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COMPUTER ESTIMATION OF DISSOCIATION CONSTANTS FROM SPECTROPHOTOMETRIC MEASUREMENTS:

Part 2. A COMPARISON OF TWO TITRATION PROCEDURES OF A vs pH CURVES MONITORING

By

Milan Meloun

Chemistry Department, College of Science, University of Baghdad, Baghdad-IRAQ.

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تم تقييم التصحيح الضوئي وقورنت لتعطي نتائج دقيقة وحساسه لقياس الرقم الهيدروجيني المستحصله من الحامض المتعدد القاعده وملائمه لعمليات البرمجه الحسابيه وقد اعتبرت كلا الطريقتين سريعه ودقيقه وقد استعمل لهذا الغرض مقارنه احصائيه لثوابت الخلل pK_a و معاملات الامتصاص الجزيئي الغرامي لصيغة Methyl Orange بواسطة البرنامج DCLET .

Two photometric titration procedures are introduced, tested and compared to give accurate and precise A-pH data of protonation equilibria of polybasic acid H_JL to be suitable for further computer processing. Both techniques are considered to be fast, efficient and precise enough, allowing large sets of

data to accumulate and safely apply computer-aided multiparametric curve fitting methods. Statistical comparison of dissociation constant pK_a *and molar absorption coefficients* ε_L, ε_{HL} *of Methyl Orange being evaluated by DCLET program proves, a conclusion that both titration procedures work with the same precision and lead to the same, accurate values of* pK_a, *3.37* $\pm$ *0.01, by internal titration in the cuvette, and 3.36* $\pm$ *0.01 by external titration outside the cuvette.*

INTRODUCTION

Advances of various applications of computers in instrumental chemistry have stimulated some new approaches to traditional experimental technique. The experimental approaches involved are based on discontinuous measurements on restricted series of solution. Such discontinuous measurements or spectrophotometric titrations may be performed very simply but laboriously with ordinary cuvettes by a measurement of absorbance of solutions containing a constant total concentration of reacting species with a series of buffers at constant ionic strength. Computer application suffers form the obvious disadvantage that only a very limited data set is used for computations which are essentially based on the statistical data processing requiring a larger amount of data.

A promising solution is offered by combining microdosing techniques of reactants with the simultaneous acquisition of data from the reaction medium[1]. Two titration techniques of a measurement of an absorbance vs pH curves have been developed on the basis of many years experience in the field of photometric titrations[1].

This paper brings a comparison of two different titration procedures and statistical analysis of an influence of many factors on the value of dissociation constant and molar absorption coefficients of variously protonated forms of acid-base equilibrium.

THEORETICAL

Evaluation of the A vs pH Curve

Evaluation of the A vs pH curve by the nonlinear regression program DCLET was described elsewhere[2,3,4].

Tests for the Equality of Two Means and Two Variances

The t-distribution can be used to test a hypothesis about the difference between the means of two normal populations if the variances of the populations are equal[5,6]. More specifically, if $\bar{x}_1$ is the mean of a sample of size n_1 from a normal population with mean and variance σ^2, and if $\bar{x}_2$ is the mean of a sample of size n_2 from a normal population with mean $_2$ and variance σ^2, the random statistic variable (t_{exp}).

$$t_{exp} = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{s^2_{pool}\left(\frac{1}{n_1} + \frac{2}{n_2}\right.}} \quad \ldots\ldots\ldots (1)$$

where

$$s^2_{pool} = \frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1 + n_2 - 2}, s_j^2 = \frac{\sum_{i=1}^{n_j} x_{ji}^2 - n_j \bar{x}j}{n_j - 1} \quad \ldots\ldots (2)$$

has t distribution with (n_1+n_2-2) degrees of freedom. The hypothesis that is tested is that the means of two normal populations are equal, $H_o: \mu_1 = \mu_2$, against $H_1: \mu_1 = \mu_2$, which is the smae as testing that the difference between the two means is zero.

