Acidity Functions in 20% Ethanol–80% Aqueous Sulfuric Acid[1]

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Abstract: Although, at high acidities, sizable amounts of ethanol are converted to ethyl hydrogen sulfate in the 20% ethanol–80% aqueous sulfuric acid solvent system, this reaction comes to equilibrium quickly and the solvent appears to be generally useful for measurements on weak bases of low water solubility. Primary anilines and azobenzenes were found to show significantly different protonation behavior in this medium, and two separate acidity functions H_0 and H_{azo} were therefore set up for this solvent; the latter scale corrects and uses data from H_0', an acidity function originally based upon both primary aniline and azobenzene indicators. A completely objective analytical procedure was used in constructing these acidity functions. This demonstrated clearly that the activity coefficient postulate, upon which the acidity function method is based, is not strictly valid even for bases as similar as pairs of primary aniline indicators; implications of this are discussed.

In order to provide an acidity scale for a medium similar to concentrated aqueous sulfuric acid but in which weak organic bases might be more soluble, Jaffe[3] set up an indicator acidity function in 20% ethanol–80% sulfuric acid. This scale, called H_0', was based principally upon azobenzene indicators, but it also used two primary anilines at its low acidity end. In the decade following that work, it became increasingly apparent that the protonation behavior of azobenzenes and primary anilines in concentrated acid might be sufficiently different to invalidate an acidity function based upon a mixture of these two kinds of indicator base.[4] Some of the possible consequences of this difference were discussed recently by Stewart[5] when he constructed another acidity function, H_0'', based wholly on diphenylamines, for the 20% ethanol–80% sulfuric acid solvent system.

In connection with a study of the basicity of carbazoles,[6] many of which are very insoluble in wholly aqueous acids, we had occasion to investigate this matter. We found that azobenzenes and primary anilines do in fact show different protonation behavior and that the H_0' scale is thus an inconsistent acidity function. We, therefore, set up a genuine H_0 (primary aniline) scale for 20% ethanol–80% sulfuric acid and also used a new azobenzene indicator to redetermine that part of H_0' which had been based upon primary anilines; this created a wholly azobenzene function, H_{azo}. In the course of this work we developed an objective, analytical procedure for translating indicator measurements into acidity function values which revealed information of general relevance to acidity function theory.

Solvent System. The 20% ethanol–80% sulfuric acid solvent system used here was generated, as in previous studies,[3,5] by diluting one part of commercial 95% aqueous ethanol with enough aqueous sulfuric acid of the appropriate concentration to give a final volume exactly five times as great. This procedure, when followed using a stock ethanolic solution of indicator in place of pure aqueous alcohol, automatically gives solutions of constant total indicator concentration; this is a feature of considerable convenience in acidity function work.

Ethanol is known to be esterified by concentrated sulfuric acid.[7] Evidence that this reaction was occurring in some of the more acidic solutions used here came from the additional nmr signal, slightly displaced from that due to ethanol, which these solutions gave,[8] and also from acidimetric titers which were lower than those expected on the basis of the amount of sulfuric acid used. Quantitative estimates of the extent of esterification could be made from the relative intensities of the two nmr signals. These agreed with results obtained by titration, Table I, provided that the reaction was formulated as producing ethyl hydrogen sulfate. These data show that no appreciable esterification occurs below *ca.* 8 *M* total sulfate concentration (50 wt % H_2SO_4), but that beyond this point the extent of reaction increases markedly with increasing acidity.

Table I. Composition of More Concentrated 20% Ethanol–80% Sulfuric Acid Solutions at 25° [a]

[Total sulfate], *M*	$[C_2H_5OH]$, *M*	$[H_2O]$, *M*	$[C_2H_5OSO_3H]/[C_2H_5OH]$ By titration	By nmr
8.3	2.8	25	0.162	
9.9	2.0	21	0.636	0.56
11.3	1.7	18	0.920	0.83
12.6	1.4	14	1.30	1.30
13.8	1.1	10	2.03	2.1
14.9	0.57	6	4.77	4.0

[a] Concentrations are stoichiometric, *i.e.*, they do not allow for ionization of H_2SO_4, $C_2H_5OSO_3H$, etc.

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(2) PRF Graduate Fellow.

(3) (a) S. J. Yeh and H. H. Jaffe, *J. Amer. Chem. Soc.*, **81**, 3274 (1959); (b) H. H. Jaffe and R. W. Gardner, *ibid.*, **80**, 319 (1958).

(4) For recent reviews dealing with the effect of indicator structure on acidity functions, see R. H. Boyd in "Solute-Solvent Interactions," J. F. Coetzee and C. D. Ritchie, Ed., Marcel Dekker, New York, N. Y., 1969; C. H. Rochester, "Acidity Functions," Academic Press, New York, N. Y., 1970; L. P. Hammett, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N. Y., 1970, Chapter 9.

