Table II (*Concluded*)

Mole % ascorbic acid	M. p. range,[a] °C., uncor.	
70.0	163.0–167.0	BCD—solid addition compound saturated solution, vapor
60.0	166.0–173.0	
55.0	169.0–176.0	
50.0	179.0–182.0	
47.5	172.0–178.0	
45.0	172.0–175.0	
40.0	169.0–176.0	D—eutectic: solid nicotinic acid, solid addition compound, saturated solution, vapor
35.0	172.0–178.0	DE—solid nicotinic acid, saturated solution, vapor
32.5	174.0–178.0	
30.0	176.0–181.0	
27.5	179.0–183.0	
25.0	181.0–184.0	
20.0	184.0–190.0	
15.0	193.0–197.0	
10.0	202.0–208.0	
5.0	224.0–227.0	
Nicotinic acid	230.0–232.0	

[a] The center of the range was used to plot Fig. 2.

Acknowledgment.—The authors wish to acknowledge the fact that Mrs. Laurene Paterson Opferman, formerly of the Research Laboratory of Gelatin Products Corporation, first discovered that a reaction occurred between nicotinamide and ascorbic acid, and first made the reaction product.

Summary

1. Temperature–concentration data are presented for the system nicotinamide–ascorbic acid, and tentatively for the system nicotinic acid–ascorbic acid.

2. It has been shown that nicotinamide reacts with vitamin C to give a yellow[6] reaction product $C_5H_4NCONH_2{=}C_6H_8O_6$, m. p. 143.5 to 145.5° (uncor.).

3. Evidence is presented to show that the linkage is between the ring nitrogen and the "acceptor" molecule.

4. Molecular weight determinations indicate dissociation in solution.

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[6] Subsequent to the original presentation of this paper, it has been shown by T. H. Milhorat in *Proc. Soc. Exp. Biol. Med.*, **56**, 52 (1944), that ascorbic acid reacts with nicotinamide and nicotinic acid with the production of a yellow color.

Detroit, Michigan Received April 25, 1945

[Contribution from the American Cyanamid Company]

The Effects of Variations of *p*H on the Ultraviolet Absorption Spectra of Some Sulfonamides

By E. J. Robinson and L. F. Pekrul

That the ultraviolet absorption maxima of some sulfonamides differ both in position and intensity, depending upon whether the compounds are in alkaline or acid solution, has been shown in several recent publications.[1] No one, to our knowledge, has carried out a thorough investigation of these changes in relation to *p*H, although data from such studies are essential before one can apply the ultraviolet method to the qualitative and quantitative analyses of these drugs.

Stenstrom and collaborators[2] noted that both the positions and the intensities of the ultraviolet absorption maxima of phenol and tyrosine in aqueous media changed with *p*H. The same observation was made by Flexser, Hammett and Dingwall[3] on a series of weak acids and bases. Both groups of investigators interpreted these variations as resulting from changes in the ion: molecule ratio, and used the data in calculating the dissociation constants of the compounds studied.

The experiments reported in this communication are the outgrowth of an attempt made several years ago to apply ultraviolet absorption spectroscopy to the analysis of mixtures of sulfonamides in biological fluids.

Experimental

Stock aqueous solutions of the drugs were made up from material recrystallized twice from 50% ethyl alcohol. Samples for spectrophotometry were prepared by suitably diluting aliquots of the stock solutions with buffers of the desired *p*H. In each case an equal volume of water similarly diluted with buffer served as a blank. The *p*H determinations were made on the final buffered drug solutions using a Beckman Glass Electrode *p*H Meter. In the more alkaline solutions the special high *p*H glass electrode was used and the proper ion correction applied to the reading.

All absorption measurements were made at room temperature using a Beckman Photoelectric Spectrophotometer, Model DU. From plots of % transmission *versus* wave length, the positions of the absorption maxima were located to the nearest 10 Å. The values of the molecular extinction coefficients at the desired wave lengths were calculated in the usual way.

