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Some Technical Improvements in Adsorption Analysis

LENNART HAGDAHL

Institutes of Biochemistry and Physical Chemistry, Uppsala, Sweden

The interferometer for adsorption analysis constructed by Tiselius and Claesson [1] has shown itself to be a useful instrument which is easy to operate and which, by virtue of its accuracy and sensitivity, has given valuable results [2].

In order that the interferometer work well, it is necessary that the solution which passes through the cuvette be thoroughly mixed. This, unfortunately, is not always the case. If the filter used does not give even and horizontal fronts, the solution in the cuvette consequently becomes layered.

THE USE OF THE MIXER

In the large number of experiments performed it has been evident that the fringes, as a rule, become blurred preceding each component which is detected. The fringes become inclined and their upper portions progressively disappear until the entire fringes are obliterated. By screwing the compensator plates one can find the fringes again after several milliliters have passed. In most cases it is the upper portions of the fringes which first reappear. The fringes grow forth and become more clear until they are as clear as they were before the boundary came into the cuvette. Occasionally it happens that fringes can be seen at two different levels.

In an attempt to remedy this unsatisfactory condition, a mixer was made through which the solution passes before it enters the cuvette. The mixer consists of a metal rod, 7.5 cm long, through which a 1 mm capillary tube passes. The construction is indicated in Fig. 1. This mixer is placed between the lowest filter and the cuvette, and it is equipped with threads to fit these. The capillary is filled with quartz sand which is held in place by a little wad of cotton at each end. The volume of liquid in the capillary filled with sand is less than 0.05 ml. Because the mixer is made of metal the sample is easily brought to even temperature.

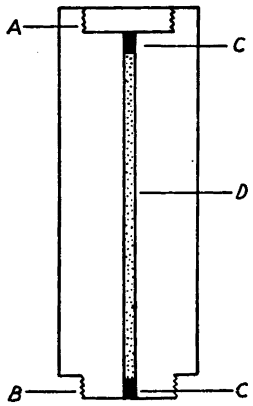

Fig. 1. Mixer.

A. Threads which fit the filter.
B. Threads which fit the cuvette.
C. Wad of cotton.
D. Capillary filled with quartz sand.

When the mixer is coupled into the system, the fringes are straight and clear as long as the solution's index of refraction remains constant. With an increase in refractive index, at least if the solution traverses the cuvette somewhat fast, the fringes bend sharply in one direction and with a decrease in refractive index they bend in the other direction. This is shown schematically in Fig. 2 where the center fringes' approximate appearance is shown throughout the experiment, placed directly over the corresponding points. The bending effect is caused by the solution streaming faster in the center of the cuvette capillary. By using the mixer one can, in most cases, follow the fringes during the entire experiment. It is only in extraordinary cases that the change in refractive index is so sudden that the fringes momentarily disappear.

When the mixer was used, the transitions between components were not found to be as sharp as expected. To ascertain the cause, several frontal analyses were performed in which the mixer was omitted but in which the eluate

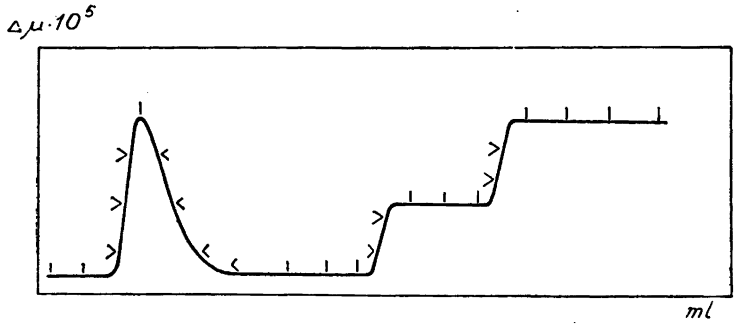

Fig. 2. The approximate appearance of the center fringes throughout the experiment. Drawn schematically.

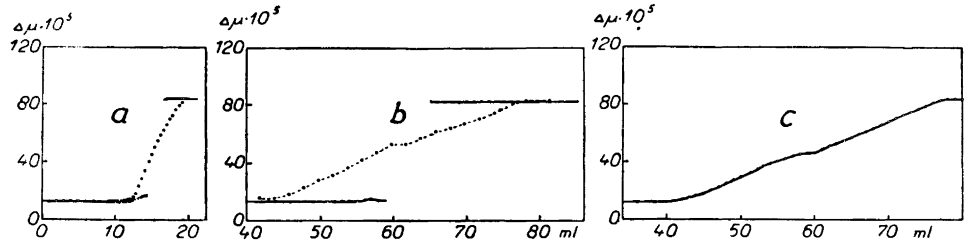

Fig. 3. Frontal analysis of 0.5 % sucrose in water.
Adsorbent: Carboraffin Supra.
Filter: a) 500 π, b) 2000 π, c) 2000 π + mixer.
a) and b) The interrupted lines show the same experiment according to the fractions taken during the experiment.

was divided into small fractions corresponding to the interferometric readings. At the close of the experiment the refractive index was measured on the fractions in the same cuvette, using the same filling of the comparison tube as was used during the experiment itself. The results are shown in Fig. 3a and 3b. The solid lines show the direct readings in the interferometer. The broken lines show the same experiment according to the fractions.

