ory adequately accounts for the errors involved in ordinary electrophoretic analyses.

TABLE I

ELECTROPHORETIC ANALYSES OF OVALBUMIN

Experiment		Protein concentration, % by wt.	Apparent electrophoretic concentration of A_1, %	Deviation of apparent electrophoretic from extrapolated concentration of A_1, %
I	1	1.41[a]	88.4	5.7
	2	0.89[a]	86.8	4.1
	3	0.72[a]	85.5	2.8
Extrapolation		0	82.7	
II	1	2.6[b]	90.3	12.9
	2	17.[b]	86.9	9.5
	3	1.2[b]	83.2	5.8
Extrapolation		0	77.4	
III	1	1.96[a]	85.3	11.8
	2	0.95[a]	79.2	5.7
Extrapolation		0	73.5	
IV	1	1.4[b]	81.1	9.4
	2	1.2[b]	79.4	7.7
	3	0.8[b]	76.8	5.1
Extrapolation		0	71.7	
V	1	1.87[a]	81.9	13.1
	2	1.05[a]	76.1	7.3
Extrapolation		0	68.8	

[a] From nitrogen determination. [b] From area of electrophoretic pattern. The relation between the protein concentration and the area of the electrophoretic pattern was determined empirically over the concentration range of 0.7 to 2%.

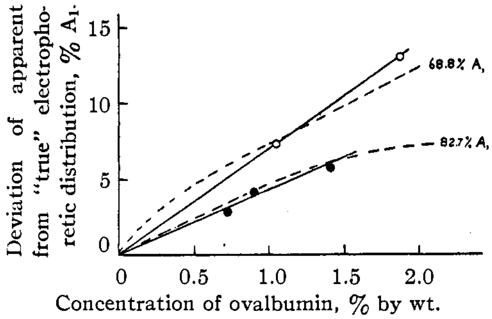

Fig. 2.—Deviations of apparent from "true" electrophoretic distributions: solid line, experimental deviations; broken curve, deviations calculated from the equations of Dole.

Acknowledgment.—The author is indebted to Dr. J. G. Kirkwood for his interest in this work and for his review of the manuscript.

Summary

The electrophoretic analysis of ovalbumin is complicated by the fact that the change of relative mobilities of the components with ionic strength is such that, while resolution into components is obtained at *p*H 6.8 and ionic strength 0.1, only slight if any resolution is found at ionic strength 0.3. "True" electrophoretic distributions of components were obtained by extrapolation of apparent distributions to zero protein concentration at constant ionic strength of 0.1. The moving boundary theory of Dole adequately accounts for the deviations of apparent from "true" distributions.

PASADENA, CALIFORNIA RECEIVED JULY 28, 1948

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TEXAS]

Spectrophotometric Studies of Complex Formation with Sulfosalicylic Acid. II. With Uranyl Ion

BY ROBERT T. FOLEY AND ROBBIN C. ANDERSON

As part of a series of studies on complex formation with sulfosalicyclic acid, it has been of interest to compare the reactions of uranium—as a member of another transition series of elements—with that of iron. Weil and Rozenblum[1] reported a solid compound of uranium and sulfosalicylate, $[C_6H_3(OH)(SO_3)(COO)]_2U$, but no observations in solution were made. Muller, Fernandes, Mai and Weinland and Hager[2] have reported complex formation between uranyl ion and salicylic acid or various salts.

In this particular investigation reactions of sulfosalicyclic acid in solutions containing uranium as uranyl ion have been studied.

Experimental

Materials.—Uranyl nitrate dihydrate was obtained by vacuum drying the hexahydrate (Eimer and Amend C. P grade) over sulfuric acid. A 0.005 *M* uranyl nitrate solution was prepared by dissolving the proper weight of the dihydrate in water at 25°.

Uranyl acetate dihydrate was also simply weighed and dissolved in water. The salt was Eimer and Amend C. P grade.

