Spectrophotometry of Stability Constant of CaC_2O_4 Based on Competition Between Murexide and Oxalate for Ca^{2+}

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By measuring the change in optical density caused by adding oxalate to solutions containing calcium and murexide, the stability constant of calcium oxalate was estimated at 2341 ± 60 (SEM) M^{-1}.

Although calcium oxalate (CaC_2O_4) is a common biologic substance and a major cause of urolithiasis, the stability constant of the calcium oxalate complex in aqueous systems has not been satisfactorily established. The literature concerning this stability constant is inconsistent. Scholder (*6*) reported two measurements of the conductivity and concentration of saturated calcium oxalate solutions at 18°C. From these measurements, Money and Davies (*4*) calculated a stability constant of 1330 M^{-1}. However, Money and Davies adopted the value of 1000 M^{-1} for the stability constant of calcium oxalate because of the large disparity between Scholder's two measurements.

The London Chemical Society compilation of stability constants (*3*) lists a value of 1000 M^{-1} at 25°C in 0.1*M* sodium chloride, citing as the source Gelles and Salama (*2*), who in turn cite Money and Davies as their source. Recently, Finlayson et al. (*1*), using calcium oxalate solubility measurements, estimated the thermodynamic stability constant of calcium oxalate at 2741 M^{-1}. In this report, a spectrophotometric technique was used to estimate the stability of calcium oxalate at 38°C.

Materials and Methods

For the set of observations made each day, 4000 ml of a working solution were prepared freshly and contained the following: ~0.1 m*M* murexide (obtained from Eastman Chemical Co. and Fisher Scientific Co. and used without further purification), ~200 m*M* sodium chloride (commercially prepared, reagent grade), ~100 m*M* trishydroxyaminomethane (Tris) (commercially prepared, reagent grade), and ~28 m*M* hydrochloric acid (commercially prepared, reagent grade). The final pH was ~8.2. This working solution was used as a solvent to prepare the following solutions: ~40 m*M* potassium oxalate (commercially prepared, reagent grade), ~40 m*M* calcium chloride (commercially prepared, reagent grade), and ~135 m*M* calcium chloride. Solutions were prepared in stoppered bottles to minimize absorption of atmospheric carbon dioxide.

Calcium, potassium, and sodium were measured with atomic absorption spectrophotometry by our own and commercially prepared standards. Analysis for potassium was used to standardize the oxalate solution. The pH was measured at 38°C with a glass-electrode pH meter that had been calibrated with commercial buffers.

The competition studies were made by placing 200 ml of working solution in a flask maintained at 38°C with a thermostating water jacket and by mixing with a magnetic stir bar. The flask was connected to a continuous-flow cell in a Gilford-modified Beckman D.U. spectrophotometer. Continuous flow was produced with a peristaltic pump. The flow cell was maintained at 38°C, and the connecting lines were thermally insulated. Increments of potassium oxalate or calcium chloride were introduced by adding 1 ml or less of the appropriate solution to the flask. Light absorption was measured at 475 nm. The electronic scale expander was set so that a full-scale deflection of the strip chart recorder (25.2 cm) equaled 0.1 optical density (OD) unit.

To ensure that adding oxalate to the working solutions would cause no change in optical density, it was necessary to soak the equipment in dilute hydrochloric acid overnight before use. Measurements of oxalate competition were obtained by adding 1 ml of ~40 m*M* calcium chloride solution to the flask, recording a base line value, and while the recorder continued running, adding 1-ml increments of ~40 m*M* potassium oxalate solution (up to a maximum of four increments). The system was allowed to stabilize for more than 20 system half-lives ($t_{1/2}$ = 10 sec) after each increment. The flask was rinsed and charged two or three times each day. At the end of the competition studies, the flask was charged with working solution, and 0.1-ml increments of ~135 m*M* calcium chloride solution were added to calibrate the murexide. Calibration measurements of murexide were analyzed as described by Walser (*7*).

Optical density perturbations caused by the competition of oxalate with murexide for calcium were analyzed with a nonlinear least-squares fitting program to give an estimate of the stability constant for the CaC_2O_4 complex.

