[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Acidity Scale in Glacial Acetic Acid. II. Buffer Solutions $-1.6 < H_0 < 3.8$

BY NORRIS F. HALL AND FRED MEYER

The measurements of H_0 in acetic acid by Paul and Hammett[1] covered a range from -3.18 to -0.88. Those of Spengeman[2] run from approximately -6 to 0. It appeared desirable to secure data in the neutral[3] and basic ranges in this solvent, and also to attempt a further correlation between H_0 and the values of "pH$^{(HAc)}$" determined potentiometrically by the earlier workers[4] and still occasionally used.[5] The availability of a convenient photoelectric instrument seemed to make possible an increase in the precision of such measurements.

Experimental

Materials.—Acetic acid, f. p. 16.34°, from the Niacet Chemical Company, was refluxed with triacetyl borate[6] and distilled in an all-glass apparatus. The fraction freezing at 16.50° or higher was used. Pure, colorless 100% sulfuric acid was prepared by the method of Hammett and Deyrup[7] by distilling sulfur trioxide into clear 95% C. P. acid and mixing the resulting fuming acid with more clear 95% acid until the freezing point of the mixture rose to a maximum (*ca.* 10.50°).

Sodium acetate, Mallinckrodt pure anhydrous grade, was recrystallized from glacial acetic acid, heated to drive off the acetic acid of crystallization, and finally dried in an oven at 150° for several days.[8] It was then bottled and kept in a desiccator.

Urea, Merck reagent grade, was used without further purification. It was dried at 110° overnight and kept in a desiccator, m. p. 132–133°.

Antipyrine, U. S. P. X. grade, was used without further purification. It was dried at 100° and kept in a desiccator, m. p. 111°.

o-Nitraniline, m. p. 72°, *p*-nitraniline, m. p. 149°, *m*-nitro-N,N-dimethylaniline, m. p. 66°, *p*-nitro-N,N-dimethylaniline, m. p. 163°, were recrystallized from alcohol and dried. *m*- and *p*-nitro-N,N-diethylaniline were prepared according to the method of Groll[9] by the nitration of diethylaniline. The para isomer, m. p. 77°, was recrystallized from alcohol, and dried at 55–61°. The meta isomer, b. p. 288–290°, was separated from the residue of an ethereal extract by vacuum distillation, since it partially decomposes when distilled at atmospheric pressure.

(1) Paul and Hammett, THIS JOURNAL, **58**, 2182 (1936).

(2) Hall and Spengeman, *ibid.*, **62**, 2487 (1940).

(3) A few measurements by Mr. Spengeman in this Laboratory, using benzeneazodiphenylamine with an assumed pK of $+1.52$, indicate that the acidity of pure acetic acid lies close to $H_0 = +1.4$.

(4) Conant and Hall, THIS JOURNAL, **49**, 3047, 3062 (1927), and later papers.

(5) Russell and Cameron, *ibid.*, **60**, 1345 (1938).

(6) Eichelberger and LaMer, *ibid.*, **55**, 3633 (1933).

(7) Hammett and Deyrup, *ibid.*, **54**, 2721 (1932).

(8) Kendall and Adler, *ibid.*, **43**, 1470 (1921).

(9) Groll, *Ber.*, **19**, 198 (1886).

Apparatus.—The instrument used in this work was the Sanford and Sheard "photelometer," made by the Central Scientific Company, Chicago.[10] A matched set of three interchangeable absorption cells, 10 mm. thick, was supplied with the instrument. Since all the indicators had about the same absorption spectrum, and the same color change, yellow to colorless, the Cenco blue filter, supplied with the instrument, was used for all readings of color intensity.

The main difficulty encountered was that the lamphouse, mounted upon the base of the instrument, causes the carriage holding the solutions to heat up in use, so that it always was 5–10° hotter than the room. This makes it difficult to obtain reproducible readings, at a fixed temperature, for those solutions whose color intensity is affected by small temperature changes.

Solutions.—All stock solutions were prepared by dissolving a weighed amount of solute in glacial acetic acid in a volumetric flask thermostated at 20.0 ± 0.2° to avoid the large concentration correction due to temperature change. All other solutions were prepared by pipetting the correct amount of the component solutions and mixing, assuming the change in volume upon mixing to be negligible since the most concentrated solutions were 0.1111 *M*.

The photelometer was calibrated for each indicator with a series of solutions prepared as follows.

The required volume of 0.010 *M* indicator was pipetted into pure acetic acid or that buffer solution in which the indicator was entirely in the basic non-ionized or colored form, and then diluting half of that solution to the original volume, and so on until a series of solutions was obtained covering the desired range.

