

## OPTIMIZATION BY ISOCHRONAL ANALYSIS

### II. CHANGES IN MOBILE PHASE VELOCITY AND TEMPERATURE, AND IN MOBILE PHASE COMPOSITION AND TEMPERATURE

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#### SUMMARY

The theory of resolution optimization by isochronal analysis is further extended to the cases involving simultaneous changes in mobile phase velocity and column temperature, and in mobile phase composition and temperature. In each case, the resolution equation, when written in terms of isochronal conditions, shows that improvements can be obtained without sacrificing analysis time. Several subcases, which relate to the dependence of the selectivity factors on the changed parameters, are described. For each system, the contributions of the various parameters to the resolution are examined. The results of these discussions are plotted as resolution surfaces, on which lines of constant analysis time are indicated. The experimental routes that chromatographers must take in order to apply isochronal analysis are described.

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#### INTRODUCTION

In a previous publication<sup>1</sup> the concept of optimization by isochronal analysis, formerly known as time normalization<sup>2-11</sup>, was reintroduced into modern high-performance liquid chromatography (HPLC). Briefly, in this mode of optimization two experimental conditions are changed simultaneously. One is altered in order to improve the resolution while the other is changed to maintain the analysis time constant. The latter requirement means that:

$$t_{RA} = t_{RB} \quad (1)$$

where subscripts A and B indicate two different experimental conditions. Eqn. 1 can be re-written as

$$\frac{L_A (1 + k'_A)}{u_A} = \frac{L_B (1 + k'_B)}{u_B} \quad (2)$$

The need to change simultaneously two experimental conditions is clearly seen from eqn. 2.

The most practical pairs of experimental conditions that can be exploited in isochronal analysis are: (1) mobile phase velocity and composition; (2) mobile phase velocity and column temperature; and (3) mobile phase composition and column temperature. The first of these was described previously<sup>1</sup>. The present paper analyses the theoretical foundations of the other two possibilities.

#### CHANGES IN MOBILE PHASE VELOCITY AND COLUMN TEMPERATURE

In this mode of optimization the temperature and the velocity can effect both the resolution and the retention time. The relative importance of each one of these two parameters is difficult to predict *a priori*. As will be shown, however, general cases can be described. The isochronal relationship between the temperature and the velocity is easily found in the following manner. The dependence of the capacity ratio on the temperature is given by the well-known van 't Hoff equation

$$\ln(k') = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln \varphi \quad (3)$$

where  $\varphi$  is the phase ratio,  $\Delta H$  and  $\Delta S$  are the enthalpy and entropy of transfer,  $T$  is the absolute temperature and  $R$  is the gas constant. Substituting eqn. 3 in eqn. 2 yields the expression for the isochronal velocity in term of the temperature

$$u = \frac{u_A}{(1 + k_A)} [1 + \varphi \exp(-\Delta H/RT + \Delta S/R)] \quad (4)$$

where it is assumed that the column length remains constant. In this expression subscript B was dropped for the sake of generality. Subscript A will indicate in the following discussion the initial experimental conditions. Eqn. 4, in fact, completely specifies the analysis time for a given solute, once the initial conditions are chosen.

The resolution equation should be examined now in terms of the simultaneous change in velocity and temperature. For that purpose, the conventional resolution expression will be used here:

$$R_s = \frac{1}{4} \sqrt{\frac{L}{H_2}} \frac{k'_2}{1 + k'_2} \frac{\alpha - 1}{\alpha} \quad (5)$$

Here,  $L$  is the column length,  $H$  is the plate height,  $\alpha$  is the selectivity (defined as  $k'_2/k'_1$ ), and subscript 2 indicates the more retained solute, *i.e.* the one whose retention time is maintained constant. Of the various contributions to the resolution the plate height is a function of both the temperature and the velocity, while the capacity factor and the selectivity are functions of the temperature alone. The plate height can be expressed as:

$$H = \frac{2D}{u} + \frac{Au^{0.33}d_p^{1.33}}{D^{0.33}} + \frac{Cud_p^2}{D} \quad (6)$$

$D$  is the diffusion coefficient of the solute in the mobile phase,  $A$  and  $C$  are the Knox

HETP coefficients<sup>12</sup> and  $d_p$  is the support particle diameter. As before<sup>1</sup>, it will be assumed here that  $A$  and  $C$  are independent of  $k'$ . While the dependence of  $H$  on  $u$  is explicit in eqn. 6, the temperature dependence is implicit via the diffusion coefficient. As in the previous publication<sup>1</sup>, a modified Perkin-Geankoplis approximation<sup>13</sup> of the diffusion coefficient will be used:

$$D = \frac{7.4 \cdot 10^{-8} T}{\eta V^{0.6}} (X_{H_2O} \Psi_{H_2O} M_{H_2O} + X_{CH_3OH} \Psi_{CH_3OH} M_{CH_3OH})^{1/2} \quad (7)$$

where  $V$  is the molar volume of the solute,  $\eta$  is the viscosity,  $X$  is the volume fraction of the appropriate mobile phase component,  $M$  is its molecular weight, and  $\Psi$  is an association factor of the component. The viscosity dependence on the temperature (and mobile phase composition) can be approximated by the expression given by Gant *et al.*<sup>14</sup>:

$$\eta = \eta_{40} \exp[2.3 \cdot 10^3 (0.65 + 0.2\eta_{40}) (1/T - 1/313)] \quad (8)$$

