method to the accurate determination of these amines in urine, both alone and in the presence of each other.

The procedure described for secondary amine determination is simple and is essentially trouble free. Although considerably less sensitive than the tertiary amine procedure, it can be made more sensitive by reading the final absorption in longer cells. The procedure for tertiary amine determination, on the other hand, is quite demanding. A high pH during the extraction or the introduction of small quantities of water after the extract is dried may result in a high blank or aberrant color development.

It should be pointed out that the specificity of the method largely depends on the pH during the initial extraction. If phenolic metabolites can be anticipated and if separation is desired, the pH should be high enough to prevent their extraction, although the high pH may introduce blank difficulties. Nonphenolic metabolites which retain the original amine character can also be expected to yield comparable color values. As pointed out by Umbreit (2), the presence of primary amines will result in approximately 1% error in the secondary amine determination, but such amines will not interfere in the tertiary amine determination if acetic anhydride is employed.

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Recorded Amperometric Tetraphenylborate Titrations of Amines, Quaternary Nitrogen Compounds, and Potassium Salts

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Improved efficiency of tetraphenylborate amperometric titrations by constant addition of titrant and continuous recording of current is reported.

AMOS AND SYMPSON (1) reported the first direct amperometric titration of potassium salts with sodium tetraphenylborate (TPB) using a dropping mercury electrode. Smith *et al.* (2) reported the electrochemical oxidation of TPB at the graphite electrode and the application of this reaction to the amperometric titrations of potassium salts. These procedures have been adapted (3) for the titration of amines.

It was found in such titrations of amines that it was not necessary to wait for a constant current value after each addition of titrant. Moreover, with the graphite electrode, stirring could be continuous during the addition of titrant. While current values are higher under these conditions, equivalence points could be obtained easily, providing equal time intervals were used between the addition of titrant and determination of current value.

At this time, we would like to report that the efficiency of TPB amperometric titrations can be significantly improved by the constant addition of titrant and continuous recording of current.

EXPERIMENTAL

Apparatus.—The titration vessel and graphite electrode were as previously described (3). A Leeds and Northrop 1199-31 calomel electrode, but with the potassium chloride solution replaced with a saturated sodium chloride solution, was used as the reference electrode. Standard potassium chloride solution was delivered to the titration vessel by a Sargent model C 10-ml. constant rate buret. Potential was applied and current recorded with a Leeds and Northrop model 62200 Electro-Chemograph.

Reagents.—The 0.1 *M* TPB, 0.1 *M* potassium chloride, and pH 4.6 buffer solutions were prepared as previously described (3). Standard and test solutions were prepared by direct weighings of reagent grade chemicals and amines dried to constant weight. Benzalkonium chloride was used as the commercially available solution.[1] At the suggestion of the supplier, hexadecyltrimethylammonium bromide[2] was dried to constant weight under vacuum without heat.

Procedure.—To standardize the sodium TPB solution, 4 ml. of this solution was added to 50 ml. of acetate buffer, followed by 2 ml. of 0.1 *M* standard potassium chloride solution. With the electrodes immersed, the solution was stirred for 10 min. A potential of 0.55 v. was established with the graphite being the positive electrode and the polarograph recorder started with a current sensitivity of 100 μamp. The excess TPB ion was titrated at 25 $\pm$ 0.1° with the standard potassium chloride solution being delivered from the constant rate buret. The titration was continued until a linear change in current with time was obtained. End points were determined by the intersection of the extrapolated linear portions of the current-time curves.

Titration of samples was accomplished by using the dried weighed compound or an aliquot of a known solution equivalent to 1×10^{-4} to 6×10^{-4} mole of compound. Samples were added to 50 ml. of buffer solution in the titration vessel at 25 $\pm$ 0.1°. While the solution was stirred, an excess of about 4 ml. of standardized sodium TPB

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[1] Marketed as Zephiran Chloride by Winthrop Laboratories.
[2] Marketed as Quatresin by The Upjohn Co.

