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On Apparatus for Chromatography

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Hellström and Borgiel [1] have given some practical advice for the manipulation of the automatic analyser for chromatographic work, designed by Claesson [2]. Some disadvantages caused by the construction of the cuvette, are discussed in the present paper and a new design is therefore suggested. In addition to this the author suggests a new type of cuvette with a measuring device, which is specifically intended as an aid for preparative rather than analytical work.

I. Cuvette for Claesson Apparatus. In Claesson's apparatus the adsorption filters are placed on a block having a horizontal boring with two plugs. The beam of light passes through the apparatus by means of a second boring in the plugs. Two round glass windows, one in each plug, and another glass window between the plugs form two cells, one for the reference liquid (solvent) and one for the liquid that issues from the filters. The latter cell is connected by borings in the plug and in the block, on one hand with the filters, and on the other to a tube through which the liquid from the filters is transferred to a collecting vessel. The plugs are cut at an angle of 45° and so the third window, situated between them, becomes oval. The plugs and this window are held together by screws which are tightened parallel to the axis of the boring. The pressure in the apparatus then operates at an angle of 45° to the surface of the oval window. The reference cell is thus quite closed, and hence the plugs and windows must be taken apart each time the reference liquid is changed. One could of course facilitate the changing of the liquid by connecting borings and tubes as in the measuring cell, but it is rather troublesome to align the borings accurately, and it is also difficult to make the two cells sufficiently tight to prevent the diffusion of liquid from one chamber to the other. This is in part due to the difficulty of finding suitable sealing material. The author believes, nevertheless, that the fundamental cause of the drawbacks in the instrument is the pressure, which, holding the cuvette together, acts at an

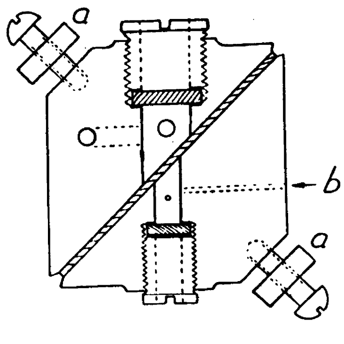

Fig. 1. The cuvette described in text. a indicates the bracket surrounding the cuvette, b the boring to the cell containing the liquid coming from the filter.

angle of 45° to the oval window and thereby develops strains which render more difficult the sealing of the apparatus.

In view of this conclusion a new cuvette was designed. A boring was made through the centre of a cubic block, at right angles to one of the surfaces. The block was then cut diagonally into two parts, as shown in Fig. 1. The corners opposite to the diagonal surfaces on both halves were chamferred, and the holes threaded *etc.* to enable circular windows to be fitted. Borings were made to and from the "inner parts" of the holes. Thus the third window corresponding to the oval window in Claesson's cuvette consist, in this apparatus, of a rectangular glass plate. Films of poly-ethylene 0.2 mm thick with suitable oval holes were used successfully as washers between the glass and metal parts. The two sections of the cube, the window and washers were held together by two screws in a loose bracket surrounding the apparatus in such a way that the screws operate at right angles to the chamferred corners, and hence the pressure keeping the apparatus together works at right angles to the oval window.

The whole cuvette assembly with its connecting tubes was mounted in a holder as shown in Fig. 2.

The tubes were connected with an upper plate threaded to hold the filters. The wide tubes through the plate in Fig. 2 were used for emptying or filling the reference cell. The narrow tubes, approx. inside diam. = 1 mm, conduct the liquid from the filter to the collecting vessel. A circular grove was also made in the plate, in order that a cylinder of any desired height might be fitted. A hole leading to a horizontal drain tube was drilled through the plate, to enable any liquid inside the cylinder to be drawn off.

In operation the apparatus was hung in the thermostat. The filters then stood on top of the thermostat. In Claesson's original apparatus the filters were immersed in the thermostat fluid, which meant that in order to change the filters the whole apparatus had to be removed from the thermostat vessel.

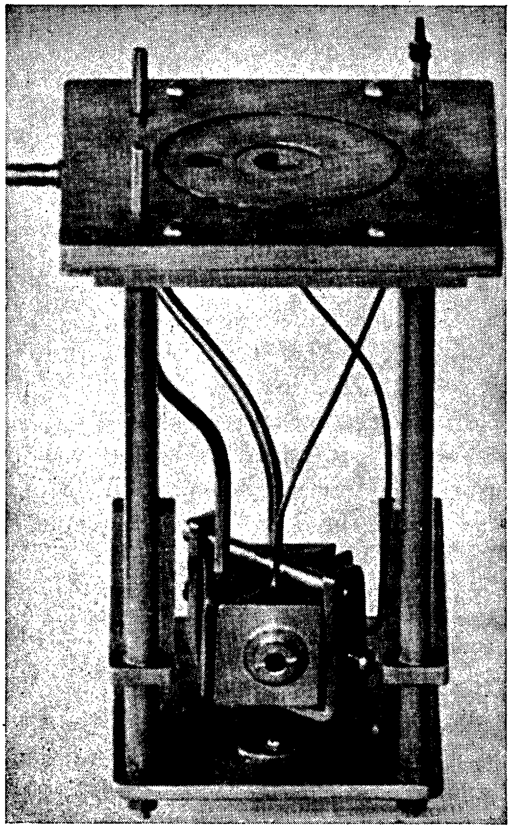

Fig. 2. The cuvette mounted in the holder with the device for the filter on the top plate.

