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STUDY OF THE PERTINENCY OF PRESSURE IN LIQUID CHROMATOGRAPHY

II. PROBLEMS IN EQUIPMENT DESIGN

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SUMMARY

The contributions of different parts of the chromatograph to peak broadening (injection, connection tubing, detector cell volume and detector response time) are discussed in terms of the recent trend in liquid chromatography to use finer particles, shorter columns and lower pressures. It is shown that although they tend to become more and more severe, the specifications are still not too drastic and that presently available chromatographs, when handled with care, are sufficient (in some cases only just sufficient) to allow the separation in a few minutes of compounds that require several thousand plates. Injection should be made on-column or just on the top of the column, very short connection tubing should be used and preference should be given to small cell detectors.

The most critical aspect at present is the detector time constant, which is too great in many instances and should not exceed 1 sec.

INTRODUCTION

The recent development of methods for the reproducible preparation of small, regular particles of supports and adsorbents that are currently used in liquid chromatography, with a well defined internal porosity and specific surface area, and of methods for packing these particles correctly, has resulted in great progress in column liquid chromatography. Firstly, very efficient columns can be made. Secondly, it has been realized that these columns can be operated at moderate flow velocities, around the optimum, so that only relatively small pressures are necessary[1,2]. Typical analyses, requiring a few thousand plates, can be achieved with 5–15-cm columns packed with 5–15-μm particles and operated at 5–20 atm in a few (up to 10) minutes[2]. Conversely, long columns, with efficiencies in the 10^4–10^5 plates range, can be achieved and operated within the present limits of technology, which permits the analysis of mixtures as complicated as those analyzed by gas chromatography on capillary columns; peak capacities[3] well above 100 can easily be achieved.

Conventional high-pressure liquid chromatography (HPLC) originated when fine particles were not available, and the columns used had high permeabilities and low efficiencies. The analysis time could be reduced by operating long columns at very large velocities, as the efficiency then decreases more slowly than the analysis time[4]. Columns packed with very fine particles could not be operated at comparable reduced velocities, and they do not need to be. It is possible to generate several thousand effective theoretical plates in 1 min, which is sufficient for most analyses[2].

The use of very short and efficient columns is not without problems, however. The band spreading during its elution through the column is small and the peak at the column outlet can be made very narrow. The contributions of the various parts of the equipment (injection port, column connections and detector) to band broadening should be kept small in comparison with the column contribution and this might result in some drastic specifications for the instrument.

It is the aim of this paper to discuss these contributions, to calculate the specifications for different experimental conditions and to estimate the impact of the new column technology on instrument design.

CHARACTERISTICS AND OPERATING CONDITIONS OF HIGH-PERFORMANCE COLUMNS

The column characteristics and the change in operating conditions that have been made necessary as a result of the use of fine particles have been discussed previously[2]. We have shown that the selection of a new optimization procedure in order to establish the experimental conditions under which the pressure is minimum, results in working at the minimum plate height, H.

Provided that the packing technique used is adequate, the reduced plate height is given by the Knox equation[4]:

$$h = \frac{2\gamma}{\nu} + A\nu^{0.33} + C\nu \qquad (1)$$

where $h = H/d_p$ and $\nu = u\,d_p/D_m$. The coefficients γ, A and C are practically independent of the particle diameter, d_p, and of the system used, and are a function of the packing method; D_m is the diffusion coefficient of the sample in the mobile phase, and u is the velocity of the mobile phase. There is a minimum in the reduced plate height, usually between 1 and 3 for regular, homogeneous packings, corresponding to an optimum reduced velocity of a few units. Using the numerical values selected in our previous paper[2], the optimum values of h and ν are 3.1 and 2.1, respectively.