TABLE I.—DETERMINATION OF COMPOUNDS

Compd.	Sample Range, mg.	Determinations, No.	Av. Recovered, %	Range Recovered, %	S.D.
Amphetamine sulfate[a]	34.3–94.5	13	99.1	4.1	±0.97
Ephedrine sulfate	30.9–107.3	13	98.3	1.4	±0.55
Acetylcholine chloride	25.6–106.1	10	99.7	4.4	±1.13
Hexadecyltrimethylammonium bromide[b]	39.0–113.1	5	100.7	3.6	±1.72
Potassium chloride[c]	15.0–22.5	9	99.3	1.3	±0.53

Compounds generously supplied by: [a] Smith Kline & French Laboratories. [b] The Upjohn Co. [c] In solution as lactated Ringer's injection U.S.P.

was added followed by 2 ml. of standard 0.1 *M* potassium chloride solution. With the electrodes immersed, the solution was stirred for 10 min. and excess sodium TPB titrated with 0.1 *M* potassium chloride, as described in the standardization procedure.

RESULTS AND DISCUSSION

The addition of a sample to excess standardized sodium TPB solution and titration of this excess was found to be useful in the previous study (3). This procedure, instead of a direct titration of the sample with sodium TPB, was retained in the present investigation. In addition, it was necessary with samples not already containing potassium salts to react a portion of this excess with a known quantity of potassium chloride before continuing the titration of the excess TPB. A period of 10 min. was found sufficient to establish the equilibrium between the amine or potassium TPB precipitate and the supernatant liquid required for reproducible results.

The electrodes were immersed in the buffer solution after the addition of sample and reagent. Stirring was maintained during the 10-min. period of TPB precipitation. This allowed a current-base line to be established rapidly when the polarizing voltage was initiated. This base line represents a maximum value which decreases to a minimum residual current with decreasing TPB ion concentration. Therefore, a large excess of TPB is not only time consuming but also may result in a change in current too large to be completely recorded. Under the experimental conditions reported here, an excess of 2 ml. of 0.1 *M* TPB gives approximately full-scale deflection at a current sensitivity of 100 μamp.

A series of titrations was used to evaluate the procedure, and the results are outlined in Table I. Amphetamine sulfate and ephedrine sulfate were selected as examples of nonquaternary amines. Amphetamine sulfate was a typical member of our series of sympathomimetic amines previously determined by amperometric titration (3). The titration of ephedrine sulfate whose TPB salt has a solubility of 6×10^{-4} *M* represented the limit of success in that study. Phenylephrine hydrochloride whose TPB salt has a solubility of 12×10^{-4} *M* was also revaluated in the present study. However, even allowing 40 min. for TPB salt precipitation at 0° resulted in only 50% recovery of known samples. These results together with those of the previous study would indicate that the present improved method would be applicable to amines in general, provided that the TPB salt formed has a solubility in the order of 6×10^{-4} *M* or less.

While the amperometric titration of quaternary compounds with TPB has not been previously reported, there would appear to be no reason why such titrations would not be successful, provided their TPB salts had the required low solubility. This is especially true since titrimetric TPB methods with end point detection based upon dye complexes with quaternary amines are well-established procedures for potassium salts (4) as well as for quaternary compounds *per se* (5). The amperometric titration of quaternary amines was successful, as indicated in Table I for acetylcholine chloride and hexadecyltrimethylammonium bromide. In addition, the titration of the mixture of benzalkonium chlorides was examined for reproducibility of results. Titration of a series of five 2-ml. samples of benzalkonium chloride solution had a standard deviation of ±0.02 ml. of potassium chloride solution used for the titration of the excess sodium TPB.

The study by Smith *et al.* (2) concerning the amperometric titration of potassium salts as well as the successful use of potassium chloride to titrate the excess sodium TPB salts in our procedures would suggest that the present method of constant addition of titrant and continuous recording of current would be of value in the titration of potassium salts. In Table I, the successful titration of potassium chloride in mixture with calcium chloride, sodium chloride, and sodium lactate in the form of lactated Ringers injection U.S.P. is reported also.

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