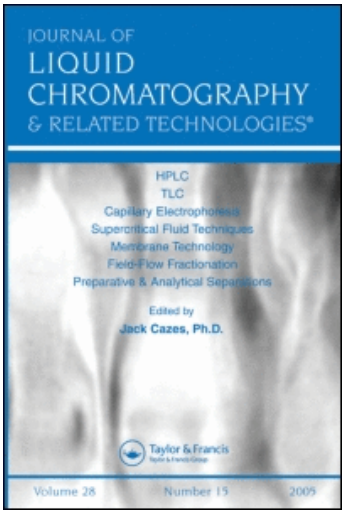

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ADVERSE EFFECTS OF LAG FILTERS USED FOR DETECTOR BASELINE NOISE SUPPRESSION IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Most detectors used for High Performance Liquid Chromatography employ a lag filter for suppression of baseline noise. This filter is characterised by its time constant, τ. At high values of τ (in excess of 0.5 sec), the lag filter seriously distorts the shape of a chromatographic peak, causing reduced peak heights, altered retention times and increased peak width. These changes result in significantly decreased resolution and decreased plate counts. These effects are investigated quantitatively.

INTRODUCTION

The efficiency of a column used for High Performance Liquid Chromatography (HPLC) is generally expressed as the number of theoretical plates, N, which is calculated according to the following general equation based on peak width and retention

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

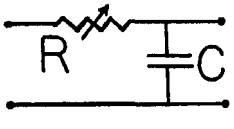
where A is a constant, t is the peak retention time and w is the peak width. The value of A is dependent on the manner used to measure w: A = 16 when w represents the baseline distance between tangents drawn to each side of the peak; A = 25 when w represents the peak width at 4.4% of peak height; A = 5.54 when w

represents the peak width at half height. The abovementioned calculation methods for N are called the 4σ method, the 5σ method and the half-height method, respectively.

The calculated value of N is used to assess the performance of a column when purchased and also as a routine assessment of its deterioration. Decreased values of N are usually attributed to voids or channels in the packing material or to extra-column effects such as excessive dead volume in connectors, tubing and the detector (1-3). Since the calculated value of N is often used as a criterion for deciding if a column is to be discarded, it is vital that the N value used truly reflects the performance of the column and is not perturbed by extra-column effects.

One factor which profoundly influences the calculated value of N is distortion of the chromatographic peak by electronic noise suppression circuitry in the detector. This distortion has been discussed in general terms (4) and we have recently given a series of recommendations for minimising peak distortion (5), however no quantitative data is available on the manner in which electronic noise filters influence calculated values of N.

A wide range of electronic noise filters is used in scientific instrumentation in order to reduce baseline noise. The most simple of these filters, and also the most widely used in UV detectors for HPLC, is the lag filter which is shown below



A fundamental property of this filter is its time constant, τ, which is defined as the time required for the amplitude of the output signal to reach 63.2% of the amplitude of a step input signal. The output signal reaches 99.7% of the input signal after a time equal to 5τ, which is sometimes referred to as the "response time". Time constant may be calculated using the formula:

$$\tau = {}^{1}/_{RC}$$

If R is in ohms and C is in Farads, then the units of τ are seconds.

In addition to smoothing the baseline by damping pulse noise, the lag filter also distorts peak shape by retarding the detector response to rapid signals occurring during the rise and fall of a peak. Since the calculation methods for N are based on peak shape, it is evident that N will be dependent to some extent on the time constant of the detector noise filter. The purpose of this paper was to quantitatively examine the relationship between τ and N and to illustrate other effects of peak distortion by lag filters.

EXPERIMENTAL

Instrumentation and Reagents

The liquid chromatograph used consisted of a Waters Model 6000 solvent pump, Model U6K injector, Model 450 variable wavelength detector and an Omniscribe Model B5217-1 recorder. When purchased, the detector was fitted with a continuously adjustable variable time constant control permitting the use of time constants from 0.1 to 1.1 secs. The time constant value indicated consisted of a residual component of 0.1 sec resulting from the detector electronics, together with a variable component (0-1 sec) resulting from the variable lag filter supplied in the detector. Because the variable filter 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 to 1.1 secs 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 (Spectrograde) and water was distilled using Millipore Milli Q Water Purification System. All other reagents were used as purchased without further purification.

