Microcomputer Automation of the Conductometric Titration of Hop Alpha-Acids[1]

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ABSTRACT

A general-purpose microcomputer was used to automate the determination of lead conductance value by titration. The microcumputer, presented with an extracted sample in a beaker, controls the addition of titrant and the taking of data, performs all calculations, and finally prints the corrected result along with a record of the titration. A number of operating parameters were studied in order to optimize the titration. The computer system performs the analysis more rapidly than a human operator and permits much more efficient use of the analyst's time.

Key words: *Computer control, Conductance value, Interface, Linear regression, Plotting, Program*

An improved system for determining α-acids in hops by using a digital conductivity meter and a programmable calculator was described previously (5). The numerical approach used was particularly well suited for complete computer automation. A general purpose microcomputer was obtained and interfaced to the necessary intruments. A program was developed to perform the entire titration procedure—controlling the addition of titrant, making conductivity observations, performing all calculations, printing the results, and plotting the curve. This article describes the system and the results of studies made to determine optimum operating parameters.

[1]Presented at the 47th Annual Meeting, Miami, FL, May 1981.
[2]A listing of this program is available from the author on request.

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EXPERIMENTAL

The analytical approach was exactly the same as described previously (5). Briefly, samples of hops were ground and 5.0 g were weighed into a flask. Toluene was added and the flask was shaken. The toluene extract was clarified by centrifugation, and a 10-ml aliquot of it was transferred to a 100-ml beaker with 50 ml of methanol and 0.5 ml of pyridine. Fixed increments of titrant were added, and an observation was made after each. The inflection point of the curve was detected, and a few points near it were discarded. Linear regression was used to fit a straight line to the remaining points on each side of the inflection point. The intercept point of the two lines defined the conductance value endpoint. From the weight of hops used, their moisture content, and the titrant strength, the α-acids content was calculated. The difference in the procedure described here is that after the sample and solvents (methanol and pyridine) have been added to the beaker, all remaining operations are controlled by the microcomputer.

Experimental Apparatus

The equipment used in this procedure is depicted in Fig. 1. Most of the apparatus was quite conventional. The X-Y recorder was a Houston Instruments model 2000. The pipetter was a Hamilton MICROLAB® P (Hamilton Co., Reno, NV) used in the repeating dispenser mode (3). The stirrer was a Talboys model 101 variable speed unit (American Scientific Products); the stirring speed was 15–20 on a 0–100 scale. The stirring rod was a straight glass tube sealed at the end.

The computer was a general-purpose type, model C2-8P, manufactured by Ohio Scientific, Inc., (Aurora, OH). This is based on a 6502 microprocessor and employs an 8K BASIC-in-ROM (*r*ead *o*nly *m*emory) operating system. In addition to the standard C2-8P configuration, a model 430B Super I/O board and 4K of additional volatile (RAM = *r*andom *a*ccess *m*emory) memory, for a total of 8K RAM, were installed. The 430B board contains eight input ports, eight output ports, an eight-channel multiplexer, an analog-to-digital (A/D) converter with eight-bit resolution (1 in 256), two eight-bit digital-to-analog converters, and a universal asynchronous receiver transmitter. The keyboard is part of the standard C2-8P. A video monitor (Leedex Video 100) and the keyboard serve as the primary methods of communication with the computer. A conventional audio cassette recorder (General Electric) was used for loading and saving programs on audio tape cassettes. A 300-baud teleprinter terminal (General Electric Terminet 30) produced printed ("hardcopy") output.

One of the computer output ports was used to trigger the pipetter to dispense a unit of titrant. A simple circuit (Fig. 2), previously described (4), was used to produce a DC voltage proportional to the solution conductivity. To protect the computer from possible damage due to the development of faults in the circuit, and to prevent a ground loop condition, an isolation amplifier (Analog Devices 288J with 947 driver) was used to obtain electronic isolation (Fig. 3). The isolation amplifier also adjusted the voltage range of the input signal, which was useful in deriving maximum benefit from the fairly limited resolution (eight-bit) of the A/D converter. The signal was fed through the multiplexer, although in this application (where only one analog input channel was used) this would not be required. The A/D converter very rapidly produces a digital number proportional to the applied voltage. The result was observed by the computer and stored in memory for later recall and computation. After all the data points were taken, the calculations were performed and the results were printed on the teleprinter. The points were plotted, when this was desired, with the X-Y recorder using the two digital-to-analog converters to produce voltages proportional to the computer's scaled output. These voltages drive the pen-positioning servomotors in the X and Y directions. An output port was used to lift and then drop the pen for movement between drawing operations.

