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ON INCREASING THE SENSITIVITY OF DETECTION IN THIN-MEDIA CHROMATOGRAPHY BY DECREASING THE DIMENSIONS OF THE SUPPORT MEDIUM

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SUMMARY

A definite possibility for increasing the sensitivity of thin-media chromatography in the detection of minute quantities of separated chromogens or fluorogens is a reduction, usually the width, in the physical dimensions of the medium. Experiments have shown, however, that the results obtained with this approach generally fall short of expectations. The principal reason for this would seem to be 'edge effects' which alter the surface properties of the medium and increase the non-uniformity of the velocity profile of the solvent, resulting in a broadening of the selected zones, a distortion of the concentration profile within the zone and frequently a decrease in separating power. These effects can largely be overcome by using a cylindrical support for the medium and very thin active layers.

INTRODUCTION

Thin-media chromatography is probably the commonest and at the same time most efficient procedure for isolating individual substances from multi-component mixtures. In many applications the amount of substance to be separated or isolated is in the microgram range. The smallest amount of substance, which under these conditions can be proven to be present and perhaps quantitated, is essentially determined by the threshold level of the methods used with respect to the particular substance involved. At present the methods most frequently used are photometric and with appropriate instrumentation it is probable that these procedures are the most sensitive ones with respect to both detection and quantitation.

The performance of any photometric method is limited not so much by the total amount of separated substance present within a particular zone, but by its concentration within that zone. In order to increase sensitivity therefore, it is essential

