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STATISTICAL APPROACH TO CHROMATOGRAPHIC THEORY

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CONTENTS

1. INTRODUCTION

The theoretical plate model continues to enjoy widespread popularity as a description of the behavior of chromatographic peaks. The plate model is generally attributed to Martin and Synge[1], but has been developed further and modified by Glueckauf[2], Mayer and Tompkins[3] and others. Giddings[4] gave an excellent discussion of the plate theory and its limitations.

In the plate theory of chromatography, a column is divided into a number of imaginary sections called plates. In each plate it is assumed that a sample substance is partitioned between the stationary and mobile phases and that equilibrium has been attained. Then the mobile phase in each plate moves forward to the next plate, carrying the sample substance in the mobile phase forward exactly one plate. A new partitioning occurs simultaneously in each plate, and it again is assumed that equilibrium is attained. This process is repeated many times, until sample constituents move along and off the column at different rates and thus are separated.

The number of imaginary sections in a column is generally called the number of theoretical plates, or theoretical plate number, and is designated as N (or n). The numerical value of N for any given column length is maximized by using very small spheres of uniform diameter, thinly coated with a stationary phase and carefully packed, as the column stationary phase. Factors such as column dimensions and

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fittings and proper eluent flow-rate also affect the value of N and hence the separation efficiency of the column.

There is no convenient way of measuring the intrinsic value of N; it must be calculated from measurable parameters, namely the retention time (or volume) of a peak and the peak width (or σ which is a function of peak width). The defining equation for this calculation is

$$N = 16\left(\frac{t}{w}\right)^2 = \frac{t^2}{\sigma^2}$$

where t is the peak retention time, w is the peak width at its base (in the same units as t) and σ^2 is the variance of the peak width.

Some chromatographers prefer to use the number of effective theoretical plates as a measure of the separation power of a column

$$N_{\text{eff}} = 16\left(\frac{t - t_0}{w}\right)^2 = \frac{(t - t_0)^2}{\sigma^2}$$

where t_0 is the retention time of a non-sorbed substance, or the "dead time" of the column. As early as 1959 Purnell[5,6] noted that N_{eff} (for which he used S, rather than N) is critical in determining the separation ability of a chromatographic column. He found that the resolution of alkanes was inconsistent with the observed value of N when the retention volumes are very small. Widely used equations show that resolution of chromatographic peaks is proportional to the number of effective theoretical plates.

The plate theory of chromatography has been criticized as lacking in physical reality because eluent flow in actual columns obviously is continuous and not a discontinuous flow from one plate to the next as portrayed by the plate theory. It is debatable whether this objection is valid because any theory that gives results consistent with experimental behavior would be useful despite differences in the actual and theoretical mechanisms.

Giddings[4] criticized the plate model on several counts and concluded that it "fails in the most important test of all — the very practical matter of predicting zone dispersion as a function of the numerous variables open to manipulation by the investigator". These variables include the particle size of the solid support, thickness of stationary phase coating, eluent flow-rate and diffusion coefficients in the stationary and mobile phases.

The rate theory of chromatography has tended to supplement, rather than replace, the plate theory. The Van Deemter equation[7] describes the effects of eluent flow-rate, longitudinal diffusion and multi-path effects within the column on plate height, H (H = column length, divided by N). For capillary columns in gas chromatography, the Golay equation[8] shows how diffusion coefficients, thickness of stationary phase coating, column diameter, flow-rate, etc., contribute to plate height, H. Giddings[4] has made major contributions to the dynamics of chromatography and has shown the effect of many experimental parameters on plate height in both gas (GC) and liquid chromatography (LC).

Although a theoretical value for H can be calculated from the diffusion coef-

ficients and other parameters contained in rate equations, the calculations often are not very accurate. For example, Desty and Goldup[9] compared experimental and calculated values for terms in the Golay equation and found good agreement for the B term but that the experimental C term was 2–6 times the calculated value. The major value of rate equations is in predicting in a qualitative manner (or semi-quantitative at best) how a change in particle size, for example, will affect chromatographic behavior.

Most chromatographers still rely on only two actual measurements to evaluate performance peak retention time and peak width The discussion in a recent book on liquid chromatography[10] is fairly typical of the way in which the dynamics of chromatography currently are handled. The authors consider that the theoretical plate number, N, of various chromatographic peaks is approximately constant and therefore they consider both N and H as measures of chromatographic efficiency. Thus, the rate theory and plate theory are in a way still tied together.

There seems to be some confusion as to how N varies (or does not vary) as the capacity factor, k, changes (recall that retention time of a peak varies directly with its capacity factor). While Snyder and Kirkland consider that N is approximately constant with k, Grob[11] states that N tends to increase with higher k and Kaiser[12] indicates that N decreases as k becomes larger Jennings[13] provides a nice experimental answer to this question Careful gas chromatographic separation of n-alkanes on a capillary column shows that N is very high at low values of k and decreases with increasing k, rapidly at first when k is small, and then more slowly at larger k values, eventually approaching an asymptotic value. Their data show that n_{eff} starts at or near zero and increases as k becomes larger N_{eff} appears to approach the same asymptotic values as N The change in N or N_{eff} with k is particularly great at k values below 1 0

In this paper we derive simple statistical expressions for calculating the mean and variance of chromatographic peaks that are still on a column (position peaks) and for these peaks as they leave the column (exit peaks). The variances of position and exit peaks as a function of capacity factor, k, are compared We also show how the major contributions to peak broadening in capillary column GC can be determined at any k value using gaseous diffusion coefficients computed from empirically determined constants for the atoms and chemical groups making up the carrier gas and the compounds separated. First, the peak variance contributions resulting from axial diffusion are calculated from a modified form of the Van Deemter equation and are subtracted from the measured peak variances. Then the remaining variance of each peak is plotted against a function of the capacity factor, k A plate number is calculated from the slope of this linear plot which is independent of k and thus provides an excellent measure of performance for a column operating under specified, fixed conditions. The intercept of this plot provides an estimate of the extra-column peak broadening of the chromatographic system.