All of the above operations were controlled by a computer program written in BASIC.[2] The flow chart of this program is shown in Fig. 4. In the initial setup phase, the operator is asked for basic information common to all samples, such as the date, titrant strength, and titrant volume increment. For each sample, the sample weight and moisture content are supplied. For each item, the computer prints a question on the monitor and the operator responds by typing a reply on the keyboard. The first operation in the data collection phase is to make an observation and store it. Each such measurement can be done either by reading the A/D converter once or by reading it a number of times and averaging the results. The voltage result is transformed to obtain a number

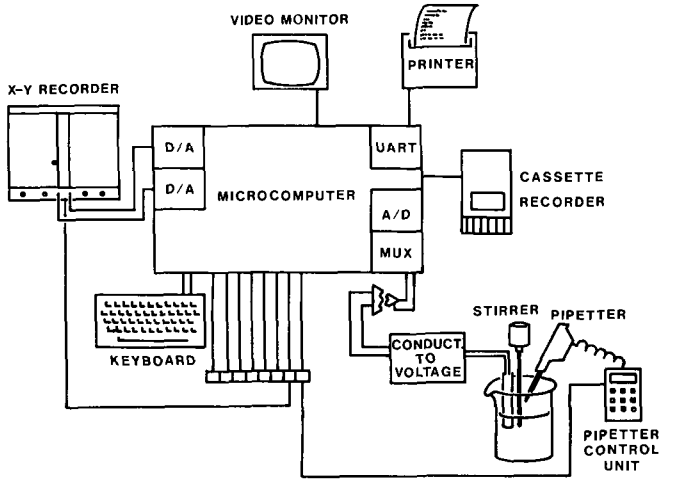

Fig. 1. Microcomputer titration system. **D/A** = Digital-to-analog converter, **A/D** = analog-to-digital converter, **UART** = universal asynchronous receiver transmitter, **MUX** = multiplexer.

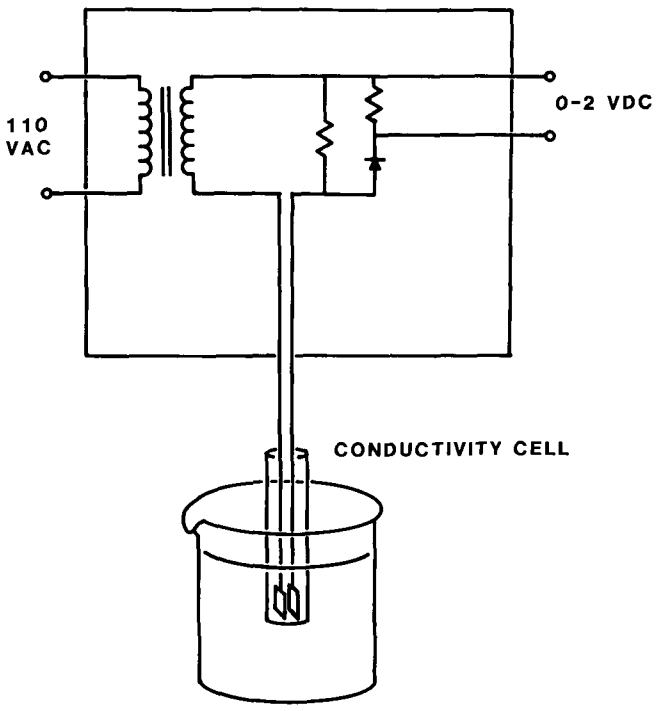

Fig. 2. The conductivity-to-voltage converter.

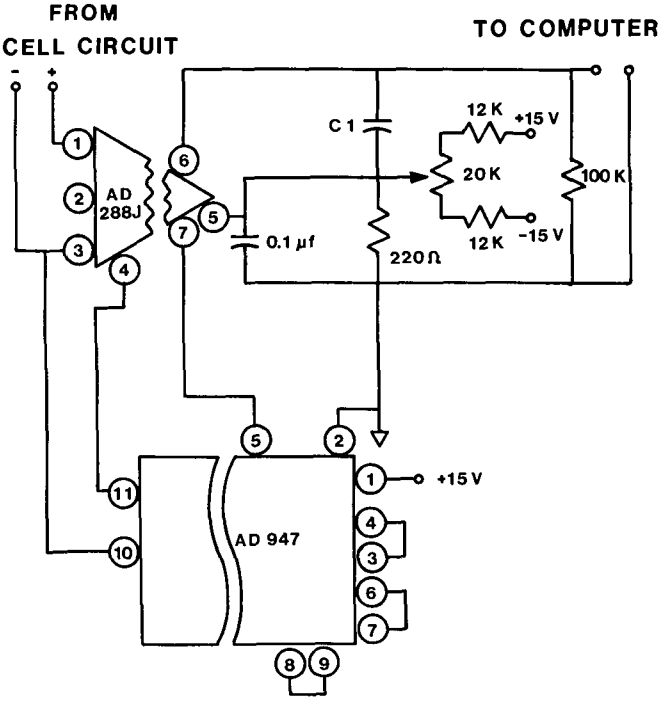

Fig. 3. Interface between the conductivity-to-voltage converter and the computer analog-to-digital converter; **C1** = capacitor.

linearly related to conductivity, as previously described (5). The program then enters a loop, in which a drop of titrant is added, time is allowed for mixing to take place, and an observation is made. After each observation, the cycle is repeated until the last point taken exceeds the conductivity of the first observation. The calculations are straightforward and have been described previously (5). The results are then printed by the teleprinter and plotted on the screen of the monitor. The titration curve is also graphed by the X-Y recorder, if this option was selected.

