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MASS LOADABILITY OF CHROMATOGRAPHIC COLUMNS

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SUMMARY

Recent developments in liquid chromatography, such as the introduction of small-bore columns, attempts to operate efficient open-tubular columns, the introduction of low-capacity selective ion exchangers and attempts to achieve very high plate numbers with techniques such as column coupling, recycling and boxcar chromatography, bring the loadability problem into focus. In many of the papers devoted to the above topics some remarks about the sample capacity are made.

Unfortunately, no theoretical framework seems to be in use for the description of peak distortion due to mass overload. As a result, experimental data apply only to the particular column used and cannot be transferred to other experimental conditions involving the same phase system and solute.

It is shown that the approximate treatments by Haarhof and Van der Linden and by Houghton can be successfully used. The results of these treatments are compared with the results obtained by computer simulations of the chromatographic transport and the very high accuracy of the approximate theories is demonstrated in this way. By suitable reorganization and simplification of the equations, a relatively simple picture of mass overload is derived. This predicts that the extra peak broadening is dependent only on the total mass of solute per gram of stationary phase contained in one plate. An important conclusion is that at very high efficiencies the maximum eluting concentration obtainable with (nearly) undistorted peaks is lower than with normal efficiencies. Another conclusion is that microcolumns are often a bad choice for trace analysis.

INTRODUCTION

The problem of the effect of high loads on the efficiency of chromatographic separations emerges as a dominating aspect in the discussion of many chromatographic techniques, such as the preparative operation of chromatographic columns[1–5], the use of microbore columns in high-performance liquid chromatography (HPLC)[6,7], open-tubular HPLC[8] and trace enrichment with columns[9,10]. Numerous studies[11–16] on the effect of hig loads on efficiency with standard columns have been carried out in liquid chromatography. Most of these were carried out with a single solute, or with mixtures of composition such that separation takes place in the very

first part of the column. Under these conditions, mutual interaction of solutes can be neglected. It must be admitted that from the point of view of separation such experiments and discussions connected with them are somewhat artificial, because in practical applications of chromatography it is the separation of adjacent peaks which limits the load that can be applied.

Especially in adsorption chromatography the displacement effect is often observed. A solute band is accelerated and sometimes contracted to a measurable extent in the presence of a large amount of another solute which moves slightly more slowly. The cause of such effects is clear; solutes compete for the adsorption sites and the molecules of the large band displace those of the first band from the adsorbent. Taken to the limits, this leads to displacement chromatography, but also under conditions of slight overload such effects may be at least as important as the distortion that would occur with the pure substances when chromatographed.

In spite of this state of affairs, the present discussion is again limited to the case of one solute, for the following reasons: it would be useful to have a theoretical framework available with which the many reported single-solute experiments can be compared; it is doubtful whether a discussion that takes the mutual interaction of solutes into account could lead to general conclusions such as those arrived at in this paper, because first very little knowledge is available about simultaneous sorption isotherms, and second many more constants are needed to describe simultaneous sorption in a realistic model; and even if the mathematical complexity involved could be overcome, the translation of the results into rules and relationships applicable to practical situations seems impossible because of the wide variety of simultaneous isotherm shapes. For the one-solute case it is possible to model the sorption isotherm in a single two-constant relationship, realistic for slight overload conditions.

We therefore decided to deal with the one-solute case exclusively. The purpose of this work was to find relationships describing loadability as a function of amount of packing, phase ratio, plate number, etc., and the distribution isotherm. Although in an absolute sense such relationships are not applicable to mixtures, in a relative way they are because a column with a high loadability for a single substance can also handle large amounts of mixtures.

To us it is surprising that the loadability, which in many instances is as important to the analytical utility of columns as is the description of the infinite dilution behaviour by plate-height theory, has been described so little in quantitative terms. Instead, one often finds intuitive and qualitative reasonings about what approximate size of the mass load would cause "unacceptable" peak deformation.

One such reasoning[2,17,18] is approximately as follows. The peak shapes are generally observed to be of the smallest width and of gaussian shape when the injection volume is small and the concentrations remain in the linear part of the isotherm. Starting with a consideration of the latter condition, a maximum value, c_m^{max}, of the mobile phase concentration, c_m, may define that linear range and the prevailing concentration at the start of the transport through the column should be lower that c_m^{max}. On the other hand, we have to consider the maximum acceptable band width at the start, because obviously an infinitely narrow band cannot contain any mass if the concentration is limited. Practice indicates and theory predicts that an initial distribution over less than $\sqrt{N}$ plates hardly affects the peak shape and width (N is the theoretical plate number of the column). Therefore, if the load, M, is applied in such a

way that at the start in the first $\sqrt{N}$ plates $c_m = c_m^{max}$, the peak will still be nearly undistorted. Taking also the distribution between the phases into account, this leads to the following condition for linearity:

$$\frac{M}{\sqrt{N}\, V_{mp}\, (1 + \kappa^{\infty})} < c_m^{max} \tag{1}$$

where V_{mp} is the volume of the mobile phase in a column length of one plate height H, equal to HA_m where A_m is the area of the cross-section of the mobile phase, and κ^{∞} is the capacity factor at infinite dilution.

If the load according to eqn. 1 is applied in a very small volume, the initial concentrations are of course larger than c_m^{max}. However, in the very first stages of the transport process the peak will broaden quickly (because of the extreme non-linearity) and it will soon occupy a number of plates of the order of $\sqrt{N}$ (which is not harmful to the peak width) and after that the transport process is linear again. Thus eqn. 1 would set the condition for (virtual) linear elution for all cases.

The difficulty in this reasoning and in eqn. 1, of course, lies, in the arbitrary choice of the criterion for linearity with which c_m^{max} is defined. It is not clear whether one should accept, *e.g.*, a 0.1 %, 1 % or 10 % deviation from linearity. Also, we note in passing that eqn. 1 suggests that the acceptable load increases with increasing column length and plate number (keeping the plate height, diameter, etc., constant), which will be shown below not to be true.

Not only the peak shapes and widths, but also the positions of peaks are influenced by the load. In a paper that is fundamental to many kinds of instrumental chromatography practised nowadays, Snyder[19] tried to correlate equilibrium data for non-linear isotherm cases obtained in batch experiments with retention data obtained with columns. He used an adaptive parameter, α. In fact, α expresses the following: if we put M mol on the column, the migration rate, at any moment and any position in the column, will be determined by the prevailing concentration, because it is this concentration which determines which fraction of the molecules (there and then) is in the mobile phase. Clearly, the "effective" concentration, corresponding to the migration rate observed macroscopically via the t_R or R_F value, is larger than

$$c_m^{eff} > \frac{M}{V_{mp}(1 + \kappa^{\infty})N} = \frac{M}{V_m(1 + \kappa^{\infty})} \tag{2}$$

because the right-hand side corresponds physically to a uniform distribution of the solute over the full length of the column, which we know from basic experiments not to be realized. In eqn. 2 c_m^{eff} is the effective concentration mentioned, and V_m is the volume of the mobile phase in the column.

