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Short Communication

Determination of thiols as sulphonic acids by capillary isotachophoresis

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Abstract

The mobilities of sulphonic acids obtained by bromine oxidation of thiols and disulphides containing COOH, NH_2 and OH groups, from pH 3 to 9, the relative charges and apparent pK values of carboxylic groups were determined by means of capillary isotachophoresis.

1. Introduction

The problem of the determination of thiols and disulphides in trace concentrations in biological materials is often of prime importance and several techniques have been developed for this purpose.

Capillary isotachophoresis is a precise and accurate method for the determination of ions [1,2], and can be directly applied to mercaptoacids and aminothiols. This approach is of limited applicability, however, because natural samples in general contain many ionic compounds in great excess, which entirely overlap the small contribution of thiols. The derivatization of thiols as suggested by Holloway [3] is hardly applicable to natural samples and does not remove the ionic interferences.

The water-soluble thiols and disulphides can be readily and quantitatively oxidized to sulphonic acids, which are much more suitable for isotachophoretic analysis. Sulphonic acids are not or only slightly extractable from aqueous solution, which can be utilized for removal of foreign acids. The possible preparation of samples can be outlined as follows: (1) hydrolysis, oxidation and evaporation, *e.g.*, cysteine, homocysteine and penicillamine in protein hydrolysates; thiols and disulphides can be identified by comparison of isotachograms obtained before and after oxidation; (2) extraction, evaporation, oxidation and stripping of interferring materials with organic solvents, *e.g.*, thioctic acid in food; (3) reduction of disulphides, extraction of thiols as tributyltin mercaptides, stripping with hydrochloric acid, oxidation and stripping with tributyltin hydroxide, *e.g.*, thiols and disulphides in urine.

2. Theoretical

The effective mobility of an ion, u_X, can be calculated from isotachophoretic results using Boček *et al.*'s equation [2]:

$$\frac{1}{u_X} = \frac{1}{u_L} + \left(\frac{1}{u_S} - \frac{1}{u_L}\right)\frac{h_X}{h_S} \quad (1)$$

where u_L = mobility of the leading ion, usually

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chloride, $u_L = 79 \cdot 10^{-5}$ cm^2 s^{-1} V^{-1}, u_S = mobility of a standard, in this paper trichloroacetate, $u_S = 36.2 \cdot 10^{-5}$ cm^2 s^{-1} V^{-1} and h_X and h_S = step heights of the ion X and of the standard ion, respectively.

Inserting the mobilities of chloride and trichloracetate, Eq. 1 can be written in the form

$$u_X = \frac{79}{1 + 1.18h} \quad (2)$$

where h = relative step height of ion X, and $h = h_X/h_S$.

The relative charge of the ion X (effective charge) can be calculated from the equation [1,2]

$$Z_X = \frac{t_X i u_X}{n_X F(u_X + u_k)} \quad (3)$$

where t_X = zone length corresponding to ion X, i = driving current, F = Faraday constant, n_X = number of moles of ion X injected on to the column and u_k = the resulting mobility of the counter-buffer ion and protons. The value of u_k can be calculated from Eq. 3 using a standard such as trichloroacetate with the charge assumed to be −1. The charge is expressed as a positive number.

For monovalent acids the charge can also be calculated as the ratio of mobility u at a given pH to the mobility u_0 at full ionization:

$$Z = \frac{u}{u_0} \quad (4)$$

The relationship between charge, the pH of the leading buffer (pH_L) and the pK of the corresponding acid is given by

$$pK = pH_L + \log\left(\frac{1}{Z} - 1\right) \quad (5)$$

In order to avoid any misunderstanding, it is necessary to discriminate between some values of pK. The thermodynamic value pK_t is calculated for infinite dilution and is really constant. The apparent values calculated from Eqs. 3 and 5, pK_Z, or from Eqs. 4 and 5, pK_u, may depend on the composition of the solution.

Table 1 gives the pK values of some monovalent carboxylic acids. The pK_t values were taken from the literature [4], whereas the pK_Z and pK_u values calculated using data at pH 3.0 summarized in refs. 1 and 4 and with Eqs. 2–5. The determined value of u_k was $65.4 \cdot 10^{-5}$ cm^2 s^{-1} V^{-1} at pH 3.0. The results indicated a linear relationship between pK_t and pK_Z expressed by

$$pK_t = 1.82\, pK_Z - 1.49 \quad (6)$$

The pK_t values calculated by means of Eq. 6 are given as pK'_t in the Table 1. The agreement is satisfactory.

