# Semiaqueous Potentiometric Determinations of Apparent $pKa_1$ Values for Benzothiadiazines and Detection of Decomposition during Solubility Variation with pH Studies

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**Abstract** □ Apparent acidity constants obtained for various benzothiadiazine diuretics by semiaqueous potentiometric titrations gave satisfactory agreement with many values obtained by aqueous potentiometry. One suitable method for determining the acidity constants of sparingly soluble drugs, the solubility variation with pH technique, does not take into account instability problems. The preparation of decomposition products and the TLC analysis of sample solutions at various time intervals during solubility studies of methyclothiazide and bendroflumethiazide indicated that decomposition takes place during agitation and equilibration. This decomposition in buffers of pH 8 and higher was confirmed with the acidified *p*-dimethylaminobenzaldehyde test for primary aromatic amines.

**Keyphrases** □ Benzothiadiazines—apparent $pKa_1$ values, semiaqueous potentiometry, detection of decomposition □ Diuretics—benzothiadiazines, semiaqueous potentiometric determination of apparent $pKa_1$ values, detection of decomposition □ $pKa_1$ values, apparent—benzothiadiazines, semiaqueous potentiometric determination □ Decomposition—benzothiadiazines, solubility variation with pH studies □ Potentiometry, semiaqueous—benzothiadiazines, determination of apparent $pKa_1$ values

In searching for the most suitable method of determining the dissociation constants of the benzothiadiazine diuretics, the utility and applicability of previously reported methods were examined first. Potentiometry in aqueous media has been commonly used in the determination of pKa values (1); however, the low aqueous solubility of benzothiadiazines precludes the use of water as the sole solvent. In such instances of solubility problems, potentiometry in mixed solvent systems has been used (2). In addition, Ågren and Bäck (3) employed the solubility variation with pH technique in determining the first acidic ionization constant of bendroflumethiazide. Their experimental conditions seemed to favor significant decomposition, which they did not appear to take into account.

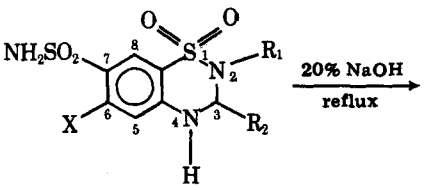
The first part of this investigation was the determination of apparent $pKa_1$ values for various benzothiadiazine diuretics, and the second phase concerned the effect of pH on the stability of representative benzothiadiazines during the solubility pKa determination procedure.

## EXPERIMENTAL

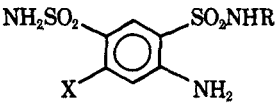
**Reagents and Buffers**—All chemicals and reagents were either ACS or reagent grade, unless otherwise specified. Buffers were prepared with glass-distilled and deionized water free of carbon dioxide. Sorensen's (4) borate and glycine buffers were used.

**Reference Standards**—Purification of the selected benzothiadiazines was achieved by recrystallization to a constant melting point from ethanol and ethanol–water mixtures.

**Semiaqueous Potentiometric Determination of Apparent $pKa_1$ Values by Extrapolation Technique**—The apparent $pKa_1$ values of various benzothiadiazines were determined by the method of Chatten




I: $R_1$ = H, $R_2$ = benzyl, X = $CF_3$
II: $R_1$ = $CH_3$, $R_2$ = $CH_2Cl$, X = Cl



III: R = H, X = $CF_3$
IV: R = $CH_3$, X = Cl

*Scheme I*

*et al.* (5) using acetone–water mixtures. Four concentrations of each benzothiadiazine (0.0005, 0.001, 0.0015, and 0.002 *M*) were prepared from 0.02 *M* acetone stock solutions. The acetone–water ratios were 5:45, 10:40, 15:35, and 20:30 ml, respectively. The solutions were titrated with 0.05 *N* NaOH, and the pH was measured[1] after the addition of each 0.1-ml increment of titrant. The apparent $pKa_1$ values then were obtained by the usual extrapolation techniques (2, 5).

**Preparation of Reference Decomposition Products**[2]—The decomposition products of bendroflumethiazide (I) and methyclothiazide (II) were prepared (Scheme I) by dissolving 1 g of the parent compound in 30 ml of 20% NaOH solution. The mixtures were refluxed for 2 hr, allowed to stand for 20 hr, and then acidified to pH 4 with 6 *N* HCl. The resulting precipitates were separated by vacuum filtration, washed with distilled water, and recrystallized from water to a constant melting point. The identity of the products (III and IV) was confirmed with IR and mass spectra.