It was then argued that the inequality in eqn. 2 is to be substituted by the equation

$$c_m^{eff} = \frac{1}{\alpha} \cdot \frac{M}{V_m(1 + \kappa^{\infty})}$$

and the proportionality constant α was adapted in such a way as to obtain reasonable

agreement between static data and retention parameters.

Now, many years later, when carrying out experiments where static and retention data are measured on the same phase system[20–22], there seems to be no better formalism for non-linear cases available for qualitatively correlating these data without the use of "fudge" factors.

In view of this very unsatisfactory situation, we decided to review and reorganize existing knowledge about non-linear, non-ideal chromatography, and complement this with some new numerical experiments.

LIMITS TO THE PRESENT DISCUSSION AND ADAPTED MODEL

Dispersion effects

In linear chromatography numerous mathematical models representing the physical reality of the chromatographic transport process exist. The plate or tanks in series model and the dispersion or Kubin and Kucera model[23,24] constitute the two main families. Specific models differ *inter alia* in the ways the finite rate of mass transfer is handled and in the boundary conditions at the beginning and the end ("open" *versus* "closed" ends[25]).

In contemporary chromatographic literature the rate model is mostly used. All linear effects within the column contributing to peak broadening, including a finite rate of mass transfer, are grouped together in the plate height, H[26,27], equivalent to a dispersion coefficient $D = Hv/2$, where v is the mobile phase velocity. We shall adapt this model as we did in another discussion of chromatographic transport[28]. An implicit approximation, made while doing so, should be mentioned: the dependence of H on the capacity factor, κ, is neglected.

Further, for reasons of mathematical convenience, we treat the "double open" column, *i.e.*, the column is considered as infinitely long on both sides. Injection is accomplished in the model by assuming a concentration $c(z,0) = I(z)$ profile at the time $t = 0$ around the length coordinate value $z = 0$. Detection is considered as monitoring the time course of the mobile phase concentration at a point $z = L$. As was implied already, the mobile and stationary phase concentrations, c_m and c_s, respectively, are considered to be in instantaneous equilibrium; any delay in this equilibrium is represented by a corresponding contribution to H or D and the dependence of this effect on κ is neglected.

This model leads to the following transport equation for the linear case:

$$\frac{dc_t}{dt} = D \cdot \frac{d^2 c_m}{dz^2} - v \cdot \frac{dc_m}{dz} \tag{3}$$

where

c_t = "total concentration", defined as $c_t = c_m + q\, c_s$;
c_m = concentration in the mobile phase;
q = volume phase ratio, V_s/V_m;
t = time;
z = length coordinate;
v = migration velocity of the mobile phase;
D = dispersion coefficient, equal to $Hv/2$;

and the initial condition

$$c_t(z,0) = I(z)$$

The one-dimensional model implies that the treatment applies to those cases only where radial inhomogeneities can be neglected. This is of special importance for the injection process. For instance, with on-column syringe injection only part of the sorbent bed is effective in the first part of the column. Therefore, comparison of our results with, e.g., those obtained by Done[29] is problematic; it is not surprising that loadabilities as found by him are much larger than the values we arrive at. In view of the present widespread use of injection valves in liquid chromatography, which does not lead to radial inhomogeneities, we consider this limitation of our treatment as unimportant.

Non-linearity effects

As soon as the assumption of linear distribution is abandoned, the number of possible models increases considerably. Again, we shall limit the discussion to one case, which we believe is the most relevant for practical applications of chromatography. Fig. 1 shows some possible shapes of isotherms. Three of these (a, b and c) approach linearity when c_m (and c_s) approach zero. This is indeed what is assumed in elementary theory, and is found experimentally in most instances. Such isotherms can be effectively represented by a power series:

$$q\, c_s = a_1\, c_m + a_2\, c_m^2 + a_3\, c_m^3 + \ldots \tag{4}$$

In this expression the left-hand side is taken as $q\, c_s$ rather than as c_s purely for reasons of mathematical convenience; note that $a_1 = \kappa^\infty$. Curves d and e, on the other hand, do not approach linearity for $c_m \to 0$. An important case is, e.g., the Freundlich isotherm, $c_s = (c_m)^n$ with $n < 0$. Such dependences cannot be properly described by a Taylor series such as eqn. 4, because derivatives at $c_m = 0$ are infinite.

We do not deny the practical relevance of curves c and d. Freundlich isotherms
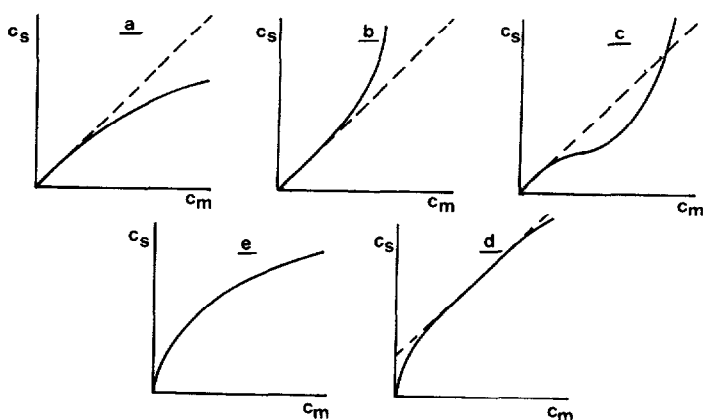

Fig. 1. Different isotherm shapes.

are frequently observed in practice. Also, curves such as that in Fig. 1d might play a role in trace analysis. Such isotherms predict virtually normal chromatographic response for high concentrations, but strongly non-linear behaviour for small concentrations, which occur when small amounts are injected. Indeed it is sometimes observed that the analytical response disappears more or less abruptly when the injected amount drops below a certain minimum[30].

Despite their importance, such cases will not be treated here, the main reason being that we are primarily interested in developing an understanding of the phenomena that we may expect when overloading chromatographic systems which give symmetrical peaks for low mass loads.

