759. *Submicro-methods for the Analysis of Organic Compounds. Part XII.* Determination of Carboxyl Group.*

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A method is described for the determination of the carboxyl group by titration in aqueous alcohol. Direct potentiometric titration is carried out with a modified glass/silver–silver chloride electrode system: a back-titration is recommended for the visual method with phenolphthalein as indicator. Samples of *ca.* 50 μg. are titrated in a total volume not exceeding 0·4 ml. with an accuracy of ±1%.

The main problems to be expected in the determination of carboxyl by titration on the submicro-scale, were interference by atmospheric carbon dioxide and, more importantly, accurate location of the end-point. The former difficulty was readily overcome by enclosing the titration assembly in a chamber through which a stream of pure nitrogen passed. Several methods for end-point location were examined: direct and back-titration with visual indicators, and direct and back-titration potentiometrically, in which two different electrode systems were used. Twelve titrations of benzoic acid were done under each set of conditions so that the standard deviation could be calculated. The results are included in Table 1.

Table 1. *Titration of* ca. 50 μg. *samples of benzoic acid with* 0·01N-*sodium hydroxide.*

Method	Standard deviation (%) for a single result
Direct titration with visual indicator	±1·3
Back-titration with visual indicator	±1·5
Direct potentiometric titration (glass/calomel)	±1·0
Direct potentiometric titration (glass/silver chloride–silver)	±0·8
Back potentiometric titration (glass/calomel)	±1·2
Back potentiometric titration (glass/silver chloride–silver)	±0·8

Uniform conditions were maintained as far as possible, but in the potentiometric titrations a slightly larger volume of solution was necessary to ensure immersion of the electrodes.

Although phenolphthalein was satisfactory in the visual back-titration (pink to colourless) it was difficult to locate its end-point in the direct titration; hence several mixed indicators were examined.[1] The best of these was α-naphtholphthalein–phenolphthalein (1 : 3) which changed from pale rose to pale green at pH 8·6 and to violet at pH 9. This indicator was used subsequently in all *direct* titrations.

Several organic acids were also titrated under all these different conditions. From these results (complete details of which have been described elsewhere [2]) the following conclusions were reached: (1) Back-titration is to be preferred for the visual method, because the end-points are more easily located and lie much closer to the potentiometric end-point. (2) In general, indirect and direct potentiometric titrations are equally satisfactory, with the glass/silver–silver chloride electrode system, but if the acid is very weak or if there is difficulty in dissolving it the indirect method is to be preferred. (3) The electrode system glass/silver–silver chloride is preferable to glass–calomel; it occupies less space and avoids risk of contaminating the solution with the bridge liquid. There is little to choose as regards stability. (3) Acids which have $pK_a > 6$ cannot be titrated satisfactorily by the recommended procedure. (5) Benzoic acid is satisfactory as a primary standard, but it is essential to titrate it immediately after the weighing, because losses due to volatilisation can prove serious on the submicro-scale. In some early experiments poor

* Part XI, *J.*, 1960, 2473.

[1] Kolthoff and Stenger, "Volumetric Analysis," Part II, Interscience Publ. Inc., New York, 1947.
[2] L. Serrano-Berges, M.Sc. Thesis, Birmingham, 1958.

FIG. 1.

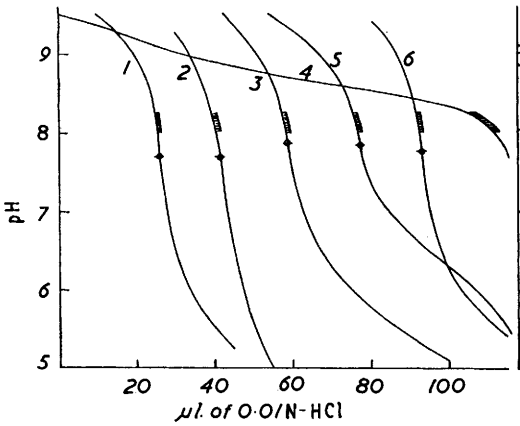
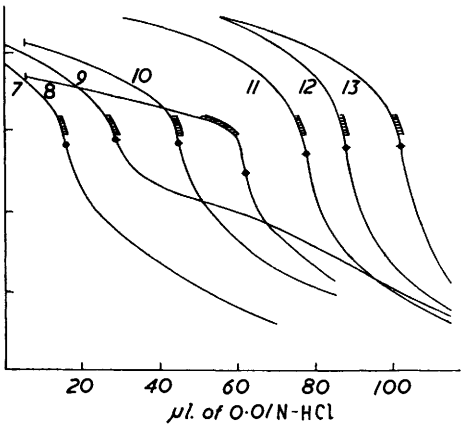


FIG. 2.



