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RESOLUTION IN NON-LINEAR CHROMATOGRAPHY

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SUMMARY

The available expressions for the statistical moments in non-linear chromatography are used to formulate an approximate expression for the resolution function in terms of column parameters.

INTRODUCTION

Chromatographic peaks resulting from a non-linear distribution isotherm are in general, asymmetrical. It has been shown that, if these peaks are fitted by bi-Gaussian distribution functions[1], the resolution function (see Fig. 1)

$$R_{msa} = \frac{\zeta_2 - \zeta_1}{2(\sigma_{12} + \sigma_{21})} \tag{1}$$

may be related to the efficiency of the separation[2]. (The efficiency criterion is taken as the impurity ratio of the smaller peak[2].) The aim of the present study is to solve the remaining problem, namely, to rewrite the resolution function in terms of column parameters. For this purpose the function R_{nl} defined by

$$R_{nl} = \frac{\langle\zeta\rangle_2 - \langle\zeta\rangle_1}{2(\sigma_1 + \sigma_2)} \tag{2}$$

was used. R_{nl} approximates R_{msa} for most cases likely to be encountered in practice

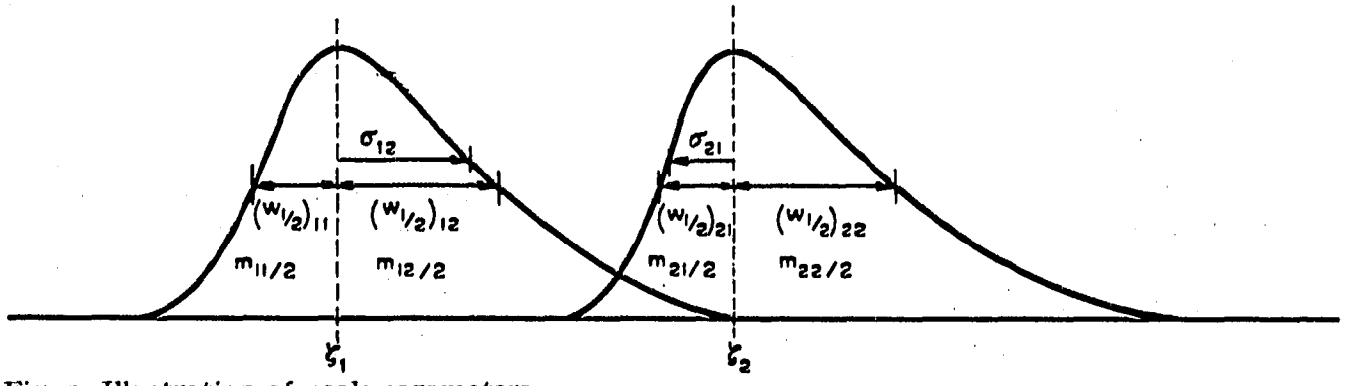

Fig. 1. Illustration of peak parameters.

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(see APPENDIX I) and is particularly convenient since analytical expressions for the moments in non-linear chromatography are available[3–5].

THEORY

According to the references cited above the zero'th and first moments may be approximated by[3]

$$m_0 = m_i \left\{ 1 + \frac{\lambda C_i}{2\sqrt{2}} \left[1 - \frac{1}{\left(1 + \frac{4\pi D_e t}{w_i^2} \right)^{\frac{1}{2}}} \right] \right\} \tag{3}$$

and

$$\langle \zeta \rangle = Ut - \frac{\lambda C_i U w_i^2}{4\sqrt{2\pi} D_e} \cdot \frac{m_i}{m_0} \left\{ \left[1 + \frac{4\pi D_e t}{w_i^2} \right]^{\frac{1}{2}} - 1 \right\} \tag{4}$$

where

$$U = u/(1 + k_1) \tag{5}$$

$$\lambda = 2k_2/(1 + k_1) \tag{6}$$

and k_1 and k_2 are defined by the non-linear isotherm

$$C_s = \varepsilon k_1 C + \varepsilon k_2 C^2 \tag{7}$$

λC_i is the non-linearity parameter, C_i is the initial solute concentration at the inlet in the mobile phase and m_i the number of moles.

The second moment will be approximated by[4]

$$\sigma_{ni}^2 = \sigma_i^2 \frac{m_i}{m_0} \left\{ 1 + \frac{\sigma_n^2}{\sigma_i^2} \right\} \tag{8}$$

where the linear contribution, σ_i^2, is given by

$$\sigma_i^2 = 2D_e t + \sigma_i^2 \tag{9}$$

and the non-linear contribution σ_n^2 is, for convenience, written in the form

$$\sigma_n^2 = \sigma_n^2(D) + \sigma_n^2(u) \tag{10}$$

$\sigma_n^2(D)$ and $\sigma_n^2(u)$ are contributions respectively associated with diffusion and flow and are given by

$$\sigma_n^2(D)/\sigma_i^2 = \frac{\lambda C_i}{2\sqrt{2}} \cdot \frac{y}{1 + y} - \frac{5\lambda C_i}{4\sqrt{2}} \cdot \left[\frac{1}{(1 + y)^{\frac{1}{2}}} - \frac{1}{(1 + y)} \right] - \frac{(\lambda C_i)^2}{8} \cdot \left[\frac{1}{(1 + y)^{\frac{1}{2}}} - \frac{1}{(1 + y)} \right] + \frac{(\lambda C_i)^2}{16} \frac{\ln (1 + y)}{1 + y} \tag{11}$$

and

$$\sigma_n^2(u)/\sigma_1^2 = \frac{\Omega}{8\pi}\cdot\frac{\lambda C_1 U w_1}{D_e}\frac{1}{2}\cdot\left[\frac{2+y}{1+y} - \frac{2}{(1+y)^{\frac{1}{2}}}\right] \quad (12)$$

where

$$y = 4\pi D_e t/w_1^2$$

Ω is a semi-empirical fitting parameter ($\Omega/8\pi \sim 10^{-2}$).

The above expressions for the moments were derived under the assumption of an equivalent Gaussian input[6] with the inlet variance given by

$$\sigma_1^2 = w_1^2/2\pi \quad (13)$$

w_1 is the width of the corresponding plug input.

In the analysis of refs. 3–5 the effective diffusion coefficient D_e was regarded as containing only terms deriving from the mobile phase. Since the nature of the stationary phase contributions is similar, however (*e.g.* ref. 7), it is expected that the approximation will also apply in this case and D_e will, in the present study, be regarded as containing contributions from both phases.

In order to simplify the above expressions for the statistical moments, the assumption of large y (either large t or small w_1 or both) will now be made, *i.e.*

$$2D_e t \gg \sigma_1^2 = w_1^2/2\pi \quad (14)$$

so that

$$\sigma_1^2 \approx 2D_e t \quad (15)$$

To the first order in λC_1, the expressions for m_0 and $\langle\zeta\rangle$ become

$$m_0 = m_1\left\{1 + \frac{\lambda C_1}{2\sqrt{2}}\right\} \quad (16)$$

and

$$\langle\zeta\rangle = Ut - \frac{\lambda C_1 U w_1}{2\sqrt{2\pi D_e}}\cdot t^{\frac{1}{2}} \quad (17)$$

In the limit of large y the non-linear contributions to the variance are

$$\lim_{y\to\infty} \sigma_n^2(D)/\sigma_1^2 = \lambda C_1/2\sqrt{2} \quad (18)$$

and

$$\lim_{y\to\infty} \sigma_n^2(u)/\sigma_1^2 = \frac{\Omega}{8\pi}\cdot\frac{1}{2}\cdot\left[\frac{\lambda C_1 U w_1}{D_e}\right]^2 \quad (19)$$

Since

$$U/D_e = 2/H_1 \quad (20)$$

where

$$H_1 = \sigma_1^2/Ut, \quad (21)$$

Eqn. 19 becomes

$$\lim_{y\to\infty} \sigma_n^2(u)/\sigma_1^2 = 2\frac{\Omega}{8\pi}\left[\frac{\lambda C_i w_i}{H_1}\right]^2. \tag{22}$$

For

$$H_1 \sim 0.14 w_i \lambda C_i \tag{23}$$

$\sigma^2(u)$ and σ_1^2 become equal while for H_1 smaller than this value, the velocity term is the dominant one.