Procedure of Testing the Difference of Two Means

1. Formulate the hull and alternative hypothesis:

   $H_o: \mu_1 - \mu_2 = 0$ versus $H_1: \mu_1 - \mu_2 \neq 0$

2. Decide on an α-significance level, look up $t_{\frac{a}{2}}(n_1+n_2-2)$ in critical tables.

3. Obtain the two random samples, calculate $\bar{x}_1$, s_1^2, $\bar{x}_2$, s_2^2, s_{pool}^2, t_{exp}.

4. Note whether t_{exp} is in the critical region and if $|t_{exp}| < t_{crit}$ accept H_o, otherwise accept H_1.

The assumptions that must be made in order to use the t-distribution to test a hypothesis about the difference between two population means are:

(1) The two populations are normal.

(2) The two populations have the same variance.

(3) The two samples are random ones, and independent.

If an experimenter wants to test assumption (2) he can use the F-distribution. If s_1^2 and s_2^2 are the variances of two independent random samples of size n_1 and n_2 taken from normal populations with variances σ_1^2 and σ_2^2, respectively, then

$$F = \frac{s_1^2 / \sigma_1^2}{s_2^2 / \sigma_2^2}, \text{ where } s_1^2 \quad s_2^2 \quad \ldots\ldots \quad (3)$$

is a value of a random variable f having the f distribution with (n_1-1) and (n_2-1) degrees of freedom. Consider the test H_o: $\sigma_1^2=\sigma_2^2$ against H_1: $\sigma_1^2 \neq \sigma_2^2$. The value $f_{exp} = s_1^2/s_2^2$ is valid when H_o is true. If H_o is true, the computed f value should be

relatively close to 1. A large value of f_{exp} will occur when s_1^2 is considerably larger than s_2^2, suggesting that $Q_1^2 > Q_2^2$.

For a level of significance equal to , we find the two critical values, $f_{1-\frac{}{2}}(n_1-1, n_2-1)$ and $f_{\frac{}{2}}(n_1-1, n_2-1)$, so that f $f_{1-\frac{}{2}}(n_1-1, n_2-1)$ and f $f_{\frac{}{2}}(n_1-1, n_2-1)$ constitute the critical region. The lower critical value is obtained from Tables[5,6] by names of the relation $f_{1-\frac{}{2}}(n_1-1, n_2-1) = 1/ f_{\frac{}{2}}(n_2-1, n_1-1)$. If the computed f_{exp} value falls in the critical region we reject H_o in favor of H_1, otherwise wer accept H_o.

EXPERIMENTAL

Chemicals and Solutions

Methyl Orange (commercial product from Lachema, Brno, Czechoslovakia) was used as a sodium slat dissolved in distilled water to give six stock solutions of the same concentration 2.0 x 10^{-3}M. The indicator purity was checked by thin-layer chromatography on Silufol using ethylalcohol-pyridine (3:1) system. Perchloric acid (1M) was obtained by dilution of 70% $HClO_4$ (p.a. Carlo Erba, Italy) with redistilled water. The titre was determined by potentiometric titration of HgO in KI medium[7]. Sodium perchlorate (1M) was prepared by neutralization of recrystallized sodium hydrocarbonate (p.a., Lachema, Brno) with concentrated perchloric acid (70% p.a., Carlo Erba, Italy) and was recrystallized twice from redistilled water. Sodium phosphate (0.03M) was prepared by weighing doubly recrystallized sodium phosphate in redistilled water. Sdoium hydroxide (2M) was prepared by dilution of 50% NaOH prepared according to Sorensen[8]. The titre was determined by potentiometric titration of oxalic acid under an inert argon atmosphere. The solution was stored in

a polyethylene bottle fitted with an ascarite tube. Sodium chloride (1M), sodium acetate (0.25M) were prepared by dissolving the substance (p.a., Lachema, Brno) in redistilled water. Standard buffers with declared pH values 4.01 and 7.00 were prepared by diluting commercial stock solution (Radiometer, Copenhagen).

