

ZONE MIGRATION IN PAPER CHROMATOGRAPHY

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INTRODUCTION

The R_F value has long been recognized as the important parameter in characterizing solute migration in chromatography. It is related to the partition of solute molecules between the mobile phase and the stationary phase. The equation which has been extensively used to describe this dependence is

$$R_F = \frac{A_M}{\alpha A_S + A_M} \quad (1)$$

where A_M and A_S are the respective cross sectional areas of mobile phase and stationary phase solvents. It will be noticed that the ratio $A_M/(\alpha A_S + A_M)$ in this equation is the ratio of the number of molecules in the mobile phase to the total number of molecules. In order that this may be considered equal to the true R_F (the distance traveled by the center of the zone divided by the distance traveled by the solvent front) two assumptions must be made. They are:

1. The average solvent velocity at the spot location must be equal to the forward velocity of the front, and
2. The ratio A_M/A_S at the spot location must remain constant irrespective of the distance migrated by the spot.

We have investigated these assumptions in the light of recent work on solvent flow in paper. Our conclusions are that assumption 1 is not true and that the local solvent velocity falls behind that of the front by a varying amount depending on the distance from the front. Concerning assumption 2, we find that this can be correct under the condition that the spot is started right at the solvent source and the flow is horizontal. Otherwise, we obtain a varying R_F , as has been commonly observed in ascending and descending flow, and when the spot is started a large distance along the paper¹⁻³. The fact that A_M/A_S is not constant along a paper strip has been often recognized in view of the concentration gradients found in the solvent concentration. This fact has not, however, been generally related to zone migration. An exception is found in the work of WOOD AND STRAIN² where these effects are theoretically considered. By virtue of recent work on solvent distribution in paper the theory of zone migration can now be given in quantitative form for any paper for which a single

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concentration profile is known. Experiments have been performed with Whatman 3 MM paper to evaluate the applicability of the theory.

THEORY

It has been established⁴ that the flow of solvent in paper obeys the diffusion equation

$$\frac{\partial w}{\partial t} = D \frac{\partial^2 w}{\partial z^2} \quad (2)$$

where w , the relative concentration, is the local ratio of solvent to dry paper weight. The solvent weight is that obtained over and above any absorbed in the pretreatment of the paper. The effective diffusion coefficient, D , is a function of w . This makes equation (2) nonlinear. Despite the difficulties of obtaining solutions to this equation, a very useful property arises with the application of the Boltzmann transformation⁵. Thus, for a one-dimensional problem such as with a rectangular sheet or strip immersed on one end, the concentration w is a function of the single variable $z/t^{1/2}$. Considering the fact that z_f , the distance to the solvent front, is merely the distance at which w approaches zero, this fact explains the parabolic flow rate law

$$z_f^2 = \kappa t \quad (3)$$

where κ is the flowrate coefficient. An equally significant deduction is that all concentration profiles in which w is plotted against z become identical when w is plotted against the reduced distance $y = z/z_f$. This has been experimentally verified using water and *n*-butanol in Whatman 3 MM, 1 and 54 papers. The separate concentration profiles are illustrated in Fig. 1, and the reduced profile in Fig. 2. Each paper-solvent system will have a characteristic reduced profile. The concept of reduced profiles is an important simplification in calculating R_F values, as will be shown. In addition to the

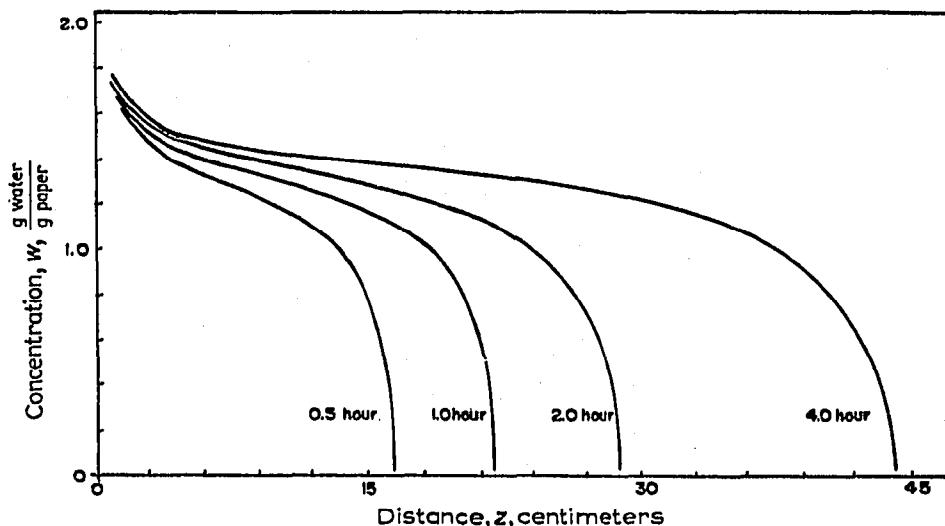


Fig. 1. Concentration profiles for Whatman 3 MM systems at various time intervals.

rectangular strips, there is good evidence that tapered strips, discs, discs with wicks, etc., all exhibit reduced concentration profiles after the passage of a short initiation period. This fact is of importance in circular chromatography, with and without wicks.