For both GC and LC columns run at a single flow-rate, a similar approach is outlined that gives another plate number that also is independent of k but which includes any peak broadening effects resulting from axial diffusion This plate number is also useful in describing the separating ability of a column under fixed conditions and in predicting the variance of peaks of different k values from those in the test chromatogram

The authors feel that the material presented here will correct some of the confusion or misconceptions that many chromatographers seem to have about plate theory. We also think that the approach presented is a simple but practical way of handling some of the dynamic effects that determine the variance of chromatographic peaks.

2. PLATE MODELS

Using a statistical approach, we shall study two classical models falling into the category of plate theories: the first is the discrete flow model and the second the continuous flow model. The characteristic feature of a plate model is that the chromatographic column is considered to be divided into a sequence of disjoint segments, or "plates". In our discussion, we shall refer to these as theoretical segments (abbreviated to TS). The sample molecules proceed from one TS to the next until they leave the last segment and exit from the column.

The purpose here is to derive statistical equations for the mean and variance of a peak that is still on the column (a position peak), and for a peak leaving the column (an exit peak); this will be done for each of the plate models. The statistical approach also leads to equations that describe the entire shape of position and exit peaks, although such equations for counter-current chromatography (CCC) are already available in the literature.

3. DISCRETE FLOW MODEL

The assumptions of the discrete flow plate model are (1) the entire mobile phase moves from each segment to the next at the end of discrete equal time intervals and (2) the sample chemical remains in equilibrium between the stationary and the mobile phases. In our analysis we shall study the movement of a single molecule through the column. In this form the equilibrium condition is expressed by the condition that the probability that the molecule is in the mobile phase is p, while the probability it is in the stationary phase is $1 - p$. Thus $p = \frac{1}{1+k}$, where k is the well known capacity factor. Thus the position of the molecule is random and depends crucially on the random "decisions" on whether to stick in the stationary phase or be free in the mobile phase during a given time unit. We shall measure the passage of time by the number of transfers (n) of the mobile phase that have occurred. We shall measure the location of the molecule by noting which segment it is in. Two quantities are of particular interest: one is the probability distribution of the position of the particle at time n, and the other the probability distribution of the time when the molecule leaves the column (the exit time).

We shall first examine the position distribution. In each time period the particle either goes to the next segment of stays in its current segment. The first event takes place with probability p and the second with probability $1 - p$. Which of these two events actually occurs in the kth time interval can be represented by the random variable

$$B_k = \begin{cases} 1 \text{ with probability } p \\ 0 \text{ with probability } 1 - p \end{cases}$$

Random variables of this type are known as "Bernoulli" random variables[14]. If we assume that our molecule starts in segment zero at time zero, then its position at time n [denoted by $X(n)$] is

$$X(n) = \sum_{k=1}^{n} B_k$$

As the B_ks are random, $X(n)$ is also random. The Bernoulli random variables in the sum are all independent and identically distributed with mean p and variance $p(1 - p)$. It follows that the expected value of $X(n)$ is

$$E[X(n)] = E\left(\sum_{k=1}^{n} B_k\right) = \sum_{k=1}^{n} E(B_k) = np \tag{1}$$

and the variance of $X(n)$ is

$$\mathrm{Var}[X(n)] = \mathrm{Var}\left(\sum_{k=1}^{n} B_k\right) = \sum_{k=1}^{n} \mathrm{Var}(B_k) = np(1 - p) \tag{2}$$

These could also be written as

$$E[X(n)] = n\left(\frac{1}{1 + k}\right) \tag{3}$$

$$\mathrm{Var}[X(n)] = n\left[\frac{k}{(1 + k)^2}\right] \tag{4}$$

The central limit theorem of probability[14] tells us that $X(n)$ is asymptotically normal, *i.e.*, the distribution of $X(n)$ approaches closer and closer to a normal, or Gaussian, distribution as n increases ($n \approx 30$ is usually considered large enough for this to be a good approximation).

Probability theory will provide even more detailed information. An elementary result tells us that the sum of n independent identically distributed Bernoulli random variables with parameter p has a binomial distribution with parameters n and p (ref. 14). It follows that

$$P\{X(n) = j\} = \binom{n}{j} p^j (1 - p)^{n-j} = \frac{n!}{(n - j)!j!} p^j (1 - p)^{n-j}$$

$$= \frac{n!}{(n - j)!j!} \frac{k^{n-j}}{(1 + k)^n} \tag{5}$$

where j is some particular TS in the column and n is the number of transfers, or the

number of time intervals that have elapsed for a substance of capacity factor k. Eqn. 5 can be used to calculate the distribution of molecules along the various TS in a column by inserting various values of j into the equation. The result calculated will be the fraction of the molecules in each theoretical segment.

Next we examine the problem of determining the exit time distribution. If we denote the exit time by T, then $T = r + \sum_{k=0}^{r-1} D_k$. In this equation r is the number of theoretical segments in the column and therefore is the minimal amount of time necessary for the molecule to leave the column if it never enters the stationary phase. D_j is the (random) time that the molecule spends in the stationary phase of segment j. D_j can take on the values 0, 1, 2, ... and it does so with probabilities p, $p(1-p)$, $p(1-p)^2$, ..., respectively. This is the distribution of a geometric random variable with parameter p (ref. 14). Thus the random variables D_j, $j = 0, \ldots, r-1$ are independent, identically distributed, geometric random variables with parameter p. The mean and variance of such a geometric random variable is $(1-p)/p$ and $(1-p)/p^2$, respectively. Thus we obtain

$$E(T) = E\left(r + \sum_{k=0}^{r-1} D_k\right) = r + \sum_{k=0}^{r-1} E(D_k) = r + r\left(\frac{1-p}{p}\right) = \frac{r}{p} = r(1+k) \quad (6)$$

$$\mathrm{Var}(T) = \mathrm{Var}\left(r + \sum_{k=0}^{r-1} D_k\right) = \mathrm{Var}\left(\sum_{k=0}^{r-1} D_k\right) = \sum_{k=0}^{r-1} \mathrm{Var}(D_k) = r\left(\frac{1-p}{p^2}\right) = rk(1+k) \quad (7)$$