The two series of buffer solutions used were prepared from 0.1111 *M* stock solutions so that the total solute strength was constant, and equal to 0.1000 *M* after the addition of the indicator solution at the time of analysis. In order that the indicators might be compared under conditions as much alike as possible, each buffer solution was prepared directly before using, in a quantity sufficient for two determinations for each indicator. Eighteen ml. samples of the buffer were pipetted into Erlenmeyer flasks, two for each indicator, and 2 ml. of an 0.01 *M* solution of the proper indicator added. The stoichiometric concentration of the buffer was then 0.1000 *M*, and of the indicator, 0.0010 *M*. The samples for analysis were allowed to stand for at least twenty minutes and not more than two hours before their color intensity was measured in the photelometer.

Procedure of Measurement.—The instrument was allowed to warm up for about twenty minutes before using, so that it would reach a steady state of operation. Pure glacial acetic acid was used as the reference standard. One of the three matched cells, 20-ml. capacity, was filled with the solvent, and placed in the central compartment of

(10) Sanford, Sheard and Osterberg, *Am. J. Clin. Path.*, **3**, 405 (1933).

the photelometer carriage. The carriage was then moved so that the cell containing the solvent was in the path of the light beam, the meter switched on, and the diaphragm adjusted so that the meter read 100.0. The solutions for analysis were poured into the remaining cells. Only one cell was placed in the carriage, and the large bulb of a thermometer graduated to tenths of a degree was immersed in the solution. Just before the temperature of the solution reached 24.7°, the standard setting was checked, and readjusted, if necessary. When the temperature reached 24.7°, the thermometer was removed, the cell placed in the path of the light beam, and the meter reading noted. The cell was then quickly removed from the light, the standard setting rechecked, and the temperature of the solution measured. If either the standard setting did not check, or if the average of the initial and final temperatures was not 25.0 ± 0.1°, the solution was allowed to cool and the measurement repeated. Although little more than the bulb of the thermometer was immersed, the correction for exposed stem was negligible because of the small difference between the temperature of the solution and the room.

Each analysis was run in duplicate and an average of the two readings, which usually differed by about 0.2 and never more than 0.3 unit, was recorded.

Factors Influencing the Accurate Measurement of Acidity.—At first, considerable difficulty was encountered in obtaining reproducible measurements of the color intensity of an indicator in sulfuric acid and buffer solutions. As already stated, this was eventually traced to the heating up of the instrument, and after a careful study the following conclusions were reached.

1. Temperature control to 0.25° is essential to the accurate measurement of intensity. The standard setting is independent of temperature in the range 20–30°. The color intensity of the indicator base in pure acetic acid is not markedly influenced by temperature in this range, but in sulfuric acid or buffer solutions, it is markedly influenced. For these solutions, the rate of change of photelometer reading is about 0.6 to 0.8 unit decrease (roughly equivalent to a decrease in acidity of 0.04–0.08 H_0 units) per degree increase in temperature.

2. There is an irreversible change in the color intensity of the indicator in the sulfuric acid and buffer solutions which is small at 25°, and only becomes appreciable either in two or three hours or with a 10° or more rise in temperature. It is of the order of 0.1–0.2 unit decrease in photelometer reading (0.01 H_0 unit decrease in acidity) per hour. The color intensity of the indicator base in pure acetic acid does not change appreciably on standing.

3. Some short time may be necessary for these sulfuric acid and buffer solutions to reach equilibrium when the indicator is added. This effect is largely masked by the time necessary to raise the solutions from room temperature to 25°.

Reproducibility of Measurements.—With the accurate control of temperature at 25.0 ± 0.1°, it was found possible to reproduce measurements within 0.25 scale unit in the photelometer reading for different solutions of the same composition at different times. The maximum accuracy in determining the concentration of any colored substance for a given reproducibility in the photelometer reading can only be obtained when the absorption follows Beer's law, and is the same for all colored substances. It can be computed that for a desired accuracy of 2%, the concentration of the colored substance would have to be such that the corresponding photelometer reading was in the range of 5–86 units. This is to be compared with the maximum visual accuracy of about 4%. If the absorption does not follow Beer's law, then this range may be determined by graphical analysis, and is smaller than the above, *e. g.*, 25–83 for *o*-nitraniline. The accuracy in determining acidity, H_0, is slightly different for each indicator, but since all indicators used have about the same absorption curves, it is about 0.02 unit for a range of photelometer readings from 95 to a point about 5 scale units above the reading for the stoichiometric concentration of the indicator base. This is equivalent to a useful range of the indicator in instrumental work, where the concentration of the colored species varies from 2–94% of the stoichiometric concentration, as compared to the 9–91% range for visual observation. The precision for *p*-nitraniline is about half of that for the rest of the indicators.