The performance of a column when using a given chromatographic system depends on nine parameters (H, u and d_p, plus the plate number, N, the column length, L, the resolution, R_s, the pressure drop, ΔP, the retention time, t_R, and the maximum concentration of the peak, $C_{max.}$), which are related by six equations. Accordingly, there are three degrees of freedom for the system, and the choice of an arbitrary value for three of these parameters determines completely the experimental conditions[2]. The most classical optimization procedure seeks the minimum analysis time at a constant plate number and particle size (usually the finest available). Now that packing materials of various sizes in the 5–50 μm range are available, it is

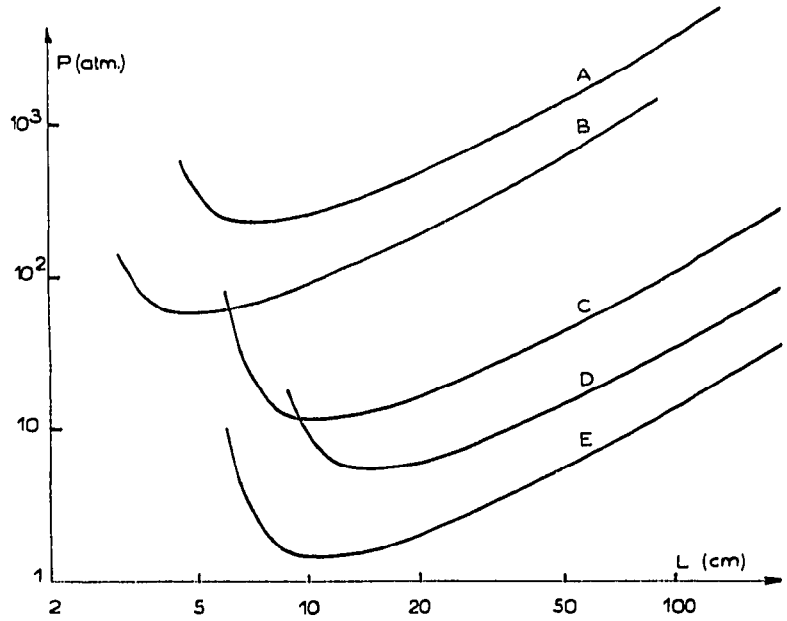

Fig. 1. Column inlet pressure *versus* column length. Each point on the various curves corresponds to a different column packed with particles of diameter increasing with L. Each curve corresponds to different performances. Numerical data are given in Table I.

Curve	*N*	t_R *(min)*
A	10,000	1
B	5000	1
C	5000	5
D	5000	10
E	2500	10

interesting to use another optimization procedure, seeking the minimum pressure drop at a constant plate number and retention time[2].

Fig. 1 shows the variation of ΔP as a function of column length for different sets of values of t_R and N that are representative of various analytical problems. It can be seen that there is always a minimum pressure for any given analysis and this optimum corresponds to the optimum velocity[2]. This minimum pressure is larger for more difficult analyses but for most conventional analyses it is much lower than the pressures that are currently used in HPLC.

It must be stressed that there is little purpose in trying to perform an analysis simply by operating the column at its optimum velocity, unless it has been especially designed for the type of analysis required. Fig. 1 can be used to calculate the column length and the pressure drop. The particle diameter is obtained from the condition that the plate height given by eqn. 1 should be minimal. Therefore, if the optimal reduced velocity is 2.1, we can derive the following from Darcy's equation[5]:

$$d_p = \left(\frac{2.1\,\eta\,D_m\,L}{k_0\,\Delta P}\right)^{1/3} \qquad (2)$$

η is the viscosity of the mobile phase and k_0 the specific permeability of the column. Variations of ΔP and L around the optimum value of d_p are small, so that usually particles that have a diameter up to 50% larger or smaller than the optimum can be used without a drastic increase in the pressure.

It has been demonstrated, both theoretically and experimentally, that such a type of column operated around the optimum conditions exhibits some intrinsic advantages over conventional columns operated at velocities high enough to take advantage of the coupling effect. These columns have a lower pressure and a shorter length. This last effect, however, makes these columns more sensitive to the extra-column sources of band spreading.

The chromatograph contributes to reduce the apparent column efficiency mainly through the injection system, the connections between the injection port and column and between the column and detector, the detector cell volume and the detector time response. These effects are discussed below.