With these compounds we have found that, at a given *p*H, the position and intensity of any maximum is constant regardless of the buffer combination used as solvent. The same is true if the desired *p*H is attained simply by the addition of acid or alkali to the unbuffered drug solution.

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[1] Böhme and Wagner, *Arch. Pharm.*, **280**, 255 (1942); Kumler and Strait, This Journal, **65**, 2349 (1943); Bell, Bone and Roblin, *ibid.*, **66**, 847 (1944); Vandenbelt and Doub, *ibid.*, **66**, 1633 (1944).

[2] Stenstrom and Reinhardt, *J. Phys. Chem.*, **29**, 1477 (1925); Stenstrom and Goldsmith, *ibid.*, **30**, 1683 (1926).

[3] Flexser, Hammett and Dingwall, This Journal, **57**, 2103 1935).

Results and Discussion

The detailed absorption curves of the compounds mentioned in this communication have been adequately presented in papers already cited. With the exception of sulfadiazine, therefore, only their changes with *p*H will be considered here.

In alkaline solutions the absorption spectra of sulfanilamide, N^1-acetylsulfanilamide, and the N^4-derivatives of both exhibit single maxima in the ultraviolet. With all four compounds the position of this maximum shifts toward longer wave lengths with little change in intensity as the *p*H of the solution is reduced to 3.0–3.5. This is interpreted as being the result of the change from the anion to the undissociated molecule. The manner in which the shifts occur is illustrated in Fig. 1.

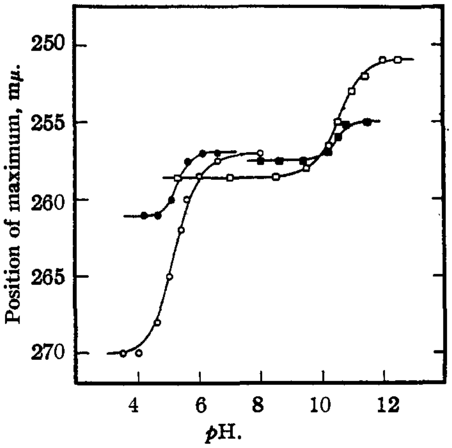

Fig. 1.—Changes in positions of maxima with changes in *p*H: □, sulfanilamide; ■, N^4-acetylsulfanilamide; O, N^1-acetylsulfanilamide; ●, N^1-N^4-diacetylsulfanilamide.

Examination of these curves reveals clearly the impossibility of using the ultraviolet method in either the qualitative or quantitative analysis of these or similar compounds without a thorough knowledge of the manner in which their absorption spectra change with *p*H. It has been found that, at a given *p*H, the spectra of mixtures of two of these compounds, each of which has but one maximum, also exhibit a single maximum. It lies between those of the two pure compounds, and its exact position depends upon their relative concentrations. This fact has already been used in the analysis of mixtures whose components were known.[4]

It can also be seen in Fig. 1 that in form these curves strongly resemble electrometric titration curves. Indeed, from them one can estimate the pK_a values of the compounds with a fair degree of accuracy. In practice this has been accomplished by drawing a smooth curve through the experimental points. The *p*H corresponding to half of the total wave length shift is then taken as equal to the pK_a value.

In the actual determination of the pK_a values, however, a different procedure has been followed. The ratio ion:molecule was calculated for a series of *p*H values using equation 4 of Flexser, *et al.*,[3] at a chosen wave length. Application of this equation requires a knowledge of the extinction coefficient of the ion and of the molecule. One can determine these values experimentally by extending the *p*H range in both directions until the calculated values of the extinction coefficient become constant. At the higher *p*H this will be the extinction coefficient of the ion and at the lower that of the molecule. Once determined, the proper values of the ion:molecule ratio and of *p*H were substituted into the Henderson–Hasselbalch equation and pK_a found directly. The values of pK_a finally given are the averages of those found at the different *p*H values.

