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Eddy Diffusion and Zone Spread in Paper Chromatography

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Summary

A velocity-independent plate-height term comparable to eddy diffusion has been described for paper chromatography. This term couples with nonequilibrium lateral diffusion and is a major contribution to zone spreading at high solvent velocities. A mechanism is proposed based on random variations in R_f values caused by the inhomogeneous structure of paper. On theoretical and experimental bases it is demonstrated that maximum resolution in paper chromatography can be obtained by control of solvent velocities to values near 1×10^{-3} cm/sec.

The investigator who discovers a paper-chromatographic system capable of providing favorable differences in R_f values of the substances he desires to separate may find the usefulness of the system diminished by an excessive zone spread. Attempts to minimize the overlap of zones by means of solvent modification, change of paper, or choice of technique (ascending, descending, etc.) may succeed only because these are methods of altering the velocity of solvent penetration of a papergram. The solvent velocity is a controlling factor in diffusion-like spreading of solute zones, and an understanding of the role of solvent velocity in each spreading mechanism is an asset to chromatographers. It is the purpose of this paper to clarify this role.

Several investigators have demonstrated that the concept of plate height is applicable to chromatographic systems that have a nonconstant solvent velocity (1–3). The fundamental plate-height expression,

$$H = A + \frac{B}{u} + Cu \tag{1}$$

has been the basis of these studies. This expression, in which A is the eddy-diffusion parameter, B the molecular-diffusion parameter, C the nonequilibrium parameter, and u the solvent velocity at the solute-zone location, must be averaged over the history of velocity variations in order to represent the average plate height observed in the completed chromatogram, H_{obs}. It has been presumed that the eddy-diffusion contribution A, at the usual low solvent velocities encountered in paper chromatography, is negligible. That this is not always true is a substantive part of this report.

De Ligny and Bax have analyzed the contribution of molecular diffusion, B, in low-velocity systems and have indicated that diffusion in both stationary and mobile phases contributes to zone broadening (2). Mallik and Giddings investigated the role of nonequilibrium in zone broadening and interpreted the results of their measurement of the C term on the basis of stationary-phase mass transfer (*1*). The author has indicated the importance of mobile-phase mass transfer in the nonequilibrium contribution to plate height (3). In the present paper an additional mechanism, arising out of the inhomogeneities of paper, is presented and combined with other studies for a more complete model of the zone-broadening mechanism in paper.

THEORY

The first term in the plate-height expression [Eq. (1)], eddy diffusion has been the center of a continuing controversy in the field of gas chromatography. The concept that individual molecules follow a streamline whose velocity persists for a packing particle diameter and then randomly alter to a new velocity has been modified by Giddings, who insists that this spreading mechanism couples with the lateral diffusion mechanism (*4*). The solute velocity may change either through persistence of the solute molecules in their streamline (changes in streamline velocity) or by diffusion to a new streamline. At low velocities the lateral diffusion is controlling, and it is on this basis that the eddy-diffusion mechanism is dismissed as negligible in paper chromatography. However, when velocity dif-

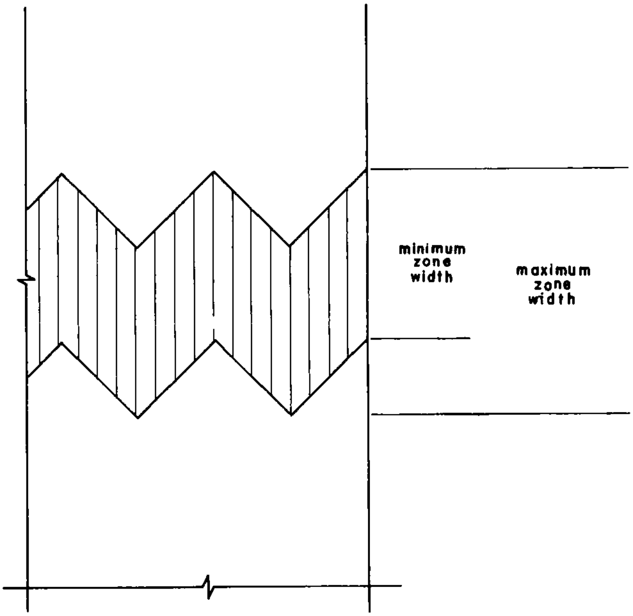

FIG. 1. When R_f fluctuations are uncoupled from lateral diffusion, a jagged edge to the solute zone is observed and the net effect is a maximum effective zone width greater than that which can be measured at a particular lateral position. Later lateral diffusion makes the maximum zone width the actual zone width.