The sulfosalicylic acid was a special sample from Eastman Kodak Co. with melting point 112-120° (reported in literature, 108-113°.[3]) Analysis for sulfate gave 0.06%. Solutions of this acid were standarized by titration against a National Bureau of Standards sample of potassium acid phthalate.

Apparatus.—Measurements of optical density were made with a Beckmann Model DU Quartz Spectrophotometer, using a tungsten lamp and Corex cells of 10-mm. light path. The cells were held in a thermostatted cell

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[1] St. Weil and St. Rozenblum, *Bull. tran. inst. pharm. (Poland)*, **1**, 1 (1902).

[2] See R. Weinland and K. Hager, *Z. anorg. allgem. Chem.*, **160**, 193 (1927).

[3] Schulze, *Apoth. Ztg.*, **51**, 319 (1936).

compartment of design similar to that of Bell and Stryker.[4] Temperature measurements were made directly on the solution in the cells, and, unless otherwise specified, were at 25 ± 0.2° for all determinations.

*p*H measurements were made with a Beckmann *p*H meter, Model G. This was calibrated against a standard buffer.

Procedure.—After each solution was prepared, it was allowed to stand one hour in a thermostat at 25°. Preliminary experiments showed that the complex reached equilibrium within one hour and did not change appreciably in the next twenty-four hours. Each sample placed in the thermostatted absorption cells was allowed to stand at least fifteen minutes to reach thermal equilibrium before measurements were made.

Results

Absorption Spectra.—In Fig. 1 are shown typical absorption curves for uranyl acetate solution and for a mixture of uranyl acetate and sulfosalicylic acid solutions.

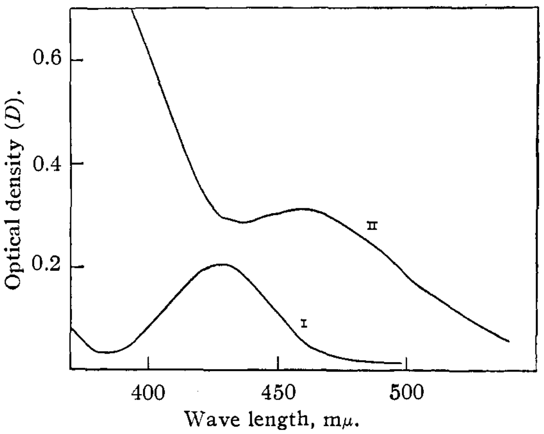

Fig. 1.

Curve I is for a solution 0.005 *M* in uranyl (II) acetate at a *p*H of 4.5. Curve II is for a solution 0.00075 *M* in uranyl acetate and 0.00425 *M* in sulfosalicyclic acid at a *p*H of 4.65. Sulfosalicyclic acid itself shows no appreciable absorption throughout this range. Since the concentration of uranyl salt is much less for curve II than for curve I, it is evident that a complex must be formed with sulfosalicyclic acid—a complex which absorbs much more strongly than uranyl acetate particularly in the region of 460 mμ and just below 400 mμ.

Uranyl nitrate solution showed essentially the same absorption curve as the uranyl acetate. (The peak at 430 mμ in absorption by these solutions possibly involves association with hydroxyl ions. Qualitative tests showed that increase in *p*H—*i. e.*, in hydroxyl ion concentration—causes an increase in intensity of the color in this wave length region. Although acetate also seems to form a complex, the complex does not absorb at this wave length).

Composition of Complex.—The composition of the complex was determined by Job's method of continuous variations.[5] In Fig. 2 are shown curves obtained when the quantity $\overline{D}$—the difference between the total optical density of the solution and that which would be shown by the uranyl salt alone if no reaction occurred—is plotted against the ratio

$$[UO_2^{++}]/[UO_2^{++}) + [C_6H_3OHCOOHSO_3H]$$

The curves in Fig. 2 are for mixtures of equimolar solutions of uranyl and sulfosalicylate at *p*H 4.65. The total molarity of uranyl plus sulfosalicylate is 0.005 throughout. Curve I is based on measurements at 400 mμ and Curve II at 460 mμ. Similar curves were obtained at 420 mμ and 440 mμ.

