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Use of the computer program EQUIL to estimate pH in model solutions and human urine

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Summary. The computer program EQUIL was designed to calculate relative supersaturations of solute components of common urinary stones. In an extended software version, quantitative consideration of charge balance for a priori or a posteriori pH estimation was added. The reliability of this computation was tested with hydrogen ion titration of buffer solutions containing HEPES [N-(2-hydroxyethyl)piperaizine-N'-ethanesulfonic acid] as well as samples of normal human urine. In the model solutions with HEPES, the difference between calculated pH values and the measured pH was smaller than 1.2% for any titration step within the buffer zone (pH 8.5-6.8). The pH values calculated for whole urine differed from the measured pH by 7% to 53%, and the calculated charge inbalance ranged from 2.6 to 9.6 mM. This net cation inbalance indicates that there is a need to account for other anionic components, including hippurate, amino acids, and isocitrate. In experimental solutions, charge balance calculations with EQUIL can be of great utility because they permit a priori estimation of pH or computation of the composition at a desired pH.

Key words: EQUIL – pH computations – Titrations – HEPES – Urine

Introduction

The computer program EQUIL has received increasing interest for experimental and clinical applications in urolithiasis research [7, 11, 14]. EQUIL is mainly used to calculate the relative supersaturations with respect to the common urinary stone salts. Relative supersaturation is defined as the ratio between activity product for each stone salt in solution and the solubility product of the salt. After assignment of pH and the total concentrations of the components in a solution, EQUIL derives the activities by numerically accounting for mass action, mass balance, and solution electrolyte behavior. Equilibration experiments with a variety of experimental solutions have already confirmed both the high accuracy and validity of such calculations [14]. Nonetheless, we are motivated to expand the computational versatility of EQUIL with respect to charge balance, temperature dependence, and range of chemical species. Accordingly, calculation of charge balance as the sum of all ionic concentrations was added to the program. Consideration of charge balance promises to be useful for a priori estimation of pH in an experimental solution and for testing the accuracy of the calculations. In well-defined hypothetical solutions, the calculated charge balance will be zero at any measured pH. With this preservation of overall electric neutrality, calculation of pH should be possible, if the total concentrations of all species are provided.

We performed titrations and computer calculations of pH with HEPES buffer [N-(2-hydroxyethyl)piperazine-N'-ethanesulfonic acid] [13] and human urine. HEPES buffer was chosen as a model solution with total concentrations and formation constants known for all components. The human urine experiments were performed to gauge the extent to which charge balance is governed by those low molecular components frequently measured clinically for the calculations of relative supersaturations [11, 14]. Such data yield estimates of driving forces favoring crystallization of calcium oxalate, apatite, brushite, struvite, and uric acid in stone patients.

Materials and methods

Reagent-grade chemicals were used without further purification. HEPES sodium salt, purchased from Sigma Chemical Co., was

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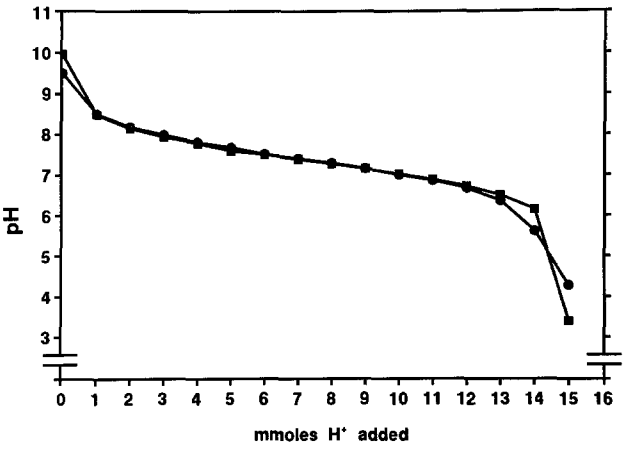


Fig. 1. Titration of 150 ml 0.10 M HEPES with 1.00 N HCl, 35°C (● measured pH, ■ calculated pH)

dissolved in deionized water to make 0.10 M buffer solutions which were kept under nitrogen in a stoppered flask at room temperature and used within a few hours. Water obtained from a Milli-Q-Reagent water system had a resistance of more than 10 MOhm. We used 1.00 N solution of hydrochloric acid from Fisher Scientific as titrant.

