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APPLICATION OF TRIBUTYLTIN CHLORIDE TO THE SEPARATION AND COLUMN CHROMATOGRAPHY OF THIOLS

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SUMMARY

Tributyltin chloride (TBT) has the properties of a liquid anion exchanger with a special affinity for sulphydryl groups. Thiols such as cysteine can be separated by extraction from aqueous solution with organic solvents in the form of TBT mercaptides. The graphs of extraction coefficient *versus* pH have maxima whose positions depend on the nature of the thiols. Thiols can be separated from each other by using TBT on polyamide as the column packing and elution with buffers of gradually increasing or decreasing pH.

INTRODUCTION

There are few methods available for the selective separation and column chromatographic resolution of thiols that permit their recovery in an unchanged state. Several of the methods suggested are based on the application of mercury compounds anchored to polymer materials. Eldjarn and Jellum[1] achieved the separation and chromatographic fractionation of SH-proteins by using SH-Sephadex treated with a bifunctional mercury compound and elution with cysteine. Thiols such as cysteine and glutathione are retained by mercury-treated phenol–formaldehyde resin[2], and by a resin prepared from ethylene, maleic acid and *p*-acetoxymercurianiline[3]. Thiols can also be separated by precipitation as the mercaptides of *p*-mercuribenzoic acid[4].

The limited use of mercury compounds in chromatographic procedures is connected with their ready decomposition in the presence of acids and mercury complexing agents.

A new approach to the separation and resolution of thiols involves their extraction in the presence of organolead compounds of the type R_3PbX, which are mercaptide-forming agents[5]. Triethyllead chloride or acetate can be used successfully for several separations, *e.g.*, cysteamine from cysteine, but cannot be recommended for the separation of thiols such as cysteine from dilute aqueous solution because the extraction coefficients are too low. This disadvantage could be overcome by using organolead compounds with higher organic radicals, but for chromatographic purposes organolead compounds are not very suitable on account of their instability in acidic solutions. Unlike organomercury compounds and organolead compounds,

organotin compounds of the type R_3SnX are resistant towards acids and very stable in solution. Although the strength of the metal–sulphur bond decreases in the order mercury > lead > tin, the stability of trialkyltin mercaptides is great enough to ensure the selective separation of thiols in the presence of an excess of foreign anions. A comparison of several organotin compounds such as tributyltin chloride (TBT), triphenyltin chloride and tribenzyltin acetate, indicated that TBT is the most suitable. It is a liquid that is almost insoluble in water but miscible with organic solvents, which can be used for spreading on carriers. TBT has already been applied to the separation of volatile thiols from gases[6].

EXPERIMENTAL

The present investigation was carried out using TBT (96% tin) supplied by EGA Chemie (Steinheim/Albuch, G.F.R.). The extraction coefficients were determined at 20° as follows. An aqueous sample containing 10 μmole of the thiol under investigation and 4 ml of buffer solution, prepared by mixing 1 *N* sodium hydroxide solution with a solution of 0.2 *M* orthophosphoric acid plus 0.2 *M* boric acid plus 0.0567 *M* citric acid, was diluted to 6 ml and equilibrated with 3 ml of a solution of TBT in isooctanol. After equilibration and phase separation, 2 ml of the organic phase, diluted with ethanol, and 2 ml of the aqueous phase, made alkaline with 2 *N* potassium hydroxide solution, were titrated with 0.001 *M* *o*-hydroxymercuribenzoic acid (HMB) using dithiofluorescein as indicator[7]. The pH of the aqueous phase was measured after equilibration. The extraction coefficient (k) was calculated as the ratio of the total thiol concentration in the organic phase to that in the aqueous phase.

The column packing was prepared by mixing a solution consisting of 2 ml of TBT and 32 ml of acetone with 10 g of polyamide for column chromatography (Woelm, Eschwege, G.F.R.) and drying. The material was filled into the tube as a slurry in water. Elution was carried out with three solutions:

(A) 0.1 *M* citric acid + 0.2 *M* boric acid + 0.2 *M* monosodium phosphate;

(B) 0.3 *M* disodium phosphate + 0.1 *M* trisodium phosphate + 0.5 g/l EDTA-Na_2;

(C) 0.1 *M* potassium hydroxide + 0.5 g/l EDTA-Na_2.