(5) D. Dolman and R. Stewart, *Can. J. Chem.*, **45**, 903 (1967).

(6) H. J. Chen, L. E. Hakka, R. L. Hinman, A. J. Kresge, and E. B. Whipple, *J. Amer. Chem. Soc.*, **93**, 5102 1971).

(7) (a) N. C. Deno and M. Newman, *ibid.*, **72**, 3852 (1950); (b) D. J. Clark and G. Williams, *J. Chem. Soc.*, 4218 (1957).

(8) D. G. Lee and R. Cameron, *J. Amer. Chem. Soc.*, **93**, 4724 (1971).

Table II. Polynomial Relationships Expressing Properties of 20% Ethanol–80% Sulfuric Acid at 25° [a]

y	x	Coefficients[b] b_0	b_1	b_2	b_3	b_4	b_5	b_6
D, g/ml	Equiv$_{H^+}$/kg (0–15.6)	9.602, −01	4.333, −02	−4.614, −03	8.833, −04	−8.393, −05	4.891, −06	−1.223, −07
D, g/ml	Equiv$_{H^+}$/l. (0–27.5)	9.612, −01	3.981, −02	−2.912, −03	2.909, −04	−1.424, −05	3.446, −07	−3.474, −09
Equiv$_{H^+}$/kg	Equiv$_{H^+}$/l. (0–27.5)	0.000, 00	1.036, +00	−3.489, −02	1.870, −03	−9.887, −05	2.880, −06	−3.087, −08
Equiv$_{H^+}$/l.	Equiv$_{H^+}$/kg (0–16.5)	0.000, 00	9.630, −01	3.137, −02	1.737, −03	−4.527, −04	5.117, −05	−1.659, −06
$-H_0$	Equiv$_{H^+}$/kg (0.2–3)	−1.348, 00	2.850 +00	−3.071, +00	2.224, +00	−9.276, −01	2.017, −01	−1.759, −02
$-H_0$	Equiv$_{H^+}$/kg (3–10)	−2.812, 00	3.363, +00	−1.459, +00	3.547, −01	−4.612, −02	3.059, −03	−8.070, −05
$-H_0$	Equiv$_{H^+}$/kg (10–16)	3.3959396, +03	−1.6135528, +03	3.1785871, +02	−3.3213543, +01	1.9420054, +00	−6.0234263, −02	7.7424208, −04
$-H_{azo}$	Equiv$_{H^+}$/kg (0.2–3)	−1.414, 00	3.242, +00	−3.823, +00	3.049, +00	−1.381, +00	3.247, +01	−3.063, −02
$-H_{azo}$	Equiv$_{H^+}$/kg (3–12.5)	−2.9565, 00	3.4186, +00	−1.2907, +00	2.6504, −01	−2.8169, −02	1.5190, −03	−3.2601, −05

[a] Equiv$_{H^+}$/kg represents equivalents of acid per kg of solution, and equiv$_{H^+}$/l. represents equivalents of acid per liter of solution: the latter is numerically equal to $N_{H_2SO_4}$ used in ref 3 and twice $M_{H_2SO_4}$ used in ref 5. [b] Coefficients of the polynomial $y = b_0 + b_1x + b_2x^2 + b_3x^3 + b_4x^4 + b_5x^5 + b_6x^6$; the last two digits of a coefficient and the sign preceding them denote the power of ten. These equations reproduce density to 1/1000th, equiv$_{H^+}$/kg or equiv$_{H^+}$/l. to 0.01, and H_0, H_{azo}, and H_0'' to 0.01–0.02; over the indicated range.

In the most acidic solution available from commercial concentrated sulfuric acid, that obtained by combining the 95% acid with ethanol directly, 80% of the available ethanol and 20% of the available sulfuric acid are used up; this solution, initially 15 M in H_2SO_4, 3 M in C_2H_5OH, and 4 M in H_2O, is at equilibrium, 12 M in H_2SO_4, 0.6 M in C_2H_5OH, 6 M in H_2O, and 3 M in $C_2H_5OSO_3H$. This steep acidity dependence of the position of equilibrium of this reaction, as well as the magnitude of the $[C_2H_5OSO_3H]/[C_2H_5OH]$ ratios observed, is consistent with results obtained in previous studies performed at lower total ethanol concentrations.[7b]

Considerable heat was evolved when the more concentrated sulfuric acid needed for the more acidic solutions was added to ethanol, and these solutions therefore had to be topped off with more acid after they had cooled and their volume had contracted. Times of the order of 1 hr were generally taken to bring these concentrated acid solutions to a given volume at constant temperature. This apparently was long enough for the esterification reaction, which is known to be rapid at high acidities,[7b] to reach equilibrium, for the nmr spectra of these solutions did not change with time and their indicator ratios were constant as well. It was nevertheless possible to characterize these solutions in terms of unhydrolyzed ethyl hydrogen sulfate by titration with standard base, provided that aliquots of solution were first quenched in equal volumes of water: alkyl hydrogen sulfates are known to react with water only slowly in dilute acid or in basic solution.[9] Under these conditions, titration end points were completely stable, in keeping with previous experience.[7a] In fact, back titration of samples added to excess sodium hydroxide showed very little (<1%) hydrolysis of ethyl hydrogen sulfate even after several days' time.

