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Observed Plate Height in TLC*

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Summary

The flow mechanism in thin-layer plates has been established as the same capillary mechanism observed in paper chromatography. A pore-size distribution of the thin-layer plate maximizes at 1×10^{-3} cm radius. The molecular-diffusion and stationary-phase mass-transfer parameters of the plate-height expression have been determined for the system Sudan Orange-Silica Gel G-chloroform to be $B = 6.0 \pm 0.4 \times 10^{-6}$ cm^2/sec and $C_s = 0.12 \pm 0.04$ sec, respectively. Estimates of the eddy-diffusion and mobile-phase mass-transfer parameters are presented.

If we may have confidence in Truter's prediction in the first volume of *Advances in Chromatography* (*1*) that thin-layer chromatography (TLC) will completely displace paper (PC) and column techniques and will relegate gas chromatography (GC) to the role of a very specialized technique, it would seem proper to pay some attention to the treatment of the more theoretical aspects of this tool. The studies and debates in the field of gas chromatography toward developing a surer and more comprehensive theory have sparked the growth of GC into a powerful analytical tool and brought it to the position of a promising new method for the determination of physical parameters such as isotherms, diffusion coefficients, and activity coefficients. Few such studies

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have been directed toward liquid chromatography. It has been the fashion to accept the generalization of theory to include all chromatographic techniques without a careful analysis of the differences in systems or in the magnitude of parameters appropriate to each system. These considerations have led the author to consider the problem of zone spreading in techniques that depend on a capillary mechanism of solvent flow and to extend these studies for this symposium to the particular case of TLC.

Paper and thin-layer chromatography are characterized by the capillary mechanism of flow which is unique in the unsaturation of the porous bed and in the solvent velocity which varies with time of development and, to a lesser extent, from point to point along the chromatogram. A reasonable study of this flow mechanism has been made for PC (*2*), and it remains to this paper to demonstrate that the models may be extended to TLC and to use them as a basis for interpreting the mechanisms leading to the final zone spread, which is observed as a plate height in a completed chromatogram.

THEORY

To provide a basis for comparison and a theoretical framework for interpretion of the results of this study, a brief review of zone spreading in PC is presented (*3,4*). The initial hypothesis for the work on PC was that the van Deemter equation, Eq. (1a), for plate height H, would be valid with the A term, eddy diffusion, being

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

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

negligible, the B term, molecular diffusion, being important at low velocities encountered in long chromatograms, and the C term, mass-transfer spreading, being important at the high solvent velocities encountered during the initial penetration of the solvent into the chromatogram. It was found necessary, however, to use the coupled form, Eq. (1b), in which the mass-transfer term is divided into mobile-phase C_m and stationary-phase C_s contributions and to interpret the A term in Eq. (1b) not as eddy diffusion but as a position-dependent fluctuation in R value due to inhomogeneity of the fiber matrix.

The plate height of Eq. (1) is a local plate height, implying that it is evaluated for a particular position or differential distance on the chromatogram dL, at which the velocity is u. As such, it may be expressed as the differential variance $d(\sigma^2)$ the zone obtains in each increment of distance it traverses:

$$H = d\sigma^2/dL \tag{2}$$

The observed plate height H_{obs}, determined from the zone at the completion of a chromatogram, is the distance average of plate heights experienced during the development of the chromatogram and is expressed in terms of the local plate height as

$$H_{\text{obs}} = \int H\, dL / \int dL \tag{3}$$

The knowledge of solvent velocity as a function of time and distance and the value of H_{obs} determined for various velocity ranges allow a determination of the system constant parameters of the local plate-height expression (1a) or (1b).

The study of zone spreading in PC and recent work in GC on the coupling of eddy diffusion suggest that Eq. (1b) is expected to hold in TLC (5). The experimental results presented below are not sufficiently sensitive to give a valid test of this equation, and the approximation represented by Eq. (1a) is used in the evaluation of results. This approximation is valid under the conditions that the A term is small compared to the mobile-phase term $C_m u$ and that the stationary-phase term $C_s u$ contributes measurably to the plate height. It was anticipated that the A term would be small, of the order of particle diameters in the thin layer, and that A-term contributions from R-value fluctuations would be negligible. The micron-size particles of the thin layer suggested that the mobile-phase C term, which is proportional to the square of the pore radius through which the solvent flows (6), would also be small; however, the study of pore sizes in the layers indicates that it is of comparable magnitude to the values found in PC and that this part of the approximation is valid.