In Fig. 2 the data for sulfanilamide are plotted. The solid line, showing the % dissociation *versus* *p*H, was calculated from the Henderson–Hasselbalch equation using the value $pK_a = 10.59$ which was found by calculation. The circles show the positions of the maximum at the various *p*H values. It is evident that the experimental points fit the theoretical curve well, particularly through the middle range where it is nearly linear.

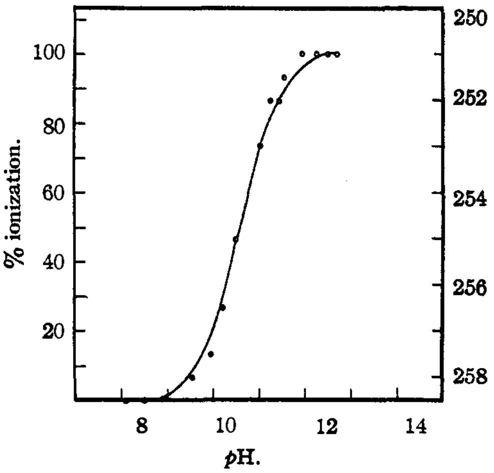

Fig. 2.—Acid ionization of sulfanilamide: solid line (ordinates at left) calculated from $pK_a = 10.59$; circles, position of maximum, mμ (ordinates at right).

The pK_a for N^1-acetylsulfanilamide was found in a similar fashion to be 5.32. When the positions of the maximum were plotted against *p*H and superimposed upon the theoretical curve as in Fig. 2, the fit was equally good.

Reference to Fig. 1 shows that the absorption maxima of the two N^4-acetyl derivatives change very much less with *p*H than do those of the parent compounds. The changes, indeed, are too small to enable one to calculate their pK_a values with any accuracy.

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(4) Robinson and Crossley, *Arch. Biochem.*, **1**, 415 (1943).

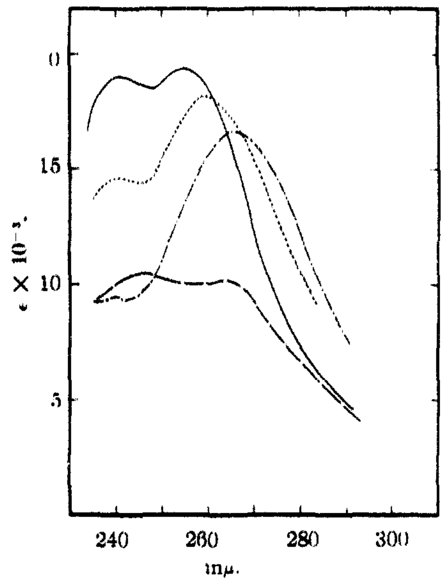

Fig. 3.—Ultraviolet absorption spectrum of sulfadiazine at several pH values: — — —, pH 8.00; - - - - - - - - - - -, pH 6.20; — - — - - - - - -, pH 4.56; ———, pH 2.00.

The ultraviolet absorption spectrum of sulfadiazine at four pH values is shown in Fig. 3. It can be seen that the spectrum of the anion ($p\text{H} > 8$) exhibits two maxima. One of these ($\lambda = 240$ mμ) decreases in intensity as the pH of the solution is lowered to about 4.0, but without change in position. The other ($\lambda = 256$ mμ) decreases in intensity and at the same time shifts to longer wave lengths. The decrease in intensity at 240 mμ was used to calculate the pK_a as already described for the other drugs. The resulting value is 6.25. When, using this value, the % dissociation–pH curve is plotted and the position changes of the other band superimposed upon it, the fit is as good as that observed with the other two compounds.

As the acidities of solutions of sulfanilamide and N¹-acetylsulfanilamide are progressively reduced below approximately pH 3, the positions of their single absorption maxima remain constant at the wave lengths shown in Fig. 1. The intensities of the maxima, however, decrease sharply. This corresponds to cationic dissociation. At about pH 1.3 in the case of sulfanilamide and about pH 1.0 for N¹-acetylsulfanilamide, the single maximum begins to show fine structure, and finally three maxima can be resolved in each case. The absorption intensities of both compounds at the wave lengths of the original single maxima continue to decrease until pH values of about 0.25 are reached.