Since these acidimetric analyses did not differentiate between the two acids, H_2SO_4 and $C_2H_5OSO_3H$, their results will be reported in terms of total acidity, as equivalents of acid per liter, equiv$_{H^+}$/l., or equivalents of acid per kilogram, equiv$_{H^+}$/kg, of solution. In order to facilitate interconversion of these two units, densities of representative 20% ethanolic sulfuric acid solutions were measured and the results were fitted to polynomial expressions. The coefficients of these polynomials, as well as others which translate equiv$_{H^+}$/l. into equiv$_{H^+}$/kg and *vice versa*, are listed in Table II.

Acidimetric titrations were also used to characterize 20% ethanolic sulfuric acid solutions in previous studies of this solvent system.[3,5] In both cases, however, no mention of the esterification reaction was made and results were reported in terms of sulfuric acid alone. Since no details of the titration methods were given, it is difficult to say whether hydrolysis of ethyl hydrogen sulfate occurred during the course of these analyses. It seems likely, however, that, even if samples were not quenched in water, rapid neutralization and dilution were effected at the beginning of a titration, and that the solutions were thereby quickly transformed into media in which hydrolysis does not occur. In the absence of further information, therefore, the sulfuric acid molarities reported by Jaffe[3] will be taken to be equal to the equiv$_{H^+}$/l. used here, and the sulfuric acid molarities used by Stewart[5] will be assumed to be equivalent to (equiv$_{H^+}$/l.)/2 (however, see below).

The H_0 Scale. This acidity function was constructed using eight of the primary aniline indicators commonly employed to measure H_0 in wholly aqueous systems. Ratios of protonated to unprotonated base concentrations, C_{BH^+}/C_B ($= I$), were determined spectrophotometrically,[10] with measurements confined to the useful range of log I, −1.5 to +1.5.[11]

The pK_a of the most basic of these indicators, *p*-nitroaniline, was measured in ethanol–perchloric acid, rather than ethanol–sulfuric acid mixtures, in order to avoid difficulties associated with incomplete second dissociation of H_2SO_4. Two methods were used to obtain this acidity constant: (1) extrapolation to infinite dilution and (2) overlap with *m*-nitroaniline, whose pK_a was itself determined by extrapolation.

The first of these methods involves extrapolating log C_{H^+}/I down to zero acid concentration.[4] This quantity differs from pK_a by an activity coefficient term, log $f_{H^+}f_B/f_{BH^+}$, which is defined as zero at zero ionic strength, μ ($\mu = C_{H^+}$ in perchloric acid solutions). This activity coefficient term will be a linear function of

(9) R. Burwell, Jr., *ibid.*, 74, 1462 (1952); J. L. Kurz, *J. Phys. Chem.*, 66, 2239 (1962); B. D. Batts, *J. Chem. Soc. B*, 551 (1966).

(10) These results appear in tables which follow these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-72-8192. Remit $3.00 for photocopy or $2.00 for microfiche.

(11) A. J. Kresge and H. J. Chen, *Anal. Chem.*, 41, 74 (1969).

μ under the reasonable assumptions that log f_B is linearly dependent upon μ and that log f_{H^-} and log f_{BH^-} can be represented by expressions of the type $-0.5\mu^{1/2}/(1 + \mu^{1/2}) + b\mu$.[12] Plots of log C_{H^-}/I *vs.* C_{H^-} were found to be linear for both *m*- and *p*-nitroaniline.

In the interest of objectivity, the intercepts of these plots (= pK_a) were evaluated analytically rather than visually. Weighted least squares were used to fit values of log C_{H^-}/I to a linear expression in C_{H^+}, with weights, w_i, assigned to each data point in inverse proportion to expected uncertainties in log I squared: $w_i \propto (\sigma_{\log I_i})^{-2}$ (C_{H^+} was assumed to be known exactly).[13a] Uncertainties in log I, $\sigma_{\log I}$, were estimated for this purpose by evaluating a recently derived[11] error function (eq 1), which takes into account the fact

$$\sigma_{\log I} \propto (1 + I^{-1})(1 + I + I^2)^{1/2} \quad (1)$$

that I can be determined most accurately when its value is near unity and that errors mount rapidly as I becomes either very large or very small. This procedure gave $pK_a = 0.705 \pm 0.008$ for *p*-nitroaniline and $pK_a = 2.273 \pm 0.004$ for *m*-nitroaniline. Each of these is significantly different from the corresponding value reported for wholly aqueous solution (1.00 and 2.46, respectively[14]), which reflects the fact that the present determinations were referred to a 20% ethanolic standard state.