Moreover, to solutions B and C, just before use, 1 g of sodium sulphite per 100 ml was added in order to prevent the oxidation of thiols by dissolved oxygen. Before use, the column was washed with the buffer, and the thiol was then placed on the column and subjected to gradient elution. The passage of the solution was assisted by gentle aspiration in order to obtain a flow-rate of 1 ml/min. The column effluent was immediately made alkaline with 2 *N* potassium hydroxide solution and titrated with 0.001 *N* HMB using dithiofluorescein or dithizone as indicator. The elution curves of thiols in mixtures were identified by comparison with the elution curves of single compounds obtained under the same conditions.

RESULTS AND DISCUSSION

It can easily be seen from Fig. 1 that the curves of log k *versus* pH each have a maximum. Neutral thiols and thiols containing both an $-NH_2$ and a $-COOH$ group have a maximum near pH 7.5. Acidic thiols (thiomalic acid) and alkaline thiols have

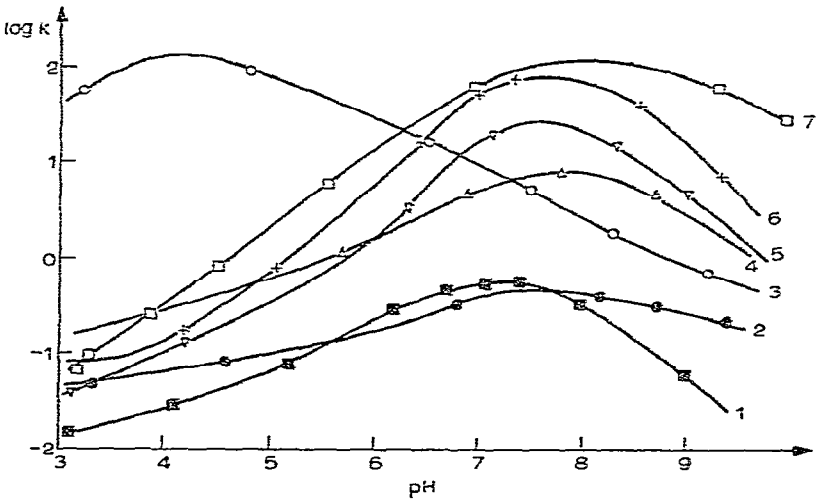

Fig. 1. Effect of pH on extraction coefficients of thiols in isooctanol–water (curve 4: benzyl alcohol) in the presence of TBT. 1, Glutathione, 0.5 *M* TBT; 2, cysteine, 0.02 *M* TBT; 3, thiomalic acid, 0.1 *M* TBT; 4, glutathione, 0.5 *M* TBT; 5, thioglucose, 0.1 *M* TBT; 6, cysteine, 0.5 *M* TBT; 7, ethyl ester of cysteine, 0.02 *M* TBT.

the maximum shifted towards the acidic or alkaline region, respectively. The strong dependence of the extraction coefficient on pH is favourable for the purposes of separation. By using an appropriate pH for extraction and re-extraction and a suitable concentration of TBT and the organic solvent, several thiols can be separated from dilute solution and from each other. For example, by using first 0.02 *M* TBT and then 0.5 *M* TBT in isooctanol, one can separate the ethyl ester of cysteine from cysteine (Fig. 1, curves 2 and 7), and subsequently cysteine from glutathione (curves 1 and 6). Glutathione itself can be separated by using 0.5 *M* TBT in benzyl alcohol, and higher SH-peptides by saturation of the aqueous phase with ammonium sulphate. It is important that the solutions of TBT mercaptides in organic solvents should remain unchanged when kept for at least 1 month. The mercaptides can be decomposed by treatment with an acid, an alkali or sodium sulphide.

The TBT-complexing equilibrium of thiols in the acidic, neutral and alkaline regions can be described by the reactions

$$Bu_3SnX + RSH + H_2O \rightleftarrows Bu_3SnSR + X^- + H_3O^+$$
$$Bu_3SnOH + RSH \rightleftarrows Bu_3SnSR + H_2O$$
$$Bu_3SnOH + RS^- \rightleftarrows Bu_3SnSR + OH^-$$

Consequently, the increase in concentration of X^- and H_3O^- ions in the acidic region and of OH^- ions in the alkaline region results in a decrease in the extraction coefficient. Moreover, the carboxyl and amino groups in thiol molecules have a great influence on the extraction equilibrium.

