





























of a particular component. Reverse phase chromatography is commonly coupled with mass spectrometry in an effort to quantify the protein that is eluted from the column.

### **Stages in Reversed Phase Chromatography**

The stages in Reversed Phase Chromatography are very similar to the stages found in Ion exchange Chromatography. Once again, the process can be summarized in four steps.

1. Equilibration
2. Sample Application
3. Elution
4. Regeneration

#### **Equilibration**

In this step the hydrophobic column is primed by applying the specific sample buffer. Because the column is hydrophobic, water molecules tend to be ordered at the junction between the column and the buffer.

#### **Application of the Sample**

In this step, the sample protein is injected into the system. Proteins in the mixture that have a high percentage of exposed hydrophobic amino acid residues will be adsorbed to the hydrophobic stationary phase. Other proteins in the mixture will be washed out.

#### **Elution**

This stage in the process involves changing the buffer conditions to elute the bound hydrophobic protein. The most common way to do this is to use a gradient that slowly increases the hydrophobicity using an organic buffer.

#### **Regeneration**

This stage involves washing off any remaining protein from the stationary phase and returning the conditions back to the way they were at the start of the process. This involves creating a less hydrophobic environment.

### **Conclusion:**

There are a variety of techniques used to separate and characterise proteins. Separation techniques rely on the differences in the solubility, size, charge, and adsorption characteristics of protein molecules. Ion-exchange chromatography is used to separate proteins on the basis of charge. Affinity chromatography utilises ligands, such as enzyme inhibitors, coenzymes, or antibodies, to specifically bind proteins to a solid support. Size of the Protein can be separated by using size exclusion chromatography. Electrophoresis can be used to separate proteins from complex mixtures on the basis of size and charge.

From the above, we can conclude that HPLC and FPLC are widely used techniques for the separation of proteins.

### **Acknowledgements:**

I express my sincere thanks to my guide **Smt. K. Sujana**, Assistant professor, College of Pharmaceutical Sciences, Acharya Nagarjuna University for her guidance and encouragement for this seminar. I take this opportunity to express my gratitude to **Acharya Nagarjuna University** for providing the necessary facilities and infrastructure.

I am also grateful to **Prof. B. Syama Sundar**, Principal, College of Pharmaceutical Sciences, Acharya Nagarjuna University, **Prof. A. Prameela Rani** and other Teaching, Non-Teaching staff for their support. I thank all my Classmates for their cooperation.

### **References:**

- [1] A text book of Organic Chemistry- Arun bahl Bs bahl, Edn. 2006. Pg.no:1, 21,389.
- [2] A text book of Biochemistry – U. Satyanarayana, Edn. 2008. Pg.no:569-579.
- [3] Voet and Voet, Biochemsitry John Wiley and Sons, 1995.
- [4] Nelson & Cox, Lehinger. *Principles of Biochemistry*, 3<sup>rd</sup> ed.
- [5] <http://GE HealthCare Life Sciences>.

- [6] <http://books.google.co.in/books?id=JM>
- [7] [http://en.wikipedia.org/wiki/Affinity\\_chromatography](http://en.wikipedia.org/wiki/Affinity_chromatography)
- [8] <http://www.biochem.arizona.edu/classes/bioc462/462a/462a.html>
- [9] [www.museumstuff.com/learn/topics/Protein\\_purification::sub::Chromatographic\\_Methods](http://www.museumstuff.com/learn/topics/Protein_purification::sub::Chromatographic_Methods)
- [10] [www.siemens.com/answers](http://www.siemens.com/answers)
- [11] [https://mail.google.com/mail/h/qshfwobfxvu7/?view=att&th=12db368bfa5c9fle&attid=. . .](https://mail.google.com/mail/h/qshfwobfxvu7/?view=att&th=12db368bfa5c9fle&attid=.)
- [12] <https://mail.google.com/mail/?ui=2&ik=e287648c98&view=att&th=1>
- [13] <https://mail.google.com/mail/h/11z7prfplyscq/?view=att&th=12dbc5b33709fe63&attid=0.1....>
- [14] [mhtml:file://E:\Documents and Settings\Guest\My Documents\nature of aa\\_docx.mht](mhtml:file://E:\Documents and Settings\Guest\My Documents\nature of aa_docx.mht)