The results of the experiment shown in Fig. 3b are surprising, for the change of refractive index has proceeded for more than 15 ml, without movement of the fringes. They merely become blurred and obscured. The upper portions progressively disappeared, to be sure, but the rest of the fringes did not advance.

An experiment was performed with the same filter and the same solution as in Fig. 3b but with the difference that a mixer was placed between the filter and the cuvette. The curve for this experiment, shown in Fig. 3c, corresponds closely with the brokenlined curve in Fig. 3b, showing the course of the concentration change in the solution which actually comes out of the apparatus.

The horizontal portions on the broken lines in Fig. 3b and 3c (between 60—62 and 58—60 ml respectively) were caused by turning off the pressure approximately half a minute for replenishing the solution. It is then clearly important that the stream velocity be the same throughout the course of the experiment if the best possible results are to be obtained, and that turning off the pressure temporarily is disastrous, since one runs the risk of getting steps and irregularities which are artifacts.

The cause for the unsharp transition zones between the different components is the unevenness of the fronts in the column or the fact that these fronts often become inclined (not horizontal). This is due to the difficulty in

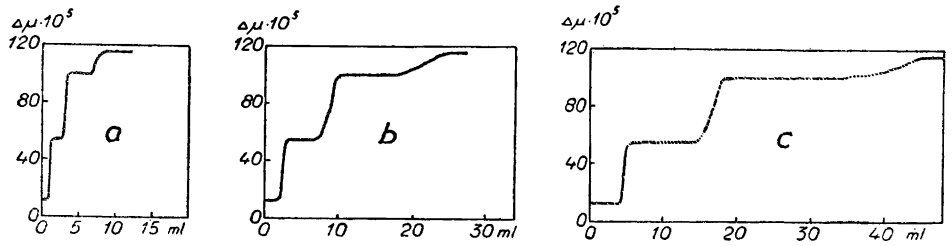

Fig. 4. Frontal analysis of (0.25 % glucose + 0.25 % sucrose + 0.25 % raffinose). Adsorbent: Carboraffin Supra. Filter: a) 100 π + mixer, b) 250 π + mixer, c) 500 π + mixer.

packing the adsorbent evenly — the direct obstacle in all chromatographic work, which is particularly disturbing when one wants to investigate adjacent zones closely, as in frontal and displacement analysis. The uneven adsorption front in the column nevertheless is usually quite sharp. When using the interferometer without mixer one tends to follow a system of fringes which corresponds only to one of the simultaneously streaming solutions in the channel.

The effect is most marked with the broad filters (*e. g.* 2000 π mm³, height 20 mm, diameter 20 mm) and is less pronounced with narrow filters where the fronts are relatively more even (see further below).

THE USE OF COUPLED FILTERS

Since it is impossible to pack an adsorption filter homogeneously, the solution's front always becomes more or less irregular. These irregularities increase with the size of the filters, but even such small filters as 500 π mm³ give quite poor fronts with large retention volumes. In Figs. 4a, b, and c some frontal analyses with three components are shown. It can be seen there that the lack of sharpness in the fronts increased partly with the size of the filter and partly with the increased retention volumes. The blurring of the fronts which increases with the retention volume cannot merely be due to poorer fronts in the filter itself. It must be due to the relationship of the components' slower movement and the consequently increased volume between the first appearance of the front at the filter's lower side and the saturation of the entire filter.

In an adsorption filter a substance in low concentration wanders more slowly than it does in high concentration. If one, therefore, presses a solution into a filter and afterward introduces a higher concentration of the same substance, the weaker front is overtaken by the stronger, if the filter is suffi-

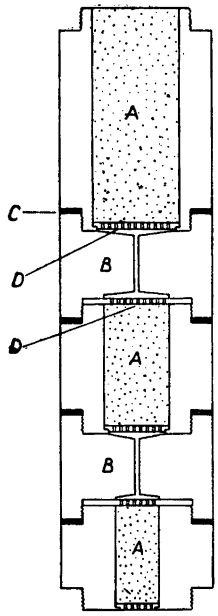

Fig. 5. Coupled filter.

