fertilizers Corporation, India); and benzoyl peroxide (Loba Chemie). All other chemicals were of Analytical grade from local suppliers.

Synthesis of Polymer Beads

The synthesis, by suspension copolymerization, was conducted in a double-walled cylindrical polymerization reactor of 11 cm diameter and 15 cm height. The continuous phase comprised of 34% (w/w) aqueous solution of calcium chloride. The discontinuous organic phase consisted of methacrylic acid, 1 mol; ethylene glycol dimethacrylate, 0.025 mol; acrylic acid, 0.05 mol; and the indicated volume of kerosene. The ratio of aqueous to organic phase was 2.75:1.00. The initiator, benzoyl peroxide, was dissolved in the monomers and introduced to the aqueous calcium chloride solution. Stirring was started under a nitrogen overlay and the temperature was raised to 80°C. Polymerization was allowed to proceed for 2 h. The polymer beads were separated by decantation, rinsed with

water and suspended for 24 h in 0.1*M* sodium hydroxide followed by washing with distilled water till the pH value of the filtrate was neutral. The beads were dried under vacuum at 60°C, and the beads passing through a 20 mesh screen and retained on a 40 mesh screen were evaluated for immobilization purposes.

Immobilization of Enzyme

Equilibration of beads, adsorption of penicillin G acylase, and cross-linking of adsorbed enzyme were performed as described (*2*).

Analytical Methods

The pore size distribution was studied by mercury intrusion porosimetry using 33 mercury porosimeter from quantachrome (USA), up to a pressure of 33,000 psia.

The assay of soluble penicillin G acylase was run as described previously (*3,4*).

For the determination of activity of immobilized penicillin G acylase, 0.5 g of immobilized enzyme was suspended in 100 mL of 0.2*M* phosphate buffer, pH 7.8 in a beaker of 500 mL capacity and agitated (100 rpm) at 40°C. In a separate beaker 8 g of K penicillin G was dissolved in 100 mL of 0.2*M* phosphate buffer, pH 7.8. The solution was immersed in water bath to attain the temperature of 40°C. The reaction was initiated by addition of K penicillin G solution to immobilized enzyme slurry. The 6-APA formed at the end of the 30 min was estimated by 4-dimethylamino benzaldehyde method (*4*). One unit of enzyme activity is defined as the quantity of enzyme required to liberate 1 μmol of 6-APA in 1 min. Units adsorbed indicate the amount of enzyme adsorbed on a resin. Immobilized enzyme assay indicates the amount of enzyme expressed after immobilization. The expression of the adsorbed enzyme is defined as the activity of the immobilized enzyme as compared to that of the enzyme adsorbed onto the matrix.

RESULTS AND DISCUSSION

Immobilization of enzymes onto porous polymer beads offer advantages of higher enzyme loading capacities, as compared to nonporous beads. However, diffusional limitations are encountered in catalytic reaction of immobilized enzymes. The diffusional effects include external diffusion limitations resulting from a stationary layer around the particles of immobilized enzyme as well as pore diffusion limitations arising from effective availability of the enzyme immobilized inside the pore (*5*). Mathematical modeling of enzyme immobilization studies in porous sup-

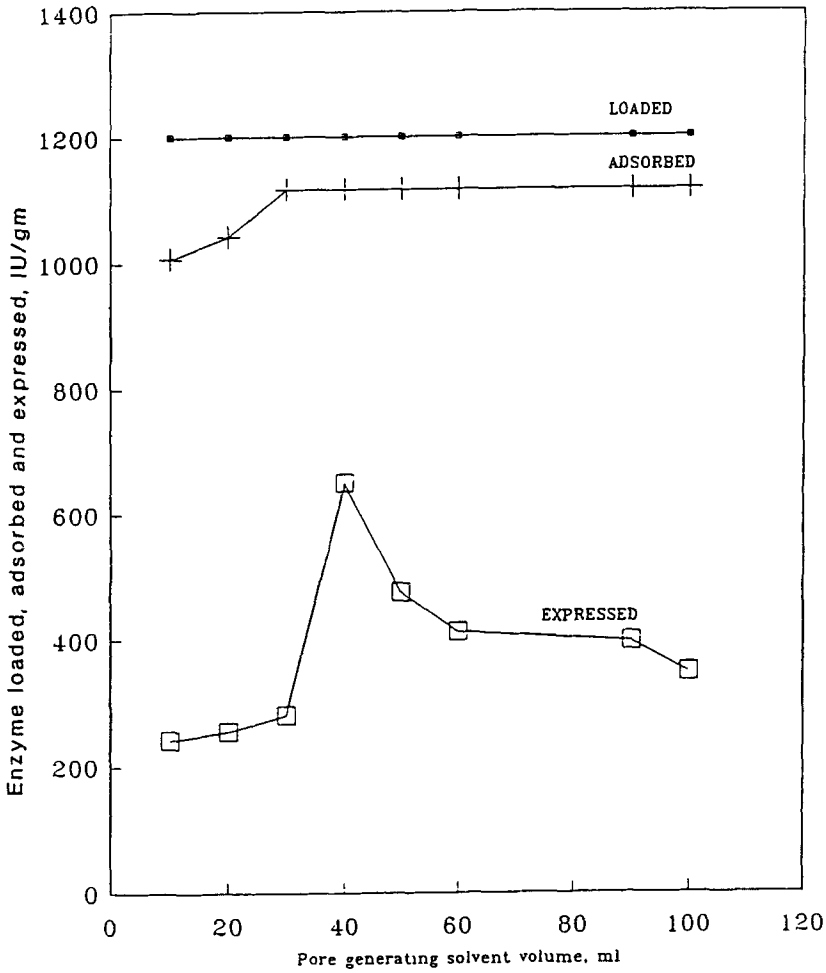

Fig. 1. Effect of volume of pore-generating solvent (kerosene) on adsorption and expression of penicillin G acylase immobilized on methacylate polymer beads.

ports are well documented (*6–8*). The external diffusion limitations are overcome by increased agitation. It is difficult to circumvent the pore diffusion limitation, which is related to the distance that the substrate must diffuse through to the enzyme active site (*5*).