Techniques of A vs pH Data Measurement

The determination of dissociation constants by ultraviolet and visible spectrophotometry can be summarized in three main operations as follows:

(a) Spectrophotometric measurement of A vs pH data (as demonstrated here).

(b) Approximative evaluation of pK_{ai} by graphical or simplified numerical method (following contribution[9,17] of that series).

(c) The exact final refinement of pK_{ai} value by computer program (previous contribution[4] of that series and elsewhere[2,3,17]).

The combined pH-photometric titration[1,10,11,13] represents a convenient approach to the data measurement which is essential for the interpretation of protonation reactions in solution. There are two possible experimental alternatives: the absorbance values are measured at a constant wavelength for varied pH or the spectrum is scanned over a specified wavelength range for the adjusted pH values of the solution. The concentration of the reagent studied, the ionic strength of the solution, and the temperature should be kept constant. For both approaches the microtitration technique with simultaneous measurement of the light absorption and pH can be applied with advantage.

(A) Internal Titration Performed in the Cuvette

One of the possible experimental arrangements is illustrated in Fig. 1. The measuring cell of about 10-30 ml is placed in the path of a monochromatic beam. All other necessary devices, e.g. a propeller stirrer (though magnetic stirring may also be used), a pH-cell, a thermometer and a capillary tip of a microburette, etc. are supported from above and are immersed in the solution being measured so that they do not interfere with the beam of radiation. This geometric arrangement of all inlets should ramin unchanged during the course of the whole measurement. If necessary, polyethylene inlet tubes are also inserted into the cap to provide flushing of the cell compartment with a solvent-saturated stream of argon.

For the adjustment of pH, a dilute buffer system is formed directly in the solution by titrating a sodium salt of a weak acid (or a mixture giving a suitable polybasic system) with 1M perchloric acid. The initial pH vlaue of the solution thus represents the highest attainable pH ($\approx$ 10), whereas the lowest value is practically limited by the dilution of the strong acid in the solution at the end of the experiment (pH 3-2). The ionic strength of the solution is adjusted to a given value by calculated addition of a strong electrolyte. Since the strong acid added during the titration is consumed to protonate basic ionic species, the ionic strength remains practically constant up to a point where an excess of the acid begins to accumulate in the solution. The reference solution should have the same composition except the reagent under study is absent.

(B) External Titration Performed Outside the Cuvette

A 150 ml double-mantle thermostated titration vessel (V) was connected with the photometer cuvette (C) through polyethylene tubes (PT). One of the capillaries in cuvette was connected to the titration vessel and the other one was connected either to a nitrogen (or argon) cylinder or to a syringe outlet tip (SI), as shown in Fig. 2.

The preparation of solutions with various pH values was carried out in the titration vessel by titration with solutions of different acidity followed by stirring by inert gas (IG) or by the propeler stirrer (S). After establishment of equilibrium in the vessel, the pH of solution was measured by the cell of glass electrode (GE) and reference electrode (RE) and transported from the titration vessel into the photometer cuvette by overpressure of the inert gas or by use of syringe injection (SI). The cuvette was rinsed several times with the solution from the titration vessel, the absorbance values for different wavelengthes were measured and the solution was transpored back into the vessel. The pH was mwasured again. Then the value of pH was changed again by adding solution from microburettes (MB), and the whole procedure was repeated with a new solution.

Instruments

The pH was measured using PHM-4d pH meter (Radiometer, Copenhagen), with a G202B glass electrode (Radiometer, Copenhagen) and saturated calomel electrode.

Spectral curves were orientatively recorded at various pH on a Specord recording spectrophotometer (Zeiss, Jena, GDR) with a TAD titration attachment[11] empolying procedure (A). Absorbance value for computer evaluation were measured on a compensation single-beam spectrophotometer, VSU2-G (Zeiss, Jena,

GDR) provided with an external titration (B). The A vs pH curves were obtained by pH-photometric titration on a single-beam Spekol spectrophotometer with an amplifier (Zeiss, Jena, GDR) using a TAL adapter[11,14,15,16].

