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EFFECT OF ANALYSIS TIME ON OPTIMUM COLUMN LENGTH IN TIME NORMALIZATION CHROMATOGRAPHY

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SUMMARY

The effect of the carrier velocity on the optimum length in time normalization chromatography (or analysis at constant retention time) was investigated. It was found both theoretically and experimentally that at any given carrier velocity (or analysis time) the resolution can be optimized. The length at which the optimization occurs is a function of the plate height dependence on the temperature. In general, an absolute maximum resolution occurs when operating at carrier velocities around the Van Deemter plot minimum. It appears that a prediction of the column length can still be made using the approach suggested by us previously. Theoretically, in the case of a capillary column, one can operate at twice the minimum velocity without a large loss in the resolution but at a saving in the analysis time.

INTRODUCTION

Time normalization is a technique which can be employed to improve chromatographic resolution without loss in analysis time. The name time normalization was first coined by Karger and Cooke^{1,2}. Although a misnomer, since no normalization in the mathematical sense of the word is done, the name remains with us due to historical reasons. Perhaps, a better name would have been "separation under the constraint of constant analysis time". In the past few years, several papers appeared which use this technique for the enhancement of chromatographic separation³⁻¹⁰.

As is well known, two operational parameters are changed simultaneously in this method. One parameter affects the resolution while the other keeps constant the retention time of the last solute. The system most extensively studied to date is the one where both the column length and its temperature are changed concurrently. It is well known now that both these parameters can be manipulated to give a maximum resolution. Grushka and Guiochon⁹ have discussed the effect of column permeability on the optimum column length (or capacity ratio) and temperature. It was found that when taking the relative retention α to be temperature independent the optimum capacity ratio k'_{opt} is between 2 and 3. Removing the restriction of constant α , Grushka⁶ derived an equation which allows the prediction of the optimum column conditions (*i.e.*, optimum length and temperature). That equation was recently verified experimentally¹⁰. In the above treatment, however, it was assumed that the analysis is done

at carrier velocity, $U_{\min.}$, around the Van Deemter minimum of the initial column put in the chromatograph, so that the plate height H is not a strong function of the temperature. Since the carrier velocity is the same for all columns, this procedure limits the analysis to a specific analysis time, which very much depends upon the nature of the solutes and the stationary phase. It is, consequently, desirable to see how the carrier velocity affects the column length and temperature that produce the optimum resolution, if such exists at the velocity chosen. In other words, rather than working at the minimum velocity (in the Van Deemter sense), the investigator may wish to speed up or slow down the analysis. The question to be asked then is: Can the resolution still be maximized at this new analysis time? If it can, how would the optimum column length and temperature be changed at the new velocity?

THEORY

The equation governing time normalization (or analysis at constant time) is simply

$$\frac{L_A}{U_A} (1 + k'_A) = \frac{L_B}{U_B} (1 + k'_B) \quad (1)$$

Subscript A indicates one system, while B denotes that of a longer column. L is the column length, k' is the capacity ratio of the most retained solute, and U is the average linear carrier velocity. We choose to use average velocity rather than outlet velocity simply because the former is more easily determined experimentally. The ramification of using average over outlet velocity was discussed by Grushka and Guiochon⁹. From eqn. 1, two conclusions can be drawn: (a) k' and L can be used interchangeably, and (b) as the length L_B increases, one must increase the temperature in order to decrease k'_B and thus keep eqn. 1 a true equality.

The resolution equation can be approximated by the well known relation

$$R_s = \frac{1}{4} \sqrt{\frac{L}{H_2}} \left(\frac{k'_2}{k'_2 + 1} \right) \left(\frac{\alpha - 1}{\alpha} \right) \quad (2)$$

H is the plate height, α is the relative retention, and subscript 2 indicates quantities obtained from the second solute, *i.e.* the solute whose retention time is kept constant. Eqn. 2 must be now related to time normalization. It is convenient, from a computational point of view, to rewrite the resolution expression in terms of capacity ratio rather than length. This can be done as follows.