*4-Amino-6-trifluoromethyl-1,3-benzenedisulfonamide (III)*—Compound III was prepared from bendroflumethiazide (I), giving fine white crystals (yield 0.52 g or 68.0%), mp 245–247° dec. [lit. (6) mp 246–247°].

*4-Amino-2-chloro-5-(methylsulfamyl)benzenesulfonamide (IV)*—Compound IV was prepared from methyclothiazide (II), giving fine, off-white crystals (yield 0.61 g or 72.6%), mp 170° dec. [lit. (6) mp 168–170°].

**Solubility Determinations**—An excess of pure drug was shaken with a constant volume of buffer and agitated[3] at constant temperature for 15 min. After 5 min of equilibration, the suspensions were filtered[4] and the clear filtrate was centrifuged[5]. The pH[1] of each solution then was obtained, and the absorbances[6] for II were read at 267 nm.

[1] Fisher Accumet model 320 expanded-scale pH meter fitted with glass and calomel electrodes.

[2] Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 267 grating IR spectrometer as potassium bromide disks. Mass spectra were taken on a Hewlett-Packard 5981A spectrometer.

[3] Dubnoff metabolic shaking incubator (GCA/Precision Scientific) maintained at 25 ± 1°.

[4] Whatman No. 1 filter paper.

[5] Servall automatic-refrigerated centrifuge maintained at 25 ± 1° (15,000 rpm for 10 min).

[6] Beckman model 25 spectrophotometer.

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**Table I—Comparison of the Apparent Acidity Constants ($pKa_1$) of Certain Benzothiadiazines Obtained by Semiaqueous Potentiometric Titration with Literature Values**

| Benzothiadiazine | Literature[a] | Semiaqueous Potentiometric Titration |
|---|---|---|
| Flumethiazide | 6.44 (13) | 6.3 |
| Hydrochlorothiazide | 7.0 (14), 7.9 (15), 8.6 (16), 8.8 (13) | 8.7 |
| Hydroflumethiazide | 8.9 (14), 8.45 (13) | 8.5 |
| Cyclothiazide | — | 8.8 |
| Trichloromethiazide | — | 6.9 |
| Methyclothiazide (II) | — | 9.5 |
| Polythiazide | — | 9.1 |

[a] Aqueous potentiometric titrimetry.

*Methyclothiazide (II)*—After the initial solubility determination in 25 ml of glycine buffer, the procedure was repeated at 1-hr intervals for 6 hr and then again at 24 hr. Once decomposition was detected at pH 8.6, 9.0, and 9.4, the suspensions stood at room temperature for 22 days without agitation.

*Bendroflumethiazide (I)*—An excess of pure drug was shaken with 10 ml of borate buffer at pH 8.4, 9.0, and 9.6 and agitated[3] continuously for 3 hr (3, 7) at constant temperature.

**Detection of Decomposition by TLC**—The II suspensions were monitored for decomposition at 0.25, 0.5, 0.75, 1, 2, 3, and 24 hr and also at 22 days following the initial exposure to the buffers. The I suspensions were monitored at the same time intervals for up to 3 hr.

At the appropriate interval, a 50-$\mu$l aliquot of each sample was removed and diluted to 150 $\mu$l with acetone. The acetone–water aliquot then was applied to a silica gel-coated chromatographic sheet[7] and developed in ethyl acetate–benzene (8:2) (8–10). The sheets were examined under shortwave (254-nm) UV light[8] and then sprayed with acidified *p*-dimethylaminobenzaldehyde[9].

## RESULTS AND DISCUSSION

Most benzothiadiazine diuretics are sparingly soluble in water, and a nonaqueous solvent must be added to ensure that the compounds remain in solution. Thus, semiaqueous potentiometry is a logical choice for investigating acidity constants if apparent values for relative acidities must be obtained rapidly. The term "relative acidities" refers to the apparent ionization constants obtained in semiaqueous media for a particular series of structurally related compounds (11). Dissociation constants obtained by potentiometric titration in semiaqueous media are not thermodynamically rigorous because there is no satisfactory way of correlating activity coefficients in different solvent systems. The liquid junction potential between two solvents also is a problem. Many references attest to these difficulties (*e.g.*, 1, 11, 12).

