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The Separation of Nonionic Organic Compounds

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I

INTRODUCTION

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1-1 Intended Coverage

At one time or another, adsorption chromatography has been applied to the separation of almost every type of sample mixture. In the early days of chromatography this was natural, since chromatography and adsorption chromatography were then synonymous. Yet even today, with numerous competitive separation methods available, few sample types are automatically excluded from the possibility of separation by adsorption chromatography. In addition to the broad applicability of adsorption chromatographic separation per se, adsorption effects frequently play an important or even dominant role in separations by related chromatographic procedures (e.g., ion exchange, paper chromatography). On a lesser scale fundamental and applied studies of surfaces are now being carried out by adsorption chromatographic techniques. Against this background of the broad applicability and widespread importance of adsorption chromatography (and adsorption in chromatography), this book will concentrate on an apparently narrow area: the separation of nonionic organic compounds of intermediate molecular weight and predominantly hydrophobic character, by chromatographic systems which utilize only adsorption forces. Our subject matter will nevertheless encompass most of adsorption chromatography.

To better appreciate the intention of our boundary lines, let us briefly review the relationship between sample type and the best chromatographic method for the separation of a particular sample. Low molecular weight compounds which are stable at temperatures up to 150°C below their boiling point are normally best separated by gas chromatography. Separation sharpness and speed are typically orders of magnitude better for this method, compared to other chromatographic procedures, and gas

chromatographic instrumentation has achieved a higher level of convenience and sophistication. High molecular weight compounds (e.g., polymers, proteins) are now separated mainly by exclusion chromatography on porous gels, or by ion exchange or electrophoresis when applicable. For samples of intermediate molecular weight, ion exchange and electrophoresis are often preferred procedures for ionic (or ionizable) samples. Partition chromatographic methods traditionally have been preferred for highly polar, hydrophilic compounds. Such samples are well adapted to conventional partition (i.e., column or paper) chromatographic techniques and tend to be poorly separated by many adsorption systems. Remaining sample types (i.e., nonionic, hydrophobic samples of intermediate molecular weight) are best separated by adsorption chromatography. Compared to analogous partition methods, adsorption procedures are generally faster and more convenient, give better separations, and can be more easily scaled up for preparative separations.

Although this classification of separation procedures according to sample type is accurate from a general standpoint, it represents an oversimplification for many individual cases. Gas chromatography can be broken down into gas–solid (i.e., adsorption) and gas–liquid techniques. Gas–solid chromatography (GSC) is used to some extent in the separation of the lower boiling hydrocarbons, and its possible application to higher boiling organic samples has received—and continues to receive—considerable attention. At the present time, however, GSC is of minor importance in the separation of organic mixtures. Its discussion in this book is restricted to a single, brief chapter (Chapter 9). Many of the solids used in adsorption chromatography possess ion exchange capability, permitting their application in the separation of ionic samples. Aside from the fact that such separations are now of minor importance, these mixed adsorption–ion exchange systems are much more complex and difficult to understand than corresponding "pure adsorption" systems. Consequently, little attention is given in this book to ion exchange effects in adsorption chromatography. With the recent development of thin-layer chromatography, this method is now applied to many of the hydrophilic sample types previously reserved for paper chromatography. This is largely the result of the greater speed and separation sharpness of thin-layer chromatography. However, these latter advantages are not fundamental in character, and partition methods may again achieve supremacy in this separation area. For this reason and because hydrophilic samples require aqueous or hydrophilic solvents which are quite difficult to treat theoretically (relative to normal solvents for adsorption chromatography), the

separation of hydrophilic samples by adsorption chromatography will not be stressed. The so-called "Molecular Sieves," although they are basically adsorbents with pores of molecular dimensions, are not treated in this book. The extent of their application in the separation of organic samples is relatively minor, and separation is based on principles which are generally unimportant in conventional adsorption chromatographic systems.