COMPUTATION

The computations were carried out on the Honyewell Bull 60/Level 66/6000 computer at The National Centre in Baghdad using the DCLET program.

RESULTS

To compare two different titration procedures of A vs pH curve measurement, an acid-base indicator Methyl Orange was used to obtain a simple sigmoidal A-pH curve of protonation equilibrium $HL \rightleftharpoons H^+ + L^-$. Dissociation constant and molar absorption coefficients ε_{HL} and ε_L of Methyl Orange were determined by nonlinear regression of A vs pH curve. For six different solutions of nearly the same concentration of Methyl Orange A vs pH curves were measured by internal and external titration procedure. From each titrated solution three paramers, $\varepsilon_L \pm s(\varepsilon_L)$, $\varepsilon_{HL} \pm s(\varepsilon_{HL})$, $pK_{al} \pm s(pK_{al})$ were estimated, shortly weritten as $x \pm s(x)$. The titration was n times repeated, $2 \leq n \leq 5$, (an example of such titration reproducibility for n=5 brings Table 1). For each parameter the mean of n repeated measurements might be calculated, $\bar{x}$ with the standard deviation, s, having been calculated as:

$$s = \sqrt{\frac{\sum_{i=1}^{n}(\bar{x} - x_i)^2}{n-1} + \left[\frac{\sum_{i=1}^{n} s(x_i)}{n}\right]^2}$$

where the first member represents the variability among the various x values and the second member represents a variability inside each measurement, i.e. the spread of experimental points along each calculated A-pH curve. A survey of averages of six repeated measurements brings Fig. 3. The accuracy and precision of both titration procedures are expressed by resulting averages and standard deviations of each parameter being at left side for internal titration and at right side for external titration. A histogram of parameters values for every solution is in the middle part of Fig. 3. Both techniques give the same values of all three parameters. External titration made using the spectrophotometer VSU2-G seems to be more precise than the internal one made on simple photometer Spekol.

The 20 values of the standard deviation of dissociation constants having been evaluated from each A vs pH curve, $s(pK_{al})$, leads to the averages $\bar{s}\ (pK_{al}) = 0.0062$. Using the equation $s(pK_{al}) = 0.000 + 1.846\ s_{inst}$ (taken from previous contribution[4] of this series), the instrumental error of Spekol can be guessed, $s_{inst} = 0.0034$. Analogously, $\bar{s}(pK_{al}) = 0.0043$ and $s_{inst} = 0.0023$ for 21 measurements of external titration may be enumerated.

At the end of every curve fitting process, the standard deviation of dependent variable (here the absorbance) is calculated, according to, $s(A) = \sqrt{U/(n - m)}$, where U is the error square sum, n is the number of experimental points and m stands for number of parameters. From 20 values of internal titration and 21 values of external one the average value was calculated $s(A) = 0.0040$ for internal and $s(A) = 0.0023$ for external titration, respectively. Both values are in good agreement with those estimated above to a equation from previous paper[4], for both spectrophotometers. This agreement means that all measured A vs pH curves were free of systematic errors and contained only random errors

Both titration procedures were compared if they give the same experimental results. Table 3 brings hypothesis test about equality of two averages and two variances. Both experimental techniques give A vs pH curves of the same spread of experimental points or similar precision of measurement because f-test mostly confirmed a hypothesis about the same variance $\sigma_1^2 \approx \sigma_2^2$

Both experimental procedures give A vs pH curves of the same shape and the same accuracy because t-test mostly confirmed a hypothesis about the same values of dissociation constant.

