Evaluation of Automatic Potentiometric Titrator in Nonaqueous Titrations

By THOMAS MEDWICK and ELY KIRSCHNER

A procedure is reported for the evaluation of the behavior of an automatic recording titrator in nonaqueous titrations and is applied to an instrument composed of commercially available units. First, the behavior of the purely instrumental features was determined and the experimental errors were compared with calculated maximum anticipated relative values. The instrument's function in this case was within expectation. Then, the response of the titrator was evaluated from titration curve properties of two compounds in acetic acid and two other compounds in isopropanol. The following maximum relative errors, with instrumental errors discounted, were observed: range, 1.5 per cent; end point potential, 4.4 per cent; analytical results, 1.0 per cent; slope of the rapidly rising portion, 11.4 per cent. On the basis of satisfactory instrumental response and reasonable titration curve errors, the relatively inexpensive unit used here is found reliable for general laboratory use. The procedure described is recommended for use in evaluating any automatic potentiometric recorder.

TITRIMETRIC analysis in various nonaqueous media enjoys success and acceptance. The biannual reviews of the literature pertaining to this work attest to this fact (1). Inspection of the official compendia (2, 3) indicates that this type of determination is useful in pharmaceutical analysis. If potentiometric end point detection is required, the use of a dependable automatic recording titrator permits convenient and rapid analyses. It is possible to build a titrator from published schematics, but this involves expense and considerable labor. Several varieties of commercial automatic recording titrators are available or, if cost is a consideration, commercial components may be simply assembled. If not already available in the laboratory, the required components are a potentiometric recorder, a suitable automatic buret, and a pH meter. No matter which of these instruments is chosen, proper evaluation of the automatic recording titrator setup should be carried out to determine performance under the specific conditions to be imposed during use.

This paper reports on a detailed evaluation procedure which examines titrimetric performance in nonaqueous media where high solution resistance makes potential measurements difficult; the titrimetric behavior of two compounds in glacial acetic acid solvent and two other compounds in isopropanol is studied. Since titration behavior yields useful acid-base information as well as purely analytical data, various features of the resultant curves are measured. In addition, the known determinate errors inherent in the various measurements are estimated in an attempt to evaluate the ability of the described instrumentation to present data reproducibly. The automatic recording potentiometric titrator used is composed of commercially available components. Wilson and Munk (4) have written about a similar titrator but did not carry out an evaluation of its performance.

Received June 13, 1966, from the College of Pharmacy, Rutgers—The State University, Newark, N. J. 07104.

Accepted for publication August 1, 1966.

The authors are grateful to Miss Barbara Ann Nemeth for technical assistance. Discussions with Mr. Leonard Bailey, Mr. Stephen Baron, and Mr. Gerald Kaplan proved very useful.

This work is supported in part by a grant from the Research Council of Rutgers—The State University.

EXPERIMENTAL

Chemicals and Solutions.—All chemicals not otherwise described were reagent grade. 1,2,3-Triphenylguanidine (Eastman yellow label) was recrystallized from 95% aqueous ethanol, m.p. 145–147° uncorrected. *m*-Nitrobenzhydrazide (Eastman white label) was recrystallized from water, m.p. 152–155° uncorrected.

Benzoic acid, primary standard (J. T. Baker) 99.99% assay. *p*-Hydroxybenzoic acid (Eastman white label). Hexadecyltrimethylammonium bromide (Eastman blue label). Acetous perchloric acid, about 0.1 *N*, was prepared according to Fritz (5).

Hexadecyltrimethylammonium hydroxide, about 0.1 *M*, was prepared according to the method described by Cundiff and Markunas (6) with the exception that isopropanol was the only organic solvent used.

Apparatus.—Sargent model SR recorder, 125 mv. full scale, equipped with a 5.0-mv. range plug, a 1 in./min. chart drive motor, and wired with a switching arrangement such that it may be turned on synchronously with the constant rate buret.

Leeds and Northrup pH indicator, model 7401, equipped with a 10 or 20-ohm precision resistor (±0.05%).