The overlap method equates the difference in pK_a of two indicators with the difference in their log I values evaluated at constant C_{H^-}.[4] The assumption here is that activity coefficient ratios of the form f_{BH^+}/f_B for the two bases are equal in a given solution and that the last term of eq 2 is therefore zero; this condition may be recognized by the constancy of log $I(1)$ − log $I(2)$ (= Δ log I). This assumption is not always valid, even

$$pK_a(1) - pK_a(2) = \log I(1) - \log I(2) + \log f_{BH^+(1)} f_{B(2)} / f_{BH^+(2)} f_{B(1)} \quad (2)$$

when both indicators are primary anilines (*vide infra*), but it did prove to be reasonably good in the solutions used here for *m*- and *p*-nitroanilines. Thus, it was possible to use this method to base the pK_a of *p*-nitroaniline upon that obtained for *m*-nitroaniline by extrapolation.

Again, for the sake of objectivity, the interpolations needed to obtain log $I(1)$ and log $I(2)$ at the same acid concentration were done analytically rather than visually. Values of log I for each indicator were fitted by weighted least squares to polynomials (sixth order) in acid concentration (equiv$_H$/kg), with weights assigned in accord with eq 1. Interpolations were performed by evaluating these polynomials. This generated a series of Δ log I values, each based on one measured and one interpolated log I, which were then combined into a weighted average. Weights were assigned here in proportion to $(\sigma_{\Delta \log I})^{-2}$, using $\sigma_{\Delta \log I}$ values obtained by propagating errors[13b] in log $I(1)$ and log $I(2)$. Errors in the constituent log I values were taken to be proportional to the product of $\sigma_{\log I}$, calculated according to eq 1, and SD, the standard deviation in log I from its polynomial interpolation function.[15] The latter factor was introduced to allow for differences in $\sigma_{\log I}$ from indicator to indicator, produced, for example, by differences in the spectral change attending protonation.

In addition to being an objective method of evaluating Δ log I, this procedure provides error estimates which can be used to determine whether or not Δ log I is constant over the overlap interval. In the present case, for example, 12 values of Δ log I were determined; these rose from 1.53 to 1.63 and then dropped back to 1.53 as the acid concentration was increased. The standard deviations estimated for these values, however, ranged from 0.03 to 0.07, which is comparable to the range of Δ log I observed, 0.10. Thus, it would seem reasonable to question the significance of the systematic variation in Δ log I and to accept its mean value, 1.58 ± 0.04, as valid within the stated error limits. This leads to a pK_a of 0.69 ± 0.04 for *p*-nitroaniline, which is in good agreement with the value of 0.705 ± 0.008 determined by extrapolation.

The weighted mean of these two values, 0.704 ± 0.008, was taken as the best pK_a for *p*-nitroaniline in 20% aqueous ethanol, and the pK_a values of the other, less basic, H_0 indicators were based upon this. The overlap method as described above was used for this purpose. In the more concentrated acid solutions needed for this purpose, however, systematic changes in Δ log I two to three times that observed for *m*- and *p*-nitroaniline, and four to five times the maximum estimated uncertainty in Δ log I, were not uncommon.

Systematic deviations of this kind are especially easily seen in plots of log $I(1)$ *vs.* log $I(2)$ for overlapping indicators, for slopes of such plots must be unity as long as log I is constant.[16] Plots of this kind were constructed for the indicators used here, and their slopes were evaluated by weighted least squares. The results, presented in Table III, show all slopes to be significantly different from unity; in three cases the deviations amount to 20–25%. It is apparent, therefore, that the activity coefficient postulate upon which the overlap method is based is not strictly valid in this system, even for bases as much alike as primary anilines, and that the

Table III. Primary Aniline Indicators Used to Construct H_0 in 20% Ethanol–80% Sulfuric Acid

Indicator	d log I_{i+1}/d log I_i[a]	pK_a[a]
1. *p*-Nitroaniline	0.947 ± 0.014	0.704 ± 0.004
2. *o*-Nitroaniline	0.932 ± 0.014	−0.585 ± 0.024
3. 2,5-Dichloro-4-nitroaniline	0.759 ± 0.044	−2.270 ± 0.028
4. 2,6-Dichloro-4-nitroaniline	0.798 ± 0.013	−3.810 ± 0.074
5. 2,4-Dinitroaniline	1.108 ± 0.007	−4.663 ± 0.103
6. 2,6-Dinitroaniline	0.971 ± 0.009	−5.703 ± 0.108
7. 2-Bromo-4,6-dinitroaniline	0.793 ± 0.011	−6.924 ± 0.108
8. 3-Methyl-2,4,6-trinitroaniline		−8.173 ± 0.124

[a] Error estimates are standard deviations of mean values.

