

AUTOMATIC REGISTRATION OF ORGANIC ACIDS IN COLUMN CHROMATOGRAPHY

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Column chromatographic analysis of organic acids generally requires laborious titration of a large number of fractions. In our laboratories an automatic apparatus has been developed by means of which the different fractions of organic acids are eluted in water immediately after they leave the column, whereby the pH of the solution is lowered below a set value for which the apparatus is adjusted. The lowering of the pH in the solution starts an automatic piston burette which adds NaOH until the pH of the solution again reaches the set value. The movements of the piston burette correspond directly to the amount of NaOH used to neutralize the acids and are recorded automatically.

APPARATUS

The following apparatus was used for the recording and the titration of the organic acids:

pH-stat: Titrator, Model TTT, Radiometer Co., Copenhagen, the pH was measured using a combined electrode GK 202.

Piston burette: Piston burette, Model E 298, Metrohm, Herisau, equipped with a built-in special resistor.

Recorder: Brown potentiometer, Honeywell, Minneapolis.

Magnetic stirrer: Model E 184, Metrohm, Herisau.

PROCEDURE

Chromatography.

The organic acids were placed on a silica gel column and separated according to the method of DONALDSON *et al.*¹. The acids were eluted with a constantly increasing amount of *n*-butanol in chloroform according to the method of WREN².

Elution. In order to make titration of the organic acids possible these had to be eluted in water. A glass cup with an overflow pipe was used as extraction vessel (Fig. 1). When the *n*-butanol-chloroform dropped into the water, a magnetic stirrer kept the liquid surface in the extraction vessel in constant movement; the surface was further disturbed by an air current which passed through the vessel. Without

this air current breaking the surface, chloroform accumulated, markedly impairing the results of the extraction. Distilled water was continuously added to the extraction fluid at a speed twice that by which *n*-butanol-chloroform left the column, thus inhibiting the formation of buffers which might influence the pH changes of the water. A combined glass electrode was also placed in the extraction vessel.

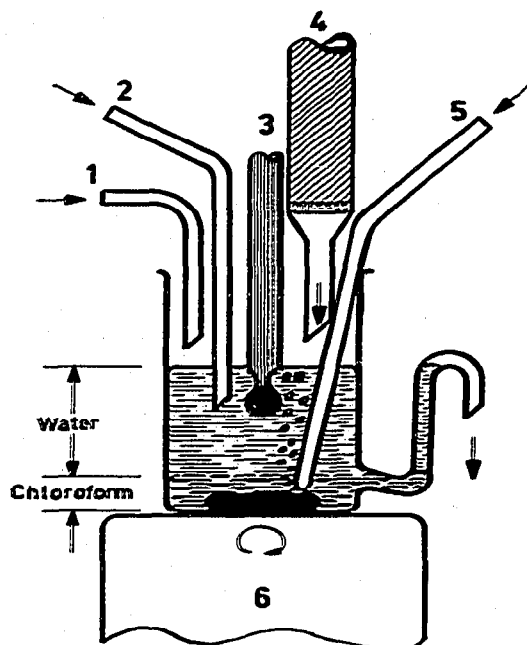


Fig. 1. Extraction vessel used for eluting in water the organic acids leaving the column. The vessel contains a glass electrode (3) for registering the acidity of the solution, one delivery tube for the titration fluid (2), one for the air current (5) and one for the addition of water (1), and a magnetic stirrer (6). The chromatography column (4) also empties into the vessel.

Titration and registration. When the acids are eluted and the pH of the solution has decreased below the threshold value at which the pH-stat is adjusted, an electric circuit is closed which starts the piston burette and 0.005 *N* NaOH is added until the initial pH is reached. The pH-stat was usually adjusted in such a way that the titration started when the pH fell below 6.9. Fig. 2 shows schematically the whole set-up of the apparatus for elution, titration and registration.

A pH meter with adjustable set point, and provided with an integral relay, was employed for pH measurement and automatic titration. As the voltage ratings of controller and piston burette were different, the latter was connected with the control output of the pH meter through another relay, which was added to the pH meter. Thus, manual control of the piston burette also remained possible. For the purpose of recording the position of the piston at a given time (and thus also the added NaOH volume) a 5000-ohm linear precision resistor coil (R_1) was mounted in the piston burette housing, its sliding contact rigidly connected with the piston feed mechanism, so that a well-defined position of this contact on the resistor is obtained for each piston position. This resistor transmitter (R_1) is supplied with direct current from a

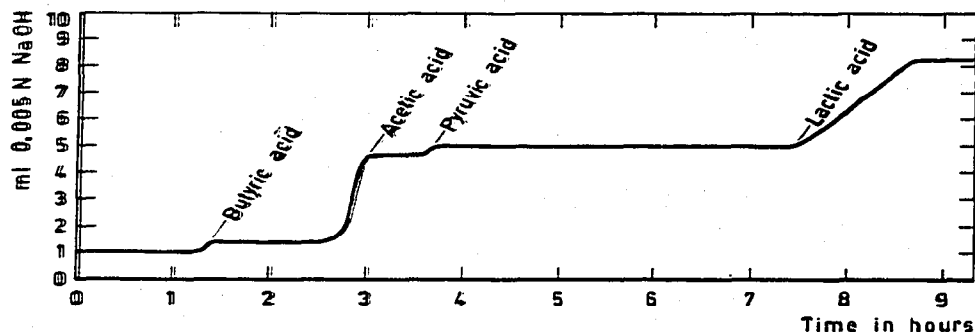


Fig. 3. Example of an automatic recording of organic acids formed in rat liver slices incubated for 2 h in physiological saline solution.

SUMMARY

An automatic apparatus for the registration of organic acids separated by column chromatography is described. An example is given of the determination of organic acids from a physiological incubation fluid. The method can be applied for the registration of other titratable acids and bases.

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