Sargent model C constant-rate buret, delivering 10 ml., equipped with a Teflon stopcock and a Teflon sleeve-covered ground-glass connection, and connected to a 2-L. titrant reservoir *via* ball socket joints and glass tubing.

Magnetic stirrer, used with Teflon-coated magnetic stirring bars.

Titration vessel, for titrations requiring protection from the atmosphere, consisting of a beaker 7 cm. in height, 7.1 cm. in diameter, with a female

71/15 ground-glass joint, and a cover with a male 71/15 joint, and with four openings to admit the reference electrode, the indicating electrode, the buret tip, and a nitrogen gas tube.

For titrations in both acetic acid and isopropanol solvents, a Beckman glass electrode (40498) was used as the indicating electrode. The reference electrode used for acetic acid was a Beckman inverted sleeve calomel electrode (43462), the saturated aqueous KCl solution being replaced by 0.1 *M* acetous LiCl; for isopropanol, the same type of calomel electrode was employed but with 0.1 *M* hexadecyltrimethylammonium bromide in isopropanol replacing the saturated aqueous KCl.

Procedure.—A sample of the compound to be titrated, listed in Table I, column 1, was accurately weighed (column 2) and dissolved in 50 ml. of the proper solvent (column 3). When acetic acid solvent was used, a 400-ml. beaker was used as the titration vessel. However, when isopropanol was to be the solvent, the titration vessel was the cell previously described which provided for the use of nitrogen as an inert atmosphere. After the proper electrodes were immersed and the stirrer turned on, the solution was automatically titrated with the specified titrant (column 4) until the total volume of about 10 ml. was delivered by the buret.

The following electrical grounding arrangement was used. The negative input terminal of the recorder was grounded to its chassis and was also connected to the "ground" terminal of the pH meter. The "ground" terminal of the pH meter, the automatic constant-rate buret chassis, and the magnetic stirrer chassis were all connected to a common water pipe ground. No titration solution ground was used.

Measurement of Titration Curve Parameter.—The recorded titration curves are plots of millivoltage (ordinate) as a function of titrant volume (abscissa). For each titration curve, the following measurements as shown in Fig. 1 were made using a metric ruler: the length of chart representing the complete titration, distance X; the voltage represented by a centimeter of abscissa distance in terms of the voltage equivalent to distance Y divided by distance Y; the range, in millivolts, or distance AB; the potential at the end point, point C; the slope of the rapidly rising linear portion or the tangent of angle ADE; the end point according to the method described by Blaedel and Meloche (7). In addition, determinate errors involved in the measurement of graphical features and found in the instrumental specifications were treated according to the usual concepts of error propagation (8).

RESULTS AND DISCUSSION

An automatic recording titrator setup may be conveniently divided into two parts: (*a*) the instrumental portion composed of the automatic buret, the pH meter, and the potentiometric recorder; and (*b*) the titration cell portion containing the sample solution together with the electrodes. If the instrumental portion is found to be functioning correctly and reproducibly, then it can properly receive and represent the signal being produced by the titration cell portion. On this basis, the two sections which follow deal first with the instrumental operation and behavior, and second, discuss the entire instrument, including, of course, the titration cell portion.

Instrumental Operation and Behavior.—The components of this titrator were chosen with the aim of achieving flexibility. The recorder is one with a large chart (25 cm.), variable ranges, and variable chart speeds. However, any good potentiometric recorder may be adapted. Similarly, any good pH meter, with a recorder output, may be used. The pH meter chosen presented such advantages as stability, ease of variation of the output signal *via* precision resistors (9), and adjustability of the "zero" millivolts position. This latter advantage is extremely important when titrations are to be carried out using various solvents and different strength acidic and basic samples. The last and only component which need be obtained specifically for this titrator is the automatic constant-rate buret. The one used here was chosen since it could be cleaned easily and was adaptable to an all glass titrant delivery system.

A factor which is extremely important in obtaining instrumental stability, particularly in the titrations involving solvents of low dielectric constant, *e.g.*, acetic acid, is that of electrical grounding. If grounding is not proper then any extraneous signals will be superimposed on the desired response resulting in unsatisfactory, erratic titration curves.