The subject of adsorption chromatography can logically be divided into the separation processes which occur within the adsorbent bed and the various steps which take place outside the bed. In this book we will be talking mainly about the separation process itself, and we will be stressing principles at the expense of experimental detail. The manipulative technique of adsorption chromatography has been dealt with in a number of recent books (see Section 2-5A) and it is not our intention to overlap this coverage where it can be avoided. However, a discussion of certain general aspects of technique and some evaluation of competitive procedures has seemed desirable. Chapters 2 and 13 cover these areas. The major lack in the previous literature of adsorption chromatography has been a systematic theory of the separation process as a function of separation conditions. This book will succeed or fail to the extent that it fills this void.

1-2 Historical Perspective

The history of chromatography and adsorption chromatography, from its earliest pre-Christian antecedents to its explicit discovery by Tswett and its subsequent development by more recent workers, has been covered in great detail by other writers [e.g., Refs. (*1–4*) for reasonably complete coverage]. These historical reviews emphasize the experimental side of adsorption chromatography almost to the exclusion of theoretical contributions, because advances in the method have been for the most part advances in technique. In the present brief section we will attempt to focus attention on the historical development of an *understanding* of the adsorption chromatographic process.

At the turn of the twentieth century both Tswett (in Russia) and Day (in America) discovered that the percolation of a liquid mixture through a bed of porous solid could result in the separation of that mixture according to the varying adsorption affinities of its constituents. Tswett's contribution was much more significant, since he both developed the technique in great detail and possessed a good qualitative understanding

of the basis of the chromatographic process. For a variety of reasons the discoveries of Tswett and Day lapsed into obscurity following their deaths, and the value of the chromatographic method was not generally apparent until the historic separation of the carotene isomers by Kuhn, Lederer, and Winterstein in 1931. The 10 years following this work were marked by an explosive growth in the application of adsorption chromatography to diverse separations, but the understanding of the basic separation process did not advance much beyond the level attained by Tswett. Notable practical developments in this period were the discoveries of elution chromatography by Reichstein and co-workers and of thin-layer chromatography by Izmailov and Shraiber. The significance of the latter discovery was not appreciated for another 20 years, however. The books of Strain (*5*) and Zechmeister and Cholnoky (*6*) summarize what was known of adsorption chromatography at the beginning of the 1940's. These books are filled mainly with details of technique, preparation of adsorbents, and apparatus, plus experimental examples of separation. A few empirical relationships on the role of sample and solvent structure in determining separation were beginning to emerge at that time.

Some attempts at the theoretical description of the chromatographic process were made in the early 1940's. However, it was Martin and Synge (*7*) who first cut through the apparent complexity of the chromatographic process with a brilliant theoretical analogy: the theoretical plate model of chromatography. By means of this hypothesis chromatographic separation could be described quantitatively in terms of two independent experimental parameters: the equilibrium distribution of sample between bed and solvent and the number of equivalent theoretical plates in the chromatographic bed. The Martin–Synge model provided a foundation for the development of quantitative theories of partition, ion exchange, and gas chromatography during the following 20 years. Unfortunately, the Martin–Synge treatment was largely inapplicable to adsorption chromatography as practiced in the period 1940–1960, since the adsorption isotherms in practical separation systems were generally nonlinear (see Chapter 4). Consequently, theoretical treatments of adsorption chromatography during this period proceeded for the most part along other lines, based on the assumed nonlinearity of the adsorption isotherm. The practical problems of isotherm nonlinearity in adsorption chromatography, notably drastic tailing of sample bands and poor separation, also prompted development of new experimental techniques: frontal analysis, displacement, gradient elution, and coupled columns. Unfortunately, the detailed theoretical treatment of nonlinear isotherm separations by these and other

techniques was of little value in subsequent applications of adsorption chromatography, since these moved increasingly in the direction of linear isotherm separation. During the period before 1960 a large number of qualitative data were obtained concerning the dependence of separation on both experimental conditions and the molecular structure of sample components. Many useful empirical generalizations gradually emerged from these data, along with a variety of contradictory theories on the fundamental factors which determine separation. Cassidy's books (*8,9*) summarize progress along these lines through the early 1950's. LeRosen and his group were particularly active in this period, proposing a detailed, quantitative theory of adsorption chromatographic separation. Unfortunately, all these attempts at constructing a general theory were doomed to failure by the lack of quantitative, linear isotherm data on adsorbents of reproducible properties for sample compounds of systematically varied structure.