It follows from eqns. 2 and 8 that, for $m_1/m_0 \sim 1$ for both components,

$$R_{n1} = \frac{\langle\zeta\rangle_2 - \langle\zeta\rangle_1}{2\sigma_1}\left[\left(1 + \frac{(\sigma_n^2)_1}{\sigma_1^2}\right)^{\frac{1}{2}} + \left(1 + \frac{(\sigma_n^2)_2}{\sigma_1^2}\right)^{\frac{1}{2}}\right]^{-1} \tag{24}$$

and for $\sigma_2 = \sigma_1$,

$$R_{n1} = \frac{\langle\zeta\rangle_2 - \langle\zeta\rangle_1}{4\sigma_1}\left[\left(1 + \frac{(\sigma_n^2)_1}{\sigma_1^2}\right)\right]^{-\frac{1}{2}} \tag{25}$$

R_{n1} will now be calculated for the instant when the mean of peak 1 reaches the detector, *i.e.* when

$$\langle\zeta\rangle_1 = l \tag{26}$$

The time corresponding to eqn. 26 is therefore the solution of the equation (see eqn. 17)

$$t - B(\lambda C_i)_1 t^{\frac{1}{2}} - l/U_1 = 0 \tag{27}$$

where the subscript 1 denotes component (peak) 1 and the notation k_{11} (for k_1) and k_{21} (for k_2) is used (see LIST OF SYMBOLS). B is given by

$$B = w_i/(2\sqrt{2\pi D_e}) \tag{28}$$

(The subscript 1 is omitted for B, since it is assumed that D_e will be approximately the same for two solutes eluted close to each other.) The solution of eqn. 27 is

$$t^{\frac{1}{2}} = \tfrac{1}{2}B(\lambda C_i)_1\left\{1 + \sqrt{1 + \frac{4l}{B^2(\lambda C_i)_1^2 U_1}}\right\} \tag{29}$$

i.e.

$$t = \frac{l}{U_1} + \tfrac{1}{2}B^2(\lambda C_i)_1^2\left\{1 + \sqrt{1 + \frac{4l}{B^2(\lambda C_i)_1^2 U_1}}\right\} \tag{30}$$

$\langle\zeta\rangle_2$ corresponding to $\langle\zeta\rangle_1 = l$ is obtained by using eqn. 30 for t in the expression for $\langle\zeta\rangle_2$, *i.e.*

$$\langle\zeta\rangle_2 = \frac{U_2}{U_1}l + \tfrac{1}{2}B^2(\lambda C_i)_1 U_2\{(\lambda C_i)_1 - (\lambda C_i)_2\}\left\{1 + \sqrt{1 + \frac{4l}{B^2(\lambda C_i)_1^2 U_1}}\right\} \tag{31}$$

so that the distance between the means of the peaks is:

$$\langle\zeta\rangle_2 - \langle\zeta\rangle_1 = l\left(\frac{U_2}{U_1} - 1\right) + \tfrac{1}{2}B^2(\lambda C_i)_1^2(1 - \beta)\left\{1 + \sqrt{1 + \frac{4l}{B^2(\lambda C_i)_1^2 U_1}}\right\}$$

i.e.

$$\langle\zeta\rangle_2 - \langle\zeta\rangle_1 = \frac{lk_{12}(\alpha - 1)}{1 + k_{12}} + \tfrac{1}{2}B^2(\lambda C_i)_1^2 U_2(1 - \beta)\left\{1 + \sqrt{1 + \frac{4l}{B^2(\lambda C_i)_1^2 U_1}}\right\} \tag{32}$$

where

$$\alpha = k_{11}/k_{12} \tag{33}$$

and

$$\beta = (\lambda C_i)_2/(\lambda C_i)_1 \tag{34}$$

If H_1 is defined as the plate height for component 1, eluted according to a linear isotherm, then (see eqn. 21)

$$\sigma_1^2 = H_1 U_1 t \tag{35}$$

and (from eqns. 15 and 35)

$$D_e = \frac{H_1}{2} U_1 \tag{36}$$

$U_1 t$ will now, according to eqn. 30, be approximated by l so that

$$\sigma_1^2 = H_1 l \tag{37}$$

From eqns. 28, 32, 36 and 37 it then follows that

$$\frac{\langle\zeta\rangle_2 - \langle\zeta\rangle_1}{\sigma_1} = \sqrt{\frac{l}{H_1}}\frac{k_{12}(\alpha - 1)}{(1 + k_{12})} + \\ + \frac{w_i^2}{8\pi}\frac{1}{H_1^{\frac{1}{2}} l^{\frac{1}{2}}}\left(\frac{1 + k_{11}}{1 + k_{12}}\right)(\lambda C_i)_1^2(1 - \beta)\left[1 + \left(1 + \frac{16\pi}{w_i^2} lH_1 \frac{1}{(\lambda C_i)_1^2}\right)^{\frac{1}{2}}\right] \tag{38}$$

An approximate expression for R_{n1} may therefore be obtained by using eqn. 38 together with the appropriate expressions for the non-linear σ^2 contributions in either eqn. 24 or 25. At large values of y, R_{n1} is approximated by (eqns. 18, 22 and 24)

$$R_{n1} = \frac{\langle\zeta\rangle_2 - \langle\zeta\rangle_1}{2\sigma_1}\left\{\left[1 + \frac{(\lambda C_i)_1^2}{2\sqrt{2}} + 2\frac{\Omega}{8\pi}\left(\frac{(\lambda C_i)_1 w_i}{H_1}\right)^2\right]^{\frac{1}{2}} + \right. \\ \left. + \left[1 + \frac{(\lambda C_i)_2^2}{2\sqrt{2}} + 2\frac{\Omega}{8\pi}\left(\frac{(\lambda C_i)_2 w_i}{H_1}\right)^2\right]^{\frac{1}{2}}\right\}^{-1} \tag{39}$$

for $\sigma_2 \neq \sigma_1$, or by eqns. 18, 22 and 25

$$R_{n1} = \frac{\langle\zeta\rangle_2 - \langle\zeta\rangle_1}{4\sigma_1}\left\{1 + \frac{(\lambda C_i)_1^2}{2\sqrt{2}} + 2\frac{\Omega}{8\pi}\left(\frac{(\lambda C_i)_1 w_i}{H_1}\right)^2\right\}^{-\frac{1}{2}} \tag{40}$$

for $\sigma_2 = \sigma_1$, with $(\langle\zeta\rangle_2 - \langle\zeta\rangle_1)/\sigma_1$ given by eqn. 38.