Results and Discussion

A typical record showing the optical density change owing to adding oxalate to the calcium murexide systems is presented in Figure 1. Results of the oxalate–murexide competition studies are listed in Table I, with the associated measurements needed to estimate the stability constant of the calcium oxalate complex. In principle, analysis of the results requires consideration of at least five complexes, which are defined by the following mass action equations:

$$K_1 = [CaC_2O_4]/([Ca^{2+}][C_2O_4^{2-}]F^2) \quad (1)$$

$$K_2 = [Ca_2C_2O_4^{2+}]/([Ca^{2+}][CaC_2O_4]) \quad (2)$$

$$K_3 = [Ca(C_2O_4)_2^{2-}]/([CaC_2O_4][C_2O_4^{2-}]) \quad (3)$$

$$K_4 = [NaC_2O_4^{-}]/([Na^{+}][C_2O_4^{2-}]F) \quad (4)$$

$$K_5^* = [\text{Ca·murexide}]/([Ca^{2+}][\text{murexide}^{2-}]) \quad (5)$$

In Equations 1–5, square brackets denote concentration, and F is the activity coefficient for a single divalent ion; the asterisk indicates that K_5^* is not a thermodynamic stability constant. By combining Equations 1–5 with conservation of mass equations, a simultaneous set of spanning equations may be derived:

$$[Ca^{2+}] = \text{total } [Ca]/(1 + K_1F^2[C_2O_4^{2-}] + 2K_1K_2[Ca^{2+}][C_2O_4^{2-}] + K_1K_3[C_2O_4^{2-}]^2 + K_5^*[\text{murexide}^{2-}]) \quad (6)$$

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Table I. Average Change in Optical Density Listed by Increment Number for Each Day's Determination

Day	n^a	Increment no.	$(\langle\Delta OD\rangle \pm SD) \times 10^3$
1	2	1	-1.725 ± 0.002
	3	2	-1.583 ± 0.010
	3	3	-1.726 ± 0.042
	1	4	-2.270 ± 0.018
2	2	1	-3.120 ± 0.001
	2	2	-2.960 ± 0.039
	2	3	-2.725 ± 0.130
3	4	1	-2.300 ± 0.313
	4	2	-1.725 ± 0.023
	4	3	-1.825 ± 0.016
4	4	1	-1.310 ± 0.005
	4	2	-1.450 ± 0.010
	4	3	-1.265 ± 0.010
5	3	1	-1.533 ± 0.214
	3	2	-1.470 ± 0.091
	3	3	-1.640 ± 0.101
	3	4	-1.797 ± 0.027
6	3	1	-2.140 ± 0.443
	3	2	-2.170 ± 0.409
	3	3	-1.987 ± 0.236
	2	4	-1.885 ± 0.018

[a] Number of increments used in the average.

$$[C_2O_4^{2-}] = \text{total } [C_2O_4]/(1 + K_1F^2[Ca^{2+}] + K_1K_2[Ca^{2+}]^2 + 2K_1K_3[Ca^{2+}][C_2O_4^{2-}] + K_4F[Na^+]) \quad (7)$$

$$[Na^+] = \text{total } [Na]/(1 + K_4F[C_2O_4^{2-}]) \quad (8)$$

$$[\text{murexide}^{2-}] = \text{total } [\text{murexide}]/(1 + K_5^*[Ca^{2+}]) \quad (9)$$

$$T^2 = (4([Ca^{2+}] + [C_2O_4^{2-}] + [Ca_2C_2O_4^{2+}] + [Ca(C_2O_4)_2^{2-}]) + [Na^+] + [Cl^-] + [H\cdot Tris^+])/2 \quad (10)$$

$$F = \exp\left[-4.808\left(\frac{T}{1+T} - 0.286\,T^2\right)\right] \quad (11)$$

Equations 6–11 can be solved iteratively and were used as a function to calculate calcium–murexide complex concentration in a nonlinear least-squares fitting routine to estimate K_1. The optical density change caused by oxalate was found by computing [calcium–murexide] before and after addition of an increment of oxalate to the flask and multiplying the difference by $\Delta OD/\Delta$ [calcium–murexide], the latter having been obtained from the murexide calibration. Recent solubility studies (*1*) indicate $K_2 = 71.6\ M^{-1}$. Our analysis of data by Nydahl (*5*) resulted in the estimate $K_3 = 6.1\ M^{-1}$ at 25°C; this value was used as representative of 38°C. (The effect of using these values of K_2 and K_3 is to increase the estimate of K_1 by 1.7% compared with its value of $K_2 = K_3 = 0$.) The value of K_4 has been estimated (*1*) at $13.3\ M^{-1}$.

