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Some Remarks on Chromatography

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The chromatography has been promoted by Tiselius[1] introducing refined methods for measuring changes in refractive power, *e. i.* variations in concentration, by means of an interferometer apparatus. Claesson[2] has designed a selfrecording apparatus in which changes in refractive power of a solution leaving the filter with the adsorbent versus the amount of solution are given as a curve on a photographic paper in an incontrovertible documentation. Such a selfrecording apparatus is of course very valuable especially when running analyses are performed. However, to use the apparatus for such purposes the inconveniences due to certain characteristics of the apparatus must be reduced to a minimum. But, unfortunately difficulties in interpreting experimental results have arisen during our work, and also in other laboratories. Therefore we think some of our experiences with the first specimen put on the market by Fabriks AB LKB-Produkter, Stockholm, could be of some interest to workers in this field.

There are evidently three principally different sources from which the difficulties in reading curve may originate: imperfectness in apparatus design, unsuitable handling of the machine and faulty performing of the actual experiment, if of course they are not due to the problems under investigation. Therefore the investigator must be aware of from which source the difficulty may originate to be able to eliminate undesired complications.

As for the details concerning the construction of the apparatus (Fig. 1) and instructions how to perform different kind of experiments we refer to the description given by Claesson[2]. In following lines we have restricted our communications only to some factors which now and then bring us more or less troubles and which at times are difficult to eliminate. As the reading of the curve is important part of the work with the apparatus we illustrate our examples with the original curves.

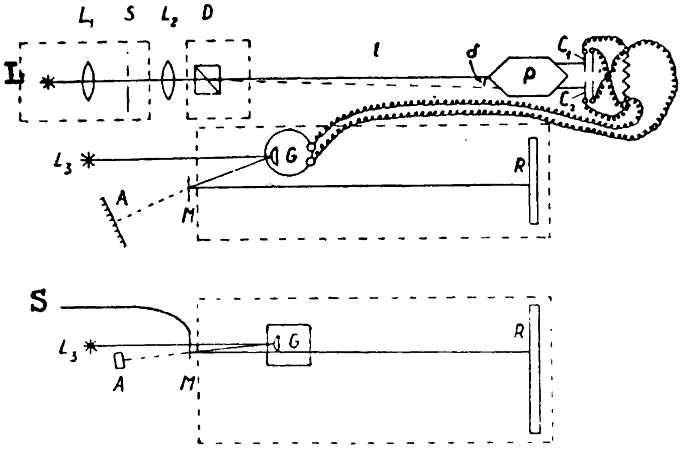


Fig. 1. Sketch of apparatus, S. Claesson (2).

L: Tungsten ribbon lamp.
L_1 and L_2: Lenses.
S: Slit.
D: Thermostat with cuvette doubble prism.
δ: Deviation of light beam due to changes in refractive power of the liquid in one of the halves of the doubble prism.
P: Hexagonal prism.
C_1 and C_2: Photo element, selenium cells.
G: Galvanometer.
S: Spring carrying the reciever for liquid from the cuvette.
M: Mirror attached to the spring S.
R: Screen or photografic paper.

The apparatus is very sensitive to outer disturbances like vibrating of the floor, draught, deviations of the room temperature in relation to that of the thermostat. To check the absence of any disturbances in the apparatus it is not advisable to carry out a full experiment but to observe the light spot directly on the screen for a longer time with the cuvette in the thermostat or — and better — without the cuvette but with a suitable aperture reducing the intensity of the light from the tungsten ribbon lamp, as too high intensity of the light spoils the selenium cells. It is advisable to provide the room with constant temperature adjusted to the thermostat temperature. This favourable condition enables to start an experiment almost immediately after the cuvette has been plunged into the thermostat.