Freshly voided midstream urine portions were collected from three healthy men and processed individually. pH was measured immediately at 37°C with a glass electrode and a PHM 64 research pH meter (Radiometer, Copenhagen). An aliquot of each urine portion was refrigerated under nitrogen without further treatment until colorimetric analysis for uric acid [6] and coulometric analysis for chloride. The remainder of the urine portions were acidified with 1.00 N HCl to a pH of 1–2 and then refrigerated until further analysis within a few days. We used an atomic absorption spectrophotometer (Perkin-Elmer Model 306, Norwalk, Connecticut) to measure sodium, potassium, magnesium, and calcium; colorimetric techniques to measure oxalic acid [8], citric acid [9], ammonia [3], and phosphorus [12]; and turbidimetry to measure inorganic sulfate [1].

Titrations

150 ml of the 0.10 M HEPES solution were pipetted into a 250-ml jacketed beaker kept at the experimental temperature with a circulating bath (Forma Scientific, Marietta, Ohio). Nitrogen flowed over the solution throughout the experiment. HCl (1.00 N) was added automatically with an ABU 80 autoburette and a TTT 80 titrator (Radiometer, Copenhagen) at a titration speed of 1.264 ± 0.002 (mean ± SD) ml/minute until the pH dropped below 5. pH measurements were recorded continuously with a REC 80 Servograph (Radiometer, Copenhagen). During the titration, the solution was stirred with a magnetic bar and temperature was monitored with a YSI Tele-thermometer (Yellow Springs Instruments Co., Yellow Springs, Ohio). Titrations were performed at 15°C, 25°C, 35°C, and 45°C. For every temperature, the pH meter was calibrated with commercially prepared buffer standards immediately before titration. The three urine specimens were titrated in the same manner at 37°C until the pH dropped below 2. The titration volume was 150 ml in samples 1 and 3, but 75 ml in sample 2. For comparison and calculation of the titration curves, the volume of sample 2 was converted arithmetically to 150 ml.

Calculations

pH values were evaluated with the ion speciation program EQUIL at the four temperatures over the appropriate titration range. The concentrations of HEPES, sodium, and chloride were adjusted for titration steps of 1 ml each and then entered into the program with a variety of guessed pH values. By iterative calculations, the value giving a calculated charge balance of zero was found and was defined as the calculated pH. For the computation of the HEPES data, the temperature dependence of the formation constant (K_2) for HEPES was taken into account. Considering mass action and mass balance, the following equations were included in EQUIL:

$$K_2 = [\text{H-HEPES}]/([\text{HEPES}^-][\text{H}^+]) \quad (1)$$

$$[\text{total H}] = [\text{H}^+] + [\text{H-HEPES}] \quad (2)$$

$$[\text{total HEPES}] = [\text{HEPES}^-] + [\text{H-HEPES}] \quad (3)$$

The ionic strength (I) was calculated as followed:

$$I = 1/2([\text{HEPES}^-] + [\text{H}^+] + [\text{Na}^+] + [\text{Cl}^-] + [\text{OH}^-]) \quad (4)$$

We should note that EQUIL is designed to compute the relevant activity coefficients (f_i) for the i-th component using the Davies [4] modification of the Debye-Hückel approximation:

$$-\log f_i = 1.202 z_i^2 [I^{1/2}/(1 + I^{1/2}) - 0.286I] \quad (5)$$

z_i is written for ionic charge.

Calculations for the three human urine specimens were performed at 37°C in the same manner with species concentrations corrected for dilution due to acidification.

Results

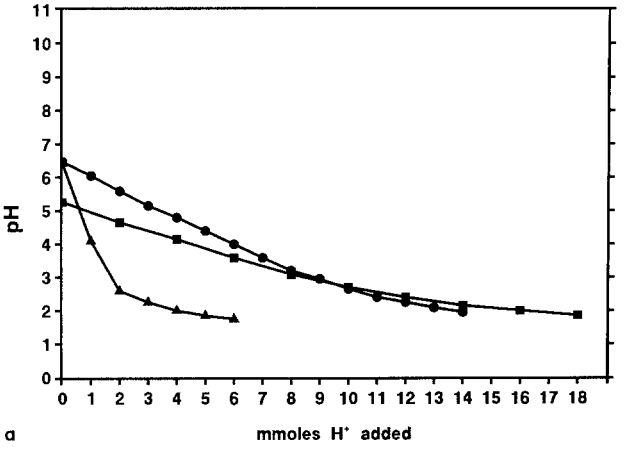
Model solution behavior

Unlike typical pH titration calculations using pK values alone, we were motivated to analyze the feasibility of ion charge balance calculations to estimate pH. In this regard, the titration experiments provide a loci of points representing a broad range of ionized and protonated species for such charge balance calculations. HEPES solutions were chosen to probe such model solution behavior. In particular, we wanted to work with a buffer that was maximally effective in the pH range from 6.5 to 8.5. This sulfonic acid derivative exhibits two pK's, with the second pK (pK_2) of 7.565 at 25°C [13]. As shown in Fig. 1, the calculated and measured pH titration curves at 35°C for 0.10 M HEPES solution were virtually superimposable. Indeed, over a range of 1.5 pH units, the measured and calculated values differed by less than 1.2% for any titration step at the four temperatures (Table 1). This finding indicated that the charge balance subroutine in EQUIL can yield reliable estimates of a solution's acid-base titration properties.