It is convenient to characterize each of those broadening effects not only by its contribution to the zone variance at the detector level, but also by the fraction of column efficiency that is lost as a result. The overall loss is the sum of the individual losses, as the effects considered are independent of each other, and the effective total zone variance, σ_T^2, can therefore be written as

$$\sigma_T^2 = \sigma_c^2 + \Sigma\sigma_a^2 \tag{3}$$

where σ_c^2 is the volume variance due to band broadening inside the column and the σ_a^2 terms are the various contributions of the chromatograph. We calculate the specifications for all critical parts of the equipment so that each of the corresponding contributions is smaller than $0^2\,\sigma_c^2$, where 0^2 is an arbitrary factor, taken as 1% in numerical applications.

Calculations have been made for four typical cases of liquid chromatography. In conventional high-pressure chromatography, where the flow velocity is very high, the reduced velocity is above 50 (usually between 100 and 200). We have accordingly calculated the contributions under experimental conditions where the reduced velocity is 50 and 150. The recent trend towards the use of short columns packed with micro-particles makes it much more attractive to work at the optimum velocity (approximately $\nu = 2.1$), as explained above. Finally, for further reference, we selected an intermediate value of the reduced velocity ($\nu = 8.5$), which corresponds to the minimum pressure gradient necessary to achieve a given separation and so corresponds to the minimum contribution of the heat effect[2].

In order to allow an easier, more meaningful comparison, calculations have been made in each instance for the experimental conditions corresponding to different levels of performance. The characteristics of the columns calculated as described previously[2] are given in Table I. Easy analyses are those in which the separation of the important compounds requires 2500 or 5000 plates and the analysis time is 10 min for compounds with $k' = 2$ (k' is the capacity ratio). Very difficult analyses are those in which the separation requires 5000 or 10 000 plates and the analysis time is 1 min ($k' = 2$). An intermediate case corresponds to 5000 plates generated in 5 min. These conditions cover most practical cases. For further comparisons, it can be noted that as liquids are not compressible, 20 000 plates can be generated in 20 min, using the

TABLE I

CHARACTERISTICS OF LIQUID CHROMATOGRAPHIC COLUMNS GIVING SOME TYPICAL PERFORMANCES

Solvent viscosity, $4 \cdot 10^{-3}$ P. Diffusion coefficient, $3.5 \cdot 10^{-5}$ cm²/sec. Total porosity: 0.85. Specific permeability, $k_0 = 8.46 \cdot 10^{-4}$. Knox coefficients: $\gamma = 0.9$, $A = 1.7$, $C = 5 \cdot 10^{-2}$, $k' = 2$.

N	t_R (min)	Variable	$\nu = 2.1$	$\nu = 8.5$	$\nu = 50$	$\nu = 150$
10,000	1	ΔP (atm)	232	400	1800	6400
		L (cm)	7	15	55	130
		d_p (μm)	2.2	3.8	6.4	7.8
5000	1	ΔP (atm)	58	100	450	1600
		L (cm)	5	11	39	93
		d_p (μm)	3.1	5.4	9	11
5000	5	ΔP (atm)	11.6	20	92	320
		L (cm)	11	25	88	208
		d_p (μm)	7	12	20	25
5000	10	ΔP (atm)	6	10	46	160
		L (cm)	15	35	124	295
		d_p (μm)	10	17	28	36
2500	10	ΔP (atm)	1.5	2.5	11.5	40
		L (cm)	11	25	88	208
		d_p (μm)	14	24	40	50

same packing particles and the same velocity used to generate 5000 plates in 5 min but with a column four times longer and a pressure four times greater.

EFFECT OF THE SAMPLE VOLUME

The various effects of sample size on the performance of a chromatographic analysis have been discussed earlier[6]. One of the most important results is that the sensitivity depends little on the length of the column used at constant N if the maximum sample size is used in all instances. It is usually assumed that the contribution to the band variance of the sample introduction into the stream of carrier liquid is given by the equation

$$\sigma_s^2 = \frac{V_s^2}{K^2} \tag{4}$$

where V_s is the sample volume and K is a constant for each injection technique. For a plug injection, K is $\sqrt{12}$ (*ca.* 3.5), but under experimental conditions, however, K is usually about 2 (ref. 6).