During the production of 6-APA, pH of the reaction must be maintained between 7.8–8.0 by alkali addition to neutralize the phenylacetic acid generated. The rate of reaction decreases at lower pH. A pH gradient across the bead of up to 4.5 is reported for penicillin G acylase immobilized on porous beads (*9,10*). Thus, it is desirable that the pore-size distribution should be such that the enzyme is adsorbed in close proximity to the surface of porous beads to ensure that the pore-diffusion limitations are minimized.

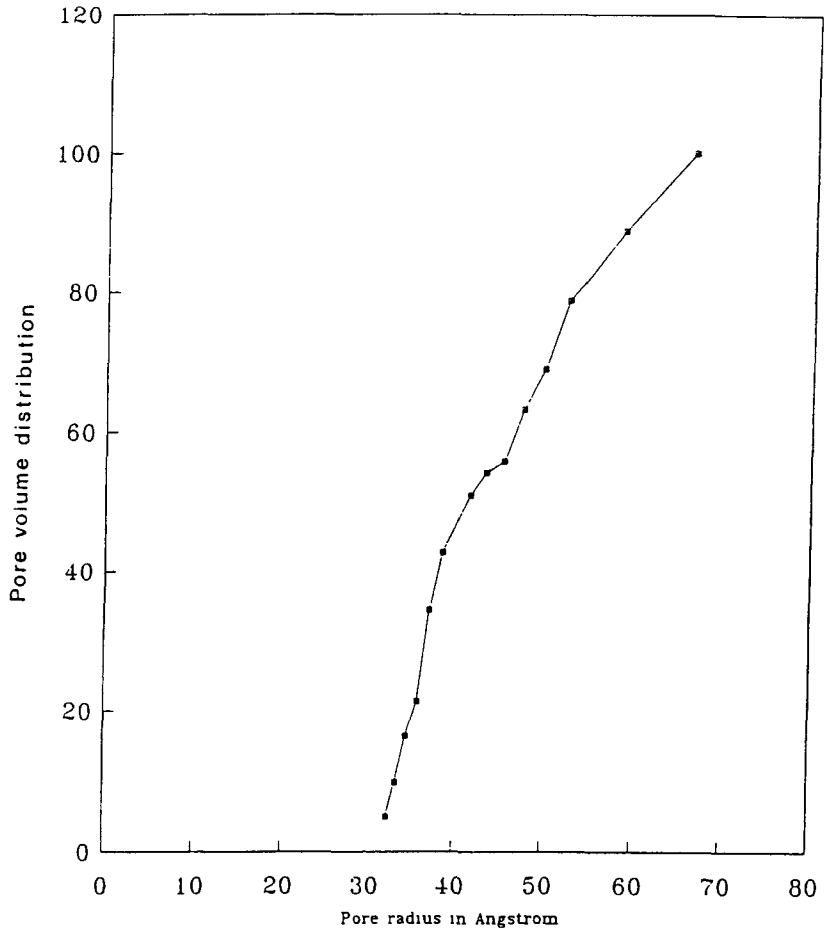

Fig. 2. Normalized pore-volume distribution in methacrylate polymer prepared with 40 mL of kerosene.

We have prepared a series of methacrylate copolymer beads of similar compositions but differing pore volumes, induced by varying the concentration of kerosene used as the pore-generating solvent. Adsorption and expression of penicillin G acylase onto these matrices was studied with an objective of optimizing pore-size distribution for a particular system. This approach leads to the identification of beads with tailored porosity suited to minimizing the pore-diffusion limitations. Maximum expression of adsorbed enzyme represents minimum pore-diffusion limitations for that system.

The results are summarized in Fig. 1. The adsorption of enzyme increases with increase in kerosene volume up to 30 mL due to increased surface area. Further increase in adsorption was not observed at higher volumes of kerosene, since the adsorption capacity of the beads had reached the saturation limit. On the other hand, the immobilized enzyme

activity initially increases, passes through a maxima corresponding to 40 mL of kerosene and decreases with further increase in the kerosene volume. Initial increase, up to 40 mL of kerosene, in the expression of adsorbed penicillin G acylase indicate decreased pore-diffusion limitations. However, further increase in kerosene volume has decreased the expression. This behavior may be attributed to the adsorption and immobilization of penicillin G acylase at the deep interior of the pore thereby increasing the pore-diffusion limitations. The possibility of decreased expression of penicillin G acylase immobilized onto beads generated with higher kerosene volume due to pH effects across the beads is ruled out since identical results were obtained when the assays were conducted using 0.1, 0.2, and 0.25*M* phosphate buffer, pH 7.8. Thus, with the porous methacrylate polymer beads, the optimum porosity for maximum expression of adsorbed penicillin G acylase is reached with 40 mL of kerosene.

The volume of macropores (pore radius >32 A°) in the polymer prepared by incorporating 40 mL of kerosene was 0.13 mL/g. The normalized pore size distribution is presented in Fig 2. It bears out that the ideal pore-size distribution should be narrow to circumvent pore-diffusion limitations.

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