$\eta_{40}$  is the viscosity at 40°C, which is given by:

$$\eta_{40} = 0.64 + 2.1X - 2.32X^2 \quad (9)$$

Substituting eqns. 3, 4, and 6–9 into eqn. 5 yields the resolution behaviour under isochronal conditions when the velocity and temperature are the manipulated parameters:

$$R_s = \frac{(L)^{1/2}}{4} \left[ \frac{(2) (7.4 \cdot 10^{-8}) T (1 + k'_A) \Omega}{u_A V^{0.6} \eta [\varphi \exp(\Gamma) + 1]} + \frac{A d_p^{1.33} u_A^{0.33} [\varphi \exp(\Gamma) + 1]^{0.33} V^{0.22} \eta^{0.33}}{(1 + k'_A)^{0.33} (7.4 \cdot 10^{-8})^{0.33} T^{0.33} \Omega^{0.33}} + \frac{C u_A [\varphi \exp(\Gamma) + 1] V^{0.6} d_p^2 \eta}{(1 + k_A) 7.4 \cdot 10^{-8} T \Omega} \right]^{-1/2} \left( 1 - \frac{\varphi \exp(\Gamma_1) + 1}{\varphi \exp(\Gamma) + 1} \right) \quad (10)$$

where

$$\Omega = (X_{H_2O} \Psi_{H_2O} M_{H_2O} + X_{CH_3OH} \Psi_{CH_3OH} M_{CH_3OH})^{1/2}$$

$$\Gamma = -\Delta H/RT + \Delta S/R$$

and  $\eta$  is the viscosity given by eqn. 8. Subscript 1 indicates quantities related to the first solute in the pair to be separated.

Eqn. 10 gives the resolution at a constant analysis time as a function of the mobile phase velocity and the column temperature. Since it is a transcendental equation, it is difficult to find analytically the exact velocity and temperature that will maximize the resolution. For that goal, numerical procedures should be employed, and in the present communication the resolution will be plotted as a function of the

velocity and the temperature. This results in a resolution surface on which isochrons (*i.e.* lines of constant analysis time) will be calculated with the above equations and plotted. From these lines the behaviour of the resolution under isochronal conditions can be predicted. The retention data for the analysis is obtained, for the most part, from the data of Gant *et al.*<sup>14</sup>. Their work allows the determination of  $\Delta H$  and  $\Delta S$  for several solutes in three different water-methanol mixtures. Other values used in the calculations were:  $\psi_{\text{H}_2\text{O}} = 2.26$ ;  $\psi_{\text{CH}_3\text{OH}} = 1.9$ ;  $A = 1$ ;  $C = 0.05$ ;  $d_p = 5 \cdot 10^{-4}$  cm; and  $L = 15$  cm. The correct value of the phase ratio remains, to a great extent, an enigma since its exact dependence on the temperature, or on the mobile phase composition in reversed phase, is not known. For the sake of the present discussion, however, a typical value of 0.15 was used in the calculations for all the conditions.

The surface obtained by the above procedure can have any shape, depending on the nature of the solutes and their temperature dependence. In general, however, the resolution is ultimately limited by the behaviour of the selectivities. In the following analysis emphasis will be placed on three general types of behaviour: (1) the selectivity is independent of the temperature; (2) the selectivity increases with increasing  $T$ ; and (3) the selectivity decreases with increasing  $T$ .

Before proceeding with the analysis it should be pointed out that in order to maintain the simulation within a practical range, the temperature was allowed to vary between 10 and 70°C, and the velocity between 0.02 and 0.6 cm/sec.

#### *Selectivity independent of temperature*

This case means that the enthalpies of transfer of the two solutes are identical. The only two solutes from the data of Gant *et al.*<sup>14</sup> that come close to fulfilling this requirement are phenetole and toluene when the mobile phase was a 60:40 methanol-water mixture. The relevant data for this pair of solutes, as calculated from the original work, are:

$$\text{For phenetole } \ln(k') = 3154/RT - 4.88/R + \ln \phi$$

$$\text{For toluene } \ln(k') = 3144/RT - 4.56/R + \ln \phi$$

For these solutes, in the temperature range of 283 K to 343 K, the selectivity changed from 1.156 to 1.159.

Fig. 1 shows the resolution surface between these two solutes as a function of the temperature and the velocity. The curvature of the surface is most obvious at low velocities. At constant, but high temperatures, the resolution has a velocity-dependent maximum. This maximum is the manifestation of the HETP dependence on the mobile phase velocity. In fact, it can be shown that the maximum in the resolution here coincides with the minimum in the plate height. At constant, but low temperatures, the maximum in the resolution occurs at the lowest velocity. This shift is due mainly to the effect of higher capacity factors and lower plate heights (because of lower diffusion coefficients). Examination of the low velocity ( $u = 0.02$  cm/s) border of the surface shows that as the temperature decreases the resolution improves. This is because of the increasing capacity factors and decreasing plate heights. At the other velocity border ( $u = 0.6$  cm/s) the resolution passes through a shallow maximum as the temperature is changed between its two limits. Unlike the previously discussed border, the efficiency here improves with increasing temperature. The capacity fac-

