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Ion-Exchange Chromatography of Proteins

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Preface

Ion-exchange chromatography (IEC) has been widely employed for the separation and purification of enzymes and proteins. In addition, recent advances in biotechnology require a large process-scale efficient method for the separation and purification of proteins. For this purpose, process-scale IEC will be employed more frequently. However, the separation mechanism in IEC of proteins is a rather complicated process. Therefore, it is not easy to make a proper choice of the design of the column dimension and the chromatographic conditions, unless the fundamental knowledge of this method is available.

In this book, we describe the separation mechanism of proteins in IEC by stressing the unique characteristics of IEC of proteins. Both theoretical and experimental works concerning this method have been reviewed. We have also briefly described the experimental method and the apparatus commonly employed, and some special operational procedures and apparatus. Particular emphasis is placed on the design and operation of the large-scale IEC. This will be informative to the reader involved in biotechnology.

Although we have tried to emphasize physical significance rather than mathematical manipulations, the mathematics involved in the theoretical parts of this book may be unfamiliar to some readers. We have attempted to guide the reader toward an understanding of the basic concepts, and to help him to acquire or improve the technique of IEC of proteins without calling for a deep understanding of the mathematics. Therefore, the reader may skip such mathematical sections. But we hope that the

reader understands the physical significance and meaning involved in the equations. This will help in understanding the experimental results.

As described in this book, IEC as a method of separating and purifying proteins is a relatively new technique and is undergoing continuous, rapid development. The literature on this method is expanding, and we have tried to include as many important references as possible. We also recognize that the scope of this book is limited to the methods that we wish to recommend and the experimental results that we found important, and that these choices are based on our backgrounds and personal preferences. So, if there are readers who feel that certain subjects deserve further exploration in a future volume, we would be grateful to have their suggestions.

Shuichi Yamamoto
Kazuhiro Nakanishi
Ryuichi Matsuno

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1
Introduction

An integral part of the study of biochemical science is devoted to the separation and analysis of a particular substance. Therefore, if we look behind revolutions in life science, we find highly developed separation techniques. For instance, in the field of genetic engineering, which has been progressing very rapidly for the last decade, the first step is the isolation of genes. Although there are several different methods for this step, each method is supported by various types of effective and sophisticated separation techniques. If the basic science thus established is directed to the production of a useful compound, the productivity may be sometimes expected to be more than a hundred times or more compared with that using conventional methods. However, the overall productivity of the production process cannot be increased to as high a level as we expect from conventional production processes, and often industrialization of the new process is abandoned. This may be partly due to the high

costs of energy and raw materials, since for the same amount of final product, the minimum energy and raw materials necessary for production are the same. However, the separation steps that follow the production step are often critical to the overall efficiency. The separation step is called a downstream treatment whose importance has been gradually recognized in the practical application of biotechnology.

Thus, we clearly see the importance of separation processes in both basic science and engineering. We should also realize that separation in engineering is much more difficult than that in basic science since the energy efficiency must be taken into consideration.

We constantly seek novel ideas of separation based on new principles. The techniques of the separation methods available at present must be improved. Finally, every separation technique should be systematized so that anyone can use it without detailed knowledge or much experience.

It is common that a theory is introduced in the systematization process, but this theory is sometimes so difficult that many users who are unfamiliar with such theoretical treatment often hesitate to be involved in such difficulties. Therefore, the theory must be as simple as possible. However, one also should recognize that theory greatly reduces the number of trial-and-error experiments that must be done to find the optimal operational conditions. In a theory, a large number of variables are often grouped with a small number of nondimensional variables. If the number of the original variables and of the grouped nondimensional variables are n and m, respectively ($m < n$), the

number of experiments can be reduced by a factor of k^{m-n}, where k is the number of experiments per one variable or non-dimensional variable.