Chromatographic Procedures

Separation by HPLC was accomplished using a μ-Bondapak C18 column (30 cm x 3.9 mm O.D., Waters Associates). The mobile phases used were acetonitrile-water (60:40 v/v) for the first test solution and (50:50 v/v) acetonitrile-water for the second test mixture. The mobile phase flow rate was 2.5 ml/min producing a back pressure of 2000 psi and the detector was operated at 254 nm with a sensitivity setting of 0.1 AUFS. The first test solution contained 26 mg acenaphthene in 100 ml of 60:40 v/v methanol/water, 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 40:40 v/v methanol/water. 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 inches/minute. Each data point on the figures shown in the results section is the mean of four replicate injections.

When the column was removed from the system, the inlet and outlet tubes to the column were 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 detector wavelength was 254 nm, the sensitivity was 0.1 a.u.f.s. and the recorder chart speed was 12 cm/min.

For the study of the effects of time constant on resolution, 10 μl of the second test mixture (described above) was injected, using a mobile phase of 70:30 CH_3CN:H_2O, a chart speed of 10 cm/min and a detector sensitivity of 0.1 a.u.f.s.

The number of theoretical plates, N, was calculated using the formula discussed previously, and the peak asymmetry, S, was calculated according to Saunders (6).

RESULTS AND DISCUSSION

Fig. 1 shows the effect of time constant on peak shape and height. As detector time constant was increased, peak height was

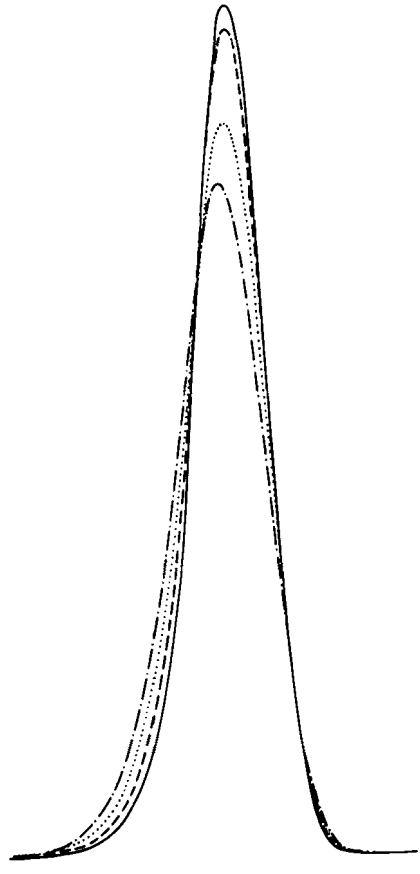


FIGURE 1. Effect of detector time constant on peak shape.
(—— τ = 0.1, --- τ = 0.4, ... τ = 0.7,
—·— τ = 1.1 sec).

reduced, peak asymmetry was increased and the position of the peak maximum was also altered.

Initially, the column was removed from the system and acetone in methanol was injected using a mobile phase of methanol and with varying settings of time constant. The retention times, asymmetry and heights of the resulting peaks were measured and the results