The relationship between pK_Z and pK_u is expressed by the equation

$$pK_Z = 0.98\, pK_u \quad (7)$$

For the calculation of the apparent dissociation constant of a carboxylic group in the presence of a sulphonic group, full ionization of the sulphonic group above pH 2.8 may be assumed which results in the following equation for the pK of the carboxylic group:

$$pK = pH_L + \log\left(\frac{1}{Z - 1} - 1\right) \quad (8)$$

With two sulphonic groups in the molecule, the difference $Z - 2$ should be introduced.

On the basis of the established relationships, the identification and determination of unknown acids is possible by adopting the following steps:

Table 1
pK values of some monovalent carboxylic acids

Acid	pK_t	pK_Z	pK_u	pK'_t
Acetic	4.76	3.40	3.55	4.70
Benzoic	4.20	3.14	3.22	4.22
Butyric	4.82	3.47	3.55	4.83
Chloroacetic	2.87	2.39	2.41	2.86
Formic	3.75	2.87	3.02	3.73
Glyceric	3.74	2.87	2.94	3.73
Glycolic	3.89	2.96	3.06	3.90
Lactic	3.86	2.96	3.04	3.90
Levulinic	4.60	3.35	3.43	4.61
Nicotinic	4.82	3.44	3.54	4.77
p-Nitrobenzoic	3.52	2.74	2.79	3.50
Phenylacetic	4.41	3.25	3.33	4.42

pK_t = thermodynamic value; pK_Z = calculated from the charge at pH 3.0; pK_u = calculated from the mobility at pH 3.0; pK'_t = pK_t value calculated from pK_Z.

determination of u_X and t_X at pH 3.0 and u_0, calculation of pK_u with Eqs. 4 and 5, pK_Z and Z_X with Eq. 5, n_X with Eq. 3 and pK_t with Eq. 6. The knowledge of pK_t can be evaluated for identification.

3. Experimental

Relative step heights were measured using an isotachophoretic analyser from Labeco (Slovak Republic), at a concentration of leading chloride ions of 0.01 *M* and a driving current of 50 μA in the analytical column. The mobilities were calculated from relative step heights using Eq. 2. The leading electrolytes were prepared from β-alanine (pH 3.0 and 3.5), aminocaproic acid (pH 4.5), histidine (pH 6.0), imidazole (pH 7.0) and methyldiethanolamine (pH 9.0), using 0.2% of PEG 200 as an additive. The terminating electrolytes were prepared from caproic acid (pH 3.0, 3.5 and 4.5) and 2-(N-morpholino)ethanesulphonic acid (MES) (pH 6, 7 and 9).

The sulphonic acids were prepared by oxidation of thiols in aqueous solution of known concentration by shaking with a 1 *M* solution of bromine in carbon tetrachloride until a stable yellow colour was just formed. The sample was evaporated under vacuum, the residue dissolved in water and an aliquot of the solution applied to the column. The method was applied to the determination cystine and cysteic acid in wool, as described in Section 4.1.

4. Results and discussion

Table 2 summarizes the mobilities determined for sulphonic acids obtained by oxidation of listed thiols. The mobility of a sulphonic acid not containing a carboxylic group (No. 1) does not depend on pH. The presence of sulphonic and carboxylic groups (Nos. 2–9) results in an increase in mobility with increase in pH, but tending to a constant value corresponding to complete ionization. The presence of one carboxylic and one amino group in the sulphonic acid (Nos. 10, 11 and 12) results in a slight increase in mobility from pH 3 to 7 and a strong increase at higher pH. The presence of two carboxylic groups and one amino group (No. 13) is reflected by a marked increase in mobility from pH 3 to 6, small change between pH 6 and 7 and a strong increase at higher pH. The results

Table 2
Mobilities (10^{-5} cm^2 s^{-1} V^{-1}) of sulphonic acids obtained by oxidation of thiols as a function of pH_L

No.	Parent thiol	Sulphonic acid	pH_L					
			3.0	3.5	4.5	6.0	7.0	9.0
1	Mercaptoethanol	2-Hydroxyethanesulphonic acid	39.4	39.5	39.3	39.6	39.7	39.4
2	Mercaptoacetic acid	Sulphoacetic acid	44.7	48.4	58.4	62.1	63.8	64.0
3	Thiolactic acid	2-Sulphopropionic acid	37.9	40.7	51.2	54.3	56.9	57.0
4	3-Mercaptopropionic acid	3-Sulphopropionic acid	36.1	37.9	47.6	53.3	55.7	56.0
5	Mercaptolactic acid	3-Sulpholactic acid	43.5	45.5	56.0	57.2	57.0	57.1
6	Thioctic acid	6,8-Disulphooctanoic acid	41.4	42.1	44.3	48.1	52.3	52.5
7	N-Acetylcysteine	N-Acetylcysteic acid	37.3	40.7	44.5	47.0	47.6	47.6
8	N-(2-Mercaptopropionyl)-glycine	N-(2-Sulphopropionyl)glycine	35.4	38.5	45.0	46.0	46.8	47.0
9	Captopril	1-(3-Sulpho-2-methyl-1-oxopropyl)-proline	30.0	32.7	37.8	38.6	38.8	39.0
10	Cysteine	Cysteic acid	32.0	33.4	33.8	33.9	34.5	46.2
11	Homocysteine	Homocysteic acid	28.6	29.5	30.0	30.7	31.2	41.8
12	Penicillamine	Dimethylcysteic acid	27.6	29.2	29.5	30.0	31.0	41.5
13	Glutathione	γ-Glutamylsulphoalanylglycine	28.8	31.4	36.5	38.0	38.3	40.8

