[Contribution from the Department of Radiation Biology, School of Medicine and Dentistry, University of Rochester]

The Uranyl–Citrate System. I. Spectrophotometric Studies in Acid Solution[1]

By Isaac Feldman and W. F. Neuman

An analysis of absorption spectra and an application of the method of continuous variations indicate that, from *p*H 2.0 to 6.0, uranyl and citrate ions combine to form complexes of two combining ratios, represented empirically by UR and U_2R, the former ion being the more stable form. The effect of *p*H on the stability of these ions is discussed.

Because of the importance of citrate in uranium toxicology,[2] a program of study of the behavior and characteristics of citrate complexes with uranyl ion was undertaken. The results of polarographic and titrimetric investigations will be reported subsequently. Spectrophotometric data on the uranyl–citrate system in acid solution are reported here.

Experimental

Materials.—All chemicals used were Baker C.P. analyzed grade. The 0.1 *M* stock solution of uranyl nitrate was standardized by the gravimetric determination of uranium after ignition to the oxide, U_3O_8. The solutions of citric acid monohydrate were checked by titration with sodium hydroxide. All solutions were prepared with conductivity water.

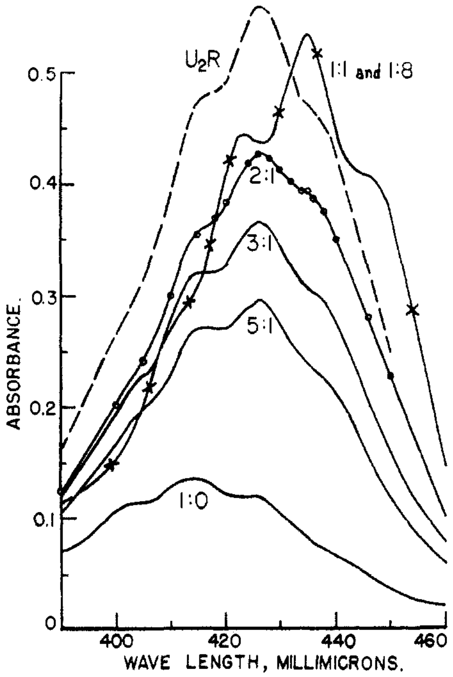

Fig. 1.—Absorption spectra of uranyl salt and of uranyl salt–citric acid mixtures at *p*H 3.43. The numerical ratio associated with each spectrum represents the initial uranium/citrate molar ratio of the solution studied. The curve for the 5:1 mixture was obtained by dividing the absorbance of a 0.025 *M* total uranium solution by the factor two. The hypothetical U_2R spectrum (dashed line) and the open circles which fall on the 2:1 curve were calculated as described in the text. All other curves, including the 2:1, were determined experimentally using mixtures 0.0125 *M* in total uranium. Each solution contained 0.08 *M* sodium chloride.

Procedure.—The solutions under study were adjusted to proper *p*H by small additions of carbonate-free, 50% sodium hydroxide (always less than 1% of the total volume). A Beckman Model G *p*H meter was employed in these adjustments. After standing 18–24 hours (an arbitrary procedure) in the dark at room temperature, each solution was studied in a Beckman model DU spectrophotometer using corex cells of 10 mm. light path to determine the absorbance[3] ($A = \log_{10} I_0/I$) at various wave lengths.

In the subsequent discussion the symbol A^* refers to the absorbance of the indicated mixture or complex ion in a system having a total uranium content of 0.0125 *M*.

Because of partial hydrolysis, the absorbance of uncomplexed uranium does not strictly obey Beer's law under the experimental conditions employed. Hence, its absorbance, A_U, was in every case obtained from a working curve. Beer's law is valid for the absorbance of uranyl chloride in an excess of citrate; therefore, it has been assumed that Beer's law applies to the *undissociated* uranyl citrate complexes described below.

Results

Absorption Spectra.—In Fig. 1 are presented the absorption spectra of uranyl salt (sodium chloride was present to minimize variations in ionic strength; therefore, solutions containing no citrate will be referred to as uranyl salt) and various uranyl salt–citric acid mixtures at *p*H 3.43. The marked changes in absorption spectra produced by the addition of citrate indicate complex formation.