If the injection should not increase the zone variance by more than a fraction 0^2, then

$$\sigma_s^2 = \frac{V_s^2}{K^2} \leqslant 0^2 \sigma_c^2 = 0^2 \cdot \frac{V_R^2}{N} \tag{5}$$

by definition of the plate number. The maximum sample size is thus

$$V_{sM} = \frac{0K\,V_R}{\sqrt{N}} \tag{5}$$

V_{sM} is a function only of the retention volume and the column efficiency. In terms of column parameters, this may also be written as

$$V_{sM} = 0K \cdot \frac{\pi}{4} \cdot d_c^2 \varepsilon_m \cdot (1 + k') \cdot \frac{L}{\sqrt{N}} \tag{7}$$

where ε_m is the total packing porosity.

If a given analysis has to be performed (constant N) and columns of different lengths are used, the sampling volume is proportional to the column length (constant N). As it has been shown previously that under such conditions the concentration at the peak maximum decreases in proportion to the column length if a constant-volume sample is injected[6], then, provided that the maximum sample size is used in all instances, the sensitivity will remain the same. The use of short columns does not seem to result in more sensitive trace analysis.

Numerical results calculated for different conditions typical of those encountered in HPLC, using either high pressures, long columns and large flow-rates or short columns operated at the optimum velocity, are reported in Table II. Clearly, these volumes are greater than those used in most instances. As stated previously, it seems that analysts tend to use sample sizes similar to those injected in gas chromatography, although there is no reason for this[6]. Even with short, very efficient columns, rather large sample sizes (in the 5–20 μl range) can be used, so that there appears to be no limitation to the use of presently available sampling valves or syringes.

The maximum sample sizes given in Table II depend only on the retention time, which might seem to be in contradiction with eqn. 6. The reason is that the retention volume is proportional to the column length and, as shown in Table I, L varies largely with the required performance. In fact, it has been shown[2] that for

TABLE II

MAXIMUM SAMPLE SIZES* (μl)

Column diameter: 4 mm. $0K \cdot \pi/4 \cdot d_c^2\, \varepsilon_m\, (1 + k') = 0.064$ (eqn. 7). *N.B.*, as shown in eqn. 16, the maximum sample size is twice the maximum detector volume assuming total mixing in the detector cell.

ν	$N = 10{,}000$, $t_R = 1$ min	$N = 5000$, $t_R = 1$ min	$N = 5000$, $t_R = 5$ min	$N = 5000$, $t_R = 10$ min	$N = 2500$, $t_R = 10$ min
2.1	4.5	4.5	10	14	14
8.5	10	10	22	31	31
50	35	35	79	112	112
150	84	84	188	267	267

* The maximum sample size is the sample volume that results in a 1% loss in peak efficiency if injected as a narrow plug.

columns operated at constant reduced velocity, L is proportional to $\sqrt{N}\, t_R$; V_{sM} is thus proportional to $\sqrt{t_R}$ (*cf.*, eqn. 6) and is independent of N.

The time during which the sample should be injected is also important. It is easy to show that this time, t_{sM}, is given by the equation

$$t_{sM} = \frac{0K\, t_R}{\sqrt{N}} \tag{8}$$

Consequently, in most practical cases (t_R = a few minutes, N = a few thousand plates) t_{sM}, which does not depend on the column length or the inlet pressure but only on the performance, is of the order of 1 sec. This is not too difficult to achieve, and it is certainly easier when the inlet pressure is low than when it is at the 100 atm level. However, in practice, a compromise has to be found, as too rapid an injection results in an extra-broadening of the peak due to the formation of eddies around the needle tip.

Finally, a word of caution is necessary. The constant K is related to the shape of the injection band at column inlet, but does not describe it completely. The profile of this band is immaterial in practice when the sample size actually injected is 100 times smaller than the maximum, as is often the case with long columns. When the ratio V_s/V_{sM} increases, this profile becomes more and more important. At large sample sizes, the column would merely soften the injection profile. Consequently, the injection technique is more critical when using short columns. This problem will be discussed elsewhere[7].