The length can be expressed as

$$L = \frac{L_A(1 + k'_A)}{(1 + k')} \quad (3)$$

Subscript B was dropped for the sake of generality. Parameters with subscript A will indicate the original condition of the system, that is, the first column put in the oven at a particular temperature, T_A . Note that eqn. 3 is velocity independent. This is, no matter what velocity the researcher chooses, it must remain the same in all columns, irrespective of their length, if the retention time is to be kept constant by a change in the temperature. Eqn. 3, again, indicates that as longer (or shorter) columns are in-

roduced to the oven, the capacity ratio on these columns must decrease (or increase). Karger and Cooke¹ derived the required expression for the temperature which will change k' to the correct value.

$$T = \frac{T_A}{1 - (R/\Delta H)T_A \ln(k'/k'_A)} \quad (4)$$

T_A is the temperature (in absolute degrees) at which column L_A was run, R is the gas constant, and ΔH is the heat of solution of the last solute.

The relative volatility term in the resolution equation is usually temperature dependent and it can be approximated as¹¹

$$\frac{\alpha - 1}{\alpha} = \frac{a}{T} - b \quad (5)$$

where a and b are constants. By using eqn. 4 the change in the relative retention term with k' , or with L , can be obtained.

The plate height H is a complicated expression that in general, for a gas chromatographic system, can be written as

$$H = 2\lambda d_p + \frac{2\gamma D_m}{U} + f(k', U) \frac{d_p^2 U}{D_m} + \frac{qk'd_f^2 U}{(1 + k')^2 D_s} \quad (6)$$

D_s is the diffusion coefficient of the solute in the stationary phase, d_f is the film thickness, $f(k', U)$ is a function of the capacity ratio and, at time, of the velocity (*i.e.* the coupled equation or variation thereof), d_p is the support diameter, and λ , γ , and q are packing characteristic. In eqn. 6, $f(k', U)$ is difficult to write explicitly for packed columns. See, for example, the recent treatment of Knox and Saleem¹². In the case of a capillary column $f(k', U)$, γ and q are known and eqn. 6 is simply the Golay expression

$$H = \frac{2D_m}{U} + \frac{(1 + 6k' + 11k'^2)r^2 U}{24(1 + k')^2 D_m} + \frac{2}{3} \frac{d_f^2 k' U}{(1 + k')^2 D_s} \quad (7)$$

where r is the tube radius.

Although our experiments were done with packed columns, the theoretical model uses the plate expression for open tubular columns. The reason is the mathematical simplicity of the latter system. The conclusions reached using this model should be applicable, at least in general trends, to packed columns as well.

Ideally, the resolution expression should be written in terms of length and temperature, since in this method of analysis the experimentally variable parameters are these two quantities. But, as mentioned previously from a computational point of view, it is easier to write the resolution as a function of temperature and capacity ratio. The latter parameter is dependent by eqn. 3 on the column length. Thus, we have

$$R_s = \frac{1}{4} \left[\frac{L_A (1 + k'_A)}{\frac{2(1 + k')^2 D_m}{U} + \frac{(1 + 6k' + 11k'^2)r^2 U}{24 D_m} + \frac{2k'd_f^2 U}{3D_s}} \right]^{\frac{1}{2}} \frac{k'}{(1 + k')^{\frac{1}{2}}} \left\{ \frac{a[1 - (R/\Delta H)T_A \ln k'/k'_A]}{T_A} - b \right\} \quad (8)$$

Eqn. 8 was obtained by utilizing eqns. 3 to 6. At $k' = 0$ the resolution is zero. At $k' = \infty$ L'Hopital's rule indicates that again R_s goes to zero. Hence, there is an optimum k' or an optimum length, and temperature which will yield an optimum resolution irrespective of the carrier velocity used. Once the velocity is chosen to suit the analyst's needs, the optimization of the resolution at that velocity can proceed in the usual manner. From eqn. 8, it is obvious that indeed the velocity at the Van Deemter minimum yields the maximum possible resolution of the system. However, if the time associated with this velocity is too short or too long, the velocity can be changed and the resolution can still be optimized. This communication shows the effect of the velocity on the optimizing parameters for various simulated and experimental systems.

EXPERIMENTAL

Theoretical studies

All theoretical calculations were done with a CDC-6400 computer using the FORTRAN IV language.

