sometimes be more convenient). Similar experiment with DHPPA and HIPA have not been performed since these substances are even more labile than the preceding ones. Billek who has synthesized DHPPA used $SO_2$ to retard decomposition.[10]

Unless kept in a borate containing solution HIPA turns pink on standing and gives a dark residue when the ether extract is evaporated.


1. Meister, A. In Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*, Academic, New York 1957, p. 404.
2. Lin, E. E. C., Pitt, B., Civen, A. and Knox, E. *J. Biol. Chem.* **233** (1958) 668.
3. Woolf, L. I. and Goodwin, B. L. *Clin. Chem.* **10** (1964) 146.
4. Haavaldsen, R. *Nature* **196** (1962) 577.
5. Bray, G. A. *Anal. Biochem.* **1** (1960) 279.
6. Schwartz, K. *Arch. Biochem. Biophys.* **92** (1961) 168.
7. Holcomb, I. J., McCann, D. S. and Boyle, A. J. *Anal. Chem.* **37** (1965) 1657.
8. Pitt, B. *Nature* **196** (1962) 272.
9. Haavaldsen, R. and Norseth, T. *Anal. Biochem.* **15** (1966) 536.
10. Billek, G. *Monatsh.* **92** (1961) 343.


Received March 9, 1967.

# Studies on Sulfinic Acids

## II b.* Supplementary Note on the Titrimetric Determination of Aromatic Sodium Sulfinates with Perchloric Acid

BERNT J. LINDBERG

*Research Division, Pharmacia AB, Uppsala, Sweden*

In previous papers of this series the titration of aromatic sodium sulfinates with perchloric acid has been described.[1,2] When a series of substituted aromatic sodium sulfinates were titrated with 0.1 N perchloric acid in glacial acetic acid it was found that the half neutralization potentials could be satisfactorily correlated with the Hammett equation. The result is shown in Fig. 1. Since the absolute value of the half neutralization potential has no useful or direct significance, no measures were taken to establish it, and hence only the potentital shifts are given in the figure. The correlation also holds for *ortho* substituted members, if the polar substituent constants given by Taft [3] are used.

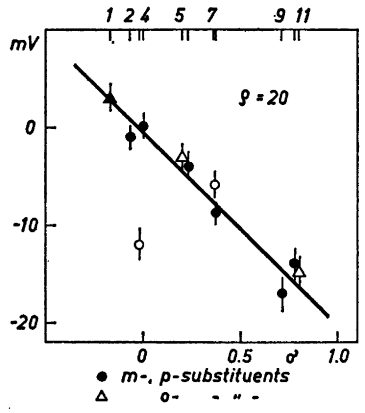


*Fig. 1.* Plot of half neutralization potentials against Hammett substituent constants for some aromatic sodium sulfinates titrated with perchloric acid.

Substituents: 1, *p*-$CH_3$, *o*-$CH_3$; 2, *m*-$CH_3$; 3, *p*-$CH_3CONH$; 4, none; 5, *o*-Cl; 6, *p*-Cl; 7, *m*-COOH; 8, *m*-Cl; 9, *m*-$NO_2$; 10, *p*-$NO_2$; 11, *o*-$NO_2$.

Ritchie *et al.* have previously shown that the p$K$'s for some *p*-substituted sulfinic acids measured spectrophotometrically could be correlated with the Hammett equation.[10] The relationship between p$K$'s and substituent constants can be expected to parallel that between the present half neutralization potentials and substituent constants. Thus it should be possible to predict the acid strengths of aromatic sulfinic acids knowing the acid strength of a few members. A summary of the dissociation constants of sulfinic acids found in the literature is given in Table 1. The large discrepancies found between the values reported by various authors may be partly due to the difficulty in determining disso-

* Part II: *Acta Chem. Scand.* **17** (1963) 383.

*Table 1.* Dissociation constants of aromatic sulfinic acids.

| Substituent | Dissociation constant | | Method | Ref. |
|---|---|---|---|---|
| H | $K \times 10^2$ | 3.5 | Conductivity | 4 |
| | | | Conductivity | 5 |
| | $K \times 10^2$ | 3.0 −3.8 | Classical | |
| | $K \times 10^2$ | 2.9 −3.3 | Thermodyn. | |
| | $K \times 10^2$ | 4.46 | Conductivity | 6 |
| | $K \times 10^2$ | 0.96−1.28 | Potentiometry | 7 |
| | $pK$ 20° | 1.29 | Potentiometry | 8 |
| | $pK$ 25° | 1.84−2.16 | Potentiometry | 9 |
| | p$K$ | 1.21 | UV | 10 |
| | p$K$ | 1.58 | UV | 11 |
| $p$-$CH_3$ | $K \times 10^2$ | 2.5 | Conductivity | 4 |
| | | | Conductivity | 5 |
| | $K \times 10^2$ | 1.9 −2.8 | Classical | |
| | $K \times 10^2$ | 2.1 −2.4 | Thermodyn. | |
| | $K \times 10^2$ | 1.48−2.0 | Potentiometry | 7 |
| | p$K$ 25° | 1.99 | Potentiometry | 9 |
| | p$K$ | 1.24 | UV | 10 |
| | p$K$ | 1.70 | UV | 11 |
| $o$-$CH_3$ | $K \times 10^2$ | | Conductivity | 5 |
| | $K \times 10^2$ | 2.2 −5.2 | Classical | |
| | $K \times 10^2$ | 2.6 −4.1 | Thermodyn. | |
| $p$-Cl | p$K$ 25° | 1.81 | Potentiometry | 9 |
| | p$K$ | 1.14 | UV | 11 |
| $p$-Br | p$K$ 25° | 1.89 | Potentiometry | 9 |
| | p$K$ | 1.09 | UV | 10 |
| $m$-$NO_2$ | p$K$ 25° | 1.88 | Potentiometry | 9 |
| | p$K$ | 0.48 | UV | 10 |
| $p$-$NO_2$ | p$K$ 25° | 1.86 | Potentiometry | 9 |
| | p$K$ | 0.64 | UV | 10 |

ciation constants of very strong acids accurately.