In the late 1950's the popularization and perfection of thin-layer chromatography by Stahl revolutionized both the practice and the understanding of adsorption chromatography. On the one hand this technique permitted much faster and sharper separations of extremely small samples, with much easier interpretation of experimental results. This permitted the rapid acquisition of numerous data on the factors affecting sample separation. Joined to these advantages was the fact that thin-layer separations, because of the experimental conditions used, normally exhibit linear isotherms. Consequently, for the first time a large body of quantitative adsorption data became available for use in developing a general theory of adsorption chromatography. At the same time conditions which would assure linear isotherm separation in columns were being discovered, and studies on linear isotherm adsorption chromatography in columns were simultaneously contributing to the development of a general theory of adsorption chromatography [e.g., Ref. (*10*)].

Almost all these recent studies have been concerned with the variation of sample distribution between bed and solvent as a function of separation conditions and sample molecular structure. The problem of the number of theoretical plates in the bed (as a function of separation conditions) has been approached from other directions. In the field of gas chromatography the experimental and theoretical unraveling of this problem proceeded quite rapidly after 1955. At the present time our knowledge in the latter area is beginning to have application to liquid chromatographic separations, since the underlying theory for both systems is basically similar. Recent experimental studies of bed plate numbers in

adsorption chromatographic systems have further advanced our understanding of this subject and rounded out the general theory of adsorption chromatography.

1-3 A Comment on the Following Pages

In the remainder of the book we will move from basic principles to idealized models and finally to actual chromatographic systems. Our approach will be essentially mathematical, since we are aiming at a quantitative treatment. However, the text can be followed in most cases on a descriptive level, and much of the value of any theory of chromatography is qualitative in nature. The main practical questions usually involve the direction in which particular experimental variables should be changed (if at all) so as to improve separation. Where final mathematical relationships based on idealized models are presented, for application to actual chromatographic separation, the reader can be assured that these equations have been corroborated for a variety of representative separations. We will avoid theoretical speculation based on abstract principles, unless it has already been established that such an approach converges on experimental results. The equations derived from idealized models always involve certain empirical parameters which must be obtained experimentally. Values of these parameters are tabulated when they are available (see Appendix V for a review). Where exceptions to an idealized model exist, owing to the complexity of real chromatographic systems, they are covered as fully as possible immediately following the description of the model. We will refer to separation effects predicted by idealized models as primary effects, and exceptions or corrections to these idealized models as secondary effects.

We begin in Chapter 2 with a discussion of the chromatographic process, developing the separate concepts of (1) equilibrium distribution of sample between adsorbent and solvent (or gas) and (2) bed efficiency or theoretical plate number. These two factors are then related in a general way to the problem of separation, and the various techniques of adsorption chromatography are introduced in terms of the different separation problems they are intended to solve. Chapter 3 provides a general discussion of adsorption, emphasizing those fundamental concepts which will be necessary in the discussions of later chapters. The effect of sample size on separation is treated in Chapter 4, particularly the factors which affect isotherm linearity. Chapter 5 provides a complete treatment of bed efficiency in liquid–solid systems. The distribution of sample

between adsorbent and solvent as a function of separation conditions is dealt with in Chapters 6, 7, 8, and 12, and the role of sample molecular structure in affecting this distribution is covered in Chapters 10 and 11. Chapter 9 provides a separate discussion of gas–solid chromatography. Finally in Chapter 13 we leave the separation process per se to discuss certain incidental considerations.