It is important to control that the deflections of the light spot caused by changes in refractive power of the liquid in the cuvette, or by turning the

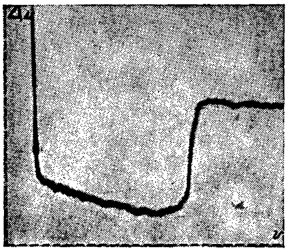


Fig. 2. Frontal analysis. Inperfect washing. Note the inclined volume line befor the step.

micrometer screw operating the hexagonal prism (P in Fig. 1), are at (an almost) right angle to the deviations caused by loading the spring (M in Fig. 1) carrying the receiver for liquid leaving the cuvette. For correction in this respect the level of the table supporting the apparatus may be altered and the position of the mirror (M in Fig. 1) attached to the spring.

Further, there is no stirring in the inner thermostat (D in Fig. 1) and a slight decrease or increase in temperature during especially long experiments is more or less unavoidable. It may cause a change of the refractive power as if in concentration of the solute, which is of course apparent. For instance, if there is a long retention volume period the volume line on the curve is not longer perpendicular to the concentration axis (Fig. 2). Such an systematic error is rather difficult to distinguish from an imperfect washing of the adsorbent.

There are some factors which have great influence upon the sensitivity of the apparatus. If a sensitivity is desired to a certain value a good rheostat should be substituted for the small radio potentiometer in the selenium cell-galvanometer curcuit. Air bubbles in the cuvette chambers (Fig. 3) and its window pockets are to be thoroughly eliminated. In order to avoid air bubbles in the pockets the deeper one is to be filled with the liquid from the inner

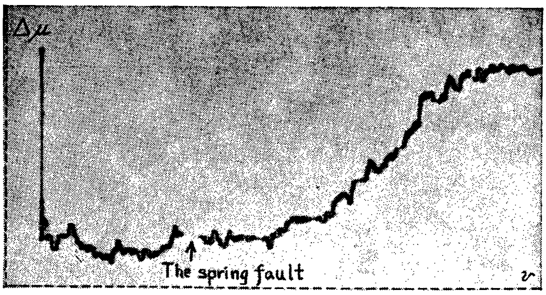


Fig. 3. Frontal analysis. Air-bubbles inside the cuvette. (On spring troubles cf. Fig. 3.)

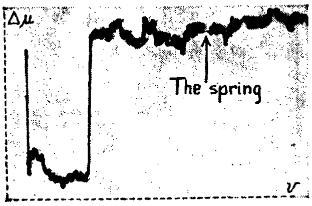


Fig. 4. Frontal analysis. Tungsten ribbon lamp troubles. (On spring troubles cf. Fig. 7.)

thermostat and the cuvette immersed in the position a little inclined to prevent loosing the liquid. If the latter precautions failed and the air bubbles are detected in the window pockets, they may be removed by means of a special syringe provided with a long tip which is bent at the end at a right angle. In order to keep windows in the cuvette and the thermostat clean, the liquid in the inner thermostat is replaced rather often. We have found it better to have water than 50 % alcohol-water mixture in the inner thermostat.

To get a convenient commodity for inspection the cuvette while in thermostat, the tube between the hexagonal prism (P in Fig. 1) and the thermostat (D in Fig. 1) is provided with a hole through which the examination may be made by means of a mirror. It may be possible to use a half reflecting mirror for inspection, which enables examination during the whole experiment, in which case, however, the sensitivity must be determined with the mirror in the tube.

We perform the determination of the sensitivity twice, firstly before the photografic paper is put on place, secondly when it is on place by turning the micrometer screw a certain angle. In the latter case we automatically get the refractive power, e. i. the concentration axis on the paper (Fig. 2).

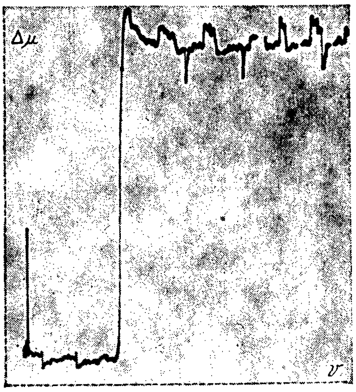


Fig. 5. Frontal analysis. [Tungsten ribbon lamp troubles or bad contacts in selenium cell — galvanometer curcuit.