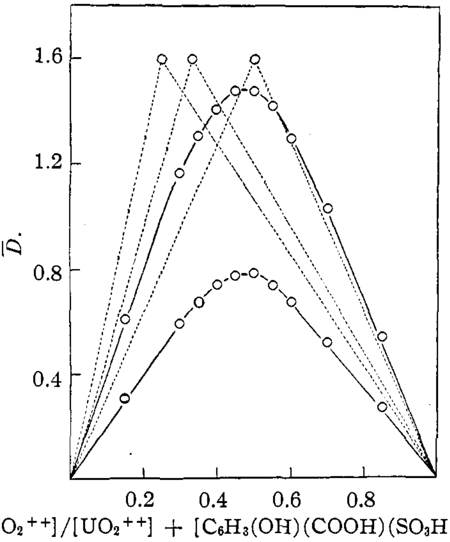

Fig. 2.

The dotted lines with peaks at ratios of 0.25, 0.33 and 0.5 represent theoretical curves which would be obtained for complexes having formulas MR_3, MR_2, and MR (where R might be sulfosalicylic acid or one of its ions), respectively—if the association reaction to form the complex were complete.

The experimental data clearly indicate that under these conditions a complex containing uranyl and sulfosalicylate in the ratio 1:1 is formed. The fact that the peak of the experimental curve is lower than that of the theoretical indicates that the association reaction is not complete, *i. e.*, that the complex is appreciably dissociated.

Measurements at *p*H 5.1 and 6.1, using acetate buffers, gave maxima at ratios between 0.45 and 0.5, thus indicating that the ratio of uranyl to sulfosalicylate is 1:1 in these regions also. However, measurements at higher *p*H values are more difficult because of the lower absorption.

Effect of *p*H Change.—The solution of the complex shows maximum absorption at a *p*H of about 4.5–4.7 and decreases markedly in absorption if the *p*H is increased or decreased, as shown in the curve of Fig. 3 (for λ = 440 mμ).

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[4] P. H. Bell and C. R. Stryker, *Science*, **105**, 415 (1947).

[5] P. Job, *Ann. chim.*, **9**, 113 (1928); **11**, 97 (1936); *cf.* also R. T. Foley and R. C. Anderson, THIS JOURNAL, **70**, 1195 (1948).

Since the pK value for the second dissociation constant of sulfosalicylic acid is about 2.86 at 25°,[6] the effect of pH in the range 2 to 4.7 suggests complex formation must occur between UO_2^{++} and $^-O_3SC_6H_4OHCOO^-$ ions. In this region increasing association to $^-O_3SC_6H_4OHCOOH$ might interfere with complex formation. The decreased complex formation at pH values above 4.67 may result from formation of a complex such as UO_2OH^+. The tendency of uranyl ion to form basic salts indicates this should be probable, but there is no conclusive evidence on this possibility at present.

Effect of Acetate.—It was noted in the experiments using acetate buffers that there was a marked decrease in color of the complex when the buffer was added. Such an effect might result from a change in the ionic strength or from a specific reaction of the acetate. Comparisons were made by adding acetate to one sample of a solution (0.002 M in uranyl and sulfosalicylate) containing the complex and chloride to another so as to produce corresponding changes in ionic strength. These showed that the effect of the acetate is specific, being much greater than the effect of change in ionic strength as shown by the solutions containing chloride (see Table I).

TABLE I

ACETATE EFFECT

Concn. added salt, moles/l.	Optical density at 460 mμ acetate added	chloride added
0	0.643	0.643
0.18	.242	.620
.36	.103	.607

Apparently the acetate ion competes with the sulfosalicylate in complex formation but forms a complex which does not absorb in this region of the spectrum. Ghosh and Mitter[7] have reported evidence from spectroscopic studies for the existence of such a complex.

