The siphon tube has an internal diameter of 4 mm and a hole 6 mm from the open end. The siphon is placed in position and the second eluant is poured into it until the meniscus is at its highest level, without siphoning over. The first eluant is then poured into the column, covering the open end and the hole of the siphon tube. A further volume of second eluant which will activate the siphon when the hole in the tube is unblocked to allow the trapped air to escape, is then placed in the reservoir. This volume is previously determined by trial and error. When the level of the first eluant in the column falls below the hole, the second eluant automatically siphons over. Two or more such siphons can be used simultaneously in the column if the holes are arranged to be at different levels.

2. *The siphon balance* (shown in Fig. 2 in our adaptation) consists of a metal rod mounted on a ball bearing to swing in a vertical plane and carrying at one end a stirrup which holds a chromatographic column. To the other end a length of polythene or nylon tubing is attached which delivers washing fluid from a reservoir to a second column. The "balance" is arranged to tilt when the eluant in the first column is almost down to the packing, thus allowing the wash solution to siphon over into the second column. A mercury switch can be attached to the "balance" rod to actuate a fraction collector, if desired.

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## A continuous flow-meter in automatic quantitative liquid chromatography

The need for a second amino-acid analyzer prompted us to build a second instrument based on the well known principles given by STEIN AND MOORE[1] and applied in, *e.g.*, the Beckman-Spinco amino-acid analyzer in use in our laboratory.

This provided us with the opportunity of improving the existing analyzer by the addition of some accessory apparatus, controlling the constancy of the flow during an experiment, and also overnight, without the necessity of regular personal supervision.

The flow-meter described here can be used in every experiment in liquid chromatography where a high accuracy in the constancy of the flow is essential.

The flow velocity in an amino-acid analyzer following the principle of STEIN AND MOORE is determined by measuring the time which an air bubble, introduced into the flow, needs to travel a certain calibrated distance. These processes, (a) introducing the bubble, and (b) measuring the time, have been automated.

The time is printed out by means of a digital printer and the flow velocity is thus measured every ten minutes.

*Experimental*

The outline of the apparatus is given in Fig. 1. The essential parts are described separately.

A lamp ($B_1$) and a photo-conductive cell ($C_1$) are placed so that only part of the radiation from the lamp reaches the cell. The photocell lies in the plane passing through the centre of the lamp and the tube, but somewhat below the vertical from the lamp to the tube. As soon as a bubble appears in the horizontal tube, in the vicinity of the said vertical, the intensity of the radiation reaching the photocell suddenly increases considerably as the beam of the radiation is reflected (total reflection) strongly at the glass–liquid–air boundary of the lower part of the bubble (Fig. 2). The photo-resistance of the cell ($C_1$) is lowered and a relay ($E_1$) is activated *via* the transistor amplifier system ($D_1$). The pulse forming unit ($F_1$) gives a pulse which starts the counting apparatus (G). After some time has elapsed the bubble reaches the area of the second circuit where the situation is completely analogous to that in the first circuit (A–$F_1$) and the pulse forming unit $F_2$ gives a pulse which stops the counter (G). The time needed for the bubble to travel the calibrated distance is then printed out on the printer (H) and the counter (G) is automatically reset.

*Description of the essential parts of the apparatus*

*The flow-tube and the bubble introducer (A).* The flow-tube is a glass tube (outer diam. 8.7 mm, inner diam. 2.7 mm). The length of the calibrated distance is 147 mm. The air inlet (Fig. 1) is placed at the top of the tube. It is connected with the laboratory air pressure system, *ca.* 0.2 atm, *via* a solenoid valve, Gebr. Müller, Ingelfingen, type 100/TII. A clockwork mechanism serves as a pulse which opens the solenoid