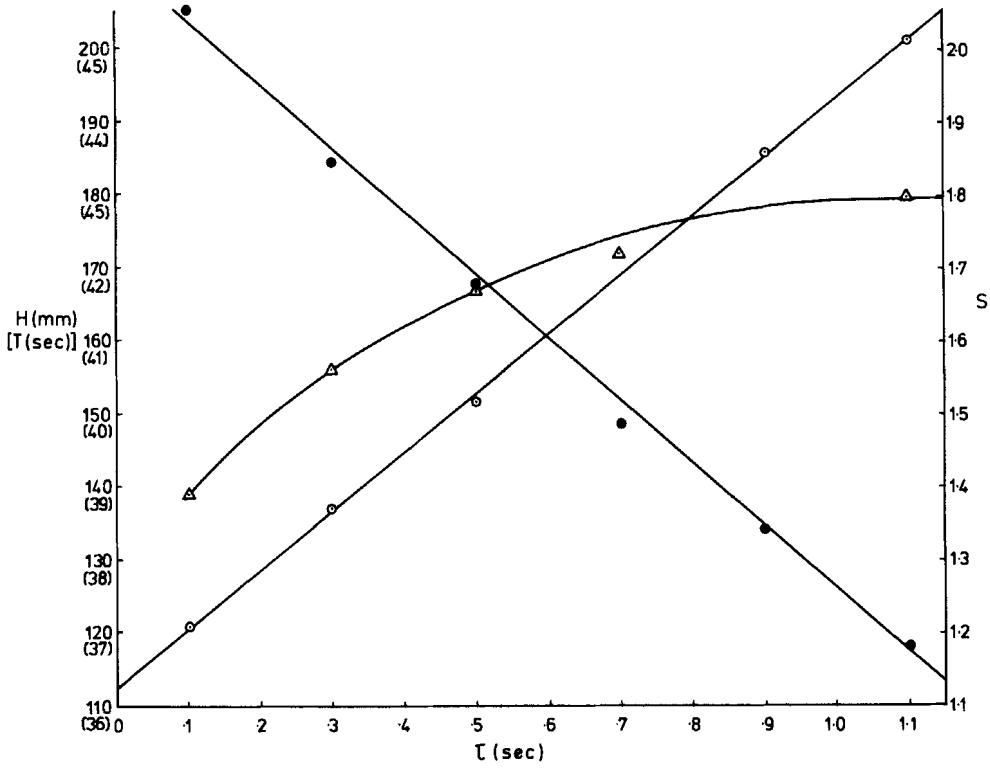


FIGURE 2. Variation of peak height (H), asymmetry (S) and retention time (T) with time constant. The column has been removed from the system.

Δ retention time (sec)
● peak height (mm)
O peak asymmetry

are shown in Fig. 2. Of particular interest was the behaviour of peak asymmetry, S, which when calculated with the column removed, is frequently used to assess peak broadening resulting from extra column effects. Fig. 2 shows that S almost doubled in value as τ was increased from 0.1 to 1.1 sec. The linear relationship between τ and S facilitates extrapolation to zero time constant to obtain

the true value of S, which can then be used to assess the magnitude of extra column effects.

The column was replaced in the chromatograph and a series of injections of acenaphthene was made using 60:40 v/v MeOH/water as mobile phase and with varying settings of τ. The resulting peaks were used for the measurement of peak heights, asymmetry and for calculation of the number of theoretical plates. The results are shown in Figs. 3 and 4 from which it can be seen that peak height again decreased linearly with time constant, while peak asymmetry increased with τ. It is noteworthy that a sharp increase in asymmetry resulted at higher values of τ, that is, those values preferred for heavy damping of baseline noise.

The number of theoretical plates (N) was calculated using each of the methods discussed previously and the results are shown in Fig. 4. As expected, the highest value of N at a particular value of τ was obtained using the half-height calculation method, while the lowest was obtained using the 5σ method. The plot of N *vs.* τ for each method was linear and the slopes of the plots were very similar, indicating that each method of calculation was influenced to the same extent by changes in τ. The linear relationship between N and τ for a lag filter facilitates negation of the effect of time constant on calculated efficiency by extrapolation to zero time constant. This can be achieved conveniently only when the detector is fitted with a calibrated, variable time constant control. This feature is not commonly provided. The data provided in Fig. 4 enables a correction factor to be applied to the plate count values obtained with detectors having a known, fixed time constant. While not supplying as reliable a result as that provided by extrapolation to zero time constant, this method does give a satisfactory estimate of N.