A $(1-\alpha)100\%$ confidence interval for the mean given by $\bar{x} \pm t_{\alpha/2} . s/\sqrt{n}$, where s is the standard deviation of a sample of size $n < 30$ and $t_{\alpha/2}$ is the value of the t-distribution, with (n-1) degrees of freedom will be for a dissociation constant 3.366 ± 0.014 for internal titration ($n=20$, $\alpha = 0.05$, $t_{\alpha/2}=2.093$, $s = 0.029$) and 3.361 ± 0.011 for external titration ($n=21$, $\alpha=0.05$, $t_{\alpha/2} = 2.086$, $s = 0.023$).

CONCLUSIONS

For collecting data with which to evaluate dissociation constants of weak light absorbing acids or bases, titrations are often prefered because of their capability of furnishing a large amount of experimental information from the interaction of a small number of standardized solutions. A controlled volume of a solution is titrated and simultaneously monitored with the aid of a suitable combination of electrochemical and photometric instruments. Temperature control, inert gas inlets, and efficient stirring are provided so that a homogeneous composition

of the carbon dioxide-free solution is immediately attained after each addition of the acid (or base). The manual reading of pH-meter and spectrophotometer takes only 40 s, allowing large sets of data to be accumulated for further data processing. The composition of the studied substance, which may be of high purity and often quite expensive, is relatively small. The protonation equilibria can thus be studied on submilligram amounts of substance which are obtained, for example, by preparative paper chromatography.

A statistical comparison of dissociation constants measured by two titration techniques leads to a conclusion that A vs pH curve measurement should be reproduced and also repeated for different solutions of a similar concentration of studied substance. More precise spectrophotometer gives lower value of achieved standard deviation of absorbance, s(A), and therefore more precise estimation of dissociation constant. Both titration procedures work with the same precision and lead to the same accurate values of dissociation constant.

Calculated values of dissociation constant of Methyl Orange evaluated from 20 measurements on 95% probability level is 3.37 +_ 0.01 for internal titration and evaluated for 21 measurements on 95% probability level is 3.36 $\pm$ 0.01 for external titration. Both values are in an agreement with published values[18] for I = 0.1, 25°C, they are within 3.29 - 3.39.

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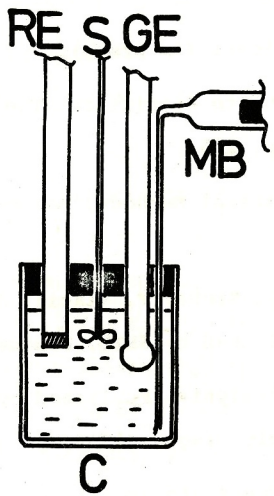

Fig. 1: Experimental arrangement of an internal titration performed in the cuvette.

Table 1: Results of nonlinear regression of A vs pH curves of Methyl Orange measured by an Internal titration using TAL attachment+Spekol spectrophotometer and an External titration using VSU2-G spectrophotometer. Measurement was repeated 5 times. Experimental conditions: 25°C, pH(standard)= 4.01 and 7.00, 0.005M sodium acetate + NaCl (I=0.1), 510 nm, ε_L($mol^{-1}.l.cm^{-1}$), ε_{HL}($mol^{-1}.l.cm^{-1}$).

Technique	Internal titration (TAL+Spekol)					External titration (VSU2-G)				
Concentration, cuvette length	4.04E-6 M, 49.99 mm					2.01E-5 M, 10.01 mm				
Reproducibility	1	2	3	4	5	1	2	3	4	5
ε_L	11230	10841	11359	10956	10878	11267	11358	11204	11344	11095
ε_{HL}	40779	40124	41179	41410	41632	41999	42134	41830	42249	41793
pK_{a1}	3.357	3.360	3.337	3.353	3.345	3.371	3.349	3.357	3.349	3.380
$s(\varepsilon_L)$	149	55	114	63	52	115	42	38	24	21
$s(\varepsilon_{HL})$	189	69	139	95	63	121	42	38	27	26
$s(pK_{a1})$	0.012	0.005	0.008	0.005	0.005	0.009	0.003	0.003	0.002	0.002
points	22	23	23	23	80	22	23	21	22	22
s(A)	0.0058	0.0023	0.0043	0.0036	0.0047	0.0046	0.0017	0.0014	0.0010	0.0009
$\bar{e}$	-2.4E-7	-1.7E-7	1.2E-7	7.8E-7	7.3E-7	3.0E-8	1.5E-7	8.5E-9	-2.7E-7	-1.6E-7
s_e	0.0054	0.0022	0.0040	0.0034	0.0046	0.0043	0.0015	0.0013	0.0009	0.0009

