Liquid Chromatography Column Theory

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Preface

The foundations of liquid chromatography column theory were laid down many years ago by Martin and Synge, who developed the Plate Thoery, and Van Deemter *et al.*, who developed the Rate Theory. In the intervening years, the Plate Theory has been extended by Said but, other than that, these two theories have remained virtually unchanged for over 25 years. They are still the basis on which modern column theory has been built. During the interim period a number of other rate theories have been put forward. Careful experimental measurements, however, have shown that the Van Deemter equation describes band dispersion in a column most accurately in terms of the absolute physical properties of the column and distribution system, particularly in the neighbourhood of the optimum velocity where all columns should be operated.

This book has been written in such a way that the skill required on the part of the reader to understand the mathematical arguments has been reduced to a minimum. Although some of the algebra may be a little extensive, the vast majority of the book will be understood by any scientist or student with a good 'A' level knowledge of algebra and calculus or the equivalent. The treatment starts with very simple concepts, and by employing the Plate Theory, equations are developed to describe the parameters of the chromatogram and the properties of the column together with variables that control its separating power. The results from the plate theory are then applied to many other chromatographic phenomena such as column overload, peak capacity and thermal effects in a column. The Rate Theory is developed to show how the mechanism of peak dispersion is related to column geometry and the properties of the phase system and how dispersion can be controlled. The two theories are then combined to show how a particular column (analytical, open tubular or preparative) may be designed to effect a specific separation in the minimum time. Simple computer programs are provided for the design of packed column, open tubular columns and preparative columns.

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September 4th 1991 Raymond P.W. Scott

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Contents

Chapter 1

Introduction to the Liquid Chromatographic Column

The column is the heart of the liquid chromatograph. It is a tube, usually a few centimeters long and less than a centimeter wide, packed with particles a few micron in diameter. In fact, the column used today is little changed from that originally used by Tswett (1), the inventor of chromatography, nearly a century ago. Tswett would probably recognize the modern liquid chromatography (LC) column for what it was, though it is doubtful if any other parts of the modern chromatograph would make sense to him. However, the contemporary LC column, though smaller and packed with different materials from those used by Tswett, gives a performance many orders of magnitude greater. The reasons for this improvement are somewhat subtle, but it must be emphasized that it has been realized as a result of the development and application of liquid chromatography column theory -the subject of this book. The modern LC instrument is, indeed, impressive, with its fully automatic multi-solvent programer and sample injector, together with its variable wavelength detector, recorder and associated computer. However, all these complex devices are there, merely to serve the column. Without the column the carefully designed equipment would have no purpose and no value. In fact, the clever instrument design, that has resulted in the high performance that is characteristic of the modern liquid chromatograph, has been a direct result of the practical application of LC column theory.

In his original work, Tswett examined a number of different column packings, but the vast majority of packing materials that are in use in LC today, are based on silica gel. This is true, even for the recently introduced carbon based packings (2) as they also require silica gel for their fabrication. Without silica gel LC would have a very limited performance and very limited areas of application. Furthermore, until some substance is

found that is more effective than silica gel, or some other column type that requires no packing is developed to a state of general practical use, silica gel will remain the one common component of most practical LC column systems. It is true that alumina can be employed as a stationary phase, but the performance of alumina columns have, until recently, proved to be grossly inferior to those of silica gel. Moreover, stable bonded phases (the packing most widely used in LC today) cannot, apparently, be formed by direct reaction with alumina. However, a recent product, consisting of spherical particles composed of fused porous alumina plates coated with polybutadiene has shown promising signs of being the first really effective alumina-based LC packing. This is a unique material that has not been fully evaluated at this time . A micrograph of this material is shown in figure (1).

Figure 1

Micrograph of an LC Packing Consisting of Polybutadiene Coated on Fused Alumina

By the courtesy of BIOTAGE Inc.

Silica gel has properties that are uniquely suitable for use as a column packing in LC and consequently, its introduction was one major factor

responsible for the great difference in performance between modern LC columns and those of Tswett. The effect of column dimensions and particle diameter on column performance is less obvious and , as will be seen later, will require some extensive discussion.