Table 1. Titration of 0.10 M HEPES with 1.00 N HCl between pH 8.5 and 6.8

Temp. (°C)	Δ pH (%)[a]	*N*
15	0.15 ± 0.20	11
25	−0.03 ± 0.22	11
35	0.26 ± 0.38	11
45	0.68 ± 0.40	11

[a] Difference between measured and calculated pH as percent from the measured values. Mean ± SD



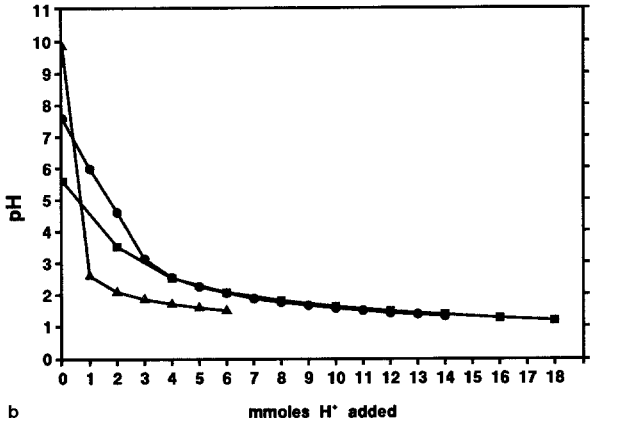


Fig. 2a, b. Titration of 150 ml human urine with 1.00 N HCl, 37°C (● sample 1, ■ sample 2, ▲ sample 3). **a** Measured pH. **b** Calculated pH

Titration experiments with whole urine

We chose next to extend these charge balance computations and titrations to consider solutions containing many more ionic species. Urine offers such an opportunity because EQUIL was designed to account for relative supersaturations with respect to the common urinary stone salts. The experimental pH titrations and calculated pH profiles for three urine samples are shown in Fig. 2. For these calculations, we used the analytical compositions listed in the footnotes of Table 2. These three urine samples displayed a range of buffer capacity, and the observed and calculated values of initial pH for each are also listed in Table 2. Sample 2 yielded pH values differing by about 7%, whereas less gratifying results were obtained for samples 1 and 3. In these cases, the discrepancy was a high as 53%. The difference in the experimental and calculated pH values could be eliminated if there were a means to account for the tabulated ion charge imbalances of 2.6 to 9.6 mM. Thus, these observations suggest that other ionic species, beyond those incorporated in EQUIL, must contribute to the overall conservation of electrolyte neutrality.

Table 2. Calculations of initial pH in human urine specimens

Sample	Measured pH	Calculated pH	Charge Inbalance (mM)
1[a]	6.476	7.58	+9.6
2[b]	5.263	5.61	+2.6
3[c]	6.425	9.86	+3.3

[a] Analytical composition (total molar concentrations): sodium = 6.18×10^{-2}; potassium = 8.98×10^{-2}; calcium = 2.27×10^{-3}; magnesium = 3.00×10^{-3}; ammonia = 3.00×10^{-2}; phosphate = 1.62×10^{-2}; sulphate = 1.15×10^{-2}; oxalate = 4.37×10^{-4}; citrate = 2.83×10^{-3}; urate = 3.68×10^{-3}; chloride = 9.40×10^{-2}

[b] Analytical composition (total molar concentrations): sodium = 4.36×10^{-2}; potassium = 1.21×10^{-1}; calcium = 8.46×10^{-3}; magnesium = 4.81×10^{-3}; ammonia = 3.93×10^{-2}; phosphate = 2.03×10^{-2}; sulphate = 1.85×10^{-2}; oxalate = 9.01×10^{-4}; citrate = 6.02×10^{-3}; urate = 4.64×10^{-3}; chloride = 1.13×10^{-1}

