577. *Submicro-methods for the Analysis of Organic Compounds. Part IX.*[1] *Titration of Organic Bases and Amine Hydrohalides in Glacial Acetic Acid.*

By R. BELCHER, J. BERGER, and T. S. WEST.

Procedures are described for the submicro-determination of organic bases, amine hydrohalides, quaternary ammonium salts, and primary aromatic amines and their hydrohalides by titration with 0·01N-perchloric acid in acetic acid in glacial acetic acid. Potentiometric and visual indicator methods (with Crystal Violet) are described. Hitherto undetected inaccuracies in macro-methods have shown on the submicro-scale and have been accounted for. The results obtained for primary aromatic amines and their hydrohalides are invariably high because of acetylation. An accuracy of *ca.* ±1% is usual.

VARIOUS factors which influence the titration of organic compounds as bases in glacial acetic acid on the submicro-scale of working have already been discussed.[1] We now apply such procedures to analysis.

(a) *Titration of Bases.*—Many organic compounds behave as bases in glacial acetic acid, *e.g.*, salts of carboxylic acids. Other substances can readily be converted into derivatives which behave as bases, *e.g.*, alcohols can be converted into xanthates and titrated with perchloric acid.[2] Carboxylic acids which are difficult to isolate pure can be determined similarly, *via* their *S*-benzylthiuronium salts,[3] whilst alcohols and alkyl halides can be dealt with as their *S*-alkylthiuronium picrates.[4] Table 1 summarises the results for several types of compound, and compares potentiometric and visual-indicator values on the submicro-scale with those obtained by macro-analysis. Submicro-procedures are satisfactory and a precision of ±1% is easily obtainable. Most compounds in Table 1 are easily dissolved in glacial acetic acid, but difficulty was encountered with the amino-acids. Since their perchlorates are much more soluble [5] they were dissolved in a slight excess of perchloric acid in acetic acid and the excess was determined by titration with sodium acetate. A similar back titration was used to analyse potassium ethyl xanthate since its decomposition, giving titratable potassium acetate, was too slow in acetic acid alone.

$$EtO{\cdot}CS_2K + Me{\cdot}CO_2H \longrightarrow EtO{\cdot}CS_2H + Me{\cdot}CO_2K$$

In most cases, a 50—60 μg. sample was used, but to ensure a reasonable titre, larger samples were taken of narcotine whose equivalent weight is much higher than that of the other substances examined.

[1] Part VIII, Belcher, Berger, and West, preceding paper.
[2] Berger, *Acta Chem. Scand.*, 1952, **6**, 1564.
[3] *Idem, ibid.*, 1954, **8**, 427.
[4] Schotte and Veibel, *ibid.*, 1953, **7**, 1357.
[5] Nadeau and Branchen, *J. Amer. Chem. Soc.*, 1935, **57**, 1363.

A blue comparison solution [1] was used for all the visual end-points except for that with lithium benzoate where the blue-green solution was used. In the potentiometric titration of lithium benzoate, the first derivative was considerably smaller than in the titration of the other compounds. The macro-titrations were preferred with visual indication of the end-point.

(b) *Titration of Amine Hydrohalides and Quaternary Ammonium Compounds.*—Whereas the salts of organic bases with oxalic acid, picric acid, etc., can readily be titrated as bases

TABLE 1.

Compound (M.W.)	Range of sample wts. (μg.)	No. of detns.	Equivalent wt. (average) Visual indicator	Potentiometric	Macro	Range of errors (%) Visual	Potentiometric
Sodium acetate trihydrate (136·1) ...	47·64—82·49	10	137·1	135·0	136·3	0·3—1·6	0·0—2·0
Sodium benzoate (144·1)	48·62—71·06	6	144·3	144·3	144·8	0·3—0·8	0·3—1·2
Lithium benzoate (128·05)	46·94—63·10	6	131·0	130·3	129·3	1·2—3·4	1·6—2·8
Ammonium succinate (152·15)	41·51—94·30	6	77·4	78·0	76·8	0·5—2·3	1·7—2·4
Potassium ethyl xanthate (160·29) *	43·66—69·65	6	158·0	158·3	160·6	1·0—3·3	1·6—2·8
S-Benzylthiuronium valerate (268·4) ...	55·54—82·49	6	267·5	271·2	268·7	0·4—3·2	1·3—3·5
S-Ethylthiuronium picrate (333·29) ...	92·85—142·3	6	335·6	334·0	333·4	2·1—2·6	0·2—2·3
Narcotine (413·41) ...	109·2—200·0	8	412·8	413·4	413·7	0·1—1·0	1·5—4·4
Atropine (289·36) ...	91·0—120·9	6	287·3	292·1	289·8	0·1—5·5	1·6—4·1
DL-α-Alanine (89·09)	47·24—72·90	10	87·9	87·8	88·9	0·2—2·3	0·5—1·8
Glycine (75·07)	45·8—73·38	8	73·9	73·8	75·3	0·7—1·5	0·0—2·7