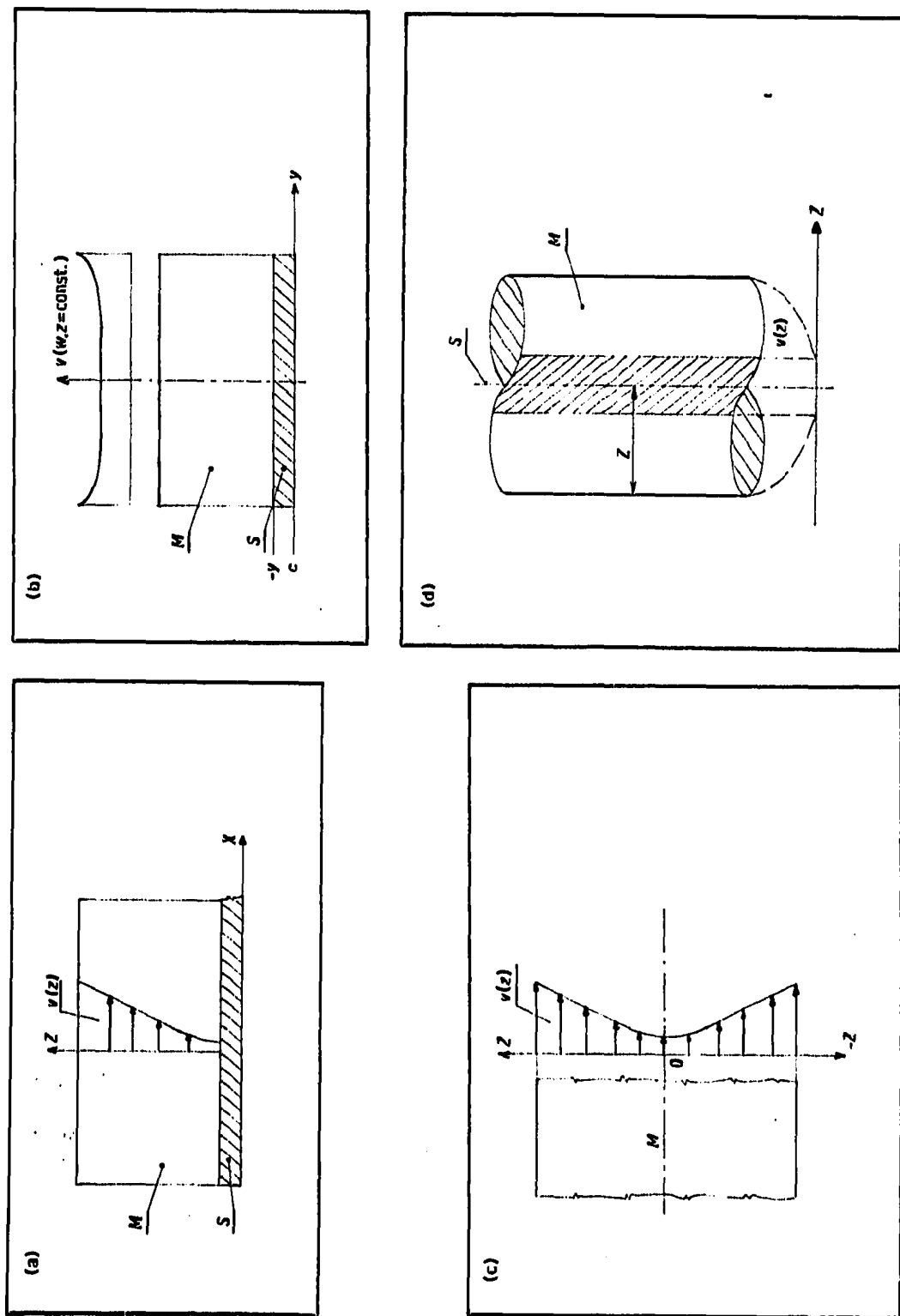


Fig. 1. Solvent velocity distributions in the chromatographic medium (diagrammatically). (a) Longitudinal section of planar medium; (b) transversal section of planar medium; (c) medium without substrate (i.e., paper) longitudinal section; and (d) cylindrical medium. M = Chromatographic medium; S = mechanical support.

that the spatial concentration of substance, that is the ratio of the total amount of separated solute in a zone to the area of that zone, be increased. A quite obvious approach to this problem is to reduce the width of the chromatographic medium. The results of this simple approach are, however, disappointing once a certain minimum width has been exceeded. The reasons for this phenomenon and ways by which it might be overcome are considered below.

WIDTH OF THE CHROMATOGRAPHIC MEDIUM

It has been shown in the past that the concentration profile of a separated substance is with reasonable approximation Gaussian. The width of the zone depends mainly upon the character and the volume relations of the components of the stationary phase and also upon the distance from the origin, where the solute was originally applied. These facts are of course well known. Expressed mathematically they lead to the expression:

$$c(x,t) = \frac{B}{\sqrt{t}} \exp \frac{-[x-vHt]^2}{Lt} \quad (1)$$

In this equation B, H, L are constants characteristic of the separation system used. v is the average velocity of solvent flow and t the time of development and x is the distance from the origin. The product

$$X = v \cdot t \cdot H \quad (2)$$

determines the mean distance an element of solute has travelled from the origin.

The width of an individual zone, the distance between different zones and the kind of substances to be isolated can be controlled by the judicious choice of the critical parameters of the system (quality of, nature of support and solvent system etc.). Even under the most favourable conditions, however, diffusion prevents reduction of any zone width below a certain minimum. The simple solution, reducing the dimensions of the medium (*i.e.*, making it narrower) and/or area of solute application, thus increasing the concentration of separated solute in a given separated zone applies only to a limited extent. If the width of the medium is decreased below a certain limit the separating power of the medium deteriorates and contrary to an improvement the situation becomes less suitable for the detection of very small amounts of solute. Some aspects of this phenomenon are analyzed below.

EDGE EFFECTS

Fig. 1 shows both a longitudinal and a transversal cross-section of the medium with the respective velocity profiles of the solvent. The solvent flows through the pores of the medium with a principal direction of flow parallel to the longitudinal (x -axis) axis of the medium. The flow velocity of the solvent is, however, far from uniform even on a macroscopic scale. In general the highest average velocity will occur at the boundary surface between medium and the surrounding air-vapour mixture, while the lowest velocity will occur at the contact surface between medium and substrate¹. The resulting longitudinal velocity profile is shown diagrammatically in Fig. 1a. If the medium is self-supporting without a substrate (*i.e.*, paper) a velocity

distribution of the type shown in Fig. 1c results. The well known reasons for this phenomenon are, of course, internal and external frictional forces acting upon the moving solvent and surface interactions between the moving and the stationary phases. Transversally a similar non-uniformity of the velocity of the mobile phase is observed (Fig. 1b). At the free edges of the medium the velocity will in general be larger than in the center part of the layer.

