576. *Submicro-methods for the Analysis of Organic Compounds. Part VIII.* Factors associated with Submicro-titration in Glacial Acetic Acid.*

By R. Belcher, J. Berger, and T. S. West.

Factors associated with the submicro-titration of organic compounds as bases in glacial acetic acid, and the evolution of a technique of potentiometric and visual submicro-titration in such a medium, are studied. Those which require careful control are the method of use of burettes with organic solvents, the weighing and dissolving the sample, the choice of the correct visual indicator shade to mark the equivalence-point, the use of colour comparison solutions, the method of determining the blank (control) analysis, and the water content of the solvent.

Many organic compounds can be titrated as acids or bases, but water is not generally a suitable medium. Consequently they are frequently determined by titration in non-aqueous solvents, *e.g.*, glacial acetic acid for bases, and basic solvents such as butylamine for acids. Many such determinations have been described on the macro-, semimicro-, and micro-scales. Since the submicro-titrations hitherto described in this Series have been carried out in aqueous media and the technique of non-aqueous titrimetry differs substantially, it was necessary to examine various factors associated with the use of organic solvents on this scale before developing analytical methods. These investigations are now described, and the analytical methods will be described later.

Titration of Organic Bases in Glacial Acetic Acid.—The use of glacial acetic acid as solvent for the titration of organic bases is well established on the conventional scales of working.[1] Perchloric acid in acetic acid is most frequently used as titrant since it is the strongest acid in this medium and is very stable. The reagent is readily prepared from *ca.* 70% aqueous perchloric acid by dilution with acetic acid. The water content can be measured by titration with Karl Fischer reagent and its concentration suitably adjusted by addition of acetic anhydride.

Primary Standards.—Potassium hydrogen phthalate was selected as primary standard. Although objections have been raised to its use in macro-titrations because of its low solubility it was found that 50 μg. samples dissolved readily (with stirring) in the amounts of solvent required and no visible precipitate of potassium perchlorate was produced immediately. In macro-titrations, high equivalent weight is desirable in a primary standard, but on the submicro-scale, where weighings can be done comparatively more accurately than titration (*i.e.*, with commercial syringe pipettes), the position is reversed.

Titration Equipment.—A suitable volume for the titration is 400 μl. (0·4 ml.) and a convenient shape of vessel approximates to a small test-tube, 11 mm. in internal diameter and 30 mm. long. When it is necessary to stopper such vessels, ordinary cork protected by thin Polythene sheet should be used, since the water in cork or in rubber is extracted by anhydrous acetic acid and interferes. The burettes used were identical with those used previously, but phenomena scarcely noticed with aqueous solutions became of prime importance with acetic acid solutions. Thus, after a syringe had been filled, the liquid was observed to rise quickly in the delivery tip of the capillary. It was found that the solvent evaporated round the plunger and not from the tip of the burette. Thus one burette lost 4·4 μl. in 90 min. Accordingly this effect must be guarded against and the time required for two titrations should not differ by more than 3—5 min.

Volatile Samples.—Samples were weighed as described previously, but although some compounds can be stored for days after weighing without appreciable loss, *e.g.*, potassium

* Part VII, *J.*, 1959, 2585.

[1] Round Table Discussion of Amer. Chem. Soc., *Analyt. Chem.*, 1952, **24**, 310.

hydrogen phthalate, others lose weight steadily, *e.g.*, *p*-bromoaniline, 1-naphthylamine, benzoic acid, and *p*-anisidine (see Table 1), and some, *e.g.*, *p*-toluidine, are so volatile that they cannot be weighed on the sensitive submicro-balance. It is therefore necessary to dissolve samples immediately after weighing.