(12) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Butterworths, London, 1959, Chapter 9.

(13) C. A. Bennett and N. L. Franklin, "Statistical Analysis in Chemistry and the Chemical Industry," Wiley, New York, N. Y., 1954, (a) p 243, (b) p 49.

(14) A. I. Biggs and R. A. Robinson, *J. Chem. Soc.*, 388 (1961).

(15) SD = Σ_i[log I_i(obsd) − log I_i(calcd)]$^2/(n + 1)$, where n is the order of the polynomial.

(16) C. D. Johnson, A. R. Katritzky, and S. A. Shapiro, *J. Amer. Chem. Soc.*, **91**, 6654 (1969).

pK_a values determined here (Table III) can be no more than approximate estimates of true values.

These pK_a values were used to calculate H_0 according to the defining relationship, $H_0 = pK_a - \log I$,[4] for 80 solutions spaced at regular intervals over the range 0–27 equiv$_{H^+}$/l. Values of log I were obtained from the polynomial interpolation functions. For each solution, the calculation was performed using data for each indicator within whose measured range of I that solution fell. The results were then combined into weighted averages using uncertainties propagated from constituent quantities. Representative values of H_0 are listed in Table IV, and the coefficients of polynomials which relate H_0 to equiv$_{H^+}$/kg are given in Table II; these polynomials, used in conjunction with a programmable desk calculator, provide a particularly convenient method of evaluating H_0 for any given solution.

Table IV. Acidity Function Values for 20% Ethanol–80% Sulfuric Acid at 25°

Equiv$_{H^+}$/kg[a]	Equiv$_{H^+}$/l.[b]	H_0[c]	H_{azo}[d]
0.2039	0.202	0.881 ± 0.012	0.89
0.4078	0.402	0.557 ± 0.012	0.56
0.612	0.604	0.366 ± 0.013	0.33
0.816	0.810	0.209 ± 0.014	0.16
1.020	1.020	0.075 ± 0.015	0.02
2.039	2.106	−0.360 ± 0.024	−0 52
2.855	3.028	−0.624 ± 0.024	−0.86
3.671	3.994	−0.891 ± 0.024	−1.13
4.486	5.01	−1.165 ± 0.020	−1.42
5.30	6.07	−1.449 ± 0.020	−1.77
6.93	8.35	−2.060 ± 0.029	−2.66
8.16	10.24	−2.569 ± 0.029	−3.44
9.38	12.30	−3.200 ± 0.029	−4.31
10.20	13.79	−3.680 ± 0.063	−4.97
11.42	16.22	−4.611 ± 0.063	−6.11
12.24	17.96	−5.196 ± 0.076	−6.95
13.05	19.79	−5.948 ± 0.087	
13.87	21.69	−6.745 ± 0.080	
14.68	23.62	−7.557 ± 0.085	
15.50	25.53	−8.372 ± 0.112	
16.31	27.34	−9.384 ± 0.151	

[a] Equivalents of acid per kilogram of solution. [b] Equivalents of acid per liter of solution; this is equivalent to $N_{H_2SO_4}$ used in ref 3 and twice $M_{H_2SO_4}$ used in ref 4. [c] Error estimates are standard deviations of the mean. [d] This scale beyond equiv$_{H^+}$/kg = 4 is H_0' of ref 3 + (−0.53).

H_{azo}. The most basic azobenzene indicator which Jaffe employed to set up the H_0' scale, 4-methoxy-4′-hydroxyazobenzene, did not allow measurements much below equiv$_{H^+}$/l. = 3. He therefore filled in the region down to dilute acid with *o*- and *p*-nitroaniline in the expectation that these primary anilines would show the same protonation behavior as the azobenzenes, *i.e.*, that f_{BH^+}/f_B for the two classes of compound would be the same. The resulting acidity function, however, undergoes a marked change in gradient at equiv$_{H^+}$/l. = 3, which suggests that this expectation was not fulfilled.