RESULTS AND DISCUSSION

The approach was simply to automate the manual procedure and then consider refinements later if appropriate or necessary. The conductivity-to-voltage converter box had been previously used with a digital voltmeter to perform the titration, and this had produced stable indications. Much filtering of the signal did not seem likely to be needed, so a 1-μF capacitor was installed at C1 in Fig. 3. A sample was placed in the beaker and 100 successive measurements were made rapidly with a BASIC program. The observations were plotted and showed wide but systematic variation. This appeared to be caused by the nature of the signal derived from the circuit (Fig. 2). An AC voltage at 60 Hz was applied across the cell (Fig. 5A). The diode clipped the voltage in one leg so that it appeared as shown in Fig. 5B.

The results observed by the microcomputer followed this pattern, indicating that the 1-μF capacitor provided virtually no smoothing of the waveform. Capacitor values of 20, 100, 250, and 500 μF were then substituted for the 1-μF capacitor (Fig. 6). In each case the variation became smaller until at 500 μF it was quite small; this value capacitor was used in all further testing because it resulted in a more representative value for a single observation and did not slow the system response appreciably. The speed of observation could be assessed by counting the peaks when smaller capacitor values were used. With the smallest capacitor, 24 discernible peaks resulted in the time necessary to make 100 observations. Because 60 peaks should be produced in a second (60 Hz), this means that 24/60 or 0.4 sec elapsed during the observations.

Establishing the Time Required for Mixing

The initial work was done with a magnetic stirrer. After the addition of a drop of titrant, complete mixing was needed, and the necessary time delay had to be established. When 100 points were collected immediately after an addition, almost no change was evident. A time delay between observations was then provided by programing the computer to count to 10 after each sampling of the A/D converter. This time delay was sufficiently long to show a lag phase before a change was sensed, followed by a rapid shift that gradually slowed to reach a plateau (Fig. 7A).

Another approach, judged to be simpler, was then tried. After the addition of a drop of titrant, a time delay was programed to allow mixing to occur. As many observations as desired were then made as rapidly as possible. Various time delays were tried by causing the computer to count to 400 (Fig. 7B), 800, 1,600, 2,400 (Fig. 7C), 3,200, and 4,000. Counting to 4,000 corresponded to about 13 or 14 sec. This time is highly likely to be a function of the stirring equipment used; stirring bars of larger volume displacement (a "flea" type, 3 mm diameter × 10 mm length, was used here) or higher stirring speeds would probably require less mixing time. At any rate, the standard delay was chosen as the time required for the computer to count to 3,200. Later a top stirrer was substituted for the magnetic stirrer, which made changing beakers much simpler and also produced more rapid mixing.

The results of a typical titration are shown in Fig. 8. The plot of a titration curve produced by the X-Y recorder is shown in Fig. 9.