We can once again apply the central limit theorem of probability to conclude that T is asymptotically normal, i.e., as r becomes large the distribution of T approaches the normal distribution. It is even possible to obtain the exact probability distribution of T. To do this we utilize an earlier result $P\{X(n) = j\} = \binom{n}{j} p^j (1-p)^{n-j}$.
We obtain the following

$P\{T = n\} = P\{X(n-1) = r-1$ and that the molecule is in the mobile phase in the next time interval$\} =$

$P\{X(n-1) = r-1\}\, P\{$mooecule is in mobile phase in next time interval$\} =$

$$\left[\binom{n-1}{r-1} p^{r-1} (1-p)^{n-r}\right] p = \binom{n-1}{r-1} p^r (1-p)^{n-r}$$

Thus

$$P\{T = n\} = \frac{(n-1)!}{(n-r)!(r-1)!} \frac{k^{n-r}}{(1+k)^n} \quad (8)$$

The result in eqn. 8 is the fraction of molecules of capacity factor k that leaves a column, containing r theoretical segments, during the nth time period

4 CONTINUOUS FLOW MODEL

The basic assumptions of this model are as follows: (1) eluent flows at a constant rate from one segment to the next; (2) the mobile and stationary phases remain in equilibrium at all times; and (3) there is perfect mixing in the segments. As we shall be looking again at only a single molecule, we interpret condition (2) to mean that the probability that the molecule is in the mobile phase at any given time is $\frac{1}{1+k}$ and the probability that it is in the stationary phase is $\frac{k}{1+k}$. "Units" are set up as follows. The amount of eluent in one segment is one unit of volume. The amount of time it takes for one unit volume to flow from one segment to the next is defined to be one time unit. Thus, in a time interval Δt, an amount of eluent Δt flows from each segment to the next.

Suppose we have r segments (numbered 0, 1, . . , $r-1$). Looking at some molecule of sample as it moves through the segments, we see that.

(1) It spends a random amount of time, D_i, in segment i.

(2) These "delay times", D_i, are independent, identically distributed random variables.

(3) The exit time for the molecule is $\sum_{i=0}^{r-1} D_i$.

(4) The TS the molecule is in at time t, $N(t)$, is given by

$$N(t) = \left\{\text{minimum value of } n \text{ such that } \sum_{i=0}^{n-1} D_i \leqslant t < \sum_{i=0}^{n} D_i\right\} \tag{9}$$

(Note the above D_i is not to be confused with the one used in the discrete flow model.)

Clearly, our first task must be to determine the distribution of the random variables, D_i. To do this we look at a single segment. We wish to calculate the probability that a molecule (which is in this segment) leaves the segment in the next time interval Δt (Δt is assumed to be small). For this to happen it is necessary that the molecule (a) be in the mobile phase, and (b) be in that particular Δt volume of eluent that is leaving. This probability is

$$P\{a\}\ P\{b\} = \left(\frac{1}{1+k}\right)\left(\frac{\Delta t}{1}\right) = \Delta t\left(\frac{1}{1+k}\right)$$

This result is characteristic of an exponential delay time with parameter $\lambda = \frac{1}{1+k}$ (ref. 14). The density of such a random variable is $\lambda e^{-\lambda t}$ $(t \geqslant 0)$.

With this piece of information together with some standard results of probability theory, we can now determine the exit time density and the probability distribution of the molecules' position at time t. First, the distribution of the exit time is the distribution of the sum of r independent identically distributed exponential random

variables It is known that this distribution is a gamma distribution with parameters r and λ (ref 14) The density function is

$$f(t) = \frac{\lambda(\lambda t)^{r-1} e^{-\lambda t}}{(r-1)!} \quad (t \geq 0)$$

Now we use the fact that the mean and variance of our exponential random variables are $1/\lambda$ and $1/\lambda^2$ respectively Let T denote the exit time [which will have the density $f(t)$ above]. Then.

$$E(T) = E\left(\sum_{i=0}^{r-1} D_i\right) = \sum_{i=0}^{r-1} E(D_i) = \frac{r}{\lambda} = r(1+k) \tag{10}$$

$$\mathrm{Var}(T) = \mathrm{Var}\left(\sum_{i=0}^{r-1} D_i\right) = \sum_{i=0}^{r-1} \mathrm{Var}(D_i) = \frac{r}{\lambda^2} = r(1+k)^2 \tag{11}$$

Once again we can apply the central limit theorem to conclude that the distribution of T is asymptotically normal (as $r \to \infty$)

It will be noted that the mean exit time (eqn. 10) is the same for both models but that the variance of the exit peak is $r(1+k)^2$ for the continuous flow model and $rk(1+k)$ for the discrete flow model.

To obtain the distribution of the position of sample molecules at time t, we note from eqn. 9 that D_i being identically distributed random variables makes $N(t)$ a Poisson process with parameter λ (ref. 15) [for the definition of $N(t)$, see eqn. 9]:

$$P\{N(t) = k\} = \frac{(\lambda t)^k e^{-\lambda t}}{k!} \tag{12}$$

for $k = 0, 1, \ldots, r-1$

It can be shown by a slightly more sophisticated application of the central limit theorem that the distribution of $N(t)$ is asymptotically normal (as $t \to \infty$) (this result also requires that r be large enough that the molecule will not yet have left the column at values of t large enough to yield a good normal approximation)

5 THEORETICAL PLATE NUMBERS IN THE TWO MODELS

The theoretical plate number, N, and the effective theoretical plate number, N_{eff}, are of course widely used in chromatography. These may be defined by

$$N = \frac{[E(T)]^2}{\mathrm{Var}(T)} \tag{13}$$

and

$$N_{\mathrm{eff}} = \frac{[E(T) - t_0]^2}{\mathrm{Var}(T)} \tag{14}$$

where t_0 is the exit time for a non-sorbed substance and $E(T)$ is the expected exit time for a sample substance.

