Journal of Chromatography, 398 (1987) 63–71
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 531

ASSESSMENT OF THE HEIGHT EQUIVALENT TO A THEORETICAL PLATE IN LIQUID CHROMATOGRAPHY

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(First received October 14th, 1986; revised manuscript received March 3rd, 1987)

SUMMARY

A number of methods for determination of the height equivalent to a theoretical plate particularly in reversed-phase liquid chromatography, are discussed. The methods are based on measurements of the peak width at half height, points of inflection, Gaussian curve fit, moments and on the exponentially modified Gaussian function. Data from 180 chromatograms obtained using the same column, together with additional data from other columns, were computer processed and applied to the different methods, the Gaussian curve fit and peak width at half height being found to be the methods of greatest merit. A significant deviation from earlier published curve forms of the Van Deemter plot is presented.

INTRODUCTION

The chromatographic process, in a column in liquid or gas chromatography, has been described theoretically or empirically by sets of equations or observations. One of the important parameters is the HETP value (height equivalent to a theoretical plate), defined as the column length, L, divided by the number of theoretical plates, N, for a particular compound

$$H = L/N = L/4(t_R/B)^2 \quad (1)$$

where t_R is the retention time of a compound and B is the peak width, measured between the points of inflection.

Van Deemter *et al.*[1] deduced a relationship between the flow-rate and the HETP value, showing clearly that a minimum HETP value existed for a particular flow-rate. The simplest form of this equation is

$$H = a + b/u + cu \quad (2)$$

where u is the linear flow-rate and a, b and c characterize different kinds of band-spreading effects. A number of simplifying assumptions had been made in order to

0021-9673/87/$03.50

achieve eqn. 2 and the authors emphasized that "its acceptability will have to be checked experimentally". Later, a number of authors modified eqn. 2 by adding non-linear terms (involving u) and by deducing complex expressions for the constants[2–5], while others have discussed various ways of theoretically deducing the plate height[6–11]. For comparative purposes, dimensionless variables, so-called reduced parameters, have been introduced in order to describe the shape of the Van Deemter relationship, free from a number of parameters.

On the one hand, a number of extensive theoretical studies of the separate constants of a modified Van Deemter equation have been made and compared to experimental values[3]. On the other hand, purely theoretical studies of computer-simulated symmetric and tailing chromatographic peaks have been made in order to deduce the most accurate way of calculating the HETP value under given conditions[12]. In addition, practical aspects have been presented and the value of the Van Deemter parameters seriously questioned[13,14]. Each of these approaches will be discussed in the following.

A most important task when developing new, or refining old, theories is to check continuously its applicability by experimental measurements, and is emphasized by most authors. This paper will deal with the practical aspects of checking the applicability of formulas expressing the relationships between flow-rates and HETP values and discuss methods for the measurement of HETP values.

ACCURACY AND PRECISION

If the logarithm of eqn. 1 is calculated

$$\ln H = \ln L - \ln 4 - 2 \ln t_R + 2 \ln B \tag{3}$$

and differentiated

$$\mathrm{d}H/H = = \mathrm{d}L/L - 2\mathrm{d}t_R/t_R + 2\mathrm{d}B/B \tag{4}$$

This can be estimated in absolute terms as ($\mathrm{d}L = 0$):

$$\Delta H/H \leqslant 2|\Delta t_R/t_R| + 2|\Delta B/B| \tag{5}$$

It follows that the relative error of the HETP value is proportional to twice the relative error of the determined peak width and twice the relative error of the measured retention time. If accurate trends in the Van Deemter relation are to be determined in liquid chromatography, the relative error of the HETP value should be less than 1%. When $\Delta H/H \leqslant 1\%$, both $\Delta t_R/t_R$ and $\Delta B/B$ must be less than 0.5%. For a fictitious but realistic HPLC chromatogram with $t_R = 300$ s and $B = 5$ s, the retention time must be measured with a maximum error of 1.5 s and the half-peak width must be measured with a maximum error of 0.025 s! If an ideal amplifier and chart-strip recorder are used for this purpose, and if the error of measurement corresponds to 1 mm, the speed of the paper fed must be more than 2.4 m/min or at least 12 m for the whole experiment. This indicates that most of the manual assessments of the Van Deemter plots made up to the present day probably contain errors

of measurement, which eliminates the possibility of the empirical discovery of small deviations from the assumed curve form.