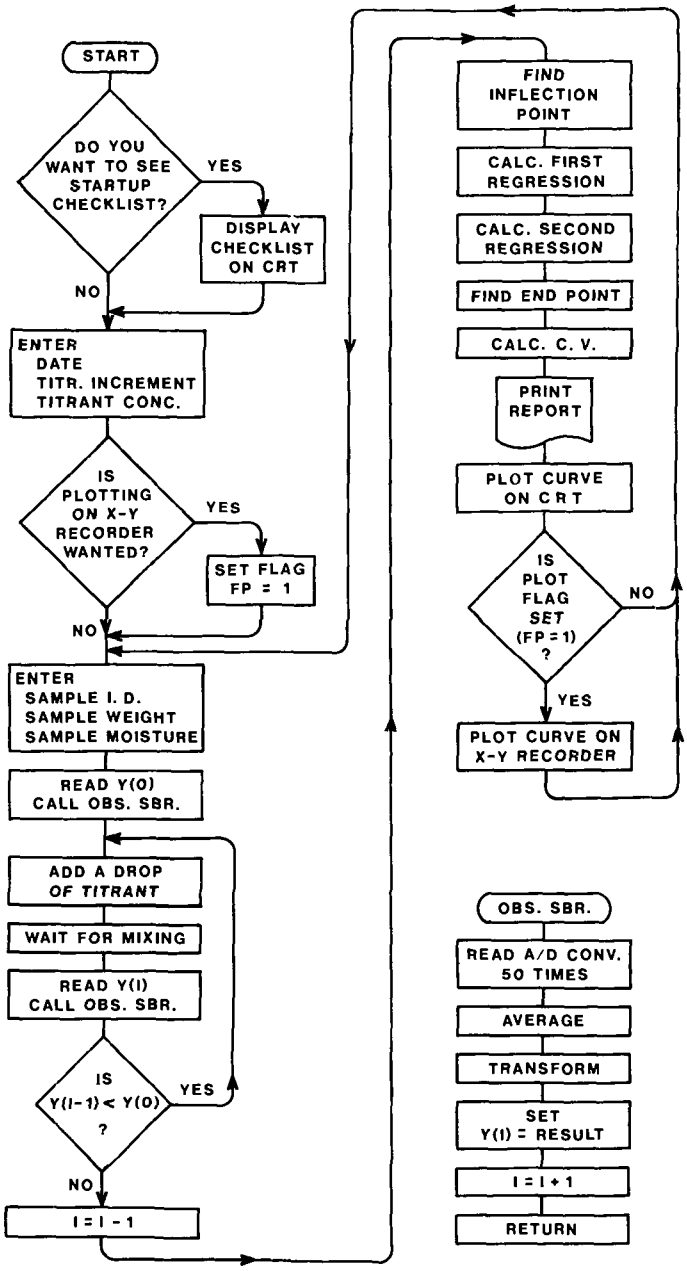

Fig. 4. Flow chart of the BASIC computer program.

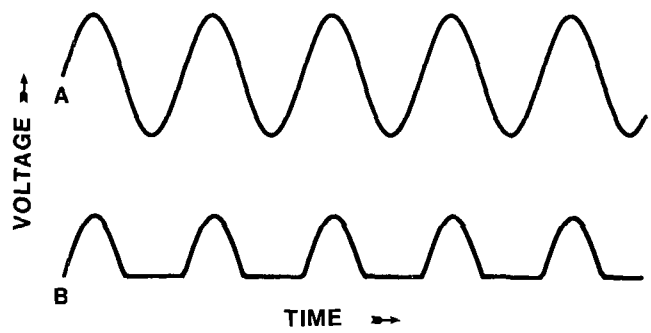

Fig. 5. A, AC voltage impressed across the cell; B, output waveform from the conductivity-to-voltage converter.

In this case, after each drop of titrant was added, several observations were made in rapid succession and averaged to obtain one data point. This cost very little time, especially compared with the time spent waiting for mixing to occur. Averaging points is beneficial because it reduces the influence of noise, such as voltage transients from the power line. In cases like this, in which a low-resolution A/D converter is used, the smallest voltage change measurable with a single point is fairly large, as shown, for example, in Fig. 7A. The A/D has a resolution of eight bits, or 2^8, which is one part in 256. Its range is from −0.5V to +0.5V, or 1.0 VDC. The smallest change that can be indicated is therefore 1.0 V/256 = 3.9 mV. With a somewhat wandering signal (such as we have here due to the AC component), a much better estimate of the actual voltage is obtained by averaging many points. This statistically improves the resolution of the A/D converter.

Effect of Averaging Various Numbers of Observations per Data Point

An experiment was done to assess the benefit of averaging various numbers of observations to obtain each data point. The computer program was modified so that it would list the result for the first observations after each titrant addition as well as the averages of the first 2, 5, 10, 20, 50, and 100 data points. Five aliquots of the same sample were titrated, with the results shown in Table I. As more observations were averaged, the results tended to change less. This was clearly apparent in the coefficient of variation figures. Conductance value is reported to the nearest 0.1% in the official ASBC method (1). When 50 observations per data point were used, the results obtained with each sample were within 0.1% of the mean conductance value. The standard procedure chosen was the averaging of 50 observations per data point; this requires about 0.2 sec.

Effect of Titrant Volume Increment

Obtaining a better estimate of the lines and therefore of the line formulas in the titration appeared possible by using smaller titrant increments and therefore taking more data points. This would require more time, primarily waiting for mixing, because of the greater number of titrant additions per determination and it would raise the problem of reduced pipetter accuracy at small delivery volumes. The MICROLAB® P has a dramatic decrease in the accuracy of the volume delivered when each increment is less than about 2% of the syringe volume (Fig. 10). In this case a 5,000-µl syringe was used, so significant error would be expected with increments near 100 µl (2%). A series of titrations was performed with each of several increments (Table II). The determinations made with 100-µl increments had a large coefficient of variation compared with those of the other increment sizes. This is presumably due to the inaccuracy of the delivered volume. Examination of the plots obtained with 100-µl increments showed some obvious deviations from a straight line. The 200- and 300-µl increment sizes appeared approximately equal in precision of results. An increase in titrant volume above this level led to a somewhat larger coefficient of variation (Fig. 11). This would be expected, because the estimates of the line formulas become based on fewer and fewer points and thus are less precise. Because 200- and 300-µl increments gave similar results, 300 µl was chosen for the standard condition because it takes less time (roughly 66% as much as with 200-µl increments, because most of the time is spent waiting for mixing to be complete).