ferences persist over long ranges, perhaps greater than a millimeter, eddy-diffusion-like processes occur which are of a different order of magnitude, and these cannot be neglected, even at the low velocities normally encountered in paper methods. This is particularly true where the coupling must occur interchannel rather than intrachannel. Such a situation has been discussed by Giddings (5).

Despite the high quality of chromatographic paper commercially available, these papers have an inhomogeneity that can contribute to the spreading of solute zones. This inhomogeneity leads to fluctuations in the relative velocity, *R* value, for a solute as it migrates along a line parallel to the flow direction. At high velocities of solvent flow these fluctuations are essentially uncoupled from lateral diffusional effects because of the large distances involved, and result in a ragged edge to the zone. Although prior to extensive lateral diffusion the zone width at any particular flow line is not greatly enhanced, the jagged edge does lead to a gross widening of the zone, which decreases separation (see Fig. 1). Since the establishment of these uneven edges is essentially uncoupled from lateral-diffusional effects, it would be possible to treat the contribution to plate height as an unusual boundary condition for later molecular diffusion. This is mathematically unwieldy and does ignore the gross loss of resolution in the early, high-velocity development of chromatograms. It is, then, practical to treat this effect on the gross spreading basis in much the same manner as transcolumn effects in preparative GC columns (6).

As a model it is presumed that there is a random sequence of domains in paper of average length $\bar{l}$ in which there is an average difference in relative solute velocity, ΔR. The length of a stochastic step can be formulated as $(\Delta R/R)\bar{l}$ and the number of steps as $R(z_i - z_0)/\bar{l}$, where $(z_i - z_0)$ is the difference between the final and initial locations of the solute zone on the papergram. These combine to give an eddy-diffusion plate height:

$$H_A = A = \frac{\Delta R^2}{R^2}\bar{l} \tag{2}$$

As discussed above, this term will couple with the mobile-phase lateral diffusion in the form of the mobile-phase nonequilibrium term $C_m u$:

$$H = \left(\frac{R^2}{\Delta R^2 \bar{l}} + \frac{1}{C_m u}\right)^{-1} \tag{3}$$

Since the coupled eddy-diffusion term is a function of velocity and the velocity is not constant with time in paper chromatography, the zone spread, as a variance, must be summed over the history of the zone and reduced by the distance of travel of the zone to yield an expression for the final observed plate height, H_{obs}. In the absence of amplification of any of the increments of spread due to pressure gradients, as can occur in gas-chromatographic systems, the calculation is essentially a distance average of the local plate height. For the special case of horizontal flow (and short distances in ascending techniques), the solvent velocity may be approximated as $u = k/z_f$, where k is a characteristic parameter of the particular paper-solvent system chosen (7) and z_f is the distance the solvent front has advanced from the solvent reservoir. Carrying out the averaging for this velocity dependence, $H_{\text{obs}} = \int H\, dz_i \Big/ \int dz$, leads to an expression for the coupled eddy-diffusion contribution to the gross zone plate height in the final chromatogram:

$$H_{\text{obs}} = \frac{kC_m}{z_f(z_f - z_0)} \ln \frac{C_m kR^2 + \bar{l}(\Delta R)^2 z_f}{C_m kR^2 + \bar{l}(\Delta R)^2 z_0} \tag{4}$$

where z_f is the distance to the solvent front from the solvent reservoir. In this formulation the nonequilibrium term, C of Eq. (1), has been split into the respective contributions of the mobile and stationary phases (i.e., $C = C_m + C_s$). That the mobile-phase contribution should be a large part of this term has been argued elsewhere (*3*). The complete local plate-height expression can now be written in an expanded form:

$$H = \left(\frac{1}{A} + \frac{1}{C_m u}\right)^{-1} + \frac{B}{u} + C_s u \tag{5}$$

The contributions of each of these three terms to the total plate height are illustrated in Fig. 2 as a function of solvent velocity.