An improved approximation for R_{n1} may be obtained by approximating l, *i.e.* $\langle\zeta\rangle_1$ (see eqn. 4), by

$$l = U_1 t - \frac{(\lambda C_i)_1 U_1 w_i}{2\sqrt{2\pi D_e}} t^{\frac{1}{2}} + \frac{(\lambda C_i)_1 U_1 w_i^2}{4\sqrt{2\pi D_e}} \tag{41}$$

from which t follows as

$$t = \frac{l}{U_1} - \sqrt{2} B^2 (\lambda C_i)_1 + \tfrac{1}{2} A^2 (\lambda C_i)_1^2 \left\{ 1 + \sqrt{1 + \frac{4l}{B^2 (\lambda C_i)_1^2 U_1} - \frac{4\sqrt{2}}{(\lambda C_i)_1}} \right\} \tag{42}$$

The separation of the means of the two peaks is now given by

$$\frac{\langle \zeta \rangle_2 - \langle \zeta \rangle_1}{\sigma_1} = \sqrt{\frac{l}{H_1}} \frac{k_{12}(\alpha - 1)}{(1 + k_{12})} + \frac{w_i^2}{8\pi} \frac{1}{H_1^{\frac{1}{2}} l^{\frac{1}{2}}} \left(\frac{1 + k_{11}}{1 + k_{12}} \right) (\lambda C_i)_1^2 (1 - \beta) \times$$

$$\times \left[1 + \left(1 + \frac{16\pi}{w_i^2} l H_1 \frac{1}{(\lambda C_i)_1^2} - \frac{4\sqrt{2}}{(\lambda C_i)_1} \right)^{\frac{1}{2}} \right] - \frac{\sqrt{2} w_i^2}{4\pi} \frac{1}{H_1^{\frac{1}{2}} l^{\frac{1}{2}}} \left(\frac{1 + k_{11}}{1 + k_{12}} \right) (\lambda C_i)_1 (1 - \beta) \tag{43}$$

which may be used in the above expressions for R_{n1}.

EVALUATION OF THEORETICAL RESULTS

The validtiy of the approximations involved in the derivation of the expressions for R_{n1} and the relationship between R_{n1} and R_{msa} were evaluated by means of computer simulation (*e.g.* ref. 3) of non-linear elution chromatography. The simulation program provided values for R_{n1} according to eqn. 2. These were then compared with the theoretically predicted values from eqn. 24. The comparison between R_{msa} and R_{n1} was effected by measuring the parameters required for the calculation for R_{msa} in eqn. 1 from the plotted simulation peaks (see Fig. 1); the componen-

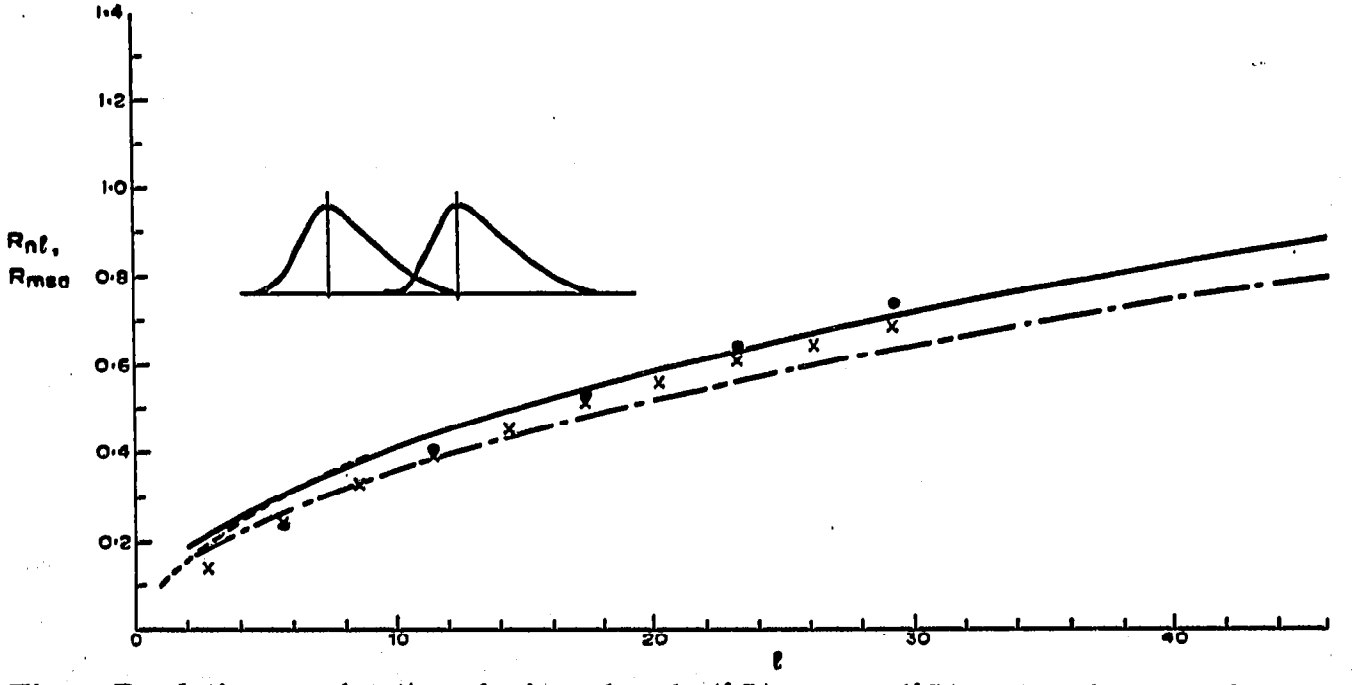

Fig. 2. Resolution as a function of column length. $(\lambda C_i)_1 = 0.5$, $(\lambda C_i)_2 = 0.5$, $k_{11} = 30$, $k_{12} = 20$, $D_e = 0.01$, $w_i = 4.8$ cm, $u = 1$ cm/sec. Simulation results: ×, R_{n1}; ●, R_{msa}.

peaks for such pairs were obtained by superposition of individually simulated peaks). σ_{12} and σ_{21} are obtained from $(w_{1/2})_{12}$ and $(w_{1/2})_{21}$ in terms of the bi-Gaussian[1] approximation, *e.g.*,

$$\sigma_{12} = (w_{\frac{1}{2}})_{12} / 1.177 \tag{44}$$

The calculations were carried out for $\alpha = 1.5$ and various combinations of D_e, $(\lambda C_1)_1$ and $(\lambda C_1)_2$. These results are graphically represented in Figs. 2–6. Each