One of the problems with which the author has struggled in this book is that of symbols. Unfortunately, this struggle has been largely unsuccessful. As a result there are too many symbols, the symbols used are occasionally ambiguous, and in many cases they differ from the usage of other writers. My only excuse is that this is a common problem for chromatography in general. To soften the impact of "symbolitis" on the reader, a list of symbols is given at the end of each chapter, and I have attempted throughout to specify units for each symbol used.

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2

THE CHROMATOGRAPHIC PROCESS AND TECHNIQUES OF SEPARATION

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To understand the essential features of adsorption chromatography, we must consider first the principles which underlie chromatographic separation in general. We must likewise examine the special characteristics and advantages of various general techniques of chromatography. For the experimentalist any discussion of the basic theory of chromatography may seem a formidable and even painful task. Certainly this impression is supported by the hundreds of original papers and dozens of books on chromatographic theory which have appeared within the past 10 years. The numerous variations in the technique of adsorption chromatography (i.e., elution, gradient elution, development, displacement, frontal analysis, etc.) further complicate any general discussion of chromatographic theory. Nevertheless, it is possible to describe the important elements of the chromatographic process briefly and simply, particularly if we concentrate on the more practical separation schemes and avoid mathematical detail where possible. For a more complete treatment or for different approaches to chromatographic theory, a variety of recent books are available [e.g., Refs. (*1–7*)].

2-1 Sample Migration in the Adsorbent Bed

Adsorption chromatographic separation begins with the preparation of a porous bed of finely divided solid—the adsorbent. The adsorbent is either poured into an open tube (column chromatography) or shaped into the form of a rectangular sheet (film chromatography, or, more commonly, thin-layer chromatography). The sample is introduced at one end of the adsorbent bed and induced to move through the bed by means of pressured flow or capillary action. In the vast majority of practical separations today, a small quantity of sample is initially applied and washed through the bed by means of solvent (or gas) flow. As the sample moves through the adsorbent bed, its various components will be held (adsorbed) at the adsorbent surface to a greater or lesser extent, depending upon the chemical nature of the component. Those molecules which are strongly adsorbed will spend most of their time on the adsorbent surface, rather than in the moving solvent (or gas) phase. Consequently, they will move through the adsorbent bed relatively slowly. Components which are adsorbed only slightly, on the other hand, will move through the bed rather rapidly. To these differences in average migration rate of different *compounds* are added differences in the migration rates of individual *molecules* of the same compound. On a molecular basis the process of migration through the bed is not a continuous one, but a series of jumps (when the molecule is unadsorbed) interspersed by periods of rest (when the molecule is adsorbed). Numerous factors randomly affect both the time spent by a molecule in an individual rest or jump period and the rate of travel of a molecule during one of the jump periods. As a result the total distance traveled along the bed by different molecules of the same compound is not constant. The overall chromatographic process thus gives rise to two separate phenomena: an average migration of different compounds along the bed, which varies according to compound structure and relative adsorption, and a range of migrations for individual molecules of the same compound. This process is illustrated in Fig. 2-1 for various stages in the hypothetical separation of a three-component sample mixture.

A simple but realistic model of the above process is readily constructed. Let us imagine a uniform adsorbent bed of length L, with total weight of adsorbent W, and free volume V^0 (for a column filled with adsorbent, V^0 is the volume of the column minus that of the adsorbent, i.e., the column volume accessible to solvent). A small quantity of a compound **X** (sample) is added to one end of the bed, the solvent is washed through the bed, and the process is stopped when the solvent front reaches the other end of the bed. At equilibrium the relative extent of adsorption of **X** is given by

the distribution coefficient K, equal to the concentration of sample in the adsorbed phase $(X)_a$ divided by that in the unadsorbed phase $(X)_u$† The units of $(X)_a$, $(X)_u$, and K will be chosen as moles per gram, moles per milliliter, and milliliters per gram, respectively. We will assume that K and the ratio of adsorbed to nonadsorbed phases is constant at any point within the adsorbent bed behind the advancing solvent front and at any time during passage of solvent through the bed. The distance traveled by