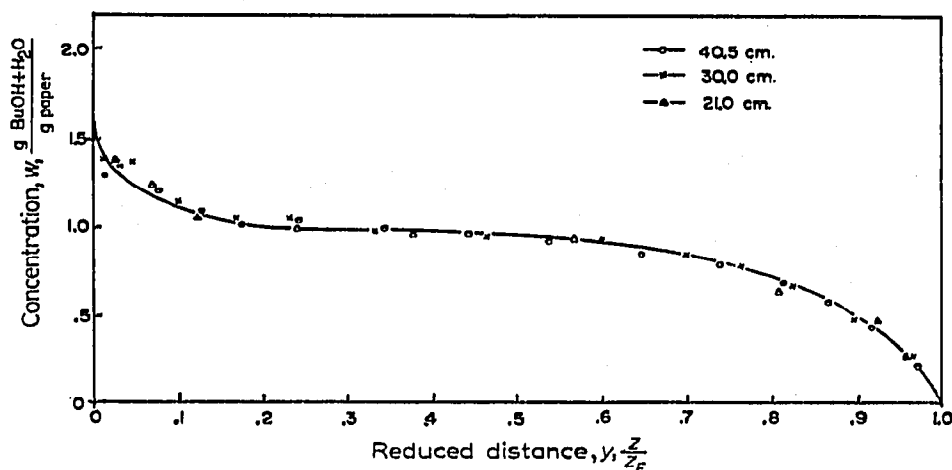


Fig. 2. Reduced concentration curve for linear, horizontal flow of *n*-butanol (H_2O saturated) on Whatman 3 MM paper.

In order to describe zone migration it is necessary to know the fraction of molecules in the mobile phase as a function of the concentration w . This ratio, given in eqn. (1), will hereafter be referred to as R since the ratio is not necessarily equal to the R_F value. If we make the very reasonable assumptions that A_M is proportional to w , and that A_S is constant (this assumes an adequate pre-exposure to saturated vapor), it is possible to rewrite the ratio in (1) as

$$R = \frac{w}{w + c} \quad (4)$$

where

$$c = \frac{aw}{A_M/A_S} \quad (5)$$

the constant c will vary from one paper-solvent system to another, but will remain essentially constant in a given system for either strips or discs.

Since a fraction R of the molecules are in the mobile phase, the mean velocity, \bar{u} , of a zone will be

$$\bar{u} = Rv \quad (6)$$

where v is the average value of the local solvent velocity. This velocity can be obtained as the ratio of the solvent flux, q , to the concentration w

$$v = q/w \quad (7)$$

the flux q must be calculated in terms of the grams of solvent passing in one second through a normal, cross sectional area just large enough to contain 1 g of dry paper per cm of length (or equivalent units in another system). Substituting eqns. (7) and (4) into (6) we obtain

$$\bar{u} = \frac{q}{w + c} \quad (8)$$

As long as the flow process maintains a reduced concentration profile, the flux is given by⁴

$$q = v_f A = v_f \int_0^w y \, dw \quad (9)$$

where v_f is the forward velocity of the solvent front, dz_f/dt , and A is the shaded area obtained from the reduced concentration profile as shown in Fig. 3. The value of A

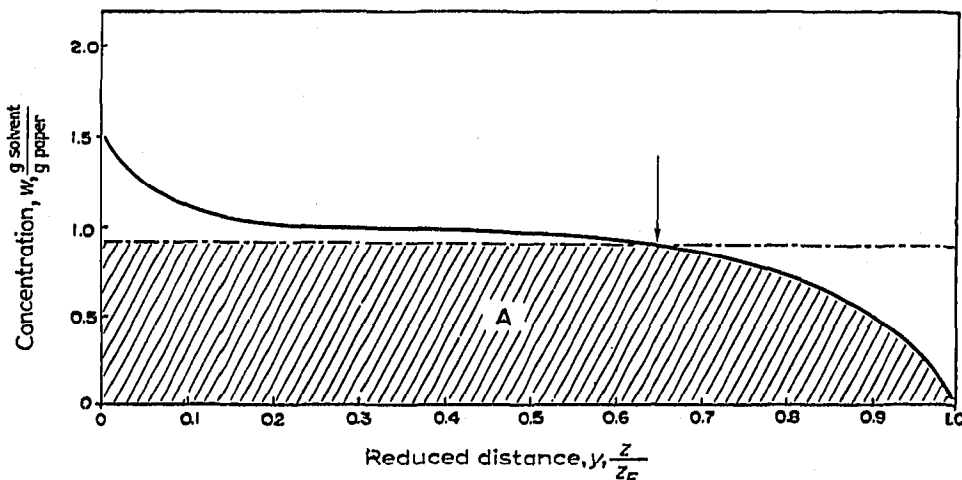


Fig. 3. The area term, $A = \int_0^w y \, dw$.