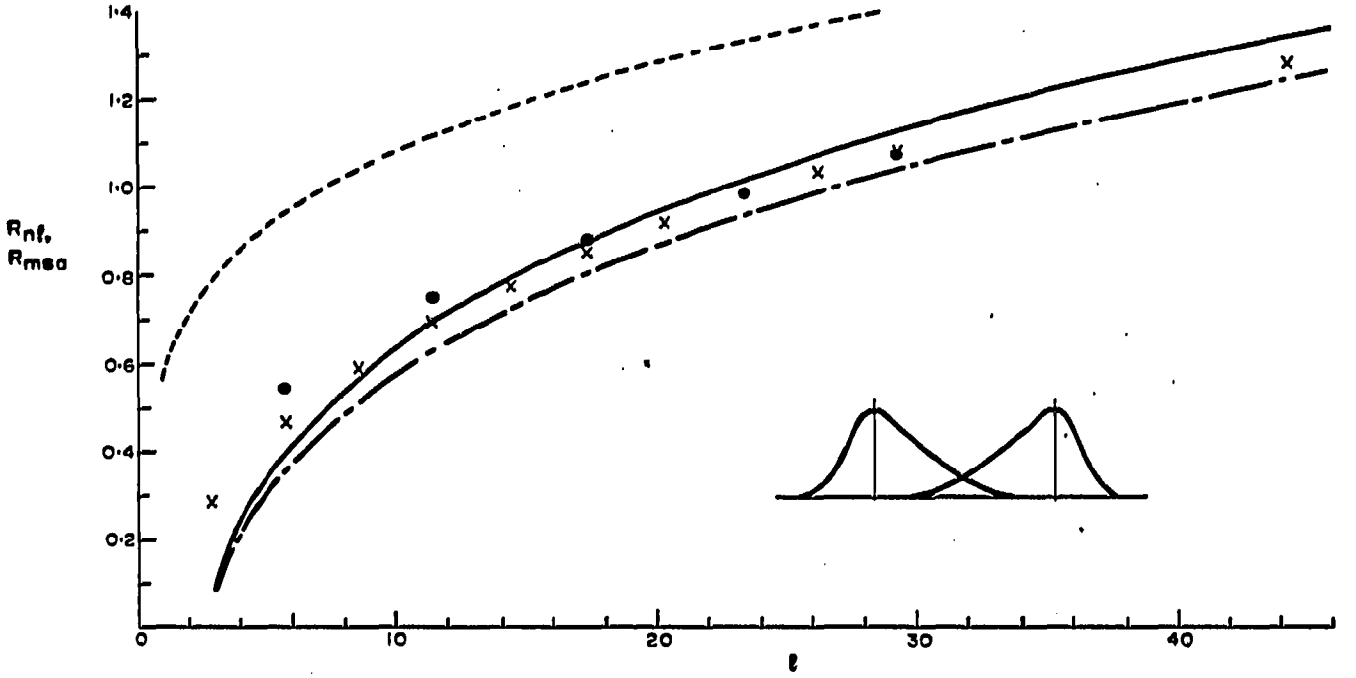

Fig. 3. Resolution as a function of column length. $(\lambda C_1)_1 = 0.5$, $(\lambda C_1)_2 = -0.5$, $k_{11} = 30$, $k_{12} = 20$, $D_e = 0.01$, $w_1 = 4.8$ cm, $u = 1$ cm/sec. Simulation results: ×, R_{nl}; ●, R_{msa}.

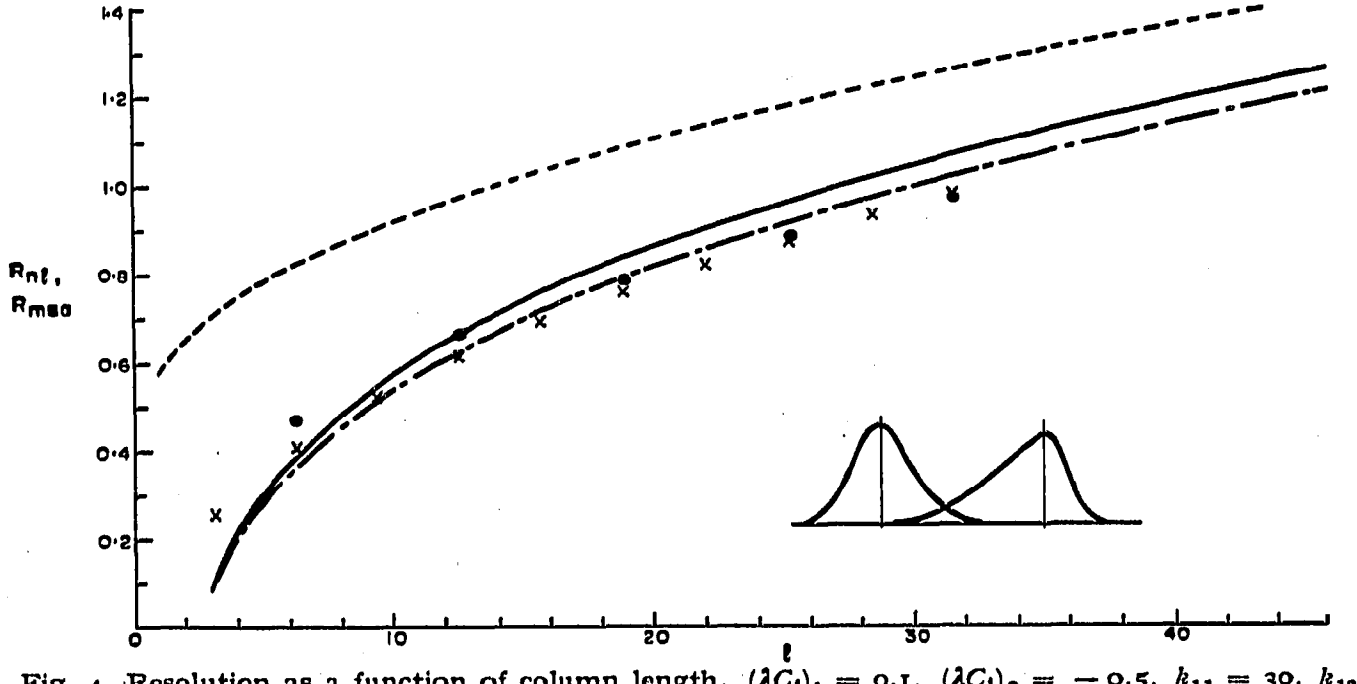

Fig. 4. Resolution as a function of column length. $(\lambda C_1)_1 = 0.1$, $(\lambda C_1)_2 = -0.5$, $k_{11} = 30$, $k_{12} = 20$, $D_e = 0.01$, $w_1 = 4.8$ cm, $u = 1$ cm/sec. Simulation results: ×, R_{nl}; ●, R_{msa}.

figure is accompanied by a schematic representation of the peak pair which it describes. In all cases R_{msa} and various approximations of R_{n1} are represented as functions of column length, l. Three approximations of R_{n1} are given: (i) eqns. 24 and 38 (– – – –); (ii) eqn. 24 and the improved $(\langle\zeta\rangle_2 - \langle\zeta_1\rangle)/\sigma_1$ value from eqn. 43 (————); and (iii) eqns. 39 and 43 (— — — — —).