The fact that no changes in the absorption spectrum are observed in lowering the uranium/citrate molar ratio from 1:1 to 1:8 is evidence that at *p*H 3.43 there exist no complexes with a combining ratio of less than unity.

When the uranium to citrate ratio is 2:1, the maxima at 424 and 435 mμ, characteristic of the 1:1 mixture, disappear and a new peak appears at 426 mμ. This curve possesses slight shoulders near 415 and 435 mμ. For the 3:1 and 5:1 mixtures, the maximum at 426 mμ remains dominant. The shoulder beginning at 435 mμ increases in breadth extending to 447 mμ. The 3:1 curve is horizontal from 414 to 419 mμ, where a hump existed in the 2:1 spectrum. This flat portion gives way to a very small, but real, maximum at 416 mμ in the 5:1 curve. In addition, a new hump appears in the vicinity of 403 mμ in the spectra of the 3:1 and 5:1 mixtures.

Examination of the spectrum of uranyl salt, 1:0, aids in interpreting these spectral changes which occur as the citrate concentration is lowered. The 1:0 curve shows a hump in the vicinity of 403 mμ, a peak at 414 mμ, a slope over the range of 415–420 mμ which is opposite in sign to that in the 2:1 curve.

These data are adequately explained by proposing the existence of two combining ratios of uranyl and citrate ions: a 1:1 combination, repre-

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[1] This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

[2] F. L. Haven and C. Randall, *J. Biol. Chem.*, **175**, 737 (1948).

[3] The terminology in this report is that recommended by W. R. Brode, *J. Optical Soc. Am.*, **39**, 1022 (1949).

sented empirically by UR, which dissociates only slightly and a more highly dissociated 2:1 combination designated by the empirical formula, U_2R. Thus, increasing the ratio of uranium to citrate to a value greater than 2:1 does not yield a complex of combining ratio greater than two, but rather produces an effect, through mass-action, on the dissociation of the U_2R. The resulting spectrum is modified, therefore, less by the UR and more by the excess uncomplexed uranium.

A curve for the 2:1 mixture may be calculated to provide a quantitative test of this interpretation. If our explanation is correct, the absorbance, A^*_{UR}, of UR may be obtained directly from the 1:8 curve. Also, the hypothetical absorbance, $A^*_{\mathrm{U_2R}}$, of U_2R can be obtained from the absorbance of the 5:1 mixture when corrected for the contribution of uncomplexed uranium. In this case, negligible dissociation of U_2R is to be expected because of the mass-action effect of excess uranyl ion. The expected absorbance of the 2:1 mixture, $A^*_{2:1}$, is given, then, by the relation

$$A^*_{2:1} = A_{\mathrm{U}} \text{ for } \left(\frac{1-x}{2} \cdot 0.0125\ M\right) + A^*_{\mathrm{U_2R}} \cdot x + A^*_{\mathrm{UR}} \cdot \frac{(1-x)}{2}$$

where x is the fraction of the total uranium in the U_2R form.

The results of such calculations, assuming a value of 0.53 for x, are given in Fig. 1 as open circles. The maximum deviation from observed results was less than 1% over the wave length range, 390–450 mμ. Such consistency lends support to the assumptions made.

The existence of but two combining ratios, a stable UR and a more dissociated U_2R, received further confirmation from an application of the method of continuous variations.[4a,b]

Method of Continuous Variations.—Results of the application of this technique are shown in Fig. 2. The absorbance values were obtained for a series of solutions prepared by mixing uranyl salt and citric acid in such a way that the sum of their molar concentrations remained constant at 0.024 M but the molar ratio, $X = UO_2^{++}/(UO_2^{++} + \text{citric acid})$, varied from zero to one. The pH of each solution was adjusted to 3.48. The ionic strength varied only from 0.10 to 0.13 because of the presence of 0.08 M sodium chloride. The curves in Fig. 2 were obtained by plotting Y, the difference between the observed absorbance value and that which would be given by uranium, if no complexing occurred, *versus* the ratio X.

