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EFFECTS OF PEAK TAILING ON COMPUTER OPTIMISATION PROCEDURES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

I. CHARACTERISTICS OF TAILED PEAKS UNDER OPTIMISATION CONDITIONS

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SUMMARY

The effects of peak tailing were studied by sequential generation of exponentially modified Gaussian peaks and observation of the trends between percentage area overlap, resolution, and the τ to σ ratio (where τ is the time constant and σ the standard deviation of the peak). It was found that only tailing of the first peak in a peak pair increased the percentage area overlap, whereas in other cases the area overlap was slightly decreased. When the heights of the component peaks in a peak pair were unequal, a swamping zone of total area overlap was introduced and the resolution (R_s) was required to attain a threshold value before separation in terms of area overlap was observed.

The dependence of peak tailing on the nature of solvent modifiers was determined experimentally, using five sympathomimetic amines as solutes and mixtures of methanol, acetonitrile, and tetrahydrofuran with water as solvents. When binary mobile phases of water with one modifier were considered, the amount of tailing exhibited by a peak was independent of the concentration of the modifier in the eluent, but was dependent on the nature of the modifier. For ternary mobile phases, formed from linear combinations of the above binary mixtures, the degree of tailing followed an approximately linear relationship with mobile phase composition.

INTRODUCTION

It is generally agreed that the occurrence of purely Gaussian peaks in high-performance liquid chromatography (HPLC) is rare[1–3], due to intra-column and extra-column factors which contribute to peak asymmetry. Intra-column factors include overload, system mismatch, and poorly packed columns, whereas extra-column asymmetry generally results from sample dispersion created by movement through the sample injector, detector cell, or the tubing between the column and these units. More detail on these factors can be found elsewhere[3–5]. The peak shapes occurring in HPLC may be accurately modelled by using the exponentially modified Gaussian (EMG) function. The EMG function consists of a Gaussian peak profile,

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convoluted with an exponential decay function. The development, characterisation, and experimental justification of this model has been thoroughly reviewed[2,3].

Computer optimisation procedures for HPLC can be defined as computer-based methods for selection of the optimal chromatographic conditions pertinent to a desired separation. Such procedures are currently undergoing intensive development and hold enormous potential for simplifying the process of chromatographic methods development. In most cases, computer optimisation is directed towards selection of the optimal mobile phase composition, and a crucial step in this process is the evaluation of the quality of chromatograms arising from all mobile phases to be considered in the optimisation. This evaluation is accomplished with the aid of a mathematical criterion which assigns a numerical value to a chromatogram as an indicator of its quality.

Many criteria are based on an initial calculation of the resolution between all the peak pairs in a chromatogram, which is then followed by appropriate mathematical manipulations in order to calculate the desired criterion. If this calculation of resolution is inaccurate, the error will be propagated to the calculation of the criterion and may result in the final selection of a mobile phase composition which is not optimal. Our experience suggests that optimisation procedures are prone to failure when one or more solutes in the mixture to be separated exhibit peak tailing. This failure arises from the fact that peaks are assumed to be Gaussian, and the area overlap of adjacent peaks is consequently assumed to be less than that existing in practice.

In this paper, we report a study of the effects that tailed peaks have on optimisation procedures in which criteria calculations are based predominantly on resolution values. Detailed calculations are made for the degree of area overlap between peak pairs which show tailing, and the manner in which peak tailing varies with changing mobile phase composition is also examined. These results form the foundation for a modified optimisation procedure which accommodates peak tailing, and this procedure is described in a subsequent paper[6].

THEORY

Computer generation of peak profiles

In order to study the area overlap characteristics of tailed peaks, it was necessary to computer-generate both Gaussian and tailed peaks. Gaussian peaks[7] were generated from eqn. 1

$$h(t) = h_{\max} \exp\left[\frac{-1}{2}\left(\frac{t - t_G}{\sigma}\right)^2\right] \tag{1}$$

where $h(t)$ is the peak height at time t, t_G is the time of the peak maximum, $h_{\max}$ is the maximum peak height, and σ is the standard deviation of the peak. Peak profiles were generated by plotting points at constant time intervals of 0.01 min, with $\sigma = 1$ min and a maximum peak height of 1 unit.