TABLE 1. *Volatilisation of* p-*anisidine during storage in a platinum boat inside a micro-desiccator, removed only for weighing.*

Storage period (hr.)	0	1	2	3	19	43
Wt. of sample (μg.)	72·6	70·2	68·3	66·1	50·9	30·9
Loss (%)	—	3·3	5·9	9·0	29·9	57·4

Visual Indicators.—The choice of indicator is extremely important. We examined most of those recommended for use on the macro-scale, *viz.*, Crystal Violet, Methyl Violet, Ciba Blue BZL, Oracet Blue B, and Neutral Red. We cannot recommend the last three for submicro-titration because of the indistinct nature of the colour change and the high indicator correction. Crystal Violet and Methyl Violet are equally good, but the former is preferred since it can be used in lower concentration and, as will be seen later, this is important. Ellerington and Nicholls [2] described the colour change of Crystal Violet at the end-point as follows: [Base in excess] Violet → blue-violet → blue → green-blue → blue-green → green → yellow-green → green-yellow → yellow [acid in excess]. In submicro-titration the whole colour change from violet to green requires only 0·2—0·4 μl. of 0·01N-perchloric acid. Adjustment to a *fixed* colour can be done very accurately, particularly when a comparison solution is used.

Colour-comparison solutions. The colour-comparison solutions recommended by Ellerington and Nicholls are insufficiently stable for use in submicro-titration, but Gremillion's observation [3] that the titration curve of urea in acetic acid is virtually straight provided the necessary means of devising one. The colour of comparison solutions thus made is independent (visually) of the water content from 0·02 to 0·2% of water.

The selection of the correct shade for the indicator at the end-point, *i.e.*, the correct pH at which to stop titration, is of the utmost importance. In theory, it is necessary to establish the correct shade for each individual base, but we found by submicro-potentiometric titrations of very many bases that the colour of Crystal Violet at equivalence is near to royal blue. Accordingly a colour-comparison solution of this colour was prepared by addition of 0·1N-perchloric acid to urea in glacial acetic acid. This colour is not the *exact* colour observed with *all* bases, but it is sufficiently near to eliminate serious error. Even for an individual base, the blue colour at the equivalence point is slightly different for 40 μg. and 60 μg. samples. For a base which is weak in glacial acetic acid, *e.g.*, lithium benzoate, the colour change in a submicro-titration is near to blue-green and the change is spread over a fairly wide range.

Volume of Medium and Determination of "Blank."—The consumption of 0·01N-perchloric acid in a submicro-titration is 30—50 μl. If a large amount of solvent (1—2 ml.) is used, the blank analysis will be *ca.* 10% of the total titre, the visual end-point will be poor and the potential change in the potentiometric titration small. The smallest possible volume which we could use in the potentiometric titration was 0·4 ml., at which (see Table 2) the potentiometric blank is 1 μl. According to Kolthoff and Bruckenstein [4] the equivalence potential (or pH) in the titration of a base in glacial acetic acid is independent of the concentration of the base or salt so that the blank titration is established by titrating to the appropriate pH. The "blank" value increases with the amount of solvent. The values are reproducible ±0·1 μl.

Conant and Werner [5] noted that ionic strength considerably affects the colour behaviour

[2] Ellerington and Nicholls, *Analyst*, 1957, **82**, 233.
[3] Gremillion, *Analyt. Chem.*, 1955, **27**, 133.
[4] Kolthoff and Bruckenstein, *J. Amer. Chem. Soc.*, 1957, **79**, 1.
[5] Conant and Werner, *ibid.*, 1930, **52**, 4436.

of indicators in glacial acetic acid. We observed that whereas 50 μg. of sodium acetate titrated with 0·01N-perchloric acid give a blue colour with Crystal Violet at the end-point, 300 μg. titrated in the same way to the same pH (and with the same equivalence) give a blue-green end-point. The potentiometric blank remained unchanged but the visual

TABLE 2. *Dependence on the amount of solvent of visual and potentiometric blanks.*

Acetic acid (*ca.* 0·02% H_2O) (ml.)	0·2	0·4	0·6	0·8	1·0	1·2
Potentiometric blank (μl.)	—	1·0	1·5	2·0	2·4	2·6
Visual blank (blue) (μl.)	0·9	1·2	1·8	2·4	3·2	3·4

blank had increased. Kolthoff and Bruckenstein derived a quantitative expression and found that the ratio between the concentrations of the acid form and basic form of the indicator is proportional to the square-root of the concentration of the salt.

This effect which is not found in aqueous titrimetry means that even if a visual blank is determined for the solvent, it will not be reliable. Table 2 shows that the difference between potentiometric and visual blanks increases with increasing amounts of solvent. Such problems do not arise in the macro-scale since no significant blank is found.