Instrumentation

The instrumentation, as well as the reagent used, were described by us previously¹⁰. Briefly, a Hewlett-Packard Model 700 gas chromatograph was used (Hewlett-Packard, Avondale, Pa., U.S.A.). Various lengths of $1/8$ -in. O.D. columns packed with 15% w/w Apiezon L coated on 80-100 mesh AW-DMCS Chromosorb W were used. Two test mixtures were used. One was made of *sec.*-butylbenzene and *tert.*-butylbenzene in benzene and the other was *n*-pentanol and isoamyl alcohol in acetone. For further details, see ref. 10.

Procedure

With the alcohol mixtures the 5-m column at a temperature of 158.3°C (chosen arbitrarily) was taken as the reference column. The minimum velocity of the Van Deemter plot was located on the 5-m column and experimental runs were made at that velocity, and at one half and at twice U_{min} . The same procedure was followed with the butylbenzenes mixture with the exception that the reference column was 1 m at 120°C (chosen arbitrarily). At each velocity the column length was changed between 5 and 1 m (decreased in the case of the alcohols and increased in that of the butylbenzenes). The normalizing temperature was computed according to eqns. 3 and 4. At each velocity, variation in the retention time was usually kept to about 1-2%.

RESULTS AND DISCUSSION

Computer simulation

Eqn. 8 was solved numerically for different velocities. In all cases, a capillary column whose length was 1500 cm operated at 350°K was taken as the initial column. The film thickness of the stationary phase was taken as 0.5 μ m. Four cases were studied. (a) At 350°K the capacity ratio k'_A of the solute whose retention time is kept constant was 1. The relative retention term was a strong function of T . (b) The same k'_A value but weaker dependence of the relative retention term on T . (c) k'_A at 350°K was 10. Strong dependence of α on T . (d) Same as (c) but weaker dependence of α on

T. Regarding the dependence of α on T , we took one case where at 350 °K α was 1.110 while at 370 °K α was 1.100. In the second case at 370 °K α was 1.105. The latter is considered to have the lower dependence on T . In all four cases, it was assumed that at 350 °K, D_m was 0.5 cm²/sec, D_s was 1×10^{-5} cm²/sec and ΔH was -10 kcal/mole. The diffusion coefficient in the gas phase was assumed to be dependent on the temperature to the 1.75th power. The dependence of D_s on the temperature was taken to be exponential with an activation energy of 8 kcal/mole. It was also assumed that H is independent of the column length. Although there are some indications that H can be a function of L (refs. 13–16), in general, the dependence is not too strong and to a first approximation the above assumption is not a serious one.

In the computer simulation, a constant retention time of the last solute was chosen and the resolution optimized in each of the four cases at that time. The retention time was then increased by 10 sec and the optimization calculation repeated. Ideally, one should present the results in a three-dimensional figure. We, however, plotted optimum resolution *versus* optimum length at each velocity. In Fig. 1 cases (a) and (b) are plotted. That is, the initial k'_A was 1. In curve A the dependence of α on T was larger than in curve B. The points on the curve represent the analysis time which was kept constant. In the figure, the points vary from 10 to 200 sec. The time increment between two points is 10 sec. It should be stressed again that at each analysis time, the resolution in Fig. 1 is the optimum for that system at that particular time. The required temperature at each length is not given in the figure (it can be easily calculated from the previous equations).

Both curves A and B go through a maximum at about 40 sec. Curve A is displaced toward shorter column length and it has a slightly higher absolute maximum