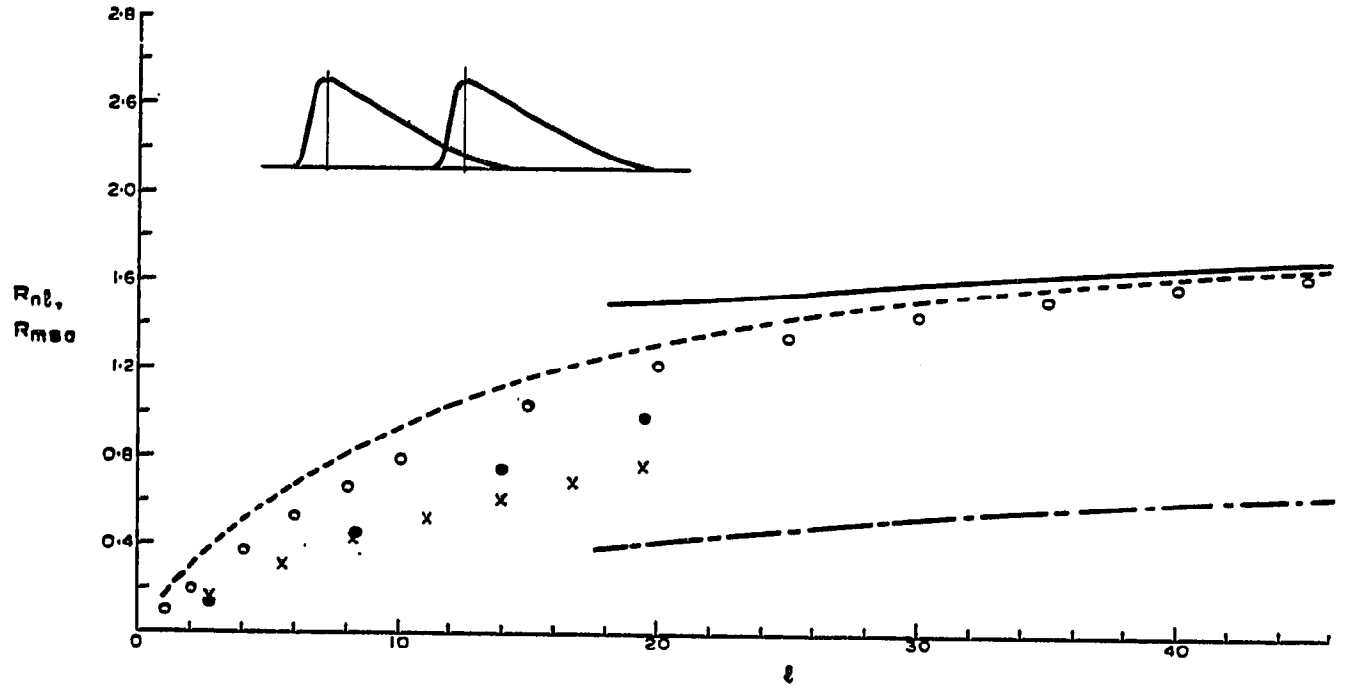

Fig. 5. Resolution as a function of column length. $(\lambda C_1)_1 = 0.5$, $(\lambda C_1)_2 = 0.5$, $k_{11} = 30$, $k_{12} = 20$, $D_e = 0.001$, $w_1 = 4.8$ cm, $u = 1$ cm/sec. Simulation results: ×, R_{n1}; ●, R_{msa}.

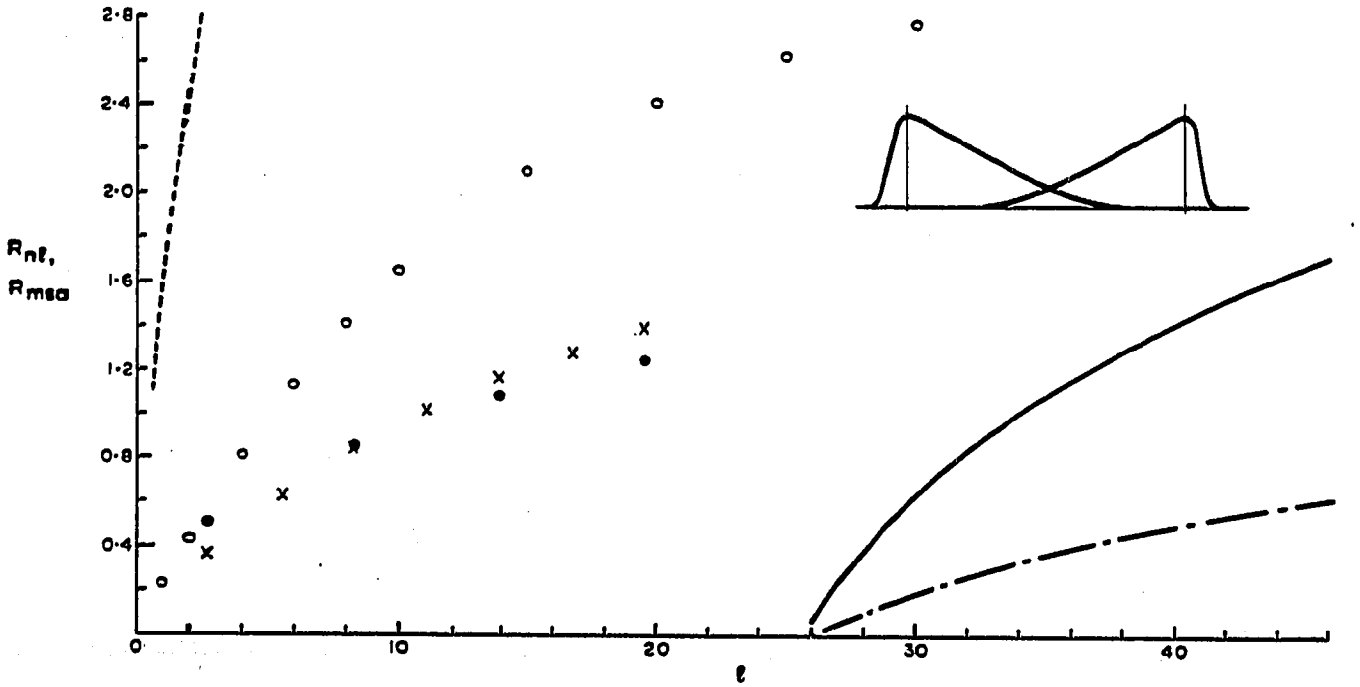

Fig. 6. Resolution as a function of column length. $(\lambda C_1)_1 = 0.5$, $(\lambda C_1)_2 = -0.5$, $k_{11} = 30$, $k_{12} = 20$, $D_e = 0.001$, $w_1 = 4.8$ cm, $u = 1$ cm/sec. Simulation results: ×, R_{n1}; ●, R_{msa}.

TABLE I

THEORETICAL RELATIONSHIP BETWEEN R_{nl} AND R_{msa}

Peak pair[a]		R_{msa} *range* (*simulation*)	A (*simulation*)	R_{nl}/R_{msa} (*simulation*)	R_{nl}/R_{msa} (*eqn. I-7*)
Peak 1	*Peak 2*				
$C_1 = 0.1$[b]	$C_1 = 0.5$	0.12–0.55	0.1338	1.57–1.0355	1.6687–1.0536
$C_1 = 0.5$[b]	$C_1 = 0.5$	0.236–0.7375	0.0476	1.045–0.9338	1.1086–1.0038
$C_1 = 0.1$[b]	$C_1 = -0.5$	0.4711–0.9749	−0.1642	0.8678–1.0	0.8629–1.0353
$C_1 = 0.5$[b]	$C_1 = -0.5$	0.5474–1.0789	−0.2107	0.8460–1.0	0.8769–1.0
$C_1 = 0.5$[c]	$C_1 = 0.5$	0.1368–0.98	0.0888	1.2113–0.7823	1.3958–0.9850
$C_1 = 0.5$[c]	$C_1 = -0.5$	0.5086–1.2458	−0.4105	0.7277–1.1251	0.6010–1.2492

[a] $k_{11} = 30$, $k_{12} = 20$, $\alpha = 1.5$, $w_1 = 4.8$ cm, $u = 1$ cm/sec.
[b] $D_e = 0.01$.
[c] $D_e = 0.001$.