We can use the result we have obtained to calculate these quantities for both the discrete flow and continuous flow models. For the discrete flow mode:

$$N = \frac{[E(T)]^2}{\mathrm{Var}(T)} = \frac{[r(1 + k)]^2}{rk(1 + k)} = r\left(\frac{1 + k}{k}\right) \tag{15}$$

$$N_{\mathrm{eff}} = \frac{[E(T) - t_0]^2}{\mathrm{Var}(T)} = \frac{(rk)^2}{rk(1 + k)} = r\left(\frac{k}{1 + k}\right) \tag{16}$$

For the continuous flow model:

$$N = \frac{[E(T)]^2}{\mathrm{Var}(T)} = \frac{[r(1 + k)]^2}{r(1 + k)^2} = r \tag{17}$$

$$N_{\mathrm{eff}} = \frac{[E(T) - t_0]^2}{\mathrm{Var}(T)} = \frac{(rk)^2}{r(1 + k)^2} = r\left(\frac{k}{1 + k}\right)^2 \tag{18}$$

Thus, only in one of the four cases is the plate number result independent of k.

6 SOME CONSEQUENCES OF THE DISCRETE FLOW MODEL

In the discrete flow model the mean and variance of the location, $X(n)$, are expressed in units involving TS (here theoretical segments indicate the *position* of the molecule). On the other hand, the mean and variance of the exit time, T, must be expressed in time units. The fundamental time unit is the time needed for eluent to flow from one TS to the next. We wish to show that simple unit conversions can be used to relate results for peak locations to those for peak exit times. We define T_j as the time when a sample molecule enters the jth theoretical segments (note that $T_r = T$). Reasoning by analogy with eqns. 6 and 7, we have $E(T_j) = j(1 + k)$ and $\mathrm{Var}(T_j) = jk(1 + k)$. Using eqns. 3 and 4 to calculate $E[X(n)]$ and $\mathrm{Var}[X(n)]$ when $n \approx j(1 + k)$

$$E[X(n)] = j(1 + k)\,\frac{1}{1 + k} = j \tag{19}$$

$$\mathrm{Var}[X(n)] = j(1 + k)\cdot\frac{k}{(1 + k)^2} = j\,\frac{k}{1 + k} \tag{20}$$

Now we see that $E(T_j) = E[X(n)](1 + k)$ and $\mathrm{Var}(T_j) = \mathrm{Var}[X(n)](1 + k)^2$. In other words, we can convert between means and variances of position and exit time random variables simply by multiplying by the conversion fact $(1 + k)$ for the means and $(1 + k)^2$ for the variances.

Counter-current chromatography (CCC) is a good system for illustrating the practical results of the equations derived. In CCC the solute molecules attain equilibrium between the mobile and stationary phases prior to each transfer to the next tube in the series Thus, each tube is by definition a theoretical plate, according to the plate theory of chromatography, or a theoretical segment in our nomenclature. It has been recognized by several authors that CCC also is a reasonable model for explaining elution behavior in column chromatography, provided that axial diffusion, multipaths and other factors that contribute to peak broadening in column chromatography, but which are non-existent in CCC, are also taken into account. This will be done in the next section, but first let us consider only the peak broadening resulting from inter-phase broadening effects, with CCC as a model.

Fig. 1 shows the distribution of a solute along the series of 36 tubes ($r = 36$) in a CCC apparatus as a function of the number of transfers, n The peaks were plotted for $k = 1.5$ from eqn. 5 with the aid of a programmable calculator. Note that the peak has become well defined and approximately Gaussian after only a few transfers and that the peak broadens and decreases in height as it moves along the series of tubes. Fig. 2 shows the same peak, calculated from eqn. 8, as it leaves the CCC apparatus and passes through an imaginary detector. The peak that was symmetrical in its position distribution of molecules now has a distinct tail The explanation for this is that the latter part of the peak continues to broaden after the earlier portions have left the "column". This effect is of some significance because it demonstrates that in columns of low "performance" some peak tailing is predicted mathematically and is not necessarily caused by a poorly functioning column.

The tailing of an exit peak decreases and the peak becomes more Gaussian as the number of theoretical segments (r) in a column becomes larger The tailing, or skewness, of a peak is predicted by the following equation:

$$\text{Coefficient of skewness} = \frac{1}{\sqrt{r}} \frac{2k^2 + 3k + 1}{(k + 1)^2[k/(k + 1)]^{1/2}} \tag{21}$$

(see ref. 14 for a definition of the coefficient of skewness). The second term in this equation has a value of just over 2 from $k = 1$ to $k = 10$, but increases rapidly as the value of k becomes very small.

It is well known that later eluting peaks are much broader than those with shorter retention times. From the exit peak variance (eqn 7) it will be seen that the peak width (4σ) is proportional to $\sqrt{k(1 + k)}$ and thus increases in approximately linear fashion with k (except at very low values of k). It is interesting to compare this with the way the width of a peak still on a column varies with k. This may be done by calculating the peak widths when the peak maximum is located in the jth theoretical segment in a column containing r theoretical segments. We shall choose j to be near the end of the column, say $j = 0\,9\,r$. From eqn. 20 for the variance of a position peak we see that $4\sigma = 4\left[\frac{jk}{1 + k}\right]^{1/2}$ In Table 1 we see that the widths of peaks centered at j vary only slightly with increasing k, much less than the exit peaks (of course it takes longer for peaks of higher k to arrive at j). The reason that elution peaks are so much broader when k is high is that such peaks are moving more slowly and take longer to pass through the detector.