FIG. 1.

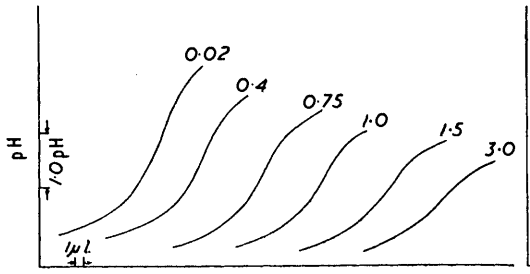
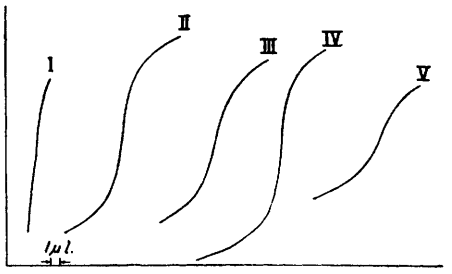


FIG. 2.



0·0/N-$HClO_4$

FIG. 1. *Potentiometric titration of* ca. 50 μg. *of* p-*anisidine in* 0·4 *ml. of glacial acetic acid of specified water content* (%). *Silver–silver chloride/glass electrode system. Salt bridge electrolyte* 0·02N-*sodium perchlorate in acetic acid.*

FIG. 2. *Experimental procedure as in Fig.* 1, *but with water content controlled at* ca. 0·1%.
I, Blank on 0·4 ml. glacial acetic acid. II, Titration of *ca.* 50 μg. of potassium hydrogen phthalate. III, Titration of *ca.* 50 μg. of sodium acetate trihydrate. IV, Theoretical curve for sodium acetate (no volume change). V, as III but without salt bridge.

Permissible Water-content of Solvent.—In macro-titrations, acetic acid containing up to 1% of water can be used safely as a solvent for the titration of most bases. According to Kolthoff and Bruckenstein the basic dissociation constant of water in glacial acetic acid is pK = 12·53 (cf. sodium acetate, pK = 6·68). The effect of water therefore becomes most pronounced near the end-point when it competes with the indicator (a weak base) for the perchloric acid. On the submicro-scale, we have found that water only slightly

TABLE 3.—*Dependence on the water content of glacial acetic acid of visual and potentiometric blanks.*

Water content of 0·4 ml. of acetic acid (%)	*ca.* 0·02	0·2	0·5	0·75	1·0	1·5	2·0
Potent. blank (μl.)	1·0	1·0	1·1	1·1	1·2	1·2	—
Visual blank (blue colour) (μl.)...	1·2	1·4	1·9	2·3	3—3·5	4—5	6—7
Remarks on the visual end-point	Good	Fairly good	Indefinite	Indefinite	Bad	Bad	Very bad

increases the potentiometric blank but adversely affects the slope of the curve and the magnitude of the potential break (cf. Table 3 and Fig. 1). The practical upper limit for water content is *ca.* 1·5%.

On the other hand, the visual blank increases considerably with concentration of

water and the colour change is drawn out. It was finally concluded that the water content should not greatly exceed 0·15—0·2% in submicro-work.*

The coefficient of cubic expansion of glacial acetic acid is *ca.* four times that of water. It is therefore desirable to apply a normality correction if the temperature varies by more than 2° from that at which the solution was standardised. This correction is 0·1% per °C.[6]

Comparison between Experimental and Theoretical Data.—According to the calculations and experimental data of Kolthoff and Bruckenstein,[4] the pH of 0·01N-perchloric acid in glacial acetic acid is 3·44 and of 0·01N-sodium acetate 10·11, *i.e.*, a pH interval of 6·67. In our experiments the difference was found to be 6·2. Fig. 2 shows, *inter alia*, the calculated and experimental curves for the titration of 0·001N-sodium acetate with 0·01N-perchloric acid. The experimental curve was obtained by use of a glass indicator against a silver–silver chloride reference electrode. The stability of the glass electrode increased considerably if it was stored permanently in glacial acetic acid solution. The difference between the experimental and the theoretical data is attributed to the uncertainties introduced by using glacial acetic acid with a higher water content than used by Kolthoff and Bruckenstein (<0·01%) and the use of a glass electrode which has been stored in glacial acetic acid for some months.