EFFECT OF THE CONNECTING TUBING

Connection volumes have an adverse effect on the width of chromatographic bands and should be avoided or limited as much as possible. Any dead volume absorbs solute when the zones move nearby and the concentration gradient is large and releases them at a much lower velocity when the concentration gradient is small, resulting in a strongly tailing peak. Tubing swept by the mobile phase has a less detrimental effect. However, the Poiseuille velocity profile builds up a radial concentration gradient, which can be relaxed only by diffusion. As this process is slow, especially in liquids, considerable band broadening results.

One of the great advantages of microsyringe injection is that it allows the sample to be placed on the top of the column, even in the uppermost layers of the packing if desired. The residue in the syringe needle when the syringe is withdrawn is not injected and therefore no connecting tubing between the injection port and the column is needed. This is not so with a sampling valve and the corresponding contribution should be calculated as shown below.

A connecting tube is usually necessary between the column and the detector. In well designed equipment, however, connections could be limited to narrow tubing a few inches long. The Golay equation:

$$H = \frac{2\, D_m}{u} + \frac{r^2\, u}{24\, D_m} \tag{9}$$

permits the calculation of the volume variance contribution, σ_a^2, of tubing of length l and radius r:

$$\sigma_a^2 = (\pi r^2)^2 \, l \, H \tag{10}$$

the length variance being related to the plate height by the equation $\sigma^2 = l \, H$.

Thus, if F is the mobile phase flow-rate:

$$\sigma_a^2 = \frac{2\pi^3 \, D_m \, r^6 \, l}{F} + \frac{\pi r^4 \, l \, F}{24 \, D_m} \tag{11}$$

D_m in liquids is very small (usually 10^{-6}–$5 \cdot 10^{-5}$) and, as shown later r is of the order of 0.1–0.5 mm, so that the first term on the right-hand side of eqn. 11 can be neglected.

The condition that the variance contribution should be less than 1 % of the peak width at the column outlet gives:

$$r^4 \, l \leqslant \frac{24 \, 0^2 \, D_m}{\pi} \cdot \frac{1}{N} \cdot \frac{V_R^2}{F} \tag{12}$$

or

$$r^4 \, l \leqslant 60^2 \, D_m \, \varepsilon_m \, (1 + k') \, d_c^2 \, L \cdot \frac{t_R}{N} \tag{13}$$

This equation is similar to that derived earlier by Scott and Kucera[8], who also verified experimentally that the variance contribution is proportional to r^4 and to l. It shows that the contributions of tubing with identical volumes are not the same as sometimes assumed, but that for such tubes they decrease as the square of their radius. Eqn. 13 also shows that the diameter of the tubing can be increased in proportion to the square root of the column diameter. Short chromatographic columns, however, are more demanding than the conventional longer columns, as shown by eqn. 13 and by the data given in Table III. This is because the factor $H/u = t_R/N$ is kept constant during the comparisons made here, all of the columns compared in each column of Table III giving the same performance.

The design of the equipment necessary for use with columns packed with small particles, which are those which give the best performance, is certainly more

TABLE III

MAXIMUM VALUE OF THE PRODUCT $r^4 \, l^*$ (cm^5)

ν	$N = 10{,}000$, $t_R = 1$ min	$N = 5000$, $t_R = 1$ min	$N = 5000$, $t_R = 5$ min	$N = 5000$, $t_R = 10$ min	$N = 2500$, $t_R = 10$ min
2.1	$3.4 \cdot 10^{-8}$	$4.9 \cdot 10^{-8}$	$5.6 \cdot 10^{-7}$	$1.6 \cdot 10^{-6}$	$2.2 \cdot 10^{-6}$
8.5	$7.8 \cdot 10^{-8}$	$1.1 \cdot 10^{-7}$	$1.3 \cdot 10^{-6}$	$3.6 \cdot 10^{-6}$	$5 \cdot 10^{-6}$
50	$2.8 \cdot 10^{-7}$	$4 \cdot 10^{-7}$	$4.5 \cdot 10^{-6}$	$1.3 \cdot 10^{-5}$	$1.8 \cdot 10^{-5}$
150	$6.6 \cdot 10^{-7}$	$9.6 \cdot 10^{-7}$	$1.1 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	$4.3 \cdot 10^{-5}$