Dissociation Constant and Free Energy.—Using optical densities for solutions containing a large excess (9:1 or more) of sulfosalicylic acid, so that as a first approximation the concentration of complex may be taken to be equal to that of uranyl added, one may determine values for the extinction coefficient of the complex.

Typical approximate values (for solutions 0.0005 M in uranyl) are shown in Table II.

TABLE II

APPROXIMATE EXTINCTION COEFFICIENTS

T, °C.	Coefficient, mμ 440	460
23.4	404	432
29.1	413	438
34.3	417	444

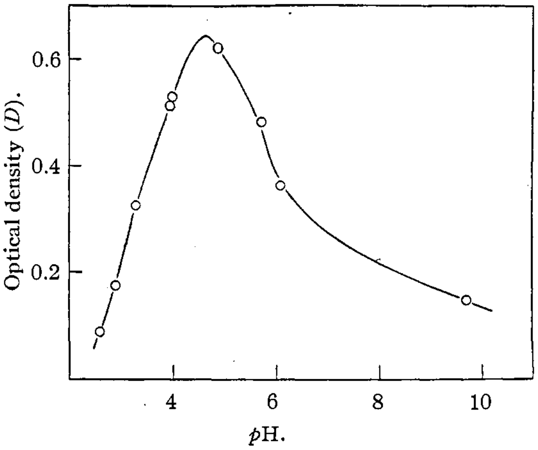

Fig. 3.

From these values one can calculate concentrations of the complex in various mixtures and thus calculate the dissociation constant. The concentration of uranyl ion is then calculated, and correction for its absorption gives a better value for the extinction coefficient of the complex. Successive approximations by this method from data for the various temperatures give for the extinction coefficient at 460 mμ, an average value of 451 (within ±2%). For 440 mμ the average value is 423. From determinations of the equilibrium constant calculated using equilibrium concentrations determined by this method an average value was obtained of

$$K = \frac{[UO_2^{++}][R]}{[UO_2^{++}R]} = 1.3 \times 10^{-4} \pm 0.8 \times 10^{-4}$$

The calculations above involve the initial assumption in each step that Beer's law may be applied for the color of the solution containing uranyl ion and the complex. This is an assumption which cannot be accurate except in very large excess of one of the reagents.[8] The variations in Table II show that it is not entirely accurate here.

A better method is to base calculations of equilibrium constants on comparisons made in solutions of equal or closely similar optical densities. A solution containing uranyl and sulfosalicylate in 1:1 ratio was diluted at constant ionic strength, until solutions were obtained having the same optical densities (after correction for UO_2^{++} absorption by using the known approximate value of K to determine UO_2^{++} concentrations) as those at other ratios such as 2:8, 3:7, etc. In this manner pairs of solutions may be prepared which have the same optical density (from the complex) and thus contain equal concentrations of complex but different total concentrations of each reagent. Since the dissociation constant is the same for both members of a given pair, the concentration of

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(6) Private communications, Dr. R. G. Bates, Nat. Bureau of Stds.

(7) J. C. Ghosh and B. N. Mitter, *J. Ind. Chem. Soc.*, **4**, 353 (1927).

(8) *Cf.* for example, A. K. Babko, *Zavodskaya Lab.*, **23**, 9 (1947), or *Chem. Abs.*, **41**, 7175 (1947).

complex and thus the value of K may be calculated from the relation

$$K = \frac{(a_1 - x)(b_1 - x)}{x} = \frac{(a_2 - x)(b_2 - x)}{x}$$

where the a's and b's denote initial concentration of uranyl and sulfosalicylate, respectively, and x denotes the concentration of complex formed.

K values calculated by this method are of the same order of magnitude as those obtained by the first method but show greater precision. The average K from several determinations is

$$K = 1.93 \times 10^{-4} \pm 0.08 \times 10^{-4} \text{ (at } 25°)$$

The free energy of *formation* of the complex may be calculated readily from the relation $\Delta F = RT \ln K = -5060$ cal./mole (where K denotes the *dissociation* constant as determined above).