Tailed peaks were generated from the EMG function[2], according to the equation

$$h(t) = \frac{A}{\tau} \exp\left[\frac{1}{2}\left(\frac{\tau}{\sigma}\right)^2 - \frac{(t - t_G)}{\tau}\right] \int_{-\infty}^{Z} \exp\left[\frac{(-x^2/2)\mathrm{d}x}{\sqrt{2\pi}}\right] \tag{2}$$

where A is the peak area, and Z is given by the expression

$$Z = \left[\frac{(t - t_G)}{\sigma} - \frac{\sigma}{\tau}\right]$$

The EMG function produces a Gaussian profile which has been distorted by an exponential function of time constant τ. The distorted (tailed) peak has the same peak area as its parent Gaussian curve, but it is characterised by a shift in area toward the trailing edge of the peak. Other characteristics include a decrease in peak height and a shift of the peak maximum toward the trailing edge of the peak, relative to that observed for the parent Gaussian peak. The extent of tailing is determined by the ratio between the time constant, τ, and the standard deviation, σ, of the respective peak. The greater the τ to σ ratio, the greater the degree of tailing of the peak.

In order to generate EMG peaks with a large negative Z value ($Z < -4$) or small τ values, it was necessary (because of computer memory limitations) to use an approximate asymptotic relationship[8] for the error function integral. Thus, for the leading edge of the peak, eqn. 2 reduces to

$$h(t) = \frac{h_{\max}\sigma}{\tau} \exp\left[\frac{1}{2}\left(\frac{t - t_G}{\sigma}\right)^2\right] \Big/ \left[\frac{\sigma}{\tau} - \frac{(t - t_G)}{\sigma}\right] \tag{3}$$

Also, for large positive values of $Z (Z > 10)$, the following approximation[8] was used

$$h(t) = 2h_{\max}\frac{\sigma}{\tau}\sqrt{\frac{\pi}{2}} \exp\left[\frac{1}{2}\left(\frac{\sigma}{\tau}\right)^2 - \frac{(t - t_G)}{\tau}\right] \tag{4}$$

The τ to σ range covered in this study was 0 to 10.0.

Calculation of R_s and percentage area overlap

In this work, resolution (R_s) was calculated from

$$R_s = 2(t_{R_{i+1}} - t_{R_i})/(4\sigma_i + 4\sigma_{i+1}) \tag{5}$$

where t_R is the time of the peak maximum (*i.e.* t_G in the case of a Gaussian peak). For calculation of R_s when either peak was tailed, the time of the peak maximum, calculated from the EMG function, was used rather than t_G for the parent Gaussian function.

The percentage area overlap (%AO) between adjacent peaks was determined by first locating the point of intersection of the peaks, and then calculating the area fraction of each peak involved in the overlap, expressed as a percentage of total area

$$\%\text{AO} = 100\left(\frac{O_i}{A_i} + \frac{O_j}{A_j}\right) \tag{6}$$

where O_i is the area of peak i which is overlapped, and A_i is the total area of peak i. Symbols with the subscript j have similar meanings. The value of %AO was calculated by integration over discrete intervals of 0.01 min.

Fig. 1. Measurement of B/A at a peak height fraction of 0.1. A is the width of the leading half, and B is the width of the trailing half of the peak.

Experimental determination of τ to σ ratios

The simplest way to determine the degree of tailing exhibited by a peak is to measure the width of the leading half (A) and the trailing half (B) of the peak, and to calculate the ratio B/A. This ratio may be measured at any peak height level, but previous authors[2,9] have concentrated on 0.1, 0.3, and 0.5 of the maximum peak height, with the majority of their calculations centering on 0.1 of the peak height. The ratio calculated in this way is denoted $B/A_{0.1}$, and Fig. 1 shows the manner in which measurements are taken. Throughout this work, only $B/A_{0.1}$ ratios were used.

$B/A_{0.1}$ ratios can be converted to τ/σ values by using eqns. 7–10[9]. These equations can be used to construct a calibration curve for simple, graphic conversion of $B/A_{0.1}$ ratios to τ to σ values.

$$W = A + B \tag{7}$$

$$M = \frac{W^2}{\left[7.35 + 22.6 \exp\left(-0.708\frac{B}{A}\right)\right]} \tag{8}$$

$$\sigma = \frac{W}{\left(3.38\frac{B}{A} + 0.969\right)} \tag{9}$$

$$\tau = \sqrt{M - \sigma^2} \tag{10}$$

EXPERIMENTAL

Instrumentation

The peak generation work was performed on an Apple II+ microcomputer (Apple, Cupertino, CA, U.S.A.), containing a Digicard 80-column expansion card (Maclagan Wright Assoc., Eltham, Australia), fitted with twin floppy-disk drives, and interfaced to a BMC Model BX80 dot matrix printer (BMC International, Japan).