Results and Discussion

Urea–sulfuric acid solutions were prepared from 0.1111 *M* solutions of (a) urea, (b) urea hydrogen sulfate (a solution containing one mole of urea to one mole of sulfuric acid) and (c) sulfuric acid by mixing definite volumes of (a) with (b), and (b) with (c), so that the acidity decreased progressively while the solute concentration remained constant, and was equal to 0.1000 *M* after the addition of the indicator, at the time of analysis. Table I gives the composition of

TABLE I

MOLARITY OF THE BUFFER COMPONENTS AT THE TIME OF ANALYSIS

Buffer	Urea	Urea hydrogen sulfate	Sulfuric acid
U 1			0.1000
U 2		0.0111	.0889
U 3		.0222	.0778
U 4		.0333	.0667
U 5		.0444	.0556
U 6		.0556	.0444
U 7		.0667	.0333
U 8		.0778	.0222
U 9		.0833	.0167
U10		.0889	.0111
U11		.0944	.0056
U12		.1000	
U13	0.0056	.0944	
U14	.0111	.0889	
U15	.0167	.0833	
U16	.0222	.0778	
U17	.0333	.0667	
U18	.0444	.0556	
U19	.0556	.0444	
U20	.0667	.0333	
U21	.0778	.0222	
U22	.0889	.0111	
U23	.1000		

each member of this series of solutions. The antipyrine buffers were prepared from solutions of antipyrine and antipyrine hydrogen sulfate in precisely the same way, and the composition of this series, A12–A23, is exactly the same, solution for solution, as the urea series, U12–U23, where antipyrine replaces urea.

TABLE II

PHOTELOMETER READINGS 25.0 ± 0.1°

Buffer	Ind. 1	Ind. 2	Ind. 3	Ind. 4	Ind. 5	Ind. 6
U 1	86.5					
U 2	86.3					
U 3	85.0					
U 4	83.5					
U 5	82.0					
U 6	78.8					
U 7	75.2	85.8				
U 8	68.2	84.1				
U 9	63.8	81.9		88.9		
U10	57.6	79.3		85.6		
U11	50.0	76.3		80.8		
U12	41.8	73.3		74.1		
U13[a]	35.2	73.5		69.2		
U14	31.3	71.2		61.6		86.9
U15	29.3	69.2		55.9		82.9
U16	27.8	67.1		51.0		78.2
U17	26.1	63.8		44.1		69.7
U18	25.2	61.2		39.8		60.9
U19	24.6	57.9		35.7		53.0
U20	24.1	55.4		32.8		45.4
U21	24.1	52.6	88.0	30.8		38.4
U22	24.1	49.4	80.5	29.0		30.7
(U22–U23)			(70.0)		(83.9)	(26.2)
U23		47.8	32.7	27.6	24.4	20.4
17.5 U + 0.5 NaAc		47.8		27.6		20.3
A12				41.3		66.3
A13				27.1		37.2
A14			83.1	24.9	88.7	29.8
A15			78.0	24.0	85.3	26.0
A16			73.3	23.8	82.9	23.8
A17			65.3	23.7	78.3	21.0
A18			57.8	23.6	74.3	20.0
A19			51.1	24.0	69.2	19.3
A20			45.1	24.3	63.3	19.2
A21			39.4	24.9	55.0	19.0
A22			35.0	25.3	41.1	19.1
A23			30.7	26.2	20.9	19.2
17.5 A + 0.5 NaAc			30.7	26.2	20.9	19.2

[a] Measurements for U13–U23 were made with different solutions of 0.010 *M* indicator than those for U1–U12. Consequently the series of readings are not exactly continuous.

Ind. 1—*o*-nitraniline
Ind. 2—*p*-nitraniline
Ind. 3—*m*-nitro-N,N-dimethylaniline
Ind. 4—*p*-nitro-N,N-dimethylaniline
Ind. 5—*m*-nitro-N,N-diethylaniline
Ind. 6—*p*-nitro-N,N-diethylaniline.

The color intensities of all the indicators used which assumed an intermediate shade in each buffer were measured with the photelometer at the same time and at 25.0 ± 0.1°. Photelometer readings are shown in Table II for both series of solutions. The indicator concentration in the solutions was always 0.0010 *M*.

No calculations were made for Ind. 3 and Ind. 5 in urea solutions, because they underwent a practically complete color transition between the last buffer solution, U22, and pure urea, U23.

