(IReactional Outober 24th), 1960).

(Column dimonstrographic analysis off organic acids generally requires laborious trituation off a large number off fractions. In our laboratories an automatic apparatus has been developed by means off which the different fractions of organic acids are clutted in watter 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 off the pHH in the solution stants an automatic piston burette which adds NaOH until the pHH off the solution again reaches the set value. The movements of the piston burettte convespond directly to the amount off NaOH used to neutralize the acids and are recorded automatically:

### ARRAN SAMUS

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

measured using a combined electrode GK 202.

Piston lumitte:: Piston lumettte;, Modell E298;, Metnohm, Herisau, equipped with a lbuilt-in special mesister.

Reconder: Brown pottentiiometter, Honeywell, Minneapolis.

Magnuttic stionar:: Modell E 1854, Metholim, Henisau.

## BROCEDURE:

## (Chromatogna#llay

Illhe arganic adids were placed on a silica gell column and separated according to the method off IDaxwidsons at al.<sup>11</sup>. Ille adids were eluted with a constantly increasing amount off *m*-buttand in abbroform according to the method of WREN<sup>2</sup>.

**Electricon.** In order two marker tituration off the organic acids possible these had to the electrical in watter. A glass cup with an oxenflow pipe was used as extraction vessel ((Fig. I)). When the *m*-butanoll-allohofform dropped into the water, a magnetic stirrer lkept the liquid sufface in the extraction wessel in constant movement; the surface was flutther disturbed by an air current which passed through the vessel. Without

515

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.

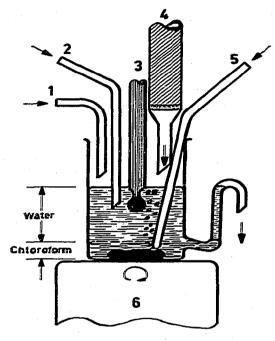


Fig. 1. Extraction vessel used for eluting in water the organic acids leaving the column. The vessel contains a glass electrode (3) for registering the acidity of the solution, one delivery tube for the titration fluid (2), one for the air current (5) and one for the addition of water (1), and a magnetic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

1. Chromatog. 5 (1061) 515-518

1.5-V battery through resistor  $R_3$  and voltage divider  $R_2$ . The latter is used to adjust the voltage across resistor  $R_1$  and therefore also serves to position the pen of the connected potentiometer recorder at a predetermined value prior to the experiment. When the piston burette is put into operation by the pH controller and by the intermediate relay, the sliding contact of the transmitter resistor will move and cause a

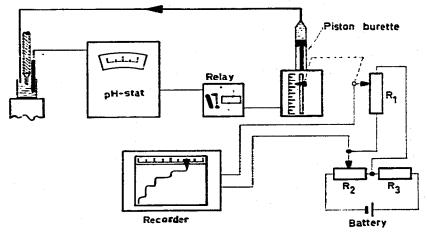


Fig. 2. Schematic outline of the apparatus for automatic registration of organic acids separated by column chromatography.

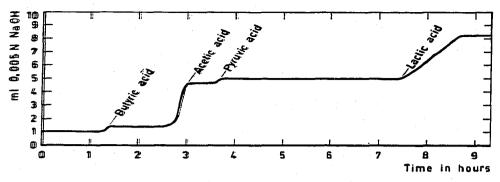
voltage corresponding to the piston position to appear at the terminals of the recorder. The latter will then continuously record the NaOH consumption in terms of time during the titration process, which lasts about 8 hours.

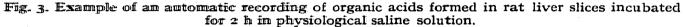
The recorder was adjusted to give maximum deflection when the piston burette moved from o to IO ml. The burette could be replaced by others with greater or smaller volumes according to the amounts of acids present. This method has advantages over one where the concentration of the NaOH is altered, since a strong base easily causes overtitration.

This apparatus can be applied for the registration of basic or acidic compounds separated by column chromatography. A similar apparatus for purposes other than chromatography has been described by JACOBSEN et al.<sup>3</sup>.

# Organic acids in physiological solutions

In our laboratories the above apparatus has been used for determinations of organic acids in blood and physiological incubation fluids. The material to be analysed was deproteinized with perchloric acid, neutralized with potassium hydroxide and the organic acids eluted from the solution with ether according to SWIM AND UTTER<sup>4</sup>. The acids were then neutralized with NaOH and evaporated to dryness in a vacuum exsiccator at room temperature. The dry residue was acidified with  $0.1 N H_2SO_4$ , adsorbed on I g silicic acid and placed on a silica gel column<sup>1</sup>. The eluted acids were titrated with 0.005 N NaOH. Fig. 3 shows an example of a chromatographic analysis of organic acids after an incubation of rat liver slices for 2 hours in a physiological medium.





### 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.

### REFERENCES

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<sup>4</sup> H. E. SWIM AND M. F. UTTER, in S. P. COLOWICK AND N. O. KAPLAN, Methods in Enzymology, Vol. IV, Academic Press, Inc., New York, 1957, p. 587.

J. Chromatog., 5 (1961) 515-518