

**PROBING PROTEIN-POLYMER INTERACTIONS  
IN PRECIPITATION AND AQUEOUS TWO-PHASE  
PARTITIONING**

**by**

**VANDANA GUPTA**

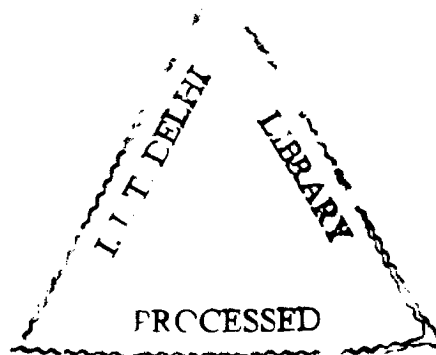
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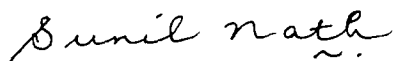
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## CERTIFICATE

This is to certify that the thesis entitled “**Probing Protein-Polymer Interactions in Precipitation and Aqueous Two-Phase Partitioning**” being submitted by **Vandana Gupta** to the Indian Institute of Technology Delhi, for the award of degree of Doctor of Philosophy, has been prepared under our supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi. The research report and the results presented in this thesis have not been submitted to any other University or Institute for the award of any degree or diploma.



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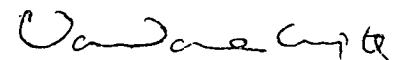
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## ABSTRACT

Polyelectrolytes are water-soluble charged polymers that interact specifically with oppositely charged proteins. In our study, we have selected polyacrylic acid and polyethylenimine as negatively and positively charged polymers, respectively to study the purification of proteins in precipitation and aqueous two-phase partitioning processes. Precipitation of different model proteins e.g., bovine serum albumin,  $\beta$ -glucosidase by polyacrylic acid as well as by polyethylenimine was studied in detail to understand the basic principles controlling the precipitation process by charged polymers. An improved assay to monitor protein precipitation after eliminating interference by polymer was developed and successfully applied. The effect of different parameters like pH, salt concentration, protein and polymer dosage and polymer molecular weight was explored. Charge neutralization of the polyelectrolyte by protein and/or salt ions was found to be of central importance in the precipitation process. Shifts in precipitation curves as a function of the process variables were studied and explained based on mechanistic considerations. Upto 50% enhancement in protein recovery by precipitation was obtained under optimal conditions. In aqueous two-phase partitioning, phase behavior and partitioning of proteins in polyelectrolyte-polyethylene glycol based systems were studied. The phase behavior of polyelectrolyte based aqueous two-phase systems was observed to be greatly affected by parameters influencing the structure/state of water. Two phases could be obtained with polymer concentrations as low as 0.5% w/w. The partitioning of pure proteins (single as well as simulated mixtures) was studied in detail and was found to be controlled primarily by electrostatic interactions. This was confirmed by measuring the electrostatic potential difference ( $\Delta\phi$ ) between two phases. Ag-AgCl electrodes were constructed for

this purpose. A consistent, inverse relationship between  $\Delta\phi$  and the protein partition coefficient was found for all systems studied. Purification of alcohol dehydrogenase and papain from crude extracts was performed. It was shown that high specificity and yields in polyelectrolyte based aqueous two-phase systems in one/two-stage processes could be obtained. This appears to hold great promise for large scale application.

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