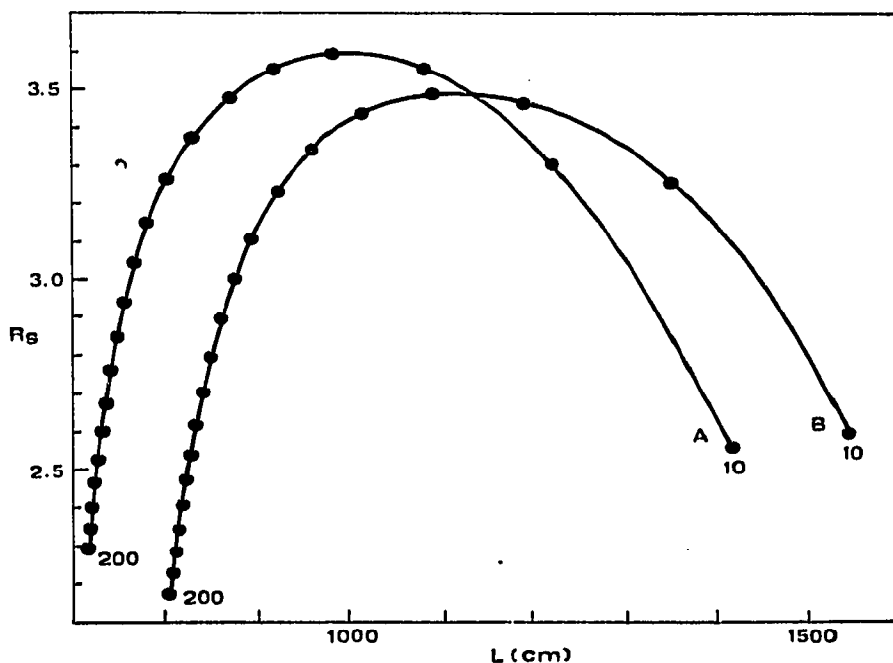


Fig. 1. Resolution *versus* optimum length. Initial $k'_A = 1$.

than curve B. The general behavior of the curves is easily understood. Eqn. 3 indicates that no matter what analysis time (or carrier velocity) is chosen, any given length must be operated at the same particular temperature associated with it. This length may not be the optimum length for all analysis times, but irrespective of the analysis time it must be operated at the same temperature and hence it will yield the same capacity ratio for the last solute and the same relative retention value. At a given length, the only quantity that changes with the choice of the retention time is H , the plate height. The optimum resolution as well as the optimum length at each analysis time is consequently due to the variation in H . Table I shows the various values calculated in the case of 10-sec and 40-sec analysis times for curve A. This table shows the variation

TABLE I

BEHAVIOR OF CHROMATOGRAPHIC PARAMETERS IN THE VICINITY OF THE OPTIMUM RESOLUTION AT TWO CARRIER VELOCITIES

Initial conditions are: $T_A = 350$ °K, $L_A = 1500$ and $k'_A = 1$.

L (cm)	k'	T (°K)	R_s	H (cm)	H_{min} (cm)	U_{min} (cm/sec)
$U = 300$ cm/sec; $t_R = 10$ sec						
1267	1.367	342.6	2.596	0.04111	0.02206	87.31
1300	1.315	343.5	2.600	0.04021	0.02184	88.85
1324	1.266	344.3	2.604	0.03977	0.02162	89.89
1352	1.219	345.2	2.606	0.03844	0.02140	91.24
1380	1.174	346.1	2.608	0.03757	0.02118	92.62
1408	1.130	347.0	2.608	0.03671	0.02095	94.04
1436	1.088	347.9	2.608	0.03586	0.02073	95.50
1465	1.048	348.9	2.606	0.03502	0.02050	97.00
1493	1.010	349.8	2.604	0.03419	0.02027	98.54
1521	0.9724	350.7	2.600	0.03337	0.02004	100.1
$U = 75$ cm/sec; $t_R = 40$ sec						
891.6	2.365	330.2	3.603	0.02480	0.02479	72.87
911.5	2.291	330.9	3.608	0.02466	0.02466	73.53
931.3	2.221	331.6	3.612	0.02452	0.02452	74.20
951.1	2.154	332.3	3.614	0.02438	0.02438	74.88
970.9	2.090	332.9	3.615	0.02425	0.02425	75.57
990.7	2.028	333.6	3.616	0.02411	0.02411	76.28
1100	1.969	334.3	3.616	0.02398	0.02397	76.99
1030	1.912	334.9	3.614	0.02384	0.02383	77.70
1050	1.857	335.6	3.612	0.02371	0.02368	78.44
1070	1.804	336.2	3.608	0.02358	0.02354	79.19

of the resolution with length and temperature around the optimum parameters at each velocity. Also included in the table are the plate heights for each column length and temperature that give the constant t_R . For each column length and temperature, we also calculated what the minimum HETP and velocity would have been if the analysis time was not restricted.