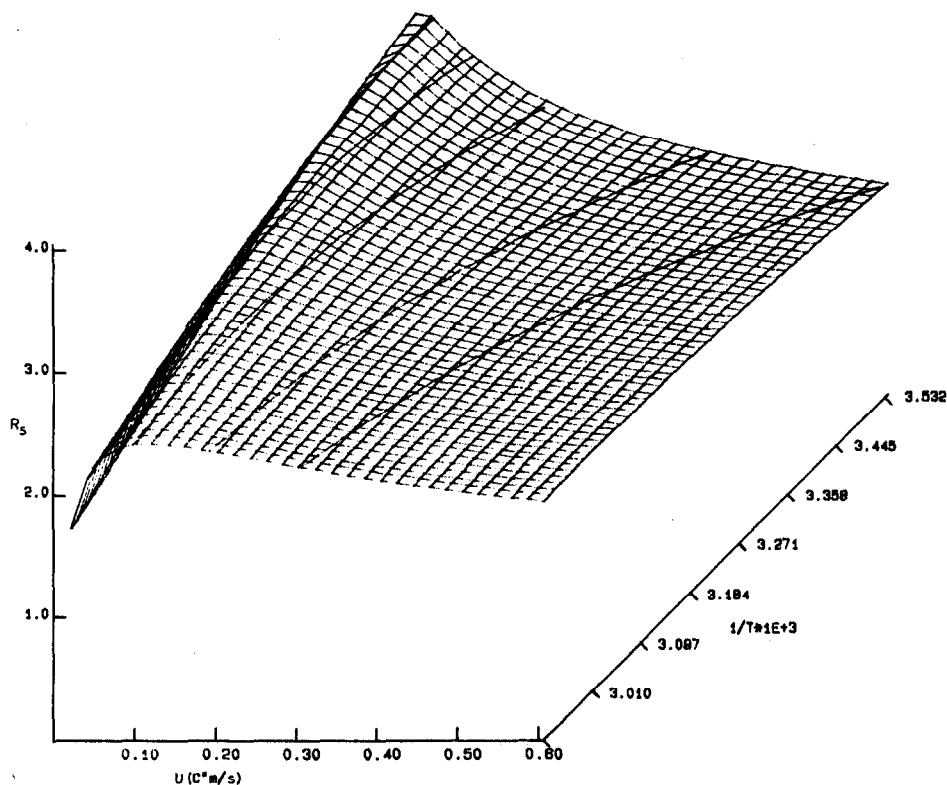


Fig. 1. The resolution, as a function of the column temperature and the mobile phase velocity, between phenetole and toluene. This is the case where  $\alpha$  is independent of the temperature. The non-grid lines on the surface are the isochrons.

tors, as before, decrease within the same temperature change. The net effect is the observed maximum in the resolution, which occurs at  $T = 334$  K. In general, the resolutions at equivalent velocities are higher at the higher temperatures. This, again, is the result of the decreasing diffusion coefficients.

The surface in Fig. 1 completely defines the resolution behaviour between phenetole and toluene. The absolute global resolution maximum can, of course, be obtained from it. In the present example,  $R_{s,\max}$  is *ca.* 3.1 and the associated retention time is 3825 s. For shorter analysis times or lower required resolutions other optimization techniques are used. We shall use the surface in Fig. 1 to demonstrate isochronal optimization.

On the surface are five isochronal lines, or isochrons. These are the (non-grid) lines that, in general, run from the bottom to the top in a left-to-right sense. The line farthest to the left is visible only at the upper left-hand side of the surface; most of the line is hidden by the curvature of the surface. The retention times corresponding to each of the lines were chosen somewhat arbitrarily: these are, from right to left; 100, 200, 400, 800, and 1600 s. At the shortest isochron, the maximum in the resolution occurs at the highest temperature studied, *i.e.* 343 K. In other words, if a retention time of 100 s is desired, and if the starting temperature is less than 343 K,

TABLE I

## LOCII OF OPTIMUM RESOLUTION BETWEEN PHENETOLE AND TOLUENE

These isochrons are for the case of  $T$ - $u$  analysis.

$t_R$ (s)	$R_s$	$u$ (cm/s)	$T$ (K)	$k'_1$	$k'_2$	$\alpha$	$H$ ( $\mu$ )
100	2.12	0.38	343	1.33	1.54	1.16	14.5
200	2.38	0.22	328	1.65	1.88	1.16	13.3
400	2.62	0.13	313	2.37	2.75	1.16	12.5
800	2.84	0.075	299	2.59	3.00	1.16	12.0
1600	3.03	0.045	286	3.31	3.82	1.15	11.7

then the resolution can be improved by increasing the temperature and decreasing the mobile phase velocity. With the 200-s isochron the maximum in the resolution does not occur at the surface boundary but on the surface itself, at  $T = 328$  K and a velocity of 0.216 cm/s. The maximum is the result of two opposing effects: an increase in the plate number and a decrease in the capacity factor with increasing temperature.

As the isochronal time becomes longer, the maximum in the resolution shifts to lower temperature and velocity. This can be seen in Table I, which gives the locii of maximum resolution of the five isochrons in Fig. 1, and the relevant isochronal velocities and temperatures. Also listed are the capacity ratios of the two solutes and the plate height of the isochronal solute. Examination of Table I shows that as the analysis times become longer, the HETPs at the isochronal optimum conditions decrease, while the capacity factors increase. Therein lies the reason for the shift in the maximum resolution. It is due to the improvement in HETP associated with the lower velocities needed to obtain longer isochronal times. As the plate height values decrease, the countering effect of the decreasing capacity ratios occur at higher  $k'$  values: hence the shift in optimum resolution to lower temperatures. Note also the lack of a selectivity effect.

Fig. 1 and Table I indicate that once the retention time has been chosen, the chromatographic resolution can be improved via isochronal optimization. Whether the analyst will have to increase the column temperature and lower the velocity, or *vice versa*, depends to a great extent on the initial conditions of the analysis.

#### Selectivity increases with increasing temperature

This behaviour requires that the enthalpy of transfer for the isochronal solute be greater (less negative) than that for the first solute. From the data of Gant *et al.*<sup>14</sup> it is found that the solutes methyl benzoate and benzene meet the above condition when the mobile phase is methanol-water (60:40). The relevant data are

$$\text{For methyl benzoate } \ln(k') = 2907/RT - 5.032/R + \ln \varphi$$

$$\text{For benzene } \ln(k') = 2582/RT - 3.874/R + \ln \varphi$$

Benzene is the isochronal solute in the present example. It should, perhaps, be mentioned again that the phase ratio  $\varphi$  was assumed to have the value of 0.15.