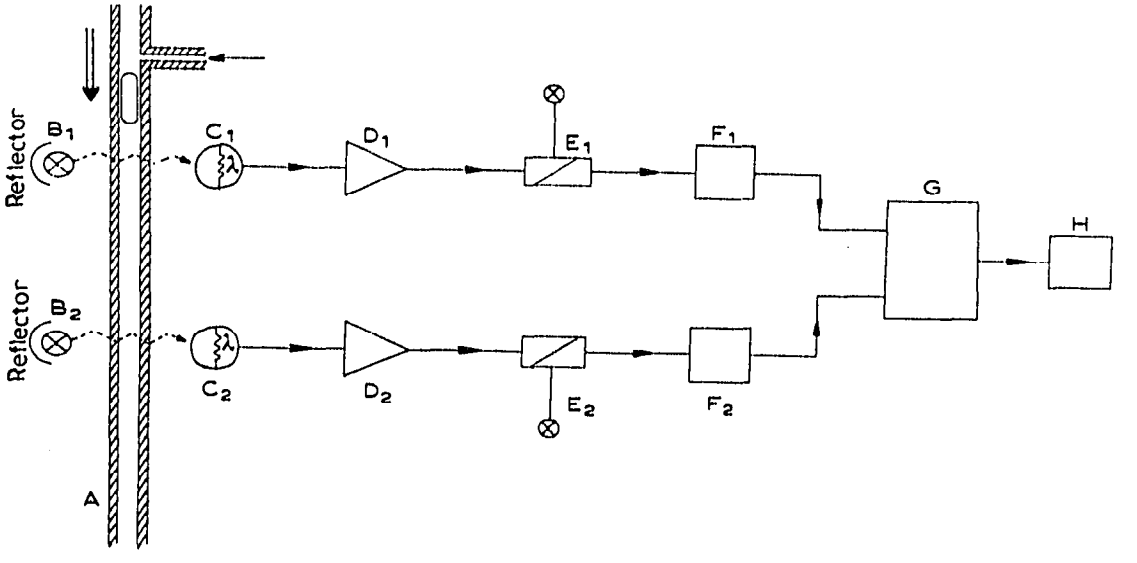


Fig. 1. Scheme of the continuous flow-meter. A = Glass tube, outer diameter 8.7 mm, inner diameter 2.7 mm; $B_1$, $B_2$ = lamp, Osram, 6.5 V, 1.4 A; $C_1$, $C_2$ = photoconductive cell, Philips ORP 62; $D_1$, $D_2$ = transistor amplifier; $E_1$, $E_2$ = relay, Siemens Trls 154c TBv 65417/93d; $F_1$ = pulse-forming unit (start); $F_2$ = pulse-forming unit (stop); G = Philips counting equipment, PW 4200 series; H = printer, PW 4202/01; ⟶ indicates direction of flow.

valve every 10 min for 0.1 sec. A bubble with a length of about 1 cm is then formed. When the flow-rate is 45 ml/h the bubble needs 75 sec to travel the precalibrated distance.

*The lamp, Osram, 6.5 V, 1.4 A and the photoconductive cell ($C_1$,$C_2$), ORP 62.* The lamp used was one of the type used in a Mettler balance. The black paint on the lamp was completely removed and a reflector was placed behind it. The photoconductive cell, ORP 62, has a sensitivity area of 1.5 mm$^2$. The optimal angle under which the photocell has to be placed is found by trial to be about 100°. Fig. 2 illustrates the holder of the flow-tube and the photoconductive cell. By shifting the cell holder up and down, the position of the cell can be adjusted accurately. The beam of light is transmitted *via* a small slit.