The liquid chromatograph consisted of a Waters Millipore (Milford, MA, U.S.A.) Model M590 pump, Model U6K injector, Model M441 UV detector (operated at 254 nm), and a Model M730 data module. The column was a Waters reversed-phase C_{18} Nova-Pak column (150 × 3.9 mm I.D.).

Reagents

Chromatography-grade solvents were used for the preparation of all mobile phases. The required amounts of organic solvents and water were measured by burette, and the resulting solution was mixed thoroughly, filtered through a 0.45-μm membrane filter, and degassed in an ultrasonic bath before use. All mobile phases contained 5 mM sodium heptanesulphonate (Ajax Chemicals, Sydney, Australia) and 1% acetic acid, and were prepared freshly as required.

Amphetamine sulphate (AMPH) was obtained from U.S.V. Pharmaceuticals (Sydney, Australia), and 2-phenethylamine hydrochloride (PEA) and phenylpropanolamine hydrochloride (PPA) were obtained from Sigma (St. Louis, MO, U.S.A.). N-Methyl-2-phenethylamine hydrochloride (N-MePEA) and N,N-dimethyl-2-phenethylamine hydrochloride (N,N-diMePEA) were synthesised and checked for purity as previously reported[10].

RESULTS AND DISCUSSION

Area overlap characteristics for peaks of equal height

R_s values should indicate the extent of separation between two adjacent peaks, as determined by the degree of area overlap present. When R_s is calculated by using eqn. 5, it is assumed that the peaks are Gaussian in shape. However, if peak tailing is present the R_s value calculated in the above manner may not be indicative of the actual degree of area overlap existing between the two peaks concerned. This area overlap, and not the calculated R_s value, is the decisive factor which ultimately determines the quality of separation.

The effects of peak tailing on area overlap were determined by generating peak pairs at different R_s values and with differing degrees of peak tailing. For a particular pair of peaks, the possible combinations of peak shapes are: (i) both peaks Gaussian (G–G); (ii) a Gaussian peak followed by a tailed peak (G–T); (iii) a tailed peak followed by a Gaussian peak (T–G); (iv) both peaks tailed (T–T). The area overlap for each of these possibilities is discussed below, using the particular case where the heights of both peaks under consideration are the same.

(i) Both peaks Gaussian (G–G). This case is used as a reference for the determination of changes in the amount of area overlap resulting from the tailing of one or both peaks. The results of the G–G peak combination are represented by solid lines in Figs. 2 and 3, and a zero value for the τ to σ ratio indicates a Gaussian peak. The solid line in Fig. 2 shows the relationship between R_s (calculated according to eqn. 5) and the percentage area overlap of the two Gaussian peaks in a peak pair. The area overlap is reduced to zero for $R_s > 1.5$.

(ii) Gaussian peak with a tailed trailing peak (G–T). Fig. 2 also shows the relationship between R_s and area overlap as the τ to σ ratio for the second eluted peak is increased. Comparison of these results for the G–G peak combination illustrates that tailing of the second peak results in a decrease in the amount of overlap of the peak pair. The extent of this reduction in area overlap increases with increased tailing of the second peak. This effect is more pronounced in the low resolution region (R_s = 0–0.6), where a larger fraction of the area of each peak is involved in overlap. At higher values of R_s (0.6–1.5), the effects of tailing of the second peak are much less evident.

(iii) Tailed peak with a trailing Gaussian peak (T–G). With this peak combination, the expected trend is an increase in area overlap, resulting from the area shift of the leading tailed peak toward the overlap region. This behaviour is illustrated in Fig. 3. The increase in area overlap is governed by the degree of tailing imposed on

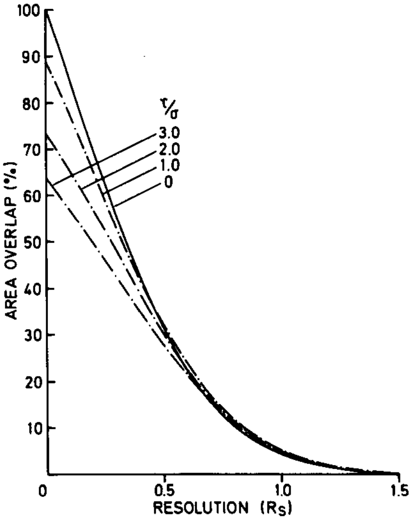


Fig. 2. Observed trends in percentage area overlap and resolution (R_s) for varying degrees of tailing imposed on the trailing peak in a peak pair (G–T case). First peak: Gaussian. Second peak: varying distortions. The solid line represents the case where both peaks are Gaussian (G–G).