Calibration of the Instrument.—A calibration curve was made for each indicator by plotting on semi-logarithmic paper the photelometer readings for a series of solutions of the colored indicator base against the concentration. The series of indicator solutions was prepared by dilution in a medium in which the indicator was definitely fully colored. This medium was selected from the series of photelometer readings for each indicator given in Table II. Thus for the urea solutions, *o*-nitraniline was calibrated in buffer U22, whereas the other indicators were calibrated in urea to which a small definite amount of sodium acetate had been added (0.50 ml. of 0.1111 *M* sodium acetate to every 17.50 ml. of 0.1111 *M* urea). A typical calibration curve, *o*-nitraniline, is shown in Fig. 1. The curve for *p*-nitraniline differs

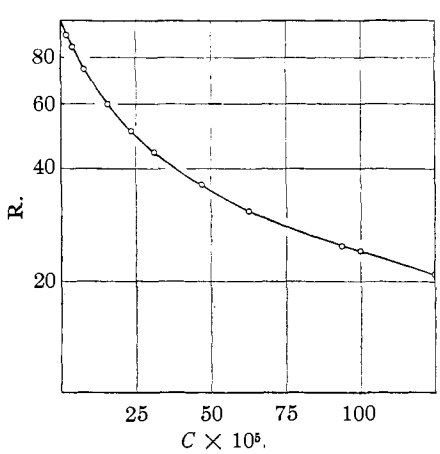

Fig. 1.—A typical calibration curve on a semi-log plot. Data obtained for *o*-nitroaniline at 25.0°, using Buffer U22 as a diluting medium. Ordinate, photelometer reading.

from all the others obtained in that it is flatter over a much larger range of concentrations and therefore the photelometer is much less sensitive to changes in the concentration of *p*-nitraniline than of the other indicator bases. This may be due to the fact that the maximum for the absorp-

tion band for *p*-nitraniline[11] lies on the limit of wave lengths, about 380 mμ, which the filter and the glass absorption cell transmit.

In antipyrine buffers, both *p*-nitro-N,N-dimethylaniline and *p*-nitro-N,N-diethylaniline show a minimum photelometer reading in the series which is quite marked for the former indicator and just at the limit of experimental error for the latter. For these indicators, as the composition of the series changes, becoming more basic and probably decreasing in ionic strength from A12–A23, the photelometer reading decreases, reaches a minimum, and finally increases. The latter increase corresponds to a decrease in the "specific color intensity of the base" as defined by Hammett.[7] This change must be attributed to a change in the nature of the medium and may be caused by either increasing basicity or decreasing ionic strength of the buffer series. This is exactly the same effect as Hammett and Deyrup observed for sulfuric acid–water mixtures, and Spengeman[2] observed for sulfuric acid–acetic acid mixtures, and operates in the same direction with respect to increasing acidity.

These investigators corrected for this effect by calculating the ionization ratio, $(BH^+)/(B)$, of the indicator from two specially defined terms, the "stoichiometric color intensity relative to the solvent," I_w, and the "specific color intensity of the colored form relative to the solvent," S_w. When there is no medium effect, S_w is equal to one. Since the determination of S_w cannot be made independently of I_w *when the indicator is partly ionized, it is necessary to assume that "S_w is independent of the change of medium incidental to the change of acidity" in this region.* It can be shown readily that this method of computation is equivalent to a colorimetric comparison of each solution of any one indicator with that solution of the same indicator which has the maximum I_w.

The equivalent correction can be made for the photelometric comparisons by simply calibrating the instrument with solutions of the indicator in that buffer solution of the series which gives the minimum photelometer reading. This was done for *p*-nitro-N,N-dimethylaniline. In the case of *p*-nitro-N,N-diethylaniline, the medium effect was so slight that it was found sufficiently accurate, in calculating the ionization ratio of the indicator, to use the calibration curve made in an antipyrine solution to which a little sodium acetate had been added (0.50 ml. of 0.1111 *M* sodium acetate to every 17.50 ml. of 0.1111 *M* base), if an "apparent value" (0.001015 *M*) the stoichiometric concentration read from the calibration curve corresponding to the minimum photelometer reading was used in place of the actual value (0.001000 *M*).

The remaining indicators, *m*-nitro-N,N-dimethylaniline and *m*-nitro-N,N-diethylaniline, were calibrated in the above-mentioned antipyrine and sodium acetate solution.

Although the composition of the buffer in the indicator dilution series remained the same, the total solute concentration did not remain constant at 0.1000 *M* but varied from 0.104 to 0.111 *M*. For indicators which did not show any medium effect, or else a very small one, this does not introduce any appreciable error. In the case of *p*-nitro-N,N-dimethylaniline, buffer A18 was used as the dilution medium, so that the salt concentration in the dilution series varied from 0.580 to 0.617 *M*. If the medium effect can be entirely attributed to the change in ionic strength and that can in turn be attributed to the salt concentration alone, then as far as the medium effect is concerned the indicator solutions are comparable to a buffer midway between A17 and A18. Since the photelometer readings for *p*-nitro-N,N-dimethylaniline for buffers A17 and A18 differ by only 0.1 unit for a 0.001 *M* indicator concentration, the error introduced by variation in ionic strength in calibrating a dilution series where the indicator concentration varies from 0.00125 to 0.000008 *M* is negligible.

