MULTIPARAMETRIC CURVE FITTING—XIII

RELIABILITY OF FORMATION CONSTANTS DETERMINED BY ANALYSIS OF POTENTIOMETRIC TITRATION DATA

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Summary—The formation (protonation) constants $\log K_i$ of the acid H_jL are determined by regression analysis of potentiometric titration data when common parameters ($\log K_i, i = 1, \ldots, j$) and group parameters ($E^{0'}, L_0, H_T$) are refined. The influence of three kinds of error on the protonation constants has been investigated: error from the strategy of minimization, random error, and error from uncertain estimates of group parameters. An analysis of variance of the $\log K_i$ matrix was made for 7 identical titrations and 8 computational strategies, or of 7 identical titrations and 8 different options of group parameters to be refined. The influence of the standard potential E^0 of the glass-electrode cell on the systematic error in $\log K$ is greater than that of the acid concentration (L_0) or the concentration of titrant used (H_T). The ill-conditioned group parameters should be refined together with the common parameters (K_i), otherwise the estimates of $\log K_i$ are not accurate enough. Two ways of calibrating the glass electrode cell were compared. *Internal* calibration (performed during titration) was more accurate than *external* calibration done separately. Of the programs tested ESAB and ACBA are the most powerful because they permit refinement of group parameters and internal calibration. Citric acid was chosen as model substance.

The protonation constants of acid(s) can be estimated by analysis of acid–base titrations, and the methods have been reviewed.[1-3] In simple cases normalized graphs might give a fair estimate, but if the influence of various systematic errors has to be taken into account computer methods are necessary.

The first attempt to use least-squares methods for refining both formation constants (common parameters) and analytical concentrations (group parameters) was made by Sillén et al.[4] More recently non-linear regression programs for analysing potentiometric data for both common and group parameters have been constructed such as ACBA,[5] ESAB[6] and SUPERQUAD.[7] In this paper the notation introduced by Sillén et al.[4] is used. Common parameters are those that are the same for all the experiments, such as formation constants. Group parameters are those that vary from one experiment to another, such as E^0, analytical concentrations, calibration of the electrode used. To limit the number of group parameters the experiments must be done as titrations, each with its own set of group parameters. Batch experiments create too many group parameters to be handled by present-day methods. In selecting the group parameters to be refined, Sillén[4] kept constant those having little or no influence on the common parameters (formation constants) searched for, since if these are varied, rather large compensating errors may arise in them and in other par-

ameters, and sometimes quite unrealistic values are obtained. For that reason group parameters are sometimes termed *dangerous* parameters.[7] Only when the correct chemical model has been identified can these hitherto constant parameters be refined. For acid–base titrations the chemical model is often known and the group parameters can be refined directly. On the other hand for systems where the model is unknown and only guessed, this difficulty should be kept in mind and group parameters refined after the correct model has been obtained by graphical or numerical methods.

The reliability of formation constants obtained by regression analysis of potentiometric data is dependent upon (*i*) calibration of the glass electrode cell, (*ii*) the algorithm used, (*iii*) the parameters selected for refinement.

In order to have a well-known but not too trivial system, citric acid was chosen as the test substance. From the literature[8,9] the following values for the protonation constants were chosen:

$$\log K_1 = 5.65$$

$$\log K_2 = 4.34$$

$$\log K_3 = 2.87 \quad (I = 0.1, 298 \ K)$$

In the treatment below these constants are formally taken as "true" values.

THEORY

The emf of a cell containing a reference electrode (half-cell) and a glass electrode (in the measuring half-cell) can be written

$$E_{cell} = E_H + E_j - E_{ref} = E^0 + \frac{RT}{F} \ln h + \frac{RT}{F} \ln \gamma_H$$

$$+ j_a h - j_b K_w/h - E_{ref} = E^{0\prime} + S \log h \quad (1)$$

where E^0 is the standard potential of the glass electrode plus other constant terms such as the asymmetry potential, etc., $h = [H^+]$, E_j is the liquid-junction potential $(j_a h - j_b K_w/h)$, and S is the slope of the electrode response, $RT/F \ln 10$, for Nernstian response.

An explicit equation for the titration volume, expressing the relation between the volume of titrant added, v_i, monitored emf, $E_{cell, i}$, and the common $(\boldsymbol{\beta})$ and group parameters $(\boldsymbol{p})$, is given by

$$v_i = f(E_{cell, i}; \boldsymbol{\beta}, \boldsymbol{p}) \quad (2a)$$

in which the vector of common parameters $\boldsymbol{\beta} = (\beta_1, \ldots, \beta_m)$ contains the formation constants of the acid $H_j L$ or a sum of acids. There is also a vector of group parameters

$$\boldsymbol{p} = (E^{0\prime}, S, K_w, E_j, L_0, L_T, H_0, H_T) \quad (2b)$$

containing, besides the constants of the Nernst equation, the total ligand concentration, L_0, and the hydrogen-ion concentration in the titrand, H_0, as well as the corresponding quantities for the titrant, L_T and H_T. Note that for the titrant, the concentration of hydroxide ions is expressed as a negative hydrogen-ion concentration. K_w is the operational ion product of water. In most cases group parameters cannot be determined independently with sufficient accuracy. However, in work with high ionic concentration media of constant ionic strength, K_w, j_a and j_b may be determined by separate experiments.

Group parameters can be refined individually or with certain constraints introduced in the computation.

Algorithms

In most regression programs for treating emf data the task is to find the model and set of formation constants that give the "best" fit to the experimental data. In ESAB[6] (or ACBA[7]) the parameters $\boldsymbol{\beta}$ and $\boldsymbol{p}$ are refined by minimizing the residual-square sum (U_v)

$$U_v = \sum_{i=1}^{n} w_i (v_{exp, i} - v_{calc, i})^2 = \text{minimum} \quad (3)$$

where w_i is the statistical weight, in which ACBA is set equal to unity, whereas in ESAB the following expression is used

$$w_i = \frac{1}{\sigma_i^2} = \frac{1}{\sigma_v^2} + \left(\frac{\partial v}{\partial E}\right)_i \sigma_E^2 \quad (4)$$

In MINIQUAD[2a,10] only the parameters $\boldsymbol{\beta}$ are refined, by minimizing the residual-square sum U_c, given by

$$U_c = \sum_{i=1}^{n} w_i (C_{exp, i} - C_{calc, i})^2 = \text{minimum} \quad (5)$$

where C_i is the total concentration of ligand (L) or proton (H) at the ith point of the titration curve.

In PSEQUAD[2b] only the parameters $\boldsymbol{\beta}$ are refined, by minimizing with respect to volume (U_v) or emf (U_E) or both.

Accuracy of the protonation constants

The value of a certain protonation constant (K) from a certain $(i$th) titration can be written

$$\log K(i) = \log K(t) + \epsilon_{cell} + \epsilon_{conc} + \epsilon_{alg} + \epsilon_i \quad (6)$$

where $\log K(t)$ is the "true" value of K in a statistical analysis of variance, often denoted by μ, and ϵ_{cell} is a systematic error due to an imprecise estimate of the group parameters, $E^{0\prime}$, S, j_a, j_b and K_w. These parameters may be improved by calibration of the glass electrode. ϵ_{conc} is a systematic error due to estimates of the group parameters L_0, L_T, H_0 and H_T, which might be evaluated by independent chemical analysis. ϵ_{alg} is a systematic error due to poor quality of the minimization procedure in the algorithm used, and ϵ_i is the random error in the ith titration.

