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Note

Counter-current distribution of hydrophilic thiols in the presence of tributyltin hydroxide

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The influence of pH on the distribution of hydrophilic thiols between water and organic solvent in the presence of tributyltin hydroxide (TBT), and the application of TBT to column chromatography of thiols have been described in a previous paper[1]. The application of TBT to the counter-current distribution (CCD) of thiols, presented in this paper seems to be very suitable for the resolution of complex mixtures of hydrophilic thiols such as cysteine peptides on a preparative scale.

EXPERIMENTAL

The stock solution of TBT was prepared by shaking together 50 ml of tributyltin chloride, 200 ml of butanol and 100 ml of 2 *M* aqueous KOH, followed by separation and filtration of the upper phase. The final concentration of TBT was determined by titration of a sample, diluted with methanol, with 0.2 *M* hydrobromic acid using methylorange as indicator.

The buffer solutions were: Buffer A, 0.3 *M* citric acid and 0.675 *M* KOH pH 5.0; Buffer B, 0.04 *M* sodium sulphide and 0.005 *M* sodium pyrosulphite, pH 7.2, fresh daily; Buffer C, 0.04 *M* sodium sulphite and 0.013 *M* sodium borate, pH 9.3, fresh daily. The thiol contents were determined by titration with *o*-hydroxymercuribenzoic acid (HMB)[1,2].

Before measurements were taken, the buffer solutions were equilibrated with butanol containing TBT. Then the buffer solution containing added thiol was equilibrated with a butanol solution of TBT followed by separation and titration of the aqueous phase. The procedure was repeated again and again using the same butanol phase, which was titrated after the last equilibration. The distribution of thiol between the two phases after each step could then be calculated.

RESULTS AND DISCUSSION

The process of the formation of a complex from a thiol and TBT and its extraction into the organic phase can be expressed as one heterogenous equilibrium:

$$RSH_{aq} + (C_4H_9)_3SnOH_{org} \rightleftarrows (C_4H_9)_3SnSR_{org} + H_2O$$

Consequently, the heterogenous equilibrium constant, K, can be written as

$$K = \frac{b}{c(B-b)} \qquad (1)$$

where b is moles of tributyltin mercaptide in the organic phase, B is total moles of TBT and c is the concentration of thiol in the aqueous phase. The value of b is found by titration of the organic phase and that of c by titration of the aqueous phase.

The plot of $b/(B-b)$ against c, at constant B, should thus be a straight line. With some exceptions this is indeed the case (Fig. 1). Cysteine, homocysteine and penicillamine satisfy eqn. 1 at every pH, acetylcysteine satisfies eqn. 1 at pH 5 and 9 but not at pH 7, and thiomalic acid does not satisfy eqn. 1 at either pH 5 or pH 7. The volume of the organic phase and volume phase ratio do not influence the equilibrium constant, and the influence of TBT content results in only a small shift