Eqn. 1 shows that the (theoretical) concentration profile of a zone is Gaussian, and that the axis of the zone is displaced from the origin by the distance X . If v is non-uniform over the cross-section of the medium the total concentration profile of the zone is produced by the superposition of partial zones shifted by various distances X . Diffusion tends to reduce these phase differences but it cannot cancel them altogether. The overall concentration profile of a zone can then be determined, in a very general way, from eqn. 1.

$$c(x,t,y) = \frac{B}{\sqrt{t}} \int_0^Z \exp \frac{-[x - Htv(y,z)]^2}{Lt} dz \quad (3)$$

Z is the thickness of the coating layer and x, y, z are here the longitudinal, transversal and thickness coordinates of the medium, respectively (see Fig. 1). The effect of diffusion along the y and z axis is, in first approximation, incorporated into the dependence $v(y, z)$ of the velocity of flow upon the y and z coordinates. If $v(y, z)$ is a linear function of z , that is if

$$v(y, z) = z \cdot v(y) \quad (4)$$

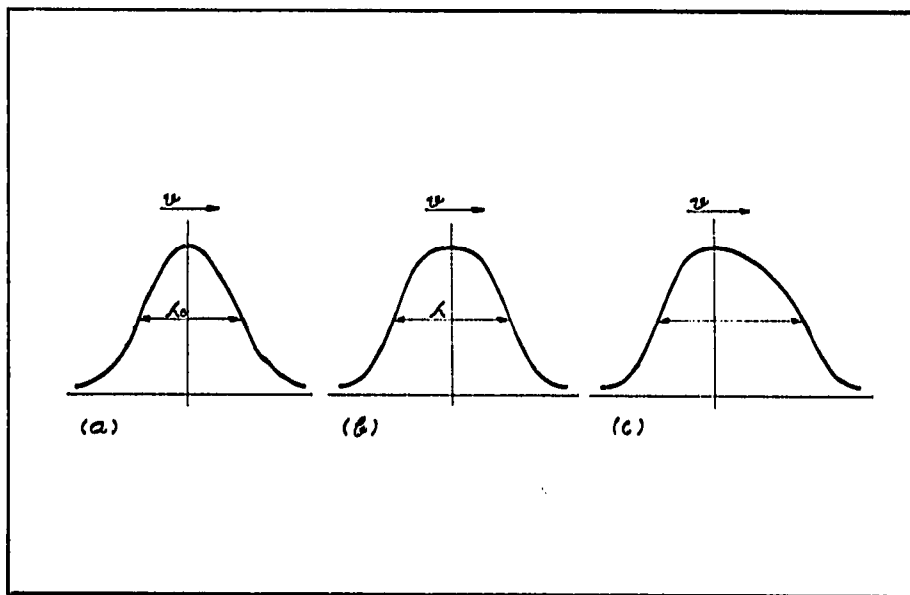


Fig. 2. The change in concentration profile due to non-uniform velocities in the z -direction. (a) Gaussian curve (v independent of z); (b) integrated Gaussian curve (v linear function of z); and (c) asymmetrical curve (v general function of z).

it is easy to see (see also eqn. 1) that the concentration profile (x,t,y) broadens, but still retains symmetry around its axis (Fig. 2b). If this is not the case, that is if $v(y,z)$ contains terms with higher powers of z , the concentration profile will in general assume an asymmetrical shape (Fig. 2c). The broadening of the zone $\Delta\lambda$ is in first approximation determined by the variation of X .

$$\Delta\lambda = X_{\max.} - X_{\min.} \quad (5)$$

The dependence of v upon the y coordinate should now be considered. If v is independent of y and changes in the effects of partition and adsorption in the vicinity of the edges of the medium can be neglected, the concentration profile is also inde-

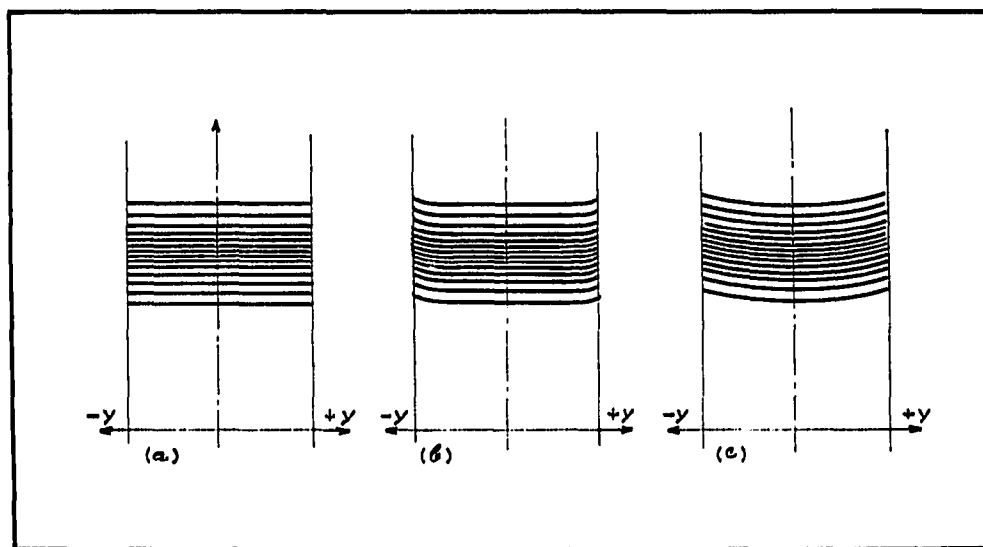


Fig. 3. The change in shape of a zone due to a higher velocity of solvent flow at the edges. (a) v independent of y ; (b) slight increase of v at the edges; and (c) v varying with y over the whole width of the medium.