One further simplification is obtained when we take only the first two terms in the expansion 4 into account. Substitution of this into the transport eqn. 3 yields a differential equation which was dealt with by Haarhof and Van der Linden[31] and Houghton[32]. They showed that an approximation can be solved analytically, and the approximation has a high accuracy that increases with increasing plate number. In order to discuss their results, it is useful to first discuss the load in amount in relation to expansion 4. If we plot qc_s as a function of c_m, as in Fig. 2a, the slope at $c_m = 0$ represents the capacity factor at infinite dilution, κ^∞.

If eqn. 4, with two terms, describes the isotherm over a certain range, the fractional departure in the ratio qc_s/c_m from κ^∞ is proportional to c_m in that range. This is illustrated in Fig. 2b, where the value of qc_s/c_m is plotted against c_m. Such a linear dependence of the capacity factor on the concentration is similar to that used successfully by Snyder[19], although in the latter instance the stationary phase concentration (θ) rather than c_m was used.

Extrapolating the linear plot in Fig. 2b to larger values of c_m, we can define a concentration, $\bar{c}_m$, equal to $(1 + \kappa^\infty)/a_2$, where the value of qc_s/c_m has been changed by $1 + \kappa^\infty$. This value $\bar{c}_m$ should be given a formal significance mainly as in many instances a two-term truncation of eqn. 4 is not accurate at this c_m value. However, the significance of $\bar{c}_m$ lies in the fact that for not too high c_m values the relative deviation in the retention time [ca. $1 + q(dc_s/dc_m)$] is equal to $2c_m/\bar{c}_m$*. As we are interested in slight deviations in κ (small overload), this is all we need. $\bar{c}_m$ measures the linearity of the phase system; the higher $\bar{c}_m$, the better the linearity.

The load in amount (moles or grams), M, applied on the column should of course be compared in some way with the thermodynamic parameter $\bar{c}_m$. A first intuitive attempt is to distribute the load M hypothetically in the equivalent of one
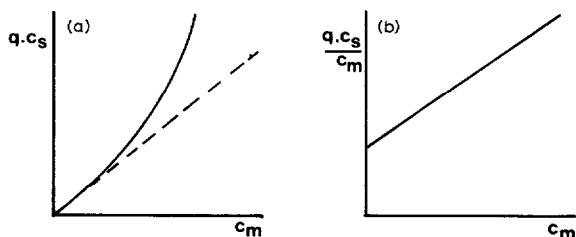

Fig. 2. Isotherms that can be described by a quadratic expression in c_m.

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* $\bar{c}_m$ is equal to $2/\lambda_i$ with λ_i as defined in an earlier paper[4].

plate, *i.e.*, the mobile phase volume $V_{mp} = HA_m$ and the associated stationary phase. The resulting concentration in the mobile phase can be calculated, assuming a linear distribution* as

$$c_m = \frac{M}{V_{mp}(1 + \kappa^\infty)}$$

and the relative change in retention would be

$$\frac{\Delta\kappa}{\kappa + 1} = \frac{2c_m}{\bar{c}_m} = \frac{M}{\frac{1}{2} V_{mp}(1 + \kappa^\infty)\bar{c}_m} \tag{5}$$

The factor 2 is related to the differentiation involved in the migration equation[33,34]. The denominator of eqn. 5 is the amount of material contained in one plate when the factor $1 + \kappa^\infty$ is changed by 100% as a result of overload. As an example we treat the Langmuir isotherm:

$$qc_s = \frac{SQc_m}{1 + Qc_m} \tag{6}$$

in which S is the saturation uptake per volume unit mobile phase with Q being an additional parameter describing the capacity factor at infinite dilution. For low c_m values eqn. 6 can be approximated by

$$qc_s = SQc_m - SQ^2c_m^2$$

or

$$qc_s/c_m = SQ - SQ^2c_m$$

Hence

$$\bar{c}_m = (\kappa^\infty + 1)S^{-1}Q^{-2}$$

Substitution in eqn. 5 leads to

$$\frac{\Delta\kappa}{\kappa^\infty + 1} = \frac{2c_m}{\bar{c}_m} = \frac{2M}{V_{mp}S^{-1}Q^{-2}(1 + \kappa^\infty)^2} = \frac{2M}{S\,V_{mp}} \cdot \frac{(\kappa^\infty)^2}{(1 + \kappa^\infty)^2} \tag{7}$$

This relates the relative deviation in retention to the ratio of the load M and the capacity of the fully saturated stationary phase in one plate, $S\,V_{mp}$. The factor $(\kappa^\infty/1 + \kappa^\infty)^2$ accounts for the fact that non-linearity is unimportant when there is no retention.

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* This may appear odd and inconsistent with the assumed isotherm. However, this is simply a case where a higher order approximation is calculated with lower order estimates.

THE HAARHOF–VAN DER LINDEN AND DUNCKHORST AND HOUGHTON TREATMENT

Returning to the more general eqn. 4, it can be stated that the right-hand side measures the load on a "natural" scale, determined by the linearity range of the isotherm, as indicated by $\bar{c}_{im}$, and the volume of one plate, V_{mp}, corrected by $1 + \kappa^{\infty}$ for the amount which can be accommodated by the stationary phase.

A similar expression emerges in the work by Haarhof and Van der Linden[31] and Houghton[32]. They introduced a parameter of dimension unity, m, reading in our terms

$$| m | = \left| \frac{2M}{V_{mp}(1 + \kappa^{\infty})\bar{c}_m} \right| \tag{8}$$

which is in accordance with eqn. 5. The sign of m was chosen to be positive for convex and negative for concave isotherms.

The elution curve found in refs. 31 and 32 was

$$c(L,t) = \frac{1}{2\sqrt{N}} \cdot \frac{Z(\tau)}{(e^m - 1)^{-1} + Z^*(\tau)} \cdot \frac{\bar{c}_m}{1 + \kappa^{\infty}} \tag{9}$$

where

$$
\begin{aligned}
L &= \text{column length;} \\
N &= \text{plate number, } L/H; \\
\tau &= t - t_R^{\infty}/\sigma^{\infty} \\
  &= \left[t - \frac{L}{v}(1 + \kappa^{\infty}) \right] / \sigma^{\infty}; \\
Z(\tau) &= e^{-\tau^2/2}; \\
Z^*(\tau) &= \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\tau} e^{-v^2/2}\, dv; \\
\sigma^{\infty} &= \text{standard deviation of the peak at infinite dilution} = t_R^{\infty}/\sqrt{N}.
\end{aligned}
$$

Eqn. 9 allows the calculation of elution functions for isotherms that are of quadratic shape in the concentration region of interest (well below $\bar{c}_m$). However, the derivation of eqn. 9 contains some assumptions and approximations that are difficult to verify, some of which might be especially inaccurate at low plate numbers. The question therefore arises of how many plates are to be passed by the solute before the accuracy is sufficient. Also, the derivation of eqn. 9 and its translation into practical parameters involves complex mathematics, where errors could easily occur. It was therefore considered useful to check these results in an independent way by means of a numerical approach.