The accuracy of $K(i)$ can be expressed by the systematic deviation $e(\log K)$ given by

$$e(\log K) = (\log K_{lit} - \log K_{exp}) \quad (7)$$

where $\log K_{lit}$ is the "best" value available in the literature, for the experimental conditions used. In the case of simulated data $\log K_{lit}$ is the preselected value from which the data have been generated.

Precision of the protonation constants

The protonation constant K (one of the protonation constants of the acid $H_j L$) is affected by an error which is randomly distributed between the titrations performed rather than between individual titration points. Braibanti et al.[11] proved that $\sigma_K^2 \approx \sigma_{tit}^2$ in the relation

$$\sigma_K^2 = \sigma_i^2 + \sigma_{tit}^2 + \sigma_{lab}^2 \quad (8)$$

where σ_K is the standard deviation of $\log K$ as determined experimentally, σ_i is the intratitration (point-to-point) standard deviation, σ_{tit} is the inter-titration (titration-to-titration) standard deviation, and σ_{lab} is the intralaboratory standard deviation.

Analysis of variance applied to n points of the kth titration in one laboratory may prove whether (i) all the points in each titration belong to the same data population; (ii) all the titrations in each laboratory belong to the same data population; (iii) the algorithm used has no influence on the parameters estimated; (iv) a certain number of group parameters (to be refined) has no influence on the common parameters. Equation (8) can be extended to include also

the influence of the algorithm used, σ_{alg}^2, and the computation strategy for the parameters to be refined, σ_{par}^2, i.e.,

$$\sigma_K^2 = \sigma_i^2 + \sigma_{tit}^2 + \sigma_{lab}^2 + \sigma_{alg}^2 + \sigma_{par}^2 \qquad (9)$$

The precision of the constant $\log K$ can be expressed by the standard deviation $s(\log K)$ found by the regression algorithm used.

EXPERIMENTAL

Reagents

Citric acid, $0.030M$, and sodium citrate, $0.030M$, were made from analytical-grade chemicals that were not further purified. Sodium hydroxide, $1M$, was prepared from metallic sodium and carbon dioxide-free water under cooling and vigorous stirring with argon. This solution was standardized by titration against a solution of potassium hydrogen phthalate by using the Gran method in the MAGEC program[12] or by the non-linear regression program ACBA.[5]

Perchloric acid, $1M$, was prepared by dilution of the 70% acid *p.a.* quality, with distilled water, and standardized against HgO and KI, with a reproducibility of $\pm 0.2\%$. Demineralized or doubly distilled water was used in the preparation of the solutions.

Apparatus

All emf measurements were made at 298.0 ± 0.1 K, by means of an OP-208/1 digital voltmeter (Radelkis, Budapest) with a G202B glass electrode (Radiometer, Copenhagen) and an OP-0830P SCE reference electrode (Radelkis, Budapest). A waterjacketed 100-ml glass vessel, closed with a Teflon bung carrying the electrodes argon inlet, thermometer, stirrer and the microburette capillary tip, was used for the titrations.

During the titrations a stream of argon was bubbled through the solution both for stirring and for maintaining an inert atmosphere. The argon was passed through the pure ionic medium before entering the equilibrium solution.

The burettes used were home-made syringe microburettes of 2500 μl or 1250 μl capacity, with a 25.00 mm micrometer screw. The polyethylene capillary tip of the microburette was immersed in the solution during addition of titrant and then pulled out to avoid leakage of titrant during the pH reading.

The microburettes were calibrated by weighing water delivered from them, with a precision of $\pm 0.015\%$ over the volume range.

Calibration of glass electrode cell

The potentiometric titrations of citric acid with sodium hydroxide were performed with use of two pH scales.

(i) The proton activity (pH) was obtained by calibrating (by Irving's method[13]) with standard buffers (S1500, 1510, 1316, Radiometer, Copenhagen), assigned the pH values 6.865, 7.410 and 4.010 at 298 K. The operating temperature and Nernstian slope, S, were compensated for by adjustment of the pH-meter.

(ii) The hydrogen concentration $[H^+] = h$ was known from the preparation of the solution and the measured emf, E_{cell}. From equation (1), $E_{cell} = E^{0\prime} + S \log h$, and with a set of experimental data (E_{cell}, h) obtained by titrating a known concentration of perchloric acid with standard sodium hydroxide, the group parameters $E^{0\prime}$ and S can be estimated in the range where E_j is practically constant and can be included in $E^{0\prime}$.

Two calibration methods were used: *internal* and *external* calibration, cf. Fig. 1. Both the precision and accuracy of the protonation constants of citric acid can be investigated. The actual values of the group parameters H_0, H_T, $E^{0\prime}$, S and pK_w are estimated by the MAGEC program from a separate
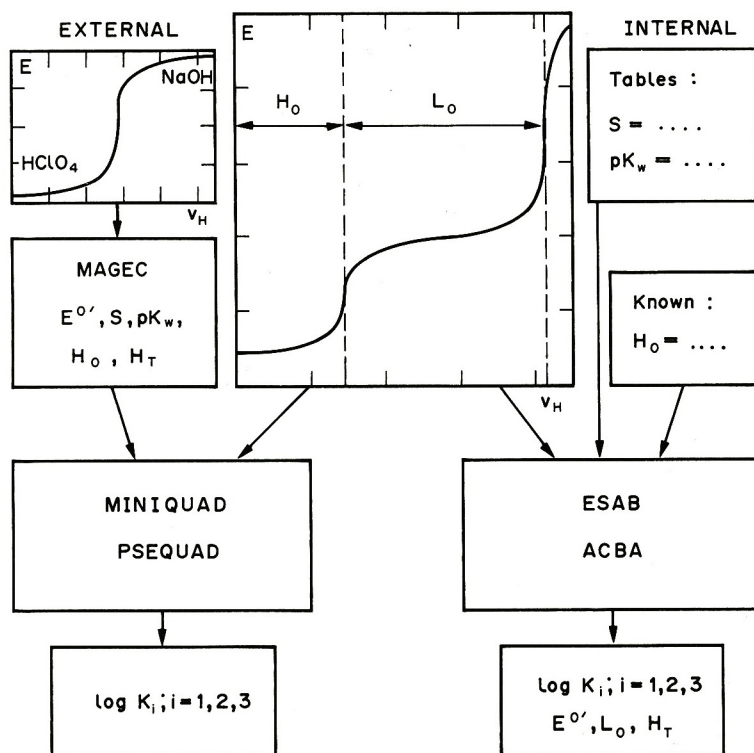

Fig. 1. Scheme of external and internal calibration of glass electrode and an estimation of the common parameters $\log K_1$, $\log K_2$, $\log K_3$ of citric acid simultaneously with the three group parameters L_0, H_T and $E^{0\prime}$.

acid–base titration, the *external* calibration, and the program ESAB estimates H_T, L_0 and $E^{0\prime}$ from the actual titration of a mixture of citric and perchloric acids with sodium hydroxide, the *internal* calibration.