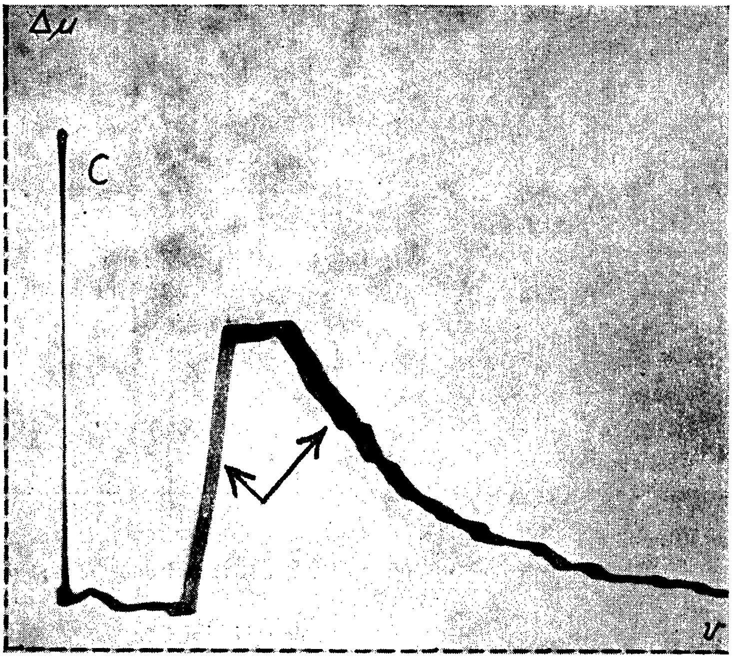
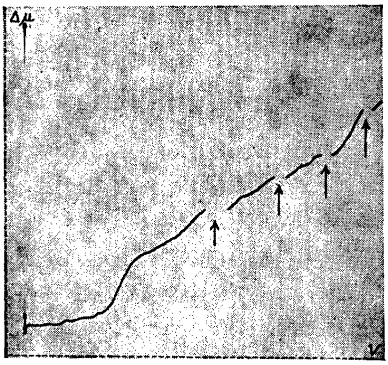


Fig. 6. Eluation analysis. Without aluminium foil. Note the swingings and broad lines in parts parallell with the concentration axis c.

The tungsten ribbon lamp (L in Fig. 1) causes at times difficulties. Originally a lamp holder was provided, which is however objectionable, and we recommend to have the lamp soldered to the wires. But even with this arrangement now and then difficulties arise from the lamp. As far as we have found they occur a longer or shorter time before the lamp is worn out and are due to

Fig. 7. Frontal analysis. Reciever holder hanging not frictionless. Note intervals in the curve.