NUMERICAL EXPERIMENTS

The numerical simulation of the chromatographic transport process is by no means easy. The main difficulty encountered is the occurrence of the so-called numer-

ical dispersion. It is usual to sample the concentration $[c(n)]$ in a number of equidistant points on the length axis. The changes with time in the $c(n)$ values are calculated in successive passes by means of estimates of the differential quotients in eqn. 3 derived from neighbouring $c(n)$ values.

In such a procedure it is difficult to simulate pure migration; for stable algorithms the migration is mostly accompanied by artificial dispersion, known as "numerical dispersion". There are mainly two methods to deal with this. The first is the use of a moving frame, well known in analytical[26,35] approaches to dispersion, and also applied in numerical work[36,37]. However, for the non-linear case it is impossible to give the frame of reference the right velocity, as the migration velocity is not constant. The second is the use of closely spaced samples. This requires a large memory and computation time. Both limitations are the most pronounced if the plate numbers are large. As one of the objects of this work was to study the relationship between plate number and admissible load, the limitations can be considered serious.

An excellent implementation of this approach was elaborated by Smit *et al.*[37,38]. In the Results section of this paper some data obtained by them will be used as well.

We devised an alternative approach to the simulation. It was considered that instead of calculating the changes in c at particular positions (moving or not) as a function of time, $c(z,t)$, it could be more effective to calculate the positions where certain values of c occur, and observe the change of these positions z as a function of time, $z(c,t)$; in other words, we take c rather than z as the independent variable. According to this, eqn. 1 is rearranged to

$$\left(\frac{dz}{dt}\right)_{c_t} = v \cdot \frac{dc_m}{dc_t} + D \cdot \frac{dc_m}{dc_t} \cdot \frac{d^2 c_t/dz^2}{dc_t/dz} \tag{10}$$

For numerical experiments, eqn. 10 has the important advantage that the term in v, the convention term, does not lead to numerical dispersion. The change in z, Δz, during a time Δt is approximated by

$$(\Delta z)_{c_t} = \left[v + D \cdot \frac{d^2 z/dc_t^2}{(dz/dc_t)^2} \right] \frac{dc_m}{dc_t} \cdot \Delta t \tag{11}$$

z is sampled for equidistant values of c_t. The remarkable simple eqn. 11 can be readily applied in this form, with suitable approximation of the derivatives by difference quotients, to the breakthrough fronts in non-linear chromatography. However, for peaks two complications occur. First, $z(c_t)$ is double valued (see Fig. 3), and the
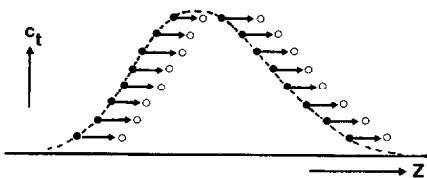

Fig. 3. Illustration of the simulation procedure used. Positions z for given values of c_t; ●, before the time step; ○, same after the time step.

cross-over from the front edge $z^+(c_t)$ to the rear edge $z^-(c_t)$ is troublesome. Second, the (equidistant) values for c_t have to be chosen along the c_t range at the start (injection), but after a certain development time most of these become useless because the peak maximum concentration is steadily decreasing. This causes a waste of memory and loss of fine structure. Moreover, the concentration decrease in this numerical representation is manifested by the "crossing over" of points in the peak top, *i.e.* front points z^+ become smaller than rear point z^-, and this causes problems in the algorithm.

We succeeded in developing a stable algorithm, which produces plausible results by reorganizing the grid after each pass in such a way that the c_t values are again evenly distributed over the concentration range of the peak at that moment, *i.e.*, the Δc_t value was decreased after each pass. Details of the program and full listing and corresponding derivations are available on request.

As computational facilities were restricted (HP 41C, Hewlett-Packard, Palo Alto, CA, U.S.A.), the isotherm was adapted to these experiments in order to simplify calculations and bring run times down to a practical level. In view of the occurrence of dc_m/dc_t in eqn. 11 and the choice of c_t as the variable, we adopted

$$R = \frac{dc_m}{dc_t} = R_0 + b\,c_t \tag{12}$$

($dc_m/dc_t = R_F$ for linear chromatography). This corresponds to

$$qc_s = \frac{R_0}{b}\left(-1 + \sqrt{1 + \frac{2c_m b}{R_0^2}}\right) - c_m$$

or

$$qc_s = \frac{1 - R_0}{R_0}\cdot c_m - \frac{b}{R_0^3}\cdot c_m^2 + 3\cdot\frac{b^2}{R_0^5}\cdot c_m^3 + \ldots \tag{13}$$

and leads to

$$\bar{c}_m = \frac{2R_0^2}{b}$$

and

$$m = Mb/RV_{mp} = Mb(1 + \kappa)/V_{mp} \tag{14}$$

Eqn. 13 corresponds to eqn. 2; however, the third and higher terms differ from zero. This was expected not to be significant, and in a few experiments where eqn. 4 or 6 was used, this was indeed corroborated.

NUMERICAL AND ANALYTICAL RESULTS

The capacity factor at infinite dilution was generally taken as 7/3, corresponding to $R_F = R_0 = 0.3$. Eqn. 12 was the isotherm with $b = 1$. This limited c_t to 0.7, because otherwise $R_F > 1$ would occur. Large amounts therefore had to be "injected"

by increasing the injection width. Injection was carried out by giving c_t a given value over a length w in the column. The velocity, v, and dispersion coefficient, D, were both unity.

Validity of the results

Mathematical proof of the validity cannot be given as yet. The procedure is a numerical *simulation*, in the sense that the numerical system behaves in limiting cases (ideal chromatography, linear chromatography) in virtually the same way as the system with differential equations. We take this as sufficient indication that the results are valid for non-ideal, non-linear cases.

For linear chromatography (results not shown), gaussian position curves are produced. The position of the peak is exactly equal to that predicted by the dispersion model. The peak second moment increase on migration is equal to that predicted, except for the fact that H values are 6 % low when twenty c_t levels are used and 11 % low with only ten c_t levels. In view of the trend in the deviations we assume that this simply reflects the rather crude sampling (20–40 points in a peak). Also, a similar sampling applied to a theoretically calculated gaussian curve gives about the same error.