A suggestion commonly encountered in instruction manuals for detectors fitted with variable time constant controls is that the time constant used should not exceed one tenth of peak width. This rule of thumb is proposed to minimise peak distortion for

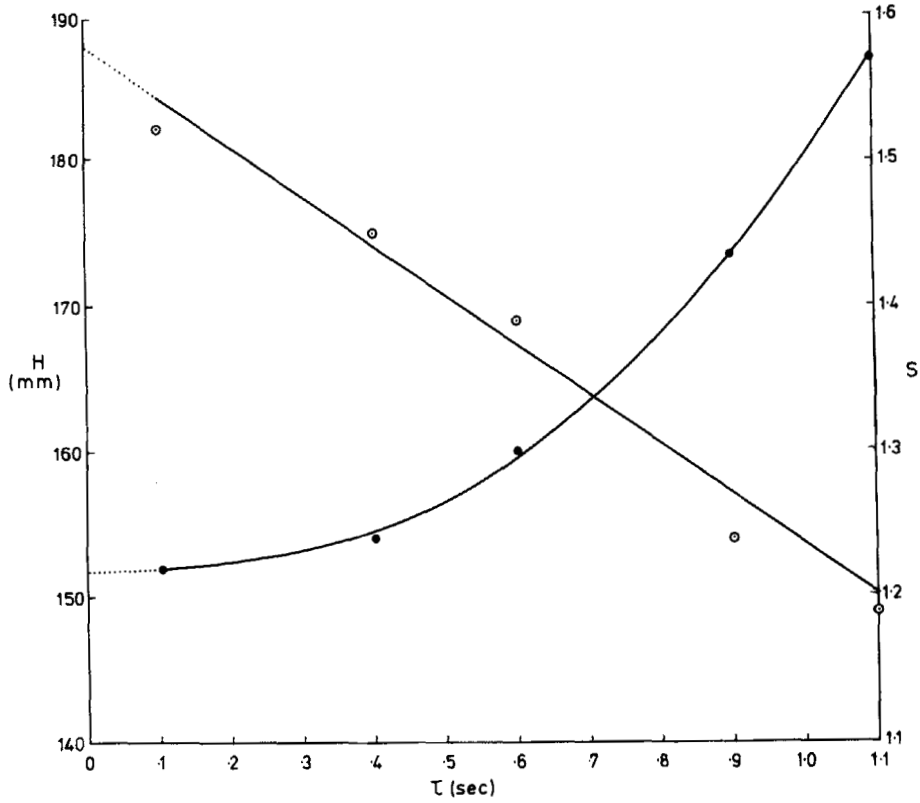


FIGURE 3. Variation of peak height (H) and asymmetry (S) with time constant. The column is included in the system.

O peak height
● peak asymmetry

general chromatography. We have examined the special case where the peak produced was to be used as the basis of an efficiency calculation. The results are shown in Fig. 5 which illustrates the dependence of normalised peak height and plate count on the ratio of τ to peak width w. It can be seen that values of τ/w in excess of 0.01 gave more than 10% error in the calculated value of N, which indicated that the abovementioned rule of thumb was most