Remark: 1.0E-5 means 1.0×10^{-5}.

Table 2: Statistical test of equality of two means and two variances by the t-test and by the f-test.
f-test: H_o: $s_1^2 = s_2^2$ against H_1: $s_1^2 \neq s_2^2$, f_{crit} is $f_{0.025}(n_1-1; n_2-1)$.
t-test: H_o: $\bar{x}_1 = \bar{x}_2$ against H_1: $\bar{x}_1 \neq \bar{x}_2$, t_{crit} is $t_{0.025}(n_1+n_2-2)$. Experimental conditions: see Table 1

		Internal titration			External titration				f-test			t-test		
Solution	Parameter	n_1	$\bar{x}_1$	s_1	n_2	$\bar{x}_2$	s_2	s_{pool}	f_{exp}	f_{crit}		t_{exp}	t_{crit}	
1	μK_{a1}	5	3.351	0.011	5	3.361	0.014	0.0126	1.620	6.39	H_o	1.256	2.306	H_o
	ε_{HL}		40980	556		42002	204	419	7.428	6.39	H_1	3.858	2.306	H_1
	ε_L		11052	242		11254	123	192	3.871	6.39	H_o	1.664	2.306	H_o
2	μK_{a1}	2	3.347	0.024	2	3.368	0.016	0.0204	2.250	161	H_o	1.029	4.303	H_o
	ε_{HL}		41258	950		42362	227	691	17.51	161	H_o	1.207	4.303	H_o
	ε_L		11225	107		11199	125	116	1.365	161	H_o	0.224	4.303	H_o
3	μK_{a1}	2	3.344	0.011	2	3.401	0.006	0.0089	3.361	161	H_o	6.433	4.303	H_1
	ε_{HL}		41799	201		41851	147	176	1.870	161	H_o	0.295	4.303	H_o
	ε_L		11007	156		10932	95	129	2.696	161	H_o	0.580	4.303	H_o
4	μK_{a1}	3	3.353	0.012	5	3.339	0.030	0.0255	6.250	19.2	H_o	0.752	2.477	H_o
	ε_{HL}		41755	315		41961	270	286	1.361	6.94	H_o	0.987	2.477	H_o
	ε_L		11207	90		10930	105	100	1.361	19.2	H_o	3.785	2.477	H_1
5	μK_{a1}	3	3.420	0.020	3	3.353	0.011	0.0161	3.306	19.0	H_o	5.084	2.776	H_1
	ε_{HL}		42451	118		41979	68	96	3.011	19.0	H_o	6.002	2.776	H_1
	ε_L		11328	74		10841	44	61	2.828	19.0	H_o	9.794	2.776	H_1
6	μK_{a1}	5	3.380	0.011	4	3.342	0.013	0.0119	1.397	6.59	H_o	4.760	2.365	H_1
	ε_{HL}		43306	390		42075	133	307	8.598	9.12	H_o	5.970	2.365	H_1
	ε_L		11731	309		10817	105	243	8.660	9.12	H_o	5.598	2.365	H_1
Average	μK_{a1}	20	3.366	0.029	21	3.361	0.023	0.026	1.590	2.12	H_o	0.613	1.960	H_o
	ε_{HL}		41970	808		42038	174	578	21.56	2.12	H_1	0.377	1.960	H_o
	ε_L		11258	260		10996	186	225	1.954	2.12	H_o	3.725	1.960	H_1