the leading peak. At any given value of R_s, the area overlap of the peak pair increases with increased tailing of the leading peak. Thus, to obtain the same degree of area overlap for a T–G peak pair and a G–G peak pair, the distance between the peak maxima of the T–G pair must be increased. As the amount of tailing in the first peak is increased, greater R_s values are required to obtain zero area overlap. This can be

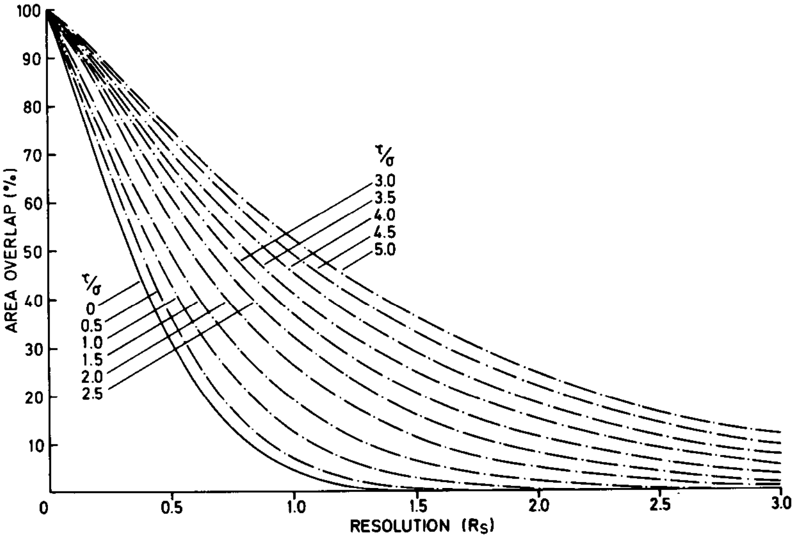


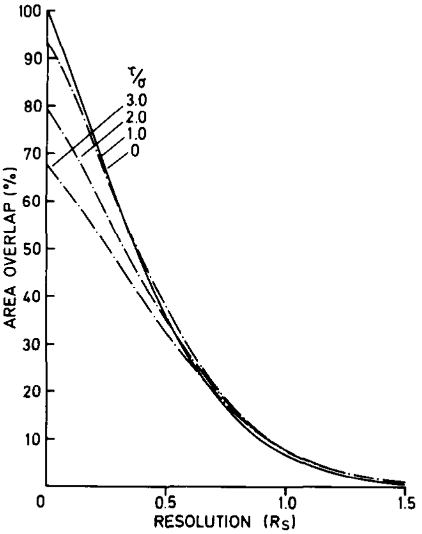
Fig. 3. Observed trends in percentage area overlap and resolution (R_s) for varying degrees of tailing imposed on the leading peak in a peak pair (T–G case). First peak: varying distortions. Second peak: Gaussian. The solid line represents the case where both peaks are Gaussian (G–G).

illustrated with an example from Fig. 3: the G–G pair of peaks are fully resolved at $R_s = 1.5$, but when the first peak is tailed by $\tau/\sigma = 1.0$, the R_s value needs to reach 2.15 before the T–G pair of peaks is fully resolved.

(iv) Both peaks tailed (T–T). In this instance, the trends in area overlap *versus* R_s (Figs. 4 and 5) were a mixture of those observed for the G–T and T–G cases above. At lower values of R_s (0–0.6), Fig. 4 shows that the amount of overlap was determined by the degree of tailing of the second peak. When the distortion of this second peak exceeded that of the first peak, there was a decrease in area overlap, resulting from simultaneous area shifts of the two peaks into and out of the overlap region. In the case shown in Fig. 4, the shift of the second peak out of the overlap region was greater than the shift of the first peak into the overlap region. At $R_s > 0.6$, the opposite trend occurred, and area overlap was slightly increased. Note that the solid line in Fig. 4 represents a peak combination where the leading peak has $\tau/\sigma = 0.5$ and the trailing peak is Gaussian.

Fig. 5 shows the case where the first peak has $\tau/\sigma = 3.0$. The solid line shows the area overlap characteristics when the trailing peak is Gaussian, and the broken line is the T–T case where $\tau/\sigma = 3.0$ for both peaks. Only very slight differences are observed between these two and this further illustrates that the τ to σ ratio for the first peak must exceed that of the second peak for increased area overlap to be observed.

