DEAE 离子交换层析分离血清蛋白质

【教学对象与学时】

教学对象：临床医学五年制、七年制学生

学时：8 学时

【预习要求】

蛋白质的基本理化性质

血清蛋白的组成及其理化性质

【目的要求】

教学目的: 熟悉层析的基本原理与分类、掌握离子交换层析的原理及操作教学要求: 利用离子交换层析对血清蛋白进行分离并对分离所得各组分性质进行比较、实验前预习，实验后写出实验报告。

【重点和难点】

重点：离子交换层析分离蛋白质的实验原理。

难点：DEAE 纤维素处理的原理与操作。

【教学过程设计】

一、布置预习内容。

1、复习蛋白质的基本理化性质，重点是蛋白质的两性电解性质及由此引申出来的蛋白质表面电量与溶液 PH 值之间的关系。

2、蛋白质的紫外吸收性质。

3、血清蛋白的组成与分类。

二、课堂教学过程

1.复习层析概念

2.交待离子交换层析概念，并提出引导性问题。

3.进行实验操作第一个环节——DEAE 纤维素的处理，在处理间歇期穿插实验理论的讲述。

3.1 膨润阶段讲述内容:

3.1.1 离子交换层析的本质—化学反应平衡，引申出离子交换层析的分类与应用范围;

3.1.2 复习蛋白质表面电量与溶液 PH 之间的关系，引申出 PH 值梯度洗脱的意义；

3.1.3 讲解双电层理论，引申出离子强度梯度洗脱的意义；

3.1.4 离子交换介质处理的理想状态，初步理解交换层析介质处理的要求；

3.1.5 待分离蛋白质与交换剂的结合，引申出离子交换层析的分离范围概念。

3.2 转型阶段讲述内容:

3.2.1 离子交换层析的分离理论，以及 PH 值梯度洗脱与离子强度梯度洗脱的不同意义；

3.2.2 离子交换剂处理的原理及其对实验结果的影响

3.2.3 仪器的连接与使用方法

4.平衡阶段进行仪器的调试等上样前的准备

5.上样

6.梯度洗脱

7.中午轮流休息

8.实验结果与结果分析

【实验报告要点】

1.离子交换层析的原理

2.实验操作步骤

3.实验结果与结果分析

【思考题】

1.阴阳离子交换剂如何选择？

2.离子强度梯度洗脱的意义？

3.本实验中，判断依次被洗脱的蛋白质性质差异？

【专业英语选读】

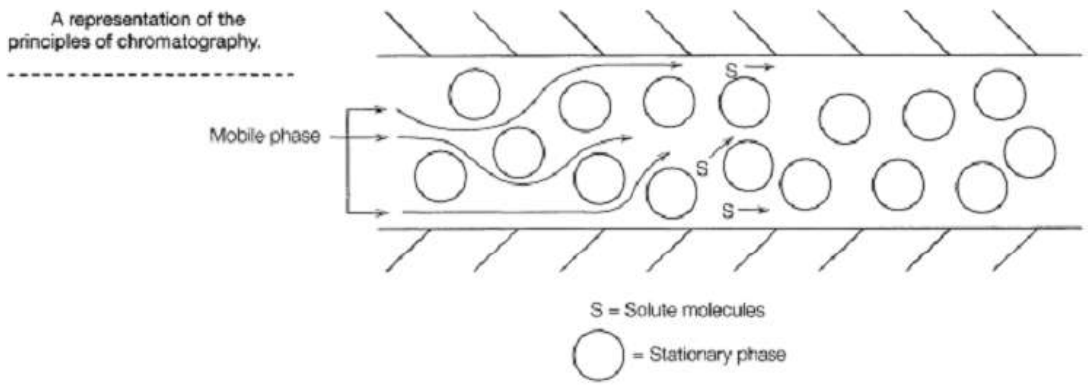
The molecular details of a biochemical process cannot be fully elucidated until the reacting molecules have been isolated and characterized. Therefore, our understanding of biochemical principles has increased at about the same pace as the development of techniques for the separation and identification of biomolecules. Chromatography has been and will continue to be the most effective technique for isolating and purifying all types of biomolecules. In addition, it is widely used as an analytical tool to measure quantitative properties.