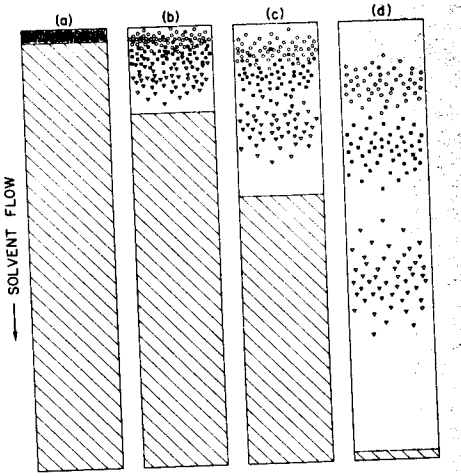

Fig. 2-1. Hypothetical separation of a three-component sample mixture by bed development. Calculated for $N = 33$ and R_F values of 0.15 (○), 0.30 (■), and 0.60 (▽).

the average molecule of sample is seen to be equal to the product of the distance traveled by the solvent front L and the fraction of **X** which is unadsorbed f_u; f_u is equal to $V^0(X)_u/[V^0(X)_u + W(X)_a]$. Thus for **X** completely desorbed ($f_u = 1$) the distance traveled by **X** is $1.0 \cdot L$; i.e., **X** travels with the solvent front. If the distance traveled by **X** divided by the distance traveled by the solvent front (L) is defined as R_F, then R_F is seen equal to f_u; i.e.,

$$R_F = V^0(X)_u/[V^0(X)_u + W(X)_a] = 1/[1 + (W/V^0)K] \quad (2\text{-}1)$$

† The distribution coefficient K for adsorption systems is essentially equivalent to the partition coefficient K' for two contiguous liquid phases 1 and 2 (or a gas and a liquid phase): $K' = (X)_1/(X)_2$, where $(X)_1$ and $(X)_2$ refer to the equilibrium concentrations of sample **X** in phases 1 and 2, respectively. K' is normally constant for small values of $(X)_1$ and $(X)_2$.

The variation in the distance migrated by individual molecules of **X** can be derived in various ways that are more or less simple to follow. The Craig distribution model of Keulemans (*3*) will be described here. According to Keulemans, a chromatographic column is likened to a Craig distribution apparatus, consisting of a series of interconnected (but distinct) bed sections or theoretical plates. Assuming N theoretical plates in the entire bed, each plate N_i contains a weight W/N of adsorbent and a free volume V^0/N. The total sample is assumed to be contained initially in a volume V^0/N of solvent which is placed in the first plate N_0. Passage of solvent through the bed is visualized to take place in steps, as in the operation of the Craig apparatus. In each step the solvent (plus any unadsorbed sample) in plates N_0, N_1, N_2, etc., shifts over to the next higher number plate and fresh solvent enters plate N_0. After each step or solvent transfer, the contents of each plate are assumed to equilibrate, with redistribution of sample between adsorbed and solvent phases according to the distribution coefficient K. This process is illustrated in Table 2-1

Table 2-1
Migration of Sample **X** in Craig Distribution Model

Transfer number	Total amount of **X** in each plate after solvent transfer						Amount of **X** transferred from each plate during following transfer					
	N_0	N_1	N_2	N_3	N_4	N_5	N_0	N_1	N_2	N_3	N_4	N_5
0	1	0	0	0	0	0	1/2	0	0	0	0	0
1	1/2	1/2	0	0	0	0	1/4	1/4	0	0	0	0
2	1/4	2/4	1/4	0	0	0	1/8	1/4	1/8	0	0	0
3	1/8	3/8	3/8	1/8	0	0	1/16	3/16	3/16	1/16	0	0
4	1/16	4/16	6/16	4/16	1/16	0	1/32	1/8	3/16	1/8	1/32	0
5	1/32	5/32	10/32	10/32	5/32	1/32	1/64	5/64	5/32	5/32	5/64	1/64