The resolution for these two solutes is shown in Fig. 2, as a function of the

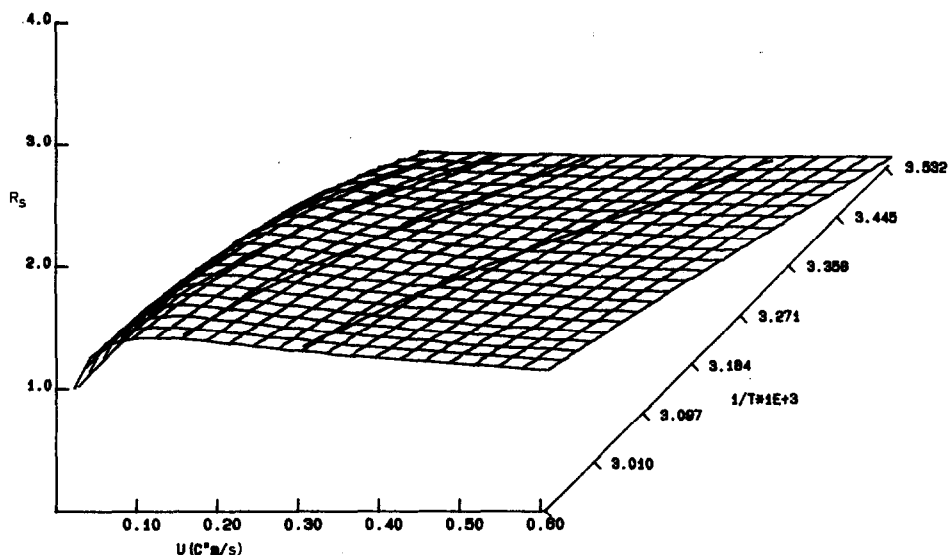


Fig. 2. The resolution, as a function of the column temperature and the mobile phase velocity, between methyl benzoate and benzene (60:40 methanol-water mobile phase). This is the case where  $\alpha$  decreases with increasing temperature. The non-grid lines on the surface are the isochrons.

column temperature and the mobile phase velocity. Some of the features of this surface are similar to those of Fig. 1. For example, at high temperatures the resolution has a maximum in the velocity sense, which is due to the minimum in the plate height-velocity relationships. Here too, at low temperatures the resolution decreases continuously as the velocity increases from 0.02 to 0.6 cm/s. On the whole the surface in Fig. 2 is rather smooth, with a general upward trend as the temperature increases. The trend is due to the increase in the selectivity, which changes from 1.005 at 283 K to 1.112 at 343 K. This relatively large change in  $\alpha$  is sufficient to overcome the decrease in the capacity ratios and, in the low velocity region, the deleterious effects of increasing  $H$  values (molecular diffusion controlled broadening), which are associated with increasing temperature. The improvement in the resolution with increasing  $T$  is more pronounced in the high-velocity region since here, in addition to the increasing selectivity, the plate heights decrease as the temperature rises (mass transfer controlled broadening).

The three non-grid lines on the surface are the isochrons, which were chosen somewhat arbitrarily. The times of analysis associated with these lines are, from right to left: 100, 200, and 400 s. In each of the lines the optimum in the resolution occurs at the highest temperature and lowest velocity allowed by the surface for that particular time of analysis. In other words, at least for relatively short analysis times, the lower border of the surface, at  $T = 343\text{K}$ , defines the conditions for optimum resolution. For longer analysis times the optimizing temperatures can be less than 343 K, and the optimum resolution is on the surface and not on its border. For very long analysis times the left-hand side border, at  $u = 0.02/\text{s}$ , is the position of optimum resolution. Table II gives the locii of maximum resolution for five isochrons, including the three on the surface in Fig. 2. It is seen that there is a maximal optimum reso-

TABLE II

## THE LOCI OF OPTIMUM RESOLUTION BETWEEN METHYL BENZOATE AND BENZENE

These isochrons are for the case of  $T-u$  analysis. The mobile phase is methanol-water (60:40). The isochronal solute is benzene.

$t_R$ (s)	$R_s$	$u$ (cm/s)	$T$ (K)	$k'_1$	$k'_2$	$\alpha$	$H$ ( $\mu$ )
100	1.28	0.29	342	0.86	0.96	1.11	13.3
200	1.38	0.15	342	0.86	0.96	1.11	11.5
400	1.37	0.073	342	0.86	0.96	1.11	11.6
800	1.21	0.037	342	0.86	0.96	1.11	14.9
1600	0.98	0.019	334	0.95	1.05	1.10	18.4

lution, which is due to the dependence of  $R_s$  on the velocity (see the lower border of the surface in Fig. 2).

The isochrons in Fig. 2 are quite straight, much straighter than those in Fig. 1. The span of velocities for each line in Fig. 2 is narrower than that in Fig. 1. Thus, a small change in the velocity requires a large change in the temperature. Roughly speaking, a change of 0.01 cm/s necessitates a change of anywhere from 2 K for the

