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Note

Extraction chromatography of alkanethiols

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Thiols are formed as by-products in many petrochemical and carbochemical processes. Low-molecular-weight alkanethiols also occur as odours, flavours or metabolites. For this reason, their isolation and determination have been the subject of numerous investigations[1], and still constitute an important analytical and technological problem[2–4].

Due to the high volatilities of simple alkanethiols, their quantitation is mainly based on gas–liquid chromatography, especially since the introduction of sulphur-sensitive detections[3–8]. This high volatility, and the sensitivity to oxidation, has meant that the liquid chromatography of free alkanethiols has so far been the subject of few chromatographic reports. Thus, free alkanethiols have been separated by reversed-phase thin-layer chromatography (RP-TLC)[9], by TLC ligand chromatography[10] and also by high-performance liquid chromatography (HPLC)[8,11,12]. Möckel undertook an extensive study of the HPLC partition chromatography of alkanethiols and other sulphur compounds, together with theoretical considerations.

Some procedures used for the liquid chromatography of alkanethiols were based on their prederivatization as 2,4-dinitrophenyl thioethers[4], 2,5-dinitrobenzoyl thioesters[4], mixed disulphides with 2,4-dinitrothiophenol[13] and 5-mercapto-2-nitrobenzoic acid[14], 4-alkanethiaaldehydes[4] and S-alkylthioglycolic amides[4].

The possibility of separation of alkanethiols by counter-current distribution and distribution chromatography, in which the extraction equilibria of thiols in hydrocarbon–alkaline phase systems were taken into consideration, was also been described by us[15–17]. As a continuation of this research program[15,17], we present here an attempt to correlate the pH-dependent extraction equilibria of thiols (between an alkaline mobile phase and hexadecane stationary phase systems) and their mobilities in partition chromatography. The results enable the separation of low-molecular-weight ($R \leqslant C_6$) alkanethiol mixtures on the millimol scale.

EXPERIMENTAL

All chemicals and supports were commercially available (Merck). The aqueous alkaline buffer solutions were prepared by mixing standardized solutions of 0.1 *M* glycine and 0.1 *M* sodium hydroxide. The stock solutions of thiols (1 and 0.2 *M*)

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were prepared by dilution of thiols in methanol. Determinations of thiols were performed by thiomercurometric titration according to the Wroński procedure[18].

The elution isotherms of thiols were determined by reversed-phase partition chromatography, using the apparatus illustrated in Fig. 1. The column was made of glass, double stopped with screw-threaded joints SQ-18 tubes (250 × 15 mm I.D.)[19]. The packing, 43.5% hexadecane on a polyamide support, gave reproducible results for in the range of 10–1000 μmol thiols, with average recoveries of about 80–90%. Packing of hexadecane on inorganic supports (silica gels, Chromosorbs) was stable only at low concentrations (up to 5%) of hexadecane and exhibited low extraction abilities.

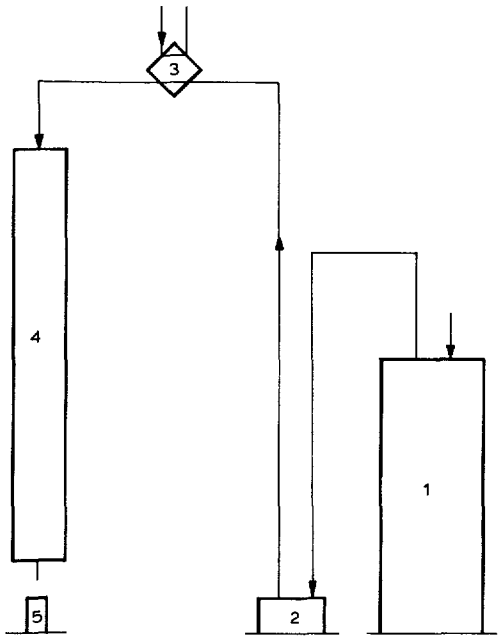

Fig. 1. The chromatographic system applied. 1 = Reservoir of mobile phase; 2 = peristaltic pump; 3 = sample-loop system; 4 = column; 5 = fraction collector.

The thiols were introduced on the column by means of a sample-loop system (100 μl). The flow of mobile phase through the column (5 ml min^{-1}) was exerted by means of a peristaltic pump Model Unipan 394 M.