Since the magnitude of a potentiometric "break" is a function of the acid or base strength of the compound being titrated, certain compounds will give rise to a smaller potentiometric break than others. In order to make the recorded titration curves as large as possible and thereby take advantage of the large recorder chart, the sensitivity of the instrument was varied. In all instances, the recorder range was kept at 5.0 mv. and the pH meter operated in the 0–1400 mv. range. However, the pH meter output was varied by using different output resistors (9). Table II, column 2, is a listing of the resistors used in each case while columns 3 and 4 show the anticipated effect of the resistor on the recorder response and instrument sensitivity. It is seen that in the study of *m*-nitrobenzhydrazide, a relatively weak base, the highest instrument sensitivity was used. The sensitivity was not increased when *p*-hydroxybenzoic acid was titrated, in spite of the weakly acidic phenol which is present, in order to titrate both acidic groups within the potential range represented by a single width of recorder chart.

It was desirable to test the reliability of the instrumental performance for every titration. The chart abscissa, in terms of centimeters of chart travelled for a complete titration, is an index of how well the automatic buret motor, the recorder chart drive motor, and the synchronous switch operate. The abscissa mean values and their standard deviation, listed in Table II, column 7, show a maximum relative standard deviation of about 0.2%. The determinate portion of this uncertainty may be ascribed to two sources. The first source, the instrumental error, arises from the automatic constant rate buret motor whose specifications list an accuracy of 0.1% in delivering the total volume (10). The recorder chart motor is not expected to contribute to the determinate error since it is activated *via* a synchronous switch and shuts off

TABLE I.—TITRATION BEHAVIOR

1	2	3	4	5	6	7	8	9
Compd.	Sample Size, meq.	Sample Solvent	Titrant	Samples, No.	Range, mv.	End point Potential, mv.	Slope, mv./ml.	Analytical Result
1,2,3-Triphenylguanidine	0.545 ± 0.009	Acetic acid	Acetous $HClO_4$	5	304, S = 4[b] (1.3%)[c]	−585, S = 7 (0.85%)	1512, S = 199 (13.2%)	N = 0.1059 S = 0.0003
m-Nitrobenzhydrazide	0.571 ± 0.009	Acetic acid	Acetous $HClO_4$	4	201, S = 6 (3.0%)	−668, S = 4 (0.30%)	306, S = 19 (6.2%)	99.8% S = 0.5
Benzoic acid	0.497 ± 0.002	Isopropanol	$HDTMA^+OH^-$ in isopropanol[a]	5	433, S = 5 (1.2%)	+286, S = 19 (5.9%)	3050, S = 457 (15.0%)	N = 0.0731 S = 0.0002
p-Hydroxybenzoic acid (as carboxylic acid)	0.322 ± 0.002	Isopropanol	$HDTMA^+OH^-$ in isopropanol	4	163.5, S = 0.7 (0.43%)	+222, S = 8 (2.7%)	314, S = 16 (5.1%)	98.0% S = 1.0
p-Hydroxybenzoic acid (as phenol)	0.322 ± 0.002	Isopropanol	$HDTMA^+OH^-$ in isopropanol	4	111, S = 2 (1.8%)	+465, S = 9 (1.5%)	183, S = 14 (7.7%)	98.5% S = 0.9

[a] $HDTMA^+OH^-$ is the abbreviation of hexadecyltrimethylammonium hydroxide. [b] In each instance, S indicates the standard deviation. [c] The figures in the parentheses are relative standard deviations.

automatically when the complete titrant volume is delivered (11). The second source of error stems from the chart measurements using a metric ruler. Distance measurements made with a metric ruler are no more accurate than about 0.02 cm. which in a distance of about 26 cm. represents 0.1% relative uncertainty. Thus, from these two sources, 0.2% total relative determinate uncertainty are calculated. This is in good agreement with the 0.2% observed uncertainty.