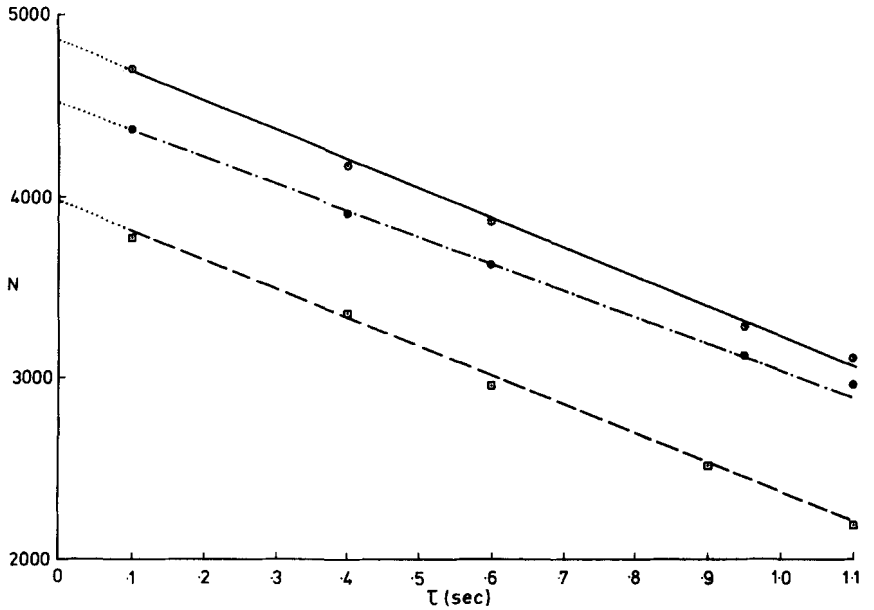


FIGURE 4. The dependence of the number of theoretical plates (N) on time constant.

O N calculated using peak width at half height
● N calculated using the 4σ method
⊡ N calculated using the 5σ method

inappropriate when the peak was to be used for an efficiency calculation. In this case a value of τ/w of 0.01 should be used.

The peak distortion introduced by a lag filter resulted in an apparent change in retention time, since the peak maximum was shifted towards longer retention. The magnitude of this effect is shown in Fig. 2. It is expected that shifts in peak maxima would be most pronounced for peaks eluting at very short retention times, since these peaks are usually sharp and so would be most prone to distortion by a lag filter. Indeed the same can be said of any of the measurable effects of increased time constant, such as reduced peak height, increased asymmetry and low values of the number of

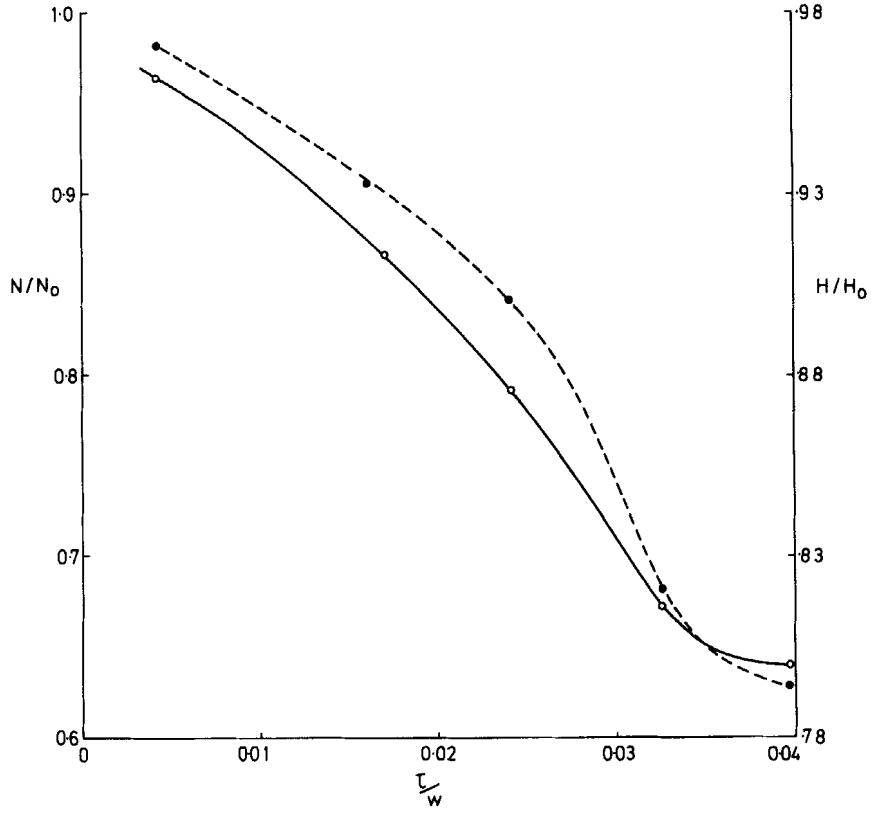


FIGURE 5. Variation of normalised peak height and number of theoretical plates with the ratio of time constant to peak width.