The LC column performs a dichotomy of purpose which must be completely understood before attempting to start any derivation of LC column theory. During the development of a chromatographic separation, two processes occur simultaneously and to large extent independently. Firstly, the individual solutes in the sample are moved apart in the column as a result of their different affinities for the stationary phase. Those solutes that interact strongly with the stationary phase being retained in the column to a greater extent than those solutes that interact more strongly with the mobile phase. Secondly, as the bands are moved apart, they spread or disperse and tend to merge together, blurring the separation that has been obtained. The column, by appropriate design, must minimize this dispersion, so that, having been moved apart and separated, the individual solutes enter the detector as individual bands. Thus, to obtain maximum resolution, the column must move the bands as far apart as possible but, at the same time, keep each band as narrow as possible.

The factors that control separation and dispersion are quite different. The relative separation of two solutes is solely dependent on the nature and magnitude of the interactions between each solute and the two phases. Thus, the relative movement of each solute band would appear to be independent of column dimensions or particle geometry and be determined only by the choice of the stationary phase and the mobile phase. However, there is a caveat to this statement. It assumes that any exclusion properties of the stationary phase are not included in the term 'particle geometry'. The pore size of the packing material can control retention directly and exclusively, as in exclusion chromatography or, indirectly, by controlling the access of the solute to the stationary phase in normal and reverse phase chromatography. As all stationary phases based on silica gel exhibit some exclusion properties, the ideal situation where the selective retention of two solutes is solely controlled by phase interactions is rarely met in practice. If the molecular size of the solutes differ, then the exclusion properties of the silica gel will always play some part in solute retention.

The Factors that Control Retention and Selectivity

The theory of solute retention, as controlled by molecular interactions between the solutes and the phase system is, in fact, not germane to the subject of this book. Nevertheless, as distribution and distribution coefficients together with retention volumes and capacity ratios will be discussed or used in the subsequent theoretical development of column theory, the basic principles of molecular interaction will be given.

The mechanism of solute retention is best considered by first defining a chromatographic separation. Defined in the classical manner, a chromatographic separation is achieved by the distribution of the solute mixture between two phases, a stationary phase and a mobile phase. Those solutes distributed preferentially in the mobile phase will pass through, or from the system, more rapidly than those substances that are distributed preferentially in the stationary phase. As a consequence the solutes will be eluted in the order of the magnitude of their distribution coefficients with respect to the stationary phase. This definition, although perfectly correct, tends to obscure the basic process of retention in the term *distribution*. A solute is distributed between two phases as a result of the relative magnitude of the molecular forces that exist between the solute molecules and those of the two phases. Consequently, the stronger the forces between the solute molecules and those of the stationary phase the more the solute will be retained. Conversely, the stronger the interactions between the solute molecules and the mobile phase the more rapidly will the solute pass through the column. Thus, solute retention is controlled by molecular forces of which there are three basic types, *ionic forces* , *polar forces* and *dispersive forces*. There could be considered a third type of intermolecular interaction, *hydrogen bonding*, but for the purpose of this discussion, forces due to hydrogen bonding will be classed as strong polar forces.

Ionic Interactions

Ionic interactions result from permanent electrical charges that exist on molecules, for example ionic materials such as salts. Such interactions are exploited in ion exchange chromatography ,in particular, for the separation

of organic acids and bases. It follows that to retain anionic materials in a chromatographic system, the stationary phase should contain cations. Conversely, to retain cationic materials, the stationary phase should contain anions. The stationary phase can consist of an ion exchanger proper, such as an ion exchange resin or can take the form of an adsorbed ion exchanger on the surface of a reverse phase, such as an alkyl sulphonate.

Polar Interactions

Polar interactions between molecules arise from permanent or induced dipoles existing in the molecules and do *not* result from *permanent* charges as in the case of ionic interactions. Examples of polar substances having permanent dipoles would be alcohols, ketones, aldehydes etc. Examples of polarizable substances would be aromatic hydrocarbons such as benzene or toluene. It is considered that, when a molecule carrying a permanent dipole comes into close proximity to a polarizable molecule, the field from the molecule with the permanent dipole induces a dipole in the polarizable molecule and thus electrical interaction can occur. It follows that to selectively retain a polar solute, then the stationary phase must also be polar and contain, perhaps, hydroxyl groups. If the solutes to be separated are *strongly* polar, then perhaps a polarizable substance such as an aromatic hydrocarbon could be employed as the stationary phase. However, to maintain strong polar interactions with the stationary phase (as opposed to the mobile phase) the mobile phase must be relatively *non-polar* or dispersive in nature.