As expected from theory,[4b,5] at 450 mμ where the ratio $(A^*_{\mathrm{UR}} - A^*_{\mathrm{U}}/(A^*_{2:1} - A^*_{\mathrm{U}})$ calculated from Fig. 1, is greater than 1.33, a sharp peak is obtained at $X = 0.5$ confirming the existence of UR. At 435 mμ the ratio $(A^*_{\mathrm{UR}} - A^*_{\mathrm{U}})/(A/^*_{2:1} - A^*_{\mathrm{U}})$ is also greater than 1.33 and again a peak is obtained at $X = 0.5$ the combining ratio of the 1:1 form. This is evidence that the U_2R complex-type is highly dissociated. Otherwise a peak would have been observed at $X = 0.67$ since $(A^*_{\mathrm{UR}} - A_{\mathrm{U}})/(A^*_{\mathrm{U_2R}} - A^*_{\mathrm{U}})$ is less than 1.33. There is sufficient contribution from U_2R, however, to produce a definite asymmetry in the curve.

The ratio $(A^*_{\mathrm{UR}} - A^*_{\mathrm{U}})/(A^*_{\mathrm{U_2R}} - A^*_{\mathrm{U}})$ is 0.73 at 427 mμ and 0.82 at 418 mμ, and as expected, a peak at $X = 0.67$ is observed at both wave lengths substantiating the existence of the 2:1 ratio, U_2R. At 400 and 390 mμ A^*_{UR} is 30% less than $A^*_{2:1}$ and the presence of the stable 1:1 complex seriously depresses the values for Y in the region

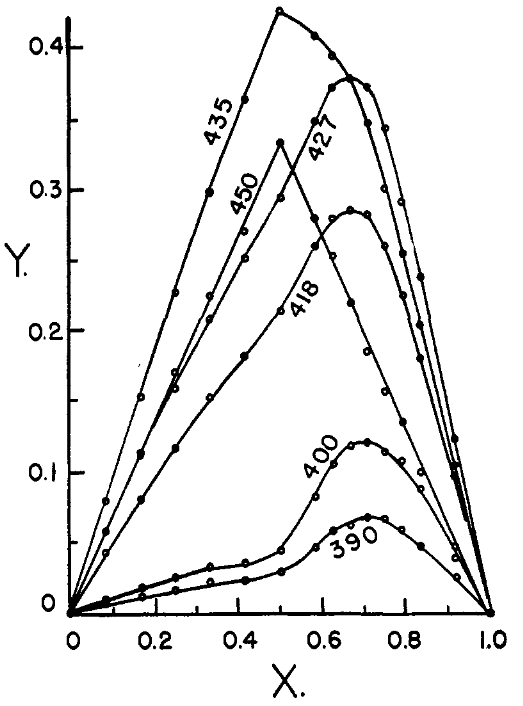

Fig. 2.—Method of continuous variations applied to the uranium–citrate complexes. See text for explanation of graph.

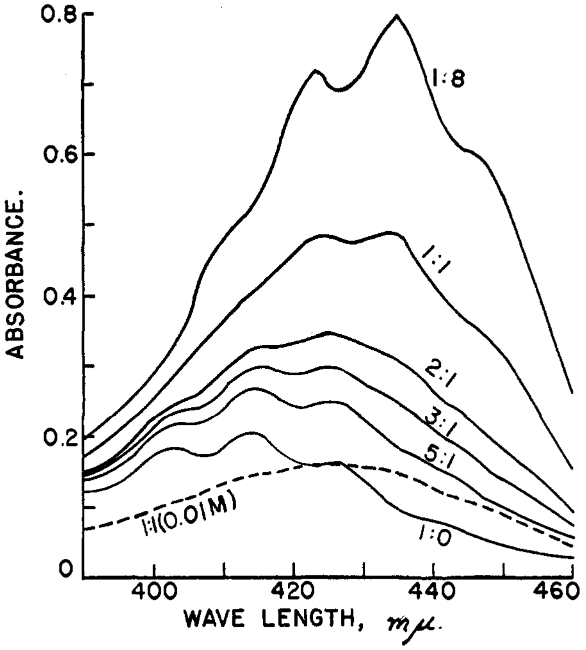

Fig. 3.—Absorption spectra of several uranyl–citrate systems at pH 2. The ratio accompanying each curve represents the initial uranium/citrate molar ratio of the solution studied. The total uranium concentration was 0.01 M for the 1:1 (0.01 M) solution, represented by the dashed-line curve. All other solutions represented by solid-line curves, contained 0.025 M total uranium. Each solution contained 0.08 M sodium chloride.

