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Note

Simple determination of cysteine and cystine in dilute solutions by high-performance liquid chromatography with electrochemical detection using a DC-TAST polarograph

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The biologically very important cysteine (RSH)–cystine (RSSR) system has been the subject of many investigations, including polarographic studies and amperometric titrations[1,2]. The very simple polarographic method permits the simultaneous determination of both compounds; at positive potentials of the dropping mercury electrode, RSH undergoes the following anodic reaction:

$$Hg + 2\,RSH \rightarrow Hg(RS)_2 + 2\,H^+ + 2\,e^-$$

while at negative potentials RSSR is reduced to RSH:

$$RSSR + 2\,H^+ + 2\,e^- \rightarrow 2\,RSH$$

Nevertheless, polarographic method gives poor results in dilute solutions of RSH and RSSR and in the presence of some other substances in a sample. Indirect polarographic methods for the determination of cystine in more dilute solutions have been also elaborated on the basis of the catalytic wave formed in the presence of cations such as Co^{2+} and Ni^{2+} in an ammonia buffer[3]. Recently a high-performance liquid chromatographic (HPLC) method with fluorescence detection of RSSR after its derivatization[4] and an electrochemical flow-through detector[5] were elaborated. Neither of these methods, however, distinguishes cystine from cysteine.

This paper reports a direct and sensitive method for the rapid determination of RSH and RSSR in the dilute solutions and in the presence of other substances. The method is based on the resolution of RSH and RSSR peaks in an ion-pair chromatographic system and their polarographic detection with a DC-TAST polarograph at two suitably chosen potentials of the dropping mercury electrode.

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EXPERIMENTAL

Reagents

L-Cysteine (puriss) was obtained from Fluka (Buchs, Switzerland) and L-cystine from Reanal (Budapest, Hungary). All other materials were of analytical-reagent or laboratory grade and were used without further purification.

Apparatus and procedure

Chromatographic experiments were performed using a Type 302 apparatus equipped with a Type PP2 flow-through detector[6] and a Type PPW1 DC-TAST polarograph[6] (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland). For HPLC, stainless-steel columns (250 × 4.5 mm I.D.) packed with 10 μm LiChrosorb RP-18 (E. Merck, Darmstadt, F.R.G.) were used.

All measurements were carried out at 20 ± 0.2°C and a constant flow-rate of 0.7 cm^3/min.

Aqueous mobile phases containing various concentrations of *p*-toluenesulphonic acid (TSA) or *d*-camphor-10-sulphonic acid (CSA) and suitable buffer com-

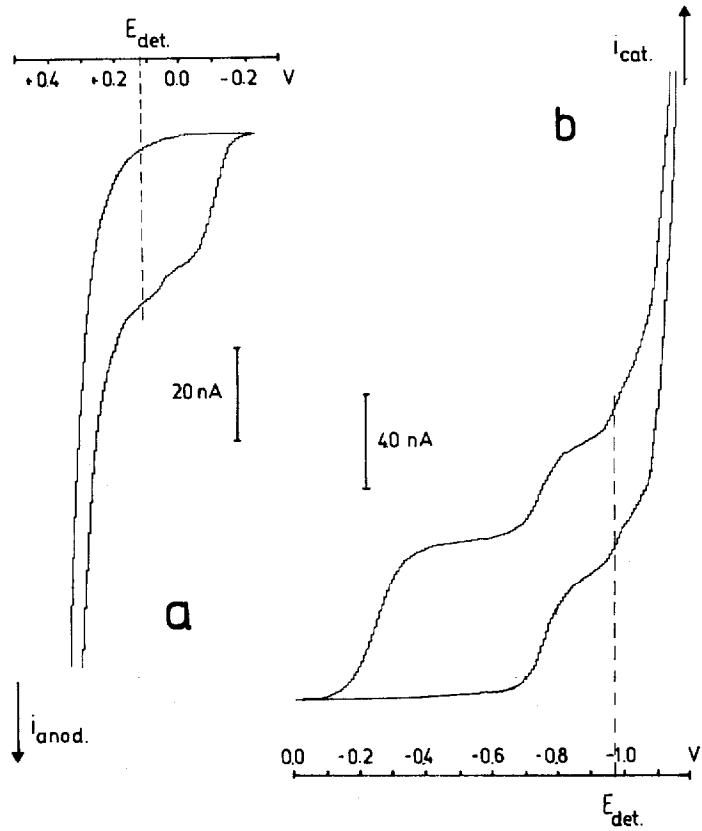
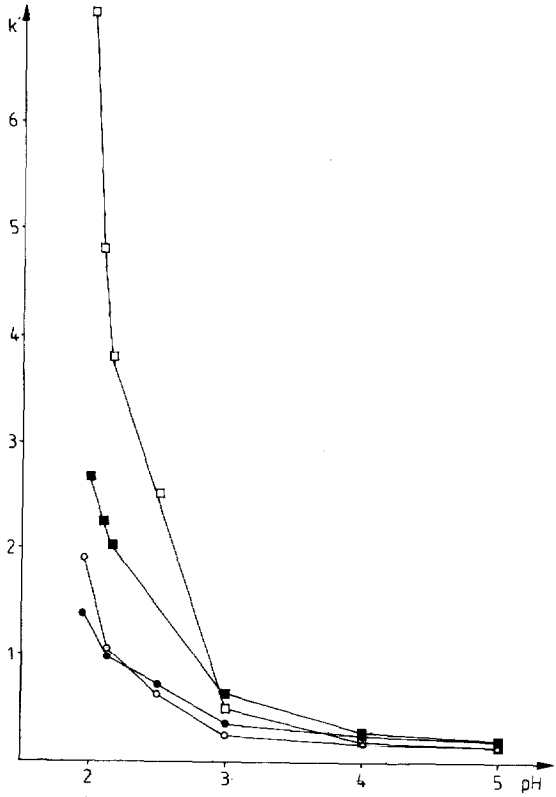