Dispersive Interactions

Dispersive interactions are more difficult to describe. Although electric in nature, they result from charge fluctuations rather than permanent electric charges on the molecule. Examples of purely dispersive interactions are the molecular forces that exist between hydrocarbon molecules. n-Heptane is not a gas due to the collective effect of all the dispersive interactions that hold the molecules together as a liquid. To retain solutes selectively, solely on the basis of dispersive interactions, the stationary phase must not contain polar or ionic substances but only hydrocarbon-type materials such as the reverse-bonded phases now so popular in LC. It follows that to allow dispersive selectivity to dominate in the stationary phase, the mobile phase

must be polar and significantly less dispersive. Hence the use of methanol-water and acetonitrile-water mixtures as mobile phases in reverse-phase chromatography systems. It should be pointed out that it is rare, that in any distribution system, only one type of interaction is present and, if it is, it will certainly be dispersive in nature. Polar interactions are always accompanied by dispersive interactions and ionic interactions will, in all probability, be accompanied by both polar and dispersive interactions.

The Physical Nature of the Column

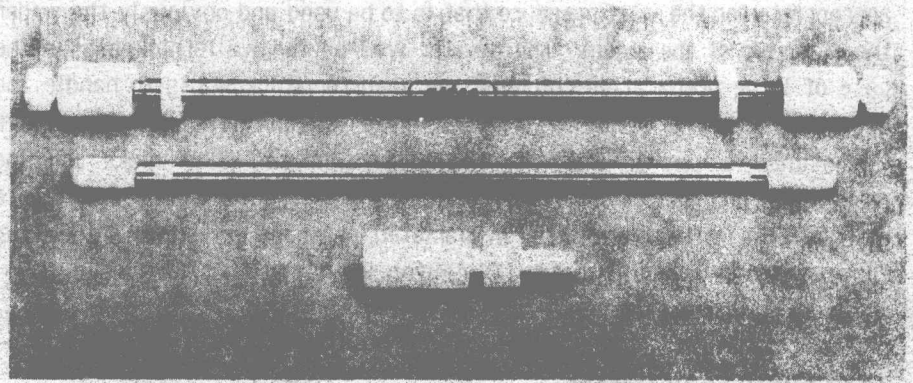
All practical, contemporary, LC columns are tubular in shape and all commercially available columns, at the time of writing this book, are *packed*. Work is being carried out to develop a satisfactory open tubular column for LC but, so far, none has exhibited the necessary loading capacity or resolving power. They have been used with some success in super critical fluid chromatography (SFC), however, the subject of SFC is outside the scope of this book. At present the use of capillary columns in LC is severely handicapped by, firstly, the lack of a high sensitivity, low dispersion detector, and secondly, by the lack of associated apparatus that has sufficiently low extra column dispersion. It will be seen later, that the width of a solute band, eluted from a capillary column, in terms of volume of eluted mobile phase, is extremely small. Consequently, any extra column dispersion that occurs must be reduced to a fraction of a microliter or less if the resolution obtained from the column is not to be lost. This problem will be subsequently discussed in detail when dealing specifically with the subject of *extra column dispersion*. However, at this point, it can be said that practical capillary LC columns may *not* become a viable commercial product for some time to come and, even when available, may have a very limited range of application when compared with the packed column of today.

The body of the LC column is normally made of stainless steel to withstand the high pressures that will be required to force the mobile phase through the interstices of the packing. Furthermore, the column is usually fitted at either end with stainless steel unions which allow connection to the injection system and the detector. However, more recently, columns with

inert plastic unions, have been designed (3) that will also cope with high pressures. These columns employ very fine threads so that the unions can not only seal satisfactorily at high pressures, but also be *hand* tightened. This renders column installation or replacement a very simple procedure. A photograph of a column with plastic end fittings is shown in figure 2. The very small diameter columns that are being developed at this time (4,5) are sometimes made of glass or fused silica. As these columns are often less than a millimeter in diameter, their walls can be fairly thin and still cope with the pressure necessary for their satisfactory operation.

Figure 2

An LC Column with Plastic Terminal Unions



By the courtesy of ASTEC Inc.

Some of these columns are pre-packed before drawing and thus, as a result of the drawing procedure, the packing is fairly open. This reduces the flow impedance and permits relatively low inlet pressures to be employed. Small diameter glass or quartz columns are not, as yet, generally available and it may be some time before they can be developed into a commercially useful product. As the plastics industry develops stronger and more inert plastics, so the totally plastic column may become a realistic possibility. Plastic columns would be much less expensive to manufacture and very easy to pack and assemble in the chromatograph. Such columns might well become available in the not too distant future, probably initially for use with the