Table 4 reveals that the standard deviation on a series of standardisations of perchloric acid with potassium hydrogen phthalate is *ca.* ±0·5%, whilst for visual titration it is ±0·7%. The normalities determined by these methods differ by 1%. This divergence is attributed to the difference in blank value and to the time required to complete a titration (2 min. visually, 8 min. potentiometrically). The effect of the time factor on such titrations has already been mentioned. From these data we conclude that it is necessary to establish two normalities, one for visual and the other for potentiometric use.

TABLE 4. *Standardisation (normality* ×100) *of* 0·01N-*perchloric acid against potassium hydrogen phthalate* (50—70 μg.)

Potent.	1·041	1·037	1·036	1·037	1·028	1·034	1·031	1·040
Visual	1·041	1·057	1·045	1·059	1·041	1·053	1·058	1·041
Potent.	1·036	1·040	1·040	1·047.		Average 1·037; s.d. ± 0·0050		
Visual	1·045	1·050	1·042	— .		Average 1·048; s.d. ± 0·0073		

Stability of Solutions.—Data for the stability of perchloric acid in acetic acid are not available in the literature, though several workers have commented favourably on the reagent. Ellerington and Nicholls [2] reported that 0·1N-reagent requires standardisation every 3 days (but more recently they obtained solutions of satisfactory stability [7]). In view of this, the present study included stability tests on 0·1N- and 0·01N-reagents stored in 1 l. volumetric flasks with the glass stoppers covered with Polythene sheet. Several times each week small samples were analysed. The 0·1N-solution gave an unchanged titre during nine months, and the 0·01N-solution can be used for at least 1—2 months without standardising.

The stability of 0·01N-sodium acetate in acetic acid was also satisfactory. . A change of only 0·3% occurred during 35 days. The colour comparison solutions faded gradually on standing in daylight for several weeks, but a solution stored in darkness for 2 months showed no visual difference in shade from a fresh one.

EXPERIMENTAL

Reagents.—(1) Acetic acid with water content adjusted to 0·02—0·15%. This is the " glacial acetic acid " used. (2) Potassium hydrogen phthalate, " Analar," finely powdered.

* Because of the influence of water all apparatus must be rinsed out thoroughly with glacial acetic acid containing Crystal Violet indicator and sufficient perchloric acid just to yield the green colour. The washings must be continued till the green colour of the solvent persists. A last washing must be done with pure glacial acetic acid.

[6] Seaman and Allen, *Analyt. Chem.*, 1951, **23**, 592.
[7] Ellerington and Nicholls, personal communication.

(3) Crystal Violet indicator solutions, 0·01% w/v and 0·5% w/v in glacial acetic acid. (4) 0·1N-Perchloric acid in acetic acid: 8·50 ml. of "AnalaR" 72% perchloric acid are added to 500 ml. of glacial acetic acid and 20 ml. of "AnalaR" acetic anhydride with agitation below 60—70°. The solution is diluted to 1 l. with glacial acetic acid and after standing for 24 hr. the water content is adjusted to 0·02—0·15%. (5) 0·01N-Perchloric acid in acetic acid, prepared by dilution of (4). (6) Blue comparison solution: 0·6 g. of urea in 70—80 ml. of glacial acetic acid treated with 3·3 ml. of 0·01% Crystal Violet indicator and 3·0 ml. of 0·1N-perchloric acid in acetic acid and diluted to 100 ml. (7) Blue-green comparison solution; prepared as above except that 6·0 ml. of 0·1N-perchloric acid in acetic acid are added. (8) Salt bridge electrolyte: 0·02N-sodium perchlorate monohydrate in glacial acetic acid.