A. INTRODUCTION TO CHROMATOGRAPHY

All types of chromatography are based on a very simple principle. The Sample to be examined (called the solute) is allowed to interact with two physically distinct entities-a mobile phase and a stationary phase. The mobile phase, which may be a gas or liquid, moves the sample through a region containing the solid or liquid stationary phase called the sorbent. The stationary phase will not be described in detail at this time, since it varies from one chromatographic method to another. However, it may be considered as having the ability to "bind" some types of solutes. The sample, which may contain one or many molecular components, comes into contact with the stationary phase. The components distribute themselves between the mobile and stationary phases. If some of the sample components are preferentially bound by the stationary phase, they spend more time in the stationary phase and, hence, are retarded in their movement through the chromatography system. Molecules that show weak affinity for the stationary phase spend more time with the mobile phase and are more rapidly removed or eluted from the system. The many interactions that occur between solute molecules and the stationary phase bring about a separation of molecules because of different affinities for the stationary phase. The general process of moving a solute mixture through a chromatographic system is called development.

The mobile phase can be collected as a function of time at the end of the chromatographic system. The mobile phase, now called the effluent, contains the solute molecules. If the chromatographic process has been effective, fractions or “cuts” that are collected at different times will contain the different components of the original sample. In summary, molecules are separated because they differ in the extent to which they are distributed between the mobile phase and the stationary phase.

Throughout this chapter and others, biochemical techniques will be designated as preparative or analytical, or both. A preparative procedure is one that can be applied to the purification of a relatively large amount of a biological material. The purpose of such an experiment would be to obtain purified material for further characterization and study. Analytical procedures are used most often to determine the purity of a biological sample; however, they may be used to evaluate any physical, chemical, or biological characteristic of a biomolecule or biological system.



Partition versus Adsorption Chromatography

Chromatographic methods are divided into two types according to how solute molecules bind to or interact with the stationary phase. Partition chromatography is the distribution of a solute between two liquid phases. This may involve direct extraction using two liquids, or it may use a liquid immobilized on a solid support as in the case of paper, thin-layer, and gas-liquid chromatography. For partition chromatography, the stationary phase in Figure consists of inert solid particles coated with liquid adsorbent. The distribution of solutes between the two phases is based primarily on solubility differences. The distribution may be quantified by using the partition coefficient, KD.

$$K_D = \frac{\text{concentration of solute in stationary phase}}{\text{concentration of solute in mobile phase}}$$

Adsorption chromatography refers to the use of a stationary phase or support, such as an ion-exchange resin, that has a finite number of relatively specific binding sites for solute molecules. There is not a clear distinction between the processes of partition and adsorption. All chromatographic separations rely, to some extent, on adsorptive processes. However, in some methods (paper, thin-layer, and gas chromatography) these specific adsorptive effects are minimal and the separation is based primarily on nonspecific solubility factors. Adsorption chromatography relies on relatively specific interactions between the solute molecules and binding sites on the surface of the stationary phase. The attractive forces between solute and support may be ionic, hydrogen bonding, or hydrophobic interactions. Binding of solute is, of course, reversible.

Because of the different interactions involved in partition and adsorption processes, they may be applied to different separation problems. Partition processes are the most effective for the separation of small molecules, especially those in

homologous series. Partition chromatography has been widely used for the separation and identification of amino acids, carbohydrates, and fatty acids. Adsorption techniques, represented by ion-exchange chromatography, are most effective when applied to the separation of macromolecules including proteins and nucleic acids.

In the rest of the chapter, various chromatographic methods will be discussed. You should recognize that no single chromatographic technique relies solely on adsorption or partition effects. Therefore, little emphasis will be placed on a classification of the techniques; instead, theoretical and practical aspects will be discussed.