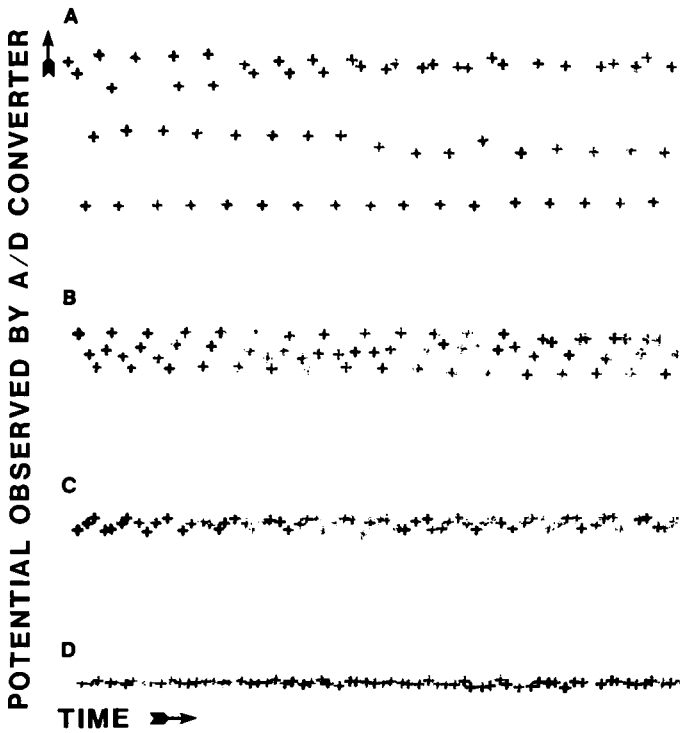

Fig. 6. Effect of various capacitor values for C1 in Fig. 3. **A**, 20 µF; **B**, 100 µF; **C**, 250 µF; **D**, 500 µF.

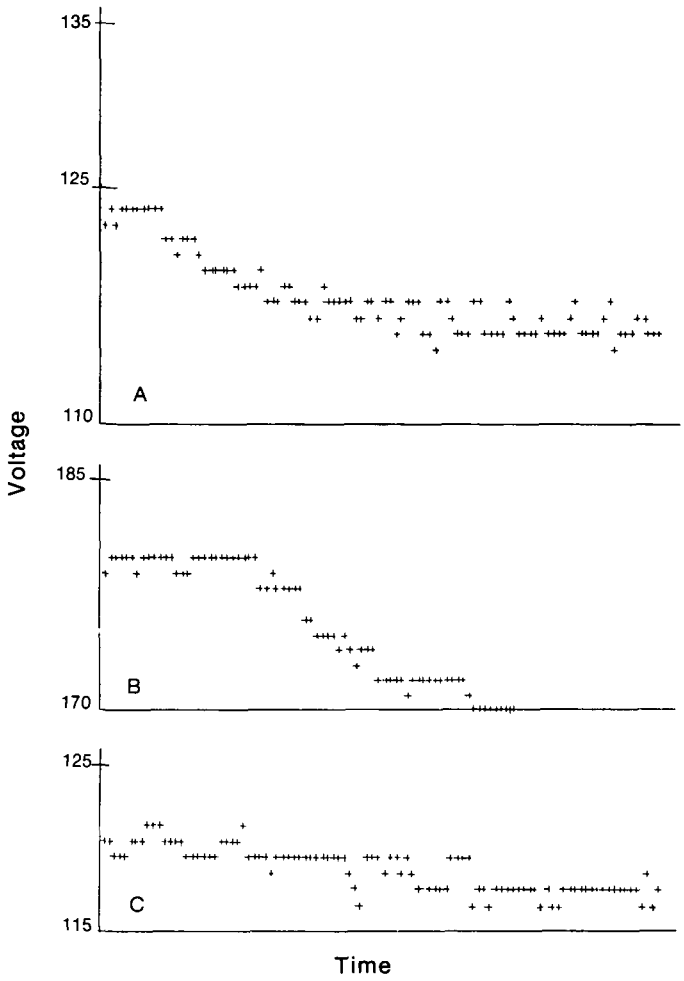

Fig. 7. Analog-to-digital converter observations of voltage made after addition of one increment of titrant: **A**, 100 observations with a 10-count delay after each; **B**, 100 observations made after a 400-count delay; **C**, 100 observations made after a 2,400-count delay.