A. Filters with adsorbent.
B. Constricted couplings.
C. Packing.
D. Perforated disks.

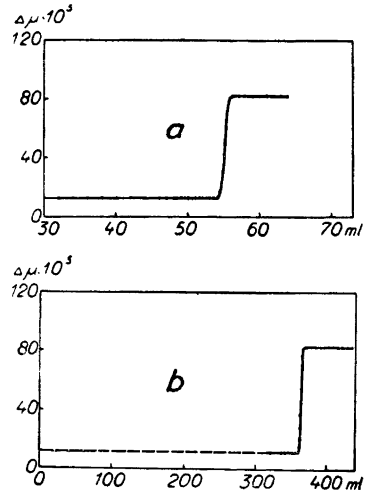

Fig. 6. Frontal analysis of 0.5 % sucrose. Adsorbent: Carboraffin Supra.
Filter: a) 875 π + 500 π + 250 π + 175 π + 100 π + mixer (total 1900 π).
b) 8000 π + 4000 π + 1250 π + mixer (total 13250 π).

ciently large. Then a single front is formed having the same concentration as the stronger solution's front.

When a frontal analysis is performed on a substance, it happens that solvent and solution emerge simultaneously when the uneven front emerges from the filter. By letting the emerging mixture pass through a capillary where it is mixed, and then by allowing the diluted solution to start on a new filter, a new front is formed. Because this corresponds to a weaker concentration and consequently moves more slowly, the chief front eventually overtakes the first and thus only one front is obtained. If the filter is smaller, and in addition has suitable proportions between height and diameter, a better front is formed. This can be repeated until one comes down to a filter which gives a satisfactory front.

An arrangement of this kind was introduced by Claesson [3] particularly for use with large amounts of adsorbent. The present author, independently,

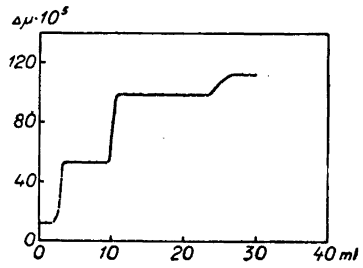

Fig. 7. Frontal analysis of (0.25 % glucose + 0.25 % sucrose + 0.25 % raffinose). Adsorbent: Carboraffin Supra. Filter: 175 π + 100 π + mixer.

observed the favorable influence on the evenness of adsorption fronts by introducing constricted couplings between successive filters with different adsorbents, used in group separations of amino acids [4]. It was also found that this type of filter coupling is very valuable for straightening out fronts in adsorption analysis with quantities commonly used for analytical separations.

In Fig. 5 is given an example of a coupled filter which has been found suitable. The important feature to be noted is the special type of coupling. This consists simply of metal rods with threads and depressions in the form of two flat cones whose points are joined by a small canal. (The depression volume in the coupling pieces ought to be as small as possible.)

Any number of filters can be coupled together without harming the fronts if one only takes care that the filters do not increase in size faster than the chief front can overtake the weaker front in each filter it traverses. If it is found advantageous, filters of the same size can be coupled together under each other. Therefore one is not bound to certain filter sizes but can choose those which seem most suitable.

In Figs. 6a and 6b two examples are shown of frontal analysis with one component, where such coupled filters were used. If, instead, all these filters had been coupled together to one continuous filter, a front only 2 ml broad would have been obtained in a retention volume of about 415 ml. But then a considerable increase in the duration of the experiment should be expected as the final filter, because of too small a diameter, would limit the rate of flow.

Frontal analysis with several components gives similar satisfactory results (compare Fig. 7 with Fig. 4b). Even with displacement analysis coupled filters give the same excellent results with several components as with one. (Fig. 8, a, b, c.) In Fig. 8c the free component's front was so sharp that the fringes temporarily disappeared. However, the falling concentration on the free component's back side could be read.

In Fig. 8d is shown an experiment performed on a 500 π mm³ filter with the same solution and displacer as in the experiment 8c. To permit a strict comparison of the two experiments even the same stream velocity was used.