depends upon the reduced coordinate y . In the figure the A is to be associated with a y directly below the intersection (arrow) of the reduced profile and the area enclosure line.

Combining eqns. (8) and (9) we have the ratio of the zone velocity to the solvent front velocity

$$\frac{\bar{u}}{v_f} = \frac{A}{w + c} \quad (10)$$

Since A and w depend only upon the reduced distance y , the above ratio depends only upon y and the system constant c . This ratio of velocities is plotted in Fig. 4 using a water-saturated butanol solvent on Whatman 3 MM paper.

An interesting result can be obtained from eqn. (10) by considering the case $c = 0$. Here $R = 1$ and $\bar{u} = v$, the local solvent velocity.

$$\frac{v}{v_f} = \frac{A}{w} \quad (11)$$

This ratio is always less than unity for the rectangular sheets and strips now under consideration. In Fig. 3 the ratio A/w is shown as the ratio of the shaded area to the area enclosed by the dotted line. This departs from unity more and more as y becomes smaller as shown by the top line in Fig. 4. This fact by itself makes eqn. (1) incorrect as a description of R_F .

We wish now to examine the R_F value on a horizontal paper strip and describe

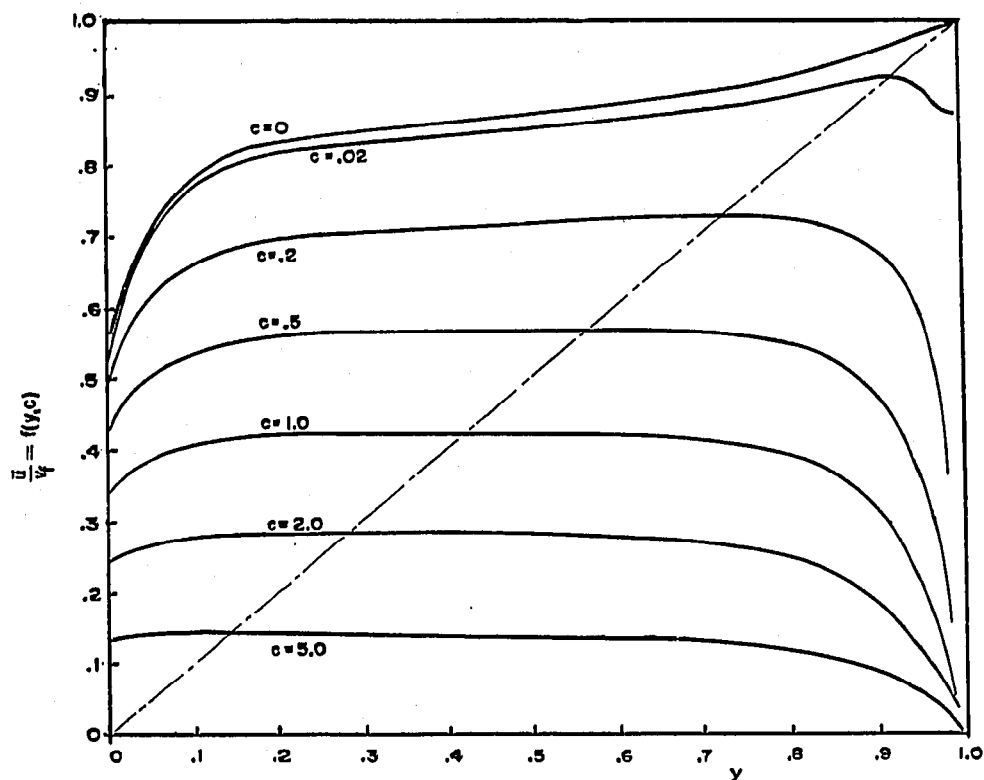


Fig. 4. Relative velocity function, as a function of fractional distance to the solvent front, y , for various values of the partition parameter, c , in a n -butanol (H_2O saturated) Whatman 3 MM system.