Fig. 1. Polarographic curves of (a) 1 × 10^{-5} *M* cysteine and (b) 1 × 10^{-5} *M* cystine solutions in 0.03 *M* phosphate buffer (pH 2.1) 2 · 10^{-2} *M* CSA and the curves of the same supporting electrolyte. Reference electrode: SCE.

ponents were deaerated with argon. Samples introduced onto the column contained oxygen or were deoxygenated in the syringe by very simple procedure described by Lloyd[7] and modified by Bylina[8]. Chromatograms were obtained as follows: cysteine, which is eluted as the first peak from the reversed-phase column, was detected at a potential of +0.250 V (*vs.* an Ag/AgCl electrode). Then, after a rapid shift of potential to −0.850 V (*vs.* an Ag/AgCl electrode), the later eluted cystine peak was detected. The examples of the separate polarographic curves of pure cysteine and cystine are given in Fig. 1. Polarographic curves were obtained with a saturated calomel electrode (SCE) as the reference electrode, and for detection in chromatography an Ag/AgCl electrode was used: $V_{Ag-AgCl} - 130\ mV = V_{SCE}$.

RESULTS AND DISCUSSION

Figs. 2 and 3 show the capacity factors (k') of RSH and RSSR as functions of pH and TSA or CSA concentration in the mobile phase. Each k' value was de-



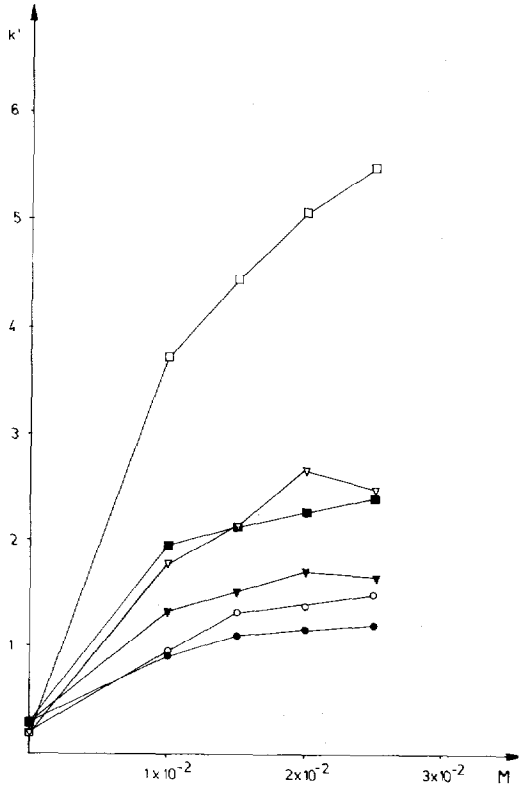


Fig. 2. Plot of capacity ratios (k') of cysteine (■, ●) and cystine (□, ○) *versus* pH of the mobile phase at a fixed concentration (0.02 *M*) of the counter ion (■, □, CSA; ●, ○, TSA). Stationary phase:LiChrosorb RP-18.