The reproduction of the solution of the ideal, non-linear case is shown in Fig. 4. The complete absence of dispersion effects cannot be realized for reasons of numerical stability (as is the case in other simulation procedures). However, it can be seen in Fig. 4 that the results of this very time-consuming experiment approach well the analytical[33,34] solution.

Non-ideal, non-linear case

Figure 5 shows the results of calculations for an intermediate load ($m = 8.33$) according to the Haarhof and Van der Linden expression[31], the simulation and for comparison the results for a linear case with the same retention at infinite dilution. The close agreement between the analytical and simulation results is noteworthy.
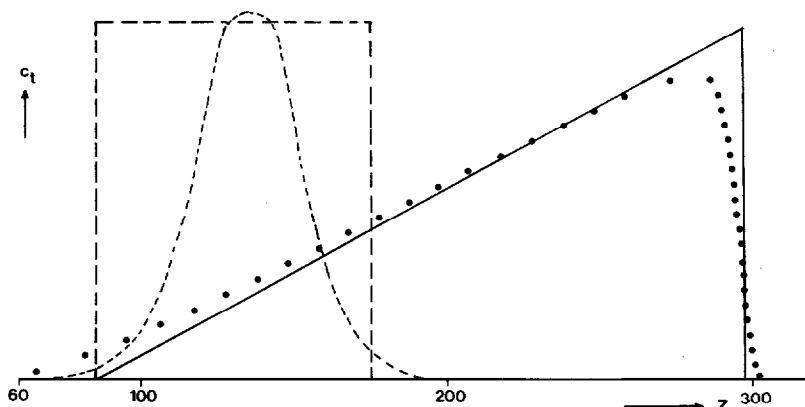

Fig. 4. Convergence of the simulation result to the solution for non-linear ideal chromatography. Peak place functions after a time lapse of 450. Isotherm shape $R = 0.3 + c_t$. Amount injected, 462 mass units in 50 plates ($m = 83.3$). -------, Gaussian, linear peak with capacity factor of 2.33333 and pulse injection; ——————, injection block displaced over 135 z units; ●, numerical results; ————, peak shape calculated for ideal chromatography[33,34].
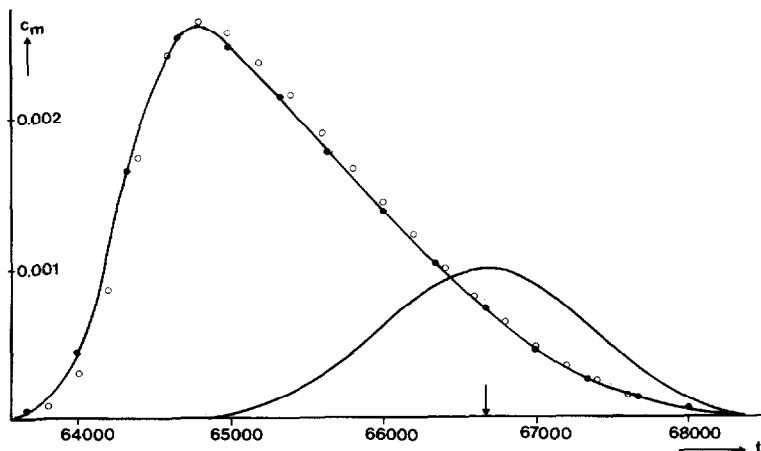
Fig. 5. Elution function as calculated by the numerical experiment (○) and the Haarhof and Van der Linde treatment (—●—●—). A linear elution curve with the same capacity factor at infinite dilution is shown for comparison (————). Amount injected, 46.2 mass units in a uniform distribution over five plates ($m = 8.333$). Column length, $2 \cdot 10^4$ (10^4 plates). Isotherm shape as in Fig. 4.

Note that both calculations are absolute, no scaling or shifting in the vertical or horizontal direction being applied.

Further proof of the agreement is shown in Fig. 6A and B. Capacity factors for the peak maximum are given. Only below $t = 5120$, corresponding to approximately 750 plates passed by the solute, do appreciable deviations occur. The open circles give the positions of the peak maximum as calculated from $\kappa = q(dc_s/dc_m)$ at the observed c_m value in the peak maximum. The good agreement of these values with the full simulation indicate that the assumption made by Huber and Gerritse[33] used in the determination of isotherms (peak maxima method) is jusitified, also when there is appreciable dispersion.

Fig. 7 gives the position peak shapes in the column at various times after injection. The first three or four plots still show the effect of the injection width. During this stage of development the plate height increase due to the injection width is still of importance, although of decreasing magnitude. Around $t = 640$, corresponding to an elution distance of about 100 plates, there is a minimum in the observed plate height, which is rather flat and as yet unexplained. However, the last four plots in Fig. 7 illustrate the main point of this work. The peaks are virtually of constant shape when they elute down the column, and the relative increase in peak width (expressed by H values given in Fig. 7; H should be 2 for a linear case) is also constant. The relative importance of thermodynamic and dispersion broadening at a fixed load does not depend on the length passed through. It is determined exclusively by the load in relation to the volume of one plate and thermodynamic intensive parameters.