Table II. Concentrations of All Solutions Used (Listed by Day) and Pertinent Data Calculated from Calibration of System[a]

Day	[Na]	[Tris]	pH, working solution	[Murexide]	$[Ca]_1$	$[Ca]_2$	$[C_2O_4]$	K_5^*	ΔOD_∞[b]	K_1 ($\times 10^{-3}$)
1	0.2040	0.1004	8.24	9.32×10^{-5}	0.0420	0.111	0.0490	513.9	0.746	2.412
2	0.1270	0.1010	8.25	6.79×10^{-5}	0.0355	0.127	0.0121	702.0	0.452	2.388
3	0.2050	0.1003	8.16	9.09×10^{-5}	0.0374	0.134	0.0427	500.8	0.672	4.311
4	0.2008	0.1005	8.35	8.60×10^{-5}	0.0366	0.130	0.0440	401.3	0.915	2.161
5	0.2011	0.1002	8.29	8.50×10^{-5}	0.0350	0.114	0.0410	292.4	1.440	2.497
6	0.2006	0.1001	8.27	1.25×10^{-4}	0.0336	0.109	0.0426	724.5	0.995	2.246
								Average $K_1 = 2.669 \pm 0.332$ (SEM)		

[a] Concentrations are molar. [b] Ref. 6.

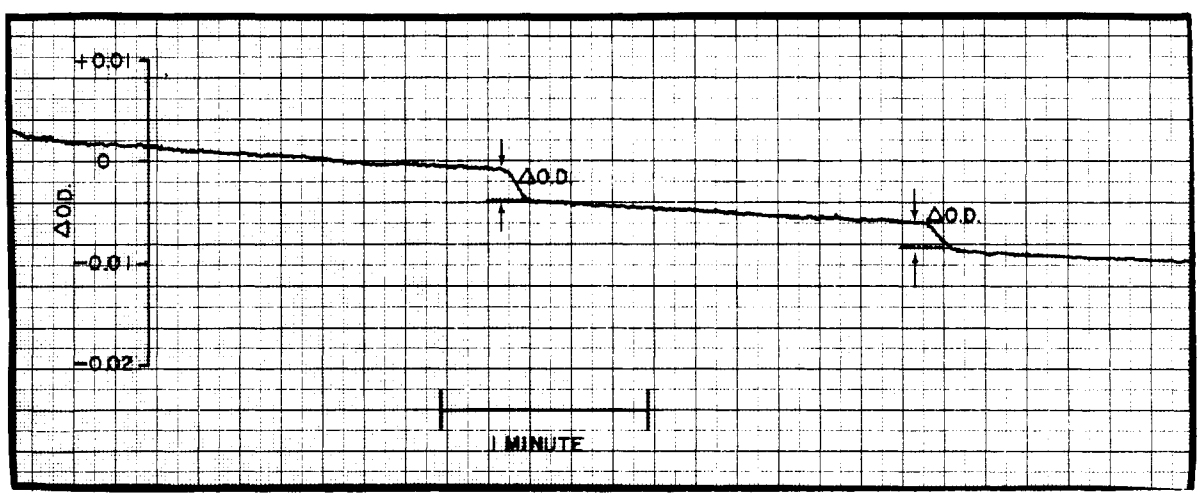

Figure 1. Typical strip chart record from an oxalate–murexide competition study

Table I shows the daily average ΔOD resulting for each oxalate increment in a sequence of oxalate increments. There is no obvious correlation between sequence position and ΔOD. A least-squares fit was made with each day's set of observations to give a fitted estimate of K_1, as shown in Table II. (Individual optical density changes were used to make the estimates instead of average optical density changes.) An average of the values of K_1 in Table II is 2669 ± 332 (SEM) M^{-1}, if the average of the different days' sets of measurements are given equal weight. Entry 3 in Table II is more than three standard deviations from the mean; excluding it lowers the average to 2341 ± 60 (SEM) M^{-1}, which we take as the estimate of K_1 resulting from this study. This stability constant, estimated by oxalate and murexide competition, is 15% less than the 2741 M^{-1} estimated by calcium oxalate solubility (*1*). The two estimates of the stability constant of calcium oxalate are essentially in agreement, and the difference between them is probably due to error in measuring the 11 measurements necessary in these competition studies.