The theoretical relationship between R_{nl} and R_{msa} as derived in APPENDIX I was also investigated by comparing R_{nl}/R_{msa} values obtained from the simulation results with those calculated from eqn. I-7. The required A values were obtained by actual measurement of the simulated peaks as were the R_{msa} values which are common to both cases. Table I summarizes these results. For each peak pair considered the variation of A with l was slight and an average $A = \langle A \rangle$ was used in the calculations.

DISCUSSION

Figs. 2–6 allow several conclusions regarding the approximations to be made. Deviations are apparently associated with small y values (*i.e.* when t and/or D_e/w_1^2 are small). Small y values can affect the predictions in a variety of ways, *e.g.*: (i) Breakdown of the mathematical approximation $y \gg 1$ in eqns. 38 and 43. (ii) The expressions for the moments themselves involve approximations in the evaluation of integrals in which the actual peak is replaced by one of Gaussian form (*e.g.* ref. 3). In cases where extreme deviations from the Gaussian occur, deviations can therefore be expected. This is particularly true for systems with small D_e values in which a triangular distribution is approached. (iii) Higher order terms become increasingly important as y decreases with constant λC_1. An indication of the relative importance of the above mechanisms is provided in Figs. 5 and 6, where the removal of restriction (i) is seen not to improve the results to the extent one would intuitively expect. (In Figs. 5 and 6 the circled values (⊙) were obtained by solving for t directly from eqn. 4 without simplifying assumptions.) Effects (ii) and (iii) can therefore have a pronounced effect. The situation represented by Fig. 5 or 6 is, however, an extreme case and for the cases represented in Figs. 2–4 the correspondence of the actual R_{nl} values and those predicted by the improved equation (eqn. 43) is generally good except for the expected deterioration with small D_e/w_1^2 values. Predictions based on eqn. 38 invariably show more marked deviations and although it may therefore be useful due to its simplicity, it should only be used for qualitative description.

The results tabulated in Table I give support to the theoretically derived relationship between R_{nl} and R_{msa}. Eqn. (I-7) can therefore be used as a means of

relating the impurity ratio via R_{msa} and R_{nl} to the column parameters. In situations where A (eqn. I-8) is not known beforehand, R_{nl} and R_{msa} may be equated to a first approximation. An idea of the errors involved in this approximation may be inferred from Fig. 7, where the ratio R_{nl}/R_{msa} has been plotted as a function of A for a representative range of R_{msa} values.

An important practical consequence of the present analysis is the possibility of predicting the column length required for a specified impurity ratio. This would involve firstly the determination of the required R_{msa} value which is consequently related to the R_{nl} value by means of eqn. I-7. The column length can then be read off from, *e.g.* Fig. 2. Consider the peaks with parameter values given in Table II. These peaks could, for instance, comprise a chromatogram region in which an improved resolution is desired. Alternatively, known components of a mixture may be injected separately to obtain these parameter values. In principle the s and m values (see Fig. 1)

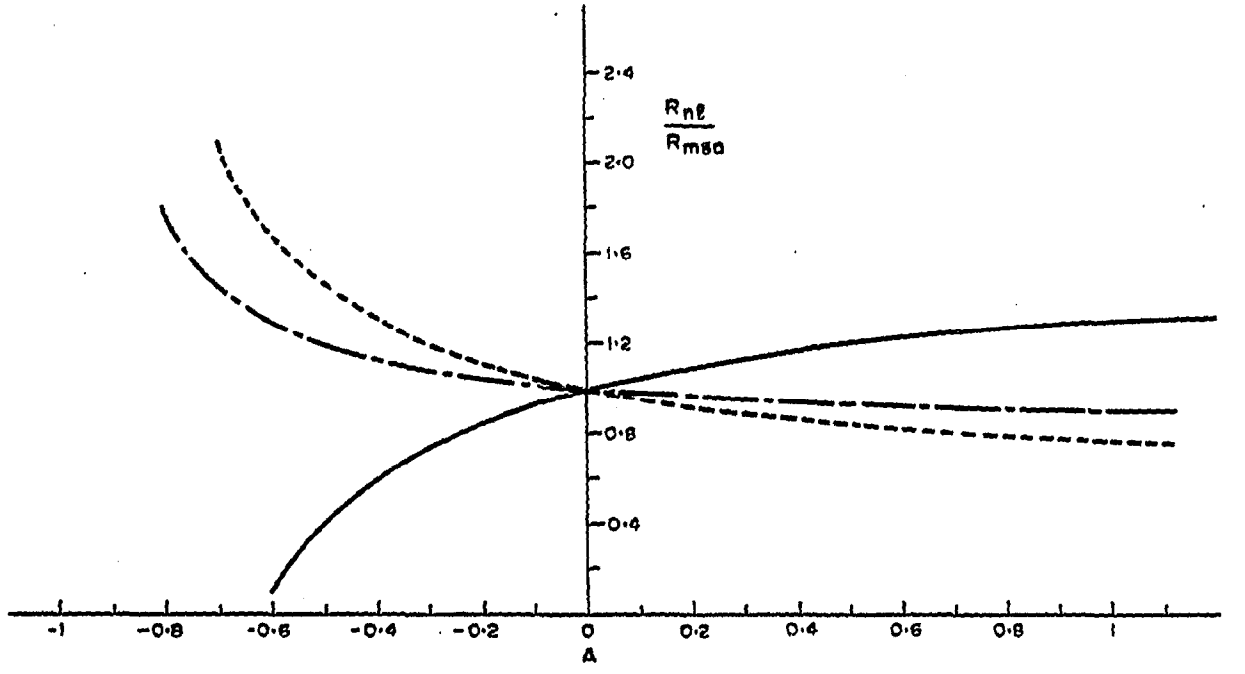

Fig. 7. Theoretical relationship between R_{nl} and A (eqn. I-7). ———, $R_{msa} = 0.5$; – – – –, $R_{msa} = 1.0$; – – – –, $R_{msa} = 1.5$.

TABLE II

PEAK PAIR CONSIDERED IN EXAMPLE

	Peak 1		*Peak 2*	
k_{11}	30	k_{12}	20	$\alpha = 1.5$
$(\lambda C_1)_1$	0.5	$(\lambda C_1)_2$	0.5	$\beta = 1$
D_e	0.01	D_e	0.01	
w_1	4.8	w_1	4.8	
$(w_{1/2})_{11}$	4.32	$(w_{1/2})_{21}$	3.87	
$(w_{1/2})_{12}$	6.6	$(w_{1/2})_{22}$	7.23	
ζ_1	27.378	ζ_2	40.499	
m_{01}	2.623	m_{02}	2.629	

$$s_p = \sigma_{21}/\sigma_{12} = (w_{\frac{1}{2}})_{21}/(w_{\frac{1}{2}})_{12} \quad (45)$$

$$s_{2a} = \sigma_{22}/\sigma_{21} = (w_{\frac{1}{2}})_{22}/(w_{\frac{1}{2}})_{21} \quad (46)$$

and

$$m_p = m_{21}/m_{12} \quad (47)$$

required to relate R_{msa} to the impurity ratio η (ref. 2) can be theoretically predicted although this is not a practical procedure. A similar situation exists in linear chromatography for the overall peak width ratio, s. Once these values are known, specification of the impurity ratio, η, uniquely defines R_{msa} in terms of η (see Fig. 8).