RESULTS AND DISCUSSION

The acidity of the sulphydryl group results in a characteristic, strongly pH-dependent distribution of thiols in aqueous–hydrocarbon biphasic systems[15,17]. The total extraction equilibrium in such a system is determined both by the partition equilibrium and by the dissociation equilibrium of the thiols, and characterized by the value of the extraction coefficient, D.

$$D = \frac{[RSH]_n}{[RSH]_a + [RS^-]_a} = k_0 \cdot \frac{1}{1 - \frac{K}{[H^+]}} \quad (1)$$

where $[RSH]_i$ and $[RS^-]_i$ represents the concentrations of thiol and thiolate anion in the non-polar (i=n) and aqueous (i=a) phase respectively, k_0 = partition coefficient of thiol, K = dissociation constant of thiol and $[H^+]$ = concentration of hydrogen ion.

Earlier[15] it was established that the low-molecular-weight thiols ($R \leqslant C_6H_{13}$) when extracted in non-polar–polar phase systems undergo characteristic distribution, and when $K \gg [H^+]$ there is a linear dependence between the concentration of hydrogen ion and coefficient D:

$$D = k_0 \cdot \frac{[H^+]}{K} \quad \text{and} \quad \log D = \log \frac{k_0}{K} - \text{pH} \tag{2}$$

On the other hand, when $K \ll [H^+]$ the extraction coefficients of the thiols are equal to their partition coefficients ($D = k_0$), which are dependent on the interaction of thiols with both phases of the extraction (partition) system. The D factors determine also the mobilities in partition chromatography, characterized in thin-layer chromatography by the coefficients R_F and R_M, and in column chromatography by the retention volumes V_R' *or* V_R[20,21]

$$R_M = \log \frac{1 - R_F}{R_F} = \log D + \log \frac{V_{st}}{V_M} \tag{3}$$

$$V_R - V_M = V_R' = \frac{1}{1 - \dfrac{K}{[H^+]}} \cdot \frac{V_{st}}{V_M} \tag{4}$$

where V_{st} = volume of the stationary (non-polar) phase and V_M = column volume of the mobile phase.

Eqn. 4 was experimentally verified for the chromatographic system with hexadecane as the stationary phase and aqueous alkaline buffer solutions as the mobile phase. The column volume of the mobile phase was determined on the basis of the elution isotherm of sodium sulphide at pH 12.9. The corresponding isotherms of thiols as a function of the mobile phase pH are presented in Fig. 2.

As a consequence of eqns. 4 and 2, the relationship between the corrected retention volumes of thiols, V_R', and the mobile phase pH is linear when $[H^+] \ll K$ (pH > 11.0), and deviates downwards for pH < 11.0 (Fig. 3). The elution isotherms of thiols, taken at different pH (Fig. 2), also suggest an optimum region of pH for separation of multicomponent thiol mixtures. Thus, the mobile phase at pH 11.0 is sufficient for the separation of propanethiol from a mixture with methanethiol and ethanethiol. Analogously, at pH 11.5, butanethiol can be removed from a mixture consisting of ethanethiol, propanethiol and butanethiol. Additionally, the use of mobile phases buffered to pH 12.0 and 12.5 enables the separation of propanethiol, butanethiol and pentanethiol (Fig. 4c), and of butanethiol, pentanethiol and hexanethiol (Fig. 4d).

However, the scope of these separations at constant pH seems to be limited to binary or three-component mixtures due to the relatively low separability of the column used, compared with HPLC systems[12]. For this reason, the separation of more