Due to our choice of the parameters in these simulated cases, the resolution, although first increasing and then decreasing, did not vary much with length. In Table I we included values which only span about ± 140 cm from the optimum length. All the values were rounded off to four significant figures. The optimum parameters are underlined in Table I. Only those quantities are plotted in Fig. 1.

We see from Table I that at 10-sec analysis time the plate heights are larger than the theoretical minima. The carrier velocity is much greater than $U_{\min.}$. At 40 sec, on the other hand, the plate heights at the optimizing length and temperature are close in value to the theoretical minimum, as is the velocity. The overall resolution is larger. In fact, as the analysis time becomes greater than 40 sec, the plate height calculated for each column length and temperature is larger than the Van Deemter minimum since the velocity is slower. Hence, the value of the optimum resolution decreases beyond 40 sec. Thus, the value of the plate height plays a major role in determining the optimum at each analysis time.

The optimum capacity ratios k'_{opt} for the maximum in curves A and B in Fig. 1 are, respectively, about 2.03 and 1.75. An important point should be made here. In all our previous treatments where H was assumed a constant it was shown that k'_{opt} must be greater than 2 for maximum resolution to occur. This is no longer correct when H is allowed to vary.

It is interesting to note in Fig. 1 that curve A has a slightly larger maximum resolution at a lower optimum length and temperature than curve B. Also, at very long analysis time the drop in the resolution is rather strong while the optimizing length does not change much. For curve A at 1800-sec analysis time the optimum length is 699 cm, not a large change from the length at 200-sec analysis time. The optimum resolution, on the other hand, at 1800 sec is 0.783. Both curves A and B indicate that, at least for the system simulated, the analysis time can be cut from 40 to 20 sec without a severe loss in the resolution. However, shorter analysis time means longer optimum columns and higher optimum temperature.

In the case where the initial k'_A was 1, we anticipated the optimum k' value to be greater than unity, as indeed was the case. Fig. 2 shows a similar plot to that in Fig. 1, with the exception that k'_A was 10. Here we anticipated the optimum value of k' to

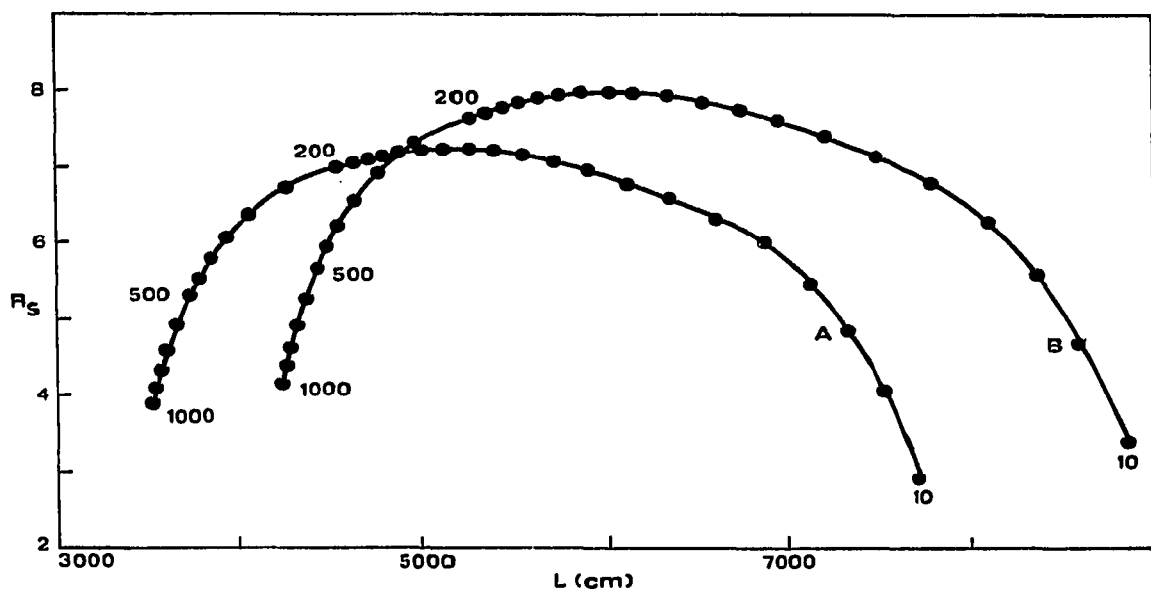


Fig. 2. Resolution versus optimum length. Initial $k'_A = 10$.