Some group parameters are given in the input data for ESAB, such as the Nernstian slope and pK_w, both of which are available from the literature, *etc.* Group parameters can be estimated by regression analysis of both branches of a titration curve or from the acid branch only, because the basic might be affected by any carbonate and silicate in the alkali.

The program MAGEC offers a choice of group parameters to be estimated. The most accurate results were obtained by estimation of only $E^{0\prime}$ and S, *cf.* part B of Table 1. With ESAB the three group parameters $E^{0\prime}$, H_0 and H_T were refined, and gave the best fit as expressed by the Hamilton R-factor, *cf.* part C in Table 1. Since $E^{0\prime}$ might slightly change from one titration to another because of E_j, the internal calibration is more accurate and to be preferred.

Titration procedure

A mixture of 20.0 ml of $0.015M$ citric acid and $0.100M$ perchloric acid was titrated with $1.00M$ sodium hydroxide and the pH or E_{cell} was read, depending on which pH-scale that was used. As mentioned earlier the temperature was kept constant at 298 ± 0.1 K.

Computation

The influence of the regression algorithm on the precision and accuracy of the three protonation constants was investigated. A set of seven titration curves was analysed by the eight regression programs: $ESAB(v_H)$, $ACBA(v_H)$, $MINIQUAD(L, H)$, $MAGEC(v_H, E_{cell})$, $MIQUV(E_{cell})$, $PSEQUAD(v_H)$, $PSEQUAD(E_{cell})$, $PKAS(pH)$. The variable(s) within brackets are those minimized in the residual-squares sum. Here v_H is the volume of acid or base added.

All computations were performed on the EC 1033 computer at the Computing Centre of the College of Chemical Technology, CS-532 10 Pardubice, Czechoslovakia.

DISCUSSION

Seven titrations ($k = 7$, *cf.* Table 3) of a mixture of perchloric and citric acids with sodium hydroxide were performed. The data were treated with seven different programs but eight computational strategies ($m = 8$, *cf.* Table 3). Besides the three common parameters (the protonation constants) the three group parameters (the concentration of citric acid in the titrand, L_0, of sodium hydroxide in the titrant, H_T, and the constant $E^{0\prime}$) were also refined.

Table 2 gives the results of one particular titration analysed by several programs, by use of both external and internal calibration. In part (A), besides the original data (v_H, E_{cell}) and $-\log h$, the statistical weight [w, *cf.* equation (4)], the Bjerrum formation function (Z), the calculated ionic strength (I) at each point, and the relative concentrations of the species HL^{2-}, H_2L^- and H_3L are given. The fourth species L^{3-} is obtained by subtracting the values in Table 2 from 100.

Part (B) compares the influence of the programs on the accuracy and precision of the estimated protonation constants. The accuracy is expressed by the systematic deviation in log K related to the "best" literature value for the experimental conditions used.

Table 1. Reproducibility of external calibration of the glass electrode cell by standardization titration of $HClO_4$ with NaOH and evaluation by programs MAGEC and ESAB; experimental conditions: $v_0 = 20.0$ ml, $H_0 = 0.09869M$, $H_T = -1.071M$, $pK_w = 13.78$, $I = 0.1$, 298 K, cell used G202B–SCE (Radiometer, Copenhagen); standard deviations (in parentheses) refer to corresponding last figures

Titration	1	2	3	4	5	6	7
Part (A): Program MAGEC, all points of titration curve used.							
v_e (ml)	1.823	1.832	1.833	1.834	1.833	1.833	1.831
$E^{0\prime}$ (mV)	375.4(5)	378.9(2)	379.8(1)	377.6(1)	378.6(1)	377.4(1)	377.4(2)
S (mV/pH)	58.50(9)	58.82(2)	58.63(2)	58.39(2)	58.53(2)	58.55(2)	58.39(3)
Part (B): Program MAGEC, points of acid branch of titration curve used only.							
v_e (ml)	1.818	1.830	1.830	1.832	1.830	1.831	1.828
$E^{0\prime}$ (mV)	389.4(11)	381.6(4)	381.8(2)	379.4(2)	380.6(1)	379.3(1)	381.3(1)
S (mV/pH)	65.54(55)	60.15(18)	59.64(12)	59.30(9)	59.52(6)	59.54(7)	60.38(7)
R-factor (%)	0.263	0.087	0.057	0.041	0.027	0.033	0.034
Part (C): Program ESAB, points of acid branch of titration curve used only.							
H_0 ($10^{-2}M$)	9.875(916)	10.013(985)	9.847(925)	9.988(874)	9.756(992)	9.981(890)	9.883(889)
$-H_T$ (M)	1.079(99)	1.088(127)	1.069(105)	1.084(96)	1.058(116)	1.084(121)	1.074(94)
$E^{0\prime}$ (mV)	378.5(24)	379.5(42)	381.0(29)	378.9(26)	380.2(28)	378.4(22)	379.2(18)
R-factor (%)	0.017	0.068	0.028	0.036	0.023	0.018	0.025

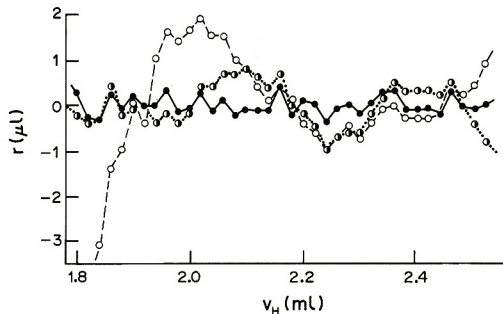v_e means volume at equivalence point.

Fig. 2. Response of an option of group parameters to be refined on the degree-of-fit and accuracy of three protonation constants of citric acid. The standard deviation $s(\log K_i)$ is written in brackets and refers to the last decimal place of $\log K_i$

Circle	$\log K_1$	$\log K_2$	$\log K_3$	$L_0, 10^{-2}M$	$-H_T$	$E^{0\prime}, mV$	$|\bar{r}|, \mu l$	$R, \%$
○	5.607(3)	4.305(3)	2.844(3)	1.517	1.091	378.5	1.47	0.068
◑	5.579(9)	4.324(7)	2.927(8)	1.453(5)	1.080(1)	378.5	1.23	0.022
●	5.631(7)	4.332(4)	2.895(4)	1.512(5)	1.092(1)	381.3(2)	0.20	0.009

Table 2. Estimation of the protonation constants of citric acid by various regression programs

Part (A): Shortened output of the results from the ESAB program.