From the above results it was concluded that peak tailing resulted in significantly increased area overlap of adjacent peak pairs only when the first peak was tailed. Tailing of the second peak often decreased the area overlap, and in instances where it



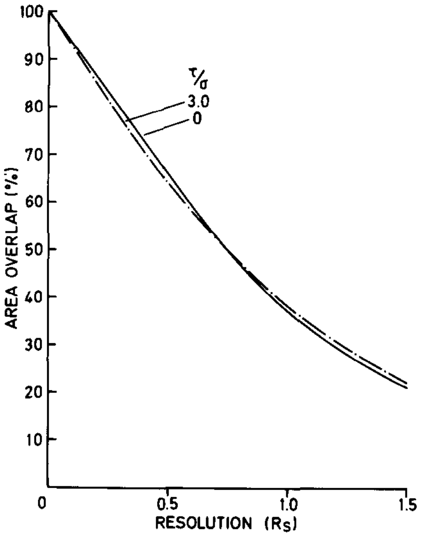


Fig. 4. Example of the observed trends in percentage area overlap and resolution (R_s) when both peaks in a peak pair are tailed (T–T case). In this instance, the tailing of the leading peak was maintained at $\tau/\sigma = 0.5$, while the degree of tailing of the trailing peak was varied as shown. The solid line represents the case where the trailing peak was Gaussian.

Fig. 5. Further example of the T–T case, where the first peak has $\tau/\sigma = 3.0$. The indicated values of τ/σ are for the second peak.

did not, the area overlap was similar to that observed when the second peak was Gaussian. These findings suggest that, when peak tailing is to be considered for optimisation purposes, increases in area overlap due to peak tailing can be assumed to arise from tailing of the first peak only.

Area overlap characteristics for peaks with varying height ratios

The above conclusions indicated that this section could be confined to consideration of the T–G case, the G–G combination again being used as a reference for comparison of results. Fig. 6 shows the relationship between R_s and area overlap for the G–G case, when the first peak is largest (Fig. 6A) and when the second peak is largest (Fig. 6B). Both figures show a noticeable swamping effect, in which R_s must be increased to a certain critical value (determined by the peak height ratio) before any separation occurs.

Similar results for the T–G case at several peak height ratios are given in Fig. 7. Fig. 7A shows that the swamping region extends to larger values of R_s when the height of the first peak is increased with respect to that of the second peak. On the other hand, when the second peak is larger, the area overlap increases with increasing τ to σ ratio of the first peak (Fig. 7B), in a similar manner to that observed for the T–G case for peaks of equal height (see Fig. 3).

The chief outcome of varying the relative peak heights is the introduction of the swamping zone at low values of R_s. However, in the region of most concern for optimisation purposes, *i.e.* R_s values of 1–1.5, the observations are very similar to those for the 1:1 peak height ratio.

Effects of solvent modifiers on peak shape

The foregoing results have highlighted the effects of peak tailing on the

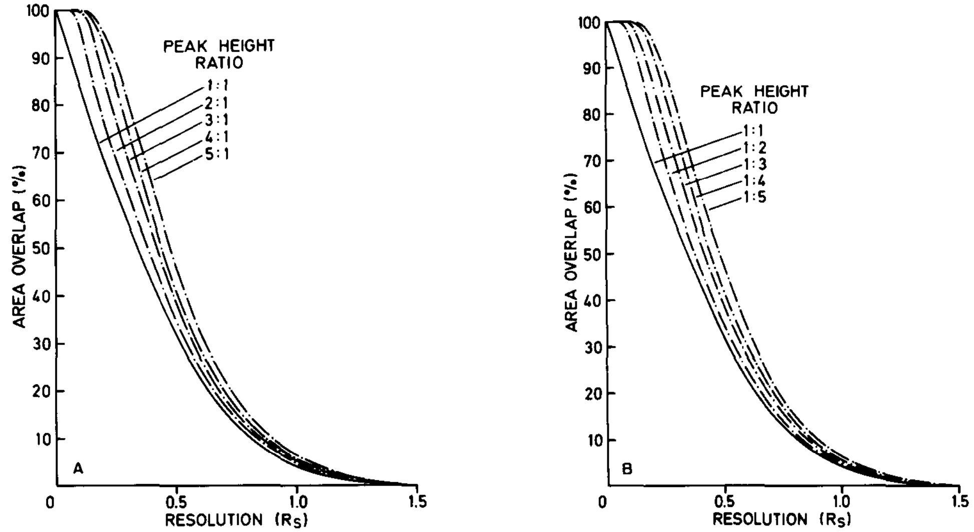


Fig. 6. Observed trends in percentage area overlap and resolution (R_s) for Gaussian peaks of varying peak height ratios, where (A) the leading peak height is largest, and (B) the trailing peak height is largest.

