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INFLUENCES OF DETECTOR TIME CONSTANT VARIATIONS ON EFFICIENCY CALCULATIONS IN THE STANDARDISATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMNS

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SUMMARY

This paper reports the influences of detector time constant variations on plate count calculations and peak retention times in the standardisation of high-performance liquid chromatographic columns. A recommendation is made here that column efficiencies should be quoted at zero time constant in order to remove the variability in plate count introduced by varying time constant. For detectors which do not have variable time constant controls, it is recommended that the calculation of plate numbers should be made on solutes having capacity factors in the region 5–6.

Further recommendations made are that the peak symmetry correlation ratio should be used to evaluate system performance only when calculated at zero time constant and that the detector time constant should not exceed one hundredth of the peak width for peaks used in the calculation of plate numbers.

INTRODUCTION

Peak asymmetry is an important aspect in relation to resolution and column performance in high-performance liquid chromatography (HPLC). The major contributions to peak tailing arise from voids or channelling in the column itself and also from extra-column effects chiefly due to solute diffusion in tube connectors, precolumns, cell volume of the detector and the injector system[1–3]. A further contribution which has not often been recognised is that improper time constant settings on UV detectors of the HPLC system can also cause peak asymmetry.

Two important terms, the "time constant" (τ) and the "response time" (t) are often referred to in the literature. The former term is the time required for the recorder to reach 63.2% of its final value and the manner in which the detector time constant affects peak shape and peak height has been briefly discussed by Stewart[4]. The latter term is the time required for the recorder to reach 99.7% of its final value and is equal to five times the time constant.

In a study of the use of a modified scanning spectrophotometer as a variable-wavelength detector for HPLC, Higgins[5] noted that HETP (height equivalent to a theoretical plate) values and peak symmetry were dependent on the time constant of

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the detector. We have explored these effects in detail and this paper presents a discussion of the serious errors which can result when column efficiency (expressed as the number of theoretical plates) is calculated using peaks which are distorted due to incorrect settings of time constant. In addition, the influence of detector time constant on retention times of solutes and their analytical sensitivities is also discussed.

EXPERIMENTAL

Instrumentation and reagents

The liquid chromatograph used consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model 6000 solvent pump, Model U6K injector, Model 450 variable-wavelength detector and an Omniscribe Model B5217-1 recorder. The detector was fitted with a continuously adjustable variable time constant control consisting of a simple first-order resistance–capacity (RC) Butterfield filter with normal roll-off characteristics of —3dB at the cut-off frequency and —6dB per octave. Because this control was difficult to set reproducibly, it was replaced by a 10-position switch with discrete resistors instead of the continuously variable potentiometer formerly fitted. Use of this switch enabled settings of time constant in the range 0.1–1.1 sec in increments of 0.1 sec. No attempt was made to compensate observed chromatographic data for the response characteristics of the recorder, since the same recorder was used for all measurements.

Analytical grade methanol was triply distilled from all glass apparatus. Acetonitrile was purchased from Ajax, Sydney, Australia (Spectrograde) and water was distilled using Millipore Milli-Q water purification system. All other reagents were used as purchased without further purification.

Chromatographic procedure

Separation by HPLC was accomplished using a μBondapak C_{18} column (30 cm × 3.9 mm O.D., Waters Assoc.). The mobile phases used were acetonitrile–water (60:40) for the first test solution and acetonitrile–water (50:50) for the second test mixture. The mobile phase flow-rate was 2.5 ml/min producing a back pressure of 2000 p.s.i. and the detector was operated at 254 nm with a sensitivity setting of 0.1 a.u.f.s. The first test solution contained 26 mg acenaphthene in 100 ml of methanol–water (60:40), and the second test mixture contained phenol (20.3 mg), *p*-cresol (19.3 mg), 2,5-xylenol (19.9 mg), anisole (20.5 mg) and phenetole (20.5 mg) in 100 ml of methanol–water (50:50). In all runs, 15 μl of the respective test solution was injected using a 25-μl syringe. All separations were carried out at 20°C with a recorder chart speed of 2 in./min.

