Coulometric Assay of Selected Medicinals Using an Arseno-Amperometric End Point Detection Technique

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The coulometric generation of bromine as a titrant was used as a basis for investigation of a coulometric method of analysis of selected medicinals. It was found that the reaction between bromine and certain medicinal agents was too slow to permit the use of a conventional amperometric end point detection technique. A residual method combining a standardized arsenite solution with amperometry was found to give excellent results. Quinidine sulfate, quinine sulfate, resorcinol, sodium secobarbital, sulfanilamide, and sulfaguanidine all gave relative standard deviations of less than 2 per cent and relative errors of less than 0.5 per cent using the arseno-amperometric end point device. The method was applied to commercial dosage forms with good results. The precision and accuracy obtained indicate this method would be of value in routine analysis.

THE ELECTROGENERATION of bromine has been used successfully in the assay of olefins and the determination of bromine numbers. Baumann and Gilbert (1) state that the generator solution used for the bromination reaction must fulfill the following three requirements. (*a*) It must be suitable for electrogeneration of bromine; (*b*) the rate of bromine addition must be greater than the rate of bromine generation; and (*c*) hydrocarbons must have moderate solubility.

Merkle and Discher (2) applied constant-current coulometric analysis to pharmaceuticals containing mercury. Electrolytically generated thioglycollic acid served as the titrant. The mercury content was determined with samples in the milligram range with an indicated deviation of less than 1%. These authors also reported a controlled-potential coulometric analysis of *N*-substituted phenothiazines (3).

Leisey and Grutsch (4) reported better than 0.5% agreement with theoretical values for olefin samples titrated with coulometrically generated bromine using an amperometric end point technique. In view of the success of these authors, an amperometric end point detection technique was used for this study.

The objective of this investigation was to utilize the coulometric generation of bromine as a titrant as a basis for a coulometric method of analysis for selected medicinals and to apply this method to the analysis of official dosage forms of these drugs where applicable. In the course of this investigation it became apparent that reproducible results, using a conventional amperometric end point technique, were not possible. It appeared this was so because the rate of generation of bromine was faster than the rate of reaction of bromine. In view of these observations the arseno-amperometric technique described in this paper was developed.

EXPERIMENTAL

Current Source.—The Sargent model IV coulometric current source was used for the generation of the titrant. The generator anode was a 1 × 1 cm. Leeds and Northrup platinum foil electrode. A platinum wire electrode, immersed in 10% sulfuric acid and separated from the titration cell by an agar salt bridge, served as the generator cathode. Figure 1 illustrates the titration assembly diagram.

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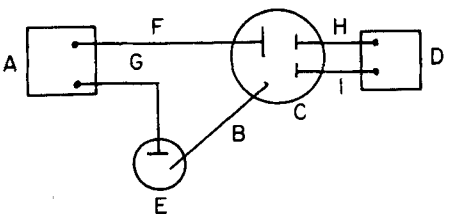

Fig. 1.—Titration assembly diagram. Key: A Sargent model IV coulometric current source; B, salt bridge (3% agar in saturated KCl); C, titration cell; D, indicator system; E, generator cathode compartment; F, generator anode; G, generator cathode; H, indicator electrode; I, indicator electrode.

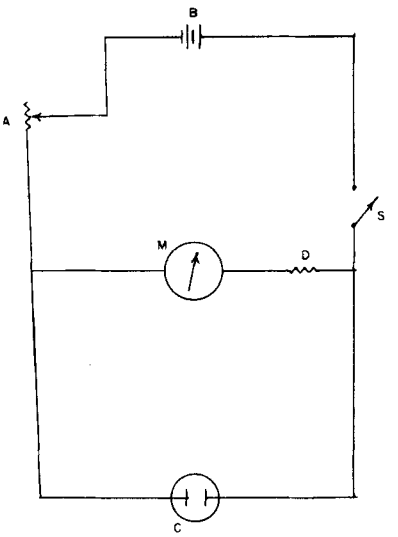

Fig. 2.—Amperometric end point detection circuit. Key: A, potentiometer, 2 w. 150 K; B, battery, 7.5 v.; C, titration cell; D, resistor, 1 w., 9 K; M, ammeter, 0–50 μamp.; S, switch, SPST.