Comparison of Methods

The results obtained with the standard microcomputer procedure were compared with those of the previously described digital meter (5). A number of hop samples were ground and extracted, and aliquots of each were titrated with the microcomputer and manually using the digital meter. Table III and Fig. 12 show the results. A t-value based on the differences (2) was calculated. This test failed to indicate a difference between the results of the two methods at the 95% confidence level; thus the methods appear to give comparable results.

A number of samples were determined in duplicate with the computer; these results are given in Table IV. A pooled coefficient of variation (2) was calculated. At 1.96, this was very similar to the result obtained in the same way (5) with the digital meter (2.02) and significantly lower (95% probability based on the F-ratio of the variances) than the result found with a conductivity bridge (3.92).

Using 300-μl increments of titrant, the times required for titrations by the three methods were determined (Table V). For a single determination, the computer approach is only slightly faster than the digital conductivity meter and programmable calculator if plotting on the X-Y recorder is done. The computer is much more productive in use than the other methods, however, because the time during the titration can be used to prepare the next sample and the time during the printing and plotting (after the titration itself is complete) can be used to clean the electrodes. In the other methods, this time is not available; the operator must use it to copy down the numbers in his notebook and later to key them into the calculator. In fact, with 300-μl increments, the computer's appetite for samples is faster than a skilled operator's ability to prepare them.

Considering ways in which to increase the speed of the determination is nonetheless interesting. This is entirely limited by the time required for mixing between the addition of a drop of titrant and the stabilization of the conductivity. As noted earlier, greater speed can be achieved through faster mixing. Yet another approach could be considered, however, which would make use of the speed of the computer. This would be to take data points

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HOP ALPHA ACIDS CONDUCTOMETRIC TITRATION

        SAMPLE:  STEINER 969 LB6
        ANALYSIS DATE:  9/25/80

        MOISTURE:              9.4 %
        SAMPLE WEIGHT:   4.999 GMS.

        EQUIV. PT.:   2023.69  UL
        ALPHA ACIDS (AS IS):   8.0601 %
        ALPHA ACIDS (DRY):   8.89635 %

INTERCEPTS:   2.28312          .234461
SLOPES:  -41.7917              49.3188
COR. COEFS.:  -.991925         .99823

   UL TITRANT    A/D RESULT    TRANSFORMED

      0            182.3         2.3462
     300           176.3         2.10633
     600           173.18        1.9947
     900           167.78        1.81935
    1200           164.24        1.71512
    1500           159.76        1.59378
    1800           155.2         1.48092
    2100           153.94        1.45144
    2400           158.02        1.54952
    2700           164.94        1.73511
    3000           169.18        1.86281
    3300           173.8         2.01624
    3600           179.34        2.22341
    3900           183           2.37662
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Fig. 8. Teleprinter output from a titration.

TABLE I
Calculated Conductometric Value (dry wt. basis) Results (in %) for Five Aliquots of a Single Sample

No. of A/D[a] Observations Averaged per Data Point	Aliquot No. 1	2	3	4	5	Mean	Coefficient of Variation
1	11.01	10.95	11.73	11.70	12.02	11.48	4.14
2	11.05	11.07	11.81	11.56	11.40	11.38	2.86
5	11.05	11.08	11.69	11.42	11.04	11.26	2.57
10	11.09	11.28	11.61	11.37	11.13	11.30	1.85
20	11.19	11.26	11.52	11.33	11.12	11.28	1.36
50	11.10	11.26	11.40	11.28	11.10	11.23	1.14
100	11.11	11.22	11.40	11.27	11.07	11.21	1.17

[a] Analog-to-digital converter.

TABLE II
Effect of the Titrant Increment Volume on Precision

Volume Increment (μl)	Result for Replicate Samples (% α-Acids) 1	2	3	4	5	Coefficient of Variation
100	4.93	5.24	5.36	5.63	6.01	7.5
200	6.24	6.09	6.03	5.97	6.03	1.7
300	6.49	6.26	6.25	6.17	6.33	1.9
400	6.85	6.27	6.42	6.67	6.66	3.5
500	5.95	5.92	5.70	5.94	6.03	2.1

TABLE III
Comparison of Conductometric Value (C.V.) Results (% dry weight) on Aliquots of the Same Extracts[a]

Sample	C.V. Given by Digital Meter	Microcomputer	Difference
1	5.2	4.5	+0.7
2	5.9	6.1	−0.2
3	7.0	7.6	−0.6
4	4.2	4.5	−0.3
5	4.2	4.4	−0.2
6	3.1	3.1	0.0
7	3.2	3.1	+0.1
8	4.2	4.3	−0.1
9	4.2	4.3	−0.1
10	3.3	3.5	−0.2
11	3.3	3.6	−0.3
12	7.3	7.3	0.0
13	7.3	7.6	−0.3
14	5.7	6.3	−0.6
15	2.9	2.7	+0.2
16	6.6	6.5	+0.1
17	7.1	7.5	−0.4
18	5.2	5.1	+0.1