The peak symmetry correlation test entailed removal of the HPLC column from the system and the inlet and outlet tubes to the column were then joined by a suitable connector. The sample solution consisted of 0.1 ml of acetone in 100 ml of methanol and 10 μl of this solution were injected. The mobile phase was pure methanol with a flow-rate of 0.5 ml/min. The wavelength of the detector was fixed at 254 nm using a sensitivity of 0.1 a.u.f.s, and the recorder chart speed was 5 in./min.

RESULTS AND DISCUSSION

Effect of time constant on peak shape, height and retention

Increase in detector time constant results in a reduction in peak height (H), an increase in peak skewness and a shift in the peak maximum towards longer retention. These effects are illustrated in Fig. 1 which shows the separation of the test mixture obtained at two extreme settings of detector time constant.

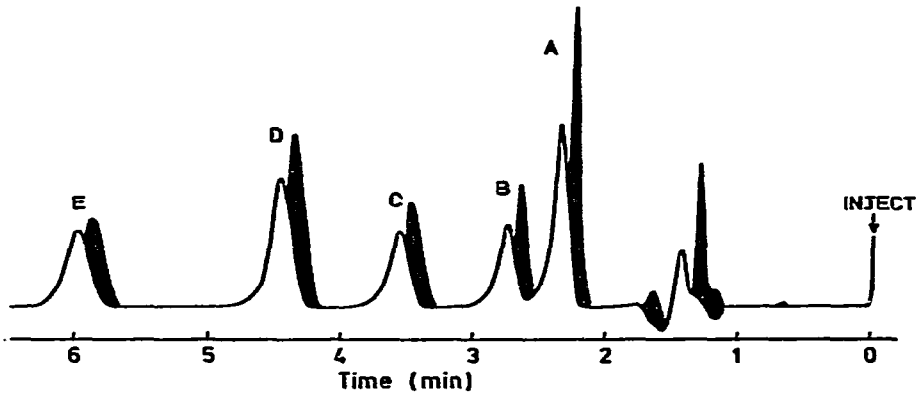

Fig. 1. Chromatogram of test mixture recorded at two different values of time constant. Dark peaks: $\tau = 0.1$; light peaks: $\tau = 1.1$. A = phenol; B = paracresol; C = 2,5-xylenol; D = anisole; E = phenetole.

The precise manner in which the above peak parameters are affected by detector time constant will be dependent on the type of filter used in the detector. In our case, the detector employed a simple Butterfield type filter and the results presented in this paper would be applicable to detectors equipped with this type of filter.

Linear relationships were observed between time constant and both peak height and capacity factor of the solute, however skewness and time constant were non-linearly related with the greatest increases in skewness being observed at the longer values of time constant, that is, those values preferred for heavy damping of baseline noise.

Effect of time constant on HETP calculation

The efficiency of the column, expressed as the number of theoretical plates (N), was calculated using the 4σ, 5σ and half-height methods with acenaphthene as solute and at various settings of detector time constant. The calculated value of N was observed to decrease linearly with increasing values of τ, in confirmation of results presented by Higgins[5]. This effect was quite pronounced. For example at $\tau = 0.1$ sec, $N = 3870$ whilst at $\tau = 1.1$ sec, $N = 2190$.

Most manufacturers of HPLC columns include in the column specifications a guaranteed minimum value of N, without specifying a corresponding value of τ. From our results, we suggest that a more logical procedure to use when reporting values of N would be to remove the time constant effect by quoting the value of N at zero time constant. To do this requires that detectors have a variable, calibrated time constant

control or an in-line RC filter (as used by Higgins) to allow at least three values of τ to be used in construction of a graph of N *vs.* τ. Extrapolation of this graph to $\tau = 0$ provides the required value of N. An alternative procedure will be suggested below for situations where modification of detector electronics is not possible.