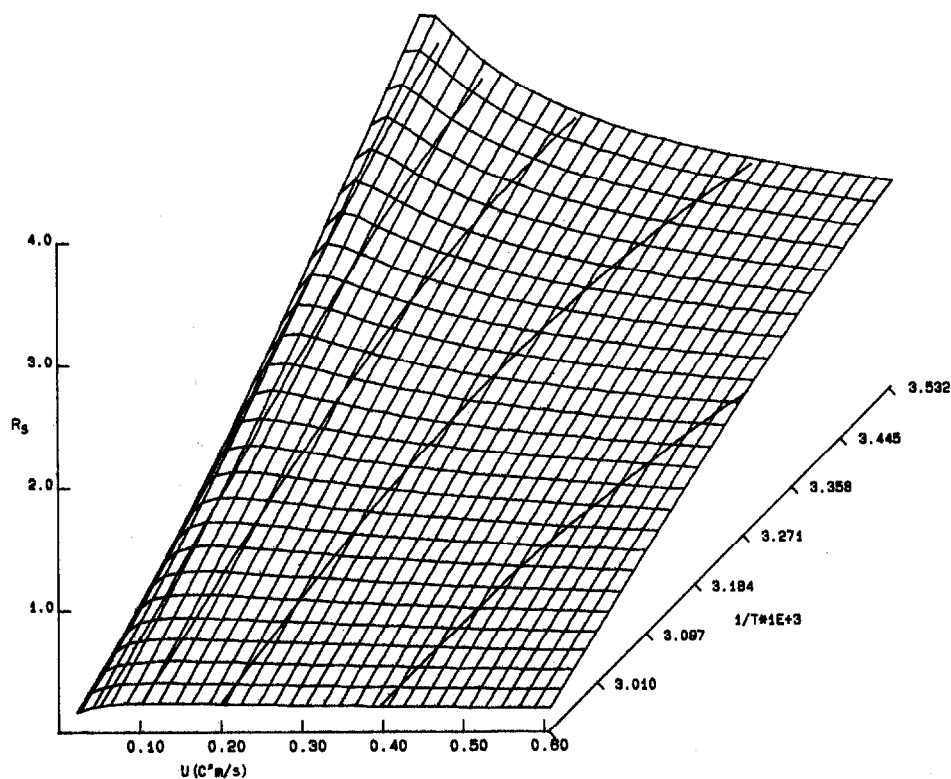


Fig. 3. The resolution, as a function of the column temperature and the mobile phase velocity, between benzene and methyl benzoate (50:50 water-methanol mobile phase). This is the case where  $\alpha$  increases with increasing temperature. The non-grid lines on the surface are the isochrons.



fast analysis times to over 20 K for the slow isochrons. The implication of this fact is obvious: the chromatographic pump must be able to maintain very accurately the flow-rates of the mobile phase, otherwise it will be rather difficult to obtain isochronal optimization.

#### *Selectivity decreases with increasing temperature*

This behaviour occurs when the enthalpy of transfer of the isochronal solute is less (more negative) than that of the first solute in the pair to be separated. The data of Gant *et al.*<sup>14</sup> indicate that benzene and methyl benzoate, in a mobile phase of 50:50 methanol-water, fit the above criterion:

$$\text{For benzene } \ln(k') = 3111/RT - 4.373/R + \ln \varphi$$

$$\text{For methyl benzoate } \ln(k') = 3510/RT - 5.511/R + \ln \varphi$$

The retention order of these two solutes is inverted compared with the previous case (methanol-water, 60:40). The importance of maintaining a constant composition of the mobile phase is, therefore, clear.

Fig. 3 gives the resolution surface for the two solutes. In general, the surface has features similar to those in Figs. 1 and 2, with the obvious exception that  $R_s$  decreases with increasing temperature. The absolute maximum in the resolution occurs at the lowest velocity and temperature of the surface.

The non-grid lines on the surface are the isochrons. Their shapes and behaviour are similar to those in Figs. 1 and 2, although the maximum resolution of each isochron occurs at the lowest allowable temperature. Table III, in which the maximum resolution locii are given, shows that the change in optimal  $R_s$  is related to velocity-induced improvement in the efficiency.

The isochrons in Fig. 3, again, indicate that a small change in the velocity may require a large change in the temperature. Thus instrumental considerations may limit, at times, this approach to optimization.

#### *Summary*

The simultaneous change of mobile phase velocity and column temperature can be used to select a resolution of choice. The resolution can be increased or decreased without loss of analysis time. Once the temperature dependence of the reten-

TABLE III

#### THE LOCI OF OPTIMUM RESOLUTION BETWEEN BENZENE AND METHYL BENZOATE

These isochrons are for the case of  $T-u$  analysis. The mobile phase is methanol-water (50:50). The isochronal solute is methyl benzoate.

$t_R$ (s)	$R_s$	$u$ (cm/s)	$T$ (K)	$k'_1$	$k'_2$	$\alpha$	$H$ ( $\mu$ )
100	1.14	0.60	306	2.78	3.03	1.09	26.4
200	1.83	0.43	283	4.16	4.76	1.14	30.8
400	2.21	0.22	283	4.16	4.76	1.14	21.0
800	2.57	0.11	283	4.16	4.76	1.14	12.2
1600	2.86	0.053	283	4.16	4.76	1.14	12.2

tion has been found, the velocity and temperature required to yield the needed resolution can be calculated.

#### CHANGES IN MOBILE PHASE COMPOSITION AND IN COLUMN TEMPERATURE

This approach is a combination of the approaches described above and in a previous report<sup>1</sup>. While probably the most versatile, it is the most complicated scheme. Complications occur for two main reasons: (1) the approximate nature of the equations describing the dependence of the retention on the mobile phase composition and temperature can lead to errors in the optimization conditions; (2) since the velocity and column length remain constant, the capacity ratio of the isochronal solute is also a constant. Hence the optimization cannot proceed via eqns. 1 and 3. The interrelationships between the isochronal temperature and mobile phase composition are complicated, and once found, must be adhered to rigorously.

The theoretical background to this variant of isochronal optimization is derived from the dependence of the capacity ratio on the temperature (eqn. 3), and on the mobile phase composition, as given by the equation

$$\ln(k') = aX + b \quad (11)$$

where  $a$  and  $b$  are constants, and  $X$  is the volume fraction of methanol in the mobile phase (see discussions in ref. 1 regarding the use of this approximation). Since these equations must hold simultaneously, then

$$\Delta H = \rho X + \gamma \quad (12)$$

$$\Delta S = \delta X + \varepsilon \quad (13)$$

The constants  $\rho$ ,  $\gamma$ ,  $\delta$  and  $\varepsilon$  are absolute constants for a given solute in a specific chromatographic system. The capacity factor can therefore be written as

$$\ln(k') = \ln \varphi - (\rho X + \gamma)/RT + (\delta X + \varepsilon)/R \quad (14)$$

This equation for the capacity factor takes into account the effects of both the temperature and the mobile phase composition. The validity of the expression depends mainly on the reliability of eqn. 11.