Whether the factor B is determined as the width at half height or as the distance between the points of inflections for the peak has no influence on the shape of the Van Deemter plot for a Gaussian peak. If a Gaussian peakshape is assumed, the detector response to an eluted peak can be written as:

$$f(t) = I_0 e^{-d(t - t_R)^2} \tag{6}$$

The value of the number of theoretical plates obtained by determinations of the inflection points, $B_{\text{inf.}}$, can be related to that calculated from the half-band width, $B_{\text{hbw.}}$, by the expression:

$$N = 4(t_R/B_{\text{inf.}})^2 = 4 \cdot 2 \ln 2 \cdot (t_R/B_{\text{hbw.}})^2 \tag{7}$$

In the following, practical methods for constructing Van Deemter plots and for determining the shape of such curves are described. Thus it becomes unnecessary to introduce reduced parameters or strictly use $B_{\text{inf.}}$ or $B_{\text{hbw.}}$ as long as a Gaussian peak shape is assumed.

The problem of empirical determination of the "true" Van Deemter relationship can thus be associated with the problem of ascertaining the peak width for each chromatographic experiment, or in the case of asymmetrical peak shapes of eluted compounds, to the choice of expression for calculating the N value. Accurate measurement of any defined peak width cannot be made by eye or by hand. Computer registration with an high sampling rate must be made in order that some kind of algorithm can be used for the estimation of the peak width within acceptable limits.

EXPERIMENTAL

The liquid chromatographic equipment consisted of a Shimadzu LC4 liquid chromatograph equipped with a variable-wavelength UV detector, and a Spectra-Physics 3500 liquid chromatograph equipped with a Schoefel variable-wavelength UV detector. All columns used for the high-performance liquid chromatographic (HPLC) experiments were polymeric or monomeric bonded ODS, used in the reversed-phase mode with acetonitrile as the eluent (250 mm × 3 mm HC-ODS or Nucleosil C_{18}). Injections were made manually with a Valco 10-μl or a Rheodyne 20-μl injector. The gas chromatograph used was a Carlo Erba 4160 HRGC equipped with a SE-54 capillary column (20 m × 0.25 mm I.D.) and with a flame ionization detector. Computer registration, calculation and storage were performed by an ABC 800 micro computer system with a 12-bit analog-to-digital converter. The software for all computer calculations and applications was written by the authors.

ASSESSMENT OF N

No eluted peak can strictly be regarded as Gaussian, thus making the calculations of N according to the inflection or half-peak width methods erroneous. According to Arnold *et al.*[15] this can be circumvented by using the moments of the

elution function, calculated as integrals and combined as a simple equation. If u_i is defined according to

$$u_i = \int_0^\infty t_i \, f(t)dt \tag{8}$$

where f(t) is the detector response (elution function) to the compound in question, the HETP value can be calculated according to:

$$N = u_1^2/(u_0 u_2 - u_1^2) \tag{9}$$

This calculation is readily made by the computer and the whole calculation is completed within a few seconds, even in BASIC. Unfortunately, the setting of the baseline is of crucial importance for the determination of the moments. Thus, small divergences in the setting of the zero line for the detector response emanating from the compounds studied may cause large variations in the calculated N. Consequently, this method was abandoned after having been tested with several series of measurements. The variations are illustrated in Table I for different methods of calculating N based on the peak shown in Fig. 1. Table II shows the variations in N for a series of chromatograms performed with the same substance under identical conditions.

Deviations from the Gaussian peak shape must thus be treated by other means or by approximations. The fact that the detector system and the recorder usually have a response time constant leads to a distortion of the true signal. Nevertheless, this time constant is necessary in order to suppress high frequency components of the detector and amplifier system. An acceptable compromise for achieving the most distortion-free signal can be found in a moderate time constant (0.1 s) at the time of