Using the method of calculation already described, these changes in intensities at the chosen wave lengths were used to determine the values of pK_b for the two compounds. These are for sulfanilamide, 11.9, and for N¹-acetylsulfanilamide, 12.6.

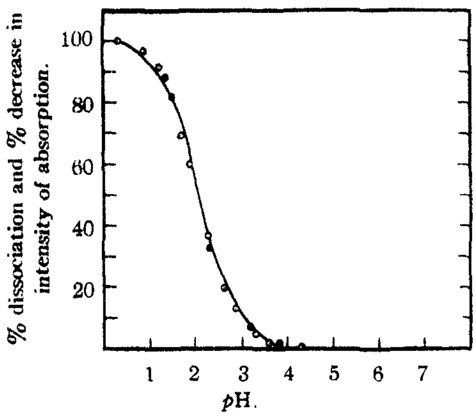

Fig. 4.—Basic dissociation of sulfanilamide: solid line calculated from $pK_b = 11.9$; for method of locating experimental points see text; ○, drug in buffer; ●, unbuffered solution made acid by addition of hydrochloric acid.

When, for either compound, the fractional decrease in the intensity of absorption at the wave length of the single maximum is plotted against pH, a smooth sigmoid curve results. In Fig. 4 the solid line shows the theoretical % dissociation–pH curve for sulfanilamide calculated from $pK_b = 11.9$. The experimental points were found by expressing the intensity decrease at any pH as a percentage of the total decrease between pH values of 4.00 and 0.25 as 100. The fit leaves little to be desired and was equally good for N¹-acetylsulfanilamide.

As has already been described, one of the absorption bands of sulfadiazine in alkaline solution has its maximum at 256 mμ. As the solution is made increasingly acid, this maximum shifts regularly to higher wave lengths. It also decreases in intensity, but this decrease is irregular and cannot be used as a basis for the calculation of pK_a. Between pH values of approximately 5.4 and 3.5 it remains constant both in position and intensity; its position is 265 mμ. Below pH 3.5 its intensity again decreases, but without change in position. This change in intensity was used as the basis for the calculation of pK_b, and the value obtained was 11.62. When these data are plotted as in Fig. 4 the fit is not nearly so good. The change in intensity–pH plot indicates a pK_b value of 12.0 which is the same as that found by Bell and Roblin[5] by a different procedure.

Table I shows a comparison of the pK values found by us spectrographically with those of Bell and Roblin determined by titration and conductivity methods. It also gives the range of the values obtained by us at the various pH levels.

The agreement between the two sets of data leaves something to be desired. A choice between them cannot be made without a thorough investigation of all procedures used, and this is beyond the scope of the present work. The precision of

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(5) Bell and Roblin, THIS JOURNAL, **64**, 2905 (1942).

TABLE I

		Present work Mean	Present work Range	Bell and Roblin
Sulfanilamide	pK_a	10.59	10.45–10.80	10.43
	pK_b	11.90	11.65–12.00	11.64
N¹-Acetylsulfanilamide	pK_a	5.32	5.25– 5.44	5.38
	pK_b	12.60	12.32–12.98	12.22
Sulfadiazine	pK_a	6.25	5.94– 6.71	6.48
	pK_b	11.62	10.93–11.98	12.00

the method outlined for the calculation of *pK* values may not be as great as that of others. The procedure has, however, proved very valuable in this laboratory in the elucidation of the properties of compounds of unknown structure, compounds of low solubility, and compounds available in limited quantity.

Acknowledgment.—The authors wish to express their appreciation to Mrs. K. H. Lees for her assistance in some of the spectrophotometric measurements.