A second criterion for instrumental performance is the reproducibility of the ordinate, in terms of mv./cm. of chart. This axis is influenced by the reliability of the meter needle (since points on the chart are marked to indicate a particular voltage reading) as well as the ability of the recorder to reproduce the same reading. The uncertainty of the entire meter scale reading is given as 10 mv. and the visual reading error is ±2 mv. (12). Thus, in the situation where the entire scale is equivalent to 700 mv., a maximum error of ±12 mv. in 700 mv. or 1.7% relative uncertainty could be recognized. The recorder is accurate to 0.25% (13) which is added to the meter relative uncertainty to yield a total of 1.95%. Finally, the metric ruler uncertainty of 0.02 cm. in a distance of approximately 25 cm. would introduce a variation of about 0.1%. Since the ordinate is expressed as a quotient, the anticipated relative determinate error is calculated to be about 1.85%. The values presented in Table II, column 6, are seen to have a maximum relative standard deviation of about 1%. This is within the calculated maximum anticipated determinate uncertainty. It should also be mentioned that the values in column 6 are all in agreement within experimental error with the anticipated values for the instrument sensitivity noted in column 4.

Titration Behavior.—In this section the ability of the entire system to sense, transmit, and reproducibly record the course of a titration in two nonaqueous media is treated. The determinate errors attributable to instrumental and measurement factors are estimated. No determinate error is assigned to the titration cell function. The four characteristics of the titration curves are listed in Table I, columns 6–9. Some variation in the concentration dependent measurements should be expected since the sample weights (column 2) are not exactly constant. Since the dilution during titration is the same for each case, no corrections are carried out.

The range values listed in Table I, column 6, are reproducible with a maximum relative standard deviation of 3%. This value is calculated from the curve by multiplying a distance (absolute uncertainty 0.02 cm.) by the proper instrument sensitivity value, Table II, column 6. For a small distance, *e.g.*, 5 cm., a relative measurement error of 0.4% may be added to a maximum sensitivity error of 1.1% to yield a total determinate relative error of 1.5%. When the individual values presented in Table I, column 6, are examined, it is noted that three of the values are within the maximum anticipated error limits, whereas one of the remaining two is 0.3% and the second is 1.5% higher than the calculated limit. It should be noted that in the last-named case the sample size varies about 1.6%. This variation would be expected to have an effect

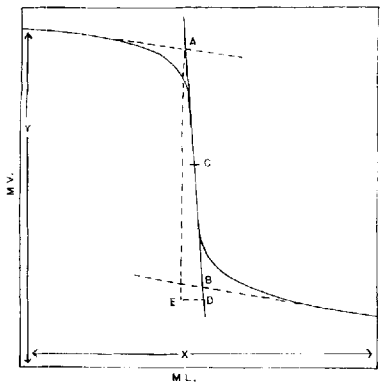

Fig. 1.—Titration curve features. Key: X, milliliters; Y, millivolts; AB, range; C, end point potential; tangent of ADE, slope.

on the range values. However, on the basis of the observed results, the titrator is able to reproduce the range value with a maximum anticipated uncertainty of 1.5% when the measurement and instrumental errors have been discounted.

The calculation of the value for the end point potential is carried out by measuring a linear distance, multiplying this distance by the recorder sensitivity, and adding the product to a reference potential on the chart. The maximum anticipated determinate error is about the same as that calculated for the range values, *viz.*, 1.5%. However, the use of a reference potential on the chart introduces an absolute uncertainty of 2 mv. in values based on this potential. In order to account for this factor, 2 mv. have been subtracted from each standard deviation (S) value reported in Table I, column 7, before the relative standard deviation is calculated. Of the 5 values reported in Table I, column 7, those for acetic acid solution are most reliable since the hydrogen-ion concentration and presumably the electrochemical potential at the end point when bases are titrated in acetic acid has been found to be independent of concentration (14). The values for 1,2,3-triphenylguanidine and *m*-nitrobenzhydrazide in acetic acid solution indicate that these potentials are reproducible within expected limits. The relative errors of the values determined in isopropanol solution are higher than the calculated limit. On the basis of the observed values, the titrator reproduces the end point potential with a maximum uncertainty of 4.4% when the measurement and instrumental errors have been discounted.