The primary requirements established for the selection of a TLC system for study were (a) a single pure solvent and (2) symmetric solute zones. The first requirement, of a single solvent, was dictated by a desire to avoid chromatographing of the solvent and to avoid the establishment of a liquid stationary phase of unknown composition and extent. The second, symmetry of the zone, was dictated by the assumptions concerning the spreading mechanism in which nonequilibrium tailing due to slow mass transfer is presumed to be absent. As will be seen in the discussion of results, these requirements do not deny the possibility of a measurable stationary-phase mass-transfer term necessary to the use of the approximation represented by Eq. (1a).

The verification of a capillary flow mechanism in TLC provides a basis for suggesting methods of controlling solvent velocity and a basis for interpreting the *A* and *C* parameters of the plate-height expression in terms of the structure of the porous bed. The capillary mechanism of flow has as its driving force the movement of frontal liquid into spaces of smaller radius in order to lower surface free energy. The driving force increases proportionally to the surface tension of the solvent. As the solvent advances it draws liquid from the bulk reservoir along the path of least resistance—through the largest available pores. The resistance to flow increases with distance to the front and with the viscosity of the liquid. In one-dimensional flow the capillary mechanism predicts that the distance of the front from the bulk solvent, z_f, is given by

$$z_f^2 = kt \tag{4}$$

where the flow parameter k is directly proportional to the ratio of surface tension to viscosity, γ/η. The concentration profile of the solvent along the chromatogram will remain unchanged if the distance is expressed as a reduced distance (reduced by the distance to the front), and the solvent velocity behind the front will be some large fraction (about 80%) of the frontal velocity (7).

The limit-of-detection method for determination of the variance of zones for the calculation of observed plates is sensitive to the relationship between total amount of solute and the limit of detection. From a knowledge of these two quantities and the zone width at the limit of detection, the variance of the zone can be argued (8). The amount of solute should be adjusted so that the change in concentration with distance allow detection of zone edges with a precision approximating the precision with which zone width can be measured. If the limit intersects roughly between an eighth and a quarter of the peak height (Fig. 1), the greatest precision can be achieved. It is well to pause here and recommend to the attention of those primarily interested in separation and purification the value of knowing the limit of detection