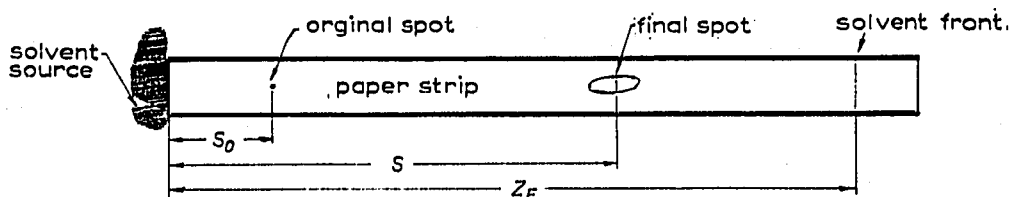


Fig. 5. The coordinates in paper chromatography.

this in the simplest way as a function of (i) the distance from the solvent source to the original spot location, and (ii) the extent of development. Fig. 5 illustrates the meaning of the various coordinates. In terms of these coordinates, $\bar{u} = ds/dt$ and $v_f = dz_f/dt$. The ratio of the two, ds/dz_f , is given in eqn. (10)

$$\frac{ds}{dz_f} = \frac{A}{w + c} \quad (12)$$

It is convenient to introduce the reduced coordinates (not to be confused with y) $S = s/s_0$ and $Z = z_f/s_0$. With this change we have

$$\frac{dS}{dZ} = \frac{A}{w + c} = f(y, c) \quad (13)$$

where we have indicated that the ratio $A/(w + c)$ is a function only of y and c .

Examination of Fig. 5, however, shows y to be equal to $s/z_f = S/Z$. Hence

$$\frac{dS}{dZ} = f(S/Z, c) \quad (14)$$

The value of S corresponding to any Z can be easily generated by a series of steps with the small finite intervals δS and δZ .

$$\delta S = f(S/Z, c) \delta Z \quad (15)$$

The initial condition is that $S = 1$ when $Z = 1$. It is to be emphasized that the function $f(y, c)$ can be easily obtained knowing only c and the reduced concentration profile.

The R_F can be evaluated at any point in the above procedure by the equation

$$R_F = \frac{s - s_0}{z_f - s_0} = \frac{S - 1}{Z - 1} \quad (16)$$

Its value will depend upon c and Z (or S), but not directly upon s_0 . Thus for a given system the R_F is expected to be the same, irrespective of changes in s_0 , as long as the same value of z_f/s_0 or s/s_0 is used for comparison.

As S becomes large (either for long developments or small s_0) the R_F approaches a constant value. This value will equal S/Z , or y . The limiting R_F can be obtained as a solution to the equation

$$R_F = f(R_F, c) \quad (17)$$

This value of $y = R_F$ can be obtained graphically from Fig. 4 using the 45° rule. Thus a straight line at 45° through the origin intersects each curve at the correct R_F value, to be read along the horizontal axis.

The method outlined here predicts zone location as a function of z_f , the distance to the solvent front. Since z_f is related to time through eqn. (3), the zone location can easily be written as a function of time.

RADIAL FLOW

Strong experimental and theoretical evidence exists⁴ which indicates that the concentration profiles obtained in radial (horizontal) flow can be reduced to a common profile after a short transient period. The reduced profile depends upon whether a wick is used or not, and upon the dimensions of the wick if one is used. Fig. 6 shows several reduced profiles for radial flow, one without a wick and the three others with wicks of varying length. It is significant that the profile becomes lower for longer wicks, providing the width remains constant. A long wick acts essentially as a large resistance to flow. For this reason discs with long wicks have the lower concentration profile shown in Fig. 6.

With the use of reduced concentration profiles in radial flow, zone migration can be calculated in much the same manner as before. Eqns. (4-8) are valid for flow in strips, radial flow, and flow in other geometries that may be used. The equation for