and Fig. 2-2 for the case where $R_F = 0.5$; i.e., at equilibrium one-half of the sample in each plate will be adsorbed and one-half contained in the solvent phase. In Table 2-1 the fractional amount of sample in each plate (adsorbed plus unadsorbed) is shown after each of the first five steps or solvent transfers. The fractional amount of sample transferred from each plate in the following transfer is also shown. Thus prior to the first solvent transfer all the sample is contained in the first plate, with half of the sample in the adsorbed phase and half in the solvent phase. After the first solvent transfer the sample originally in the solvent phase of plate N_0 is now in plate N_1, and fresh solvent has replaced the solvent originally in plate N_0.

Half of the total sample is now in plate N_0 and half in plate N_1. Equilibration of the contents of each plate gives one-fourth of the total sample in the adsorbed and solvent phases of plates N_0 and N_1, respectively. In the second solvent transfer the one-fourth of the total sample in the solvent phase of plate N_0 is moved to plate N_1, and the one-fourth of total sample in the solvent phase of plate N_1 is moved to plate N_2. The total sample fractions in plates N_0, N_1, and N_2 are now 0.25, 0.50, and 0.25. This

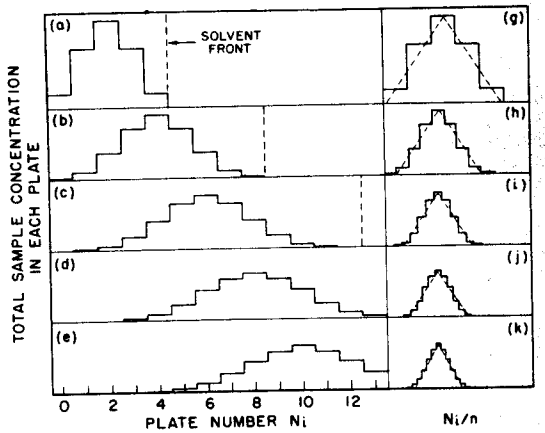

Fig. 2-2. Distribution of sample on the adsorbent bed after varying degrees of solvent transfer, according to the Craig distribution model: (a, g) 4 transfers; (b, h) 8 transfers; (c, i) 12 transfers; (d, j) 16 transfers; (e, k) 20 transfers.

process is continued to give the various sample distributions in the adsorbent bed shown in Table 2-1, as a function of the number of solvent transfers.

In Fig. 2-2(a–e) is shown the distribution of **X** on the adsorbent bed after 4, 8, 12, 16, and 20 transfers, respectively. As **X** moves down the adsorbent bed, a symmetrical concentration distribution or band is formed. The center of the band after each of these transfers moves down the bed exactly one-half as far as the solvent front, as predicted by Eq. (2-1). As the band moves down the column, it widens and eventually approaches the shape of a Gaussian curve. This is illustrated in Fig. 2-3 for the preceding example after 20 transfers. Mathematically the distribution of **X** on the adsorbent bed (compare Table 2-1) is given by the coefficients of the binomial expansion $(x + y)^n$, where n is the number of solvent transfers that have taken place. At large values of n the resulting distribution can be shown to converge to a Gaussian distribution.