be less than 10. Again curve A is the one where α has a stronger dependence on T . Between 10 and 200 sec, the points on the plot indicate 10-sec increments in the analysis time. Between 200 and 500 sec the points indicate 50-sec increments. Between 500 and 1000 sec the points indicate 100-sec increments. The reason for the general shape of the curve is the same as discussed previously. Here, however, the curves around the absolute maximum resolution are rather broad. For both curves the maximum resolution occurs at between 130 and 140 sec. Although, as in Fig. 1, curve A is to the left of B, the absolute maximum resolution here is higher for the latter case. The maximum optimum k' values were, respectively, 2.22 and 1.74 for curves A and B. Since the optimum capacity ratios were smaller than the initial one ($k'_A = 10$), the optimum column lengths were greater than the initial 1500 cm. This means operation at a higher temperature. It is interesting to note that the k' values of the absolute maximum resolution for the curves A in Figs. 1 and 2 are about the same whether $k_A = 1$ or 10. The same holds true for the curves B in the two figures.

Fig. 2 indicates that, again for the system investigated, cutting the analysis time by a half will not affect much the resolution. If lower temperatures are needed one could double the analysis time to about 260 sec and still obtain good resolution. As in Fig. 1, too long an analysis time will cut drastically the resolution but not the column length or the temperature. Theoretically, then, it is seen that an optimum resolution can be found for any analysis time by a concurrent change in the column length and its temperature. The maximum possible resolution is obtained only when the plate height at the optimum parameters is close in value to the Van Deemter minimum for that system. That is, when the analysis time is such that the velocity of the carrier is close in value to $U_{min.}$

Butylbenzenes mixture

The behavior of this system on packed columns at a velocity around the Van Deemter minimum was discussed by us previously¹⁰. The minimum velocity $U_{min.}$ was about 10.6 cm/sec. The analysis time was about 167 sec, which corresponded to the retention time of *tert.*-butylbenzene. The original column was 1 m and ran at 393°K. The capacity ratio k'_A was 15.7. The resolution as a function of the length for this analysis time is shown in Fig. 3. Also shown in Fig. 3 are the resolution *versus* column length at 5.29 cm/sec and at 20.78 cm/sec. Table II shows the relevant data for the last two velocities. It is seen that in both cases, as well as in the data given in Ref. 10, similar lengths were run at the same temperature for normalization to occur. Also at each length and temperature, irrespective of the analysis time, the capacity ratios and the relative retentions were identical to within experimental error ($\pm 5\%$). The only parameter affecting the resolution was the plate height. In general, the plate heights at 5.3 cm/sec were lower than those at 20.8 cm/sec. Hence in Fig. 3, the lowest curve was the one run at 20.8 cm/sec while the highest curve was the one run at 10.5 cm/sec, *i.e.* $U_{min.}$. This trend is theoretically expected, as discussed in the previous section. The maximum point in each curve can be plotted in one curve in a manner similar to those of Figs. 1 or 2. Fig. 3 shows that, at least for the butylbenzenes, the optimum was about the same for the $U_{min.}$ curve and for the slow velocity while it was slightly longer for the fast velocity. This is in rough agreement with the behavior depicted in Figs. 1 and 2. Overall, however, the optimum lengths at all three velocities were comparable and their prediction can be made using the approach suggested by us earlier^{6,10}. The dis-