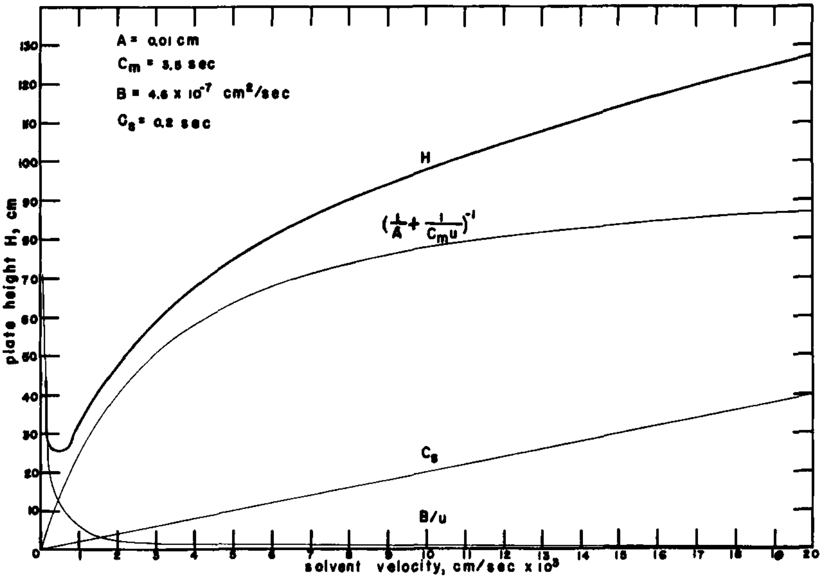

FIG. 2. The total local plate height as a function of solvent velocity is the sum of three terms: the coupled eddy diffusion, molecular diffusion, and nonequilibrium spreading.

It is recognized that in many paper-chromatographic techniques there is a considerable variation in the solvent velocity from the initial rapid penetration of the solvent to the final slow arrival of the front at some position distant from the solvent reservoir. In these experiments, zones experienced velocities ranging between 2×10^{-4} to 2×10^{-3} cm/sec. As can be seen from Fig. 2, the largest spreading occurs at very high velocities (also extremely slow flow rates) such as are met in the initial penetration of solvent into paper. Since the final zone plate height represents an average of all spreading encountered in the development of the papergram, improvement in resolution can be achieved by avoiding high solvent velocities.

EXPERIMENTAL

All results reported, unless otherwise noted, were for the system DL-alanine, butanol–water–acetic acid (60-25-15), Whatman 3MM paper. Experiments were performed as horizontal flow in glass chambers inside a vapor-saturated glove box. This was used to ensure saturation of the atmosphere. All papers were presaturated from the vapor phase for a minimum period of 1 hour.

Initial zones were mechanically streaked, 2λ of 0.02 *M* alanine, on 2.5-cm-wide paper strips in such a manner as to yield an initial zone width no greater than 0.2 cm. Zones were developed by spraying with 0.25% ninhydrin in acetone with a 12-hr room-temperature development following initial hot-air drying. Zone edges were detected using a constant back-lighting device. The same source was used in the measurement of limit of detection concentrations [see (*1*) for details of the method of limit of detection for determination of zone variance and plate height]. Zone width was measured with calipers and micrometer.

All experiments were performed in a constant-temperature room at 30°C.

RESULTS AND DISCUSSION

The existence of the ΔR_f effect discussed above is most readily discerned at high solvent velocities when there is negligible contribution from the mobile-phase term, C_m. The jagged edge of short-run zones is readily seen with the unaided eye, whereas no such effect is detected in the solvent front. Densitometer scans of several typical zones indicated the absence of tailing in these zones, and a Gaussian-like distribution existed. The normal interval of fluctuations appears to average 0.3 cm. The R_f profiles illustrated