Determination of the Ionization Ratio, $(BH^+)/(B)$.—The values of the ionization ratio were computed from the photelometer reading for each indicator for each solution in which it was used by obtaining from the proper calibration curves the corresponding concentrations of the colored non-ionized indicator base, (B), and the stoichiometric concentration of the indicator in the solutions, c_s. Thus

$$(BH^+) = c_s - (B) \qquad (1)$$

and

$$\frac{(BH^+)}{(B)} = \frac{c_s - (B)}{(B)} \qquad (2)$$

These values are given in Table III.

In Figs. 2 and 3, the values of log $(BH^+)/(B)$ for each indicator are plotted against the composition of the comparison solution. If the as-

(11) Landolt–Börnstein, "Tabellen," 5th ed., 3rd suppl., Fig. 183, p. 1394.

TABLE III

IONIZATION RATIO, $(BH^+)/(B)$, AT 25.0 ± 0.1°
STOICHIOMETRIC INDICATOR CONCENTRATION = 0.001 *M*

Buffer	Ind. 1	Ind. 2	Ind. 3	Ind. 4	Ind. 5	Ind. 6[a]
U 1	26.6					
U 2	25.7					
U 3	23.2					
U 4	20.6					
U 5	18.5					
U 6	15.0					
U 7	12.1	71.8				
U 8	8.30	56.2				
U 9	6.40	41.1		99.0		
U10	4.71	31.0		60.5		
U11	3.19	21.2		39.0		
U12	1.87	15.0		24.8		
U13	1.02	14.7		16.8		
U14	0.625	11.3		10.3		99.0
U15	.445	9.00		7.00		65.6
U16	.312	7.08		4.85		46.0
U17	.164	4.64		2.60		27.6
U18	.0885	3.24		1.59		16.4
U19	.0445	1.84		0.951		10.4
U20	.0000	1.18		.568		6.21
U21		0.656		.318		3.58
U22		.159		.133		1.61
U23		.000		.000		0.00
A12				2.88		24.4
A13				0.380		3.51
A14			7.60	.133	16.4	1.59
A15			5.20	.043	12.3	0.905
A16			3.88	.022	9.95	.558
A17			2.42	.013	7.43	.196
A18			1.54	.000	6.02	.083
A19			1.02		4.56	.021
A20			0.653		3.35	.003
A21			.363		2.22	.000
A22			.166		1.04	
A23			.000		0.00	

[a] The "apparent stoichiometric concentration" here is 0.001015 *M*.

sumption of Hammett and Deyrup is correct that

$$\log \frac{f_{B_1H^+}}{f_{B_1}} \frac{f_{B_2}}{f_{B_2H^+}} = \eta \leqq 0 \qquad (3)$$

when indicators of the same charge type are transferred from one medium to another, then

$$pK_1 - pK_2 = \log \frac{(B_1H^+)}{(B_1)} - \log \frac{(B_2H^+)}{(B_2)} \qquad (4)$$

since the ionization ratios are determined in solutions of the same acidity, H_0, and

$$pK = -\log a_{H^+} \frac{f_B}{f_{BH^+}} \frac{(B)}{(BH^+)} \qquad (5)$$

$$= H_0 + \log \frac{(BH^+)}{(B)} \qquad (6)$$

Therefore, the curves of log $(BH^+)/(B)$ should be parallel, and the difference in ordinates between any two indicators should equal the difference

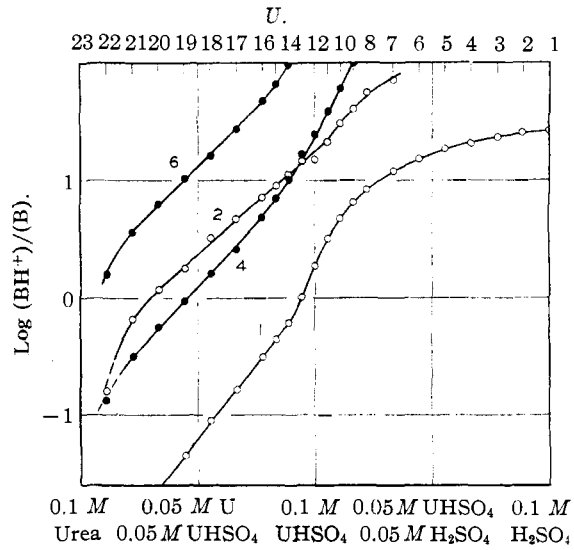

Fig. 2.—The numbers on the curves correspond to the indicators listed in Table II. Upper abscissa, number of buffer used; lower abscissa, composition as indicated.