The apparent $pKa_1$ values obtained by potentiometric titrations of selected benzothiadiazines in water–acetone mixtures are given in Table I and are compared with literature values. Despite the problems outlined previously, the apparent values obtained for the $pKa_1$ of several benzothiadiazines by semiaqueous potentiometric titrimetry compare favorably with those reported for aqueous potentiometry. This finding clearly suggests that the method is useful as a comparative means for structurally related compounds such as the benzothiadiazine diuretics, and it can provide accurate relative acidity constants.

Close *et al.* (6) observed that boiling certain benzothiadiazines with alkali readily promoted opening of the thiadiazine ring system. This type of hydrolysis was studied by Mollica *et al.* (16, 17) who investigated these effects on hydrochlorothiazide at 60° in 1 *N* NaOH. Accordingly, an experiment was designed in this laboratory to study the effect of pH on the stability of representative benzothiadiazine diuretics under the conditions outlined for the solubility variation with pH method of determining pKa values.

The investigation was initiated with the preparation of reference decomposition products that were obtained by alkaline hydrolysis of bendroflumethiazide (I) and methyclothiazide (II). Substitution at the 3-position affects the overall reaction rate (16); but when 3-substituted benzothiadiazines are refluxed in 20% NaOH, complete hydrolysis to produce the corresponding benzenedisulfonamide (III or IV) can be expected.

[7] Eastman chromagram developing apparatus and sheets (silica gel adsorbent with fluorescent indicator).

[8] Ultraviolet lamp, Applied Science Laboratories.

[9] *p*-Dimethylaminobenzaldehyde (1 g), dissolved in 95% ethanol to provide a 1% solution, was acidified by the addition of 10 ml of concentrated hydrochloric acid (9, 10).

**Table II—$R_f$ Values for Representative Benzothiadiazines and Their Decomposition Products in Ethyl Acetate–Benzene (8:2)**

| Compound | $R_f$ Value | Color Formed with Acidified *p*-Dimethylaminobenzaldehyde |
|---|---|---|
| Hydrochlorothiazide | 0.17 | — |
| I | 0.68 | — |
| II | 0.60 | — |
| III | 0.36 | Lemon yellow |
| IV | 0.46 | Lemon yellow |

TLC analysis provided $R_f$ values for both parent compounds (I and II) and the decomposition products (III and IV) (Table II). The presence of the primary aromatic amino group was confirmed by the Schiff base formation with acidified *p*-dimethylaminobenzaldehyde.

The UV spectra of the decomposition products are similar to those of the parent 3,4-saturated benzothiadiazines because the basic chromophore has not been altered sufficiently to effect a change in the wavelength of maximum absorption. Conversion of the basic amino group to a free aromatic amine may cause a shift of only a few nanometers to a longer wavelength, and this change is insufficient to distinguish between the parent 3,4-saturated benzothiadiazines and their decomposition products. Baer *et al.* (18) examined the UV absorption spectra of chlorothiazide, a 3,4-unsaturated benzothiadiazine, and its decomposition product obtained in a basic medium and observed a 30-nm shift in the wavelength of maximum absorbance.

Since the 3,4-saturated benzothiadiazines and their decomposition products have similar UV absorption spectra, the use of UV spectrophotometry to measure quantitatively the drug content of samples in the solubility variation with pH technique will not detect any decomposition taking place in the basic buffers. The pKa value ultimately derived from the solubility data may be that of the decomposition product or, more likely, a mixture of the parent benzothiadiazine and its hydrolysis product.

Samples of methyclothiazide (II) and bendroflumethiazide (I) in buffers of pH > 9 were obtained from the solubility studies and analyzed by TLC. The results of the analysis indicated the presence of the benzothiadiazines and their decomposition products, as confirmed by the $R_f$ values and the color reaction with acidified *p*-dimethylaminobenzaldehyde spray reagent. From this reaction, an interesting and unexpected result was noted. After application of the acidified *p*-dimethylaminobenzaldehyde, violet spots appeared at the same $R_f$ values as I and II, and the color intensity of these spots increased with increasing pH values for the buffers.