We obtained direct evidence bearing on this point by examining the protonation behavior of 2,4-dimethoxyazobenzene. This substance is considerably more basic than Jaffe's most basic azobenzene indicator, and we could perform measurements in dilute acid as well as the region near equiv$_{H^+}$/l. = 3.[10] In dilute 20% ethanolic H_2SO_4, the protonation of this azobenzene adheres closely to the H_0 scale, as would be expected for a region where differences in indicator behavior have not been fully developed and all scales closely approximate $-\log C_{H^+}$. For example, 24 determinations of log I(*p*-nitroaniline) − log I(2,4-dimethoxyazobenzene) made at equiv$_{H^+}$/l. = 0.01–0.5 show only random deviations from a mean value of 0.735 ± 0.019. (This fixes the pK_a of 2,4-dimethoxyazobenzene at −0.03 ± 0.02.) Above equiv$_{H^+}$/l. = 0.5, on the other hand, Δ log I changes steadily until, in the most acidic solution used (equiv$_{H^+}$/l. = 4.2), the value of I observed for the azobenzene is twice that expected on the basis of a protonation controlled by H_0. At approximately equiv$_{H^+}$/l. = 3, moreover, which is the point where H_0' switches from primary aniline to azobenzene indicators, log I for 2,4-dimethoxyazobenzene begins to parallel H_0'. It is apparent, therefore, that the protonation behavior of azobenzenes is different from that of primary anilines and that the two reactions must be described by separate acidity functions.

The inconsistency in H_0' introduced by mixing these two kinds of indicator may be removed by using the present data for 2,4-dimethoxyazobenzene to construct an acidity function extending from equiv$_{H^+}$/l. = 0.01 to 4.2. In the region where log I for 2,4-dimethoxyazobenzene was found to run parallel to H_0' (equiv$_{H^+}$/l. = 3–4.2), this new acidity function is more negative than H_0' by a constant amount, 0.53 log unit. This is the correction which converts H_0' into an azobenzene-based scale above equiv$_{H^+}$/l. = 3, and the combination of that result with the 2,4-dimethoxyazobenzene scale gives a wholly azobenzene scale over the entire region equiv$_{H^+}$/l. = 0.01–19. Representative values of this acidity function, now called H_{azo}, are given in Table IV, and the coefficients of the polynomials which relate it to equiv$_{H^+}$/kg are listed in Table II.

Discussion

The present H_0 function shows a less steep acidity dependence, *i.e.*, changes with acid concentration more slowly, than the H_{azo} function (Figure 1). This is consistent with the general belief that solvation through hydrogen bonding plays an important role in determining the acidity dependence of reactions occurring in concentrated acids.[4] The conjugate acid of an H_0 indicator, a primary ammonium ion $ArNH_3^+$, has three sites (N–H bonds) at which strong hydrogen bonds to the solvent (water or ethanol) may be formed; this offsets to some degree the strong solvation of the hydrogen ion and makes the position of equilibrium of this reaction less sensitive to the decrease in water concentration which necessarily accompanies an increase in acidity. A protonated azobenzene, on the other hand, has only one N–H bond, and its positive charge, moreover, is very probably delocalized from this nitrogen into one of the benzene rings. The position of equilibrium of this reaction will therefore be more sensitive to changes in water concentration, which is tantamount to a steeper acidity dependence. Differences of this kind between primary, secondary, and tertiary amines in wholly aqueous systems are well known.[17]

It is of interest to see whether the third acidity function available for 20% ethanolic sulfuric acid, H_0'',[5] conforms to this relationship between hydrogen bonding

(17) R. W. Taft, *ibid.*, **82**, 1965 (1960); E. M. Arnett and G. W. Mach, *ibid.*, **86**, 2671 (1964); **88**, 1177 (1966).

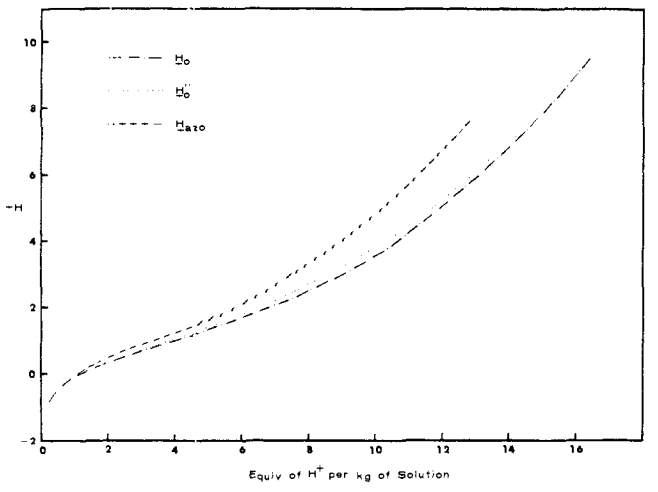

Figure 1. Acidity functions in 20% ethanol–80% sulfuric acid.

and acidity dependence. This scale is based wholly on secondary amine indicators and therefore should fall roughly midway between H_0 and H_{azo}, perhaps a bit closer to H_0 if resonance is reasonably effective at reducing the positive charge on nitrogen in protonated azobenzenes. The H_0'' scale as published is referred to a wholly aqueous standard state, but the change to a 20% ethanolic state needed for comparison with H_0 and H_{azo} may be made by using the fact that $\Delta \log I$ for *p*-nitroaniline and diphenylamine, the reference H_0'' indicator, is reported to be -0.07 ± 0.01 in dilute 20% ethanolic sulfuric acid.[5] This, combined with the pK_a determined here for *p*-nitroaniline in this medium, gives $pK_a = 0.634 \pm 0.013$ for diphenylamine in 20% ethanol. This value is more negative by 0.15 than the wholly aqueous pK_a, 0.78,[5] and H_0'' may thus be converted to a 20% ethanolic standard state by adding -0.15 to each published value.