$\log K_1 = 5.667 \pm 0.010$, $\log K_2 = 4.361 \pm 0.005$, $\log K_3 = 2.932 \pm 0.005$ (common parameters)
$L_0 = (1.499 \pm 0.007) \times 10^{-2}M$, $E^{0\prime} = 381.5 \pm 0.3$ mV, $-H_T = 1.089 \pm 0.0013M$ (group parameters)

i	$v_H, \mu l$	Residual μl	E, mV	$-\log h$	w	Z	I	$HL, \%$	$H_2L, \%$	$H_3L, \%$
1	1800	0.0	237.4	2.436	0.77	2.75	0.095	0.29	24.38	75.33
2	1820	0.1	233.2	2.507	0.81	2.72	0.095	0.38	27.49	72.13
3	1840	−0.3	228.9	2.579	0.84	2.68	0.096	0.51	30.90	68.59
4	1860	0.7	224.1	2.661	0.87	2.64	0.096	0.69	34.95	64.36
5	1880	−0.1	219.6	2.737	0.89	2.60	0.097	0.92	38.92	60.16
6	1900	−0.6	214.9	2.816	0.90	2.55	0.097	1.22	43.19	55.59
7	1920	−0.3	209.9	2.901	0.92	2.49	0.098	1.64	47.76	50.60
8	1940	−0.1	204.8	2.987	0.94	2.44	0.099	2.19	52.33	45.47
9	1960	0.4	199.5	3.076	0.96	2.38	0.100	2.93	56.87	40.20
10	1980	0.2	194.2	3.166	0.98	2.32	0.101	3.87	61.03	35.10
11	2000	0.1	188.7	3.259	1.00	2.25	0.102	5.08	64.81	30.09
12	2020	0.1	183.0	3.355	1.03	2.19	0.103	6.66	68.01	25.29
13	2040	0.0	177.1	3.455	1.05	2.12	0.104	8.68	70.45	20.82
14	2060	0.4	170.9	3.560	1.06	2.05	0.105	11.28	71.93	16.70
15	2080	−0.3	164.9	3.661	1.07	1.99	0.106	14.32	72.26	13.29
16	2100	−0.4	158.7	3.766	1.07	1.92	0.107	18.02	71.45	10.32
17	2120	0.0	152.4	3.872	1.06	1.85	0.109	22.38	69.43	7.85
18	2140	−0.1	146.4	3.974	1.05	1.78	0.111	27.06	66.47	5.95
19	2160	0.2	140.4	4.075	1.03	1.71	0.112	32.18	62.60	4.44
20	2180	0.0	134.7	4.172	1.01	1.63	0.114	37.36	58.21	3.30
21	2200	−0.5	129.2	4.265	1.00	1.56	0.116	42.50	53.46	2.45
22	2220	−0.1	123.5	4.361	1.00	1.49	0.118	47.82	48.18	1.77
23	2240	0.1	117.9	4.456	1.00	1.42	0.119	52.85	42.82	1.26
24	2260	−0.1	112.4	4.549	1.00	1.35	0.121	57.41	37.55	0.89
25	2280	−0.2	106.8	4.643	1.01	1.28	0.123	61.51	32.35	0.62
26	2300	0.2	101.0	4.741	1.02	1.20	0.125	65.00	27.28	0.42
27	2320	0.2	95.2	4.839	1.03	1.13	0.128	67.58	22.63	0.28
28	2340	0.3	89.3	4.939	1.04	1.06	0.130	69.16	18.41	0.18
29	2360	0.4	83.3	5.041	1.04	0.98	0.132	69.63	14.67	0.11
30	2380	−0.2	77.5	5.139	1.05	0.91	0.134	68.99	11.60	0.07
31	2400	0.3	71.4	5.242	1.04	0.84	0.137	67.17	8.91	0.04
32	2420	0.4	65.4	5.343	1.04	0.77	0.139	64.33	6.75	0.03
33	2440	−0.2	59.6	5.441	1.05	0.70	0.142	60.72	5.09	0.02
34	2460	−0.2	53.6	5.543	1.05	0.62	0.145	56.26	3.73	0.01
35	2480	−0.3	47.5	5.646	1.07	0.55	0.147	51.18	2.68	0.01
36	2500	−0.2	41.1	5.754	1.10	0.48	0.150	45.51	1.86	0.00
37	2520	0.1	34.2	5.870	1.15	0.40	0.153	39.31	1.23	0.00

Degree-of-fit test: $|\bar{r}| = 0.23$ μl, $s(r) = 0.28$ μl, $R = 0.013\%$.

Table 2 continued overleaf

Table 2. (contd.)

Part (B): External calibration of glass electrode cell and MAGEC evaluation of group parameters: $S = 58.40$ mV/pH, $E^{0'} = 379.5$ mV, $L_0 = 1.501 \times 10^{-2}M$, $-H_T = 1.089\,M$; systematic error in log K_i is $e_i = 10^3\, e\,(\log K_i)$, $i = 1, 2, 3$; standard deviations (in parentheses) refer to the corresponding last figures; values from literature: log $K_1 = 5.65$, log $K_2 = 4.34$, log $K_3 = 2.87$ [for $I = 0.1$ (NaClO$_4$), 298.0 K].

Program	Group parameters	e_3	e_2	e_1	$E^{0'}$, mV	L_0, $10^{-2}M$	$-H_T$, M	$s(r)$	$|\bar{r}|$	R, %
ACBA(v_H)	—	112(1)	82(1)	105(1)	379.5	1.501	1.089	0.5 μl	0.4 μl	0.023
	L_0	102(2)	54(4)	528(8)	379.5	1.480(3)	1.089	0.4 μl	0.3 μl	0.021
	H_T	65(6)	40(5)	51(6)	379.5	1.501	1.093(1)	0.4 μl	0.3 μl	0.018
	L_0, H_T	75(4)	38(3)	38(5)	379.5	1.489(2)	1.092(1)	0.3 μl	0.2 μl	0.013
	$E^{0'}$, $S = 59.31$ mV/pH	0	0	0	377.8(3)	1.501	1.089	2.0 μl	1.5 μl	0.091
	L_0, H_T, $E^{0'}$	75(5)	38(3)	37(7)	379.4(3)	1.487(6)	1.091(5)	0.3 μl	0.2 μl	0.013
MINIQUAD(L, H)		113(2)	80(2)	108(2)	379.5	1.501	1.089	1.8E-5M	1.0E-5M	0.088
MAGEC(v, E_{cell})		92(X)	91(X)	53(X)	—	1.501	1.089	not calculated		
MIQUV(E_{cell})		113(2)	81(1)	108(1)	379.5	1.501	1.089	1.88 mV	1.21 mV	1.20
PSEQUAD(v_H)		112(1)	82(1)	105(1)	379.5	1.501	1.089	0.5 μl	0.5 μl	—
PSEQUAD(E_{cell})		112(1)	82(1)	105(1)	379.5	1.501	1.089	0.1 mV	0.1 mV	—
PKAS(pH)		110(3)	81(1)	105(4)	379.5	1.501	1.089	0.002 pH	0.001 pH	0.052

Part (C): Internal calibration of the glass electrode cell using ESAB: $S = 59.159$ mV/pH, $E^{0'} = 378.5$ mV, $L_0 = 1.517 \times 10^{-2}M$, $-H_T = 1.091M$; (X) means that standard deviation could not be estimated; modified version of MINIQUAD program for estimation of $E^{0'}$ was used.