Temperature Effect and Heat of Reaction.—From the data in Table II it may be seen that increased temperature tends to increase complex formation but that the effect is not pronounced. A determination of the dissociation constant at 33.2° gave an average value of $1.48 \times 10^{-4} \pm 0.6 \times 10^{-4}$.

It is apparent that the heat of reaction must be small. It can be calculated readily from standard relations, but since the difference in K values is small the effect of experimental errors becomes very pronounced and can outweigh the temperature effect itself. Such a calculation is then of uncertain validity.

Structure of Complex.—None of the experiments using Job's method in the region of *p*H 4.6 indicate other than a 1:1 mole ratio in the complex. Since the color fades rapidly as the *p*H changes, there is no evidence for formation of more than one colored complex under the conditions of these experiments.

No attempt should be made to express a structural formula for this complex until more evidence is available, in particular concerning the presence or absence of hydrogen or hydroxide ions.

It may be noted, however, that the low free energy of formation indicates a weak association effect rather than a strong covalent bond. Extraction tests with various organic solvents such as benzene and carbon tetrachloride were unsuccessful, giving no positive evidence for the presence of any neutral, covalent grouping. The 1:1 ratio, the *p*H region in which the maximum effect occurs, and the rapid formation—without appreciable "aging" effect—indicate association of ions such as UO_2^{++} and $^{-}O_3SC_6H_3OHCOO^{-}$. This would give an apparent charge of zero, but a "zwitterion" type of structure is possible. The *p*H effect and the apparently high reactivity of the complex with either increase or decrease in *p*H are suggestive of such a structure.

The authors wish to express their appreciation to the Pan American Refining Corporation for its generous support of this work by a fellowship grant (to R. T. F.).

Summary

1. Spectrophotometric data for solutions with concentrations in the region of 0.005 *M* and over a *p*H range of 2 to 10 indicate only one complex formed with uranyl ion and sulfosalicyclic acid. This involves a 1:1 mole ratio of uranyl and sulfosalicyclic acid.

2. The effects of changing *p*H, of temperature, and of acetate ion have been studied.

3. The dissociation constant and free energy of formation of the complex have been calculated.

RECEIVED JUNE 9, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF TEXAS]

Spectrophotometric Studies on Complex Formation with Sulfosalicylic Acid. III. With Copper(II)

BY STANLEY E. TURNER AND ROBBIN C. ANDERSON

As part of a series of experiments on complex formation by sulfosalicylic acid with various metal ions in solution[1] a study of its reaction with copper (II) ions has been made.

Spacu and Macarovici[2] reported formation of a deep olive-green complex of composition $[Cu(OC_6H_3CO_2{\cdot}SO_3)_2]H_4$ in connection with experiments on copper-cobalt complex sulfosalicylates. Babko[3] has studied the copper-salicylate system and reports a complex of the type CuR from *p*H 3-5 and CuR_2 from *p*H 7–9.

Experimental

The apparatus used was the same as that described previously.[1] Preliminary absorption measurements were made with a General Electric Hardy Recording Spectrophotometer. Data for determination of composition of the complex, temperature effects, etc., were taken with a Beckmann Model DU Spectrophotometer equipped with a thermostat for temperature control in the absorption cells.

*p*H measurements were made with a Beckman model G *p*H meter.

To avoid any interference from iron, Merck Reagent Grade copper sulfate was precipitated and redissolved in ammonium hydroxide. The solution was acidified with nitric acid and evaporated almost to dryness to remove

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(1) Robt. T. Foley and Robbin C. Anderson, THIS JOURNAL, **70**, 1195 (1948).

(2) G. Spacu and G. Gh. Macarovici, *Bull. Soc. Stiintje Cluj*, **8**, 364 (1936); *cf. Chem. Abs.*, **31**, 3810 (1937).

(3) A. K. Babko, *J. Gen. Chem.* (*U. S. S. R.*), **17**, 443 (1947), *cf. Chem. Abs.*, **42**, 475 (1948).