- ● H/H_o where H is peak height at time constant τ and H_o is peak height at zero time constant.
- o N/N_o where N is the number of theoretical plates calculated at time constant τ and N_o is the number of theoretical plates calculated at zero time constant.

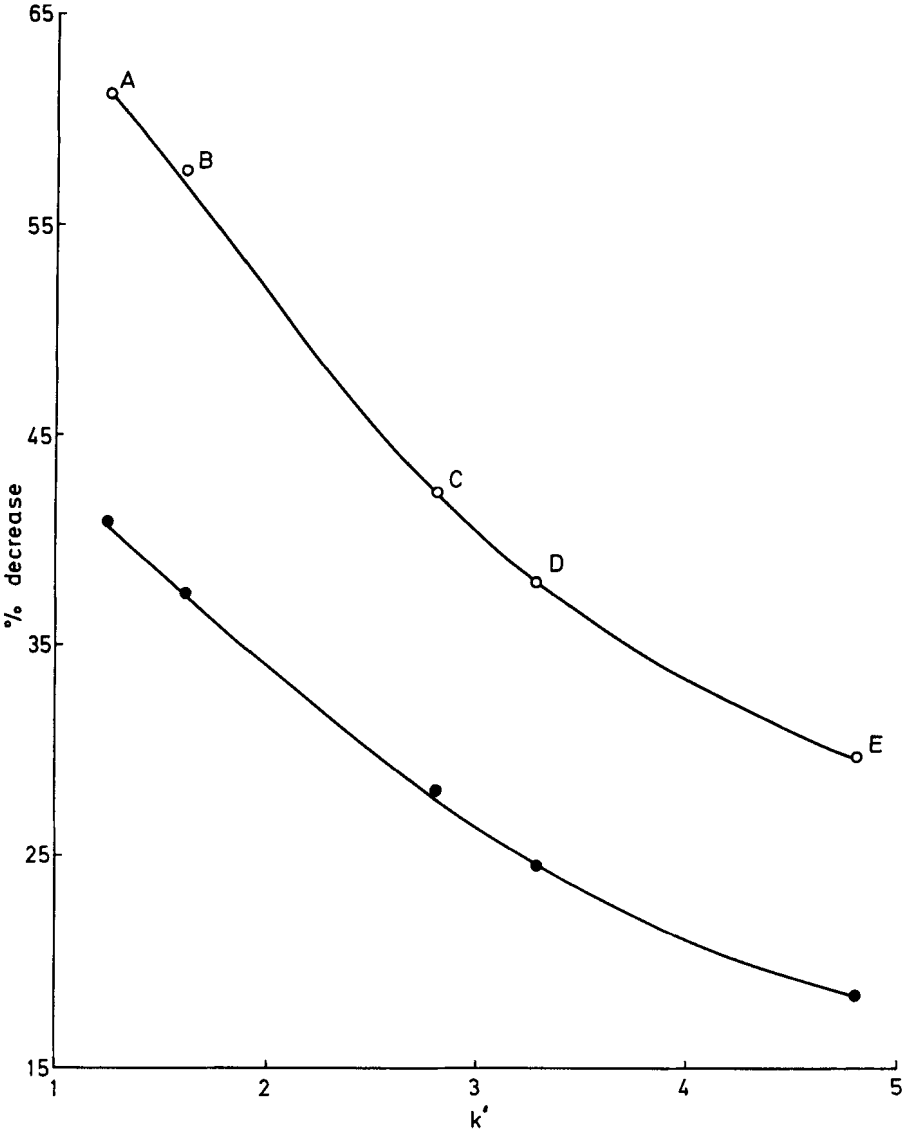


FIGURE 6. Percentage decrease in peak height (●) and number of theoretical plates (O) resulting from a time constant change from zero to 1.1 secs plotted against capacity factor of the peak (k').