For the above example ($l = 29.2$)

$$R_{msa} = 0.7375$$

$$s_p = 0.5864$$

and

$$m_p = 0.5781$$

so that it follows from Fig. 8 that

$$\eta = 0.075$$

The success of the theoretical model in predicting column lengths required

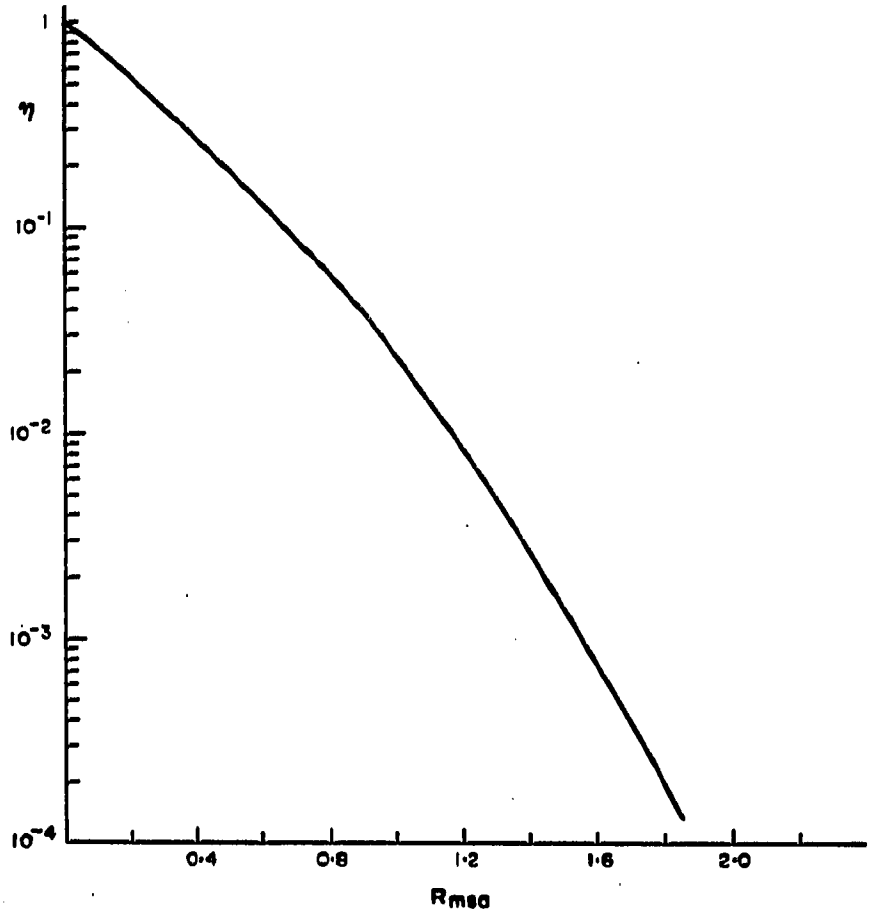

Fig. 8. Impurity ratio as a function of R_{msa} (for peak pair in Table II).

for different purity specifications (η) can now be gauged by calculating these lengths and comparing them to the actual lengths at which these purities were actually reached. For all the cases, the A value (0.0516) corresponding to $l = 29.2$ was used and the same R_{msa}–η relationship (Fig. 8) was used throughout. The results are tabulated in Table III. The correspondence is satisfactory in the light of the approximations involved.

TABLE III

COLUMN LENGTHS REQUIRED FOR SPECIFIED PURITIES

η	R_{msa} (*Fig. 8*)	R_n (*eqn. I-7*)	l (*cm*) (*Fig. 2*)	l (*cm*) (*actual*)
0.46	0.24	0.238	4.2	5.7
0.26	0.4	0.396	9.8	11.5
0.16	0.54	0.535	17	17.3
0.11	0.54	0.634	23.6	23.3
0.075	0.64	0.733	31.2	29.2

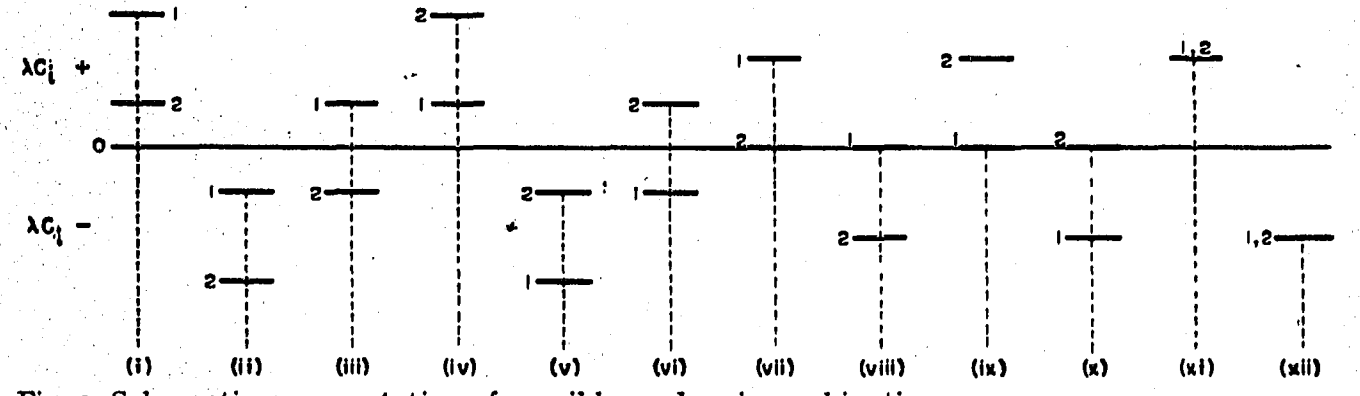

Fig. 9. Schematic representation of possible peak pair combinations.

In non-linear chromatography there are actually twelve possible combinations of $(\lambda C_i)_1$ and $(\lambda C_i)_2$, each leading to a characteristic non-linear peak pattern. These are schematically represented in Fig. 9. A systematic qualitative analysis of the behaviour of R_{n1} relative to R_n for the cited cases is made possible by an analysis of the approximate equation

$$R_{n1} = \frac{\langle\zeta\rangle_2 - \langle\zeta\rangle_1}{4\sigma_1} \tag{48}$$

with $(\langle\zeta\rangle_2 - \langle\zeta\rangle_1)/\sigma_1$ given by eqn. 38. Effects relating to non-linear second moment contributions to R_{n1} is thus neglected in the present approximation. In addition it will be assumed that

$$\left\{1 + \frac{16\pi}{w_i^2}\, lH_1 \frac{1}{(\lambda C_i)_1^2}\right\}^{\frac{1}{2}} \approx \frac{4\sqrt{\pi}}{w_i^2}\sqrt{lH_1}\,\frac{1}{(\lambda C_i)_1} \tag{49}$$

so that

$$R_{n1} - R_n = \Delta_1\left\{1 + \frac{\Delta_2}{\Delta_1}\right\} \tag{50}$$

where

$$R_n = \frac{1}{4}\sqrt{\frac{l}{H_1}}\frac{k_{12}(\alpha-1)}{(1+k_{12})} \tag{51}$$

and Δ_1 and Δ_2 are given by

$$\Delta_1 = \frac{w_i^2}{32\pi}\cdot\frac{1}{H_1^{\frac{1}{2}}l^{\frac{1}{2}}}\left(\frac{1+k_{11}}{1+k_{12}}\right)(\lambda C_i)_1[(\lambda C_i)_1-(\lambda C_i)_2] \tag{52}$$

and

$$\Delta_2 = \frac{w_i}{8\sqrt{\pi}}\cdot\frac{1}{H_1}\left(\frac{1+k_{11}}{1+k_{12}}\right)[(\lambda C_i)_1-(\lambda C_i)_2] \tag{53}$$

Application of these results to the cases depicted in Fig. 9 are summarized in Table IV. Actual inspection of the numerical computations showed that for case (v), $|\Delta_2/\Delta_1|<1$. It should be noted that R_n is only equal to the linear resolution, R_l, when $U_1 t = l$ since

$$R_l = \frac{1}{4}\sqrt{\frac{U_1^2}{H_1}}\frac{k_{12}(\alpha-1)}{(1+k_{12})} \tag{54}$$

and interpretation of the above scheme in terms of R_l thus requires a knowledge of the ratio $U_1 t/l$.