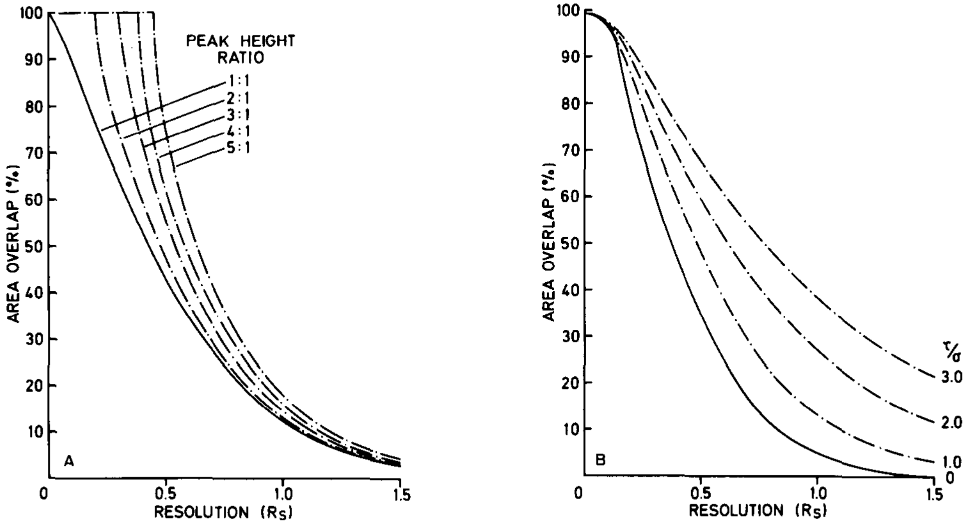


Fig. 7. Observed trends in percentage area overlap and resolution (R_s) for the T–G case with peaks of unequal height. (A) The τ to σ ratio for the first peak is constant (1.0) and peak height ratio is varied. (B) The peak height ratio is constant (1:2) and the τ to σ ratio for the first peak is varied. Both (A) and (B): second peak is Gaussian.

resolution between peak pairs. The extent of this effect is dependent on the tailing and on whether it is the leading or trailing peak that shows tailing. These findings have obvious ramifications for optimisation procedures in which Gaussian peak shapes are assumed. It is possible to devise a new optimisation criterion which gives due consideration to peak tailing effects, but the manner in which peak tailing varies under changing mobile phase conditions must first be established. Peak tailing can be ascribed to various extra-column and intra-column factors. Those factors which arise from purely physical effects can be expected to remain constant in the course of an optimisation procedure, but this may not be true of chemical factors, such as interaction of the solutes with silanol sites on the stationary phase.

Optimisation procedures for reversed-phase liquid chromatography (RPLC) generally involve the exploitation of selectivity effects occurring when mobile phases are prepared from combinations of water with methanol, acetonitrile, and tetrahydrofuran (THF). Binary (water plus one modifier), ternary (water plus two modifiers), or quaternary (water plus all three modifiers) mobile phases may be used, although ternary mobile phases usually provide sufficient selectivity to separate most sample mixtures. In order to correct successfully for peak tailing effects in optimisation processes, it is therefore necessary to examine the variation of peak tailing with changing mobile phase composition, at least for binary and ternary mobile phases.

To study these effects, careful peak shape measurements were made on five sympathomimetic amines known to exhibit tailing. The peak profile of each solute was recorded in a variety of binary mixtures of water with methanol, acetonitrile or THF. The concentration of organic solvents was varied in order to obtain peak profiles of each solute covering the approximate capacity factor range $1 \leqslant k' \leqslant 10$, which is the retention range usually applicable to optimisation procedures. Peak asymmetry was measured, and τ/σ values were calculated for each peak from eqns. 7–10. The τ to σ ratios obtained for all five solutes are listed in Table I and a graphic representation of the results for one of the solutes (N,N-diMePEA) is presented in Fig. 8. This figure shows that τ to σ ratios for a particular binary mobile phase were essentially constant as the eluotropic strength of the mobile phase (and hence the capacity factor of the solute) was varied. However, striking differences in τ to σ ratios existed between the different modifiers. They can be attributed to differences in the interaction of these modifiers with the solutes and the stationary phase. For the series of solutes studied, tailing was least in the THF–water binary and greatest in the acetonitrile–water binary mobile phases. It is expected that this trend would vary with the nature of the solutes involved. The slight scatter of points in Table I is due to the error inherent in the *B*/*A* measurements. It should be pointed out that the accuracy of these measurements decreases with decreasing capacity factor.