(A = phenol, B = paracresol, C = 2,5-xylenol, D = anisole, E = phenetole).

theoretical plates. To investigate the relationship between capacity factor and time constant effects, a test solution was injected containing a group of solutes having capacity factors ranging from 1 to 5, using several time constants. The results are shown in Fig. 6, which was prepared by comparing the peak height and number of theoretical plates measured for each solute at zero time constant with that obtained at the maximum time

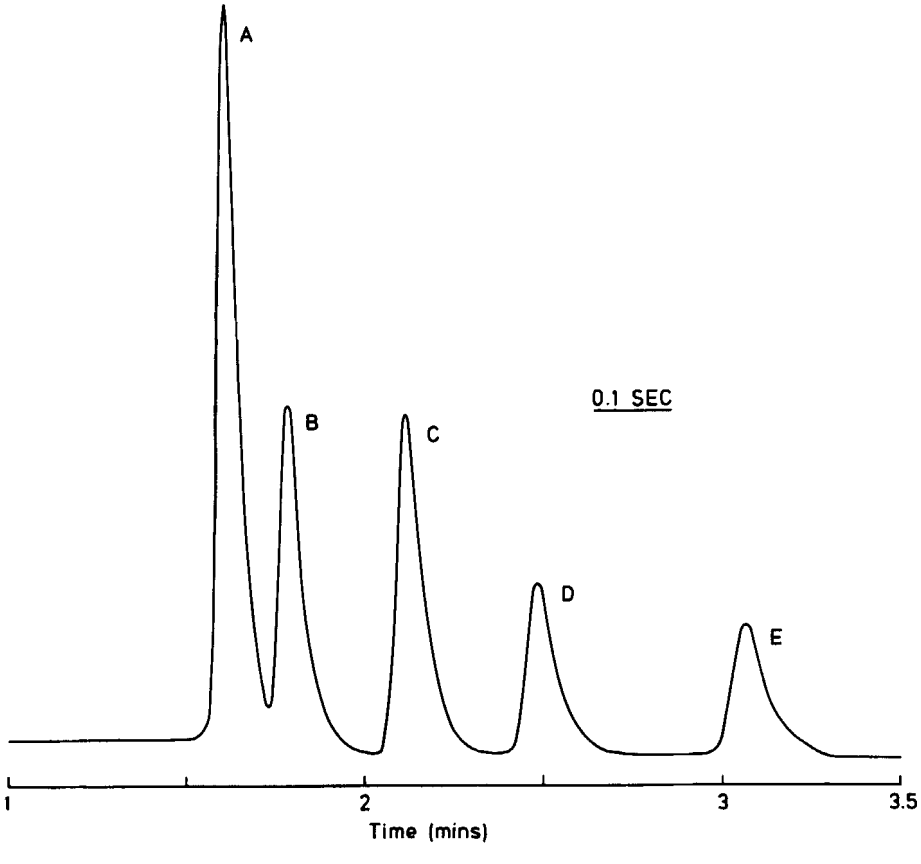


FIGURE 7. Effect of time constant on resolution. The solutes are listed in Fig. 6 and the time constant for each run is marked on the diagrams.