Table 3
Relative charges Z and apparent pK_Z values of carboxylic groups in sulphonic acids as a function of pH of the leading electrolyte

Sulphonic acid	Parameter	pH_L		
		3.0	3.5	4.5
2-Sulphopropionic acid	Z	1.12	1.24	1.77
	pK_Z	3.87	4.00	3.98
3-Sulphopropionic acid	Z	1.01	1.14	1.58
	pK_Z	–	4.29	4.36
N-Acetylcysteic acid	Z	1.31	1.54	1.81
	pK_Z	3.35	3.43	3.71
N-(2-Sulphopropionyl) glycine	Z	1.22	1.38	1.84
	pK_Z	3.55	3.71	3.78
1-(3-Sulpho-2-methyl-1-oxopropyl)proline	Z	1.31	1.58	1.91
	pK_Z	3.35	3.36	3.50

presented can be utilized to select the best pH for the separation of the sulphonic acids.

Table 3 demonstrates some charges determined using Eq. 3 and pK_Z values calculated with Eq. 8. The u_k values (10^{-5} cm^2 s^{-1} V^{-1}), found were as follows: pH 3.0, 67.4; pH 3.5, 53.3; pH 4.5, 39.6; pH 6.0, 38.9. At pH 9.0 the complete ionization results in $Z = 2$. The calculated pK_Z values are independent of pH.

4.1. Determination of cystine and cysteic acid in wool

Of many methods suggested for determination of cystine in protein hydrolysates, the most familiar is that developed by Schram *et al.* [5] based on oxidation followed by isolation of the resulting cysteic acid on an ion-exchange resin and its determination by the ninhydrin spectrophotometric procedure. Another approach involves the separation of cysteic acid using paper chromatography or paper electrophoresis and ninhydrin spectrophotometry [6].

The determination of cysteic acid using the capillary isotachophoretic method suggested in this paper simplifies the whole procedure and extends its field of application. One part of the hydrolysate is examined for cysteic acid and the other, after oxidation with bromine, for total cysteic acid, and the cystine content is calculated by difference. At the same time aspartic acid and glutamic acid can be determined and used to characterize the sample.

About 10 mg of sample was placed in a test-tube (80 mm × 10 mm I.D.), 2 ml of 6 *M* hydrochloric acid were added and the contents were heated by placing the tube in a hole in an iron plate at 120°C. After 18 h the hydrolysate was diluted to 5 ml and a 2-ml portion was transferred into an ampoule (44 mm × 17 mm I.D.) and evaporated at 65°C at normal pressure. The other part of the hydrolysate was oxidized by shaking with 1 *M* bromine in carbon tetrachloride and a 1-ml volume was evaporated to dryness at 65°C in a second ampoule. To each ampoule 1 ml of water and 1 ml of 0.1 *M* tributyltin hydroxide in octane were added and, after shaking and separation, the aqueous phase was examined at pH 3.5.

A calibration graph for cysteic acid was prepared with a standard solution of cysteine after oxidation in 0.2 *M* hydrochloric acid with bromine, evaporation and dilution. The established relationship between the zone length (t, s) and the number of nanomoles of cysteic acid applied to the column at pH 3.5 and a driving current of 50 μA (n_C) can be expressed by the following empirical equations:

$$n_C = 0.191\,t - 0.295 \quad \text{for } t < 40$$

$$n_C = 0.203\,t - 0.76 \quad \text{for } t > 40$$

The relative step heights with the use of 0.2% (v/v) isocapronic acid as terminating electrolyte were found to be cysteic acid 0.26, aspartic acid 0.49 and glutamic acid 0.72. In addition, at relative step heights of 0.19, 0.22, 0.30, 0.34 and 0.41 small amounts of unidentified acids were observed.

The results obtained for a sample of Merino wool on a moisture-free basis, with standard deviations for six independent determinations, were cystine 11.5 ± 0.2 and cysteic acid 0.48 ± 0.04%.

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