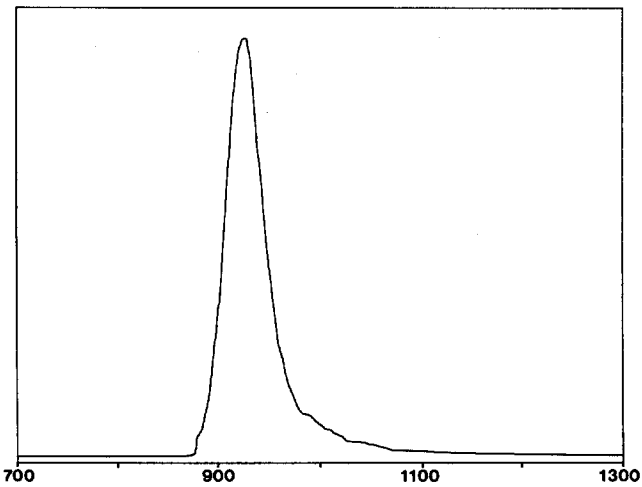

Fig. 1. Peak derived from an HPLC system with an ODS column, and used for the calculations presented in Table I. The unit of the x-axis is counts and the speed of registration is 10 counts/s.

TABLE I

METHODS OF CALCULATION OF *N versus* THE NUMBER OF DATA POINTS USED

Approximate peak width at half height: 5.5 s. Speed of registration: 10 counts/s. The peak used for calculations is shown in Fig. 1. "window" refers to the time axis in Fig. 1.

Method	*Width of window used for calculations*				*Maximum variance (%)*
	700–1300	*800–1200*	*850–1200*	*850–1300*	
Width at half height	2508	2511	2512	2511	0.16
Simulation of a Gaussian peak shape	2333	2337	2339	2337	0.23
Method of moments	555	691	695	570	20.26
Exponentially modified Gaussian peak shape	1468	1481	1483	1475	1.00

registration, followed by digital filtering of the registered signal. Digital filtering according to the original idea of Savitzky and Golay[16] also allows determination of the higher derivatives and thus determination of the point of inflection. Theoretically, this approach seems reasonable, but in practice can lead to difficulties in determining the correct value of the peak width in a given chromatogram. Thus each noise reduction performed by digital filtering ultimately leads to peak broadening and to a decrease in the HETP values. Furthermore, since this adding of errors is not systematically made from one experiment to another with respect to changes in the flow-rate, the Van Deemter plot becomes a non-coontinuous function. This effect is explained by changes in the peak widths with alterations in the flow-rates, thus making it necessary to alter continuously the band width of the digital filter. The marked effect of this is evidenced by the fact that the extreme points (maximum and minimum) of the first derivative do not coincide with the zeropoints of the second derivative. In practice, the filtering is continued until these points coincide within the range of one sampling cycle, which also emphasizes the necessity of using interpolated function values throughout the calculations, *i.e.*, the elution profile must not be re-

TABLE II

VARIANCE OF *N* DETERMINED FROM SIX CONSECUTIVE EXPERIMENTS WHEN THE TIME WINDOW FOR CALCULATIONS WAS INITIALLY DETERMINED FROM THE PEAK WIDTH AT HALF HEIGHT

Time window = (peak max. −2pw) to (peak max. + 4pw), where pw = peak width.

Method	*N (average)*	*Maximum variance (%)*
Width at half height	2021	1.09
Simulation of a Gaussian peak shape	2536	3.19
Method of moments	986	4.67
Exponentially modified Gaussian peak shape	1578	10.39

garded as a histogram. The problem associated with achieving high-quality Van Deemter plots with this method is thus strongly dependent on the judgements made by the chromatographer and the quality of the signal. The errors associated with the practical problem of finding strict rules for application of the inflection point approach to any eluted peak were found to be too large. The method was consequently not considered useful for general applications.

The most common method found in the literature for determining the HETP value is based on measurements of peak widths at half height. Again, the accuracy of the computer can be used to achieve high quality results if the registered signal is simulated as continuous, *i.e.*, the detector response is interpolated between registered points. Although, the determination of a correct baseline is still of great importance, it is considerably less crucial than in the case where N is determined from the moments of the detector signal. The Van Deemter plot determined by measurement of the peak width at half height is shown in Fig. 2 for 180 registered chromatograms from the same polymeric ODS column. Six determinations of the N value were made at each flow-rate used and the fairly large variation between the N values at the same flow-rate can be distinguished from the figure.