[a] t test: mean difference $= \bar{D} = 0.12$, standard deviation of the difference $= S_D = 0.307$, $t = \bar{D}/(S_D/\sqrt{n}) = -0.12/(0.307/\sqrt{18}) = -1.66$; $t_{0.05}$ with 17 degrees of freedom $= 2.11$.

beginning shortly after the titrant addition and before a steady state had occurred. These points could then be transformed and operated on by the same linear regression routine used for fitting the straight lines. In this way, prediction of the value at which the conductivity will plateau should be possible without actually waiting for it to occur. By taking a large number of data points, the same degree of statistical noise filtering should result. The next increment of titrant could then be added. However, because the limiting factor is currently sample preparation by the analyst, even when a repeating dispenser is used for methanol and an automatic pipet for pyridine, investing time in increasing the titration speed further does not appear worthwhile.

An attempt was made to increase the convenience of the

TABLE IV
Duplicate Conductometric Value (C.V.) Determinations with Computer[a]

Sample	C.V. (% dry weight) 1	2	Mean	D^2
1	4.48	4.44	4.46	0.0016
2	3.11	3.06	3.09	0.0025
3	4.28	4.32	4.30	0.0016
4	3.50	3.58	3.54	0.0064
5	7.25	7.59	7.42	0.1156
6	6.51	6.93	6.72	0.1764
7	8.30	8.23	8.27	0.0049
8	8.24	8.35	8.30	0.0121
9	11.10	11.26	11.18	0.0256
10	11.40	11.28	11.34	0.0144
Total			68.62	0.3611
Mean			6.86	

[a] Pooled variance $= S^2 = \frac{\Sigma D_i^2}{2 \cdot g} = \frac{0.3611}{2(10)} = 0.0181$, where D is the difference between duplicate observations and g is the number of sets of observations. Standard deviation $= S = \sqrt{S^2} = \sqrt{0.0181} = 0.134$. Pooled coefficient of variation $= \frac{0.134}{6.86} \times 100 = 1.96$.

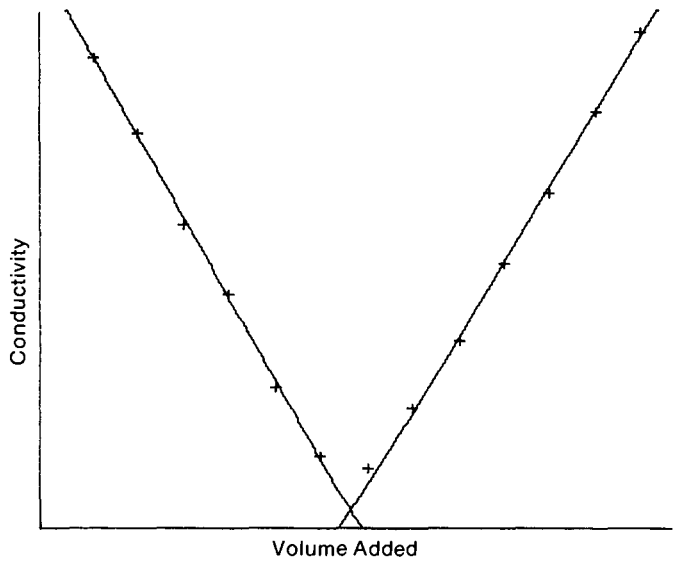

Fig. 9. X-Y plotter output from a titration.

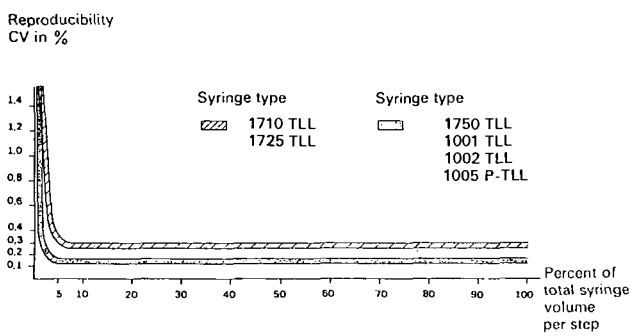

Fig. 10. Accuracy of delivered volume as a function of syringe volume (used with permission from the Hamilton MICROLAB® P Manual [3]). CV = coefficient of variation.