in their pK's. This seems to be fairly true for urea solutions, especially where the comparison solutions are really buffers, *i. e.*, mixtures of urea

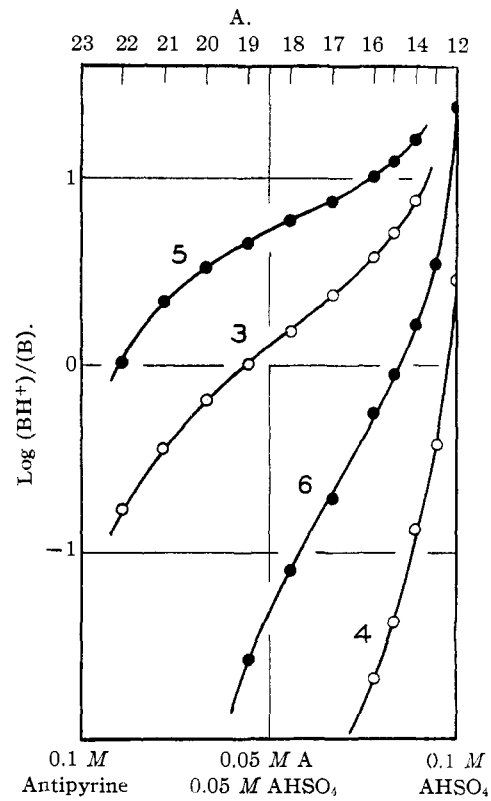

Fig. 3.—The numbers on the curves correspond to the indicators listed in Table II. Upper abscissa, composition of buffer used; lower abscissa, composition as indicated.

and urea hydrogen sulfate, except in the case of *p*-nitraniline. The deviation shown by *p*-nitroaniline may be due to several causes. First, the absorption spectrum shows a slight shift of the maximum to shorter wave lengths as the indicator base is ionized, which is equivalent to a slight medium effect superimposed upon the ionization. Second, the "colorless" ion absorbs light to an appreciable extent in the region of wave lengths 380–500 mμ, transmitted to the solution so that to a slight extent *p*-nitraniline behaves as a two color indicator. Third, it is also possible that the structure of the *p*-nitranilinium ion is different from the other indicator ions, so that the activity coefficient ratio is affected to a different degree by a change in the medium than are the coefficient ratios of the other indicators.

The log $(BH^+)/(B)$ curves in the antipyrine buffers are not as nearly parallel as the corresponding curves in the urea buffers, and this may be due in part to specific factors in ionic structure. Calculations made for the values of log $(BH^+)/(B)$ of *p*-nitro-N,N-diethylaniline neglecting the small medium effect give essentially the same curve, but the individual values do not follow the curve as closely in the more basic region as do the corrected values. This seems to justify the method of correction for the slight medium effect.

Indicator Constants.—The pK's of the indicators can be determined from Figs. 2 and 3 using equation (4). The pK's of the indicators used in urea solutions, *p*-nitraniline, *p*-nitro-N,N-dimethylaniline, and *p*-nitro-N,N-diethylaniline, were determined in the region where the solutions were buffers (mixtures of urea and urea hydrogen sulfate) by obtaining the differences in ordinates between their log $(BH^+)/(B)$ curves and that for *o*-nitraniline at fixed compositions, spaced at regular intervals, averaging these differences, and adding them to the pK for *o*-nitraniline. This value was taken as -0.17, an average of the values found for this indicator for various aqueous systems by Hammett and Paul.[12] The pK's for *m*-nitro-N,N-dimethylaniline, and *m*-nitro-N,N-diethylaniline were determined in exactly the same way using *p*-nitro-N,N-dimethylaniline and *p*-nitro-N,N-diethylaniline as the reference indicators in antipyrine buffers. The pK's of the reference indicators were taken as those found in the urea buffers. The two values for each of these indicators were then averaged, taking into account the relative number of comparison points with each reference indicator.

The final values of the pK's for the indicators are given in Table IV. From this table it is

TABLE IV

INDICATOR CONSTANTS

	pK_{HAc}	pK_{H_2O}
Ind. 1, *o*-nitraniline	(−0.17)	−0.17
Ind. 2, *p*-nitraniline	1.28 ± 0.05	1.11
Ind. 3, *m*-nitro-N,N-dimethylaniline	3.14 ± 0.11	
Ind. 4, *p*-nitro-N,N-dimethylaniline	1.08 ± 0.02	
Ind. 5, *m*-nitro-N,N-diethylaniline	3.74 ± 0.14	
Ind. 6, *p*-nitro-N,N-diethylaniline	2.10 ± 0.03	

seen that the pK of *p*-nitraniline obtained in urea buffers differs from the value obtained by Hammett and Deyrup in water by 0.17 unit. This discrepancy is not unusually great, and it is not larger than that found by Hall and Spengeman[2] in comparing the pK's of other indicators in glacial acetic acid to those in water.

