CHAPTER 5

CONCLUSIONS
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The interrelation between hydrophilic/hydrophobic balance, pore size and pore size distribution on one side and covalent binding of PGA and expression of the bound PGA on the other were investigated by synthesising a series of macroporous beaded copolymers. Five series of copolymers synthesised and the variables investigated were:

i) GMA-DVB copolymers: synthesised with varying amount of porogen, crosslinking monomer, protective and polymerisation initiator.

ii) GMA-PETA copolymers: synthesised by variation in crosslink density and type of porogen.

iii) GMA-PETA-EGDM terpolymers: subtle variation of hydrophilicity of the copolymer matrices by varying the amount of EGDM.

iv) GMA-TRIM copolymers: synthesised by varying amount of crosslinking monomer.

v) GMA-TMPTA copolymers: synthesised by varying amount of crosslinking monomer.

Broad conclusions drawn were that the important requisites for binding of PGA on porous copolymer matrices were specific pore size, pore size distribution, pore volume, surface area and optimum concentration of reactive groups. The internal structure of the porous beads was controllable by altering parameters in the copolymerisation recipe such as mole fraction of crosslinking monomer as well as the type and volume of porogen. The internal structure of the copolymers show agglomeration of nuclei to give rise to microspheres which further agglomerate to form beads. Copolymers prepared with less than 50% dilution of porogen tend to be nonporous. Polymer synthesised with higher amount of porogen show considerable porosity. Pore volume of the copolymers increase with crosslink density as well as volume of porogen. At high crosslink densities, copolymers formed in the presence of large volume of porogen display very broad pore size distribution and large number of macropores. The average pore radii in copolymers synthesised in the composition range of 10-30 % CLD are
observed to be between 100-300 \( ^o \). These pores arise from inter-microsphere spacing. Surface area is observed to increase with crosslink density due to decrease in the size of the microspheres. Initiator concentration and concentration of protective colloid do not significantly affect pore size, its distribution, pore volume and surface area. Of the five major synthesis parameters, only two, namely crosslinking density and porogen volume significantly influence the porosity. The pore volume and surface area are maximum in the GMA-DVB copolymers, followed by GMA-TRIM copolymers. The GMA-TMPTA and GMA-PETA copolymers have relatively lower pore volume and surface area.

Pore size and pore size distribution is an important factor contributing to the covalent binding and activity of immobilised enzyme, since it influences diffusional phenomena during the course of catalysis. Binding of the PGA to the copolymer matrix is dependent on pore size and pore volume, hydrophilicity/hydrophobicity of the matrix, steric effects and diffusional limitations. The expression of the bound PGA is also dependent on diffusional limitations, permeability of substrate and products, hindrances in enzyme-substrate interaction and hydrophilic/hydrophobic character of the support.

The binding of PGA, in general, on hydrophobic copolymer matrices, was low, but expression was good on polymers with low CLD. On the other hand in polymers with high CLD, the binding was almost quantitative but percent expression was very low. The binding increased with broadening of pore size distribution, larger pore volume and greater hydrophobicity of the matrices. On these hydrophobic matrices expression of bound enzyme is good at low values of CLD. Due to narrow pore size distribution and lower pore volume, large amount of PGA is not bound hence crowding of enzyme in the pores is avoided. Thus the steric hindrance between enzyme and substrate is less, resulting in better activity of immobilised PGA. In case of high amount of enzyme being immobilised inside the porous matrix (at high CLD) diffusion of substrate is a limiting factor. Hence substrate concentration in the
vicinity of the bound PGA decreases leading to lower measured activity; which accounts for low percent expression on highly crosslinked matrices. The greater hydrophobicity also leads to disruption of tertiary structure of enzyme, thus lowering the percent expression of PGA immobilised on these highly hydrophobic copolymer matrices.

In the case of hydrophilic copolymers like GMA-PETA/TMPTA the binding of PGA is reduced considerably in comparison to hydrophobic matrices with same amount of CLD. With increase in CLD the binding increases which maybe attributed to the trifunctional nature of these crosslinking agents, which generate higher crosslinking, enabling higher loading of epoxy groups and hence enzyme onto the matrix. The results of expression of PGA bound on these copolymers is opposite to those obtained in case of PGA bound on hydrophobic matrices. At high CLD the expression on these copolymers is relatively better due to their hydrophilic nature. Since PGA prefers hydrophobic environment, the involvement of PGA active site in binding or disruption of tertiary structure on increase in hydrophobicity of the matrix is not a serious problem on these hydrophilic copolymers. Hence the activity of the PGA on these matrices is better at higher values of CLD.