The relationship between the total concentration of TBT in the organic phase (*B*), the total thiol content per volume of organic phase (*A*) and the ratio of aqueous

to organic phase volume (v), can be expressed as follows. If B is greater than A, the extraction coefficient can be expressed by the ratio

$$k = \frac{b}{t} \tag{1}$$

where b is the concentration of TBT mercaptide in the organic phase and t is the concentration of free thiol in the aqueous phase. The concentrations of free thiol in the organic phase and TBT mercaptide in the aqueous phase are omitted. At constant pH, the extraction equilibrium constant, K, can be expressed by the equation

$$K = \frac{b}{(B-b)\,t} \cdot K_\gamma = \frac{k}{B-b} \cdot K_\gamma \tag{2}$$

where K_γ represents the activity coefficient of the equilibrium constituents. Inserting b, calculated from the equation $A = b + vt = b + vb/k$, into eqn. 2, one obtains

$$K = \frac{k\,K_\gamma}{B - \dfrac{A\,k}{v+k}} \tag{3}$$

For practical purposes, a half-empirical approximation can be recommended:

$$K = \frac{k}{\left(B - \dfrac{A\,k}{v+k}\right)^n} \tag{4}$$

where both K and n are constant at constant pH and constant conditions. A more detailed study on this subject will be carried out later.

The course of the separation demonstrated in Figs. 2–5 can be readily ex-

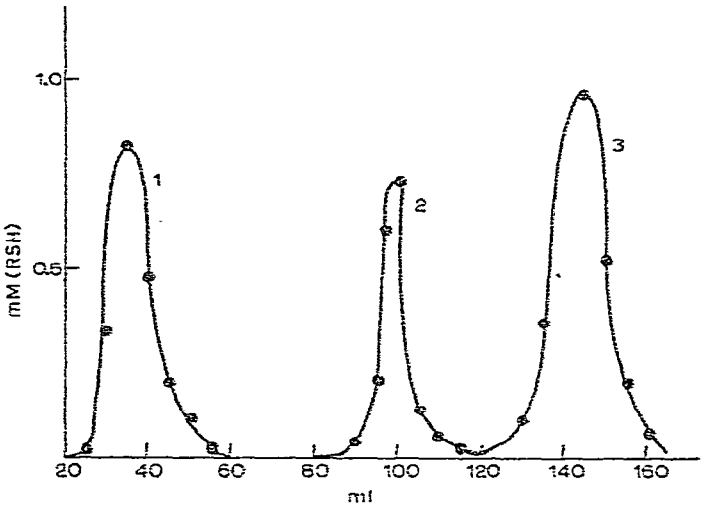

Fig. 2. Elution curves of glutathione (1), cysteine (2) and mercaptoethanol (3) using a column of 20 mm diameter containing 10 g of polyamide covered with 2 ml of TBT. The column was washed with a solution consisting of 4B + A and eluted with the same solution to volume 65 ml, followed by gradient elution by the addition of A to 25 ml of the solution 4B + A in the mixer, resulting in a decrease of pH from 8 to 2.

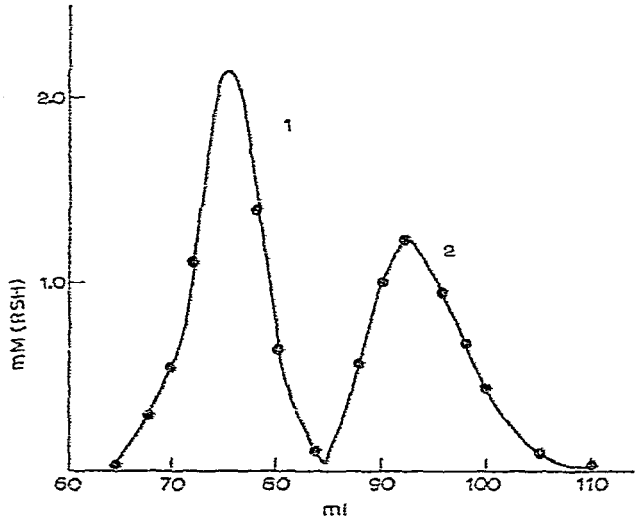

Fig. 3. Elution curves of cysteine (1) and penicillamine (2) using a column of 16 mm diameter containing 18 g of polyamide covered with 3.6 ml of TBT. The column was washed with the solution 4B + A and eluted using a pH decreasing from 8 to 4 with gradient elution by addition of A to 50 ml of the solution 4B + A.