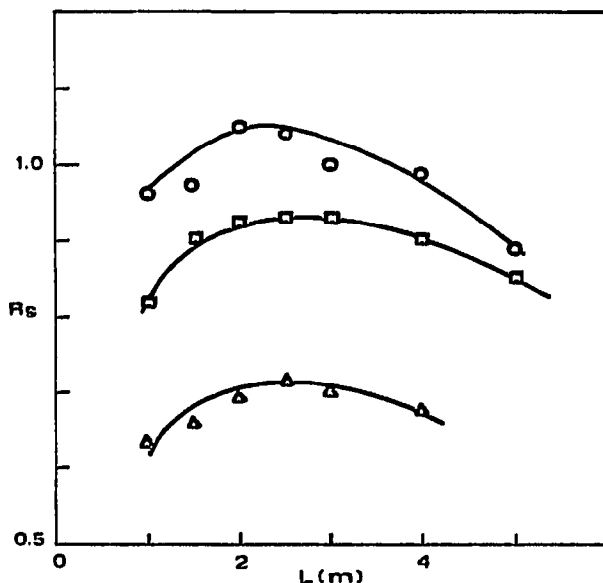


Fig. 3. Resolution *versus* column length—butylbenzenes. \square , $U = 5.29$ cm/sec; \circ , $U = 10.5$ cm/sec; \triangle , $U = 20.78$ cm/sec.

crepancy between Fig. 3 and the theoretical prediction might be attributed to the fact that the experimental work was done with packed rather than capillary columns.

Amyl alcohols mixture

In this case the reference column, L_A , was 5 m operated at 433 °K. The

TABLE II
CHROMATOGRAPHIC DATA OF BUTYLBENZENES
Normalizing times: 316.3 and 78.4 sec.

L (cm)	t_R (sec)	U (cm/sec)	k	T (°K)	α	H (cm)	R_s
$U = 5.29$ cm/sec							
100*	316.3	5.29	15.7	390	1.12	0.099	0.821
150	315.3	5.30	10.1	404	1.11	0.096	0.905
200	314.1	5.26	7.32	413	1.11	0.106	0.915
250	316.4	5.31	5.73	425	1.10	0.116	0.930
300	315.6	5.32	4.59	441	1.10	0.114	0.922
400	312.7	5.32	3.16	457	1.09	0.115	0.907
500	314.2	5.32	2.34	476	1.08	0.113	0.853
$U = 20.78$ cm/sec							
100*	78.42	20.8	15.3	390	1.12	0.178	0.640
150	79.08	21.2	10.2	406	1.11	0.168	0.660
200	78.86	20.9	7.26	415	1.11	0.182	0.691
250	78.37	21.1	5.64	427	1.10	0.169	0.719
300	78.16	21.2	4.53	441	1.09	0.182	0.700
400	79.12	21.0	3.16	458	1.08	0.189	0.677

* Reference column.

minimum in the Van Deemter plot occurred at about 7.67 cm/sec. At this velocity the analysis time of *n*-propanol, the normalized solute, was about 132 sec. The relevant data pertaining to this velocity are given in Ref. 10. Fig. 4 shows resolution *versus* column length plots for the amyl alcohols mixture at 4.64, 7.67, and 14.1 cm/sec. The data for the slow and high velocity are shown in Table III.

From Fig. 4 it seems that the maximum resolution was obtained when operating at the slowest velocity. The optimum length for the slow velocity and for U_{\min} .