In this mode of isochronal optimization, once the analysis time has been chosen, the capacity factor remains constant throughout the procedure: *i.e.*  $k' = k'_A$ . Eqn. 14 can, therefore, be rearranged to yield the required temperature for each mobile phase composition

$$T = \frac{\rho X + \gamma}{\delta X + \varepsilon + R \ln(\varphi) - R \ln(k')} \quad (15)$$

Eqn. 15, in essence, is the equivalent of eqns. 1 and 2 as used in other modes of isochronal optimization. Substitution of eqn. 15 into eqns. 7 and 8 allows the behaviour of the efficiency in the  $X$ - $T$  isochronal optimization to be examined.

The resolution will be altered by changes in  $H$  and in the selectivity, which is given by

$$\alpha = \frac{k'_2}{\varphi \exp [-(\rho_1 X + \gamma_1)/RT + (\delta_1 X + \varepsilon_1)/R]} \quad (16)$$

Subscripts 1 and 2 refer to the first and second solutes. When the dependence of  $H$  and the selectivity on the isochronal temperature and mobile phase composition are taken into account, the following resolution equation results

$$R_s = \frac{(L)^{1/2}}{4} \left[ \frac{(2) (7.4 \cdot 10^{-8}) T (1 + K'_A) \Omega}{u_A V^{0.6} \eta [\varphi \exp(Z) + 1]} + \frac{A d_p^{1.33} u_A^{0.33} [\varphi \exp(Z) + 1]^{0.33} V^{0.22} \eta^{0.33}}{(1 + k'_A)^{0.33} (7.4 \cdot 10^{-8})^{0.33} T^{0.33} \Omega^{0.33}} + \frac{C u_A [\varphi \exp(Z) + 1] V^{0.6} d_p^2 \eta}{(1 + k_A) 7.4 \cdot 10^{-8} T \Omega} \right]^{-1/2} + \left( 1 - \frac{\varphi \exp(Z_1) + 1}{\varphi \exp(Z) + 1} \right) \quad (17)$$

where  $\Omega$  and  $\eta$  are as defined above,  $T$  is given by eqn. 15 and

$$Z = -(\rho X + \gamma)/RT + (\delta X + \epsilon)/R$$

Although this equation looks complicated, it is an easy task to compute the resolution numerically.

Previous modes of isochronal optimization were studied by investigating several possible behaviours of the selectivity, as either the mobile phase composition or the column temperature varied. In these cases, the composition and temperature were not interrelated. Eventually, however, each change in one parameter necessitates a change in the second. Consequently, no easy categorization can be made, and each separation must be examined individually. To point out the complexities of the resolution behaviour, two typical cases, based on the data of Gant *et al.*<sup>14</sup>, will be described. These were chosen to represent a case where the retention goes through reversal in the methanol content axis, and a case where there are two reversals: one in the temperature axis and one in the modifier axis.

#### *Surface with a retention reversal in the methanol axis*

An example of this behaviour is provided by the solutes anisole and benzene. The relevant equations describing the retention behaviour of these solutes are as follows:

$$\text{For anisole: } \ln(k') = -(5225X - 5906)/RT + (5.08X - 7.62)/R + \ln \varphi$$

$$\text{For benzene: } \ln(k') = -(6090X - 6182)/RT + (7.16X - 8.03)/R + \ln \varphi$$

The order of elution of these two solutes changes as a function of the mobile phase composition. Benzene is the last solute at the water-rich end, whereas anisole elutes last at the methanol-rich end. Thus, as shown in Fig. 4, the resolution surface goes through a minimum, where  $R_s = 0$ . An analysis of the surface can, perhaps, be best accomplished by describing the behaviour of each of its four borders. At the lowest temperature, the resolution decreases as the methanol content is increased. The resolution goes through zero at *ca.* 82% methanol, before beginning to increase again,

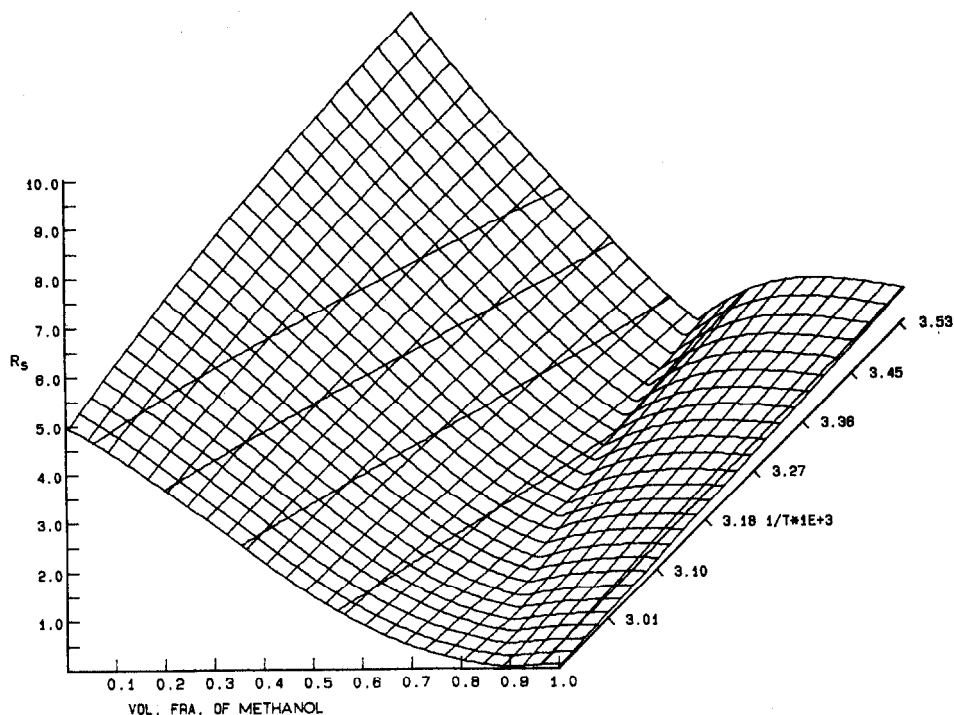


Fig. 4. The resolution, as a function of the column temperature and mobile phase composition, between anisole and benzene. The non-grid lines on the surface are the isochrons.