Some interesting effects of structure upon basic strength can be seen from this table. Substituting two methyl groups on the nitrogen of *p*-nitraniline lowers the basic strength by about 0.2 unit, but two ethyl groups increase the basic strength of *p*-nitraniline by 0.8 unit. The meta isomers are considerably stronger bases than the para, and in both cases the diethyl indicators are stronger bases than the dimethyl.

Acidity of the Buffer Solutions.—From the pK's of the indicators the acidity function, H_0, was computed from the definition

$$H_0 = pK - \log \frac{(BH^+)}{(B)}$$

for each solution of both series of buffers. These data are given in Table V, where the results are averaged for each solution. In obtaining the average acidities for urea buffers, the values obtained from *p*-nitraniline were not used, because it did not follow the primary assumption concerning activity coefficients as closely as the other indicators. A plot of acidity against composition is given in Fig. 4. From this plot it is seen that the curves are essentially titration curves, and resemble those obtained by Hall and Werner[13] for the potentiometric titrations of urea and of *o*-chloraniline, whose basic strength is very close to that of antipyrine. This resemblance is great

(12) Hammett and Paul, THIS JOURNAL, **56**, 827 (1934).

(13) Hall and Werner, *ibid.*, **50**, 2367 (1928).

TABLE V

ACIDITY, H_0, OF THE BUFFER SOLUTIONS AT 25.0°

Buffer	Ind. 1	Ind. 2	Ind. 3	Ind. 4	Ind. 5	Ind. 6	Average[a] (excluding no. 2)
U 1	−1.60						−1.60
U 2	−1.58						−1.58
U 3	−1.54						−1.54
U 4	−1.49						−1.49
U 5	−1.44						−1.44
U 6	−1.35						−1.35
U 7	−1.25	−0.58					−1.25
U 8	−1.09	− .47					−1.09
U 9	−0.98	− .33		−0.92			−0.95 ± 0.03[a]
U10	− .84	− .21		− .70			− .77 ± .07
U11	− .67	− .05		− .51			− .59 ± .08
U12	− .44	.10		− .32			− .38 ± .06
U13	− .18	.11		− .15			− .17 ± .02
U14	.03	.23		.07		0.10	.07 ± .01
U15	.18	.33		.23		.28	.23 ± .02
U16	.34	.43		.39		.44	.39 ± .02
U17	.61	.61		.66		.66	.64 ± .01
U18	.88	.77		.88		.88	.88
U19	1.18	1.02		1.10		1.08	1.12 ± .02
U20		1.21		1.33		1.31	1.32 ± .01
U21		1.46		1.58		1.54	1.56 ± .02
U22		2.08		1.96		1.89	1.93 ± .04
A12				0.62		0.77	0.67 ± .05
A13				1.50		1.55	1.53 ± .03
A14			2.26	1.96		1.90	2.14 ± .08
A15			2.42	2.44	2.65	2.14	2.44 ± .11
A16			2.55	2.74	2.74	2.35	2.62 ± .14
A17			2.76	2.98	2.87	2.81	2.81 ± .04
A18			2.95		2.96		2.96 ± .01
A19			3.13		3.08		3.11 ± .03
A20			3.33		3.28		3.31 ± .03
A21			3.58		3.39		3.49 ± .10
A22			3.92		3.72		3.82 ± .10

[a] The deviations given in the last column are the mean deviations of the mean.

despite the difference in the methods of measurement and the fact that in the indicator method the total solute strength was kept constant. The H_0 at 50% titration for urea is 0.98. The corresponding pH(HAc) value given by Hall and Werner is −0.7, which is 1.7 units more negative. Hall,[14] in titrations with perchloric acid, found $pK' = +1.45$ for antipyrine. If 1.7 is added to this value, the sum (3.15) lies close to the H_0 value for the half-titration point of this base.