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Diffusion and Viscosity in $CHCl_3$–CH_3COOH System at 25°C

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Sets of mutual diffusion coefficients and viscosity data are presented for the chloroform–acetic acid system at 25°C. The experimental data are compared with those given by Leffler's equation for diffusion coefficients. Equations for fitting density, refractive index, and viscosity data are given.

Sets of mutual diffusion coefficients and viscosity data are presented for the chloroform–acetic acid system at 25°C. The experimental data fit Leffler's equation (*5*) for diffusion coefficients in the range of dilute chloroform solutions by assuming that acetic acid is a dimer.

Experimental

Materials. Freshly distilled, reagent-grade acetic acid (A) (C. Erba, Milan) was used. The chloroform (C), reagent grade (C. Erba, Milan), was shaken several times with double-distilled water (ratio 1:2) to eliminate ethyl alcohol, kept over anhydrous calcium chloride for a few hours, and then passed through a basic alumina column to eliminate the last alcohol traces. It was used immediately for diffusion or viscosity runs. Purity was controlled by gas chromatography.

Solutions. All solutions for diffusion and viscosity runs were made by weighing both components. No correction for the weighing in vacuum was applied.

Viscosity. Viscosity measurements were made at 25° ± 0.01 by using an Ubbelhode microviscometer (volume ≃ 1 ml; running times: water, 243.6 sec; C, 97.8 sec; A, 294.4 sec; water viscosity was taken from "Handbook of Chemistry and Physics" (*4*), 0.8937 cP at 25°C. No kinetic correction was needed. Experimental results are given in Table I. Densities were computed from the data of Campbell et al. (*2*) fitted with the following equation:

$$d_{25} = 1.04405 + 0.56191\,X_1 - 0.16317\,X_1^2 + 0.03782\,X_1^3 \pm 0.00065 \quad (1)$$

where X_1 is the stoichiometric mole fraction of chloroform.

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The experimental viscosity data have been fitted with the following equation:

$$\eta = 1.1442 - 1.4576\,X_1 + 1.8221\,X_1^2 - 1.5190\,X_1^3 + 0.5435\,X_1^4 \pm 0.0046 \text{ cP} \quad (2)$$

Diffusion. Diffusion experiments were carried out at 25° ± 0.02 by using the Gouy interferometric technique (*3, 8*) with a mercury source lamp (Wratten 11 + Wratten 22 A filters, λ = 546.1 nm). A single-channel cell (*8*) was used with a Teflon-glass stopcock to avoid lubrication problems. The initial boundary was made with the syphoning technique (*3, 8*) at the level of the diffusiometer optical axis. The experimental results are collected in Table II.

From the refractive index increments given in Table II, we have computed the refractive indexes of the system A–C as a function of the C mole fraction. The results are in good agreement with Campbell's data (*2*). The $\Delta n/\Delta X_1$ experimental data fit the following equation:

$$\Delta n/\Delta X_1 = 0.08282 - 0.00380\,X_1 - 0.04562\,X_1^2 + 0.02960\,X_1^3 \pm 0.00055 \quad (3)$$

at λ = 546.1 nm

(The data of runs 8a, 9, and 17a,b were not used in computing Equation 3). By integrating Equation 3, an equation for the refractive index has been obtained which fits Campbell's data:

$$n_{25} = 1.37046 + 0.08282\,X_1 - 0.00190\,X^2 - 0.01521\,X_1^3 + 0.00740\,X_1^4 \pm 0.00020 \quad (4)$$

at λ = 589.3 nm

Campbell's data fit the following equation:

$$n_{25} = 1.37029 + 0.08821\,X_1 - 0.02919\,X_1^2 + 0.02947\,X_1^3 - 0.01551\,X_1^4 \pm 0.00014 \quad (5)$$

Our $\Delta n/\Delta X_1$ data have been measured at 546.1 nm and can fit the refractive index data at 589.3 nm because the integration constant 1.37046 corresponds to the chloroform refractive index at 589.3 nm, and the refractive