The results show H_0'' to be much closer to H_0 than expected (Figure 1). However, the breakdown of the hydrogen bonding–acidity dependence relationship which this implies may be more apparent than real, for at least two effects could be operating to shift H_0'' from its true position toward H_0. The first of these is a possibly incorrect referencing of H_0'' to the 20% ethanolic standard state. The shift of -0.15 described above is insufficient to make H_0'' coincide with H_0 and H_{azo} in dilute 20% ethanolic sulfuric acid, where all acidity functions should be equal to $-\log C_{H^+}$ and where H_0 and H_{azo} are themselves identical; the additional shift of -0.07 which is needed would move H_0'' away from H_0 toward H_{azo}. It is also possible that some hydrolysis of ethyl hydrogen sulfate occurred during the acidimetric assay of the solutions used to measure H_0''. This would have the effect of giving these solutions a greater acid concentration than would have been obtained by titrations as performed here, and allowance for this again moves H_0'' closer to H_{azo}. It is significant in this respect that H_0'' shows a steady if small divergence from H_0 up to *ca.* equiv$_{H^+}$/kg = 10, where it then begins to fall back; this is the point where extensive formation of ethyl hydrogen sulfate begins to occur.

Too fine an interpretation, however, must not be placed upon comparisons such as this, for the present H_0 scale, and presumably other acidity functions as well, is subject to considerable uncertainty. There is, first of all, the accumulation of errors characteristic of the stepwise operation used in constructing acidity functions. This is clearly visible in Table IV: the uncertainty in H_0 rises from 0.01 in dilute solution to 0.15 for the most concentrated acid used. The principal source of this mounting error is the increasing uncertainty in the pK_a values of successively weaker bases (Table III): $\log I$ can be measured with similar accuracy in concentrated acid as in dilute solution, but the pK_a of an indicator used in concentrated acid must contain all of the error in the pK_a of every base used to refer that indicator back to the dilute solution standard state.

Potentially more serious than this rising imprecision, however, is the breakdown of the activity coefficient postulate, the basic premise upon which the acidity function method is based.[4] For example, the product of the slopes of $\log I$ *vs.* $\log I$ plots given in Table III is 0.46 ± 0.08; this suggests that the value of H_0 at its high acidity end measured with the present collection of indicators, -9.4, is four log units (0.4×0.46) greater than it would have been had all of the measurements been performed with just the first indicator alone. It is not possible, of course, to cover so wide a range of acidity with a single indicator, and the present multi-indicator acidity function is a practical necessity. But the result may not be a general function which describes the protonation behavior of all primary anilines in 20% ethanolic sulfuric acid closely. In other words, substitution of another primary aniline for one of the indicators actually used might alter the numerical value of this acidity function at the point of substitution and in all more acidic solutions. For that matter, a different result could be obtained without changing the set of indicators if the number or distribution of measurements in the region of overlap for a pair of bases with strongly different f_{BH^+}/f_B ratios were altered appreciably.

It is possible, on the other hand, that the systematic decrease in acidity dependence of successively weaker bases implied by the changing slopes of Table III is a general characteristic of aniline indicators. One can argue, for example, that the anilinium ion conjugate acids of weaker bases, being stronger acids, will form stronger hydrogen bonds to the solvent, and that these indicators will, therefore, show less steep acidity dependences than their more strongly basic counterparts. This still leads to breakdown of the activity coefficient postulate, but the breakdown is now a systematic function of base strength and acid concentration. Replacement of one indicator by another of (necessarily) similar pK_a will therefore give similar results. It is interesting in this regard that an H_0 function for aqueous sulfuric acid recently constructed with fluorinated primary aniline indicators was found to be virtually identical with the conventional H_0 scale based upon nitro derivatives of aniline.[18] Unfortunately, slopes of $\log I$ *vs.* $\log I$ plots were not listed in that work. It should be mentioned that $\log I$ *vs.* $\log I$ plots were made in connection with another recent determination of H_0 for aqueous sulfuric acid, and no systematic change in individual behavior was found; the method of treating the data in that study, however, was not quite as objective as that used here.

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Experimental Section

Materials. All of the primary anilines were commercial samples except for 3-methyl-2,4,6-trinitroaniline, which was kindly provided by the late Dr. M. Jorgenson. These substances were recrystallized from ethanol or ethanol–water mixtures until they had constant melting points which agreed with accepted literature values.