Apparatus.—(1) Submicro-balance.[8] (2) Titration vessels: these must be washed as described already, dried by a stream of dry nitrogen, and stoppered with a cork covered with Polythene sheet. (3) "Agla" glass syringes: these must be used as described above. Minimum evaporation from the plunger is usually established in 15 min. (4) Magnetic stirrer used with glass-encased rotors 3 mm. long, external diameter *ca.* 1 mm. (5) "Daylight" blue bulbs (230 v, 60 w Mazda, BTH single coil) clamped, *ca.* 15 cm. above titration platform. The illumination is used only near the end-point to minimise errors due to heating of the syringe. (6) pH Meter: "Vibron Electrometer" Model 33-B and "Vibron pH Measuring Unit," Model C-33-B, Electronic Instruments Ltd., Richmond, Surrey, was used to give a pH discrimination of 0·002 pH unit. A Weston cell and circular potentiometer (1 megohm) was placed in series with the electrodes to obtain correct polarity and to adjust scale readings. The instrument was used on "negative input," and with full-scale deflection equal to one pH unit. (7) Glass electrode. Diameter of bulb 3·5 mm., diameter of stem, 3·5 mm., stem bent at right-angles *ca.* 4 cm. above bulb. This electrode was made to our specifications by Electronic Instruments Ltd. Design No. SK 605. (8) Silver–silver chloride electrode. Platinum disc 2—3 mm. in diameter sealed into 4 mm. glass tube, disc silvered and coated with AgCl by anodic treatment (total length of electrode 14 cm.). (9) Salt bridge: 7 mm. glass tubing drawn out to end in 1 cm. long capillary of 3 mm. external diameter, total length of bridge 5—6 cm. The capillary was stopped up with asbestos so that the leak rate from the assembled electrode and bridge was *ca.* 1 ml. in 4—5 days. The silver–silver chloride electrode was sealed into this salt bridge with a small rubber stopper.

Procedure.—The sample was weighed on the submicro-balance and transferred to the titration vessel in the usual way (see previous Parts).

(a) *Potentiometric method.* 0·4 ml. of glacial acetic acid was added from a 2 ml. graduated pipette, followed by one drop of 0·01% Crystal Violet and a rotor. The vessel was stoppered with a Polythene-protected cork and the contents were stirred magnetically for 3 min. The presence of the indicator warns of the approach of the end-point. The electrodes which had been stored in glacial acetic acid were wiped with filter paper and placed in a titration vessel containing 0·4 ml. of glacial acetic acid and a drop of 0·01% Crystal Violet and a rotor. The pH on the pH meter is then adjusted to *ca.* 8·5 by means of the potentiometer; this empirical value allows readings to be taken over the entire range of a submicro-titration. There may be a small drift after the adjustment as pH in pure acetic acid is not very well defined. With the titration equipment thus prepared, the glass electrode was first placed in the vessel containing the dissolved sample, then the burette tip (duly wiped with filter paper wetted with glacial acetic acid) and finally the salt bridge of the silver–silver chloride electrode.

In the vicinity of the end-point, the titrant was added in 1 μl. increments and the pH meter was read 15—20 sec. after each addition. The time required for titration carried out at a suitable rate was 7—8 min. The end-point was calculated according to the conventional second-derivative method.

Blank determinations were carried out as above with omission of the sample. The titration was begun in this instance by adding 0·4 μl. of 0·01N-perchloric acid twice and making further additions in 0·2 μl. lots. The readings were taken 30 sec. after each addition. The titration was continued well beyond the equivalence pH found in the actual determination. The blank analysis corresponded to the amount of perchloric acid required to obtain the equivalence pH. A typical value was 1·0 ± 0·1 μl. of 0·01N-perchloric acid. In each case the average of three determinations was chosen.

(b) *Visual indicator method.* The sample was weighed and dissolved as above and the

[8] Asbury, Belcher, and West, *Mikrochim. Acta*, 1956, 598.

titration was carried out till the same blue colour was obtained as that of the comparison solution which was placed in a similar vessel alongside the titration tube. The end-point was usually detected within $\pm 0{\cdot}1$ μl. especially when a small over-titration was used. The time required for a visual titration was 2 min.

The blank, which was usually reproducible within $\pm 0{\cdot}1$ μl., was determined by titrating the solvent to the same blue colour as above. Again the average of three blank determinations was taken.

One of us (J. B.) thanks the Carlsberg–Wellcome Foundation for a travelling fellowship.

CHEMISTRY DEPARTMENT, THE UNIVERSITY, BIRMINGHAM, 15. [*Received, March* 13*th*, 1959.]