As previously reported the methoxy-substituted members could not be titrated in glacial acetic acid medium. In the present series inconsistent stoichiometric results were also found for the $m$- and $p$-COONa and $p$-$CH_3CONH$ substituted members. This is due to the basicity of these substituents. The apparent half neutralization potential with the $m$-COONa substituent corresponds best to the substituent constant of the acid form of the carboxyl group. The apparent half neutralization potential with the $p$-$CH_3CONH$ substituent is far too low, indicating protonation of the substituent. The $p$-amino substituted sulfinic acid was insoluble in acetic acid and has an IR spectrum which is consistent with a zwitter-ionic structure.

The titrations were performed at 22° with a Radiometer TTT 1 automatic titrator using a Radiometer 202 B glass electrode and a modified calomel electrode as a reference. The sample size was 10−15 mg and titrations were performed in

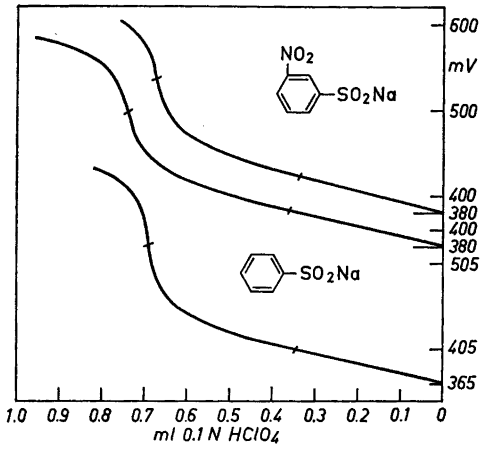


*Fig. 2.* The titration of sodium benzenesulfinate and sodium *m*-nitro-benzenesulfinate with 0.1 N $HClO_4$ in glacial acetic acid.

duplicate. The reproducibility of the half neutralization potential is indicated by vertical bars in Fig. 1. Fig. 2 shows some typical titration curves.


1. Lindberg, B. J. *Acta Chem. Scand.* **17** (1963) 377.
2. Lindberg, B. J. *Acta Chem. Scand.* **17** (1963) 383.
3. Taft, Jr., R. W. In Newman, M. S. *Steric Effects in Organic Chemistry*, Wiley, London 1956, 556, p. 619.
4. Lovén, J. M. *Z. physik. Chem.* **19** (1896) 456.
5. Coats, R. R. and Gibson, D. T. *J. Chem. Soc.* **1940** 445.
6. Schjånberg, E. *Ber.* **76** (1943) 287.
7. Forrest, T. P. and Ryan, D. E. *Can. J. Chem.* **36** (1958) 1674.
8. Rumpf, P. and Sadet, J. *Bull. Soc. Chim. France* **1958** 447.
9. Burkhard, R. K., Sellers, D. E., de Cou, F. and Lambert, J. L. *J. Org. Chem.* **24** (1959) 767.
10. Ritchie, C. D., Saltiel, J. D. and Lewis, E. S. *J. Am. Chem. Soc.* **83** (1961) 4601.
11. Kobayashi, M. and Koga, N. *Bull. Chem. Soc. Japan* **39** (1966) 1788.


Received March 22, 1967.

# Crystallization of Horse Liver Alcohol Dehydrogenase Complexes from Alcohol Solutions

E. ZEPPEZAUER, B.-O. SÖDERBERG and C.-I. BRÄNDÉN

*Department of Agricultural Chemistry I, Agricultural College of Sweden, Uppsala 7, Sweden*

Å. ÅKESON and H. THEORELL

*The Nobel Medical Institute, Department of Biochemistry, Stockholm 60, Sweden*

Large crystals of horse liver alcohol dehydrogenase (LADH) and its complexes with coenzyme and inhibitor molecules were first prepared by Yonetani and Theorell [1] by slow evaporation of concentrated solutions of the protein in weak phosphate buffer at pH 7.0. During a preliminary X-ray investigation [2] of these crystals it was found that they were very fragile and sensitive to small temperature changes. Since they were very unsuitable for accurate X-ray work we have explored other conditions of crystallizations to obtain crystals suitable for a crystal structure determination of LADH. The final conditions reported here were found after many unsuccessful attempts with high salt concentrations, different buffer systems and pH, and various solvents.

*Materials and methods.* Stock suspensions of microcrystals of homogeneous [3] LADH-e were kept at −20°C in 30 % ethanol-water solution. A suitable amount of this suspension was centrifuged at −20°C and the crystals were dissolved in 0.05 M Tris−HCl buffer at pH 9.4. This and the following treatment were made at +4°C. The solution was then first dialysed for 24 h against 0.05 M Tris−HCl buffer at pH 9.4 and then against 0.05 M Tris−HCl buffer at pH 8.4 containing 5 % of ethanol for another 24 h. Denatured protein was removed by centrifugation and the remaining solution was diluted with buffer to a final protein concentration of 0.5 or 1 % and transferred to dialysis bags each containing 1 ml of protein solution. Each bag was then placed in a bottle containing 20 ml buffer at pH 8.4 and alcohol to a concentration just below the precipitation point. Binary and ternary complexes were prepared by addition of excess coenzyme and inhibitor to the