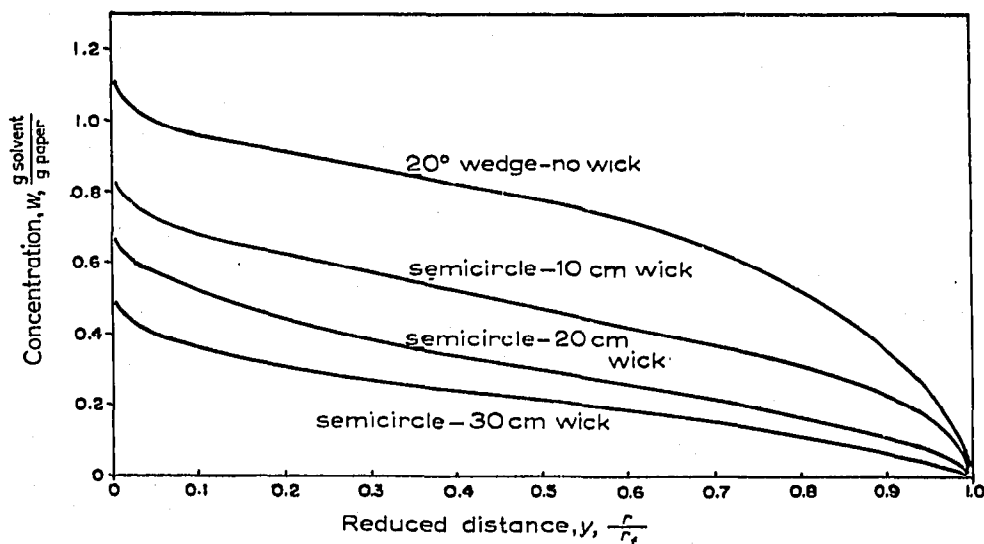


Fig. 6. Experimental concentration profiles; Whatman 3 MM, *n*-butanol (H_2O saturated).

flux, however, is different depending upon the geometry. For radial flow the expression that is equivalent to eqn. (9) is

$$q = v_f A' = v_f \int_0^w y^2 dw/y \quad (18)$$

In the radial case discussed here, A' , rather than an area, is obviously defined by

$$A' = \int_0^w y^2 dw/y \quad (\text{radial flow}) \quad (19)$$

Using A' in place of A , eqns. (10-12) are valid for radial flow. Furthermore by defining

$$f'(y, c) = \frac{A'}{w + c} \quad (20)$$

and letting z_f be the radial distance to the solvent front, eqns. (13-15) are obtained. From eqn. (15) it is possible to generate the zone migration as before. The distance migrated by the spot divided by the distance moved in the same time by the solvent front is defined as R_R . The expression for R_R is equivalent to eqn. (16)

$$R_R = \frac{s - s_0}{z_f - s_0} = \frac{S - 1}{Z - 1} \quad (21)$$

and the limiting value of R_R is found as $R_R = y$

$$R_R = f'(R_R, c) \quad (22)$$

The relationship between limiting R_R and R_F values is of considerable importance in establishing a uniform interpretation of chromatographic data. Unfortunately the R_R of eqn. (22) and the R_F of eqn. (17) are not simply related. This fact is borne out by the experimental results and is illustrated in Fig. 10. Furthermore, the R_R values vary from one another for different wick dimensions. Thus lower R_R values are

obtained with long narrow wicks than with short wide ones because of the lesser amount of solvent on the paper. This appears quantitatively in terms of a diminished value for A' .

It has often been assumed that the R_F and R_R values are related by

$$R_R^2 = R_F \quad (23)$$

This equation is correct for limiting R_R and R_F values if one can assume a uniform, equal concentration in the two papers. In actual practice it is quite inadequate as will be shown in the experimental section. The equation can be derived by assuming the concentration in both the rectangular and radial cases to be a uniform concentration, w , from the source to the solvent front. Making this assumption, the value of A , eqn. (9), is w . Using this in eqn. (13) we find $f(y, c) = w/(w + c)$. Using eqn. (17) we find also that $R_F = w/(w + c)$. In the radial case we find that $A' = w/y$ when the concentration is assumed uniform. From eqn. (20), $f'(y) = w/y(w + c)$. Using eqn. (22) we have

$$R_R = f'(R_R, c) = w/R_R(w + c)$$

or

$$R_R^2 = w/(w + c) \quad (24)$$

Hence eqn. (23) is correct for the profiles assumed above. A uniform profile, however, is a poor approximation to the actual behaviour of paper.

Gravitational and other external fields are not easily analyzed in relationship to zone migration. The principle difficulty lies in the inclusion of a term for flow due to the potential field. This term renders eqn. (2) incorrect, and the Boltzmann transformation, which leads directly to the concept of reduced concentration profiles, is no longer appropriate. The profile can, however, be developed by numerical methods along with the rate of zone migration. This problem is under investigation. It is evident that the concentration profiles for ascending flow eventually lie considerably below those for descending flow, resulting in a similar change in the R_F values.

EXPERIMENTAL

A small room was thermostated at 30° and saturated with water vapor. The chromatographic chambers were sealed and the bottoms covered with solvent to provide maximum area for saturating the interior atmosphere. Sheets of saturated paper were placed in the chambers to provide additional surface.

The chromatographic paper was desiccated over P_2O_5 *in vacuo* for about 24 h prior to use and exposed to solvent vapors prior to chromatographing. Concentration profiles were determined by sectioning the paper and weighing on an analytical balance in stoppered weighing bottles. Dry weights were ascertained after suitable desiccation and corrections for adsorbed water were made.

The solvent system was *n*-butanol saturated with water. The paper was Whatman 3 MM chromatographic sheets. The Methyl Orange used for the zone substance was applied with a hypodermic needle as a 1/3 saturated solution in the developing solvent.