Although these phenomena were not considered in detail in the present paper, it is interesting to note that for small α values (~ 1.1) certain specific $(\lambda C_i)_1$ and $(\lambda C_i)_2$ combinations (*e.g.* $(\lambda C_i)_1 = -0.5$, $(\lambda C_i)_2 = 0.5$) lead to peak pairs in which the elution sequence (as determined by the $\langle\zeta\rangle$ values) is inverted relative to that for the linear ($\lambda = 0$) case. This results when non-linearity is so pronounced that it dominates the linear peak separating ability characterized mainly by α.

TABLE IV

CLASSIFICATION SCHEME FOR NON-LINEAR PEAK PATTERNS

Case	Δ_1	Δ_2	$\frac{\Delta_2}{\Delta_1}$	$\left\|\frac{\Delta_2}{\Delta_2}\right\|$	$R_{nl}-R_n$
(i)	>0	<0	+	<1	>0
				>1	>0
(ii)	<0	>0	−	<1	<0
				>1	<0
(iii)	do (i)				
(iv)	<0	<0	+	<1	<0
				>1	<0
(v)	>0	<0	−	<1	>0
				>1	<0
(vi)	do (v)				
(vii)	do (i)				
(viii)	0	>0	—	—	>0
(ix)	0	<0	—	—	<0
(x)	do (v)				
(xi)	0	0	—	—	$=0$
(xii)	do (xi)				

APPENDIX I

Relationship between R_n and R_{msa}

R_{nl} and R_{msa} are given by

$$R_{nl} = \frac{\langle\zeta\rangle_2 - \langle\zeta\rangle_1}{2(\sigma_1 + \sigma_2)} \tag{I-1}$$

and

$$R_{msa} = \frac{\zeta_2 - \zeta_1}{2(\sigma_{12} + \sigma_{21})} \tag{I-2}$$

If the peaks are fitted by means of bi-Gaussian distribution functions, the means and standard deviations appearing in eqn. I-1 may be approximated by[1] (see Fig. 1)

$$\langle\zeta\rangle_1 = \zeta_1 + \sqrt{\frac{2}{\pi}}(\sigma_{12} - \sigma_{11}) \tag{I-3}$$

$$\langle\zeta\rangle_2 = \zeta_2 + \sqrt{\frac{2}{\pi}}(\sigma_{22} - \sigma_{21}) \tag{I-4}$$

$$\sigma_1 = \tfrac{1}{2}(\sigma_{11} + \sigma_{12}) \tag{I-5}$$

and

$$\sigma_2 = \tfrac{1}{2}(\sigma_{21} + \sigma_{22}) \tag{I-6}$$

Substitution into eqn. I-1 gives, upon rearrangement,

$$R_{nl} = R_{msa}\left\{\frac{1 + \sqrt{\frac{2}{\pi}}A}{1 + A}\right\} \tag{I-7}$$

where

$$A = \frac{\sigma_{22} + \sigma_{11}}{2(\sigma_{12} + \sigma_{21})} - \frac{1}{2} =$$

$$= \frac{(w_{\frac{1}{2}})_{22} + (w_{\frac{1}{2}})_{11}}{2[(w_{\frac{1}{2}})_{12} + (w_{\frac{1}{2}})_{21}]} - \frac{1}{2} \tag{I-8}$$

It follows from eqn. I-7 that for small values of $|A|$ and for $R_{msa} = \sqrt{2/\pi} \approx 0.8$, $R_{nl} \approx R_{msa}$. Eqn. I-7 is represented graphically in Fig. 7, where R_{nl}/R_{msa} is plotted as a function of A for various values of R_{msa}.

LIST OF SYMBOLS

A	convenient parameter, eqn. I-8
B	convenient parameter, eqn. 28
C	solute concentration in mobile phase
C_1	value of C at the inlet at time $t = 0$
C_s	concentration of adsorbed solute
D_e	effective diffusion coefficient due to mobile phase non-equilibrium
H_i	plate height for linear ($\lambda = 0$) elution chromatography (eqn. 21)
k_i	($i = 1,2$) parameters in non-linear distribution isotherm (eqn. 7)

k_{ij} ($i = 1,2$; $j = 1,2$), k_i for component (peak) j
l column length
m_1 mass of solute per unit cross-section of the mobile phase at the inlet at time $t = 0$
m_0 zero'th moment
m_{0j} ($j = 1,2$), m_0 for component j
m_{ij} ($j = 1,2$; $j = 1,2$) mass parameters for asymmetrical (bi-Gaussian) peaks (see Fig. 1)
m_p $= m_{21}/m_{12}$, convenient parameter
R_1 resolution in linear chromatography (eqn. 54)
R_{msa} resolution function related to purity specification
R_n contribution to R_{n1} (eqn. 51)
R_{n1} resolution function related to column parameters
s_2a $= \sigma_{22}/\sigma_{21}$, skewness parameter of (bi-Gaussian) peak 2
s_p $= \sigma_{21}/\sigma_{12}$, convenient parameter
t time
u carrier flow velocity
U $= u/(1 + k_1)$
U_j $= u/(1 + k_{1j})$ ($j = 1,2$), u for component j
w_1 width of plug inlet sample profile
$(w1/2)_{ij}$ ($i = 1,2$; $j = 1,2$) width-at-halfheight parameters (see Fig. 1)
y $= 4\pi D_e l/w_1^2$, dimensionless time parameter
ζ_j ($j = 1,2$) position of maximum of j'th peak (Fig. 1)
$\langle\zeta\rangle_j$ ($j = 1,2$) position of mean of j'th peak

GREEK SYMBOLS

α $= k_{11}/k_{12}$
β $= (\lambda C_1)_2/(\lambda C_1)_1$
Δ_1 convenient parameter (eqn. 52)
Δ_2 convenient parameter (eqn. 53)
ε void fraction
η impurity ratio
λ $= 2k_2/(1 + k_1)$, non-linearity parameter
σ_j ($j = 1,2$) standard deviation of peak j
σ_{ij} $= w_{ij}/1.77$ ($i = 1,2$; $j = 1,2$), (bi-Gaussian) peak standard deviation parameters (see Fig. 1)
σ_1 standard deviation of peak eluted under linear conditions ($\lambda = 0$)
σ_1^2 inlet variance
σ_1^2 variance of peak eluted under linear conditions (linear contribution to σ_{n1}^2)
σ_n^2 non-linear contribution to σ_{n1}^2
$(\sigma_n^2)_j$ ($j = 1,2$), σ_n^2 for peak j
σ_{n1}^2 total variance in non-linear chromatography
$\sigma_n^2(D)$ diffusional contribution to σ_n^2
$\sigma_n^2(u)$ flow contribution to σ_n^2
Ω convenient semi-empirical fitting parameter (see eqn. 12)

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