Comparison with respect to peak moments are possible between the Haarhof and Van der Linden expression[31], the simulation results obtained by Smit et al.[37,38] and the simulation discussed above. Fig. 8 gives this comparison for first moments. It can be seen that the agreement is good for low overloads, e.g., $m < 5$, and less good
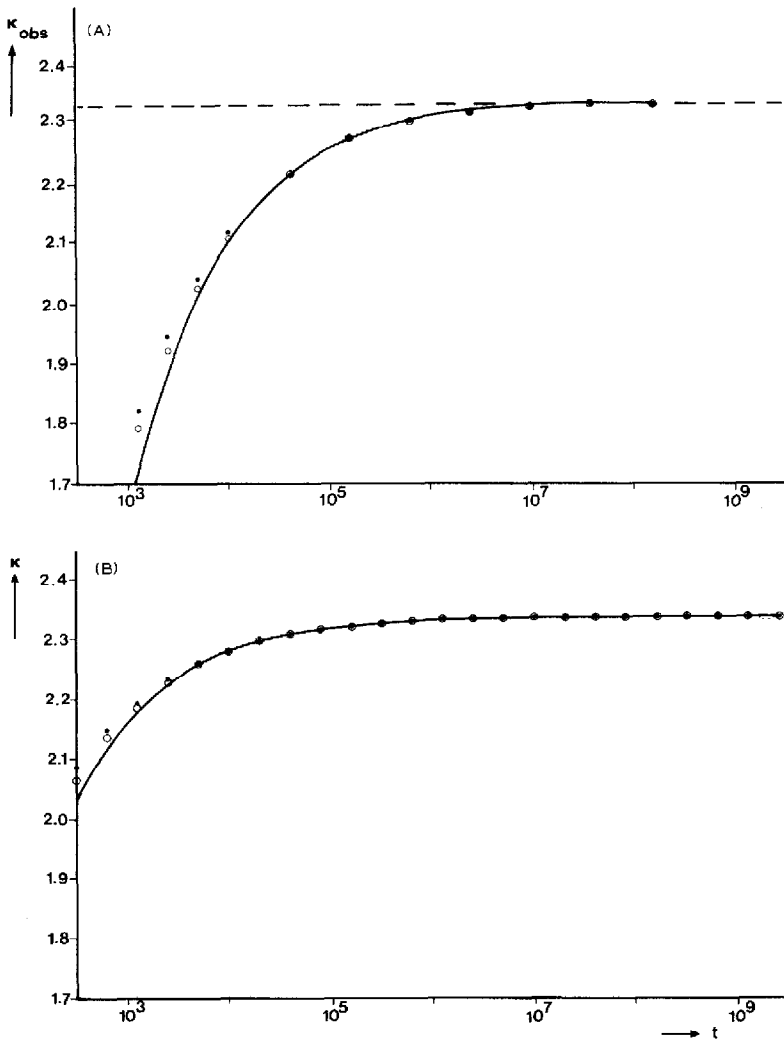
Fig. 6. Observed capacity factors for the peak maxima ($vt/z_{max} - 1$) as a function of the elapsed time t. ——, Calculated according to eqn. 9; ●, from numerical experiments; ○, calculated from $\kappa = dc_s/dc_m$; ------, capacity factor κ at infinite dilution. Loads: (A) $m = 8.333$; (B) $m = 1.667$. Isotherm shape as in Fig. 4.

for, e.g., $m = 20$. However, even there the accuracy is sufficient for estimating the order of magnitude of the effect.

Fig. 9 gives the same comparison for second moments, normalized on the infinite dilution variance, $(\sigma^\infty)^2$.

DISCUSSION

The approximate description of elution in non-linear, non-ideal chromatography by Haarhof and Van der Linden[31] is corroborated by two types of numerical

$t = 2.5$ $H = 8.5$	$t = 10$ $H = 3.85$	$t = 40$ $H = 2.79$	$t = 160$ $H = 2.64$

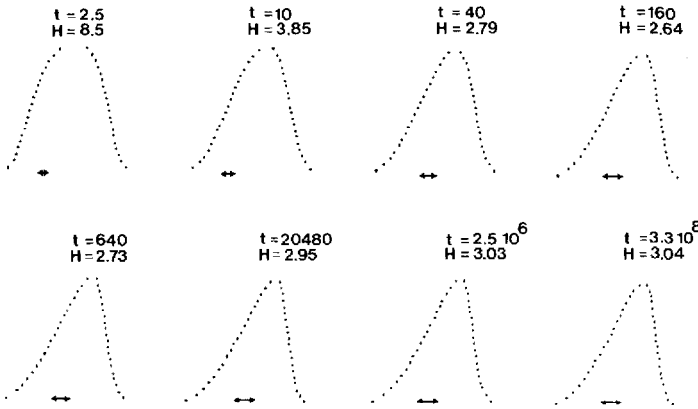| $t = 640$ $H = 2.73$	$t = 20480$ $H = 2.95$	$t = 2.5 \, 10^6$ $H = 3.03$	$t = 3.3 \, 10^8$ $H = 3.04$

Fig. 7. Shapes of peak place functions for various elapsed times t, showing the virtual invariance of the peak shape during migration through the column. Curves were obtained with the numerical experiment. Amount injected as in Fig. 6. Isotherm shape as in Fig. 4. Peak widths in the figure are normalized; the arrow indicates the length standard deviation that would be obtained in a corresponding linear elution case. Indicated H values are from the second moment, $H = \mu_{2z}/\mu_{1z}$.

simulation. There seems to be no doubt that this treatment is sufficiently accurate for estimating the detrimental effect of chromatography at or just above the limits of linearity.

However, in order to develop these concepts into a practical tool suitable for the prediction of non-linearity effects under various experimental conditions, and suitable for understanding the consequences of changes in plate number, phase ratio, capacity factor, etc., it is necessary to rearrange the equations. This is so because the
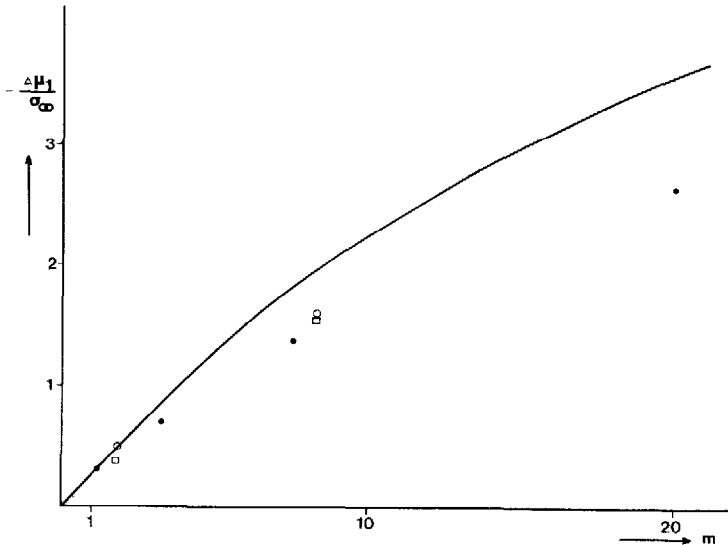