Fig. 3. Plot of capacity ratios (k') of cysteine and cystine *versus* counter ion concentration at a fixed pH. ■, RSH; □, RSSR; 0.03 *M* phosphate buffer (pH 2.1); counter ion, CSA. ▼, RSH; ▽, RSSR; 0.05 *M* phosphate buffer (pH 2.2); counter ion CSA. ●, RSH; ○, RSSR; 0.03 *M* phosphate buffer (pH 2.1); counter ion TSA. Stationary phase: LiChrosorb RP-18.

termined as the mean of five or six measurements (injections) with a relative standard deviation ($\Delta k'/k'$) of 0.1%. The investigated samples dissolved in the mobile phase were stable within particular series of experiments for at least several hours. The above relationships led to the following conclusions.

For the resolution of RSH and RSSR, CSA gives much more selective counter ions than TSA; *e.g.*, at pH 2.1 the selectivity factor, $\alpha_{RSSR/RSH}$, is 2.25 in $2 \cdot 10^{-2}$ *M* CSA solution and only 1.18 in TSA solution of the same concentration. The resolution is distinctly influenced by the concentration of the phosphate buffer, which stabilizes the pH and at the same time ensures a suitable conductivity of the mobile phase for polarographic detection; *e.g.*, at pH 2.2 in $2 \cdot 10^{-2}$ *M* CSA solution $\alpha_{RSSR/RSH} = 1.87$ in 0.03 *M* and 1.57 in 0.05 *M* phosphate buffer solution. Taking into account above relationships and the time of separation, the following conditions were determined to be the most suitable for the chromatography of cysteine and cystine mixtures: pH 2.1, 0.03 *M* phosphate buffer and 0.01 *M* CSA (Fig. 4).

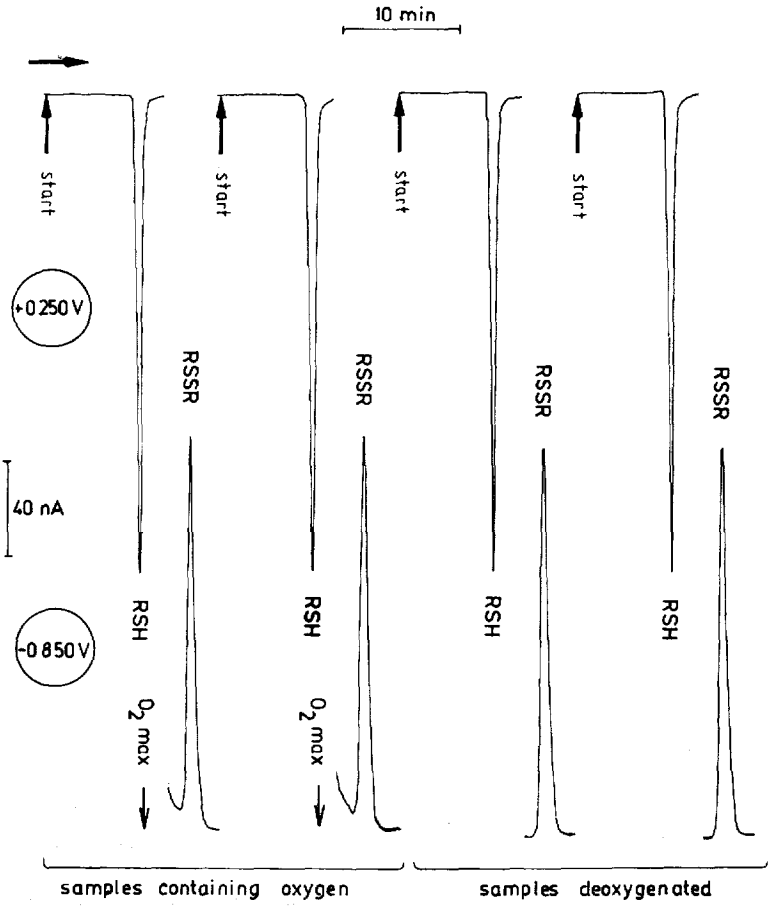


Fig. 4. Four consecutive chromatograms of a mixture of $2.5 \cdot 10^{-4}$ *M* RSH and $2 \cdot 10^{-4}$ *M* RSSR. Sample size: 10 μl. Mobile phase composition: 0.01 *M* CSA, 0.03 *M* phosphate buffer (pH 2.1). Column: 250 × 4.5 mm I.D., packed with 10-μm LiChrosorb RP-18.