Programs	Group parameters	e_3	e_2	e_1	$E^{0'}$, mV	L_0, $10^{-2}M$	$-H_T$, M	$s(r)$	$\bar{r}$	R, %
ESAB(v_H)	—	23(7)	-20(7)	-15(8)	378.5	1.517	1.091	1.9 μl	1.2 μl	0.088
	$E^{0'}$	65(3)	35(2)	48(2)	382.1(1)	1.517	1.091	0.3 μl	0.2 μl	0.015
	L_0	43(7)	-66(13)	109(27)	378.5	1.480(0)	1.091	1.6 μl	1.0 μl	0.076
	H_T	138(18)	123(16)	169(21)	378.5	1.517	1.076(2)	1.3 μl	1.1 μl	0.060
	$E^{0'}$, L_0	57(4)	21(5)	23(9)	381.9(1)	1.508(3)	1.091	0.3 μl	0.2 μl	0.013
	$E^{0'}$, L_0, H_T	63(5)	21(5)	17(10)	381.5(3)	1.499(7)	1.089(1)	0.3 μl	0.2 μl	0.013
MINIQUAD(L, H)		188(4)	23(3)	-11(2)	378.5	1.517	1.091	2.8E-5M	1.8E-5M	0.135
	$E^{0'}$	66(2)	34(1)	51(1)	372.1(X)	1.517	1.091	1.3E-5M	6.9E-6M	0.062

The precision is expressed by the standard deviation (given in parentheses after each value, and referred to the last digit of the value). The fit obtained is expressed by the mean residual, $|\bar{r}|$, the standard deviation $[s(r)]$, and the Hamilton R-factor (in %).

The lowest values of the systematic error were obtained when the program ESAB was used in combination with the internal calibration of the glass electrode [part (C) in Table 2].

Refinement of the group parameters $E^{0\prime}$, L_0 and H_T leads to the lowest systematic error in the protonation constants, as shown in Fig. 2. The most important group parameter seems to be the constant $E^{0\prime}$, as its refinement leads to $R = 0.015\%$, *cf.* Table 2(C).

Table 3. Effect of choice of group parameters to be refined, and reproducibility, on accuracy and precision of the protonation constants: $\log K_1$, $\log K_2$, $\log K_3$: the refined group parameters are, for various M: (1) none, (2) L_0, (3) H_T, (4) L_0, H_T, (5) $E^{0\prime}$, (6) $H_T, E^{0\prime}$, (7) $L_0, E^{0\prime}$, (8) $L_0, H_T, E^{0\prime}$: in brackets are the standard deviations, referring to the last figures: initial guess of group parameters: $L_0^{(0)} = 0.01517M$, $H_T^{(0)} = 1.091M$, $E^{0\prime(0)} = 378.5\,mV$, $S^{(0)} = 59.159\,mV/pH$, $pK_w^{(0)} = 13.78$, at $I = 0.1$ (NaClO$_4$) and 298 K

		Repeated titrations							Average titration	Weighted mean
	m	$k=1$	2	3	4	5	6	7		
	1	2.905(0)	2.675(8)	2.836(3)	2.844(3)	2.855(3)	2.847(4)	2.974(4)	2.847(3)	2.889(21)
	2	2.906(1)	2.657(13)	2.820(6)	2.826(5)	2.835(6)	2.827(7)	2.783(8)	2.825(6)	2.895(36)
	3	2.911(3)	3.096(28)	2.981(18)	2.950(19)	2.995(20)	3.009(18)	2.965(15)	2.970(15)	2.921(27)
	4	2.911(3)	2.963(17)	2.946(8)	2.927(8)	2.955(10)	2.961(8)	2.929(12)	2.930(7)	2.924(10)
$\log K_1$	5	2.906(2)	2.962(4)	2.916(3)	2.912(2)	2.939(3)	2.935(3)	2.874(6)	2.914(1)	2.912(8)
	6	2.911(4)	2.847(7)	2.915(5)	2.897(4)	2.919(5)	2.927(5)	2.908(11)	2.913(3)	2.907(7)
	7	2.910(3)	2.853(5)	2.914(4)	2.903(3)	2.928(4)	2.927(4)	2.906(9)	2.913(3)	2.909(7)
	8	2.911(4)	2.848(7)	2.917(6)	2.899(4)	2.916(6)	2.932(5)	2.906(11)	2.913(3)	2.907(8)
	1	4.323(0)	4.233(7)	4.305(3)	4.305(3)	4.321(3)	4.320(4)	4.233(3)	4.302(3)	4.319(7)
	2	4.326(3)	4.156(22)	4.267(13)	4.265(10)	4.274(12)	4.274(13)	4.209(16)	4.249(13)	4.309(25)
	3	4.329(3)	4.599(25)	4.433(16)	4.399(17)	4.443(18)	4.463(16)	4.381(14)	4.410(13)	4.346(38)
	4	4.330(3)	4.330(17)	4.343(8)	4.324(7)	4.345(10)	4.354(8)	4.303(14)	4.320(8)	4.333(4)
$\log K_2$	5	4.324(1)	4.342(2)	4.354(2)	4.347(2)	4.373(2)	4.375(2)	4.281(4)	4.342(1)	4.320(16)
	6	4.330(3)	4.327(6)	4.353(5)	4.333(3)	4.353(5)	4.367(5)	4.314(10)	4.342(3)	4.339(5)
	7	4.331(4)	4.328(7)	4.352(5)	4.332(4)	4.355(5)	4.361(5)	4.333(14)	4.341(4)	4.342(5)
	8	4.331(4)	4.327(7)	4.352(5)	4.331(4)	4.355(5)	4.361(5)	4.332(15)	4.341(4)	4.342(5)
	1	5.617(0)	5.555(9)	5.605(4)	5.607(3)	5.629(4)	5.635(4)	5.502(4)	5.599(3)	5.611(12)
	2	5.623(5)	5.399(45)	5.529(26)	5.525(21)	5.534(25)	5.541(27)	5.454(33)	5.497(26)	5.605(38)
	3	5.625(4)	6.018(33)	5.769(20)	5.729(22)	5.780(23)	5.819(21)	5.697(18)	5.737(16)	5.650(54)
	4	5.627(6)	5.535(32)	5.599(14)	5.579(12)	5.599(17)	5.615(13)	5.551(24)	5.571(14)	5.610(13)
$\log K_3$	5	5.619(1)	5.682(3)	5.661(2)	5.656(2)	5.689(3)	5.698(2)	5.558(5)	5.647(1)	5.645(18)
	6	5.625(4)	5.663(8)	5.660(6)	5.636(4)	5.662(6)	5.687(7)	5.604(14)	5.646(4)	5.645(8)
	7	5.630(7)	5.657(12)	5.657(9)	5.627(6)	5.656(10)	5.673(9)	5.653(25)	5.644(7)	5.644(7)
	8	5.629(8)	5.661(13)	5.655(11)	5.631(7)	5.669(10)	5.667(10)	5.651(29)	5.643(9)	5.650(3)
	1	—	—	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—	—	—
	3	1.090(0)	1.055(2)	1.078(2)	1.081(2)	1.878(2)	1.086(2)	1.075(1)	1.080(1)	1.080(6)
H_T	4	1.090(0)	1.058(1)	1.078(1)	1.080(1)	1.078(1)	1.077(1)	1.076(1)	1.080(1)	1.077(5)
(M)	5	—	—	—	—	—	—	—	—	—
	6	1.090(0)	1.093(1)	1.091(1)	1.093(0)	1.094(1)	1.092(1)	1.086(1)	1.091(0)	1.091(1)
	7	—	—	—	—	—	—	—	—	—
	8	1.091(1)	1.092(1)	1.090(1)	1.092(1)	1.095(1)	1.089(1)	1.091(3)	1.091(1)	1.091(1)
	1	—	—	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—	—
E^0	4	—	—	—	—	—	—	—	—	—
(mV)	5	378.6(1)	385.7(1)	381.7(1)	381.3(1)	381.9(1)	382.1(1)	381.7(2)	381.2(1)	381.9(12)
	6	378.5(1)	385.8(1)	381.7(1)	381.5(1)	382.3(1)	382.2(1)	381.1(3)	381.2(1)	382.0(12)
	7	378.6(1)	385.5(2)	381.7(1)	381.1(1)	381.7(1)	381.9(1)	382.2(2)	381.1(1)	381.3(9)
	8	378.6(2)	385.7(3)	381.5(3)	381.3(2)	382.5(3)	381.5(3)	382.2(6)	381.1(2)	381.3(12)
	1	—	—	—	—	—	—	—	—	
	2	1.519(2)	1.456(17)	1.485(10)	1.485(8)	1.480(10)	1.480(10)	1.498(13)	1.475(10)	
	3	—	—	—	—	—	—	—	—	
L_0	4	1.518(0)	1.348(11)	1.449(5)	1.453(5)	1.443(6)	1.440(5)	1.461(9)	1.453(5)	
$(10^{-2}M)$	6	— .	—	—	—	—	—	—	—	—
	7	1.521(2)	1.508(4)	1.515(3)	1.507(2)	1.505(3)	1.508(3)	1.551(9)	1.516(3)	
	8	1.520(5)	1.515(8)	1.512(7)	1.512(5)	1.524(7)	1.499(7)	1.550(18)	1.515(5)	
	1	0.009	0.192	0.080	0.068	0.084	0.088	0.087	0.067	
	2	0.009	0.166	0.071	0.057	0.071	0.076	0.083	0.055	
	3	0.009	0.132	0.055	0.054	0.064	0.060	0.049	0.043	
$R(\%)$	4	0.009	0.057	0.024	0.022	0.030	0.024	0.034	0.018	
	5	0.009	0.018	0.013	0.012	0.017	0.014	0.031	0.007	
	6	0.009	0.017	0.013	0.010	0.013	0.014	0.026	0.007	
	7	0.009	0.016	0.013	0.010	0.015	0.013	0.026	0.007	
	8	0.009	0.016	0.013	0.009	0.013	0.013	0.025	0.007	