Fig. 8. The deviation in the first moment from that for the infinite dilution case, and expressed as a fraction of the standard deviation for the infinite dilution case σ_∞, as a function of the load m. ————, Haarhof and Van der Linden expression[31]; ●, results from Smit et al.[37,38]; □, results from present work for a plate number N of 800; ○, same for a plate number $N = 1.5 \cdot 10^6$.
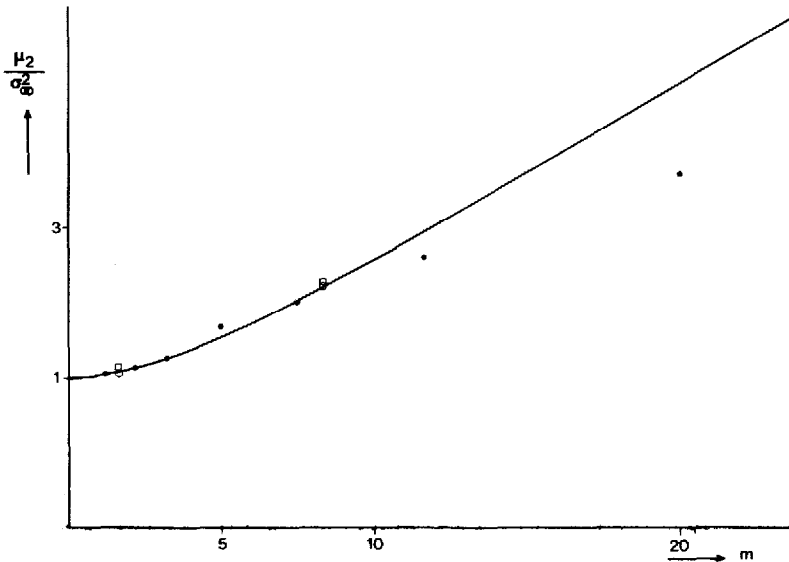
Fig. 9. The second moment of peaks as predicted by two simulation experiments and by the Haarhof and Van der Linden expression[31]. ●, Results from Smit et al.[37,38]; □, present results for a plate number $N = 800$; ○, same for a plate number $N = 1.5 \cdot 10^6$; ————, Haarhof and van der Linden expression[31].

overload thus far has been discussed in terms of mobile phase concentration, and this is impractical because in most instances concentrations in the stationary phase become too high. The conversion to stationary phase concentrations is easy when a model for the isotherm is given. For the case of the Langmuir isotherm, the conversion was already given as (eqn. 7)

$$m = \left(\frac{\kappa^\infty}{1 + \kappa^\infty}\right)^2 \frac{2M}{SV_{mp}}$$

As was shown, the peak deformation and the relative peak width increase are described by m exclusively. It is therefore possible to calculate an acceptable load if certain specifications are given. For instance, a 5% increase in the observed standard deviation (10% in the second moment) may be considered as acceptable. It can be seen from Figs. 7 and 8 that m should then not exceed 4–5. At that point (rather arbitrarily chosen, but one could take other readings from the graphs) the change in the first moment is 1.0 σ^∞ and that in the peak maximum is 1.5 σ^∞ (both for $m = 4$). These figures correspond to a relative deviation of 1.0 $N^{-1/2} \cdot 100\%$ and 1.5 $N^{-1/2} \cdot 100\%$ in the retention times of the first moment and the peak maximum, respectively. For this case ($m = 4$), eqn. 7 leads to

$$M_{max}^{10\%} = \frac{4}{2}\left(\frac{1 + \kappa^\infty}{\kappa^\infty}\right)^2 SV_{mp} = 2\left(\frac{1 + \kappa^\infty}{\kappa^\infty}\right)^2 SV_{mp} = 2N^{-1}\left(\frac{1 + \kappa^\infty}{\kappa^\infty}\right)^2 SV_m \quad (15)$$

where V_m is the volume of mobile phase in the column. SV_m is equal to the amount of

solute absorbed at the surface contained in the column when saturated. For many cases this can be estimated from specific surface areas (m^2/g), molecular dimensions (surface necessity, area required by an adsorbed molecule) and the column characteristics (plate number, grams of packing per unit volume). Apart from that it is readily determined by the break through method.

As an example, consider a 15 cm × 4.6 I.D. column, packed with 1.3 g of a 300 m^2/g material with a plate number of 10,000, and a solute with $\kappa^\infty = 3$, a molecular weight of 300 and a surface necessity of 100 Å per molecule. This leads, via eqn. 15, to

$$M_{max}^{10\%} = 2 \cdot 10^{-4} \, (4/3)^2 \cdot \frac{300 \; m^2 \cdot 1.3}{100 \cdot 10^{-20} \cdot 6 \cdot 10^{23}} = 2.3 \cdot 10^{-7} \; \text{mol} \approx 70 \; \mu g$$

which is, of course, of the right order of magnitude. The quantity SV_m is proportional to the amount of packing, W_s, in the column:

$$SV_m = \beta W_s \tag{16}$$

where β is a proportionality constant accounting for specific surface area and surface necessity.

For numerous phase systems occurring in normal- or reversed-phase adsorption, and for liquid–liquid partition, the Langmuir isotherm will not be applicable. The parameter SV_m, being the amount in the saturated stationary phase, loses its significance in these instances. However, we can still define a similar quantity M^{sat} as follows:

$$M^{sat} = \frac{M_s}{|\varepsilon|} \tag{17}$$

where M_s is the amount in the stationary phase and ε is the deviation in the capacity factor occurring when an amount M_s is sorbed:

$$\frac{M_s}{M_m} = (1 + \varepsilon) \, \kappa^\infty$$

For Langmuir behaviour $M^{sat} = SV_m$. The quantity M^{sat} will also, in other instances, be proportional to the amount of stationary phase, V_s, in volume, or W_s, in mass, and is dependent on the solute and phase system:

$$M^{sat} = \beta' V_s \text{ or } M^{sat} = \beta'' W_s \tag{18}$$

As indicated above, we have $\beta'' = \beta$ for Langmuir isotherms, and β is then determined by specific surface area and surface necessity. When overload is determined by the presence of too large concentrations in the stationary phase, it is to be expected that β'' does not vary with the phase ratio and is roughly the same for solutes of the same type chromatographed in the system. This discussion leads to a point that was one of the main objectives of this work, i.e., to make a proposal for reporting load-

ability. Taking the efficiency loss as the criterion (*e.g.*, 30 % loss in plate number), or the absolute increase in H (in mm)[29], loadabilities have been reported in micrograms or in micrograms per gram of packing. These values, however, apply to the column studied, and have the serious drawback that something which is basically a property of the distribution equilibrium is mixed up with the dispersion aspect of the column. For instance, the use of different sieve fractions of the same material in the same or different columns leads to different loadabilities.

It follows from this work that normalizing the load on the amount of stationary phase in one plate leads to the same loadability for a phase system and solute used with different plate numbers or column sizes. We therefore propose that critical loads, *e.g.*, with the criterion of a 30 % increase in H, are reported as micrograms per gram in one plate, equivalent to the usual micrograms per gram multiplied by the plate number.