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[4] (a) P. Job, *Ann. Chem.*, **11**, 97 (1936); (b) W. C. Vosburgh and G. R. Cooper, THIS JOURNAL, **63**, 436 (1941).

[5] The significance of the ratio value of 1.33 is fully discussed by A. L. Underwood, T. Y. Toribara and W. F. Neuman, THIS JOURNAL, **72**, 5597 (1950).

of $X = 0.5$. This interference accounts for the fact that the peaks for U_2R at these wave lengths are displaced to the right, $X = 0.71$, of the theoretical value, $X = 0.67$.

Effect of *p*H.—It has been demonstrated by Dounce[6] that uranyl ion combines only with the ionized form of an organic acid. One should expect, then, that lowering the *p*H would favor dissociation of both UR and U_2R by reducing the concentration of *ionized* citrate in a given mixture. That this is indeed the case is shown by the data in Fig. 3 where absorption spectra of various mixtures of *p*H 2 are given. Here, only the 1:8 curve shows the absorption spectrum characteristic of the UR-combination. The spectra of the other mixtures differ from that given by uncomplexed uranium indicating some complex-formation but the curves do not lend themselves to further analysis.

Spectra of various uranyl–citrate mixtures over the *p*H range 3.0 to 6.0 are given in Fig. 4. At *p*H 3.0, the 1:8 mixture exhibited the same spectral contour as that given by the 1:1 mixture, but showed a 5% greater absorbance. In the *p*H range 3.4 to 4.6, the spectra of the 1:1 and 1:8 mixtures were identical. There were no shifts in the characteristic peaks attributed to UR but there was a general 5% increase in the absorbance as the *p*H rose. Apparently the UR-combination is stable to some extent over the *p*H range 2.0 to 4.6.

Spectra of the 2:1 mixtures, while exhibiting the general contour attributed to U_2R, increased steadily in absorbance as the *p*H rose to 4.64. While not conclusive, this suggests that the U_2R combination is present from *p*H 3.0 to 4.64 but dissociation decreases with increasing *p*H.

Above *p*H 4.6, the spectra of all mixtures showed marked changes as the *p*H rose. The peak absorbance in all cases shifted to higher wave-lengths until at *p*H 6.0, all three mixtures (1:8, 1:1, 2:1) showed an ill-defined, broad peak in the region of 437 to 439 mμ. In addition, there were marked increases in peak absorbance as seen in Fig. 5. This suggests changes in the electronic configuration of the complexed uranium but gives no information relative to combining ratios.

Correlative data, however, are helpful in this regard. As the *p*H is raised to 6.0, *uncomplexed* uranium undergoes hydrolysis with the precipitation of the hydroxide (*cf.* Fig. 5). All three mixtures showed no spectral changes upon centrifuga-

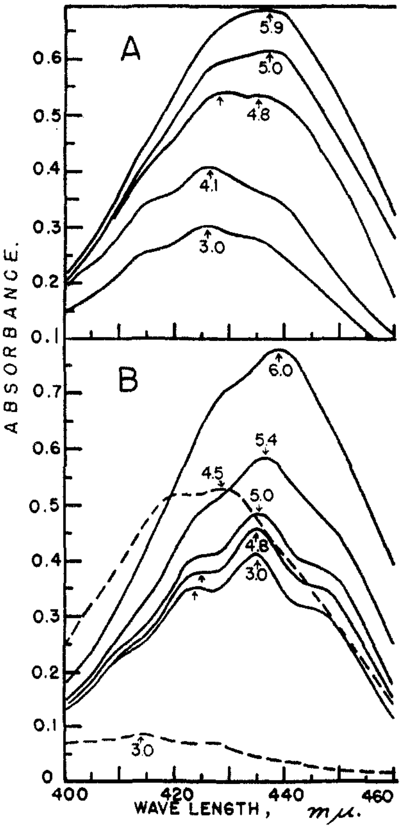

Fig. 4.—Absorption spectra of uranyl-citrate systems in *p*H range 3–6. Graph A contains spectra of 2:1 mixtures. Graph B contains spectra of 1:1 mixtures, solid-lines, and of uranyl salt (1:0) solutions, dashed-line curves. The *p*H of each solution studied accompanies its spectrum. Each solution contained 0.01 *M* total uranium and 0.08 *M* sodium chloride.