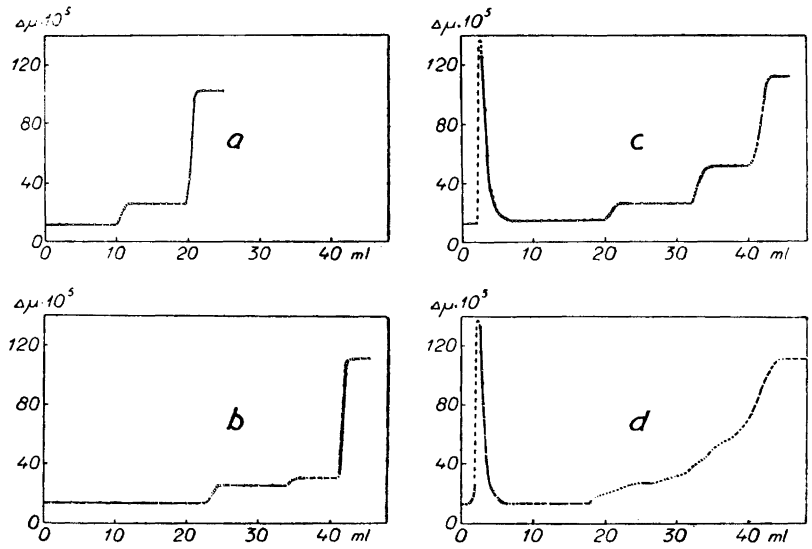

Fig. 8. Displacement development of some sugars.
Displaced substance:
a) 1.5 ml 0.5 % sucrose.
b) 2 ml (0.5 % sucrose + 0.5 % maltose).
c) and d) 1 ml (1 % glucose + 1 % sucrose + 3 % raffinose).
Developer: 0.5 % ephedrine.
Adsorbent: Carboraffin Supra.
Filter: a) 175 π + 100 π + mixer.
b) and c) 250 π + 175 π + 100 π + mixer.
d) 500 π + mixer.

The free component in this case is also very sharp, but the two other components and the displacer have such poor fronts that in the diagram it is seen that they practically form a single slowly rising front.

In elution analysis sharper fronts are obtained than previously, but the components back sides may be somewhat heightened. Since most components displace each other to a certain extent, the separation in elution experiments

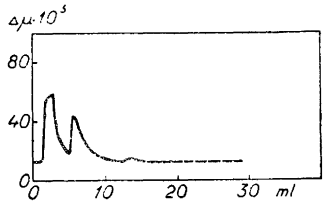

Fig. 9. Elution analysis of 2 ml (1/3 % alanine + 1/3 % valine + 1/3 % leucine) Eluent: Water. Adsorbent: Carboraffin Supra. Filter: 500 π + 250 π + 100 π + 50 π + mixer.

may be better than judged from the diagrams. Also in this case the sharpening effect of the coupled filters improves the separation.

In all the experiments described here the filters have been packed with wet charcoal. The charcoal is mixed with water and the charcoal suspension transferred to the filter by a broad-tipped pipette. During the filling of the filter excess water is sucked away by vacuum from below the filter. During the entire procedure one must be careful that the charcoal is never sucked dry so that the charcoal forms cracks. Enough charcoal is added so that a small heap is formed on top of the filter. This is then pressed into the filter with a spatula until it is filled with charcoal of a sufficiently stiff consistency. Then the excess charcoal is scraped away and the surface is made as even as possible with a spatula. This pressing of the charcoal filling is done so that it will not sink during the experiment. In experiments using coupled filters pockets must be avoided as much as possible for these can bring about unnecessarily poor results.

Through this wet packing better fronts are obtained and air is avoided in the filter. All the filters can be screwed together and washed at the same time. With dry packing each filter must be washed separately and until all air disappears before it may be screwed to another filter, for otherwise air may remain in the couplings between the various filters. These must be completely filled with liquid for best performance.

Filters with the following internal dimensions have been used.

Diam. (mm)	20	20	20	10	10	10	7.1	7.1	5	3.6
Height (mm)	80	40	20	50	35	20	20	14	16	15.5
Volume (π mm^3)	8000	4000	2000	1250	875	500	250	175	100	50

The above filters should not be considered as optimum ones for experimental work. They merely represent those models which were in use here at the time this work was under way. One of them, the 2000 π filter, is actually very poor. If possible it is better to use longer and narrower filters, but when coupled filters are used this is not so important.

SUMMARY

The advantages of using the mixer described for interferometric experiments are that the fringes can be followed during the entire experiment and that consequently the results obtained are more truly consistent than those formerly obtainable.

Through the use of coupled filters with constriction pieces, a considerable improvement of fronts in adsorption analysis is obtained, both with small and large amounts of adsorbents. The coupled filters are of advantage in all types of adsorption analysis — frontal analysis, elution analysis, and displacement development.

I wish to express my gratitude to Professor Arne Tiselius for the encouraging interest he has taken in this work, and for his helpful discussions concerning this material.

Also, I sincerely wish to thank Dr Ralph Holman for the English translation of this article.

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