* The maximum value of the product $r^4 \, l$ is that which results in a 1 % loss in efficiency. $6 \, 0^2 \, D_m \, \varepsilon_m \, (1 + k') \, d_c^2 = 0.9 \cdot 10^{-6}$ (eqn. 13); $r^4 = 10^{-8}$ for a 0.2-mm I.D. and $4 \cdot 10^{-7}$ for a 0.5-mm I.D. tube.

difficult. H/u is equal to (h/ν) (d_p^2/D_m) and for columns that operate around the optimum it decreases with d_p^2 because, as explained above, the optimum values of h and ν are relatively constant. It appears, however, that present technology should make possible the manufacture of the connections that are needed for use with the efficient, short columns that can be prepared with 3–5-μm particles. For example, a 5-cm long column that enables 5000 plates to be obtained in 1 min, which is already very good, requires a 0.2 mm I.D. connection that is shorter than 5 cm, which is acceptable.

The transition from a large diameter column (4–10 mm I.D.) to a narrow capillary tube (0.2–0.5 mm I.D.) can also result in a severe loss in efficiency. The column should be filled up to the mouth of the narrow tube in order to suppress dead volumes. A study of the optimum shape of this section has not been made yet; a cone filled with inert material is certainly acceptable.

A method to reduce drastically the contribution of connecting tubes to the peak variance would be to design the tubing in such a way that the flow is turbulent there. This seems to be possible only with columns that are wider than 1 cm.

VOLUME OF THE DETECTOR CELL

The detector cell is not a plug-flow reactor, but some mixing occurs within it. Depending on its geometry, the cell can be considered either as a connection tube, when eqn. 13 applies, or as a mixing chamber. Then the composition of the liquid is the same throughout the cell. It has been shown by Sternberg[9] that in this case, which is the most unfavourable, the time variance contribution is

$$\sigma^2 = \frac{V_d^2}{F^2} \tag{14}$$

Thus, a condition similar to eqn. 5 can be written and the maximum detector volume, V_{dM} is given by:

$$V_{dM} = 0 \cdot \frac{\pi}{4} \cdot d_c^2 \varepsilon_m (1 + k') \sqrt{LH} \tag{15}$$

Eqn. 15 differs from eqn. 7 only by a numerical constant so that the maximum sample size and the maximum detector volume are related by the equation

$$V_{dM} = \frac{V_{sM}}{2} \tag{16}$$

assuming that $K \approx 2$. V_{dM} can thus be obtained from Table II.

A recent survey of liquid chromatographic equipment[10] shows that most UV detectors now have cell volumes between 6 and 10 μl. As shown by the data in Table IV, the loss in efficiency resulting from the use of a 10-μl cell is not negligible for very demanding analyses such as those which require 5000–10 000 plates in 1 min. The loss resulting from the use of a 5-μl cell being four times smaller would be acceptable even in this case, so that most UV detector cells now commercially available can be used with short columns provided that care is taken in their interfacing. Even in

TABLE IV

EFFICIENCY LOST (%) WHEN USING A 10-μl CELL DETECTOR*

ν	$N = 10{,}000$, $t_R = 1$ min	$N = 5000$, $t_R = 1$ min	$N = 5000$, $t_R = 5$ min	$N = 5000$, $t_R = 10$ min	$N = 2500$, $t_R = 10$ min
2.1	20	20	4	2	2
8.5	4.0	4.0	0.8	0.4	0.4
50	0.3	0.3	0.06	0.03	0.03
150	0.06	0.06	0.01	<0.01	<0.01

* $\theta^2 = 9.74\ V_d^2\ N/L^2$, with $V_d = 0.01$ ml.

simple analyses, however, the use of the best differential refractometer available (overall cell volume *ca.* 30 μl) will result in very serious loss in efficiency (*cf.*, Table IV). This loss is very high with the most efficient columns.

However, as the detector cell does not work as a pure mixing chamber, there is only partial re-mixing of the solute there. With plug flow, the contribution of the detector cell volume would be $2\sqrt{3}$ times smaller. The contribution of an actual detector is usually intermediate between these two extremes. Further, the differential refractometer is a 10-μl cell connected to the column by a 30-μl tube used as a heat exchanger. The total contribution can be reduced to the contribution of the detector cell if a specially designed connecting tube with a flattened section and a twisted profile is used[11].

