NOTES.

Apparatus for Microtitrations with a Glass Electrode. By J. R. CATCH, A. H. COOK, and J. A. KITCHENER.

DETAILS are given of a simple apparatus which has been used for some years with satisfactory results for the microtitration of biological products, sulphonamides, determination of pK values, detection of latent acid groupings, etc. The titration cell consists of a tube A (Fig. 1) sealed at one end to a capillary B. The internal diameter of A is slightly larger than the external diameter of a sealed glass electrode C (Cambridge Instrument Co.); B is filled throughout its length with 2% agar gel in saturated aqueous potassium chloride and is of very narrow bore so that diffusion of solution

FIG. 1.

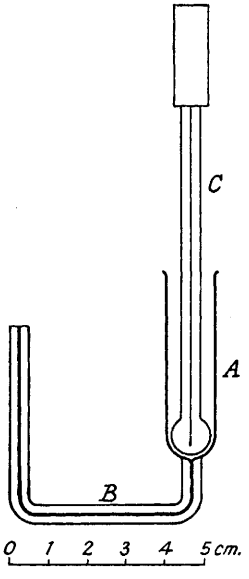
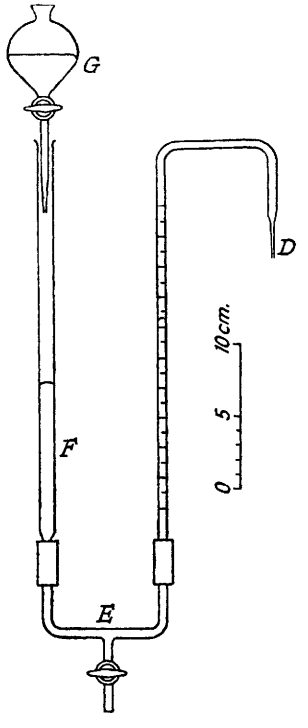


FIG. 2.



from A into B is negligible; A is thus virtually closed at its lower end and carries a conducting bridge. The titration can be carried out in an inert atmosphere if desired by passing nitrogen from a capillary through A. The sample (2—5 mg.) under examination is weighed into A and dissolved in 0·1—0·2 c.c. of water or other solvent which is sufficient to surround the bulb of the electrode. Sparingly soluble acids or bases may be titrated as a finely divided suspension: this does not affect the observed value of the equivalent, though the shape of the titration curve may be modified as the sample passes into solution. The open end of B is connected in the ordinary way through a potassium chloride salt bridge to a calomel electrode, and the e.m.f. of the whole cell is measured on a valve electrometer–pH meter.

Additions of acid or alkali are made from micro-burettes of the type shown in Fig. 2, and it is convenient to set them up in pairs so that back-titrations can be carried out without disturbing any part of the apparatus. The burettes consist of 1-c.c. graduated pipettes sealed to a tube with a fine but stout capillary tip D. When filled, the burettes are adjusted so that this tip projects into the cell A and touches the wall a little above the resting level of the bulb of C. Diffusion of acid or alkali from D into the liquid contents of A is undetectable if D is a very narrow capillary. The lower end of each burette is connected, by tubing carrying a tap E, with a wide tube F into which mercury is run from a dropping-funnel G. The apparatus is filled by first filling it with mercury and then opening E whilst D is held below the level of the acid or alkali. Expulsion from D is controlled by additions of mercury from G through its capillary outlet and there is no difficulty in delivering quantities of reagent of about 0·01 c.c. which can be read to the nearest 0·001 c.c. Rubber pressure-tubing connections at E give flexibility but in no way disturb the stability and reproducibility of the volume readings. After each addition of alkali or acid, mixing in A is effected by a few passages of C up and down the closely fitting walls of A, after which the pH readings are taken, and a titration curve constructed in the normal

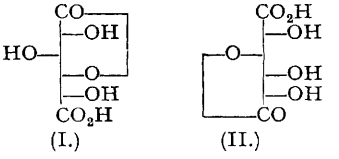
way. In using N/20-acid or -alkali to titrate or back-titrate known simple compounds such as benzoic acid, the accuracy in determining the equivalent value is about 1 in 500 and therefore compares favourably with the macrotitrimetric procedure. Of especial value, however, is the possibility of constructing complete titration curves by using only micro-quantities of material and inexpensive adjuncts.

The authors thank Professor I. M. Heilbron, D.S.O., F.R.S., for his interest and encouragement.—IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON, S.W.7. [*Received, January 20th,* 1945.]

The Constitution of the Two Lactones of d-*Glucosaccharic Acid.* By T. REICHSTEIN.

SMITH recently expressed the opinion (J., 1944, 571) that the lactone, m. p. 133°, of Sohst and Tollens (*Annalen*, 1888, **245**, 1) is a mixture of *d*-glucosaccharo-1 : 4-lactone (I) and *d*-glucosaccharo-3 : 6-lactone (II). This opinion is based on the fact that by prolonged treatment of the above-mentioned lactone with methyl iodide and silver oxide he was able to isolate the tetramethyl derivatives of (I) and (II) in moderate yields. Considerable quantities of other products were formed in the reaction, three of which were isolated in a state of purity, thus proving that the methylation process was accompanied by rearrangements and degradations.

The lactone, m. p. 133°, was long considered to possess formula (I) (Fischer and Piloty, *Ber.*, 1891, **24**, 521) until Schmidt, Zeiser, and Dippold (*Ber.*, 1937, **70**, 2402) produced strong evidence in support of formula (II), the correctness of which was proved by Schmidt and Günthert (*Ber.*, 1938, **71**, 493) by smooth cleavage with periodic acid and confirmed by Sutter and Reichstein (*Helv. Chim. Acta*, 1938, **21**, 210) by the results of reduction with sodium amalgam. The last-named authors also showed that the second *d*-glucosaccharolactone, isolated as a hydrate, m. p. *ca.* 90°, by Reichstein, Grüssner, and Oppenauer (*Helv. Chim. Acta*, 1933, **16**, 1032) very probably possesses formula (I) (only the size of the lactone ring was not definitely proved) and that (I) and (II) can be separated by fractional crystallisation, although with some difficulty; it was found that from supersaturated solutions of the pure compounds and of mixtures only that compound crystallised which had been used for inoculation, and that (I) and (II) differ greatly in solubility.



In the light of these facts, the present writer finds it difficult to assume that the lactone of higher m. p. (135—136° according to Schmidt, Zeiser, and Dippold, *loc. cit.*, and to Sutter and Reichstein, *loc. cit.*) is a mixture and inclines to the view that a partial isomerisation of (II) may have occurred during the methylation process.—PHARMAZEUTISCHE ANSTALT DER UNIVERSITÄT, BASEL. [*Received, March 1st,* 1945.]