The initial spot size was approximately 2 mm in diameter. All chromatograms were developed in the machine direction of the paper by horizontal technique. The wicks used in the semicircular cases were uniform 1 cm in width and of reported lengths.

RESULTS

From the initially determined reduced concentration profile for linear flow of solvent in paper, a set of R_F versus reduced distance of travel, S , curves were calculated by a numerical method, using relation (15) for various values of the partition parameter, c . This is shown in Fig. 7. These curves show how a chromatographic zone approaches a

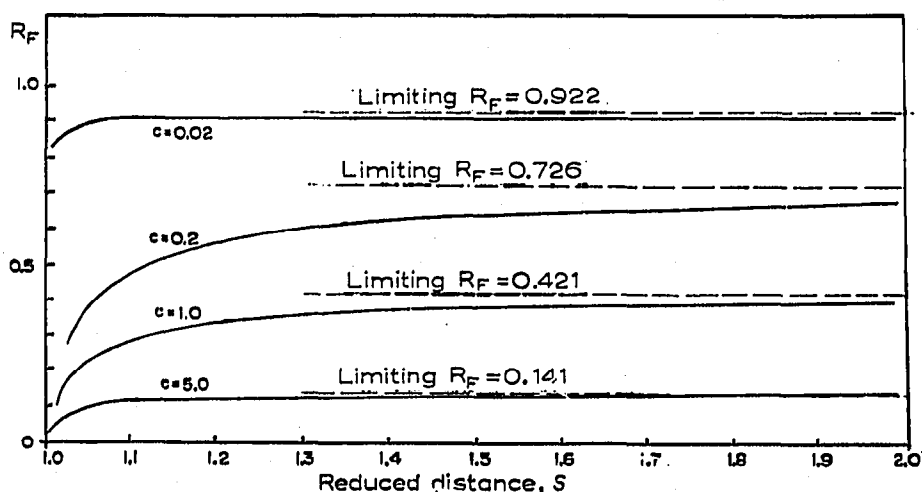


Fig. 7. R_F value as a function of reduced zone travel for various values of the partition parameter, c , for linear, horizontal flow in a *n*-butanol (H_2O saturated) Whatman 3 MM system.

limiting value asymptotically. As may be expected, this approach is quite rapid for both very high and very low values of R_F , but is an important feature with materials of intermediate R_F value.

A long developed chromatograph of Methyl Orange was made with a low initial spot placement, s_0 , to yield an approximation to the limiting R_F value for this substance. From this the partition parameter was estimated to be $c = 1.3$ and the theoretical behavior of R_F as a function of S was calculated.

Methyl Orange was then chromatographed from starting positions of $s_0 = 2, 4, 8, 16,$ and 24 cm and the R_F of the zone was observed as a function of the reduced distance. These experimental points are given in Fig. 8 in comparison with the theoretical prediction. The experimental points are in reasonable agreement with the predicted behavior, supporting the proposed theory. The experiments further confirm the expression of this behavior in terms of the reduced distance variable, S , which removes the dependence of R_F on initial zone location.

As a further test of the theory presented, it was proposed to predict R_R values for circular flow from the information gained (*i.e.* $c = 1.3$) in the rectangular flow

experiment. A wick-semicircle system was used as the concentration profiles could be varied in the semicircle by adjustment of the wick length. The concentration profiles can be obtained numerically from the rectangular profile but for this case it

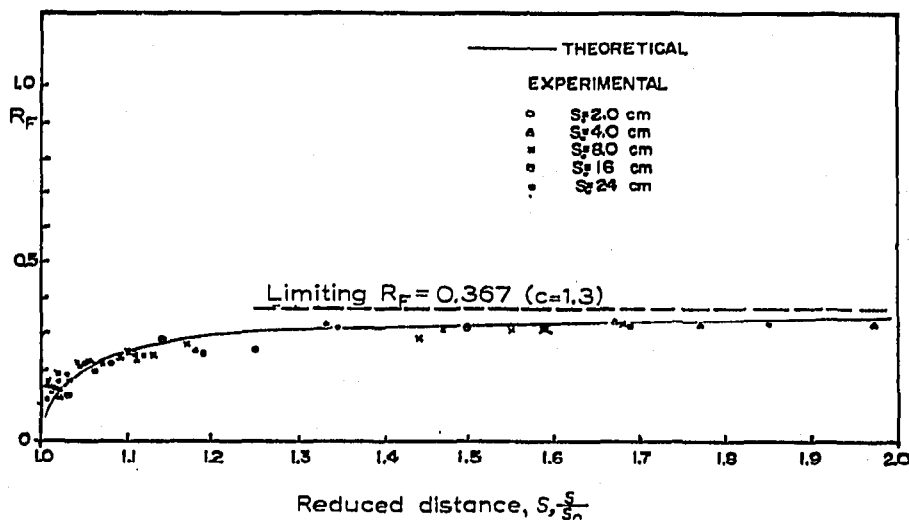


Fig. 8. Comparison of experimental R_F with theoretical prediction for Methyl Orange ($c = 1.3$) on a linear, horizontal chromatogram in a n -butanol (H_2O saturated) Whatman 3 MM system.