* Back titration.

in acetic acid, those of the halogen acids are not amenable to similar treatment. For such salts two methods have been devised: (1) The solution is boiled during titration to expel the halogen acid,[6] and (2) the compound reacts with excess of mercuric acetate: [7]

$$Hg(O_2CMe)_2 + 2BH^+Cl^- \rightleftharpoons HgCl_2 + 2BH^+,MeCO_2^-$$

In the second method, the excess of mercuric acetate is undissociated and is not titrated by perchloric acid, whereas the acetate of the base behaves as a strong base. The first procedure is less satisfactory both with respect to difficulties in the manipulation of boiling glacial acetic acid and to the possibility of decomposition of the organic compound.

TABLE 2.

Compound (M.W.)	Range of sample wts. (μg.)	No. of detns.	Equivalent wt. (average) Visual indicator	Potentiometric	Macro	Range of errors (%) Visual	Potentiometric
Cocaine hydrochloride (339·8)	72·63—127·7	9	339·6	341·6	342·8	0·1—3·5	0·6—3·4
Ephedrine hydrochloride (201·7)	57·37—75·13	6	200·0	203·6	202·2	1·2—2·5	0·7—3·4
Tetramethylammonium iodide (201·1)...	49·32—67·01	6	199·2	198·4	201·2	1·6—2·4	1·0—3·2
Di-n-butylamine hydrochloride (165·7)	46·77—61·36	6	164·4	164·4	166·1	0·8—2·0	0·4—2·5
Triethylamine hydrobromide (182·1) ...	48·40—74·83	6	181·7	183·7	182·7	0·2—2·7	0·6—2·2

On the submicro-scale we examined only the mercuric acetate method. To ensure that the reagent solution was standardised under the conditions of the determinations

[6] Higuchi and Concha, *J. Amer. Pharmaceut. Assoc., Sci. Edn.*, 1951, **40**, 173; *Science*, 1951, **113**, 210.

[7] Pifer and Wollish, *Analyt. Chem.*, 1952, **24**, 300.

S-benzylthiuronium chloride (by the mercuric acetate method) was used as primary standard.[3] The normality found by this procedure differed insignificantly from that obtained against potassium hydrogen phthalate. Although previous authors [7,8] have stressed the importance of not exceeding a one-fold excess of mercuric acetate we were unable to find any necessity for this on the submicro-scale. The results obtained on 60 μg. amounts of *S*-benzylthiuronium chloride were the same with two-, six-, and even twelve-fold excesses of mercuric acetate. Accordingly an amount corresponding (on average) to a six-fold excess was used for further submicro-titrations. The purity of the mercuric acetate is important.

The blank (control) analysis increased although the same solvent was used as in the foregoing titrations and was independent of the amount of mercuric acetate. The increase in titre was found to be due to reaction of the Crystal Violet (hexamethylpararosaniline chloride) with the mercuric acetate.

The data for visual and potentiometric analyses of five compounds are given in Table 2. The accuracy and precision are of the same order as before. Three minutes' stirring time was sufficient to dissolve most of the compounds except tetramethylammonium iodide which required *ca.* 15 min. Samples larger than 50—60 μg. were used for cocaine hydrochloride because of its high equivalent weight.

In the visual titration the blue matching solution was used to help in detection of the end-point.

(c) *Determination of Primary Aromatic Amines and their Hydrohalides.*—Keen and Fritz [10] described a micro-method for the titration of 0·2—4 mg. of aromatic amines. They were able to titrate *ca.* 400 μg. of aniline dissolved in 1 ml. of acetic acid with an error of *ca.* 0·5%. We have repeated their experiment potentiometrically with 1-naphthylamine [1] and the apparatus previously described. Table 3 shows that the result is dependent on the water content of the solvent. It is apparent that acetylation occurs to the extent of 10% if the water content of the solvent is *ca.* 0·02% (Karl Fischer determination). With a water content of 0·15% as much as 5% of acetylation takes place, but with a solvent containing 0·6—1·0% of water only slight acetylation occurs.

TABLE 3. *Equivalent weights found by potentiometric titration of* ca. 400 μg. *of* 1-*naphthylamine dissolved in* 1 *ml. of glacial acetic acid containing varying amounts of water. The titration was performed about* 5 *minutes after the sample had been dissolved. Equivalent weight found by visual macro-titration:* 144·6 (*Calc.* 143·2).

Water content:	0·02%	0·15%	0·6%	1·0%
	164·2	152·0	148·8	148·6
	162·4	153·1	147·6	147·8
			147·9	147·6
			Av. 148·1	Av. 148·0

We conclude that Fritz and Keen's procedure cannot be used safely unless the water content of the glacial acetic acid is controlled. In our procedure a water content of 0·75% is employed for titration of primary aromatic amines.