Table 3. (*contd.*)

		$k = 1$	2	3	Repeated titrations 4	5	6	7	Average titration	Weighted mean
$m = 1$		0.202	4.168	1.725	1.469	1.819	1.913	1.877	1.448	
2		0.199	3.604	1.530	1.229	1.541	1.650	1.798	1.192	
3		0.191	2.861	1.202	1.169	1.384	1.297	1.057	0.932	
$s(r)$	4	0.190	1.240	0.524	0.485	0.660	0.513	0.741	0.391	
(μl)	5	0.199	0.394	0.285	0.262	0.369	0.319	0.664	0.150	
6		0.191	0.358	0.285	0.173	0.282	0.305	0.564	0.149	
7		0.190	0.363	0.284	0.209	0.315	0.287	0.539	0.149	
8		0.189	0.357	0.283	0.203	0.277	0.279	0.539	0.149	
$m = 1$		0.153	2.600	1.092	0.962	1.203	1.182	1.244	0.891	
2		0.147	2.389	1.053	0.830	1.079	1.046	1.232	0.778	
3		0.134	2.273	1.016	0.206	1.140	1.074	0.862	0.771	
$|\bar{r}|$	4	0.133	1.024	0.445	0.425	0.556	0.433	0.528	0.338	
(μl)	6	0.152	0.331	0.218	0.204	0.310	0.253	0.518	0.123	
5		0.133	0.293	0.218	0.173	0.218	0.250	0.385	0.124	
7		0.136	0.304	0.218	0.157	0.261	0.232	0.395	0.123	
8		0.134	0.295	0.218	0.163	0.205	0.227	0.393	0.123	
$m = 1$		0.202	4.168	1.725	1.469	1.819	1.913	1.877	1.448	
2		0.199	3.604	1.530	1.229	1.541	1.650	1.798	1.192	
3		0.191	2.861	1.202	1.169	1.384	1.297	1.057	0.932	
$s(r)$	4	0.190	1.240	0.524	0.485	0.660	0.513	0.741	0.391	
(μl)	5	0.199	0.394	0.285	0.262	0.369	0.319	0.664	0.150	
6		0.191	0.358	0.285	0.173	0.282	0.305	0.564	0.149	
7		0.190	0.363	0.284	0.209	0.315	0.287	0.539	0.149	
8		0.189	0.357	0.283	0.203	0.277	0.279	0.539	0.149	
$m = 1$		0.153	2.600	1.092	0.962	1.203	1.182	1.244	0.891	
2		0.147	2.389	1.053	0.830	1.079	1.046	1.232	0.778	
3		0.134	2.273	1.016	0.206	1.140	1.074	0.862	0.771	
$|\bar{r}|$	4	0.133	1.024	0.445	0.425	0.556	0.433	0.528	0.338	
(μl)	5	0.152	0.331	0.218	0.204	0.310	0.253	0.518	0.123	
6		0.133	0.293	0.218	0.173	0.218	0.250	0.385	0.124	
7		0.136	0.304	0.218	0.157	0.261	0.232	0.395	0.123	
8		0.134	0.295	0.218	0.163	0.205	0.227	0.393	0.123	

When L_0 and H_T are also refined, R decreases to 0.013%.

When a modified version of MINIQUAD is used that allows $\dot{E}^{0\prime}$ to be refined, more accurate estimates of the protonation constants are obtained as well as an improved fit [*cf.* Table 2(C)].

When $E^{0\prime}$, L_0 H_T and S are estimated from an independent determination the log K values are still loaded by some systematic error, *cf.* Table 2(C), uppermost row for ESAB. Table 3 gives the reproducibility of log K values for various choices of group parameters.

$E^{0\prime}$ has the greatest influence on the accuracy, and hence should always be refined. As further group parameters are refined the fit is improved, as demonstrated in Table 3.

The influence of different factors on the protonation constants is obtained by analysis of the matrix of these constants (*cf.* Table 3). When this matrix for various computational strategies and repeated titrations is analysed, the influence of a given choice of group parameters and of the reproducibility on log K may be tested.

The Fisher–Snedecor test in analysis of variance (ANOVA) is applied to test the variance σ_{par}^2 arising from different sets of refined group parameters in comparison with the variance, σ_{tit}^2, from the reproducibility. The test is performed by comparing the experimental value F_{exp} with the critical value $F_{crit}[\alpha, m - 1, (m - 1)(k - 1)]$ where α is the significance level and F_{exp} is the ratio of the variance tested (σ^2) to the residual variance σ_{res}^2, and when F_{exp} is larger than F_{crit}, the variance σ^2 is significantly different from that of the residuals.