2,4-Dimethoxyazobenzene was prepared by coupling diazotized aniline with *m*-methoxyphenol and then methylating the free hydroxyl group with dimethyl sulfate.[19] This coupling reaction produced two substances, both of which gave 2,4-dimethoxyazobenzene upon methylation; that fact plus their nmr spectra identified them as the positional isomers 2-hydroxy-4-methoxyazobenzene and 4-hydroxy-2-methoxyazobenzene. 2,4-Dimethoxyazobenzene was purified by alternate recrystallization from 95% ethanol and from hexane until its melting point was constant and in agreement with the literature value.

This preparation gave a mixture of *cis*- and *trans*-2,4-dimethoxyazobenzene, but isomerization of the cis isomer to the more stable trans form is acid catalyzed and very rapid at the acidities employed for the indicator measurements.[20] Thus, these measurements refer to *trans*-2,4-dimethoxyazobenzene only.

Deionized water was purified further by distillation from alkaline permanganate in glass apparatus. All other materials were best available commercial grades and were used without further purification.

Density Measurements. Solutions were prepared by pipetting 10-ml quantities of 95% ethanol into 50-ml volumetric flasks and then filling the flasks to the mark with aqueous sulfuric acid of the appropriate concentration. With concentrated acids, considerable heat was evolved during this dilution; the acid was therefore added in small portions and the flask was cooled between additions. In all cases, final volume adjustments were made with the flask and its contents in temperature equilibrium with a bath operating at 25.0 ± 0.05°.

Densities were determined using Weld pycnometers of 10-ml nominal volume; these were filled in the recommended way[21] while suspended in the 25° constant temperature bath. Weighings were performed on a Mettler type B6 semimicrobalance and were corrected for the effect of air buoyancy.[21] Each measurement was made in duplicate with each of two pycnometers; the results are therefore averages of four separate determinations.[10] Some density measurements of wholly aqueous sulfuric acid were also made; these agreed with published[22] values to within 0.001 g/ml.

A few density measurements of 20% ethanolic sulfuric acid have been made before,[3b] but the values reported are consistently lower than the present results by *ca.* 2%; the reason for this difference is not known.

Indicator Measurements. Stock solutions of indicators in 95% ethanol were prepared at concentrations (*ca.* 10^{-3} *M*) selected to give maximum absorbance readings. Aliquots of these solutions were then diluted with sulfuric acid as described above for density measurements, and spectra were recorded from 500 to 350 nm using spectrometers (Beckman DK-2 or Cary 11) with cell compartments thermostated at 25.0 ± 0.1°. Absorbances were estimated to 0.001 from the recorded traces at absorption maxima and also at positions 5 nm to either side. The values of A so obtained were transformed into indicator ratios using the relationship $I = (A_B - A)/(A - A_{BH^+})$, where A_B and A_{BH^+} are the absorbances of solutions containing indicator completely in its basic and acidic forms, respectively. These limiting absorbances were measured at acidities at least 3 H units to either side of the indicator pK_a. The spectrum of each solution was usually recorded three times, and most values of I are therefore averages of nine measurements.[10]

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Electrochemical Studies of Heme Proteins. Coulometric, Polarographic, and Combined Spectroelectrochemical Methods for Reduction of the Heme Prosthetic Group in Cytochrome *c*

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Abstract: The detailed electrochemical behavior of native horse-heart cytochrome *c* is described. This heme protein is shown to reduce at a variety of electrode materials producing freely diffusing ferrocytochrome *c* that is fully active in the cytochrome oxidase enzyme system. Adsorption of the protein onto the electrode surface has significant influence on the observed electrochemistry, but it does not cause electrode fouling or loss of the electrode's ability to transfer electrons. On the basis of these results, it is not possible to distinguish between an electron transfer mechanism involving charge conduction through the protein fabric and a mechanism wherein electron transfer occurs only at the exposed heme edge. The relaxation techniques developed here appear suitable for electrochemical study of high molecular weight proteins in general.

Because cytochrome *c*, a heme protein distributed widely in living organisms, has a central role in the electron transfer reactions of aerobic metabolism, the mechanism of reduction and oxidation of the protein iron is of great interest. Data from heterogeneous electrochemical experiments may yield information on redox stoichiometry, on equilibrium, on the transport of electroactive species to and from the electrode surface, and on the chemical reactions occurring between the electroactive species and other components in the solution phase. These data are obtained without alterations in the atomic composition of the solutions under study, since only electrons are added or removed. For these reasons we have begun what we believe is the first systematic application of the various methods of electrochemistry to an elucidation of the behavior of native cytochrome *c*. In addition these techniques can serve as clean, synthetic methods for the production of reduced or oxidized material without the required addition of other redox reagents. Electrochemical procedures may also be combined with spectrophotometric methods to increase the amount of