Preliminary work had indicated that the dependence of both peak height and the number of theoretical plates on time constant was governed by the capacity factor of the peak used. The percentage decrease in H and N obtained by changing the time constant from 0.1 sec to 1.1 sec was calculated for each of the solutes in the test mixture (Fig. 1) and compared with the capacity factor of the solute (measured at zero time constant by extrapolation). It was found that the time constant effect on H and N, as reflected by the decrease in these parameters when τ is increased, is much less for the longer retained solutes. This is due to the fact that the broad peaks observed at high capacity factors are much less susceptible to changes in τ than are sharp, narrow peaks. It can be concluded from these results that calculations of N based on a solute with a capacity factor in the region of 5–6 would give a value closer to that obtained by the extrapolation method discussed earlier (*i.e.* the value of N at zero time constant) than for a faster eluted peak. Thus if no variable time constant control is present and electronics modification is not desirable, then selection of a solute with a high capacity factor for calculations of N would be a suitable compromise.

This contention is supported by the following values of N: 5470 (calculated by the method of extrapolation to zero time constant) and 5350 (calculated using phenotole which has a capacity factor of 5.85 in a mobile phase of acetonitrile–water (45:55) with a detector time constant of 0.6 sec).

Some authors and instrument manufacturers have suggested that suitable values of time constant can be selected by reference to the peak width. Stewart[4] recommends that time constant should not exceed one tenth of peak width (w) and this recommendation is based on treatment of the peak shape as a normal distribution. The handbook for the detector used in this study makes the same suggestion. In order to evaluate the suitability of this recommendation for data to be used in column efficiency calculations, we determined the value of N at various ratios of τ/w using acenaphthene as solute. The results showed that the plate count was reduced from its theoretical maximum (at zero time constant) as the ratio τ/w was increased. A reduction of 10% or less in plate count was achieved only for values of τ/w of 0.01 or less. The conclusion arising from this study is that the selection of time constant settings such that $\tau/w \leqslant 0.1$ is inappropriate when the chromatogram is to be used for plate count calculations. In this case, $\tau/w \leqslant 0.01$ should be used.

Peak symmetry correlation test

The symmetry correlation ratio is expressed as $R_S = 0.8\ (A/B)$ where A is the base width of a peak measured at 4.4% of the peak height and B is the base width between tangents. For a perfectly Gaussian peak, this ratio is equal to one. R_S is often used as an indicator of the efficiency of the chromatographic system with the column removed; high values of R_S generally reflect malfunctions such as cracked cell windows, excessive dead volume in the injector or tubing etc. This ratio was calculated at various values of τ and was found to increase linearly with τ. It follows that the peak symmetry correlation test can be validly applied only when the time constant effect is removed. To do this, the linear R_S *vs.* τ plot should be extrapolated to $\tau = 0$ and the

resulting value of R_S used as a measure of system performance. In our laboratory, a value of $R_S \leqslant 1.3$ indicated proper functioning of the chromatograph.

CONCLUSIONS

The effect of detector time constant on peak height, peak skewness, capacity factor, number of theoretical plates and the peak symmetry correlation ratio has been examined. The results have led to four recommendations for minimising the effect of time constant on the above parameters and these are:

(1) Column efficiencies (expressed as the number of theoretical plates) should be determined at zero time constant by extrapolation of the linear N *vs.* τ plot.

(2) If no variable time constant control is fitted to the detector, then calculation of N using a peak of a solute with capacity factor 5–6 ensures minimal time constant effect on the value obtained.

(3) The peak symmetry correlation ratio R_S should be determined at zero time constant by extrapolation of the linear R_S *vs.* τ plot.

(4) The commonly held idea that a value of $\tau/w \leqslant 0.1$ is optimal for general chromatography is inappropriate when a peak is to be used for efficiency calculations. In this case $\tau/w \leqslant 0.01$ should be used.

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