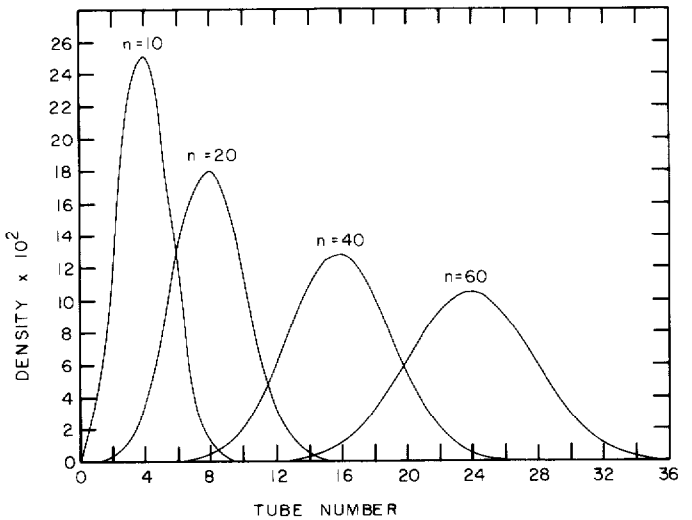

Fig 1 Position peaks for a compound with $k = 1.5$ in a CCC apparatus of 36 tubes after n transfers

Exit peaks calculated for the discrete flow model using eqn 8 emphasize a major drawback of the plate theory that has been so widely used in chromatography. In no case does either N or N_{eff} (calculated from eqns 13 and 14) agree with the actual number of plates (tubes) in CCC, which is r. The correct values for N and N_{eff} are given by eqns. 15 and 16, respectively. Fig. 3 shows the expected values of N and N_{eff} as a function of k, calculated from eqns. 15 and 16 for a CCC apparatus where $r = 1000$ The values of N are very high as $k \rightarrow 0$ and become meaningless as a measure of separating power. Similarly, a single value of H (height equivalent to a theoretical plate) is an ambiguous expression of column efficiency when H varies so widely for different peaks.

The application of eqns 5 and 8 to CCC is well documented and there would seem to be little doubt as to their correctness It is logical that eqns. 3–8 should apply also to column chromatography An examination of actual column chromatograms is now in order, to determine whether the equations do or do not apply.

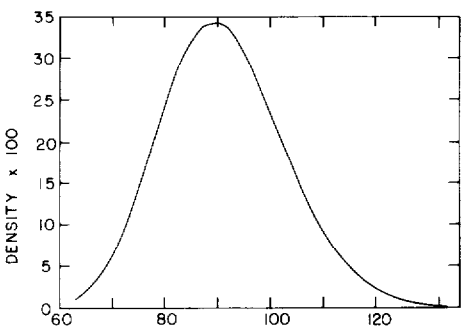

Fig 2 Exit peak for a compound with $k = 1.5$ from a CCC apparatus of 36 tubes (n = number of transfers)

TABLE I

WIDTHS (4σ) OF POSITION AND EXIT PEAKS AS A FUNCTION OF k

k	*Position width** (TS)	*Exit width*** (TS)	*Exit width**** (sec)
0 1	36	42	2 5
0.2	49	62	3 7
0 4	64	95	5 7
0 6	74	124	7 4
0 8	80	152	9 1
1 0	85	179	10 7
1 5	93	245	14.7
2 0	98	310	18.6
3 0	104	438	26 3
4 0	107	566	33 9
6 0	111	820	49 2
8 0	113	1073	64 4
10 0	114	1327	79 6
15 0	116	1960	117 6

* Calculated from $4\sigma = 4\left[\frac{jk}{1 + k}\right]^{1/2}$, where $j = 900$

** Calculated from $4\sigma = 4[rk(1 + k)]^{1/2}$, where $r = 1000$.

*** Calculated from the previous column for an eluent flow-rate of 16 67 TS/sec

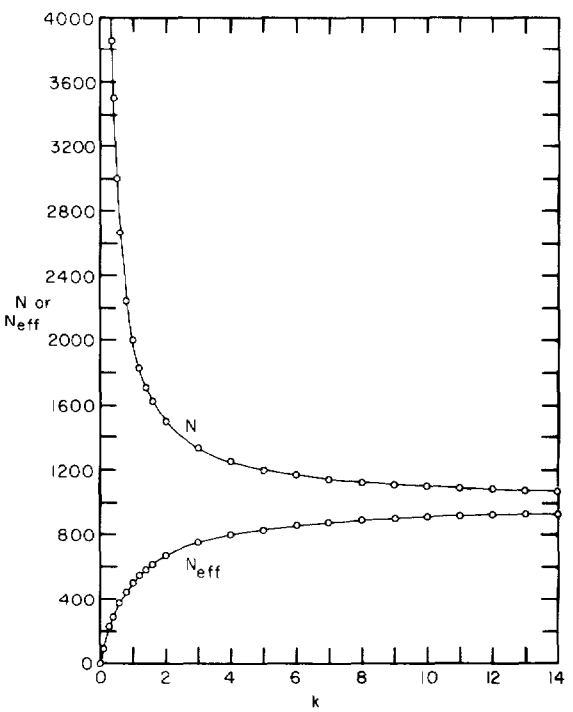

Fig. 3 Expected values of N and N_{eff} for an exit peak from a CCC apparatus where $j = 1000$

7 CALCULATION OF PLATE NUMBERS FROM CHROMATOGRAPHIC DATA

When working with actual data for column chromatography, it is necessary to consider the various dynamic effects that contribute to the width of chromatographic peaks. The major effect almost always is what is termed "resistance to mass transfer", which we shall call "inter-phase broadening". Additional peak broadening occurs through axial diffusion, multi-paths (in packed columns) and extra-column effects such as broadening in fittings, connecting tubing and the detector. Injection band broadening is apt to be significant also, as was recognized by Keulemans as early as 1959[16,17].

If we assume that these broadening effects act independently of one another, the measured peak variance is the sum of the variances of the individual broadening effects For capillary GC columns (where there is no multi-path effect), we can write

$$\sigma^2 = \sigma_{ec}^2 + \sigma_d^2 + \sigma_{ip}^2 \tag{22}$$

where ec stands for extra column, d for diffusion and ip for inter-phase. The approach taken will be first to estimate the peak variance resulting from axial diffusion and to subtract this from the measured peak variance Then a plate number representing the inter-phase broadening can be calculated from the slope of a plot of the remaining peak variance against $k(1 + k)$, which comes from the peak variance in the discrete model, $rk(1 + k)$.

7.1. *Diffusion broadening, σ_d^2*

The effect of diffusion on plate height, H, is given by the Van Deemter equation, which has the form, $H = \frac{B}{u} + Cu$, where B is the diffusion term, u is the linear column flow-rate and C is the resistance to mass transfer. In this equation, $B = 2D_g$, where D_g is the axial diffusion coefficient in the gas phase (cm^2/sec).