In the titration of the hydrohalides of primary aromatic amines 100 μl. of 0·02M-mercuric acetate in glacial acetic acid [1] must be added. Consequently the sample is dissolved in 0·3 ml. of glacial acetic acid (1·0% H_2O) to obtain a final water concentration of 0·75%.

As it is not possible to obtain accurate visual titrations in glacial acetic acid containing more than 0·2% of water because of the indefinite nature of the colour change at the end-point and the complicated relationship between ionic strength and indicator equilibrium, these titrations were done potentiometrically. Table 4 shows that in a medium

[8] Ekeblad, *J. Pharm. Pharmacol.*, 1952, **4**, 636.
[9] Beckett and Tinley, "Titration in Non-Aqueous Solvents," B.D.H. Ltd., Poole, England, 1955.
[10] Keen and Fritz, *Analyt. Chem.*, 1952, **24**, 564.

TABLE 4.

Compound (M.W.)	Equiv. found in acetic acid containing the following percentages of water 0·02	0·15	0·75	1·5	Equiv. by macro-titration
p-Anisidine (123·1)	136·4	130·9	127·0 *	126·0 *	
	139·7	131·2	Max. error 4·4 Min. error 3·3	Max. error 4·3 Min. error 2·6	124·8
1-Naphthylamine (143·2)	—	—	148·3 ‡ Max. error 5·8 Min. error 4·2	148·7 149·4	144·6
p-Anisidine hydrochloride (159·6) ...	—	—	163·8 ‡ Max. error 6·5 Min. error 2·4	—	161·2

Average of 8 determinations *, 4 determinations †, 5 determinations ‡.

containing 0·75% of water an accuracy of 1—3% is obtainable. The higher limit of error is undoubtedly due to the slight acetylation which still occurs. This argument is consistent with our observation that amongst some fifty titrations of primary aromatic amines on the submicro-scale we have never obtained an equivalent weight less than that obtained on the macro-scale where the acetylation effect is not noticeable.[11,12]

The presence of 0·75% of water in the solvent adversely affects the nature of the potential break at the end-point. To obtain a reasonable curve it is advisable near the end-point to add the titrant in increments of 2 μl. rather than the customary 1 μl.

EXPERIMENTAL

Titration of Bases.—Reagents. 0·01N-Sodium acetate prepared by dissolution of *ca.* 1·36 g. of " AnalaR " sodium acetate trihydrate in glacial acetic acid,[1] and made up to 1 l. This solution was standardised by titrating measured portions of 0·01N-perchloric acid in acetic acid with it as described previously.[1] A blank determination was made and the above titre corrected accordingly. A normality must be calculated for potentiometric and visual-indicator titrations. Other reagents were as described previously.[1] The apparatus was as described before.[1]

Procedure (Back titration). The weighed sample (50—60 μg.) was placed in the titration vessel and 0·4 ml. of glacial acetic acid, 1 drop of Crystal Violet indicator, and a slight excess (*ca.* 5—8 μl.) of 0·01N-perchloric acid were added and the contents of the vessel were allowed to dissolve under magnetic stirring (3 min. for xanthates, 8—10 min. for amino-acids). A blank determination was made and deducted from the above titre.

Other procedures were as described previously.[1] The stirring time was extended to 8 min. for *S*-ethylthiuronium picrate.

Titration of Primary Aromatic Amines and their Halides.—Reagents. 0·02M-Mercuric acetate, prepared by dissolution of *ca.* 0·8 g. of solid in glacial acetic acid [1] and diluted to 250 ml. Mercuric acetate must conform to purity tests specified elsewhere.[9] Other reagents were as described previously.[1] Apparatus was as before.[1]

Procedure. The sample was dissolved in 0·4 ml. of glacial acetic acid (water content 0·75%) and the potentiometric titration was performed as described previously, except that the titrant was added in 2·0 μl. increments near the end-point. A blank analysis was determined on the same amount of solvent. In the titration of the hydrohalides the sample was dissolved in 0·3 ml. of glacial acetic acid (1·0% water content) and 100 μl. of 0·02M-mercuric acetate were added before titration.

For the titration of other amine hydrohalides and quaternary ammonium compounds, the sample was dissolved in 0·3 ml. of glacial acetic acid,[1] and 100 μl. of 0·02M-mercuric acetate were added.

CHEMISTRY DEPARTMENT, THE UNIVERSITY,
BIRMINGHAM 15.

[*Received, March 13th*, 1959.]

[11] Fritz, *Analyt. Chem.*, 1950, **22**, 1028.
[12] Markunas and Riddick, *ibid.*, 1951, **53**, 337.