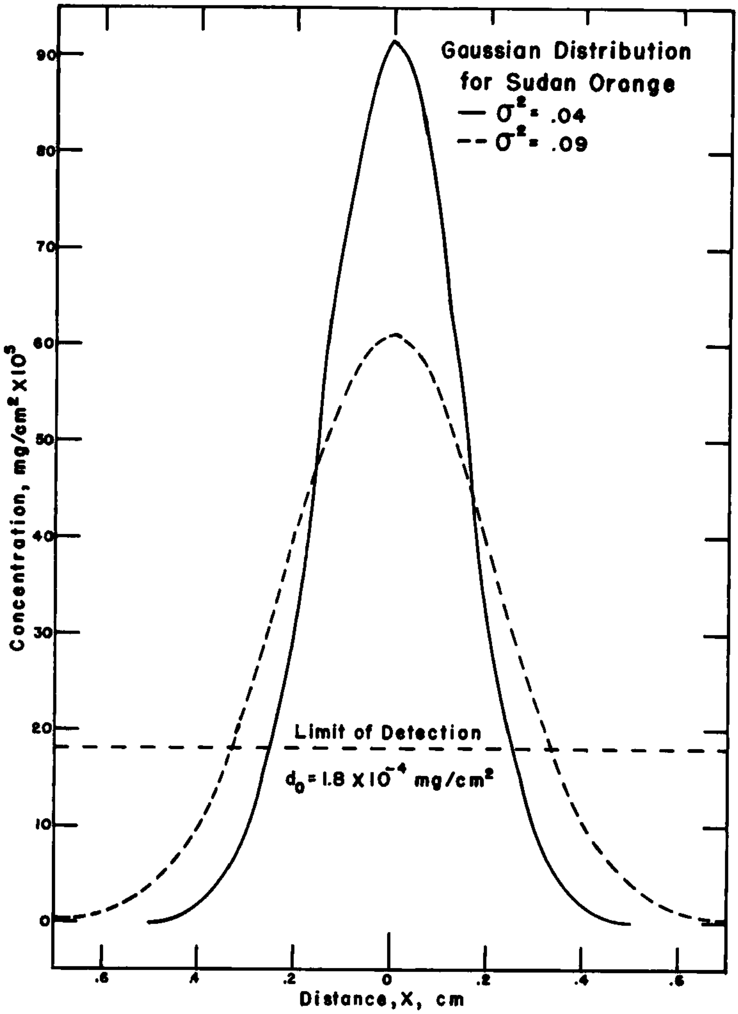

FIG. 1. Gaussian distributions of variance $\sigma^2 = 0.04$ and $\sigma^2 = 0.09$ in relationship to the measured limit of detection and zone width from which they were reconstructed. Both distributions represent the same amount of Sudan Orange dye.

in relationship to the concentrations of zones. When working near the limit, there can be an apparent resolution that bears little direct correlation to fact, as serious tailing and overlap of zones can occur entirely below the limit of detection. It is even possible to observe zone areas decreasing with increasing spread of the zone (note that this would be true if the limit of detection were above the intersection of the two distributions in Fig. 1). It hardly need be mentioned that the limit need not be the same for different materials, different methods of detection, etc., which can change apparent relationships between zones on a chromatogram. Limits of detection are seldom given when chromtograms are displayed.

EXPERIMENTAL

All thin-layer plates used in this work were spread 0.50-mm thick with a 17:40 Silica gel G-water slurry, dried a minimum of 2 hr at 140°C, and presaturated at least 1 hr in solvent vapors. Except for height-of-rise experiments, all plates were 2 × 8 or 8 × 8-in. plate glass selected for flatness and were developed by ascending technique in solvent tanks lined with solvent-saturated filter paper. Solute zones were applied with a mechanical device utilizing a 1-μl syringe. Initial zone width was held to less than 1 mm and a minimum zone length (normal to flow) of at least 5 cm. Sudan Orange dye was used exclusively and was applied in an initial line containing 4.6×10^{-4} mg/cm. Limit of detection was determined from multiple spotting of different dye dilutions to be 1.8×10^{-4} mg/cm^2. Zone edges were detected visually under constant backlighting conditions and widths measured with calipers and micrometer. Zone symmetry was tested by scanning zones with a Spinco Analytrol densitometer with microzone attachment. With zone widths 15 times slit width and limits of detection lower that visual, no asymmetry of zones could be measured with Sudan Orange chromatographed with chloroform, the solvent used for all zone-spreading experiments.

Height-of-rise and solvent-concentration profiles were determined by sampling the silica gel, weighing, drying, and weighing. All transfer operations were performed inside a solvent-saturated glove box to minimize evaporation.

All experiments were performed at 25 ± 1°C.

RESULTS AND DISCUSSION

Test of Flow Mechanism

In all plates tested the advance of the front was well approximated by Eq. (4) up to 7 cm height with a slightly greater than