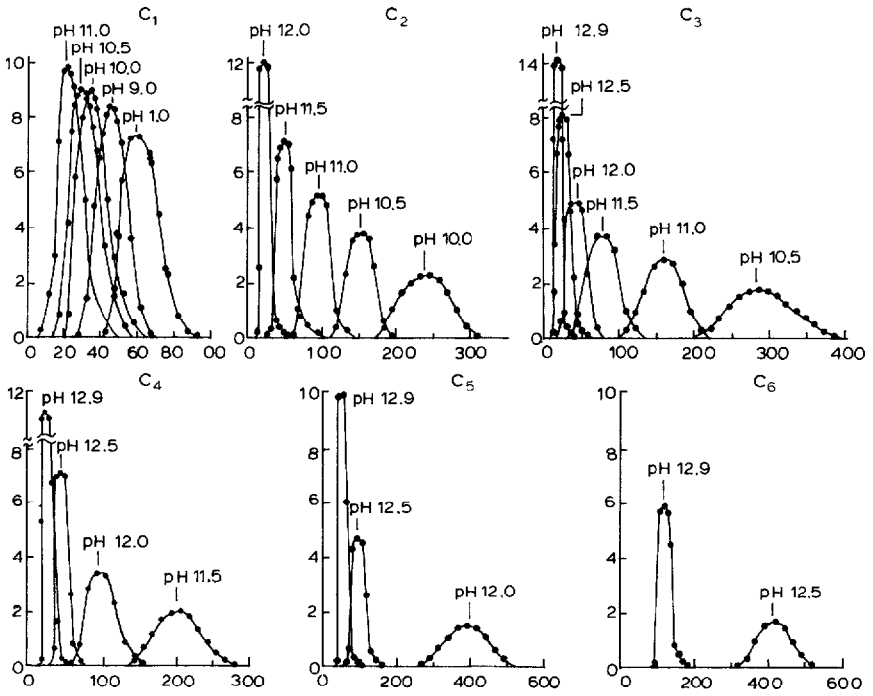

Fig. 2. Elution isotherms of thiols (μmol of RSH *vs.* ml of eluate) taken at different pH values of the mobile phase (20°C). Thiols: C_1 = methanethiol; C_2 = ethanethiol; C_3 = *n*-propanethiol; C_4 = *n*-butanethiol; C_5 = *n*-pentanethiol; C_6 = *n*-hexanethiol.

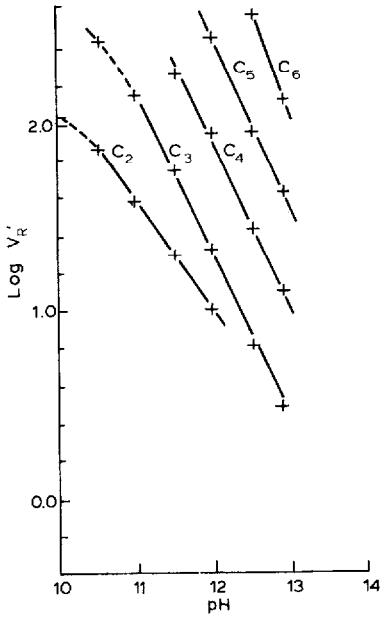

Fig. 3. Relationships of log V_R' *vs.* pH for *n*-alkanethiols, determined on the basis of the elution isotherms presented in Fig. 2.

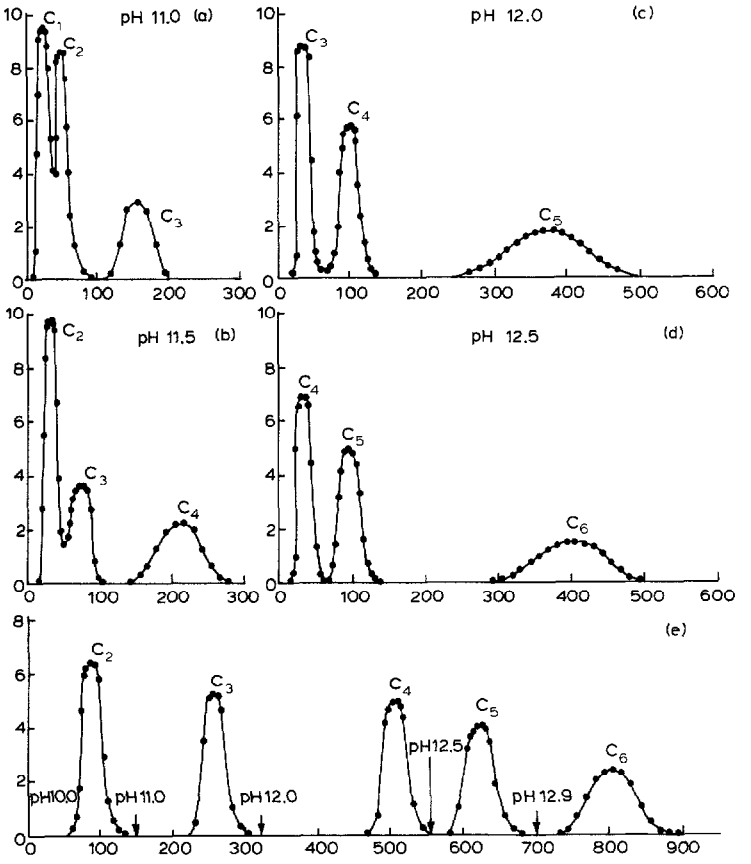

Fig. 4. Separations (μmol of RSH *vs.* ml of eluate) of thiol mixtures.

complex mixtures of alkanethiols required the further optimization of their extraction equilibria, in particular the use of a pH gradient. The elution isotherm of a five-component mixture of alkanethiols (C_2–C_6) is presented in Fig. 4e. The separation of thiols was achieved by increasing successively the pH of the mobile phase: ehanethiol was eluted at pH 10.0, propanethiol at pH 11.0, higher thiols (butanethiol, pentanethiol and hexanethiol) at pH 12.0, 12.5 and 12.9, respectively. Additionally, the high extraction capacity of the column enabled the separation of the thiols even on a millimol scale.

Further optimization of partition chromatography of high-molecular-weight alkanethiols ($R \geqslant C_6H_{13}$) and the column capacity will be attempted.

ACKNOWLEDGEMENT

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