The variation of peak tailing was also investigated, using ternary mobile phase

TABLE I

VARIATION OF τ TO σ RATIOS IN BINARY MOBILE PHASES OF WATER WITH METHANOL, ACETONITRILE OR THF

Solute identities are given in the Experimental section.

Solute	*Methanol*		*Acetonitrile*		*THF*	
	k'	τ/σ	k'	τ/σ	k'	τ/σ
PPA	0.6	2.6	0.9	3.1	1.1	2.3
	1.0	2.8	1.4	3.1	2.7	2.3
	1.3	2.6	3.5	2.9	3.9	2.3
	7.1	2.5	4.6	2.9	4.4	2.1
	11.7	2.8	11.9	3.1	9.5	2.1
PEA	0.6	3.4	1.1	5.1	1.2	2.6
	1.0	3.4	1.9	5.0	2.7	2.8
	1.4	3.5	4.6	4.8	4.4	2.8
	7.5	3.7	6.1	5.1	9.9	2.5
	12.8	3.7	15.5	4.8	14.0	2.9
N-MePEA	0.6	2.9	1.4	8.4	1.1	3.2
	1.1	3.2	2.4	8.8	2.4	3.2
	1.4	3.1	5.7	8.8	4.0	2.9
	7.3	3.1	7.5	8.7	8.8	2.9
	13.1	3.2	9.6	8.8	12.6	3.2
N,N-diMePEA	0.6	4.8	1.7	9.6	1.0	3.2
	1.2	4.7	2.8	9.5	2.2	3.4
	1.3	4.8	3.0	9.3	3.6	3.2
	6.8	4.7	7.4	9.5	7.9	3.1
	12.3	5.0	9.7	9.5	11.6	3.5
AMPH	0.6	3.7	1.4	6.5	1.4	2.1
	1.5	3.4	2.3	6.4	3.5	2.2
	1.7	3.4	2.5	6.4	6.1	1.8
	7.8	3.5	6.8	6.4	9.8	2.0
	11.4	3.5	9.1	6.4	14.7	2.1

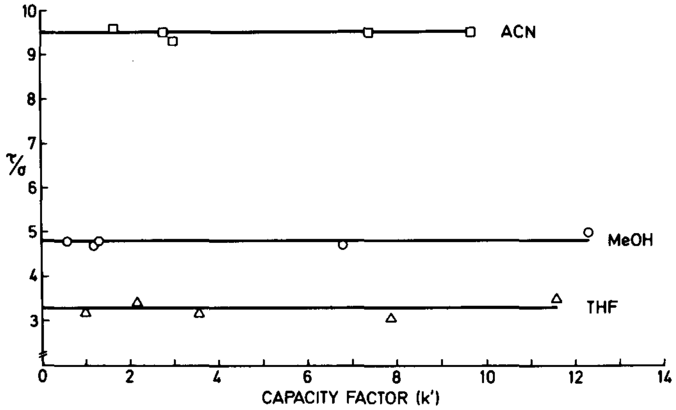


Fig. 8. Variation of τ to σ ratios for N,N-diMePEA in binary mobile phases. See Table I for information on other solutes studied. ACN = Acetonitrile; MeOH = methanol.

compositions. A hypothetical optimisation search area was defined by the three binary mobile phase compositions: methanol–water (60:40), acetonitrile–water (30:70) and THF–water (30:70). τ/σ values for the five sympathomimetic amines were determined at these three binary compositions and at ternary compositions produced from linear combinations of the binary mobile phases. Our aim was to ascertain the manner in which peak tailing varied over a typical optimisation search area.

Table II lists the mobile-phase compositions examined, together with the resultant τ/σ values for the five solutes, whilst Fig. 9 provides a graphic depiction of the results for PEA and N,N-diMePEA, which are representative of the group of solutes used. Fig. 9 shows that for ternary mobile phases τ/σ varied in an approximately linear manner with mobile phase composition. In some circumstances a slight curvature in

TABLE II

VARIATION OF τ TO σ RATIOS IN TERNARY MOBILE PHASES

Solute identities are given in the Experimental section.