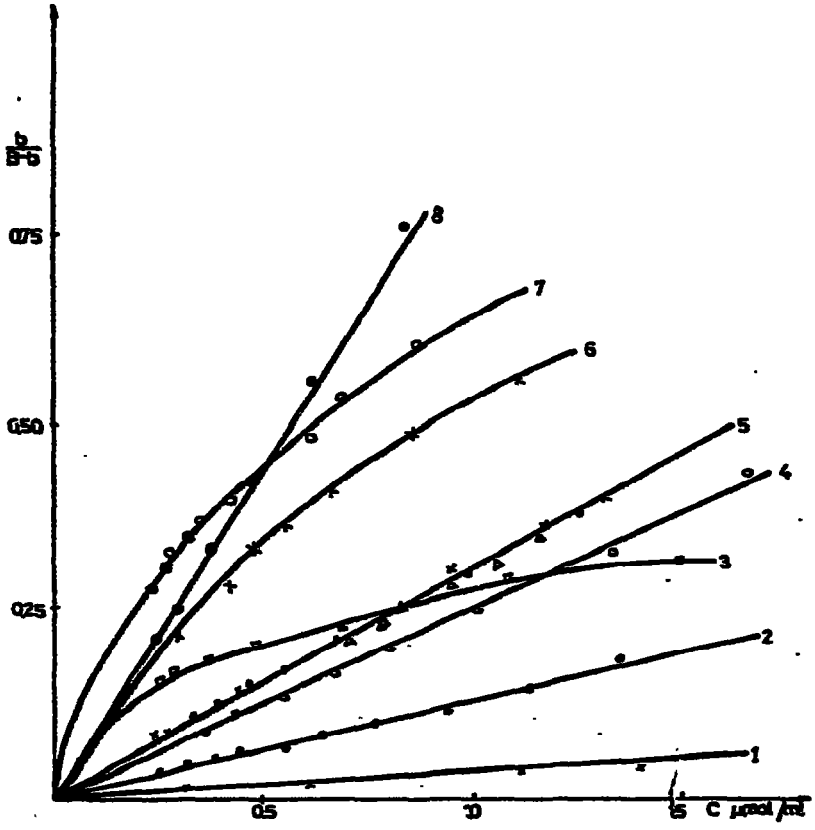

Fig. 1. The ratio of thiol content in butanol to the difference between TBT content and thiol content in butanol as function of the thiol concentration in the aqueous phase after equilibration. Upper phase 5 ml, lower phase 10 ml; B in μmol. 1 = Glutathione, pH 7.2, B = 160; 2 = acetylcysteine, pH 9.3, B = 480; 3 = thiomalic acid, pH 7.2, B = 240; 4 = penicillamine, pH 7.2, B = 160; 5 = cysteine, pH 7.2, B = 240 (△, upper phase 10 ml, lower phase 5 ml; ×, B = 120); 6 = thiomalic acid, pH 5.0, B = 240; 7 = acetylcysteine, pH 7.2, B = 240; 8 = homocysteine, pH 7.2, B = 80.

of K, which increases slightly with decreasing B; e.g. for cysteine at pH 7.2 at B values of 100, 160 and 230 μmol the calculated K values are 0.35, 0.33 and 0.31. A similar shift has been found for other thiols. The acetylcysteine equilibrium constant at pH 5 has been found at $B = 240$ μmol in the range 0.345–0.355, and at $B = 480$ μmol in the range 0.295–0.305, for eight determinations at different concentrations of the thiol. The deviation of the results from eqn. 1 is less at pH 7 for thiols that also have an acid function. It may be concluded that the carboxyl group, if not counterbalanced by an amino group, combines with TBT. The strongest deviation is shown by thiomalic acid, a moderate one by acetylcysteine, and a slight one by glutathione. The deviation from eqn. 1 will have an adverse effect on the CCD.

The determined equilibrium constants are summarized in Table I.

TABLE I

THE EQUILIBRIUM CONSTANTS OF THIOLS WITH TBT AT VARIOUS PH VALUES AT 25°

Values are in ml/μmol, corrected for $B = 100$ μmol.

Thiol	*pH*		
	5.0	*7.2*	*9.3*
Cysteine	$4 \cdot 10^{-3}$	0.35	0.13
Homocysteine	0.026	0.87	0.40
Penicillamine	$3 \cdot 10^{-3}$	0.25	0.077
Acetylcysteine	0.36	1.2*	0.14
Glutathione	—	0.08*	—
Thiomalic acid	0.94*	0.56*	$6 \cdot 10^{-3}$

* Extrapolated to $c = 0$.

The necessary number of transfers, n, for a separation of two substances by the CCD technique depends on their moving fraction in the system. The direct use of eqn. 1 for the calculation of moving fraction is very inconvenient because it changes with concentration. If $B \gg b$, then eqn. 1 can be approximated to the form $K = b/Bc$, which yields a constant moving fraction.

$$q = \frac{V}{BK + V}, \quad \text{and} \quad p = 1 - q = \frac{BK}{BK + V} \tag{2}$$

where q is the moving fraction for moving aqueous phase,
p is the moving fraction for moving organic phase and
V is the volume of aqueous phase. The value of n can then be calculated from the equation.

$$\sqrt{n} = \frac{3[(K_1B + V)\sqrt{K_2} + (K_2B + V)\sqrt{K_1}]}{\sqrt{BV}(K_2 - K_1)} \tag{3}$$