FIGS. 1 and 2. *Potentiometric back-titrations of*: (1) *oxalic*, (2) *salicylic, and* (3) *cinnamic acid*, (4) *glycine*, (5) *phthalic*, (6) *benzoic*, (7) *citric*, (8) p-*chlorobenzoic*, (9) *succinic*, (10) *tartaric*, (11) m-*trifluoromethylbenzoic*, (12) *hippuric, and* (13) *sulphanilic acid.*

The colour transition of the visual indicator is represented by the shaded zones.
⊖ *Electrometric end-point.*

FIG. 3.

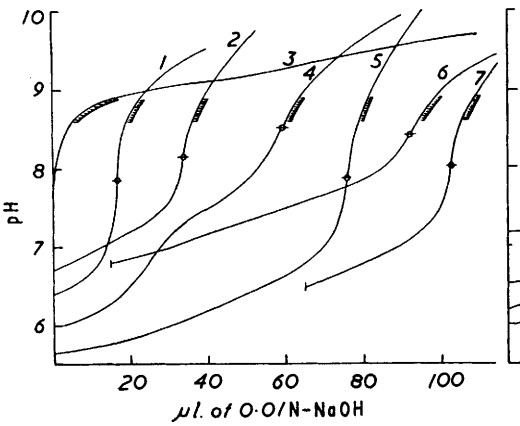
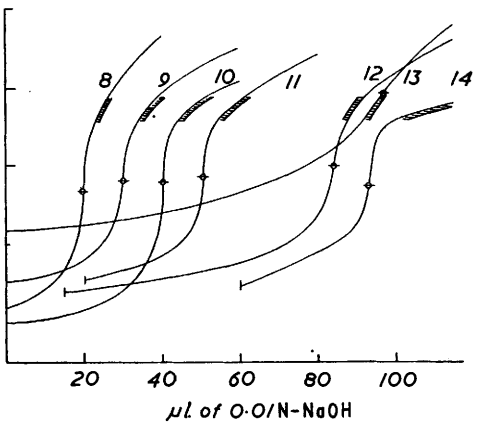


FIG. 4.



FIGS. 3 and 4. *Potentiometric direct titrations of*: (1) *potassium hydrogen tetrachlorophthalate*, (2) *cinnamic acid*, (3) *glycine*, (4) *phthalic*, (5) *oxalic*, (6) *succinic*, (7) *benzoic*, (8) *salicylic*, (9) *hippuric*, (10) *sulphanilic*, (11) m-*trifluoromethylbenzoic*, (12) *tartaric*, (13) *citric, and* (14) p-*chlorobenzoic acid.*

Shaded areas and ⊖: *see* Fig. 1.

TABLE 2. *Visual titration.*

Component	Range of wts. (μg.)	CO_2H (%) Calc.	CO_2H (%) Found	Error (%)	No. of detns.	Range of error Max.	Range of error Min.
Oxalic acid	34—63	71·42	71·12	−0·30	6	−1·45	−0·08
Succinic acid	42—66	76·25	76·66	+0·41	6	+1·29	−0·02
Tartaric acid	51—85	60·00	60·02	+0·02	6	+0·46	−0·02
Citric acid	40—82	64·27	64·96	+0·69	6	+1·36	+0·16
Hippuric acid	39—67	25·12	24·71	+0·41	5	+0·72	−0·16
p-Chlorobenzoic acid	42—64	28·75	29·28	+0·53	5	+0·97	−0·16
m-Trifluoromethylbenzoic acid	44—83	23·68	23·43	−0·25	5	−0·61	−0·11
Salicylic acid	32—67	32·59	32·71	+0·12	6	+0·65	−0·03
Phthalic acid	43—80	54·19	54·71	+0·52	6	+0·83	−0·25
Potassium hydrogen tetrachlorophthalate	38—115	13·16	13·13	−0·03	7	−0·26	0·0
Sulphanilic acid	49—61	25·99	26·31	+0·32	6	+0·58	0·0
Cinnamic acid	39—59	30·39	30·45	+0·06	6	+0·74	+0·08