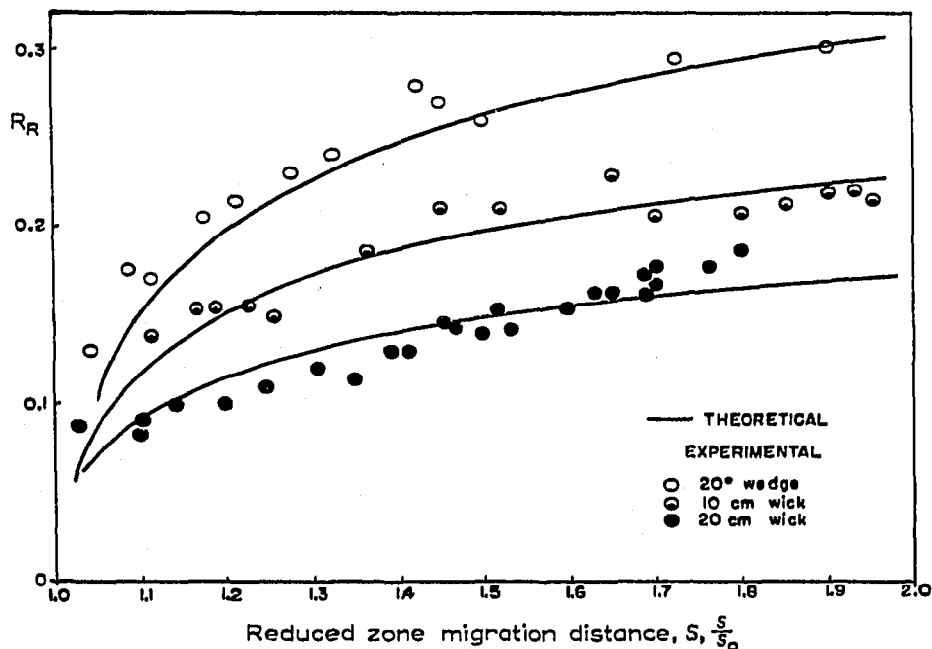


Fig. 9. Experimental and theoretical zone migration for radial cases.

is simpler to determine them experimentally. The radial concentration profiles are given in Fig. 6 for the various wick lengths used. The zero wick length profile was determined on a 20° wedge. The results are shown in Fig. 9.

Using the partition coefficient determined in the linear experiment and the concentration profile for the 20 cm wick length, the R_R and R_R^2 versus S behavior of

the zones was predicted as shown in Fig. 10. It is apparent that neither R_R nor R_R^2 can be compared directly with rectangular R_F 's. Despite the considerable experimental error of the experimental R_F values, the results are quite conclusive in support of the