The aqueous samples from the solubility studies were diluted with

**Table III—Absorbance Changes for Methyclothiazide (II) Relative to Equilibration–Agitation Periods**

| pH | Hours[a] | Absorbance at 267 nm | | |
|---|---|---|---|---|
| | | Sample 1 | Sample 2 | Sample 3 |
| 8.6 | 0.5 | 1.138 | 1.146 | 1.198 |
| | 1.0 | 1.220 | 1.231 | 1.277 |
| | 3.0 | 1.277 | 1.298 | 1.338 |
| | 4.0 | 1.297 | 1.318 | 1.354 |
| | 5.0 | 1.303 | 1.320 | 1.372 |
| | 6.0 | 1.337 | 1.343 | 1.390 |
| | 24.0 | 1.410 | 1.416 | 1.440 |
| | 528.0 | 0.105 | 0.105 | 0.110 |
| 9.0 | 1.0 | 0.938 | 0.842 | 0.893 |
| | 2.0 | 1.152 | 1.040 | 1.088 |
| | 3.0 | 1.263 | 1.133 | 1.171 |
| | 4.0 | 1.346 | 1.239 | 1.254 |
| | 5.0 | 1.412 | 1.333 | 1.324 |
| | 6.0 | 1.400 | 1.393 | 1.435 |
| | 24.0 | 1.516 | 1.571 | 1.597 |
| | 26.0 | 1.711 | 1.715 | 1.697 |
| | 28.0 | 1.790 | 1.808 | 1.745 |
| | 528.0 | 0.175 | 0.202 | 0.178 |
| 9.4 | 3.0 | 1.799 | 1.850 | 1.890 |
| | 528.0 | 0.243 | 0.280 | 0.232 |

[a] The absorbance values at 22 days of equilibration are for diluted samples (0.1 ml of filtrate plus 2.9 ml of the appropriate buffer).

acetone before they were spotted on the TLC plates, whereas the I and II reference standards were dissolved in ethyl acetate prior to TLC analysis. Since the violet color was not produced with samples from the ethyl acetate stock solutions, it can be postulated that the acidified *p*-dimethylaminobenzaldehyde only reacts with the benzothiadiazines in their ionized form. The color only appeared when buffers of pH > 9 (the region following the first pKa) were used in the solubility studies. The violet color faded soon after its development, indicating an unstable complex.

Schiff base formation with primary aromatic and aliphatic amines yields yellow, orange-red, or brown products. The only record of a violet color that the authors could find was that of the reaction of pyrrole with *p*-dimethylaminobenzaldehyde (19), and the product rearranged into a quinoidal form. The type of colored complex formed between the benzothiadiazines and *p*-dimethylaminobenzaldehyde is difficult to postulate without further work since there is no apparent record of Schiff base formation with the nitrogen of a sulfonamido group.

Decomposition during the solubility studies was evident from the TLC analyses of the solutions performed at various times. Monitoring of the solubility samples by TLC indicated that detectable decomposition had occurred after 1 hr of agitation at pH 8.6, 9.0, and 9.4 for II and at pH 8.4, 9.0, and 9.6 for I. In addition to that of the parent benzothiadiazine, a spot was visible under shortwave (254-nm) UV light that had the same $R_f$ value as the corresponding reference decomposition product (either III or IV). The spots did not react visibly with acidified *p*-dimethylaminobenzaldehyde because of the low concentration of the particular decomposition product present in the samples at that time. The color formed, and the intensity increased, as the time of exposure of the benzothiadiazines to the basic buffers was extended.

At pH 8.6 for II and at 8.4 for I, the degradation products at 1 hr of exposure were visible only under shortwave UV light; but after 3 hr of exposure, faint-yellow spots appeared upon application of acidified *p*-dimethylaminobenzaldehyde. Upon further exposure of the drugs to the basic buffers, the color of the spots increased in intensity.

If equilibration is considered to be complete when any further change in the drug concentration is <3% (1, 20), the 3-hr limit of agitation time set by Green (7) and used by Ågren and Bäck (3) is not adequate for either I or II. As shown by Table III, the concentration of II was still changing significantly once decomposition had started. The decomposition of I also began before the 3-hr agitation had been completed.

Although solubility variation with pH is an ideal method for determining thermodynamic pKa values for sparingly soluble compounds (20), the technique is not suitable for the benzothiadiazine diuretics. Under the conditions of the method, the decomposition of these drugs occurs before equilibration has been achieved. Work is continuing in this laboratory on the determination of pKa values for these compounds by other methods.

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