By this operation the thermal equilibrium in the thermostat was disturbed, and there are other disadvantages. With the apparatus designed by us, however, the filters can be changed without lifting the cuvette out of the thermostat vessel. It is true that the filters will then operate at room temperature, but by using a cylinder of suitable height and by means of a second thermostat with spiral tubing, thermostatic control of the filter can be achieved. By such a device it was possible to attain greater variation of temperature for adsorption measurements than was practicable when both filters and measuring cuvette were in the same thermostat, and were therefore, at the same temperature.

II. Cuvette with measuring device. The type of cuvette suggested in the previous section is very easy to manufacture, and a specimen was made in

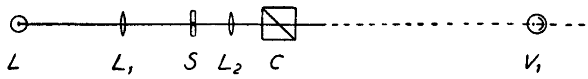

Fig. 3. The lamp L, lenses L_1 and L_2, cuvette C and phototube, V_1. The slit S (text S_1) is focussed on a slit S_2, not given in the fig., just before the phototube.

which both the measuring cell and reference cell were equally small, in order that these two cells might be connected, so that the liquid from the filters should run first through one, then through the other. A beam of light passed through such a cuvette will, disregarding certain diffusion, be deflected when the "front" enters the first measuring cell. When the "front" also passes the second cell — liquid of the same refractive index being present in both cells — the beam of light will revert to its original position. Provided that the volume of fluid passing through the cuvette is measured, one can, using the above instrument, obtain an idea of the derivative of the change in refractive index as a function of traversed solution volume. — The arrangements thus allow the use of the crossing over technique proposed by Holman [3] for the Tiselius-Claesson apparatus.

The author has been satisfied in this connection with trying to develop an automatic device that signals each time a "front" passes the cuvette. In the case under consideration a photo-tube, screened in such a way that it is illuminated by the beam of light only when the fluids in the two cells have the same refractive index, was used to this end. The photo-tube was coupled to an amplifier tube operating an electronic switch for an ordinary doorbell. The apparatus was adjusted in such a way that the bell was silent when the tube was illuminated, and a signal was obtained when the "front" entered one cell.

In Fig. 3 the relative positions of the lamp and necessary lenses is given and Fig. 4 is a circuit diagram of the apparatus with both the photo-tube and bell.

In the example shown the distance from the cuvette to the slit in front of the photo-tube is approximately 2 700 mm. (Fig. 3) A 10 mm deflection of the beam thus corresponds to a difference in refractive index, $\Delta\mu$, between the two measuring cells of nearly 0.004 units. In practice it was found that in the apparatus a deflection was obtained for a 3—4 mm movement of the beam of light, corresponding to $\Delta\mu \approx 0.0013$. When the front passes a measuring cell "schlieren" appear, causing the image of the slit, S_1, on the slit of the photo-tube, S_2, to be blurred, and thereby decreasing the intensity of light. This presents another means of increasing the sensitivity of the instrument.

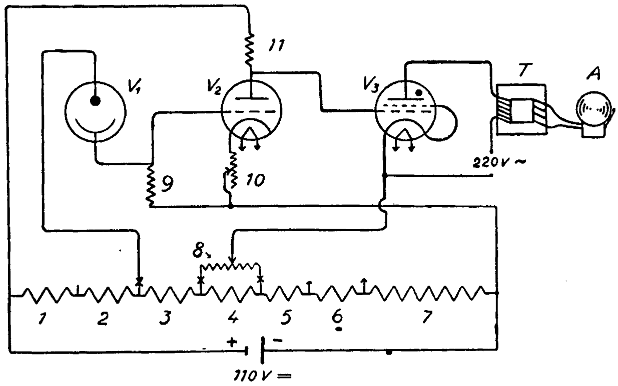

Fig. 4. Circuit diagram with the phototube, RCA 868, V_1. Triode tube, RCA 6SF5, V_2. Thyratrone tube, "Standard" G4S5, V_3. Transformer, T. Bell, A. Resistances: 1 — 4: 2 K Ω, 5: 1 K Ω, 6: 0.5 K Ω, 7: 10 K Ω, 8: 0.1 M Ω, 9: 10 M Ω, 10: 6 K Ω, 11: 10 K Ω.

The above apparatus was, as stated in the introduction, primarily intended as an aid for preparative work. As such work is generally performed at high concentrations and by the technique of "flowing chromatograms", the solvent is frequently changed. This involves a considerable change in refractive index, and the sensitivity indicated will be more than sufficient.

The apparatus has the further advantage that it should be possible to use any volume suitable to the experimental requirements between the two cells. Time may be saved in changing collecting vessels, or if desired, the volume of solvent comprising the "front" itself can be extracted. The model of the cuvette designed for experimental purposes was not equipped with permanent tube connections from cell to cell. For that reason increase in volume between the two cells mentioned above can easily be effected. The cuvette can also be connected in such a way that the liquid passes only one cell, should, for example, one only want to know when a "front" is passing.

It is evident that above apparatus can be used with a variety of measuring devices. Thus this type of cuvette may be used with an alternative registering device or the amplifier may be made to control an automatic movement of the collecting vessels. It might also be possible to combine a current apparatus that continuously changes collecting vessels with an instrument, based on the above design of cuvette, that undertakes the collection of the volume of liquid corresponding to the front itself.

SUMMARY

A new cuvette for the Claesson's self registering apparatus [2] is designed. In addition to this a cuvette with measuring device is also suggested.

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