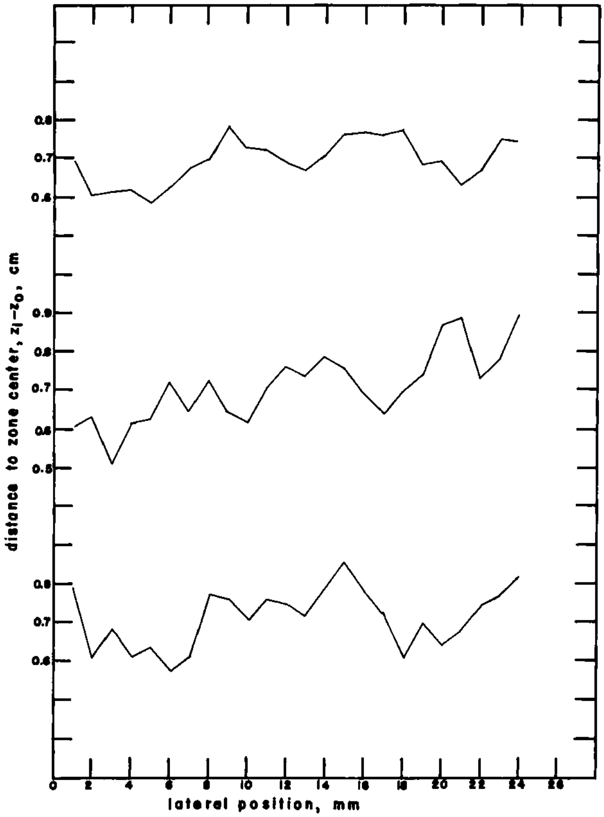

FIG. 3. Profiles of the center of three zones chromatographed 0.7 cm at high solvent velocity. Initial zone locations were 3 cm apart in the machine direction of a strip of W-3MM paper.

in Fig. 3 (measured at 0.1-cm intervals) indicate this, and the different profiles in the figure were measured at positions 3 cm apart on a strip of paper cut in the machine direction. It is evident from the figure that a particular *R* value does not persist along any single line in the paper. This allows the assumption of random-domain distribution in the model presented earlier. This figure also indicates that there can be a similar effect on a much larger scale, of the order of a centimeter, as the right-hand side of the zone is somewhat advanced over the left. The lack of coupling of such large-order effects by lateral diffusion prevents the contribution to the observed plate height. Several experiments were performed with solvent flow normal to the machine direction, and the effect was of the same order of magnitude and interval of fluctuation as in the machine-direction experiments reported here.

A survey of different chromatographic papers was made and the results are reported in Table 1. That this effect is not unique to a particular paper is evident from the data presented. The importance of this effect in various papers will depend on the flow rate of solvents. In slower papers the effect will be less important.

TABLE 1

Difference between Maximum and Minimum Widths (See Fig. 1) for Zones Chromatographed 0.6 cm on Various Chromatographic Papers

Paper	Difference in width, cm
W-1	0.48
W-2	0.42
W-3MM	0.37
W-4	0.45
W-5	0.37
W-7	0.58
W-20	0.30
W-31DT	0.45
W-40	0.44
W-42	0.32
W-44	0.40
W-50	0.49
W-52	0.21
W-54	0.37

Chromatographing zones at different average velocities by placing the initial zone location at varying distances from the bulk solvent and measuring the variation in R_f of the developed zones clearly indicates the coupling of this effect with lateral diffusional processes. The results of several experiments in which the solvent was allowed to flow 10 cm past the initial zone location are illustrated in Fig. 4, in which the standard deviation of the R_f is given as a function of the average solvent velocity. The average velocities here are too low (lateral diffusional times too great) to allow extrapolation to high velocities for a determination of the ΔR_f values need to evaluate the A term by Eq. (2).

The estimation of a value for the A term was made from the very short chromatograms reported in Fig. 3 (zone travel, 0.7 cm), where it can be assumed that the A term is essentially uncoupled from the C term by the high velocity and that the times involved leave