reaching a local maximum of 0.87 at 82% methanol. The initial decrease in the resolution is due mainly to a decrease in the selectivity. A decrease in  $k'$  and an increase in  $H$  further contribute to the deterioration in  $R_s$ . After the point of retention reversal ( $R_s = 0$ ),  $\alpha$  increases, and  $H$  improves because of the decreasing viscosity of the mobile phase. Thus, the resolution begins to improve again. However, as the methanol content approaches 100%, the very small  $k'$  values become the dominating factor, and  $R_s$  drops once more.

At the high temperature border, when the methanol content is increased, the resolution decreases for reasons similar to those described above. The retention reversal occurs here at *ca.* 88% methanol; past this point the resolution increases slightly. In general, the reversal point ( $R_s = 0$ ) shifts to higher methanol content. The local maximum is observed up to a temperature of *ca.* 334 K, above which the decrease in  $k'$ , as the methanol content is increased, negates the improvement in the selectivity.

The behaviour at the 100% water border is straightforward. The absolute maximum in  $R_s$  is found at this border, at the lowest temperature used. As  $T$  is increased the resolution drops because of decreasing  $\alpha$  and  $k'$  values. For the same reasons, the resolution at the 100% methanol border also decreases with increasing temperature.

The surface in Fig. 4 is much more complex than the surfaces discussed before, and it shows the wide range of resolutions that can be obtained by manipulating the

correct experimental conditions. This is also evident in isochronal optimization. The non-grid lines on the surface are five isochrons covering analysis times of 100, 200, 400, 800, and 1600 s. The 100-s line is very close to the 100% methanol border. As the temperature is increased (or as the methanol content is decreased) the resolution decreases continuously. This is due to the fact that  $\alpha$  decreases with an increase in  $T$ . In this time-scale, when the mobile phase is almost pure methanol, a very small change in the amount of modifier requires a very large change in the isochronal temperature.

The 200-s line goes through a minimum in resolution, at the retention reversal point (63.3% methanol, 300 K). The maximum resolution of this isochron is at the highest temperature border. A similar point of maximum resolution also occurs for the isochrons at 400, 800, and 1600 s. However, these isochrons do not go through retention inversions. As the isochrons become longer, the span in methanol change is much greater for an equivalent change in  $T$ . In all cases, the dominating factor that determines the trend in the resolution is the behaviour of  $\alpha$ . For the three slowest isochrons,  $\alpha$  increases as  $T$  is increased and the methanol content is decreased. This can be seen in Table IV, which gives the locii of maximum resolution for each isochron.

#### Surface with two retention inversions

An example of this behaviour is given by the solute pair phenetole-toluene<sup>14</sup>. The relevant data for these two solutes are as follows:

$$\text{For phenetole: } \ln(k') = -(5885X - 6607)/RT + (4.12X - 7.12)/R + \ln \varphi$$

$$\text{For toluene: } \ln(k') = -(5015X - 6168)/RT + (1.59X - 5.62)/R + \ln \varphi$$

The surface, shown in Fig. 5, is rather complex, with retention reversals in both the temperature and the modifier content axes. Except at low temperatures and in water-rich mobile phases, toluene elutes after phenetole. At the 283 K border, increasing the methanol content from zero to 100% causes an interesting change in the resolution. Initially, the resolution actually decreases as the two solutes merge. After the retention reversal, where  $R_s = 0$ , the resolution increases to a maximum value of 2.32 at 62% methanol. Past that point, the resolution decreases to a value of 0.8 at

TABLE IV  
LOCII OF OPTIMUM RESOLUTION BETWEEN ANISOLE AND BENZENE

These isochrons are for the case of  $T$ - $X$  analysis.

$t_R$ (s)	$R_s$	Methanol (%)	$T$ (K)	$k'_1$	$k'_2$	$\alpha$	$H$ ( $\mu$ )
100*	0.64	99.9	283	0.125	0.14	1.23	12.0
200**	1.17	54.6	343	1.14	1.23	1.08	11.5
400***	2.51	35.0	343	3.08	3.47	1.13	11.5
800***	3.65	19.5	343	6.78	7.91	1.17	11.3
1600***	4.62	5.2	343	13.98	16.84	1.20	11.3

\* The isochronal solute is anisole.

\*\* Retention inversion occurs on this isochron.

\*\*\* The isochronal solute is benzene.