Table 4 shows the results of an ANOVA test. It shows that while the variance from the reproducibility is not significant, but the variances from the algorithm used or the choice of group parameters are significant, the latter being the more important of the two.

The parameters can be divided into two groups: well-conditioned and ill-conditioned. Ill-conditioned parameters have little influence on the residual-squares sum function U, which makes their determination rather uncertain. This is illustrated in Fig. 3 where $(1 - U)$ is plotted against each common parameter and various ill-conditioned group parameters. As seen in Fig. 3, none of the ill-conditioned parameters $E^{0\prime}$, L_0 and H_T leads to a pronounced maximum in $(1 - U)$, so their determination is uncertain, and might sometimes lead to false estimates of the common parameters. This is the reason why such parameters are sometimes called dangerous parameters. On the other hand, well-conditioned parameters have great influence on the hyperparaboloid (U-surface), as shown in Fig. 4, where a

Table 4. Analysis of variance in the protonation constants of citric acid for: (i) influence of algorithm used in comparison with reproducibility of titration for (a) external calibration using MAGEC, (b) internal calibration using ESAB; (ii) influence of group parameters compared to reproducibility in titration: both matrices have the same size 7×8 (= $k \times m$, cf. Table 3); the values for F_{crit} are F_{crit} (0.05, 6, 36) = 2.68, F_{crit} (0.05, 6, 42) = 2.25, F_{crit} (0.05, 7, 42) = 2.17

(i) Influence of algorithm used and reproducibility, on $\log K_i$, $i = 1, 2, 3$

		Algorithm used		Reproducibility	
$\log K_1$	(a)	$F_{exp} = 5.37$	$F_{crit} = 2.68$	$F_{exp} = 0.41$	$F_{crit} = 2.68$
	(b)	2.50		4.45	
$\log K_2$	(a)	2.85		0.58	
	(b)	4.05		1.50	
$\log K_3$	(a)	6.16		2.18	
	(b)	1.11		6.20	

(ii) Influence of group parameters refined and reproducibility, on $\log K_i$

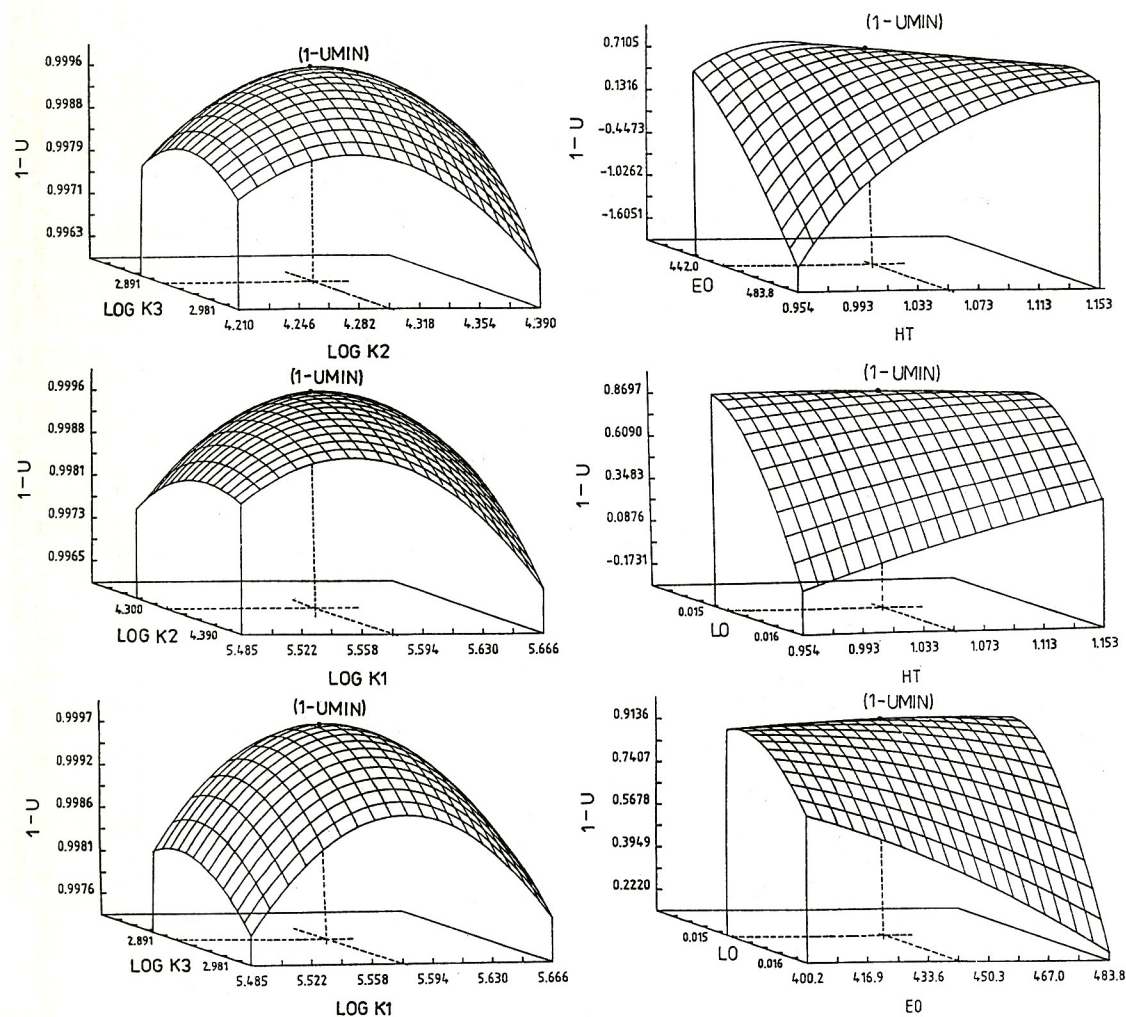|	Reproducibility		Group parameters	
$\log K_1$	$F_{exp} = 12.81$	$F_{crit} = 2.17$	$F_{exp} = 1.88$	$F_{crit} = 2.25$
$\log K_2$	10.16		1.41	
$\log K_3$	8.80		2.03	