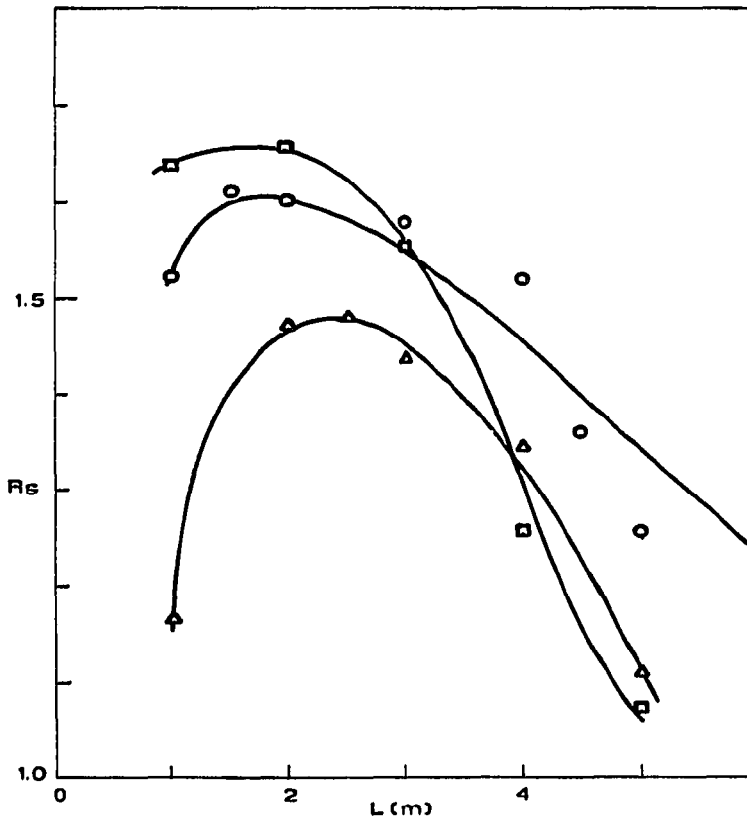


Fig. 4. Resolution *versus* column length —amyl alcohols. \square , $U = 4.64$ cm/sec; \circ , $U = 7.67$ cm/sec; \triangle , $U = 14.1$ cm/sec.

(7.67 cm/sec) was roughly equal. This behavior of the resolution is unexpected in view of Figs. 1 and 2. However, in examining Table III it can be seen that the plate height varied in a somewhat unpredictable manner. On the whole, at the fast velocity H decreased with increasing column length while the reverse was true at the slow velocity. From Ref. 10, H at U_{\min} was roughly a constant on short columns and decreased slightly on the 5-m column. Since k' and α were constant at each length and temperature for all three velocities, the resolution behavior was due to the changes in the plate height. At shorter column lengths, experiments with the slower velocity gave smaller plate height values than those with the minimum velocity. It should be remembered

TABLE III
 CHROMATOGRAPHIC DATA OF AMYL ALCOHOLS
 Normalizing times: 219.2 and 71.64 sec.

L (cm)	t_R (sec)	U (cm/sec)	k	T ($^{\circ}K$)	α	H (cm.)	R_s
$U = 4.64$ cm/sec							
100	220.6	4.74	9.47	337	1.34	0.140	1.64
200	219.6	4.68	4.17	364	1.30	0.169	1.66
300	218.6	4.68	2.42	388	1.25	0.169	1.56
400	217.0	4.70	1.56	408	1.22	0.214	1.26
500*	219.2	4.64	1.03	433	1.19	0.195	1.07
$U = 14.1$ cm/sec							
100	72.8	14.1	9.27	337	1.34	0.249	1.16
150	73.6	14.1	5.43	353	1.30	0.271	1.19
200	72.9	14.3	4.15	364	1.29	0.205	1.47
250	74.1	14.0	3.17	374	1.27	0.203	1.48
300	71.5	14.2	2.37	389	1.25	0.189	1.44
400	72.3	14.0	1.55	409	1.22	0.183	1.35
500*	71.6	14.1	1.03	433	1.19	0.179	1.11

* Reference column.

here that in the case of the amyl alcohols the reference column was 5 m. At the slow velocity the dependence of H on L might be an important factor which we chose to neglect in the theoretical discussion.

The optimum in resolution at the faster velocity was, as in Fig. 3, the lowest and it was shifted to slightly longer column lengths, as expected theoretically. On the whole, however, the optimizing length was to a first approximation equal in all three cases. This, again, indicates that our prediction formula should, in packed columns, give at least "an order of magnitude" value for the optimizing length irrespective of the velocity, provided the latter is not too far off the minimum velocity.

The experimental behavior in the case of capillary columns still remains to be studied. This paper demonstrates nonetheless that at each carrier velocity, the investigator can optimize the resolution by a concurrent change in column length and temperature. Both the theoretical results and the experimental results indicate that frequently one can operate at a velocity which is faster than $U_{min.}$. The loss in resolution is frequently not too large and, if it can be tolerated, is made up by the faster analysis time. It also seems that our approach to the prediction of the optimum length can be used at velocities different than $U_{min.}$

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