Mobile phase composition				*τ to σ ratio*				
Methanol	*Acetonitrile*	*THF*	*Water*	*PPA*	*PEA*	*N-MePEA*	*N,N-diMePEA*	*AMPH*
60	0	0	40	2.7	3.5	3.1	4.8	3.5
45	7.5	0	47.5	2.9	3.5	4.1	5.8	3.5
30	15	0	55	3.2	4.4	4.8	7.1	3.8
15	22.5	0	62.5	3.2	4.4	6.4	8.1	5.0
0	30	0	70	3.0	5.0	8.7	9.5	6.4
0	22.5	7.5	70	3.1	4.8	7.3	7.1	5.3
0	15	15	70	2.8	3.7	5.4	5.3	3.9
0	7.5	22.5	70	2.5	3.4	4.4	4.4	2.8
0	0	30	70	2.2	2.7	3.1	3.3	2.0
15	0	22.5	62.5	2.5	2.5	2.8	3.8	2.3
30	0	15	55	2.9	2.9	3.2	4.4	2.5
45	0	7.5	47.5	2.9	3.2	3.2	4.5	2.8
60	0	0	40	2.7	3.5	3.1	4.8	3.5

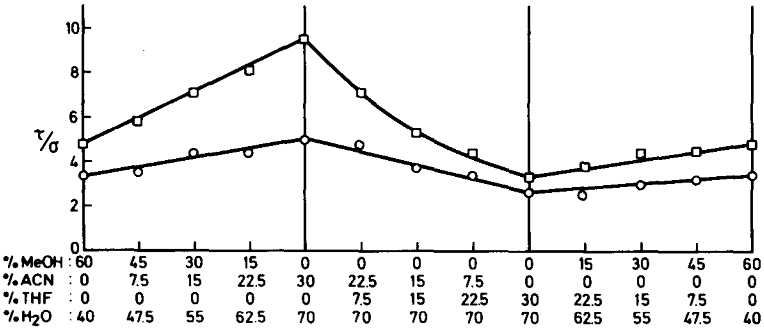


Fig. 9. Variation of τ/σ for PEA (○) and N,N-diMePEA (□) in ternary mobile phases. Data for further solutes can be found in Table II. MeOH = Methanol; ACN = acetonitrile.

the plots was observed, *e.g.*, N,N-diMePEA in acetonitrile–THF–water ternary mobile phases, but it is difficult to conclude whether this is a true trend or a propagation of the B/A measurement error mentioned earlier. In either case, the curvature is not extensive, and the relationship between τ/σ and mobile phase composition can be approximated by a straight line.

Suitability of the EMG model

The large number of chromatographic peaks generated in the above studies of the effect of solvent modifier on peak shapes provided ample data for assessment of the suitability of the EMG model for the simulation of tailed peaks. For a number of the experimental peaks, the width of the leading half (A) and the trailing half (B) were measured and used to calculate τ/σ using eqns. 7–10. The values so determined were then used to generate EMG peaks of the same height as the experimental peaks. When the experimental and computer-generated peaks were overlaid, it was found that they were virtually superimposable in every case examined. The closeness of fit obtained with the EMG model was considered to be strong evidence of the suitability of this model for simulating experimentally obtained tailed peaks.

CONCLUSIONS

This study has provided quantitative information on the degree of area overlap existing between peak pairs in which tailing is evident. From a comparison of tailed peak pairs with Gaussian peaks having the same retention times, it has been shown that area overlap in the tailed case is increased significantly, relative to the Gaussian peaks, only when the first peak in a pair is tailed. For the solutes tested, the degree of tailing exhibited was constant in binary mobile phases of water and one of the solvent modifiers methanol, acetonitrile or THF, regardless of the eluotropic strength of the mobile phase used. Differences were noted in the degree of tailing observed for the same solute when the nature of the modifier in the binary mobile phase was varied. The τ to σ ratios were found to follow an approximately linear relationship with the mobile phase composition for ternary mobile phases formed from linear combinations of the binary compositions referred to above.

These results have direct application to computer optimisation procedures for

HPLC in that they provide a basis for predicting the manner in which peak tailing can be expected to vary over the range of mobile phases to be considered in the optimisation process. In turn, this knowledge permits accurate calculation of the degree of area overlap of each sample peak with its neighbouring later-eluted peak, and therefore provides a realistic assessment of the separation achieved. A new optimisation procedure which incorporates these features is discussed elsewhere[6].

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