The relative widening of the band as it moves along the bed is less than proportional to the distance traveled. This is seen in Fig. 2-2(g–k), where the bands of Fig. 2-2(a–e) are replotted versus N_i/n (rather than against N_i); n is proportional to the distance traveled by the band center. If bandwidth were proportional to the distance traveled by the band, the widths of these latter bands would remain constant. Instead, the reduced bands of Fig. 2-2(g–k) are seen to steadily narrow with increasing distance traveled by the band center; this fact forms the basis of chromatographic

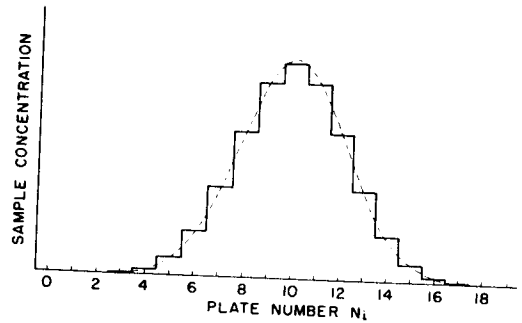

Fig. 2-3. Approach of band shape to a Gaussian distribution. Solid line, Craig distribution model after 20 transfers ($R_F = 0.5$); dashed line, Gaussian curve.

separation. The relationship between bandwidth and the distance traveled by the band can be derived as follows. After some number n solvent transfers and the attainment of an approximately Gaussian band shape as in Fig. 2-3, the bandwidth can be defined by the standard deviation σ_n of the Gaussian curve. The distance traveled by the band center will be given as d_n. Now assume an additional n solvent transfers, following which the total distance migrated by the band center will be $2d_n$. Because the distribution coefficient K is constant for different sample concentrations, the migration of the contents of each plate (after the first n transfers) during the second n transfers can be calculated independently of any intermixing of the contents of different plates. This process is illustrated in Fig. 2-4. The bands arising from the migration of the contents of individual plates after n transfers are shown as solid curves in Fig. 2-4 ("$2n$"). Each of these bands has a standard deviation equal to σ_n, since the migration process giving rise to each of these bands is equivalent to the migration of total sample in the first n transfers. The total sample band after $2n$ transfers is simply the sum of these bands for individual plates (dashed curve in Fig. 2-4). The standard deviation of this composite

band after $2n$ transfers σ_{2n} can be regarded as arising from the combination of two independent processes, each of standard deviation σ_n. For this situation an elementary theorem in statistics gives

$$\sigma_{2n}^2 = \underset{(0\to n)}{\sigma_n^2} + \underset{(n\to 2n)}{\sigma_n^2}$$

and, therefore,

$$\sigma_{2n} = \sqrt{2}\sigma_n$$

That is, while the distance traveled by the band has doubled between n and $2n$ transfers, bandwidth has increased only by the factor $\sqrt{2}$. Thus for a particular adsorbent bed, sample bandwidth σ will increase in

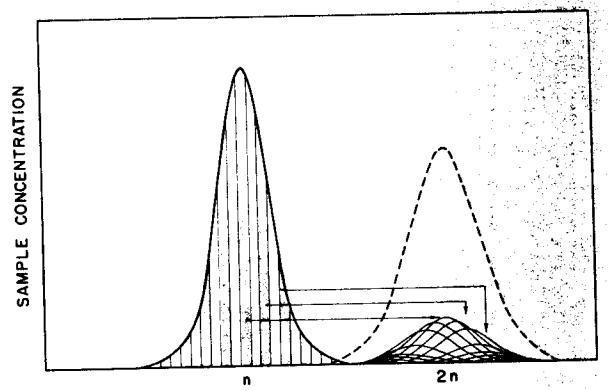

Fig. 2-4. Band widening during sample migration.

proportion to the square root of the distance d traveled by the band center. Since the distance traveled by the band center is proportional to the number of plates passed by the band center N', we have σ proportional to $\sqrt{d}$, d proportional to N', and therefore d/σ proportional to $\sqrt{N'}$. Further development of the Craig distribution model (or other models of the chromatographic process) shows that the last proportionality constant is unity, i.e.,

$$d/\sigma = \sqrt{N'} \qquad (2\text{-}2)$$

Equation (2-2) can be used to define the plate number of an adsorbent bed from the point of sample application to the position of the band center. If the band center is moved to the end of the adsorbent bed by continued solvent transfer (with some solvent eventually leaving the bed