TABLE V
Comparison of Time Required (min) for Different Implementations of Hop α-Acids Conductometric Titration[a]

	Conductometric Bridge and Calculator	Digital Conductance Box and Programmable Calculator	Computer
Titration (and notebook entry if necessary)	6–10	3.2–5	2
Calculation and printing	4–5	2	1
Plotting	(none plotted)	...[b]	(2)[c]
Total	10–15	5.2–7	3(5)

[a] In all cases, 300-μl increments of titrant were used.
[b] Plotting time is included in the 2 min for calculation and printing.
[c] Plotting on the terminal screen is always done and requires a few seconds; plotting on the X-Y recorder is optional and takes 2 min.

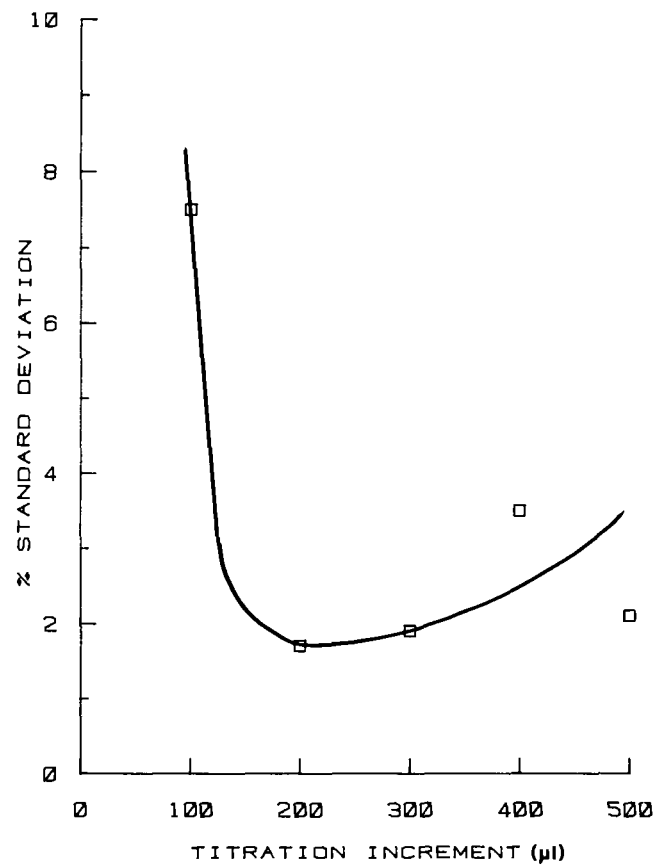

Fig. 11. Precision of results for replicate determinations as a function of titration increment volume.

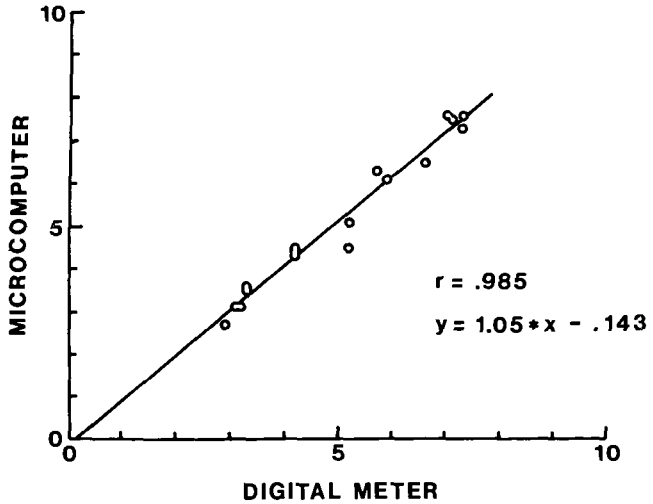

Fig. 12. Comparison of results (% dry weight) obtained when aliquots of the same sample extracts were titrated with the digital meter and the microcomputer.

determination. A check valve assembly was obtained for the MICROLAB P that permits automatic refilling of the pipetter. When the syringe is empty, the next pulse from the computer causes it to fully retract the plunger. The check valve assembly is designed to dispense titrant normally but to close off the tip and allow refilling from a titrant reservoir during a backward stroke of the syringe plunger. The computer program was modified to accommodate the refilling operation and the system appeared to work well. When a comparison of results similar to Fig. 12 was made, however, the slope was found to be near 1.2. The difficulty arose from a swelling of the check valve material in the methanolic titrant. This led to poor sealing and consequent delivery of less than the desired volume of titrant in each drop. At this point, the original procedure of refilling the pipetter after each titration was resumed. Interfacing a digital burette to the microcomputer presumably would solve this problem.

CONCLUSIONS

A microcomputer was successfully interfaced to a number of pieces of equipment in order to perform the conductometric value titration. The resulting apparatus gave results that were very comparable with those of other methods ($r = 0.985$) and at least as precise (coefficient of variation = 1.96). The time required for a typical determination was cut in half over the previous procedure if plotting on the X-Y recorder was omitted, and the analyst was able to make much more efficient use of time.

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