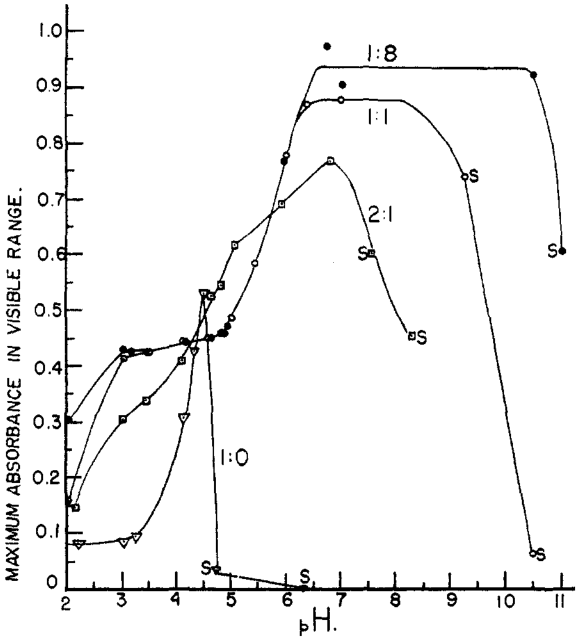

Fig. 5.—Effect of *p*H on maximum absorbance in visible range by several uranyl–citrate systems. The numerical ratio accompanying each curve represents the initial uranium/citrate molar ratio in the solution studied. Each solution contained 0.01 *M* total uranium. The 1:1, 2:1 and 1:0 mixtures contained 0.08 *M* sodium chloride. *Each* mixture was centrifuged at 16,000 × gravity for 30 minutes. When a precipitate was obtained, the supernatant liquid was studied; this is denoted on the graph by the letter *S*. The absorbance at each point was measured at the wave length of the highest peak of the appropriate spectrum; see Figs. 3 and 4. Points above *p*H 6 were taken at 440 mμ because above *p*H 6 the wave lengths and absorbances at the maxima were reproducible to only about two millimicrons and 5%, respectively.

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(6) A. L. Dounce, p. 65, in "The Pharmacology and Toxicology of Uranium Compounds," edited by C. Voegtlin and H. C. Hodge, McGraw-Hill Book Co., Inc., New York, N. Y., 1949.

tion at 16,000 times gravity for 30 minutes. In addition, the uranium in these mixtures was completely dialyzable. This is good evidence that the uranium in all three mixtures was almost completely complexed.

Since the 1:1 and 1:8 mixtures gave *identical* absorption spectra, it is not likely there exist any complexes having a combining ratio, U/citrate, of less than unity. Since the absorption spectra given by the 1:1 and 1:8 mixtures differed from that given by the 2:1 mixture, the existence of a complex of combining ratio greater than one is suggested. Were the absorbance of the 2:1 solution due only to the presence of a 1:1 complex, some precipitation would have been observed because of the insufficiency of citrate. The existence of an ion of combining ratio, U/citrate, greater than two has not been ruled out. This seems an unlikely possibility, however. Such a complex, U_3R for example, would provide each uranyl ion with only *one* functional carboxyl group of a citrate molecule. A single carboxyl group does not provide for efficient complexing. For example, uranium in a ten-fold excess of acetate does not remain complexed but undergoes hydrolysis and precipitation as the *p*H is raised to *p*H 6.0.