Summary

1. The details of the changes, with *p*H, in the ultraviolet absorption spectra of several sulfonamides are described.
2. It is shown that these changes can be used either for the calculation or graphic determination of *pK* values.

STAMFORD, CONN.

RECEIVED MARCH 24, 1945

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE

The Melting Point and Heat of Fusion of Barium Hydroxide

BY RALPH P. SEWARD

Although it is known that barium hydroxide is easily fused without decomposition, the only values recorded for its melting point are 395° for "impure" barium hydroxide by Balarew[1] and 375–380°, by Hedvall and Heinberger.[2] No value for the heat of fusion of barium hydroxide appears in the literature. The investigation reported here consisted of the determination of the freezing points of barium hydroxide and of solutions of several different salts dissolved in barium hydroxide. From these freezing points the heat of fusion has been calculated. The solutes employed were barium carbonate, sodium hydroxide, barium chloride, and barium bromide.

The latter pair of solutes, barium chloride and barium bromide, were added to see whether they would lower the freezing point of the barium hydroxide twice as much per mole added as the first pair, barium carbonate and sodium hydroxide. This question, which is discussed by Lewis and Randall,[3] has apparently never been thoroughly investigated. The experiment of Goodwin and Kalmus[4] cited by Lewis and Randall in which it was found that the freezing point of lead chloride was lowered about 70% more by lead bromide than the calculated lowering for a non-dissociated solute, has no quantitative significance since it has been shown that lead bromide forms solid solutions with lead chloride.

Experimental

Materials.—To prepare pure anhydrous barium hydroxide a technical grade of barium hydroxide containing considerable carbonate was dissolved in hot water, filtered and twice recrystallized in Corning alkali resistant glassware, precautions being taken to prevent any contact with air containing carbon dioxide. To dehydrate the crystallized octahydrate without fusion most of the hydrate water was removed at room temperature with a vacuum pump. The material was then brought to 100° and the pumping continued for two days. The barium hydroxide prepared by this treatment is a free flowing powder. Gravimetric analysis as barium sulfate corresponded to 99.5% barium hydroxide. Titration with standard hydrochloric acid indicated a barium hydroxide content of 99.3%. This hydroxide dissolved in water to an almost perfectly clear solution, showing that very little carbonate was present. From the nature of the cooling curve at the freezing point it was calculated that there was less than 0.3 mole per cent. of liquid soluble–solid insoluble impurity present. The solute materials were of ordinary C. P. quality.

Experimental Method.—A known weight, about 20 g., of barium hydroxide was fused in a nickel crucible 80 mm. high and 18 mm. diameter. The crucible fitted into a small electric furnace by means of which the rate of cooling could be regulated. Cooling curves were taken, the temperature being measured by means of a chromel–alumel thermocouple which was placed with no protection in the molten hydroxide. No corrosion or change in the temperature–e. m. f. characteristics of the couple was observed. Several cooling curves were taken with each sample, successive additions of weighed amounts of solute being added. The thermocouple was calibrated at the melting points of cadmium and zinc. Although the freezing points are recorded to tenths of a degree it is not contended that they are that accurate. Individual freezing points are probably not in error by more than 1°.

Results

The m. p. of barium hydroxide was found to be 408 ± 1°. With the solutes barium carbonate and barium bromide the eutectic halt was detected with the smallest solute concentration used. For this reason it was concluded that the solid phase was pure barium hydroxide. With barium carbonate the eutectic temperature was 374° and the composition 89 mole % barium hydroxide, with barium bromide 345° and 88 mole %.

When sodium hydroxide is added to barium hydroxide the cooling curves show an initial break at temperatures which decrease with increased

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(1) Balarew, *Z. anorg. allgem. Chem.*, **134**, 121 (1924).

(2) Hedvall and Heinberger, *ibid.*, **140**, 250 (1924).

(3) Lewis and Randall, "Thermodynamics," McGraw-Hill, New York, N. Y., 1923, p. 219.

(4) Goodwin and Kalmus, *Phys. Rev.*, **26**, 19 (1909).