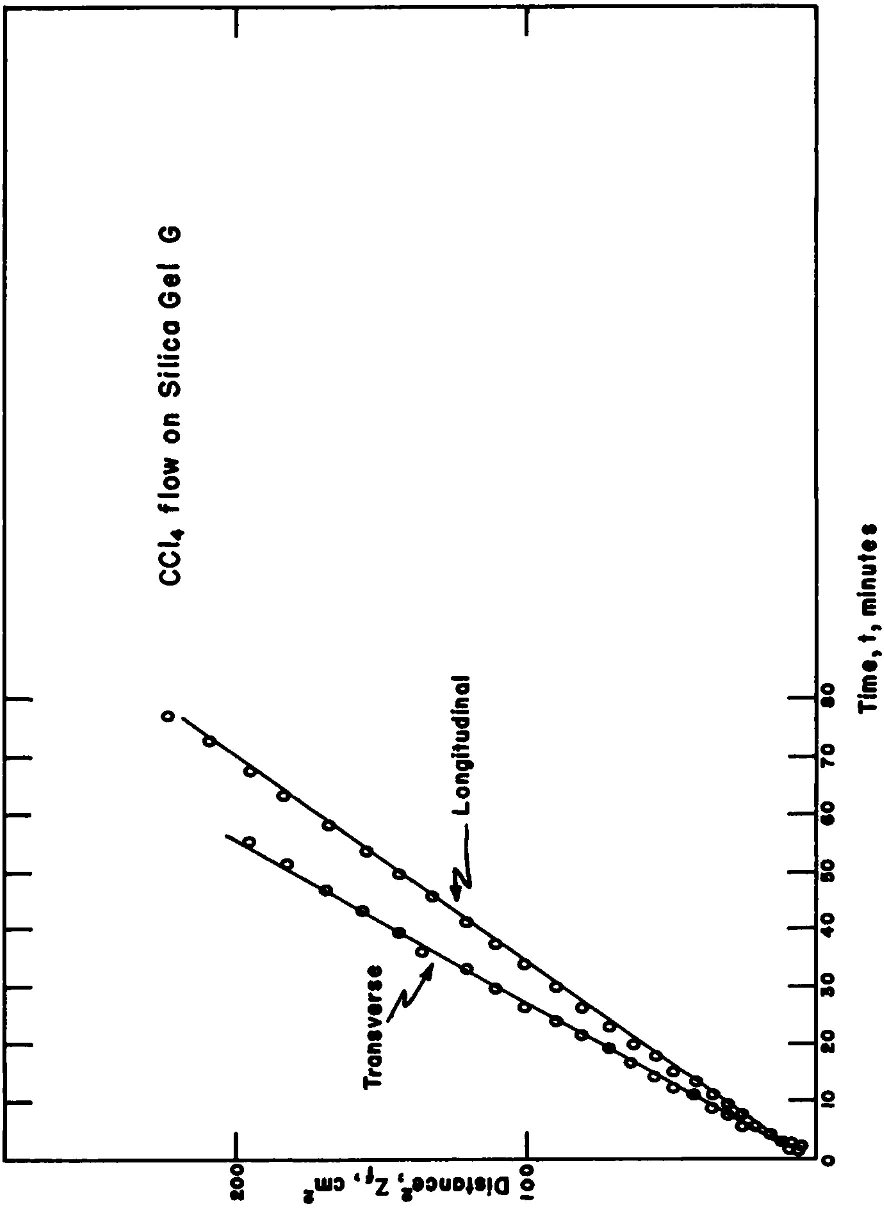

FIG. 2. Rate of penetration of CCl$_4$ into a thin-layer plate transverse to and longitudinal with spreading (machine) direction. This illustrates the flow equation $z^2 = kt$.

predicted decrease in velocity above this height, owing to the influence of gravity (Fig. 2). The flow parameter *k* of Eq. (4) varied as much as 20% from plate to plate for a given solvent. The same plate could be dried and reused with less than 3% variation in *k*. With care, plates could be prepared with less than 5% variation in *k* between machine direction and normal-to-machine direction. Point-to-point variation in *k* for a single plate in one direction was negligible.

The reducibility of concentration profiles was difficult to ascertain, owing to evaporation, short distances, and possible influence of gravity, but the results in Fig. 3 indicate that the prediction is correct.

The proportionality of the flow parameter *k* to the ratio of surface tension to viscosity is confirmed by the data in Fig. 4. All determinations of flow rate for various solvents were performed with the same thin-layer plate.