pendent of the y coordinate. That is to say, that the zone has the same concentration distribution over the whole width of the medium (assuming that the solute was deposited at the origin as a narrow band extending across the full width of the medium).

Normally the velocity of solvent flow at the edges will always be slightly larger than in the center. The result is a slightly concave shape of the zone as indicated in Fig. 3b. If the medium is now made narrower the velocity increase due to the free edges begins to be felt over a proportionately larger part of the total width of the medium. Lateral diffusion again tends to equalize the concentration everywhere along the y -axis (Fig. 3c). The zone becomes wider, making an accurate quantitative determination more difficult. As a consequence of the spreading and widening of the zone the concentration in it may eventually become smaller than would have been the case if a wider medium had been used. The spreading of the zones along the x -axis may lead to overlap of adjacent zones and subsequently to a decrease in the resolving power.

CIRCULAR MEDIA

In the light of the arguments presented above it appears necessary to remove edge effects if an attempt to increase the sensitivity of thin-media chromatography by decreasing the dimensions of the medium is to be achieved. This can be done in a simple and straightforward manner by employing a circular medium such as a glass or quartz capillary tube layered with a suitable absorbent. Experiments which will be published separately seem to confirm our expectations. It is obviously not possible to decrease the diameter of the support medium indefinitely for the following reasons. Fig. 1d illustrates the situation.

At the surface of the support rod of diameter, D , the solvent is virtually stationary. On a planar substrate the velocity of the solvent increases approximately linearly with the distance from the substrate as shown in Fig. 1a. If, however, the substrate has the shape of a cylindrical rod with radius, R , it can be shown that the velocity of solvent flow changes approximately parabolically with distance z from the axis of the rod (eqn. 2).

$$v(z) = A(z^2 - R^2) \quad (4)$$

Let the thickness of the coating layer be Z . The flow velocity at the circumference is then:

$$Z = R\gamma \quad (5)$$

$$v(Z) = AR^2 (2\gamma + \gamma^2)$$

Since the velocity at the surface of the rod is zero, eqn. 5 determines also the maximum difference in velocity across the medium. A large value in this difference will produce a broadening of the zones as previously described and will therefore largely abolish the advantage of the smaller dimensions of the medium with regard to an increase in sensitivity. If the difference in solvent velocity is very large, overlap of zones may occur and the resolving power of the method will become reduced if not altogether abolished. Diffusion in a direction perpendicular to the substrate will again tend to reduce the effective velocity differences to some extent. It should be noted that on a cylindrical support the relative thickness of the active medium is of importance. This means that on a rod with a smaller diameter a thinner layer of coating must be applied. Using conventional coating techniques there is a certain minimum thickness of coating which can be uniformly and reliably applied. In order to avoid this difficulty alternative methods of applying a chromatographically active layer to cylindrical rods are presently under consideration in our laboratory. On a planar medium it is the absolute thickness of the active layer which is important regardless of the thickness of the support. Due to the nearly linear dependence of the solvent velocity upon the distance from the support, as compared to the approximately quadratic (see eqn. 5) dependence on a circular support, the thickness of the layer is of much less importance.

An example may illustrate the above conclusions. Let us assume a zone of separated substance with effective width ± 4 mm and distance from the origin of 100 mm. These values are assumed to be nominal ones for a constant and uniform velocity of the solvent. Consider now a planar medium with a velocity spread, not compensated by diffusion, of only $\pm 2\%$. The obvious result is a broadening of the

zone to ± 6 mm and a flattening of the regular Gaussian density profile within the zone. If the velocity distribution is non-linear, the resulting concentration profile will be non-symmetrical with respect to the peak. It depends upon the sense of curvature of the velocity profile, whether it is convex or concave and whether the leading or trailing part of the zone will be wider. In most cases an approximately parabolic distribution of the type shown in Fig. 3c can be assumed. The axis of the zone then moves with a velocity larger than half the difference between maximum and minimum. Consequently the leading part of the zone will in general be the shorter one.

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