Another indication of the ability of this system to reproduce results is the analytical data which are presented in Table I, column 9. It is noted that both titrants have been standardized to values precise to about 0.3%. This is within expectations for a volumetric method. The recoveries as listed in column 9 of the other two compounds have a maximum standard deviation of 1.0%. This demonstrates that the instrument is able to yield analytical data and 1.0% maximum uncertainty.

The last criterion chosen to evaluate the behavior of the system is the slope of the rapidly rising portion of the curve. This property is also known as the sharpness index (15). The values presented in Table I, column 8, were obtained by measuring the tangent of angle ADE, Fig. 1. The calculations are carried out according to the following equation:

$$\text{tan. ADE} = \frac{(\text{AE})\ (\text{sensitivity})}{(\text{DE})}$$

The estimate of the relative errors transmitted to the final slope value was found to be a maximum of 3.6% in the case of benzoic acid. The observed relative standard deviations are larger than the estimated relative errors. On the basis of these data, the slope is reproduced with a maximum uncertainty of 11.4% (15.0–3.6) when the measurement and instrumental errors have been discounted.

The value of 11.4% which has been calculated to be the maximum uncertainty of the slope may be composed of unknown determinate as well as random errors. Blaedel and Meloche (16) discuss end point errors and particularly the random error involved in selecting the inflection point. These authors make the following statements: "In locating the inflection point, the standard error appears to be about 10% of the volume interval covered by the straight-line portion of the experimental titration curve. This figure of 10% is a rough estimate based on experience, and has no theoretical basis." The "volume interval covered by the straight-line portion" mentioned corresponds to the line DE of Fig. 1. Since DE is used in the slope calculation, its random error will be propagated according to usual concepts which dictate that the squares of the relative standard deviations

Table II.—Instrumental Performance

1 Compd.	2 Resistor at pH Meter Recorder Output, ohms ±0.05%	3 Recorder Response, Full Scale (25 cm.), mv.	4 Instrument Sensitivity Calcd., mv./cm.	5 Trials, No.	6 Instrument Sensitivity, Exptl., mv./cm.	7 Chart Travel, cm.
1,2,3-Triphenylguanidine	10	700	28.0	5	27.8, S^a = 0.3 (1.1%)[b]	26.20, S^a = 0.04 (0.15%)[b]
m-Nitrobenzhydrazide	20	350	14.0	4	13.95, S = 0.07 (0.5%)	26.16, S = 0.05 (0.19%)
Benzoic acid	10	700	28.0	5	27.9, S = 0.1 (0.4%)	26.26, S = 0.06 (0.23%)
p-Hydroxybenzoic acid	10	700	28.0	4	27.8, S = 0.2 (0.7%)	26.30, S = 0.00 (—)

[a] In each instance, S indicates the standard deviation. [b] The figures in the parentheses are relative standard deviations.

of the elements in a quotient are summed and the square root is extracted to yield the relative standard deviation of the quotient (8). When this is done with the 10% uncertainty mentioned previously, a relative random error of at least 10% is expected in the result. On this basis, only 1.4% of the original 11.4% remains unexplained. This percentage may very well be random error in AE for which no estimate is available.

During the rapidly rising portion of the curve, the sensing and recording of potentials is most difficult since the potential is changing very rapidly. Factors such as speed of recorder response, efficiency of stirring, and rate of titrant addition are all involved in this measurement. It is possible to obtain an electronic setup whereby the rate of titrant addition is slowed as a function of the rate of change of potential. This may improve the reproducibility of the slope. However, this feedback mechanism substantially increases the cost of the instrument. Commercial titrators offer this advantageous feature.

SUMMARY

1. A procedure for evaluating titration behavior of an automatic potentiometric titrator is reported.

2. The procedure consists of an instrument evaluation followed by an examination of titration curve properties. The range, end point potential, analytical results, and the slope of the rapidly rising portion of each curve are determined.

3. The maximum anticipated relative errors are calculated and compared with experimental results obtained from a relatively inexpensive titrator composed of commercially available units.