Another method of determining a stable value for N is to postulate a particulate peak shape that can be expressed in terms of mathematical functions. This particulate peak shape is brought further in to line with the eluted peak shape by means of a root mean square (r.m.s.) value. Thus if a Gaussian peak shape is assumed according to eqn. 6, d is determined by iteratively seeking the least mean squares between the theoretical and the registered peaks

$$\min \sum_{i=1}^{n} (kt_i + l + I_0 e^{-d(t_i - t_R)^2} - y_i)^2 \tag{10}$$

where I_0 is initially fixed as the peak height, t_R is set to the time coordinate at maximum peak height, $kt + l$ represents a straight baseline and y_i is the actual registered signal. Regarding the possible shapes in chromatograms, it could be assumed that eqn. 10 had only one minimum under the given conditions. Linear programming was thus used for calculation of the optimum I_0, d and t_R values. In this case the HETP value, based on the peak width at the inflection points, can be calculated as:

$$\text{HETP} = L/2dt_R^2 \tag{11}$$

The results, applied to the same 180 chromatograms discussed previously, are shown in Fig. 3. The variation in N at the same flow-rate (retention time) could be reduced if only the optimum d value was calculated (without altering the initially fixed I_0 and t_R values). The differences between the two methods, Figs. 2 and 3, are surprisingly small considering that small variations in the detector signal (noise) ought to influence the direct measurement of the half-band width much more than indirect measurements from an assumed curve form. The latter was comparable to digital filtering on a broad bandwidth basis.

The reason for the small differences recorded between direct bandwidth measurements and those made via curve modelling is primarily to be found in non-stable

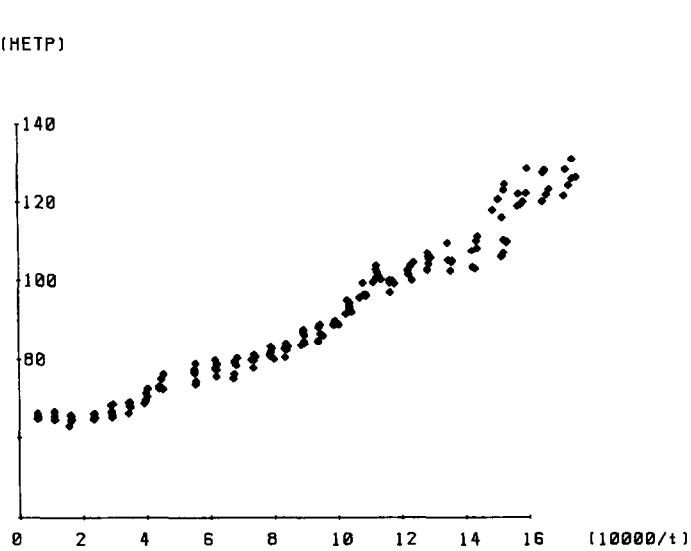

Fig. 2. Van Deemter plot for benzo[*k*]fluoranthene eluted from a reversed-phase polymeric bonded ODS column. Mobile phase: acetonitrile. Detector: UV at 254 nm. HETP calculations based on peak width at half height.

chromatographic properties. Thus variations in the curve form of an eluted peak, due to random variations in the injection, column and detector system, cause far more significant changes in the HETP value of the peak than either of the two methods used for calculation. This might be one of the reasons for Hawkes' statement[13] "The publication of Moody's[14] experiment on the evaluation of the parameters in the Van Deemter equation provokes the question why anybody would want

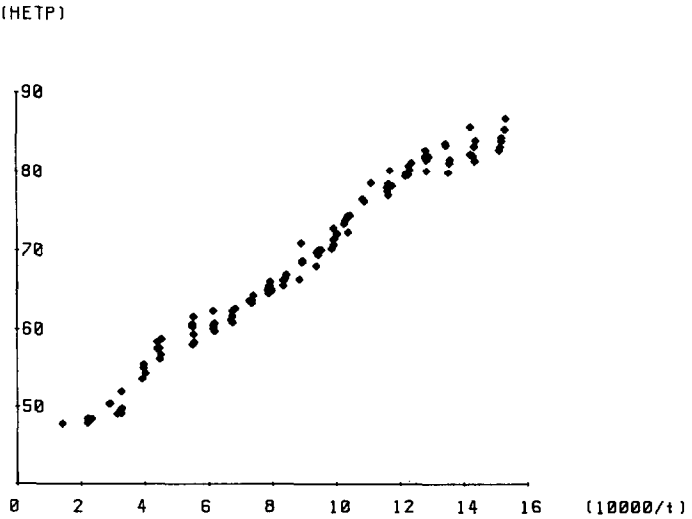

Fig. 3. Van Deemter plot for benzo[*k*]fluoranthene. Details as in Fig. 2 except HETP calculations based on the peak width between inflection points of the Gaussian function best fitted to the elution profile.