These results leave little doubt that the capillary mechanism

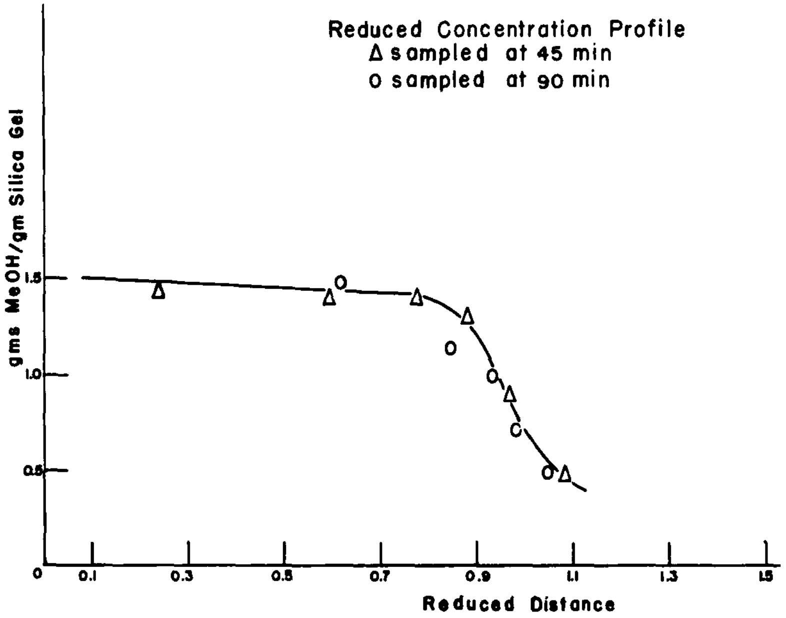

FIG. 3. Concentration profile of solvent (MeOH) in a thin-layer plate, where distance from bulk solvent is reported as the distance reduced by the distance to the solvent front.

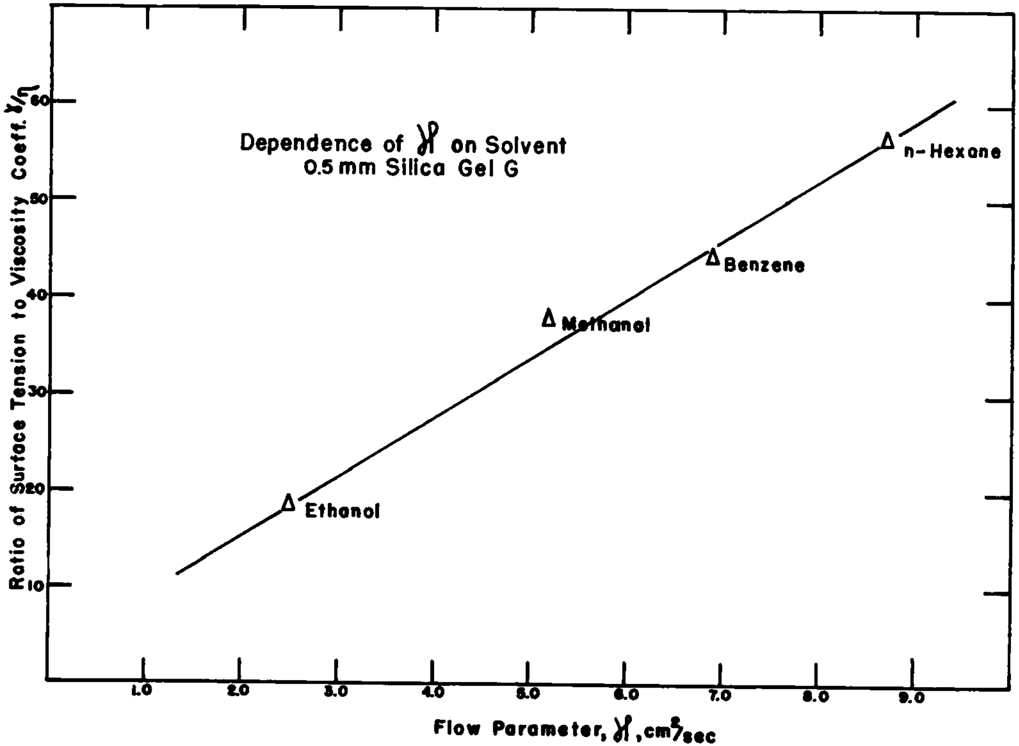

FIG. 4. Dependence of the flow parameter $k = z^2/t$ on the ratio of surface tension to the coefficient of viscosity. All data refer to the same Silica Gel G plate.

of flow model is correct for thin layers. The deviations of the velocity from predictions of Eq. (4) led the author to use numerical integration of velocity in the use of Eq. (3) but did allow the use of Ref. *7* in predicting solvent flow rates at positions behind the advancing front. Confidence in the mechanism of flow also aids in the interpretation of apparent anomalies in the pore structure of the thin layer discussed below.