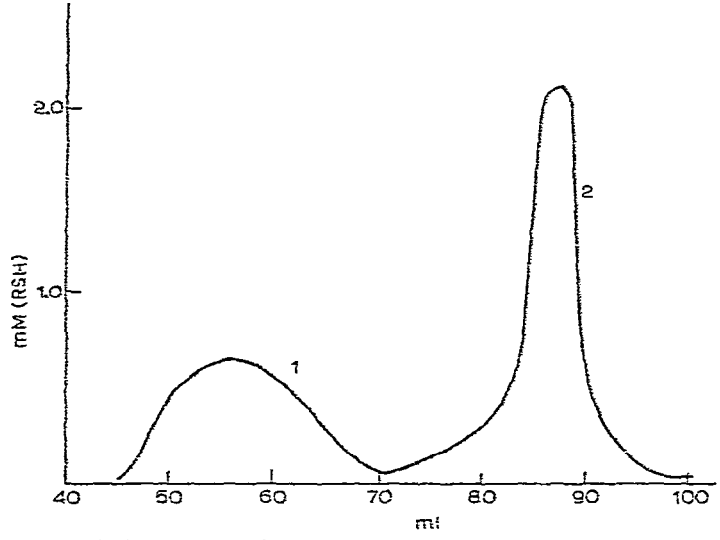

Fig. 4. Elution curves of thioglucose (1) and cysteine (2) using a column of 20 mm diameter containing 10 g of polyamide covered with 2 ml of TBT. The column was washed with 4B + A and eluted using a pH increasing from 8 to 10 with gradient elution by addition of C to 25 ml of 4B + A.

plained on the basis of the relationship between extraction coefficients and pH. Glutathione (Fig. 2) is only slightly retained by TBT and can be removed from the column without affecting cysteine. By gradually acidifying the eluent, cysteine is eluted at pH 7–6, and subsequently mercaptoethanol at pH 5–2.5 of the column

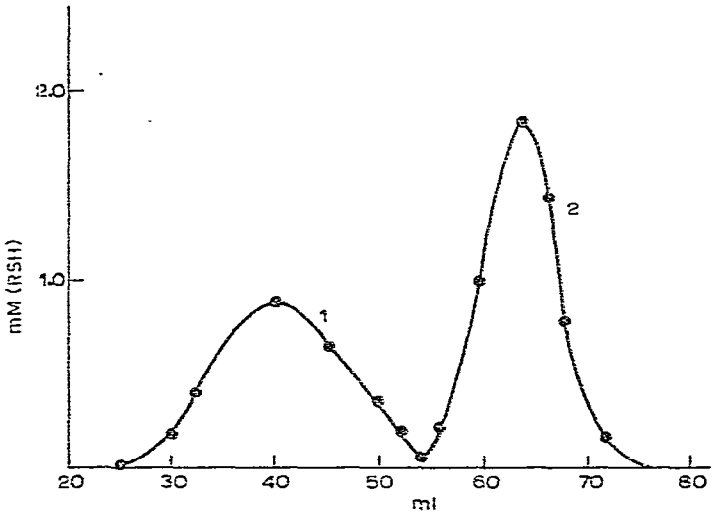

Fig. 5. Elution curves of thiomalic acid (1) and thioglycollic acid (2) using a 20 mm diameter column containing 5 g of polyamide covered with 1 ml of TBT. The column was washed with B and eluted using a pH increasing from 10 to 12 with gradient elution by addition of C to 25 ml of B.

effluent. Thus the full separation of the three thiols is possible. Fig. 3 demonstrates the separation of the closely related compounds cysteine and penicillamine using gradual acidification of the eluent. The separation of thioglucose from cysteine (Fig. 4) and thiomalic acid from thioglycollic acid (Fig. 5) can be performed by using gradient elution with increasing pH. The recovery of thiols, as found by rapid titration of the column effluent containing sodium sulphite, is almost theoretical. As the TBT is slowly washed out of the column the column material should be regenerated after several runs.

On the basis of the results obtained, one can suggest a scheme for the investigation of thiols in aqueous solutions. After extraction at pH 7.5 with a solution of TBT in a higher alcohol, a first re-extraction is carried out with water at pH 3 (neutral thiols) and a second re-extraction with 0.1 *M* potassium hydroxide solution (acidic thiols). The first re-extract is resolved by TBT column chromatography using gradient elution from pH 8 to 3, and from pH 8 to 11, and the second re-extract from pH 9 to 12. The separated thiols can be extracted again as TBT mercaptides and preserved in organic solvents for more detailed investigation.

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