Fig. 3. Three-dimensional representation of the $(1 - U)$ surface for the well-conditioned parameters (left half of figure) and ill-conditioned parameters (right half) indicates which parameters are rather uncertainly estimated by the regression program.
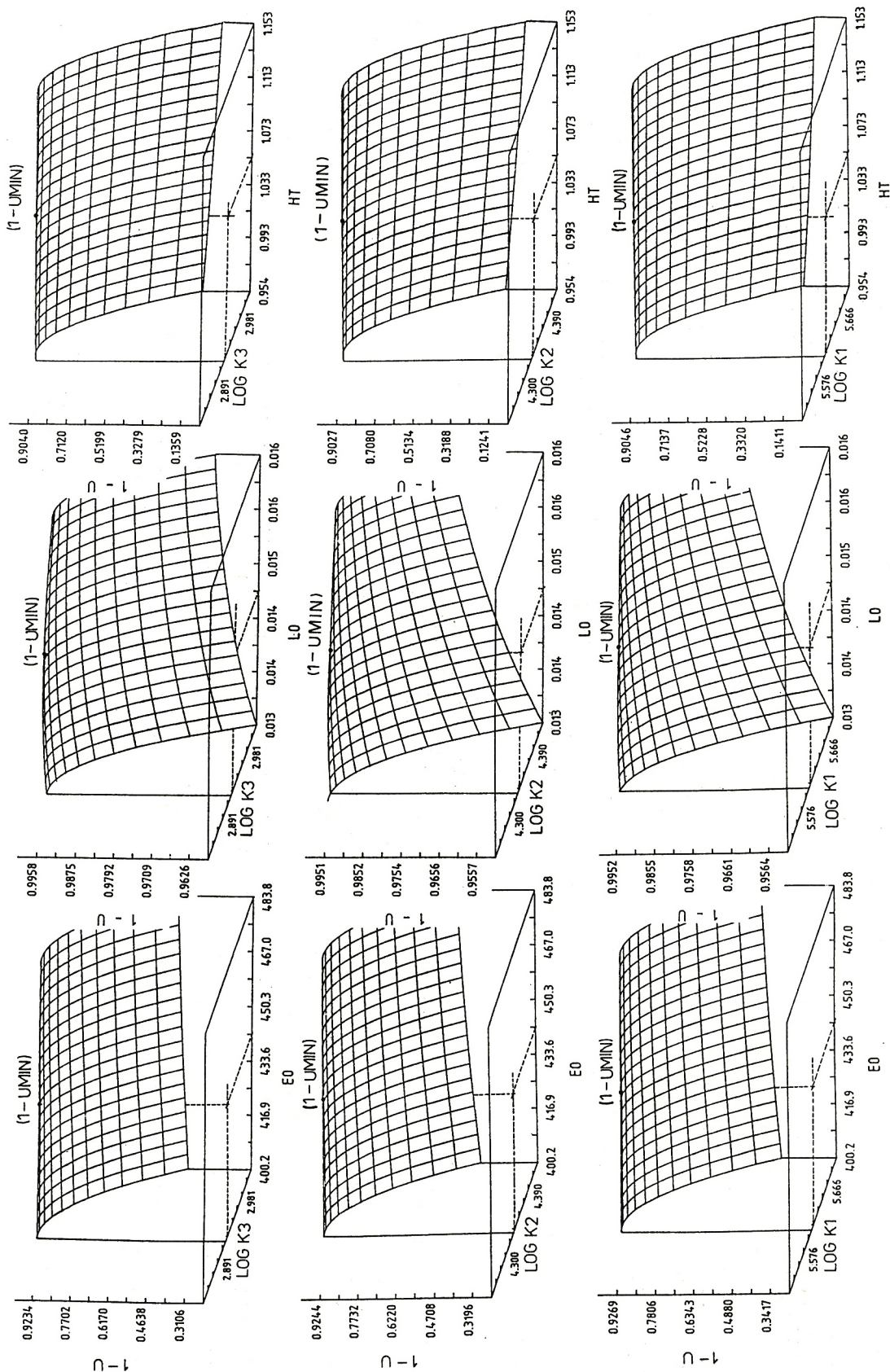
Fig. 4. The minimum of U for 6 parameters [[$\log K_i$, $i = 1, 2, 3$], L_0, H_T, $E^{0'}$] may be represented by a pit in the hyperparaboloid surface in $(6 + 1)$-dimensional space. Three-dimensional representation of the $(1 - U)$ surface is drawn for the ill-conditioned parameters where determination leads to rather uncertain estimates.
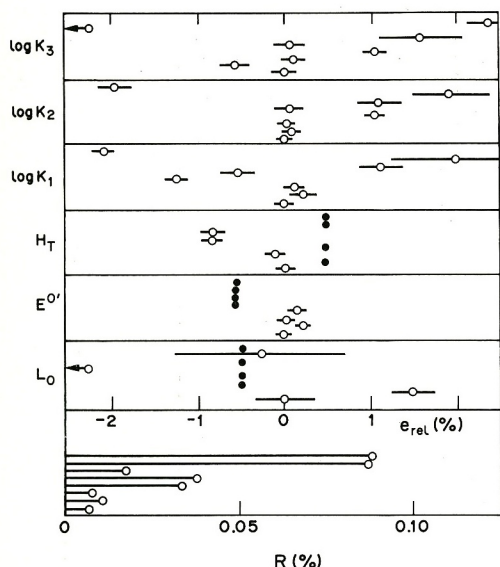
Fig. 5. Influence of group parameters on the relative systematic error defined by $e_{rel} = e (\log K)/\log K$ [cf. equation (7)]. In each part the uppermost points refer to runs 1, 2, down to 8. Parameters kept constant are marked (●). The circles (○) express the accuracy, and the linear segment the precision of each parameter. Run 1: no group parameters refined. Run 2: L_0 refined. Run 3: H_T refined. Run 4: L_0 and H_T refined. Run 5: $E^{0'}$ refined. Run 6: H_T and $E^{0'}$ refined. Run 7: L_0 and $E^{0'}$ refined. Run 8: L_0, H_T and $E^{0'}$ refined.

CONCLUSIONS

Three kinds of errors in determination of protonation constants have been investigated: error from the minimization strategy, the random error, and error from group parameters. Of these the group parameters have the greatest influence on the systematic errors in $\log K_i$. By variation of one or two etc. of the parameters such as $E^{0'}$, L_0 and H_T, the systematic errors in the $\log K$ values can be minimized or practically disappear. Of the group parameters studied, $E^{0'}$ has the greatest influence and an attempt should always be made to refine this parameter together with the formation constants, in spite of the fact that it might be ill-conditioned and therefore make the computational strategy (i.e., choice of program) important.

Of the programs tested, ESAB and ACBA are the most powerful, because they permit refinement of the group parameters. A very interesting program for the same purpose is SUPERQUAD,[7] which we intended to include in this comparison, but could not manage because the program was never received.

good maximum is obtained for the three protonation constants, whereas various group parameters are ill-conditioned in relation to each other.

Various sets of synthetic data were constructed by using random errors generated so that they should have a normal distribution, and different values were assumed for the instrumental error, $s_{inst}(E_{cell}) = 0.04$, 0.28, 2.80 mV, etc. Refinement of these simulated data gave residuals which did not show any systematic trends. Figure 5 shows the influence of various group parameters on the $\log K$ values obtained with $s_{inst}(E_{cell}) = 0.04$ mV. Group parameters, which were not refined, were kept constant at values about 0.5% from the true ones.

In the first runs there was refinement of one or more of the group parameters, and the common parameters then always had a systematic error of 1–2%. In the sixth and seventh runs two group parameters were refined and the systematic error decreased to about 0.1%. In the eighth run all three group parameters were refined and the systematic error in $\log K_i$ was practically zero. The goodness-of-fit, as indicated by the Hamilton R-factor, decreased each time and became smallest, cf. Fig. 5, for run 8.

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