Pore Size

Figure 5 illustrates the pore distribution determined from the equilibrium concentration profile of a height-of-rise experiment (9). A rather narrow distribution is observed with the mode at 1×10^{-3} cm. These pores seem extraordinarily large for a bed formed from particles reported by the manufacturer to be in the 1- to 5-μ-size range. Despite a certain lack of confidence in the height-of-rise method of pore-distribution determination, these results are supported by other evidence.

1. The flow parameter k or Eq. (4) is comparable to k values met in papers of this general pore size (9).

2. Photomicrographs of Silica Gel G slurries indicate a tendency toward flocculation of particles. If these flocs, through the drying process, grow to diameters of the order of 10^{-3} cm, they would pack to give the kind of distribution observed. The literature indicates that silicate materials of particle sizes below 20 μ show anomalous viscosity and capillary-rise phenomena interpretable in terms of flocculation (*10,11*). Photomicrographs also show, despite manufacturer's claim, about 5% boulders (up to 500μ) admixed with 1- to 5-μ material.

3. The mass-transfer coefficients reported below are predictable from a model of flocs and pores of the 10^{-3}-cm order of magnitude.

This evidence leads the author to suggest a model for the thin layer of packing of flocs about 10^{-3} cm in radius with an internal pore structure formed from 1- to 5-μ particles holding solvent as a stationary phase. The packing of the flocs forms pores of 10^{-3}-cm average radius holding solvent which constitutes the mobile phase. This model predicts a stationary-phase mass-transfer term of sufficient magnitude to allow the use of Eq. (1a) in the interpretation of observed plate heights.

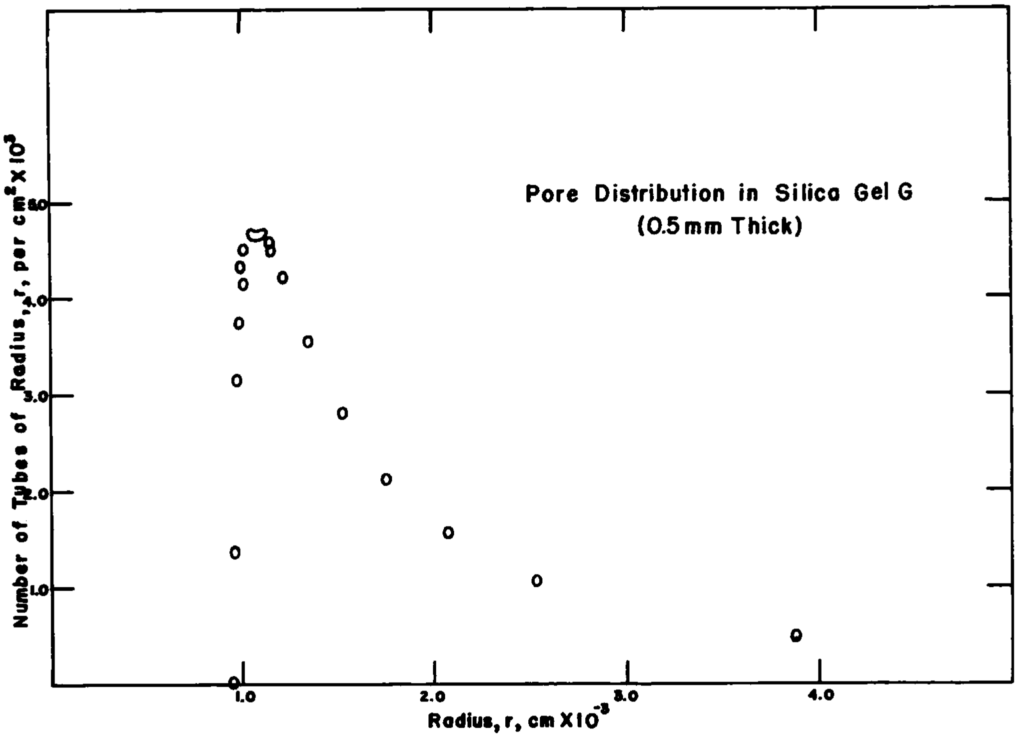

FIG. 5. Pore distribution in a 0.5-mm-thick Silica Gel G thin-layer plate determined from equilibrium height-of-rise data.