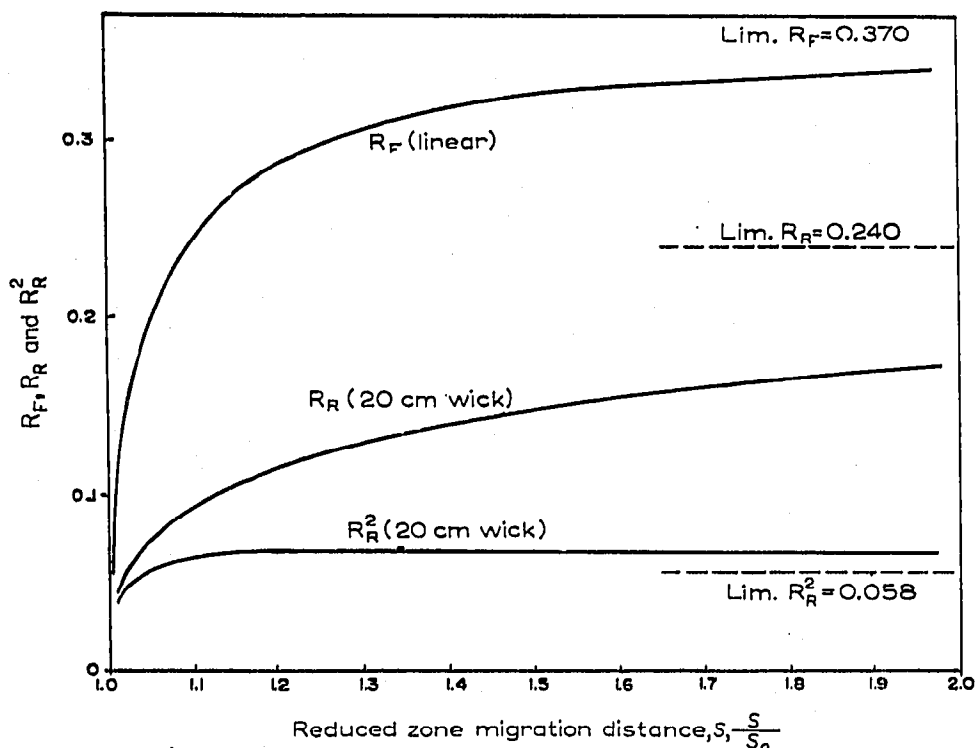


Fig. 10. Comparison of R_F , R_R and R_R^2 values.

arguments presented here. The curves do not always lie in the order R_F , R_R , and R_R^2 , however. Under some circumstances $R_R > R_F$, but whether or not this is true, the R_R^2 values are smallest of the three. The parameter which chromatographically characterizes a system is the partition parameter and not the R_F value.

DISCUSSION AND CONCLUSIONS

The theoretical and experimental results obtained above illustrate several precautions that must be taken in interpreting data in paper chromatography. The role of the concentration gradient is extremely important. Its presence vitiates theories of paper chromatography based upon uniform concentration profiles. Such theories appear deficient in two respects. First, with zones started an appreciable distance from the solvent source, a transient period occurs during which the R_F changes from an initial value of zero to some limiting value. This transition is important, as a rough rule, for a zone migration distance equal to the original distance from the solvent source, s_0 . This effect has been observed by several workers¹.

More important than the transient unsettled period is the fact that the limiting R_F values are determined by local concentrations. The procedure whereby an average

concentration is assumed for all solute zones is incorrect, and does not lead to the proper relationship between the partition coefficient and the R_F . Thus in the experimental work reported here, c has to be assigned the value 1.50 rather than 1.30 to arrive at the correct R_F value. Consequently both c and α are in error by about 15% using the classical method. This situation is rectified by observing the correct limiting R_F , and then using eqn. (17) to solve for c . Eqn. (5) can be used to obtain α from c .

Another point shown in the above results is the fact that no simple comparison exists between the different paper geometries, *i.e.*, rectangular and radial. The relationship of these to ascending, descending and centrifugal chromatography is expected to be even more complicated. The only common denominator of all these methods appears to be the partition parameters, α and c . Our present inability to define accurately the nature of the stationary phase in paper chromatography leaves a certain arbitrary factor in independent methods for determining partition coefficients. It is believed by the authors that the present analysis provides a tool for the investigation into the nature of the stationary phase. The problem is under consideration.

Several limitations are imposed on the above treatment by virtue of the assumptions made. First, a linear isotherm has been assumed such that R , the fraction of molecules in the mobile phase, depends only upon the solvent but not the solute concentration. Overloading can thus invalidate the above treatment. Also the paper must be free from any significant amount of evaporation. For long runs this problem becomes crucial since small differences from saturation can lead to significant losses. Finally, the theory, while basically correct, must be modified to handle the cases where gradients other than in solvent concentration are found.

TABLE OF SYMBOLS

α	partition coefficient
A	area shown in Fig. 3
A_M	cross-sectional area of mobile phase normal to direction of flow
A_S	cross-sectional area of immobile phase normal to direction of flow
c	partition parameter, $\alpha w A_S / A_M$
D	effective diffusion coefficient for solvent flow in paper
κ	flow rate coefficient
q	flux
R	fraction of molecules in the mobile phase
R_F	spot divided by solvent front distance for rectangular flow
R_R	spot divided by solvent front distance for radial flow
s	distance of zone from bulk solvent
s_0	initial zone position
S	reduced zone distance, s/s_0
t	time
\bar{u}	mean velocity of the solute zone
v	average local solvent velocity
v_f	velocity of the solvent front

- w concentration, (g of solvent)/(g of dry paper)
 y reduced distance, z/z_f
 z distance from bulk solvent
 z_f distance of solvent front from bulk solvent
 Z reduced solvent front distance, z_f/s_0

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SUMMARY

The influence of concentration gradients upon zone migration has been investigated theoretically and experimentally. It is shown that the equation $R_F = A_M/(\alpha A_S + A_M)$ is incorrect in relating the R_F to the partition coefficient α . The R_F shows an initial unsettled period during which it approaches a limiting R_F value. Both the limiting and transient values were calculated for the migration of Methyl Orange on Whatman 3 MM paper with a water-saturated butanol solvent.

Cases of radial flow with variable length wicks were also treated. Using the data from rectangular flow, R_R values were successfully predicted for the same Methyl Orange system. The reasons for deviation from the relationship $R_R^2 = R_F$ were discussed.

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