According to the classical definition of H and N, the diffusion contribution to H is converted to σ_d^2 as follows

$$H = \frac{L}{N} = \frac{L\,\sigma_d^2}{[t_0(1 + k)]^2} = \frac{2D_g}{u} \tag{23}$$

$$\sigma_d^2 = \frac{2D_g}{u}\,\frac{(t_0)^2\,(1 + k)^2}{L} = \frac{2D_g L(1 + k)^2}{u^3} \tag{24}$$

where L is the column length (cm) and u is the linear flow-rate (cm/sec).

One way to estimate the diffusion broadening variance is to run a chromatogram at two different flow-rates Diffusion broadening varies as the inverse cube of the flow velocity, but inter-phase broadening varies only as the inverse of the flow velocity. This difference allows us to solve for diffusion broadening by solving two simultaneous equations (the dependence of the broadening on flow velocity will be considered in the next section)

Although this method works, a more convenient way is to use the method for

estimating gaseous diffusion coefficients proposed by Giddings[18]:

$$D_g \text{ (for A,B)} = \frac{10^{-3}T^{1.75}\left(\frac{1}{M_A} + \frac{1}{M_B}\right)^{\frac{1}{2}}}{p[(\Sigma_A v_i)^{1/3} + (\Sigma_B v_i)^{1/3}]^2} \tag{25}$$

where M_A and M_B are the molecular weights of the carrier gas and a chromatographically separated substance, respectively, T is the temperature (°K) p is the pressure (bar) and v_i are empirically determined values for various atoms and groups making up the carrier gas and the separated chemical. Giddings and co-workers obtained excellent agreement (usually to within $\pm 5\%$) between D_g values calculated this way and experimentally reported values[18].

As will be shown by an actual example, we found that this method gives reasonable results when applied to column chromatography. Although the values of D_g decrease as one proceeds to increasingly higher members of a homologous series, the fact that the D_g values are multiplied by $(1 + k)^2$ means that σ_d^2 (in sec^2) actually increases as one proceeds to later eluting peaks. Despite differences in individual D_g values, a linear regression plot of calculated σ_d^2 *versus* $(1 + k)^2$ is generally obtained with a high correlation coefficient.

7 2. *Inter-phase broadening*, σ_{ip}^2

The general approach taken is to subtract σ_d^2 from the measured variance of each peak and to plot the difference, σ_{net}^2, against one of the expressions for stochastic peak variance derived earlier. This should give a straight line with a slope related to a plate number and an intercept indicative of extra-column peak variance.

However, one question is whether $rk(1 + k)$ in the discrete flow model or $r(1 + k)^2$ in the continuous flow model is correct. For many plots, both give straight lines by linear regression. This is because the ratio of$(1 + k)^2/k(1 + k)$ does not change greatly with k at higher k values (say 2.5 15) and also that linear regression is insensitive to points of lower k that might not lie on a straight-line plot.

However, C values calculated from the Golay equation where several of the k values are quite low shows that slopes of individual points (y/x for each point) are nearly constant for plots of σ^2 *versus* $k(1 + k)$ but they vary considerably for the same k values when σ^2 is plotted *versus* $(1 + k)^2$ This supports the contention that the discrete flow is correct for column chromatography and that it is correct to plot σ^2 *versus* $k(1 + k)$ for inter-phase broadening.

Using eqn 7 and making use of the conversion factor t_0/r (sec/TS) it can be shown that

$$\sigma_{ip}^2 = \frac{(t_0)^2\, k(1 + k)}{r} = \frac{L^2\, k(1 + k)}{u^2 r} \tag{26}$$

where L is the column length (cm), u is the linear flow-rate (cm/sec) and r is the number of theoretical segments in the column at the flow-rate employed. However, we know that the plate number, r, is proportional to the reciprocal of linear flow-rate.

Introducing a plate number, r', which is the number of theoretical segments in a column at u = 1cm/sec

$$r = \frac{r'}{u}$$

$$\sigma_s^2 = \frac{L^2 k(1 + k)}{u r'} \tag{27}$$

This equation for peak variance due to stochastic broadening was tested on data published by Kaiser and Rieder[19] for the separation of alkanes and aliphatic esters by capillary column GC at six different flow-rates. First, the peak variance resulting from axial diffusion was subtracted from the measured peak variance as outlined above. Then the remaining variance (σ_{net}^2) was plotted against $k(1 + k)$ and the plate number r' calculated from the slope of the straight-line plot obtained. A sample calculation is shown in Table 2. The results are summarized in Table 3, which shows a constant value for r' within a reasonable experimental error The plate number, r, at any flow-rate can be calculated simply by dividing r' by u.

The plate number, r', seems to be a convenient way of evaluating and comparing column performances It is computed easily from measured peak variances using linear regression and is independent of peak capacity factor. It is also independent of axial diffusion effects. This is good because diffusion really has nothing to do with the separating ability of a column other than contributing to the broadening of peaks. In some respects r' is similar to the C term in the Van Deemter and Golay equations. However, C is different for each peak whereas r' is not. It would appear that r' can be more easily and accurately measured from ordinary chromatographic data than can C.

At a fixed flow-rate it is convenient to obtain a plate number that includes

TABLE 2

SAMPLE DATA FROM KAISER AND REDIER[19] FOR t_0 = 146 sec SHOWING AXIAL DIFFUSION CONTRIBUTION TO PEAK VARIANCE

σ^2 (diffusion) was calculated from eqn. 24 using D_g values calculated from eqn 25 at an estimated pressure of 1 2 bar The values of the gaseous diffusion coefficient (D_g) used ranged from 0 0795 for C_9 to 0 0653 for C_{13}

*Compound**	*k*	σ^2 *(measured)* *(sec²)*	σ^2 *(diffusion)* *(sec²)*	σ^2 *(net)* *(sec²)*
C_9	1 26	1 52	0 63	0 88
E_9	1 45	1 96	0 74	1 22
C_{10}	2.38	4 15	1 33	2 82
E_{10}	2 75	4 88	1 64	3.24
C_{11}	4 51	10 41	3 38	7 03
E_{11}	5 23	13 34	4 32	9 02
C_{12}	8 55	35 34	9 65	25 69
E_{12}	9 94	43 88	12 66	31 22
C_{13}	16.2	112 7	30 0	82 7

