SORPTION STUDIES ON IMMOBILIZED METAL ION AFFINITY GELS FOR PROTEIN FRACTIONATION

by

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CERTIFICATE

This is to certify that the thesis entitled, **"SORPTION STUDIES ON IMMOBILIZED METAL ION AFFINITY GELS FOR PROTEIN FRACTIONATION"**, being submitted by **Ms. SADHANA SHARMA** to the Indian Institute of Technology, Delhi for the award of the degree of **Doctor of Philosophy,** is a record of bonafide research work carried out by her under my supervision. The results contained in this dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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कर्मण्येवाधिकारस्ते मा फलेषु कदाचनः।
मा कर्मफलहेतुर्भूर्मा मा ते सङ्गोस्त्वकर्मणि ।।४७।।

द्वितीयोध्यायः
श्रीमदभगवद्गीता

You have a right to perform your prescribed duty,
But you are not entitled to the fruits of action.
Never consider yourself the cause of the results of your activities,
and never be attached to not doing your duty.

The Shrimad Bhagwad Gita 2:47

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ABSTRACT

Immobilized metal ion affinity chromatography (IMAC) is one of the most recent techniques for the downstream processing of biomolecules. This is based upon the interaction of immobilized metal ions (metal chelates) through their surface-exposed amino acid residues such as histidine, cysteine and tryptophan. Its application for the purification of proteins was first reported by Porath in 1975. Since then, it has emerged as an essential laboratory scale method for the separation and purification of proteins. This is evident from the rapidly increasing applications of IMAC for the isolation and purification of recombinant proteins from their culture broths. However, its applications at the preparatory scale are still in infancy.

The design, optimization and scale up of a chromatographic process using IMAC demands a thorough understanding of the fundamental factors governing the various interactions between immobilized metal ions and proteins. Over the last two decades, many researchers have investigated these aspects to a considerable extent, there are still certain aspects which need deeper attention. For instance, there is dearth of information on the influence of solution environment on the metal sorption characteristics of IMA gels. Also, leaching of the metal ions from the chelated gels that has been of great concern since the inception of the technique, has not been investigated systematically. Moreover, the quantitative data on the protein sorption characteristics is available for specific systems only and is required to be developed for most protein systems. In our present effort we have tried to investigate these aspects in systematic manner.

The sorption studies were conducted on two IMA gels namely, Iminodiacetate (IDA) and Tris(2-aminoethyl)amine (TREN), bound to Sepharose 6B. The proteins identified for this investigation are viz. ovalbumin, conalbumin, lysozyme, wheat germ agglutinin, wheat germ lipase, wheat germ acid phosphatase and bovine serum albumin. The metal sorption characteristics of both, IDA and TREN, gels were systematically investigated under varying chemical environment (pH, ionic strength and feed metal concentration) for the two most frequently used metal ions namely, Cu (II) and Ni (II) and metal loading conditions were optimized. Further, the stability of these IMA-M(II) supports was examined under various solution conditions (pH, ionic strength and imidazole concentration) that are generally used during equilibration, washing and elution. This was followed by the protein adsorption studies on metal loaded IMA gels under different

operating conditions. The data was analyzed, both qualitatively and quantitatively, using various theoretical models and the relevance of this information for designing actual protein fractionation processes was highlighted.

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