Eqn. 3 has a minimum at

$$\frac{B}{V} = \sqrt{\frac{1}{K_1K_2}} \tag{4}$$

At the minimum the following equality holds:

$$(K_1B + V)\sqrt{K_2} = (K_2B + V)\sqrt{K_1}$$

If the two equilibrium constants are known, the conditions for a successful separation can be calculated. To improve the calculation however, the B value should be corrected by addition of the average b value, because the B values in the derived equations are really the difference of B and b. As the average concentration, c, in the aqueous phase is equal to the initial concentration, c_0, divided by the band width $\Delta r = 6\sqrt{n(1-q)q}$, the average b value will be given by the equation

$$b = \frac{c_0KB}{r} = c_0(KB + V)\sqrt{\frac{KB}{36\,Vn}} \tag{5}$$

Let us take as an example the separation of acetylcysteine ($K_1 = 0.36$) from homocysteine ($K_2 = 0.026$), at pH 5, $V = 10$ ml. From eqn. 4 we obtain $B/V = 10.3$, so $B = 103$ μmol. The minimum transfer number, n, calculated from eqn. 3, is 18. Assuming the initial concentrations of both thiols to be 10 μmol/ml, from eqn. 5 we obtain $b_1 = 35.6$ and $b_2 = 2.6$; the corrected B value becomes 103 + 35.6 + 2.6 = 141.2. The experimental verification of the above calculation is presented in Fig. 2.

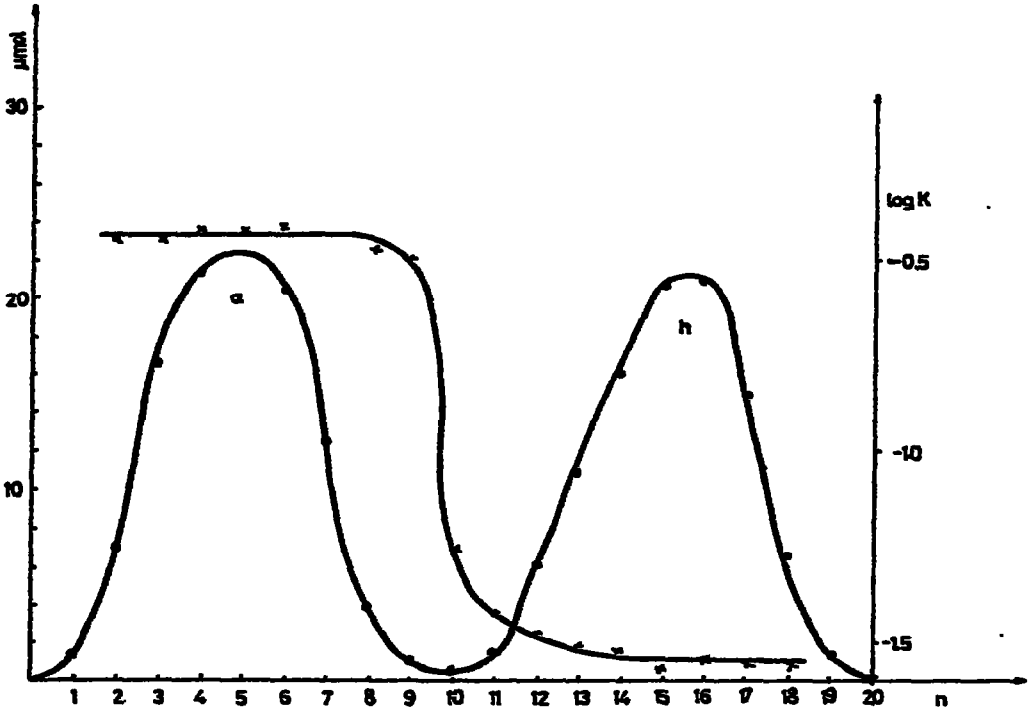

Fig. 2. CCD curves for acetylcysteine (a) and homocysteine (h) in butanol–water, 5 ml upper phase and 10 ml lower phase, at pH 5.0, B = 141 μmol in each unit, 20 transfers, and log of equilibrium constant, K, in each unit.

The CCD has been carried out in 20 separating funnels (25 ml), each one containing 141 μmol of TBT in 5 ml of butanol and 10 ml of buffer A, previously equilibrated. These resuls are in satisfactory agreement with the calculations.

REFERENCES

1 M. Wrotiski, *J. Chromatogr.*, 117 (1976) 47.
2 M. Wrotiski, *Talanta*, 24 (1977) 347.