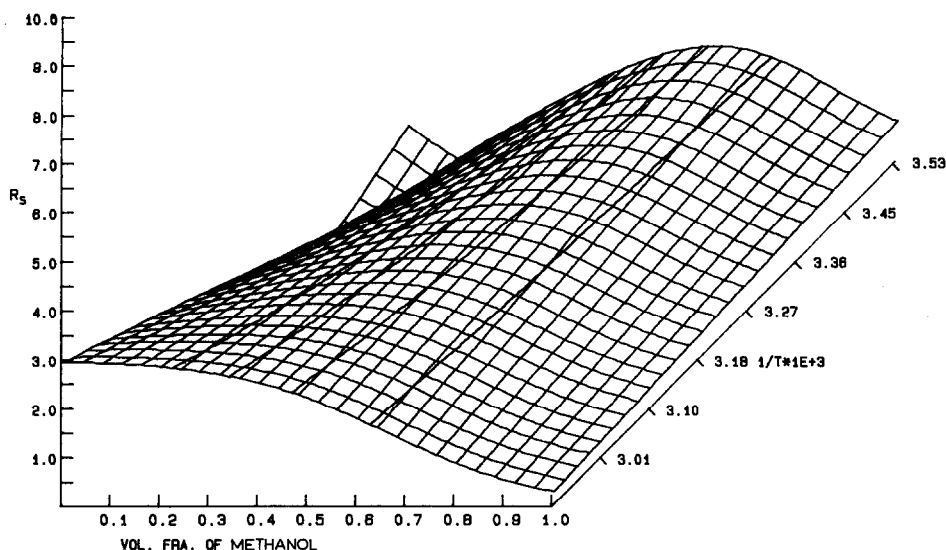


Fig. 5. The resolution, as a function of the column temperature and mobile phase composition, between phenetole and toluene. The non-grid lines on the surface are the isochrons.

100% modifier. The maximum is a result of increasing selectivity and decreasing  $k'$ . The influence of the efficiency here is much less pronounced.

At the 343 K border the resolution behaviour is less complicated. There is still a maximum, albeit very small, in the resolution, which occurs at very low methanol content (*ca.* 2%). From that point on,  $R_s$  decreases continuously and more and more rapidly, as the amount of methanol in the mobile phase increases. This behaviour is due mainly to the decrease in the capacity ratio. The effect of the selectivity at that border is minimal;  $\alpha$  changes only from 1.114 to 1.118. The influence of the efficiency is also very small at high temperatures.

At the zero methanol border the resolution goes through zero, at the point of retention reversal. The absolute resolution maximum occurs on this border *at the highest temperature studied*. Continuously increasing  $\alpha$  and decreasing  $H$  are responsible for the behaviour of the resolution at the high temperature part of this border. On the other hand, the 100% modifier border is quite simple to explain. The resolution decreases continuously with increasing temperature because of continuously decreasing selectivity and capacity factor terms. The efficiency at that border, while going through a minimum, is of little importance in affecting the resolution.

The non-grid lines on the surface are the isochrons, corresponding, from right to left, to 200, 400, 800, and 1600 s. At 200 s the optimum resolution occurs at the lowest temperature studied. The simultaneous increase in  $T$  and decrease in methanol content decrease the resolution continuously. This is a direct manifestation of the decrease in the selectivity. The 400-s isochron is similar to the previous isochron in that the optimum in the resolution occurs at the lowest temperature studied. In addition, however, this isochron goes through a minimum at 316 K and 53–54% methanol, owing to the opposing effects of slowly decreasing selectivity and improving efficiency. The 800-s isochron also has a minimum, occurring at 286 K and 50.2%

TABLE V  
LOCII OF OPTIMUM RESOLUTION BETWEEN PHENETOLE AND TOLUENE

These isochrons are for the case of  $T$ - $X$  analysis. The isochronal solute is toluene.

$t_R$ (s)	$R_s$	Methanol (%)	$T$ (K)	$k'_1$	$k'_2$	$\alpha$	$H$ ( $\mu$ )
200	2.09	74.2	283	.03	1.23	1.19	16.6
400	2.32	61.4	283	3.01	3.46	1.15	17.8
800	2.62	34.0	343	7.10	7.91	1.12	11.5
1600	2.79	22.4	343	13.08	16.81	1.11	11.4

methanol, but the optimum in the resolution is now at the highest temperature studied. The selectivity along this isochron also goes through a minimum, which occurs at 313.3 K and 42.5% methanol. The efficiency, on the other hand, improves continuously as the temperature is increased. These effects, together with the fact that the capacity factor also increases with  $T$ , are the reasons for the minimum in the resolution. The 1600-s isochron has no minimum, and the resolution increases with increasing temperature. Chiefly responsible for the increase of  $R_s$  are the increasing selectivity and efficiency. Table V shows the locii of maximum resolution, along with other relevant data, for each isochron.

Fig. 5 and Table V again indicate the importance that the time of analysis can have on determining the optimal isochronal conditions. Whereas at one analysis time the optimum resolution is achieved at lower temperature and higher methanol content than the initial conditions, the opposite can occur at another times. In either case, however, an optimum resolution can indeed be obtained isochronally. The isochrons in Fig. 5 are steeper than those in Fig. 4. Thus, precise instrumental control of the mobile phase composition is vital.

## CONCLUSIONS

The achievement of a desired resolution can be pursued via different approaches. The optimization discussed here attempts to keep the analysis time constant as the experimental conditions are altered to maximize the resolution. It is shown theoretically that an isochronal approach can present a viable method of resolution optimization. Like most, if not all, optimization techniques the method requires some preliminary experiments. In the case of the velocity-temperature scheme, the temperature dependence of the retention should be known. In the case of the composition-temperature optimization, the composition dependence of  $k'$  should be established as well. In the former case two or three experiments at different temperatures should suffice to approximate the  $T$  dependence of the capacity factor. In the second case the number of initial experiments may be as high as nine (three temperatures at three different mobile phase compositions), before a reasonable approximation of the combined  $X$ - $T$  dependence of  $k'$  is ascertained. With the present advances in computerized HPLC units these are not serious limitations, and automated data collection and reduction can be accomplished quickly and accurately. The real limitation of the isochronal approach lies in the nature of the approximations made for calculation purposes. For example, the assumption that  $\ln(k')$  is linearly related

to the amount of modifier over a wide range of compositions may introduce serious errors and disagreements between theory and experiments. A limitation of a different kind is in the expression used for  $H$  in the resolution calculations. This again will introduce errors between theory and experiments. Nonetheless, the theoretical treatment given here shows that isochronal optimization can be successful. Preliminary experimental results show that, in practice, the method can succeed in improving the resolution of a LC system. These results will be reported shortly.

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