Indicator System.—A circuit diagram of the amperometric end point technique used appears in Fig. 2. This circuit is that of Carson (5), modified by inserting a switch and by substituting a second inert platinum electrode in place of the calomel reference electrode. The cathode was a 2.5 × 3.1 cm. platinum foil electrode and a Beckman model 700 adapter indicator electrode served as the anode.

TABLE I.—RESULTS OF THE ANALYSIS OF QUINIDINE SULFATE U.S.P.

	12 Replicates
Amt. present	24.80 mg.
Range	24.69–24.92 mg.
Mean	24.77 mg.
S. D.	0.072
Rel. S. D.	0.29%
Rel. error	0.12%

TABLE II.—RESULTS OF THE ASSAY OF RESORCINOL

	10 Replicates	9 Replicates
Amt. present	2.06 mg.	5.17 mg.
Range	2.05–2.08 mg.	5.15–5.19 mg.
Mean	2.06 mg.	5.17 mg.
S.D.	0.012	0.013
Rel. S.D.	0.58%	0.25%
Rel. error	0.00%	0.00%

It should be noted that only the size of the generator anode is critical. If the current density is too great at this electrode, erratic values of current result.

Reagents.—The sodium bromide was Mallinckrodt N.F. grade and the arsenic trioxide was Mallinckrodt analytical reagent grade. The sulfuric acid used was Allied Chemical reagent grade.

General Titration Procedure.—All titrations were carried out using the Sargent model IV coulometric current source as the source of constant current. A 250-ml. beaker was used as the titration vessel. In the vessel were placed 100 ml. of generator solution (0.2 *M* NaBr, 0.05 *M* H_2SO_4) and the sample aliquot. All samples, with the exception of arsenite, gave unstable, premature, and nonreproducible end points using the conventional amperometric method previously described. Since arsenite reacts almost instantaneously with the bromine, this agent gives highly reproducible and stable end points using the amperometric technique described. It was therefore decided that a calculated excess of standardized arsenite would be added near the end point of the coulometric titration of the medicinal agents chosen for this study. The problem of deciding when to add the standardized arsenite was solved by running exploratory titrations. It was observed that excess bromine was present beyond the end point when the indicator current as observed on the meter (Fig. 2) did not increase when the current source was turned off. At this time an accurately measured excess of standardized arsenite solution was added to the titration vessel, and the titration continued until the current decreased. The final end point was taken to be the instant when the needle just started to move in a decreasing direction.

The arsenite solution was prepared by dissolving 1.2 Gm. of arsenic trioxide (accurately weighed) in 50 ml. of 10% sodium hydroxide. This solution was then made slightly acid with 10% sulfuric acid and brought to a volume of exactly 1 L. with distilled water. Ten-milliliter replicates were standardized using constant-current coulometry with an amperometric end point technique.

Quinidine Sulfate U.S.P.—The sample solution was prepared by dissolving 1 Gm. of quinidine sulfate powder (accurately weighed) in 100 ml. of 10% sulfuric acid and then adding sufficient distilled water to make a volume of exactly 1 L. Ten milliliters of this solution was taken as the sample aliquot and titrated to the predetermined excess. Exactly 5.00 ml. of arsenite solution was added, and the titration continued until the end point was reached. (Table I.)

Quinine sulfate, resorcinol, sodium secobarbital, sulfanilamide, and sulfaguanidine were all assayed by a procedure similar to the one described above.

Quinidine Sulfate Tablets, 200 mg.—All dosage forms of quinidine were extracted by the same procedure (6). The powder from 20 dosage forms was weighed accurately and quantitatively transferred to a 1-L. volumetric flask. To this flask were added 500 ml. of distilled water and 50 ml. of 10% sulfuric acid. The contents were well agitated and left to stand 12 hr. with occasional mixing. The solution was then filtered through a sintered-glass funnel and brought to volume in a 1-L. volumetric flask with distilled water. Ten-milliliter aliquots of this solution were taken for each replicate. (Tables II and III.)

Table IV compares the results of the arseno-

TABLE III.—RESULTS OF THE ASSAY OF OTHER SELECTED MEDICINALS[a]

	Sodium Secobarbital	Sulfanilamide	Sulfaguanidine
Amt. present	10.00 mg.	9.91 mg.	8.50 mg.
Range	9.85–10.02 mg.	9.90–10.00 mg.	8.46–8.56 mg.
Mean	9.97 mg.	9.94 mg.	8.50 mg.
S.D.	0.067	0.044	0.038
Rel. S.D.	0.67%	0.44%	0.45%
Rel. error	0.30%	0.30%	0.00%

[a] Five replicates.

