A CHROMATOGRAPHIC FRACTION-SAMPLING DEVICE

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In the preparative scale separation of amino acids and peptides, by the methods of HIRS, MOORE AND STEIN[1-3], the usual method for the location of peaks has been to take samples from the effluent fractions, and subject these to reaction with ninhydrin, either before or after alkaline hydrolysis. Since analysis of amino acids can now be done automatically[4-7], it is desirable also to mechanize preparative separations.

Using the apparatus of SIMMONDS[5], the device described below was devised to sample each fraction automatically, diverting the sample to the analysis machine, while the remainder of the fraction was collected separately. It is, in fact, more general in its application, and can be used whenever effluent fractions need to be divided into two portions, for subsequent analysis.

DESCRIPTION OF APPARATUS

The fraction-sampling device is shown in Fig. 1. When the body of the collecting tube is full, liquid escapes via the overflow B into a drop-counting chamber. When the required number of drops has been collected for the sample, the valve V is lifted by the solenoid S, and the main part of the fraction is delivered to the fraction collector.

The fraction volume can be adjusted by inserting extension pieces between the upper and lower parts of the collecting chamber. In this way, the volume delivered can be easily varied between say 2 ml and 20–30 ml, by the use of a graduated series of extension pieces. Preferably, the apparatus can be scaled up for larger fractions.

The sample volume can be determined in any convenient way. A conductivity-operated, electromechanical drop counter[8] has been used, but any other drop counter or volume-operated device (see *e.g.*, SIMMONDS[5]) can be used. The circuit used to lift the valve, V, and to operate the turntable is as used by SIMMONDS[5], except that the input terminals are bridged by the drop counter.

The sample may be diverted into a second row of tubes in the fraction collector, or into any suitable automatic recording device. When used with the apparatus of SIMMONDS[5] for amino acid or peptide separations, the electrical arrangement is as shown in Fig. 2. The recording machine is started by an extra set of contacts on the relay controlling valve V (Fig. 1). If required, a short alkaline hydrolysis can be done before the ninhydrin reaction, in an additional heating vessel mounted above the

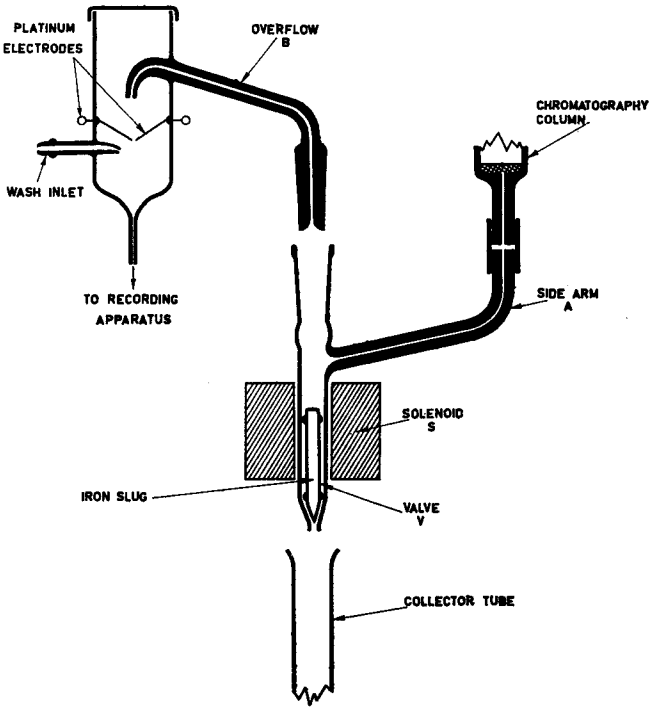

Fig. 1. Fraction-sampling device.

ninhydrin heating vessel, and controlled by an additional bank of motor-operated switches.

When working with small samples, it may be desirable to wash the sample into the recorder by means of a jet of distilled water (or other suitable liquid) from a dispenser of the type used by SIMMONDS[5], and operated in parallel with the solenoid S (Fig. 1).

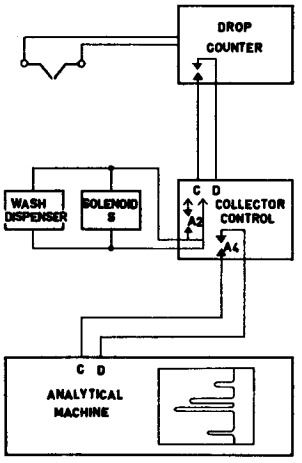

Fig. 2. Electrical arrangement for automatic monitoring of column effluent.

RESULTS AND DISCUSSION

Various amino acid separations have been done, and the fractions analysed both automatically, by using the sampling device, and manually, by taking aliquots from the main fractions, as a check on the operation of the device. Fraction sizes have been varied from 3 ml to 10 ml, and sample size from 2 drops to 2 ml. For all such combinations, a close correlation was obtained between the automatic and manual absorbance readings.

For most purposes location of peaks is all that is required, but if a quantitative estimation is needed, this can be done by measurement of drop size, and standardization in the appropriate way. This aspect has not been fully investigated, but provided peaks are spread over at least three fractions, an accuracy of better than $\pm 8\%$ appears to be obtainable, despite the fact that there is no provision for mixing.

SUMMARY

A fraction-collecting apparatus is described which automatically samples each fraction in turn as it is collected, and diverts the sample for analysis by any suitable method.

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