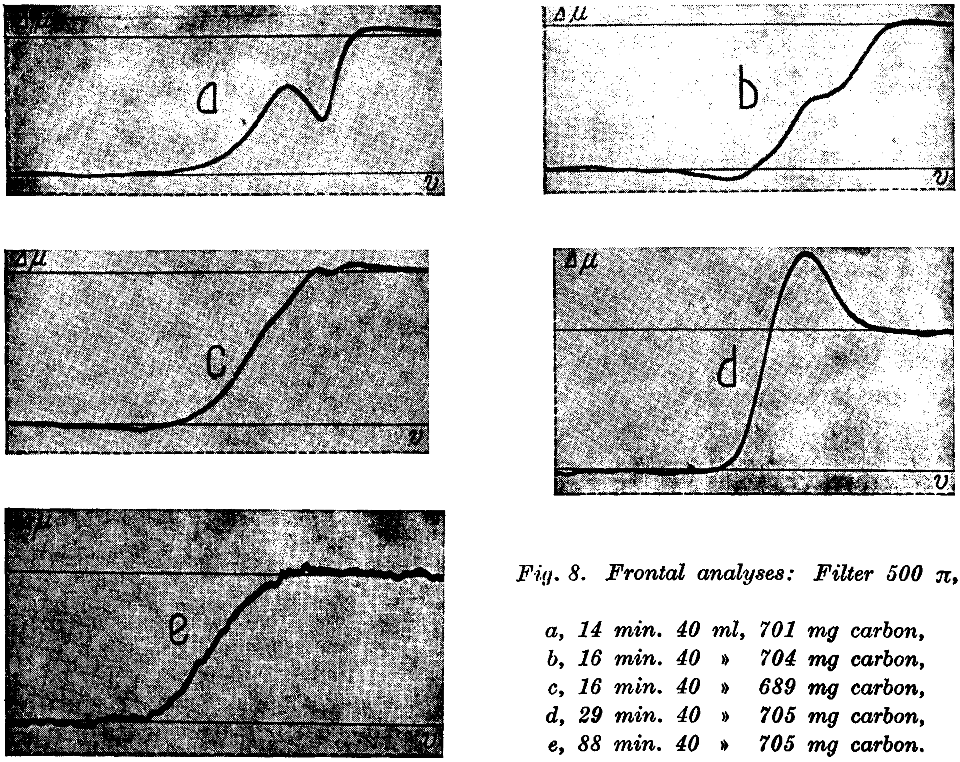


Fig. 8. Frontal analyses: Filter 500 π,

a, 14 min. 40 ml, 701 mg carbon,
b, 16 min. 40 » 704 mg carbon,
c, 16 min. 40 » 689 mg carbon,
d, 29 min. 40 » 705 mg carbon,
e, 88 min. 40 » 705 mg carbon.

small otherwise undetectable damages of the tungsten ribbon near the ribbon holder in the lamp (Figs. 4 and 5).

The galvanometer once put in order is very reliable. It may happen, that it must be removed for some reason and then, when put back again, the mirror is not working properly. The apparatus is then hypersensitive to slight knocking and vibrations, what indicates that the mirror is not hanging free.

The balance and the receiver for the liquid from the cuvette have caused a lot of troubles. We have replaced the glass receiver which has proved to be too heavy by a plastic bag put on a glass ring and hanged on the original holder attached to the spring. It is recommended to repress the oscillations of the balance caused by falling drops by a metal strip dipping into paraffin oil and what has proved to be also very advantageous in this respect a strip of aluminium foil attached to the receiver in such a manner that the falling drops slide down along this strip (Fig. 6). Much attention must be given to the way in which the receiver holder is attached to the spring. If the holder is not hanging quite free there may appear intervals in the curve (Fig. 7). In order to make the movements quite uniform and to exclude friction we have attached the receiver to the spring with rubber strings.

In the literature it is said chromatography should be performed at a low rate. As far as we are informed only Claesson [2] has stated an upper limit, in case of filter 10 mm inside diameter. (Sillén and coworker [3] have amongst others treated the rate-problem in case of ion exchange.) It is of course an advantage if the time of an analysis can be shortened, therefore we looked for rapid working carbons in order to learn a little moore about this question. Thus we met some deviations from a normal curve which proved to be reproducible — at least in case so far investigated — if the rate of flow of the solution through the filter was taken in account. From Fig. 8 a—e it is clear how the shape changes with the rate. It is also obvious, quite a »good curve» (Fig. 8 c) may be obtained, but the result may nevertheless be in a way illusive, as the »good shape» disappears when the rate of flow is increased *or* decreased. Therefore, if difficulties in reading a curve like those in Fig. 8 arise they may be due to unsuitable rate of flow. We will on another occasion give some more details on this question, but we have mentioned this here as we at first thought the bad shape was due to unproper handling of the apparatus.

SUMMARY

Some experiences from work with a selfrecording apparatus designed by S. Claesson are given. Difficulties in proper understanding of experimental adsorption curves have arisen but ways to eliminate the difficulties are shown. Further, there is stated that the rate at which the solution passes through the filter, under at least certain circumstances, has a great influence upon the shape of the adsorption curve for frontal analysis so far investigated.

LITTERATUR

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2. Claesson, S. *Diss.* Upsala (1946).
3. Sillén, L. G. *Arkiv Kemi, Mineral. Geol.* **A 22** (1946) no. 15, Sillèn, L. G., and Ekedahl, E. *Ibid.* **A 22** (1946) no. 16 and **A 25** (1947) no. 4.

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