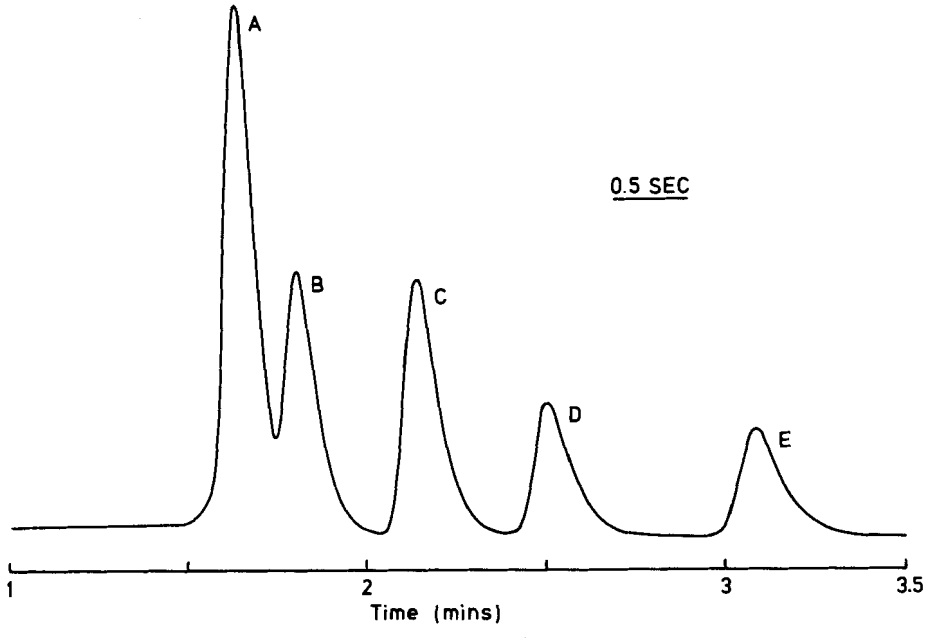


FIGURE 7B

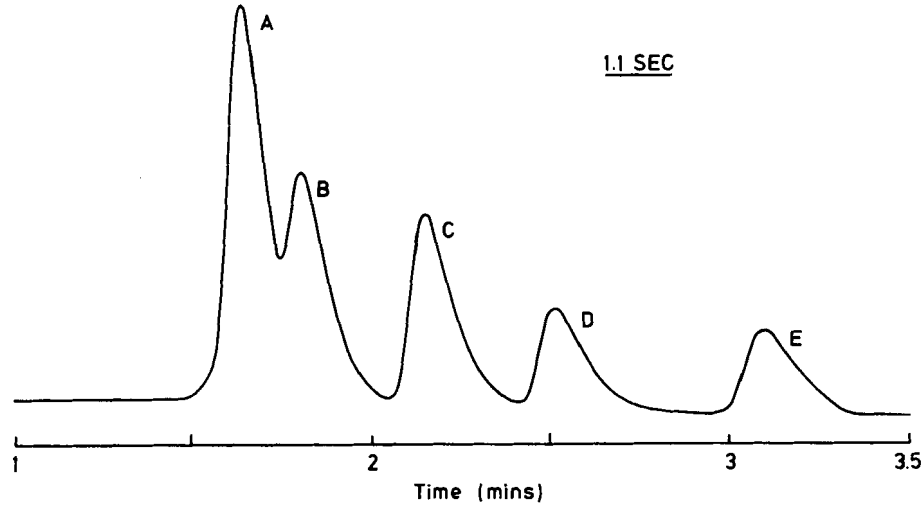


FIGURE 7C

constant of 1.1 secs. Fig. 6 shows that this increase in time constant produced smaller corresponding reductions in peak height and plate count value for longer retained peaks. Extrapolation of the plate count curve in Fig. 6 suggested that use of a solute with a capacity factor of 6 for efficiency calculations would give an error of about 25% for a detector time constant of 1.1 secs. Since many fixed time constant detectors use a time constant in the region of 1 sec and recommended procedures for efficiency calculations employ a solute with a capacity factor of 5-6, then errors in plate count of more than 25% can be expected with these detectors.

Resolution between adjacent peaks is also affected by the detector time constant due to peak distortion effects. This was investigated by injecting the same solute mixture as used for the previous section, however a higher percentage of organic modifier was used to bring the peaks closer together. The results are given in Fig. 7 which illustrates the apparent decrease in resolution between phenol and paracresol as the detector time constant was increased from 0.1 sec to 1.1 sec. Quantification of the areas of these two peaks was relatively simple at a time constant of 0.1 sec, but became very difficult at a time constant of 1.1 sec.

CONCLUSIONS

The use of a lag filter to suppress baseline noise in HPLC detectors results in appreciable peak distortion for values of time constant in excess of 0.1 sec. This peak distortion causes errors in the calculation of column efficiency and an apparent loss of resolution by the column. As a general rule, the time constant for a detector fitted with a lag filter should not exceed 0.1 sec for normal chromatographic use and should be increased only when baseline noise suppression is essential for the detection of trace amounts of solute. Values of the number of theoretical plates should be calculated at zero time constant where possible.

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