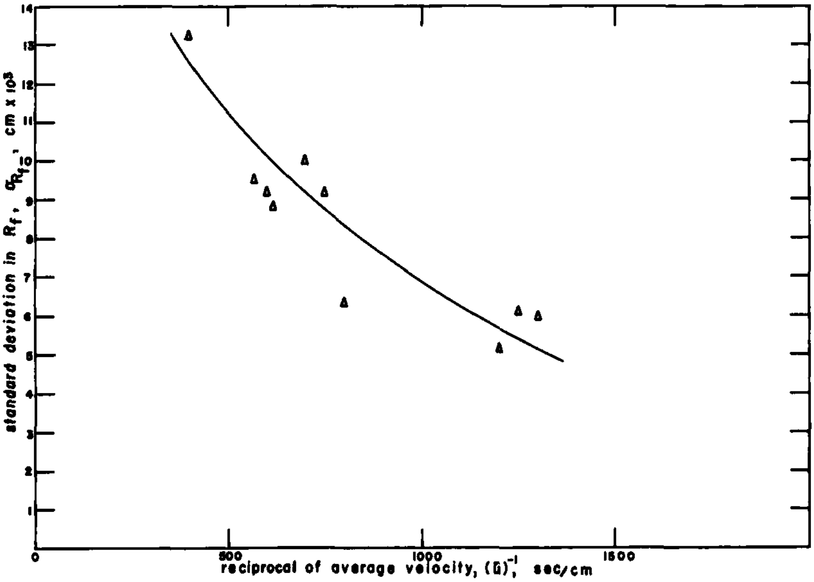

FIG. 4. The standard deviation of zone R_f values (sampling at 0.2-cm intervals normal to flow) after a zone travel of 3 cm is illustrated as a function of the reciprocal average solvent velocity. At lower velocities the fluctuations in R_f are coupled with lateral diffusional effects, lowering the dispersion apparent in R_f.

the molecular-diffusion effects negligible. The average difference in plate height as determined by the limit of the detection method between the maximum and minimum zone widths (see Fig. 1) was ascribed to the A term. The A was found to be of the order of 0.01 cm, which is equivalent to a standard deviation in R_f values of 20% for an average domain size of $\bar{l} = 0.3$ cm. This is not an unreasonable value for fluctuations in R_f.

To prepare Fig. 2, reasonable estimates of the other terms contributing to the plate height were required. Unfortunately, in this series of experiments it was impossible to maintain parabolic flow rates over long distances (i.e., $z_f^2 = kt$) or to reproduce flow rates from paper to paper with sufficient precision to determine the required parameters for an integrated form of Eq. (5).

Although the A-term contribution was not recognized in the work of Mallik and Giddings (*1*) or earlier similar work in this laboratory (3), the velocity ranges covered in their determination suggest that their value for the nonequilibrium term, C, is at least

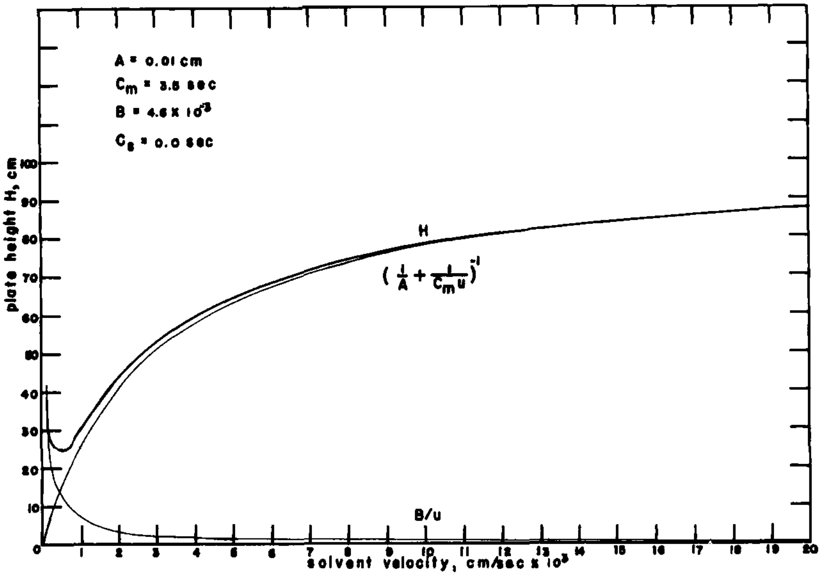

FIG. 5. The total plate height as a function of solvent velocity where there is complete coupling of the nonequilibrium term with eddy diffusion. Note how the more complete coupling leads to a leveling off of the total plate height at lower velocities then in the case of partial coupling (see Fig. 2).

correct in order of magnitude. A major question to be answered is the extent to which the nonequilibrium term couples (i.e., is mobile-phase-controlled) with the *A* term. In Fig. 5 is shown the local plate height under conditions of complete coupling of the nonequilibrium term with the R_f fluctuations. Comparison of Fig. 5 with Fig. 2, in which there is only partial coupling, reveals that extensive coupling leads to an earlier leveling of the plate height at high velocities.