Ion-exchange chromatography (IEC) is known to be an efficient technique for the separation of proteins and has been routinely used in the biochemical science laboratory. Considering the separation efficiency and energy requirements, its relatively large-scale application to industrial protein separation is expected. In this book, we summarize and make clear the developments in the study of the ion-exchange chromatography of proteins and describe how we might design the ion-exchange chromatography of proteins with the knowledge available at present. We also discuss its inherent limitations. We can thus speculate about future problems, and these may be effective for the future systematization of protein IEC.

A large number of references on protein IEC and related subjects are published every year. A review that covers the literature on various types of chromatography is given in Analytical Chemistry every 2 years. Titles on chromatography and related methods are compiled in the bibliography section of the Journal of Chromatography in each volume. These will help the reader to search for the important references. Symposium volumes in the Journal of Chromatography are also useful for surveying current topics in the liquid chromatography of proteins. In addition to the books on (high-performance) liquid chromatography (for example, Snyder and Kirkland, 1974; Heftmann, 1975; Hearn et al., 1983), the books on the downstream treatments in biotechnology (Bailey and Ollis, 1986) and purification methods in enzymology (Scopes, 1982) are informative.

1.1 TERMINOLOGY

Before beginning, we briefly describe several terms that frequently appear in this book so that readers can easily understand the text.

Figure 1.1 schematically illustrates the general operation procedure in column chromatography. Packing materials (chromatographic media) are usually packed into a cylindrical tube. Since most commercial packing materials (the ion exchanger) for ion-exchange chromatography of proteins have a gel structure, we occasionally use the term "gel" for the packing material. The term "column" usually implies the bed inside the tube. The word column in this context thus has the same meaning as "gel bed" or "fixed bed." The tube itself is called a column tube or an empty column. After a sample is applied to the column, then

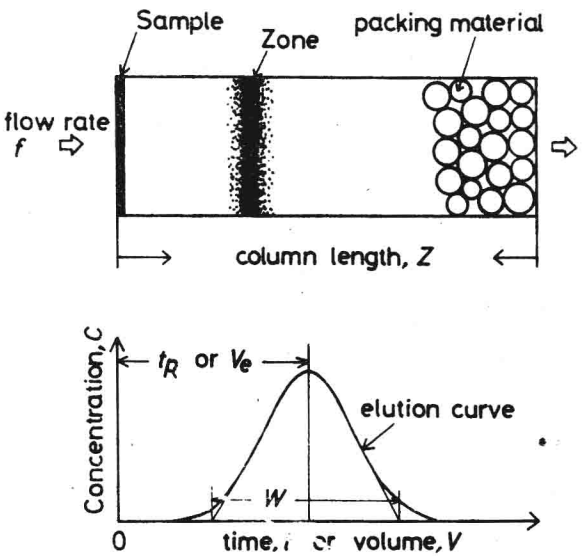

Fig. 1.1 Schematic illustration of a chromatographic column and an elution curve.

the elution is started. The term "development" is often used instead of "elution." However, we prefer to use "elution." The applied sample occupies a certain part of the column. This part, called the zone or band, gradually becomes wider (owing to several factors described in the text) as it travels down through the column. This phenomenon is called zone spreading, band broadening, or peak dispersion, which is one of the important subjects treated here.

During the elution process, the effluent (eluate) from the outlet of the column is monitored either continuously or discontinuously while solvent (eluant) is fed to the inlet of the column. Instead of the term "eluant," we very frequently use "elution buffer." When the composition of the elution buffer is equal to that of the initial buffer with which the column is equilibrated, this is "isocratic elution." In contrast, if the elution buffer is different from the initial buffer, this elution method is "nonisocratic elution." Gradient elution and stepwise elution are the two most common nonisocratic elution methods. If the concentration of the sample at the outlet of the column is plotted against the time or the volume from the start of the elution, the curve shown in Fig. 1.1 is obtained. This curve is referred to as the "elution curve," although several different terms, such as elution profile, elution pattern, and chromatogram, are also frequently used in the literature. We sometimes call the elution curve simply the peak. The position at which the elution curve is maximum is the peak position; the time and the volume from the start of elution to the peak position are the peak retention time t_R and the elution volume V_e, respectively. Characterization of the elution curve is discussed in Sec. 2.1.1 in detail.