* C = *n*-alkane and carbon number; E = alkane ester and carbon number

TABLE 3

PLATE NUMBER (r) CALCULATED FROM DATA OF KAISER AND RIEDER[19] AFTER SUBTRACTING DIFFUSION PEAK VARIANCE

t_0 (sec)	u (cm/sec)	r 10^5 (TS)
276	7 25	9 19
154 4	12 95	10 40
146 0	13 70	9 86
106 8	18 73	10 71
88 2	22 68	9 53
87 4	22 88	9 82
	Average:	9.92 (s = 0.56)

broadening resulting from axial diffusion as well as inter-phase peak broadening. This can be done simply by measuring the variance of each of the chromatographic peaks and plotting measured σ^2 against peak variance, $k(1 + k)$. The apparent plate number, r, is obtained from the slope of the linear plot. The intercept is indicative of the extra-column peak broadening plus the diffusion broadening of a non-sorbed or a very slightly sorbed peak.

This simple plotting method works because a plot of σ_d^2 *versus* $(1 + k)^2$ is linear and a plot of σ_d^2 *versus* $k(1 + k)$ is almost linear. The flow-rates that are normally used in chromatography sufficiently fast that diffusion contribution to peak width usually is not very substantial. Thus, any slight non-linearity of the σ_d^2 part of the plot is insignificant.

The equation relating measured peak variance to r and k is derived by converting the peak variance, $rk(1 + k)$, from TS units to sec^2 by dividing $\frac{r^2}{(t_0)^2}$ (t_0 is the hold-up time):

$$\sigma_{ip}^2 = \frac{rk(1 + k)}{r^2/(t_0)^2} = \frac{(t_0)^2}{r}[k(1 + k)] \tag{28}$$

where σ_{ip}^2 and t_0 are now in minutes or seconds Substitution into eqn. 22 gives

$$\sigma^2 = \frac{(t_0)^2}{r} \cdot [k(1 + k)] + \sigma_{ec}^2 \tag{29}$$

where σ, t_0 and σ_{ec} are in minutes or seconds This equation predicts that a plot of σ^2 *versus* $k(1 + k)$ for the various peaks in a chromatogram will give a straight line with slope $(t_0)^2/r$ and intercept $= \sigma_{ec}^2$

The validity of eqn. 29 was tested on a number of actual chromatograms Usually the chart speed of the recorder was 4–10 cm/min to facilitate measurement of the peak widths with a ruler and magnifying lens. The peak variance was obtained from the peak width at half of its height, $w_{1/2}$ (where $\sigma = 2.355\ w_{1/2}$) in order to minimize the effect of any tailing in the lower part of the peak.

Data for five chromatographic separations are summarized in Table 4. Chro-

TABLE 4

CALCULATION OF r FROM CHROMATOGRAPHIC DATA

Chromatogram	*Peak*	k	$k(1+k)$	σ^2	*Results*
(A) *n*-Alkanes	C_7	0.14	0 16	0 00032 (min^2)	Correlation coefficient = 0 9999
by capillary	C_8	0.8	1 44	0 00090	σ_2^2 = 0 00001
GC[13]	C_9	1 4	3.36	0 00187	Slope = 0 00518
t_0 = 9 72 min	C_{10}	2 3	7 59	0.00380	r = 182,500
Oven at 130 C	C_{11}	3 9	19 1	0 0096	
	C_{12}	6 5	48 8	0 0249	
	C_{13}	10 8	127 4	0 0661	
(B) *n*-Alkanes	C_8	0.97	1 91	4 62 10^{-5} (min^2)	Correlation coefficient = 0 9999
by capillary	C_9	2 31	7 65	14 1 10^{-5}	σ_2^2 = 2 27 10^{-5}
GC at two	C_{10}	5 42	34 8	62 8 10^{-5}	Slope = 1 676 10^{-5}
different	C_{11}	12 33	164 4	$277 \cdot 10^{-5}$	r = 85,900
temperatures*	C_8	0 60	0 96	4 06 10^{-5}	
First set,	C_9	1 44	3 51	7 21 10^{-5}	
60°C,	C_{10}	3 26	13 9	26 0 10^{-5}	
second set	C_{11}	7 19	58 9	$101 \cdot 10^{-5}$	
70°C					
t_0 = 1 20 min					
(C) Carboxylic	Acetic	1 8	5.04	0.0451 (mm^2)	Correlation coefficient = 0.9998
acids by	Propionic	3 4	15 0	0.0649	σ_2^2 = 0 0348
packed-column	Isobutyric	5 0	30 0	0.0909	Slope = 0 00196
GC**.	*n*-Butyric	6 55	49.4	0 1303	r = 2040
t_0 = 2 0 mm	Isovaleric	9 7	104	0.2426	
	n-Valeric	13 3	190	0.4057	
(D) Alkali	Li^+	4 35	23 26	51 8 (sec^2)	Correlation coefficient = 0.9988
metal ions	Na^+	6 10	43 2	83 0	σ_2^2 = 19 0
by ion	NH_4^+	8 47	80.1	148 0	Slope = 1 514
exchange***	K^+	11 45	142.6	229 8	r = 1190
50-μl sample	Rb^+	13 0	182.0	295 8	
t_0 = 42 5 sec					
(E) Alkali	Li^+	4.90	28.88	54 6 (sec^2)	Correlation coefficient = 0.9979
metals by	Na^+	6 54	49.30	62 4	σ_2^2 = 16.3
ion exchange	NH_4^+	9 44	98.47	117 2	Slope = 1 067
20-μl sample	K^+	12 51	169.0	200 0	r = 1430
t_0 = 39 sec	Rb^+	14 43	222.7	253 6	
(F) Pesticides	Sevin	0 955	1 87	0 000357 (min^2)	Correlation coefficient = 0.9996
by liquid	Prolate	1 16	2 50	0 000391	σ_2^2 = 2 11 10^{-4}
chromatog-	Ruelene	1 72	4 67	0 000786	Slope = 0 7445 10^{-4}
raphy t_0 =	Coral	1.94	5 72	0 000612	r = 2660
0 445 min§	Dursban	3.45	15 35	0 001356	
	trans-Permathrin	5 81	39 55	0 003236	
	cis-Permathrin	6 80	53 01	0 004100	