TIME CONSTANT OF THE DETECTOR RESPONSE

It does not suffice to have a narrow concentration band; it is necessary that the detector should give a signal that does not distort and broaden the actual band profile. The response time of the detector should be sufficiently small in comparison with the band width. This effect is independent of the inlet pressure, column length and particle diameter, and depends only on the actual zone width.

As shown by Schmauch[12] and by McWilliam and Bolton[13], the profile recorded with a detector that has a time constant τ is larger than the actual profile by a factor $(1 + \tau/\sigma_t)$, provided this factor is smaller than *ca.* 1.2. Therefore, if the decrease in apparent efficiency is again required to be smaller than θ^2, then τ should be smaller than

$$\tau_M = \theta \cdot \frac{t_R}{\sqrt{N}} \tag{17}$$

Numerical results are given in Table V. Except for very simple analyses, a time constant of about 0.5 sec is necessary in most instances in order to avoid an efficiency loss of more than 1%. This is not sufficient for the most difficult analyses which cannot be performed without severe loss in performance with a detector that has a time constant greater than 0.1 sec. Data acquisition with a computer becomes necessary.

This is the most serious problem at present, as the time constant of most detectors[10] is between 0.5 and 3 sec, and few have a time constant less than 0.5 sec. It seems that this problem has so far been considered to be of secondary importance.

TABLE V

MAXIMUM TIME CONSTANT*

N	t_R (min)	τ_M (sec)
10,000	1	0.06
5000	1	0.08
5000	5	0.4
5000	10	0.85
2500	10	1.2

* Loss of efficiency = 1%.

This should no longer be so and detectors should not have a time constant greater than 1 sec.

The time constant results from either the electronic or the hydrodynamic part of the detector. It seems that the time constant of sensors, amplifiers, recorders, etc., can easily be reduced to well below 1 sec, although with a possible increase in the noise level. The time constant of a mixing chamber is V_d/F, so that if the detector cell works as such, the time constant cannot be smaller. The optimum flow-rate through a 4 mm I.D. column packed with 5-μm particles is about 0.02 cm^3/sec ($\nu = 2.1$), which gives a contribution to the time constant less than 0.5 sec only if the cell volume is smaller than 10 μl. If, however, this effect determines the time constant, it does not need to be added here, as the contribution of the detector cell volume has already been accounted for and should not be included twice.

If the detector response is not linear, the peak recorded is smaller and appears to be broader than the actual peak. If the detector response is $y = k(C - aC^2)$, the deviation from linearity is $\Delta h/h = ah/k$, where h is the actual peak height[14]. Then, it can be shown that if a is small, the error in the peak variance is 0.4 ah/k. Care should be taken that measurements are made in the linear range of the detector, especially when a UV detector is used.

CONCLUSION

It seemed obvious at first that the best way to reduce analysis times is to increase the flow velocity, and thus the inlet pressure, as the efficiency does not decrease as fast as the retention time[4]. This is so, however, as long as the analyst has to work with only one packing material. If, however, particles of various sizes are available, it is possible to achieve rapid analyses at velocities around the optimum, using short columns and low pressures (5–30 atm for most practical cases), selecting the best packing for each analysis.

The use of short columns, however, makes the analytical performances much more sensitive to the quality of the apparatus used and its design becomes very critical. This is illustrated by Fig. 2, which shows the variation of the HETP of a 6-cm long column packed with 5-μm particles (Reeve Angel Partisil 5). The efficiency decreases sharply with increasing flow-rate because of the large contribution to the apparent peak width of a detector with a 0.4–0.5 sec time constant. This contribution increases in proportion to u^2 (ref. 15). If a correction is made in order to account for this effect, curve 2 in Fig. 2 is obtained, which is certainly nearer than curve 1 to the true HETP curve. In order to make correct measurements in the 1–2 cm/sec range, a time con-