The *B*, molecular-diffusion, term used in the preparation of Figs. 2 and 5 was determined for our system by a measurement of zone spreading in a nonflow situation. This value, $B = 4.6 \times 10^{-7}$, is in good agreement with predictions from the data of de Ligny and Bax (2).

A number of plate-height determinations were made by the limit-of-detection method, where solvent velocity was controlled by varying the initial zone location. The solvent front was allowed to advance 15 cm past the initial zone location in each case, and the

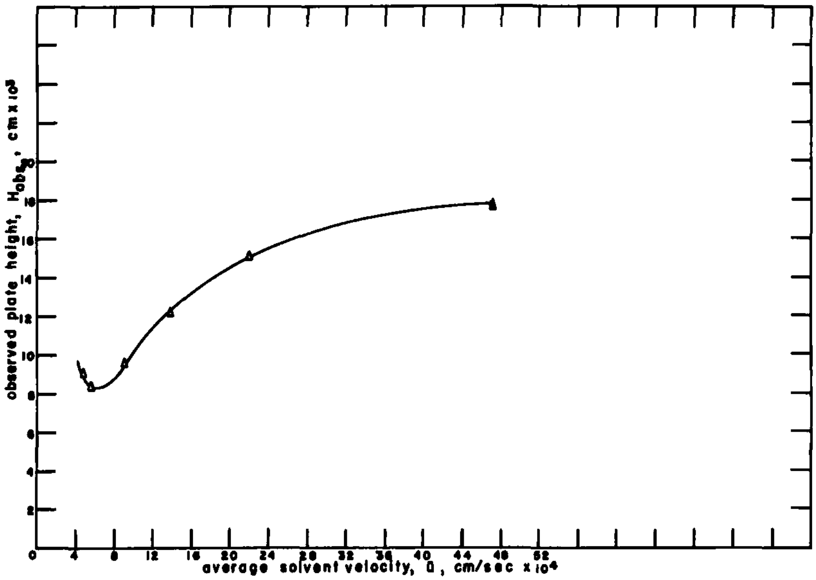

FIG. 6. The observed plate height as a function of average solvent velocity for zone travel of 4.5 cm. Solvent velocity was varied by locating the initial zone at varying distances from the solvent reservoir.

flow was approximated by the expression $z_f^2 = kt$. The observed plate heights are presented in Fig. 6 as a function of the average solvent velocity. When these data are compared with Figs. 2 and 5 it is found that they indicate a high degree of coupling between the R_f fluctuation and the nonequilibrium effects. This further indicates that the nonequilibrium effect is largely centered in the mobile phase. Figure 6 also illustrates the improvement of resolution that may be achieved by limiting the range of solvent velocities in paper chromatography. Striking improvement in resolution can be gained in the separation of alanine and glycine by moving the initial zone location far from the solvent reservoir to avoid the initial high velocities of solvent flow.

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REFERENCES

1. K. L. Mallik and J. C. Giddings, *Anal. Chem.*, **34**, 760 (1962).
2. C. L. de Ligny and D. Bax, *Z. Anal. Chem.*, **205**, 22 (1964).
3. G. H. Stewart, in *Advances in Chromatography* (J. C. Giddings and R. A. Keller, eds.), Vol. 1, Dekker, New York, 1965, p. 93.
4. J. C. Giddings, *Nature*, **184**, 357 (1959).
5. J. C. Giddings, *Anal. Chem.*, **35**, 1338 (1963).
6. J. C. Giddings, *J. Gas Chromatog.*, **1**, 12 (1963).
7. A. L. Ruoff, D. L. Prince, J. C. Giddings, and G. H. Stewart, *Kolloid-Z.*, **166**, 144 (1959).

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