Eluting concentration under various chromatographic conditions

Finally, the conclusions most relevant for analytical chromatography can be drawn. If we accept that β' or β'' values do not depend strongly on the phase ratio and type of solute, the "acceptable" (defined above) load is

$$M_{max}^{10\%} = 2N^{-1}\left(\frac{\kappa^{\infty} + 1}{\kappa^{\infty}}\right)^2 \beta' - V_s \tag{19}$$

The eluting concentration for this case is equal to

$$c_m^{top} = 0.38 \, M_{max}^{10\%} \, \sigma_V^{-1} \tag{20}$$

where c_m^{top} is the maximum concentration and σ_V is the volume standard deviation at infinite dilution. The factor 0.38 replaces $1/\sqrt{2\pi}$ valid for a gaussian distribution. Rearrangement of eqn. 20 using $\sigma_V = V_m(1 + \kappa^{\infty})N^{-1/2}$ gives

$$c_m^{top} = 0.76 \, N^{-1/2} \cdot \frac{\kappa^{\infty} + 1}{(\kappa^{\infty})^2} \cdot \beta' \cdot \frac{V_s}{V_m} \tag{21}$$

This equation applies to the case where the amount injected is already large enough to cause 10 % extra peak broadening. It is to be used for comparing c_m^{top} with the concentration detection limit of the detector. The ratio yields the dynamic range of the complete system. In this comparison it should be taken into account that the critical point is often the detection of traces in the presence of major constituents. Therefore, c_m^{top} as given in the equation should preferably be several orders of magnitude larger than the detection limit. The parameters β' and κ^{∞} in eqn. 21 are prescribed by the solute and phase system and by the requirements of rapid separation, respectively. Therefore, the remaining factors are more interesting to discuss.

High plate numbers have a detrimental effect on detectability. This is clear from eqn. 21, but it can also be explained as follows. Starting, *e.g.*, from a column as presently used, one can imagine a *p*-fold increase in its length and a resulting *p*-fold increase in the plate number, while keeping velocity, particle size, phase ratio, etc., constant. However, the tolerable amount then remains the same as relative peak

deformation is independent on the length passed. The eluting concentration is therefore smaller by a factor $p^{-1/2}$.

The equation also shows the small importance that small-bore columns can have in single-step chromatography for solving trace analytical problems (to be clearly distinguished from microanalytical problems, in which the sample amount is limited). It was shown over 10 years ago by Huber et al.[39] that consideration of volume overload leads to the conclusion that ultimately the detector sensitivity is the exclusive crucial point. In the present discussion it appears again that column dimensions per se have no effect on detectability; in eqn. 21 parameters such as length, particle size and column diameter do not occur.

The factor β' applies to the type of stationary phase. Not much can be said about it, apart from the fact that high surface areas are advantageous in adsorption systems, a fairly obvious conclusion.

Finally, eqn. 21 stresses the importance of the phase ratio $q = V_s/V_m$. Detectability is directly proportional to this factor, as long as stationary phase overload is the critical factor. In gas chromatography this factor is often very low, because of kinematic requirements and the extreme small ratio of the diffusion coefficient in the stationary and mobile phases. In liquid chromatography there is no such kinematic reason for low phase ratios; in fact, in a packed column a 1:1 ratio is perfectly feasible and can be even more favourable from speed and efficiency points of view than lower ratios. This points to renewed interest in liquid–liquid chromatography, as the bonded phase systems are limited to a V_s/V_m ratio of around 1:20.

For capillary LC the formidable requirements on the volume standard deviation due to the detector have been elegantly discussed by Knox and Gilbert[40]. However, the concentrations that can be expected were not discussed.

Eqn. 17 contains a favourable and an unfavourable factor in this respect. The plate numbers for competitive open-tubular LC will be large[40], and concentrations will be lower in proportion to $N^{-1/2}$. However, this is a general aspect of columns of high plate number. On the other hand, the V_s/V_m ratio can be favourable for open-tubular LC. It has been shown already by Tijssen et al.[8] that liquid–liquid chromatography in this geometry is possible. From the kinematic point of view there appears to be no reason to avoid d_f/R ratios as large as 0.3–0.5 and this would bring V_s/V_m phase ratios to values around 1, a factor of 20 more favourable that is feasible with present packed-column adsorption systems.

SYMBOLS

$a_1 \ldots a_n$	constants describing isotherm (eqn. 4);
b	constant describing non-linearity in eqn. 12;
c_m	mobile phase concentration;
c_m^{max}	concentration in the mobile phase at the upper limit of the linear range of the isotherm;
c_m^{eff}	effective mobile phase concentration in eqn. 2;
c_s	stationary phase concentration;
c_m^{top}	eluting mobile phase concentration at peak crest;
$\bar{c}_m$	$(1 + \kappa^\infty)/a_2$;
c_t	"total concentration" $= c_m + qc_t$;

D	dispersion coefficient of solute;
H	theoretical plate height;
L	column length;
M	amount of solute applied on the column;
$M_{max}^{10\%}$	allowable load when a 10% plate height increase is accepted;
M^{sat}	virtual saturation uptake defined by eqn. 17;
m	dimensionless form of M;
n	exponent in Freundlich expression;
N	theoretical plate number;
q	volume phase ratio, V_s/V_m;
Q	affinity parameter in Langmuir expression;
R	retardation factor for the migration rate of a concentration $= dc_m/dc_i$;
R_0	value of R at infinite dilution;
R_F	retardation factor;
S	saturation uptake of an adsorbent for the solute, per unit volume associated mobile phase;
t	time;
t_R	retention time;
t_R^∞	retention time at infinite dilution;
v	migration velocity of the mobile phase;
V_m	mobile phase volume in the column;
V_{mp}	mobile phase volume contained in one plate;
W_s	amount of packing in the column;
z	coordinate along column axis; at injection point $z = 0$;
$Z(\tau)$	$e^{-\tau^2/2}$;
$Z^*(\tau)$	$\dfrac{1}{\sqrt{2\pi}} \displaystyle\int_{-\infty}^{\tau} e^{-v^2/2}\, dv$;
α	proportionality factor according to ref. 19;
β, β', β''	proportionality factors in eqns. 16 and 17 between saturation uptake and amount of packing;
ε	fractional deviation in the capacity factor in eqn. 17;
κ	capacity factor;
κ^∞	capacity factor at infinite dilution;
$\Delta\kappa$	change in capacity factor due to overload;
μ_1	normalized first moment;
μ_2	normalized, centralized second moment;
σ_t	standard deviation at infinite dilution;
τ	reduced time, $(t - t_R^\infty)/\sigma_t$.

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