* J Kaczvinsky, unpublished results (1981)

** Data from chromatogram in an advertising brochure (1981)

*** G Sevenich, unpublished results (1981)

§ L Rice, unpublished results (1981)

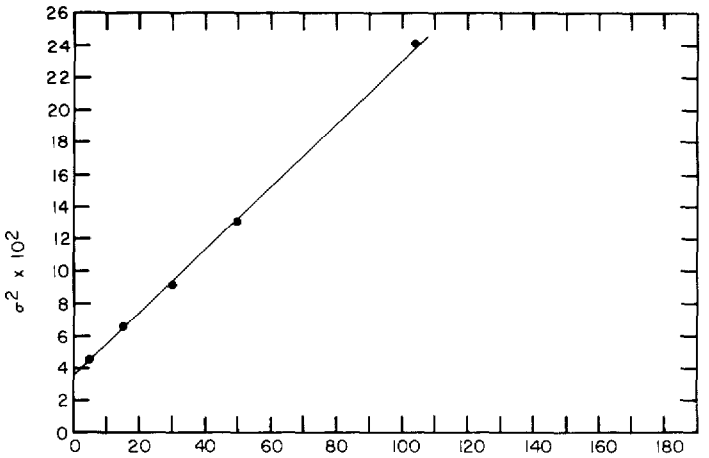

Fig 4. Linear regression plot for chromatogram C in Table 2

matogram A is unique among those tested in that the intercept, σ_{ec}^2, was essentially zero. Of course there is some error in measuring k and σ^2 for each peak, but the equipment and technique used were both known to be excellent.

The data for B in Table 4 are for two chromatograms run at different temperatures, yet all eight points lie on a straight line within reasonable experimental error. The intercept, σ_{ec}^2, is significant in this instance and constitutes nearly 50% of the measured variance of the first C_8 peak. Note that the early peaks are far more affected by σ_{ec}^2 than the latter ones if the actual peak widths (4 σ) are compared.

Chromatogram C was run on a packed GC column and shows a far greater σ_{ec}^2 relative to the measured peak variances than the chromatograms or capillary columns. Fig. 4 confirms that the experimental points actually do fall on a straight line when plotted according to eqn. 29 It is interesting to calculate the traditional plate number, N, before and after subtraction of σ_{ec}^2 from σ^2 for each peak. Before any correction is applied, N increases from peaks 1 to 6: N = 695, 1190, 1580, 1750, 1890, 2020 After subtracting σ_{ec}^2 from the variance of each peak, N decreases from peaks 1 to 6· N = 3050, 2570, 2390, 2200, 2200

Chromatograms D and E are for an ion-exchange separation of alkali metal ions. Again, the data support the validity of eqn. 28 even though some of the peaks were tailed, and accurate measurement of peak width was difficult. The intercept, σ_{ec}^2, is smaller and the value of r is larger when the sample volume is reduced from 50 to 20 μl.

Chromatogram F in Table 4 was obtained with a commercial liquid chromatograph with a 10 cm × 4.6 mm I D. column. The large value for r confirmed the excellent separating ability of the column, but the additional broadening represented by σ_{ec}^2 was large. Much of this can be attributed to the use of a small "guard" column filled with a coarser reversed-phase absorbent than that used in the chromatographic column Subsequent replacement of the guard column with a pre-column of slightly smaller diameter packed with 10-μm absorbent reduced σ_{ec}^2 by almost 50%.

While there is no direct proof that the intercept, σ_{ec}^2, of our linear regression analysis gives an accurate measure of the sum of in-column and extra-column broad-

ening that applied equally to all peaks, the underlying principle does appear sound and the results obtained from actual data are reasonable The idea of linear regression analysis of chromatographic data originated with Kaiser and co-workers[19–21], who plotted $w_{1/2}$ against k. They obtained the "real" number of theoretical plates from the slope and an indication of the extra-column peak broadening from the intercept We think that the concept pioneered by Kaiser and co-workers is a valuable one but that their method of plotting the data is not completely correct. According to Guiochon[22], a plot of $w_{1/2}$ *versus* k is not linear at all values of k and the intercept of such a plot underestimates the "extra-column" peak broadening

Smuts *et al.*[23] proposed a plot of peak variance against $(1 + k)^2$, although the purpose of this work was different from ours. This type of plot would follow if the continuous flow model [where the peak variance is $(1 + k)^2$] is correct for column chromatography. However, Smuts *et al.*[24] stated that such a plot is not completely linear Plots of σ^2 *versus* $(1 + k)^2$ for the data in Table 2 also did not give a completely straight line, although the intercept was often not greatly different from that obtained when using the discrete flow model. All of our results suggest that the discrete flow model is better for column chromatography.

8 SUMMARY

The classical plate theory, and original extensions of it, are derived by using simple concepts from the theory of probability and statistics. Each molecule of a sample chemical substance is examined separately and its motion through the column is described as a stochastic process Equations for calculating the mean and variance of chromatographic peaks as a function of capacity factor, k, are given for a discrete flow model and a continuous flow model. The variance of position and exit peaks as a function of capacity factor is compared and a simple relationship between the two is derived. The expressions for the mean and variance of chromatographic peaks are used to define plate numbers that describe the separating ability of a chromatographic column under fixed conditions and which, unlike the classical plate numbers N and N_{eff}, are independent of capacity factor, k In capillary column GC a method is given for determining diffusion coefficients in the gaseous phase that makes it possible to subtract the contribution to peak variance resulting from axial diffusion. When the remaining variance is plotted against $k(1 + k)$, linear regression indicates a straight line with an excellent correlation coefficient and an intercept that is indicative of extra-column peak broadening. A plate number, r', is computed from the slope that is independent of k and can be used to calculate a plate number, r, for any given linear flow-rate Another simple plotting method gives a plate number that includes the multi-path contribution to peak broadening for packed columns. Numerous examples are given to demonstrate the applicability of these simple concepts to actual chromatographic data.

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