Above *p*H 6.0, the spectra shift with time, the *p*H does not remain constant, and CO_2 absorption is a serious problem. Though the curves in Fig. 5 extend to the alkaline region, the data are not sufficiently accurate to permit an analysis and are included for reference purposes only. They do show that citrate is capable of preventing the precipitation of uranium even in basic solution.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, SYRACUSE UNIVERSITY]

The Crystal Structure of Sulfamic Acid[1]

BY FRANK A. KANDA AND ADEN J. KING

X-Ray studies show that sulfamic acid crystallizes in the orthorhombic system. The unit cell, comprised of eight molecules, has a_0 = 8.100 Å., b_0 = 8.049 Å., and c_0 = 9.228 Å., all ±0.002 Å. All atoms are in general positions of the space group V_h^{15}-P_{bca}. Analysis of diffraction intensity data by means of Patterson-Harker projections and Fourier refinements established the parameters. Hydrogen parameters were deduced to fit this structure. Interatomic distances within the molecule are: sulfur-nitrogen, 1.73 Å.; sulfur-oxygen (1), 1.49 Å.; sulfur-oxygen (2), 1.47 Å.; sulfur-oxygen (3), 1.48 Å. (all ±0.05 Å.). Bonding conditions are discussed resulting in the conclusion that the acid crystallizes as zwitterion molecules ($NH_3^+SO_3^-$) of a distorted tetrahedral form. Extensive hydrogen bonding of the type N-H· · ·O is shown to exist in the structure with each NH_3^+ group entering into five bridging systems. The hydrogen bond distances vary from 2.82-3.07 Å.

A survey of the literature discloses that sulfamic acid has been investigated from the standpoint of its chemical and physical properties, and its unit cell dimensions have been determined, but a complete analysis of its structure has never been made by X-ray diffraction methods. The structure of potassium sulfamate[2] and the Raman spectra[3] of the acid indicate that the ion is essentially tetrahedral, and infrared spectra[4] show N-H··O bonding in its crystalline condition. It has been suggested[5] that it may exist as a zwitterion, $^+H_3N{\cdot}SO_3^-$, in the solid state. Although little evidence in support of this is available from its properties in aqueous solution, where it functions only as a relatively strong acid, its high melting point 206°, in contrast with other sulfonic acids, strongly favors this structure. An interesting analogy exists between sulfamic acid and glycine in that their melting points are both considerably higher than similarly constituted substances in which zwitterion formation is impossible. In Table I are listed for comparison the melting points of sulfamic acid, glycine and other closely related derivatives of sulfonic and acetic acids. It is evident that the lattice forces must be much stronger in the crystals of the $-NH_2$ derivatives of these acids than in their -OH or -Cl derivatives. The fact that glycine[6] has been shown to possess a zwitterion structure is at least indicative of a similar structure in sulfamic acid.

TABLE I

MELTING POINTS (°C.) OF SULFAMIC ACID, GLYCINE AND RELATED COMPOUNDS

H_2NSO_3H	206	H_2NCH_2COOH	233
$HOSO_3H$	10.49	$HOCH_2COOH$	63(α)
$ClSO_3H$	−80	$ClCH_2COOH$	63(α)

The following report gives the results of a study of sulfamic acid by X-ray diffraction methods with the objective of determining the types of bonding which exist in its crystalline form.

The Unit Cell and Space Group.—Measurements by goniometric methods and by Laue and oscillation photographs showed that sulfamic acid crystallizes in a primitive orthorhombic cell with a_0 = 8.100 Å., b_0 = 8.049 Å. and c_0 = 9.228 Å., all values correct to ±0.002 Å. These dimensions were calculated from back reflection diffrac-

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[1] Presented at the Symposium on Properties, Structure and Thermodynamics of Inorganic Substances sponsored by the Division of Physical and Inorganic Chemistry, A. C. S., at Syracuse, New York, June, 1948.

[2] C. J. Brown, F. G. Cox and F. J. Llewellyn, *J. Chem. Soc.*, 10 (1940).

[3] S. J. Gupta and A. K. Majumdar, *J. Indian Chem. Soc.*, **18**, 45 (1941).

[4] H. E. Cupery, *Ind. Eng. Chem.*, **30**, 526 (1938).

[5] P. Baumgarten, *Ber.*, **62B**, 820 (1939).

[6] G. Albrecht and R. Corey, THIS JOURNAL, **62**, 2758 (1940).