4. On the basis of satisfactory instrumental response and of reasonable titration curve errors, the titrator used here is found to be reliable for general laboratory use.

REFERENCES

(1) Streuli, C. A., *Anal. Chem.*, **36**, 363R(1964).
(2) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965.
(3) "The National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965.
(4) Wilson, A. M., and Munk, M. E., *Anal. Chem.*, **34**, 443(1962).
(5) Fritz, J. S., "Acid-Base Titrations in Nonaqueous Solvents," G. Frederick Smith Chemical Co., Columbus, Ohio, 1952, p. 13.
(6) Cundiff, R. H., and Markunas, P. C., *Anal. Chem.*, **30**, 1450(1958).
(7) Blaedel, W. J., and Meloche, V., "Elementary Quantitative Analysis: Theory and Practice," Row, Peterson & Co, White Plains, N. Y., 1963, p. 351.
(8) Benedetti-Pichler, A. A., "Essentials of Quantitative Analysis," The Ronald Press Co., New York, N. Y., 1956, p. 12.
(9) Leeds and Northrup 7401 and 7402 pH Indicators Directions, 177166, Issue 2, Leeds and Northrup Co., Philadelphia, Pa., 1962, p. 12.
(10) Sargent Catalogue 113, E. H. Sargent & Co., Chicago, Ill., 1964, p. 165.
(11) E. H. Sargent & Co., private communication.
(12) Leeds and Northrup 7401 and 7402 pH Indicators Directions, 177166, Issue 2, Leeds and Northrup Co., Philadelphia, Pa., 1962, inside cover.
(13) Sargent Catalogue 113, E. H. Sargent & Co., Chicago, Ill., 1964, p. 838.
(14) Kolthoff, I. M., and Bruckenstein, S., *J. Am. Chem. Soc.*, **79**, 1(1957).
(15) Butler, J. N., "Ionic Equilibrium, A Mathematical Approach," Addison-Wesley Publishing Co., Inc., Reading, Mass., 1964, p. 158.
(16) Blaedel, W. J., and Meloche, V., "Elementary Quantitative Analysis: Theory and Practice," Row, Peterson & Co., White Plains, N. Y., 1963, p. 356.

Notes

Isolation of Aurantiacin from *Hydnellum caeruleum*

By M. L. MONTFORT*, V. E. TYLER, JR., and L. R. BRADY

Two procedures were employed for the isolation of aurantiacin from an ether extract of the basidiomycete *Hydnellum caeruleum*. Identification of the compound was based on its melting point, spectral properties (infrared, visible, and ultraviolet), and alkaline degradation to atromentin and benzoic acid.

POLYPORIC ACID (2,5-dihydroxy-3,6-diphenyl-1,4-benzoquinone) was shown by Burton and Cain to be the constituent responsible for the antitumor properties of the lichen *Sticta orygmaea* Ach. (*S. coronata* Müll. Arg.) (1). Subsequently, the antitumor activity of some related synthetic diaryldihydroxyquinones (2, 3) has been recorded. Recently, atromentin [2,5-dihydroxy-3,6-bis(4′-hydroxyphenyl)-1,4-benzoquinone] was identified as the anticoagulant principle in *Hydnellum diabolus* Banker (4, 5). Thus, compounds of this type have therapeutic potential, and their occurrence in nature has medicinal interest.

No diphenylbenzoquinone compound is known to occur in spermatophytes, but aurantiacin (atromentin-2,5-dibenzoate) (6), leucomelone (3′-hydroxyatromentin) (7), muscarufin [2,5-di(2′-carboxyphenyl) - 3 - hydroxy - 6 - (4′ - carboxy - 1,3 - buta-

Received April 11, 1966, from the Drug Plant Laboratory, College of Pharmacy, University of Washington, Seattle 98105.
Accepted for publication August 26, 1966.
Presented to the Pharmacognosy and Natural Products Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.
This investigation was supported in part by grant GM 07515-06 from the U. S. Public Health Service, Bethesda, Md.
* Winner of the 1966 Kilmer Prize.