TABLE 3. *Potentiometric titration.*

Compound	Range of wts. (μg.)	CO_2H (%) Calc.	Found	Error (%)	No. of detns.	Range of error (%) Max.	Min.
Oxalic acid	49—51	71·42	71·09	−0·33	5	−0·40	−0·14
Succinic acid	49—64	76·25	76·32	+0·07	6	+0·82	−0·13
Tartaric acid	43—63	60·00	59·25	−0·75	6	−0·90	−0·52
Citric acid	41—94	64·27	64·77	+0·50	6	+0·53	+0·47
Hippuric acid	54—69	25·12	24·74	−0·38	5	−0·64	−0·22
m-Trifluoromethylbenzoic acid	38—64	23·68	23·72	+0·04	5	+0·25	0·0
Salicylic acid	44—59	32·59	32·45	−0·14	6	−0·43	−0·07
Phthalic acid	53—69	54·19	54·48	+0·29	6	+0·52	0·0
Potassium hydrogen tetrachlorophthalate	38—89	13·16	13·16	0·0	6	−0·21	+0·05
Sulphanilic acid	40—59	25·99	26·04	+0·05	5	+0·42	0·0

results were obtained when dissolution and titration were done some hours after the weighing; there was a loss of 22·5% of the sample after 75 hr. at room temperature.

Potentiometric titration curves are shown in Figs. 1—4.

Results are included in Tables 2 and 3; the typical error is approximately 1%.

EXPERIMENTAL

Solvent Mixture.—It was advantageous to use a 1 : 2 mixture of ethanol and water for dissolution of the acids. For visual titrations it was convenient to include the indicator in this solution.

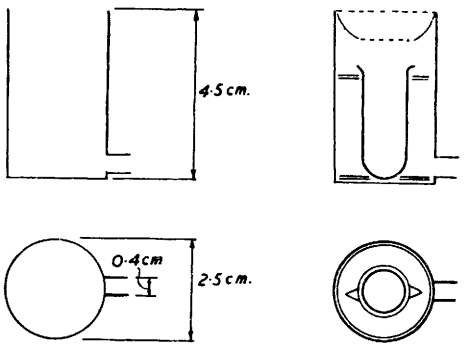


FIG. 5. *Microchamber for titration in CO_2-free atmosphere.*

Apparatus.—An " Agla " micrometer syringe burette (Burroughs Wellcome Ltd.) was used. Borosilicate glass test tubes with hemispherical bottoms, of 11 mm. inner diameter and 30 mm. long, were used as titration vessels. The magnetic stirrer had a 3 mm. long rotor. To avoid interference by atmospheric carbon dioxide a special microchamber was used, through which a continuous flow of nitrogen was passed (Fig. 5).

With this microchamber it was possible to work in an inert atmosphere without obstructing the top of the titration vessel. There was enough room to accommodate the electrodes and the syringe, and it was possible to use the same titration vessels and similar apparatus for both the visual and the potentiometric procedure.

The microchamber is a cylindrical vessel with a flat bottom made of glass (diameter 2·5 cm.; height 4·5 cm.) with a lateral tube (diameter 0·4 cm.) for the inlet of nitrogen. The titration tube was maintained vertical and well centred by means of a plastic disc of the same diameter as the microchamber. A plastic cover with a central hole of the same diameter as the titration tube reduced the space available for the outlet of the nitrogen.

The pressure of the outcoming gas was between 1 and 3 lb./sq. in. The nitrogen was freed from carbon dioxide by passage through soda-asbestos.

For visual titration, light was provided by a Daylight Blue bulb (230 v, 60 w, Mazda B.T.H. single coil), clamped about 20 cm. above the titration vessel.

Reagents.—0·01N-*Hydrochloric acid.* Prepared by dilution with distilled water from " AnalaR " hydrochloric acid (*d* 1·19).

0·01N-*Sodium hydroxide.* Prepared by dilution with freshly boiled distilled water from 0·1N-sodium hydroxide solution (prepared from Sörensen's oily-lye).

Phenolphthalein indicator solution (*Solvent Mixture*). A 1 : 2 v/v mixture of 0·1% ethanolic phenolphthalein and boiled distilled water.

α-Naphtholphthalein–phenolphthalein indicator solution. One part of 0·1% alcoholic α-naphtholphthalein and three parts of 0·1% alcoholic phenolphthalein. The *solvent mixture* in this case is prepared by mixing one part of the above with three parts of freshly boiled distilled water. This formulation was only used for *direct* visual titration.

Procedure.—Visual back-titration. The sample was weighed and transferred to the titration-vessel which was tapped gently to remove any particles adhering to the sides. A rotor was introduced and 200 μl. of the solvent mixture containing the phenolphthalein indicator (150 μl. if more than 100 μl. of alkali were likely to be consumed) were added.