[c] Analytical composition (total molar concentrations): sodium = 6.90×10^{-2}; potassium = 1.87×10^{-2}; calcium = 8.28×10^{-4}; magnesium = 8.23×10^{-4}; ammonia = 1.01×10^{-2}; phosphate = 2.05×10^{-3}; sulphate = 1.98×10^{-3}; oxalate = 1.27×10^{-4}; citrate = 6.28×10^{-4}; urate = 1.04×10^{-3}; chloride = 7.80×10^{-2}

Discussion

While maintenance of overall charge neutrality is implicit in all electrolyte solution behavior, the results represented in this report indicate that such maintenance of neutrality offers a requisite constraint in calculating pH. Any detailed account of ion charge balance must ensure that

$$\sum_{i=1}^{n} z_i[X_i^{-z_i}] = \sum_{j=1}^{m} z_j[Y_j^{+z_j}]$$

Table 3. Other anionic species in human urine beyond those incorporated in the EQUIL software

	Net charge	Concentration (mM)	Ionic Concentration (mM)
Amino Acids [5]:			
Arginine	+1	0.13	+0.13
Aspartate	−1	0.51	−0.51
Glutamate	−1	1.45	−1.45
Histidine	+0.5	1.50	+0.75
Lysine	+1	0.36	+0.36
Hippurate [5]	−1	3.55	−3.55
Isocitrate	−2.5	0.14	−0.35
Sum:			−4.62

where the z_i and z_j represent the number of unit charges characteristic of each ionic species, and the square brackets denote molar concentrations. While the EQUIL software includes a broad range of acid-base reactions, we now consider some of the factors that can make the EQUIL software even more representative of human urine samples.

With the EQUIL software, a reliable prediction of pH seems to be possible for well-defined solutions with one component or a combination of buffers as long as the pH is within 1.5 units of a pK. Outside that pH range, accuracy of the calculated estimates decreases sharply. In the case of HEPES, the acid-base behavior might be influenced by the second basic nitrogen atom and the sulfonate group which is not considered in the computation [13]. Application of charge-balance computations of pH for acidified human urine, however, do not yield good pH estimates. Quite obviously, urinary pH is affected by various substances not currently included in this analysis. The net cation charge inbalance computed for urine indicates that the computer program EQUIL should be expanded to account for other anionic substances. For example, more than twenty species of amino acids are excreted in human urine [5]. At pH 6.0, five amino acids (i.e., arginine, aspartate, glutamate, histidine, and lysine) have side-chains in ionic form. From their mean concentrations and side-chain ionizable group dissociation constants, an anionic charge concentration of 0.7 mM can be estimated for this pH (Table 3). Moreover, the mean urinary excretion of hippurate is 700 mg/24 h [5], which results in an additional 3.5 mM of anionic species. Of further note is isocitrate, a metabolite that is not determined with the colorimetric technique used for citrate [9]. In 14 stone-formers, gas-chromatographic-mass-spectrometric analysis yielded an urinary excretion of isocitrate of 0.14 mM (William C. Thomas, Jr., unpublished findings). This corresponds to about 0.35 mM anionic charge from isocitrate alone. By contrast, normal urine contains only about 70 mg/l proteins with 38% albumin and 47% α-globulin as main components [2]. From hydrogen ion titration data for serum albumin [10], we estimate a net charge of −13 per mole albumin, resulting in an anionic concentration of 5 μM in urine. Therefore, just a minimal part of the calculated charge inbalance in our urine specimens can be explained by proteins. Far more important are hippurate, several amino acids, and isocitrate which together account for about 5 mM (Table 3). This value lies in the range of the computed net cation charge inbalance (Table 2), suggesting that a detailed ion balance would bring observed and calculated pH values into good agreement.

In addition to calculating initial solution pH values, we have also computed the titration curves for urine samples. Clearly, phosphate and citrate are major contributors to buffer capacity in the urinary pH range, but other weak acids and bases may play a role in bringing the computed buffer capacity closer to that experimentally observed. A major part of the buffer capacity could be explained with the amino acids, hippurate, and isocitrate. The overall concentration of carboxyl groups from amino acids is estimated as 10 mM and from hippurate as 3.5 mM [5]. Titrations of model solutions seem to be useful to study the impact of a variety of substances on the charge balance in urine, but further investigations are obviously warranted.

Finally, we should note that in the preparation of experimental solutions, pH often is adjusted by adding acid or base which sometimes leads to adverse dilution. Urine often is acidified during collection to prevent precipitation of calcium phosphates, production of oxalic acid in vitro, or bacterial overgrowth. On an outpatient basis, pH measurement of freshly voided urine is cumbersome. For both purposes, the development of methods for a priori or a posteriori calculation of pH would therefore represent a major improvement. Consideration of overall charge balance may afford an additional route for achieving that goal.

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