TABLE IV.—COMPARISON OF THE RESULTS OF THE TWO METHODS OF ANALYSIS

	Powder[a]		Tablets[a]	
	C	As	C	As
Mean % labelled amt.	99.68	99.88	99.20	99.92
S.D.	0.177	0.072	2.18	0.57
Rel. S.D.	0.94%	0.29%	1.13%	0.29%
Rel. error	0.32%	0.12%	0.80%	0.08%

[a] C = conventional amperometric method; A_S = arseno-amperometric method.

amperometric and the conventional amperometric end point method of analysis of quinidine sulfate powder and quinidine sulfate tablets.

CONCLUSIONS

The data obtained in this investigation indicate that this method of analysis is well suited for the quantitative determination of medicinals whose reaction with bromine is too slow to allow the use of the conventional amperometric end point detection technique with good precision.

The precision and accuracy obtained in this investigation indicate it would be of great value in routine analysis.

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Quantitative Evaluation of Conduction Anesthetics in Albino Mice

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Conduction anesthetics were evaluated quantitatively using a test procedure based on the vocalization of mice in response to electrical stimulation.

MICE have been used to study surface anesthesia (1, 2) and in addition have been used to evaluate anesthetics injected directly into the tissues (3–5). A method using mice for the quantitative assessment of conduction anesthetics is described in this report.

EXPERIMENTAL

Male Swiss-Webster albino mice were used as experimental animals. A constant volume (0.05 ml.) of drug solution was injected intramuscularly or subcutaneously using a 27-gauge 0.5-in. needle. Ten minutes after injection, the control foot of each animal was stimulated rapidly and repeatedly until the animal vocalized and then continued to vocalize in response to 10 successive stimulations. Any animal that failed to respond when its control foot was stimulated was eliminated from the test. Approximately 5% of the animals had to be rejected for this reason. The stimulus was an electrical current (100 v. d.c.) delivered by a Grass model S-4 stimulator through bipolar silver electrodes. Immediately after a satisfactory response was established, the foot on the injected side was stimulated 5 times and any animal that failed to vocalize one or more times was classified as being locally anesthetized. Because of the tissue damage resulting from the intense stimuli, each animal was used only once. To facilitate conduction of the electrical current, the foot was moistened with 10% sodium chloride solution just prior to contact with the stimulating electrodes. In a series of preliminary experiments dose response curves were obtained by three methods.

Method A.—A 27-gauge 0.5-in. needle was inserted to its full length posterior to the heel, aimed proximally in such a way that the needle was close and parallel to the femur. The solution (0.05 ml.) was deposited in the muscles posterior to the femur.

Method B.—The needle was first inserted to its full length medially to the heel and parallel to the femur and 0.05 ml. of solution was deposited into muscles on the medial side of the femur. An equal amount of solution was injected also into muscles on the lateral side of the femur.

Method C.—This method consisted of injecting 0.05 ml. of drug solution subcutaneously, medially, and slightly above the heel, infiltrating the space formed by the large tendons posteriorly, and anteriorly by the tibia.

After evaluating the results obtained in the preliminary experiments, an additional series of dose response curves was obtained by *Method C* in order to obtain relative potency values. In each experiment of this series a dose response curve was obtained simultaneously for cocaine hydrochloride and a standard local anesthetic agent. Each relative potency determination was repeated in a separate experiment.

Twenty animals were used to determine each point on a dose response curve except for the preliminary experiment using *Method C*, in which each point was based on 10 animals. A minimum of three different drug concentrations was used to establish each dose response curve in all instances.

RESULTS

The anesthetic doses for 50% of mice (AD_{50}), calculated as described by Bliss (6) from dose response curves obtained by methods *A*, *B*, and C, are presented in Tables I, II, and III. Lambda (λ) values were calculated to serve as a basis for choosing between alternative procedures; the lower the value the greater the precision of the method. Table IV shows the relative potency values calculated (7) from a series of dose response curves obtained by *Method C*. It can be seen that the results of the relative potency determinations were reproducible, although the average λ value was not so low as expected on the basis of the preliminary work. It is interesting to note that the order and magnitude of potency for procaine hydrochloride and dibucaine hydrochloride relative to cocaine hydrochloride did

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