The vessel was placed inside the micro-chamber and nitrogen was passed through the latter. The mixture was stirred for 15 min. and 100 μl. of 0·01N-sodium hydroxide were added with the tip of the syringe about 1 mm. below the surface. The tip was then raised above the surface and washed with one drop of distilled water. The syringe was removed and back-titration done similarly with 0·01N-hydrochloric acid until the indicator became colourless. The same standard illumination was maintained for all determinations. The control (see Standardisation) was deducted from the titration value.

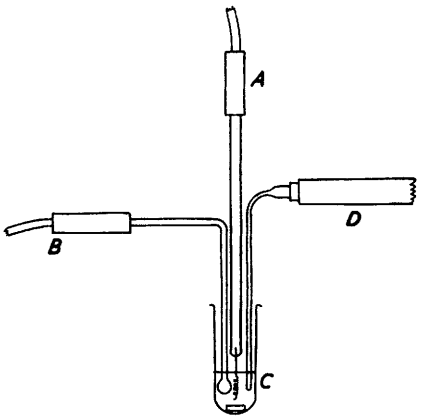


FIG. 6. *Titration assembly.*
A, Silver electrode.
B, Glass electrode.
C, Vessel and stirrer.
D, Burette.

Direct visual titration. This was as above except that the titration was carried out directly with the standard alkali and the mixed indicator was used. The end-point colour change is from pale rose through pale green at pH 8·6 to violet at pH 9·0. Comparison solutions were used to match the end-points.

Standardisation of Sodium Hydroxide and Hydrochloric Acid.—Samples of benzoic acid (*ca.* 50 μg.) were weighed and immediately dissolved in 200 μl. of solvent mixture, 100 μl. of sodium hydroxide were added, and the excess of alkali was back-titrated with hydrochloric acid which had previously been titrated against the sodium hydroxide in the same medium.

A control value on the solvent mixture was established by titrating a mixture of 200 μl. of solvent mixture and 150 μl. of boiled distilled water to the first appearance of a pink colour. The average of 10 such titrations (~1·5 μl. of 0·01N-sodium hydroxide) was deducted from the volume of alkali required by the benzoic acid.

Potentiometric Titration.—Silver–silver chloride electrode. A special electrode was used (Cambridge Instrument Co. Ltd.). To give more space above the titration vessel the stem was extended to give a total length of 14 cm. The electrode consisted of a 4 mm.-thick glass tube ending in a platinum wire spring (2 mm. in diameter and 8 mm. long). The platinum surface was silvered and covered with a film of silver chloride by anodic treatment in hydrochloric acid.[3]

pH Meter. For the potentiometric titration, a Vibron electrometer (model 33-B) was used in conjunction with a Vibron pH measuring unit (model c-33-B; Electronic Instruments Ltd., Surrey, England).

[3] Belcher, Berger, and West, *J.*, 1959, 2877.

Titration vessels. In the potentiometric titration only 0·4 ml. of solvent is used. In order to allow space for a rotor, two submicro-electrodes and the tip of the syringe (Fig. 6), the titration vessel must be exactly as specified.

Procedure for Direct Potentiometric Method.—The samples were weighed and transferred directly to the titration vessels. The dissolution was carried out, immediately after weighing, by stirring for 15 min. in 400 μl. of the " solvent mixture." The presence of the visual indicator served to give warning of the proximity of the end-point. Before starting the titration, the vessel was inserted inside a microchamber where there was a continuous flow of nitrogen.

The glass/silver–silver chloride electrode system was lowered into the solution, the bulb of the glass electrode being just immersed; the tip of the microsyringe was inserted to a depth of about 1 mm. Efficient stirring was maintained and the standard sodium hydroxide solution was added, near the equivalence zone, in portions of 1 μl. each at intervals of 1 min., to overcome variations in delivery during the titration. The variations are due to variable evaporation of solvent from the microchamber and irregularities in evaporation from the plunger of the syringe burette due to heating effects on the barrel of the syringe from the standard illumination lamp.

The potentiometric end-point was determined according to the method of second increments against reagent volume. When the second derivative is equal to the zero-point of maximum slope, the volume corresponding to the equivalence point is determined.

Standardisation of the sodium hydroxide solution. Benzoic acid (microanalytical standard, B.D.H.) was used as standard. Standardisation of the sodium hydroxide solution was carried out by the procedure previously described.

About 50 μg. of benzoic acid were dissolved in 400 μl. of " solvent mixture " and then potentiometrically titrated with 0·01N-sodium hydroxide, the system glass/silver–silver chloride being used.

The volume of reagent found experimentally was corrected by a control determined on the solvent mixture as in the visual titration.

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