Some important differences between these titration curves and those of Hall and Werner are shown by a plot of H_0, for urea and antipyrine, against log $X/(1 - X)$, where X is the per cent. of base titrated, in Fig. 5. Hall and Werner showed that such a plot was a sensitive test for a weak base, which should have a slope of unity. However, none of the bases titrated by them with perchloric acid gave the theoretical slope. In this investigation, on the other hand, the best line drawn through the experimental points for the indicator measurements gives a slope of 1.05 for urea, and 0.83 for antipyrine. Furthermore, a

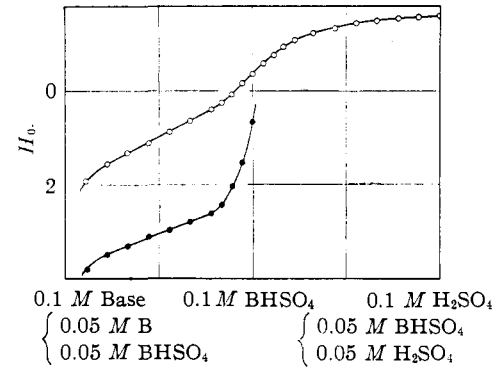

Fig. 4.—The data for urea are shown by the open circles; those for antipyrine, by the closed circles.

(14) N. F. Hall, THIS JOURNAL, **52**, 5115 (1930).

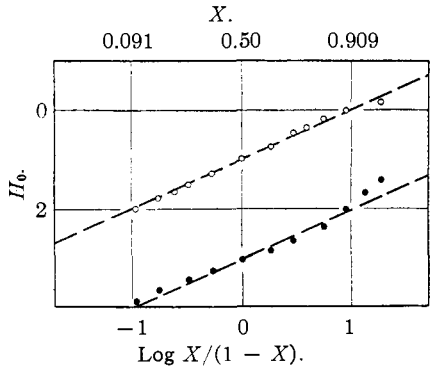

Fig. 5.—The data for urea are shown by the open circles; those for antipyrine, by the closed circles. Upper ordinate, X, the fraction of the base titrated; lower ordinate, log $X/(1 - X)$.

line drawn through the point of half-titration with the theoretical slope (the dashed lines in Fig. 5) does lie fairly close to the experimental points in both cases. This indicates that some of the errors inherent in the electrometric method such as the liquid junction potential and the change in activity coefficients with dilution tended to distort the results. It is of interest to note that the form of the antipyrine titration curve is somewhat abnormal. Hall[14] observed that the e. m. f. curve for this base was steeper than the curves for the titration of other bases of comparable strength, and that its strength in acetic acid appeared abnormally great. In these measurements, on the other hand, the abnormality is shown by a flatness of the curve and a considerable departure from the theoretical form on the *acid* side after about 80% titration.

Summary

The acidity of tenth molar urea–sulfuric acid solutions and antipyrine–sulfuric acid solutions in glacial acetic acid was studied at 25.0° with six indicators, *o*-nitraniline, *p*-nitraniline, *m*- and *p*-nitro-N,N-dimethylaniline, and *m*- and *p*-nitro-N,N-diethylaniline. The pK's of these indicators were determined in these buffers from the pK of *o*-nitraniline, taken as −0.17. The acidity function, H_0, was determined from these pK's for each solution used. The H_0 values for the points of half neutralization of urea and antipyrine were found to be 0.98 and 3.05, respectively. These values differ by about 1.7 from the pH(HAc) values found by Hall and his co-workers, and the value 1.7 for difference between the pH(HAc) scale and the H_0 scale is in fair agreement with the value 2 found by Hall and Spengeman.

MADISON, WISCONSIN RECEIVED JULY 19, 1940

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Further Studies on the Enzyme, Tyrosinase

BY DONALD C. GREGG AND J. M. NELSON

As is well known, the phenol oxidase, tyrosinase, has been regarded as an enzyme capable of catalyzing two types of aerobic oxidation. One of these is the introduction of a hydroxyl group ortho to the one already present in certain monohydric phenols. The other brings about the oxidation of certain *o*-dihydric phenols to their corresponding *o*-quinones. Since *p*-cresol has been widely used as a substrate for studying the first and catechol for the latter, the two activities will be termed cresolase and catecholase, respectively.

A convenient source for this enzyme is the common mushroom, *Psalliota campestris*. When aqueous extracts of the plant, containing the tyrosinase, are permitted to stand exposed to air, they darken and considerable enzymic activity is lost. A loss also occurs when the enzyme in the aqueous extract is subjected to the usual methods of purification, such as precipitation with cold acetone, ammonium sulfate, dialysis, etc. Upon comparing the ratio between the two final activities with that of the original fresh extract, it is found that the cresolase activity has suffered a greater loss than the catecholase activity. Thus preparations have been obtained very low in cresolase but high in catecholase. In highly purified preparations the latter activity has been found proportional to the copper content.[1,2] Due to this relatively greater loss in cresolase activity during the purification, and also due to only the catecholase activity being proportional to the copper content, certain workers[1] have concluded that the cresolase activity is not part of the en-

(1) D. Keilin and T. Mann, *Proc. Roy. Soc.* (London), **125B**, 187 (1938).

(2) B. J. Ludwig and J. M. Nelson, THIS JOURNAL, **61**, 2601 (1939).