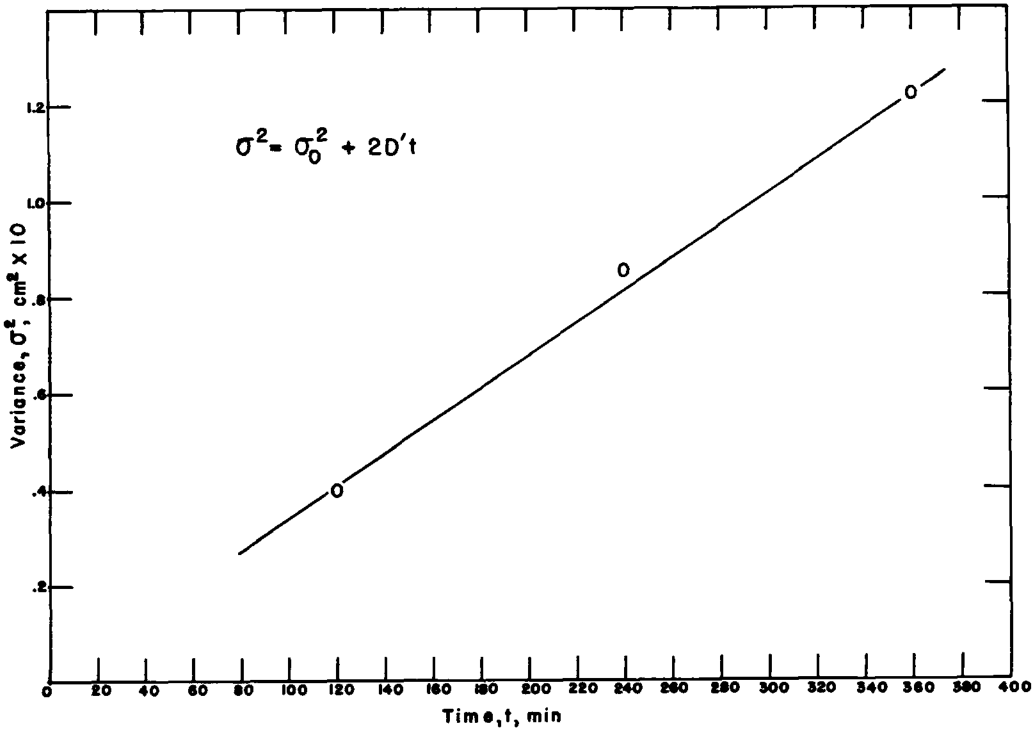

FIG. 6. Determination of the molecular-diffusion parameter $B = 2\gamma D$ from the slope of the zone variance as a function of time in a nonflowing system.

Plate-Height Parameters

The mass-transfer term of Eq. (1a) was argued by numerical integration of Eq. (3) from observed velocities. Four velocity ranges were sampled on each thin-layer plate, and separate determinations led to a mean value of C equal to 0.12 ± 0.04 sec. This is the order of magnitude predicted for C_s on the basis of the B term below and the stationary-phase flocs of 10^{-3} cm.

In the velocity ranges sampled, with an eddy diffusion A of the magnitude of floc diameters, and predicting C_m to be the same order of magnitudes as C_s, the approximation of Eq. (1a) would be valid.

The molecular-diffusion parameter, $B = 2\gamma D$, was determined in a nonflowing system by determination of zone variance at various time intervals as previously reported for PC (*4*). Figure 6 shows the results of a typical experiment interpreting the expression $\sigma_t^2 = \sigma_i^2 + 2\gamma Dt$, where the total variance σ_t^2 is the sum of initial variance σ_i^2, and variance gained through molecular diffusion. The mean value was found to be $B = 6.0 \pm 0.4 \times 10^{-6}$ cm^2/sec.

CONCLUSIONS

The homogeneous structure of thin-layer plates (silica gel) prevents extraordinary spreading due to R-value fluctuations at high solvent velocities, such as found in paper chromatography, and the pore structure of thin layers allows operation at solvent velocities high enough to avoid excessive zone spreading through molecular diffusion. The control of solvent velocity in thin-layer plates is not the important factor it appears to be in PC for control of plate heights.

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