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

by

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Thesis submitted
in fulfilment of the requirements of the degree of Doctor of Philosophy
to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI
JULY, 2001

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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ACKNOWLEDGEMENTS

I take this opportunity to express my sincere gratitude to my research supervisor Dr. Sunil Nath, for his excellent guidance and support. His great enthusiasm and constant encouragement inspired me to sail through tough phases of this work with a positive attitude. His critical approach to even infinitesimal details helped me to develop my research skills to a great extent.

I am highly thankful to my co-supervisor Prof. Subhash Chand for his admirable guidance during this work. His constant interest in the work greatly inspired me to work on the basic as well as the applied part of this project. His wide knowledge on the subject has helped me to work on few very crucial problems in this area.

I would like to express my gratitude to all members of my SRC- Prof. V. S. Bisaria, Prof S. N. Mukhopadhyay, Prof. G. P. Agarwal and Prof. A. K. Gupta. I would like to thank the Head, Department of Biochemical Engineering and Biotechnology for providing me the laboratory facilities for carrying out my laboratory work.

I am also grateful to Prof. S. K Guha, Dr. (Ms.) Koyal Roychowdhary and their laboratory staff (Centre of Biomedical Engineering, IIT Delhi) for their help during the construction of the Ag-AgCl electrodes used in this work. Thank is also due for Prof. B. K. Puri and Dr. (Ms.) Anju (Department of Chemistry, IIT Delhi) for their help during the development of electrodes. The enthusiasm and support provided by these people made me more determined to make these electrodes.

A special thank is reserved for Dr. (Ms.) Jaspreet Pannu. Her altruistic support and positive attitude has helped me tremendously during the most crucial phases of this work. I am also thankful to my senior Dr. (Ms.) Malini Seth for all her help in this period. I take this opportunity to thank Ms. Ritu Mehta for all the affection and support she has given me during this work, specially during very critical phases in this tenure. I am also thankful to Ms. Ruchi Shukla for helping me out during final stages of this work. I would like to thank Dr. (Ms.) Anu Wallecha for being there whenever I needed her all this while.

I would like to thank all the students of the department for their help during various stages of this work specially Dr. (Ms.) Vandana Goswami, Dr. Goutam Karan, Ms. Shivani Verma, Ms. Preeti Srivastava, Mr. Siddhartha Jain and Mr. Amit Bhasin. I would also like to thank Mr. Saurabh Chattopadhyay for being a wonderful friend whenever I needed his help in this tenure. A special thank is for Mr. Nishant Sharma and Mr. Jasdeep Singh Gill for their affection and wittiness that made the writing of this thesis a pleasure.

I would take this opportunity to thank Ms. Renu Sethi not only for helping me out enthusiastically during the various phases of this work but also for being a nice companion in the lab. I would also like to thank all other staff members of this department especially Ms. Neera Verma, Ms. Sunita, Mr. Bhagwan Singh, Mr. S P Rana, Mr. J A Khan, Mr. D V Sharma and Mr. Rajkumar for their ever ready help. I would like to specially thank Mr. Ramesh for washing those endless test tubes round the clock that made my work easier.

I take this opportunity to thank my family for their tremendous support and love during this research work. The constant encouragement and faith of my parents led me to overcome the most difficult phases of this work serenely. I am thankful to my brother, Mr. Vinay Sheel Gupta and sister, Ms. Deepika for being

wonderful buddies during my various mood swings while pursuing for the research work in this tenure. Their caring attitude helped me in crossing various hurdles with a light mind-set.

Finally, I would like to thank all my friends for their unconditional support in all these years.

(Vandana Gupta)

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, β-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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