to determine them". This statement must be regarded as a frustrated expression of an opinion based on actual variations even in fixed chromatographic systems. In contrast with this practical approach to the problem, the investigation made by Bidlingmeyer and Warren[12], based on computer-simulated chromatograms, concludes that the calculation technique based on, for example, the moments of the curve yields results with the most accuracy when asymmetric peaks are considered. On the contrary, this statement is based on a method which cannot be applied to any real-life chromatographic system.

Finally, a method described by Foley and Dorsey[17] was used in order to establish a Van Deemter plot theoretically based on slightly tailing peaks. The N value was calculated according to

$$N = 41.7(t_R/W_{0.1})^2/(A/B + 1.25) \quad (12)$$

where A and B are the widths of the tailing and leading peaks, respectively, measured at 10% of the peak height and $W_{0.1} = A + B$. The method is based on the exponentially modified Gaussian function for peak simulation[18–22]. The results of this HETP plot for the same chromatograms presented in Figs. 2 and 3 is shown in Fig. 4. In general, the method yields increased HETP values with increased variances within each flow-rate group. This is a result of the peak width being measuring at only 10% of the maximum peak height. The first derivative is usually small in this area compared to the first derivative measured at the half-peak height, and is thus much more sensitive to random noise and increased errors in the measurement of the HETP value.

In conclusion, for the purpose of selecting the most reproducible method for measuring the HETP value in practical experiments, the simulated curve fitting and accurate half-band width measurement methods seem to be the best of the methods

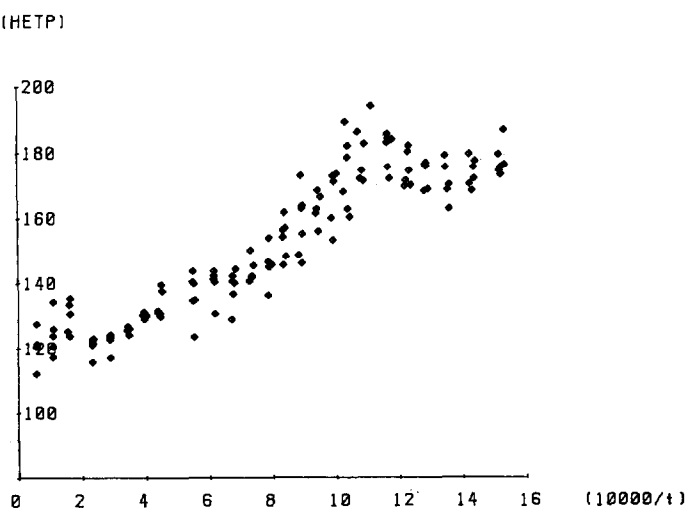

Fig. 4. Van Deemter plot of benzo[*k*]fluoranthene. Details as in Fig. 2 except HETP calculations based on exponentially modified Gaussian functions (eqn. 12).

discussed. The same could also be said of other reversed-phase HPLC columns. HETP assessment within capillary gas chromatography (GC) was also studied, but gas chromatography-specific properties such as peak splitting, which could be registered with the high-speed sampling technique used, made the problem more complex and will be discussed in a separate paper.

In spite of differences in standard deviations and in the absolute value of the HETP at the same flow-rate, all three curves in Figs. 1–3 show a distinguishable but significant form. One of the purposes in obtaining an accurate measurement of the Van Deemter plot was to test the existing theories by means of a least squares fit to the theoretical curves calculated and proposed by Van Deemter *et al.*[1], Giddings[2], Huber[4] and Done and Knox[5]. Since none of the proposed curve forms contains more than one point of inflection, the result of this test does not yield any valuable information. Thus the dependence of HETP on the linear flow-rate, for the system studied, including the injector, tubing, column system and detector, should be regarded as more complex than is taken into account by present theories.

ACKNOWLEDGEMENT

We thank Beryl Holm for reviewing the manuscript.

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