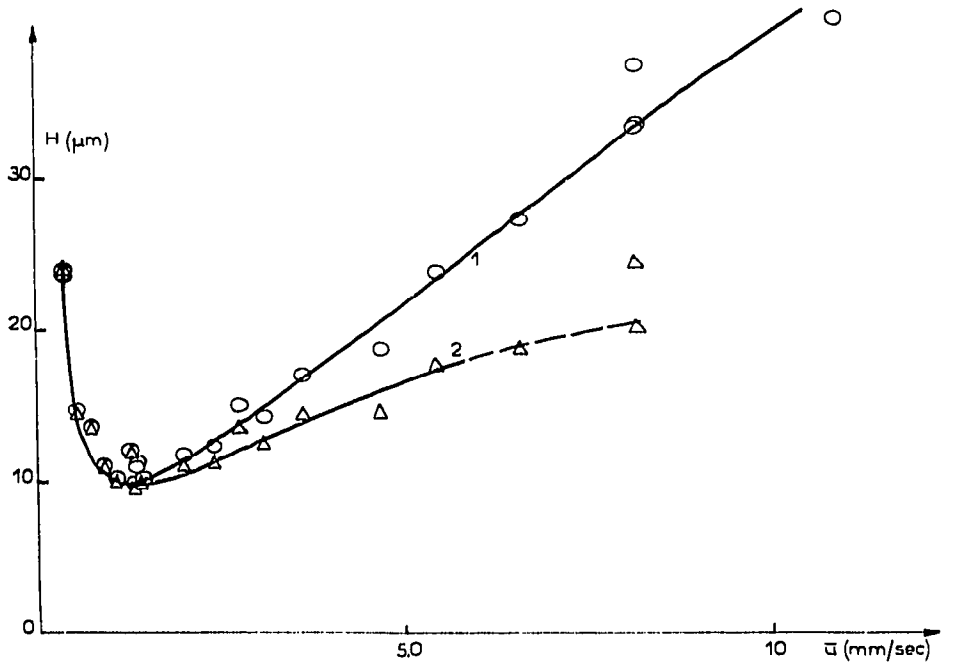

Fig. 2. *H versus u* for a 6 cm × 4 mm I.D. column packed with Reeve Angel Partisil 5. Particle size *ca.* 5 μm. Mobile phase, *n*-heptane; solute, anthracene; $k' = 2.6$; detector, Uvicord with a modified 5-μl cell. ○, curve 1: experimental results. △, curve 2: experimental data corrected for a 0.4-sec time constant.

stant of 0.05 sec would be necessary. The effect remains small and acceptable around the minimum of the curve, but this would not be so when finer particles are used.

Provided that on-column injection is possible and that the volume and length of the connection between the column and the detector are kept small enough (in most instances they need not be long, the connection being just for convenience in column handling), the only major source of trouble for the analyst is the cell volume of the detectors and their time constant. It is hoped that this will soon be overcome as the development of the necessary small fast-response detectors should not be a problem. It should be emphasized, however, that with the specifications given above the equipment already gives a 4% total loss in efficiency.

This situation is rather fortunate as the use of 5–10 μm particles appears to be simple in practice, but further progress seems unlikely in the near future. The use of smaller particles, in the 1–3 μm range, would raise great problems; it would be difficult to operate a 1-cm long column at several hundred atmospheres with 1 μl detector cell volume and a connecting tube smaller than 0.1 mm I.D. and 1 cm long.

In view of the decreasing size of columns and of the ancillary equipment, as well as the trend towards the use of only a few stationary phases, and as the specificity of the system for the sample to be analyzed is achieved, contrary to the procedure in gas chromatography, by adjusting the composition of the mobile phase, it seems probable that the design and manufacture of an integrated system, where sampling

device, column and detector cell are factory-assembled in one piece will soon be achieved. This will greatly ease the operation of high-performance systems, although it will deprive the analyst of some flexibility.

It can also be forecasted that the development of new detectors for liquid chromatography faces more difficult technological problems than ever. This is specially true for reaction detectors. Although they can be designed so that they do not behave as mixing chambers, plug flow is very difficult to achieve, even when using tubes with a special inner profile[11].

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