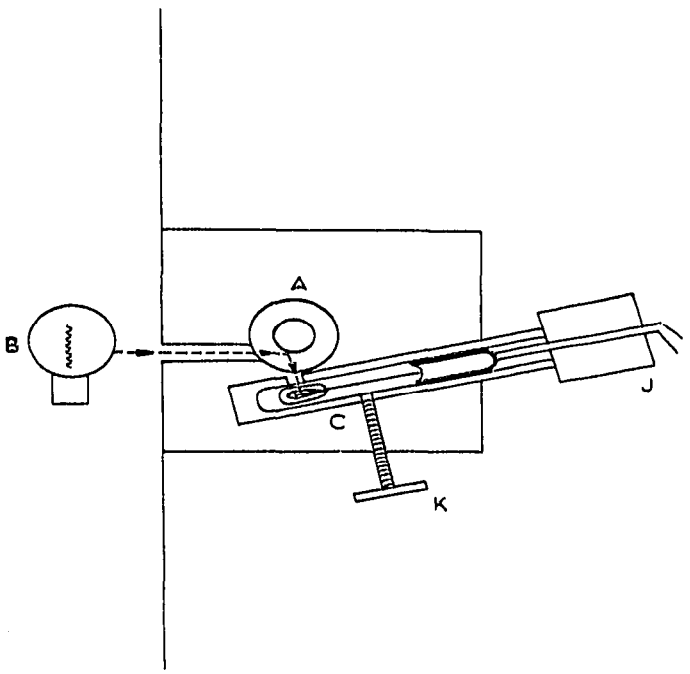


Fig. 2. Detail of the continuous flow-meter. A = Glass tube; B = lamp; C = photoconductive cell; J = adjustable cell-holder; K = fastening screw.

*The transistor amplifier ($D_1$,$D_2$), the pulse-forming unit ($F_1$,$F_2$) and the relay ($E_1$,$E_2$), Siemens Trls 154c TBv 65417/93d.* The correct values of the resistances and the numbers of the transistors are given in Fig. 3.

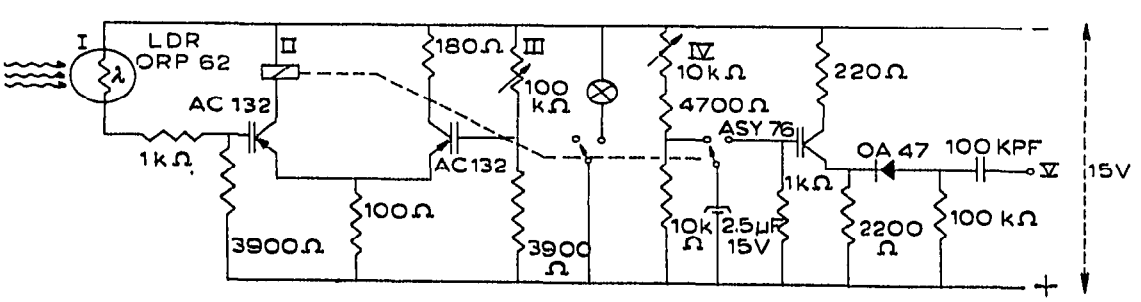


Fig. 3. Scheme of the electrical circuit. I = Photoconductive cell; II = relay, Siemens Trls 154 c TBv 65417/93d; III = potentiometer controlling sensitivity; IV = potentiometer regulating pulse height; V = pulse output.

A potentiometer of 100 k$\Omega$ is adjusted so that the relay is activated when the resistance of the photoconductive cell is below 20 k$\Omega$. (The dark resistance of the ORP 62 is *ca.* 80 k$\Omega$.)

As PbS photoconductive cells are very temperature dependant, the use of a PbS cell is not to be recommended. Consequently a CdS photoconductive cell was used. The best working temperature for the ORP 62 lies between 20 and 30°.

*The Philips counting equipment (G), PW4200.* This equipment consists of the following parts: electronic scaler PW 4230/00, electronic timer PW 4260/00, control unit PW 4201/00, LT supply 4211/00, printer control PW 4209/00 and one electric adding machine PW 4202/01 (H).

*Discussion*

When an accelerated or conventional amino-acid analysis runs smoothly, the flow velocity during the analysis remains constant. The mean standard deviation in the flow velocity was measured in eight consecutive runs which gave: 4, 5, 6, 5, 4, 5, 3 and 2‰.

The counting apparatus as described in this article gives every possibility of control. It can easily be coupled with an event marker on the recorder. When the flow velocity drifts, a correction can be made immediately. In the first approximation the area of the peak is directly proportional to the flow velocity time. The precise values of the correction will be given later.

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1 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190.

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