These conditions can be recommended with one restriction, namely that the samples should be deaerated because the elution curve of oxygen is partly superimposed on the cystine peak. With samples containing oxygen more concentrated solutions of CSA must be used in which RSSR is more strongly retained on the column, *e.g.*, $2 \cdot 10^{-2}$ *M*, and consequently the time of analysis is longer (Fig. 5).

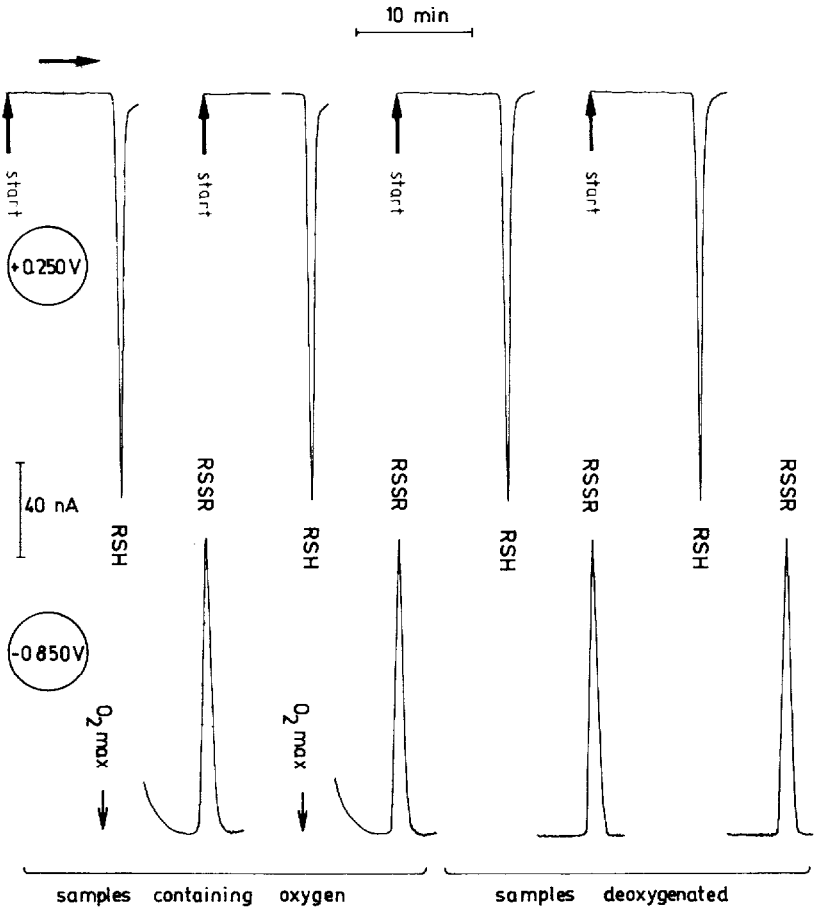


Fig. 5. Four consecutive chromatograms of a mixture of $2.5 \cdot 10^{-4}$ *M* RSH and $2 \cdot 10^{-4}$ *M* RSSR. Sample size: 10 μl. Mobile phase composition: 0.02 *M* CSA, 0.03 *M* phosphate buffer (pH 2.1). Column: 250 × 4.5 mm I.D., packed with 10-μm LiChrosorb RP-18.

We found a linear dependence of the peak height of RSH and RSSR on concentration in the concentration ranges $0\text{–}1 \cdot 10^{-3}$ *M* for cysteine and $0\text{–}5 \cdot 10^{-4}$ *M* for cystine, as can be seen in Fig. 6.

The detection limit (defined as the amount of an injected substance giving a signal twice that of the baseline noise) is $5 \cdot 10^{-12}$ mol for cysteine *i.e.*, 10 μl of $5 \cdot 10^{-7}$ *M* solution, and $20 \cdot 10^{-12}$ mol for cystine *i.e.*, 10 μ of $2 \cdot 10^{-6}$ *M* solution. These favourable detection limits with DC-TAST polarography[6] cannot be achieved

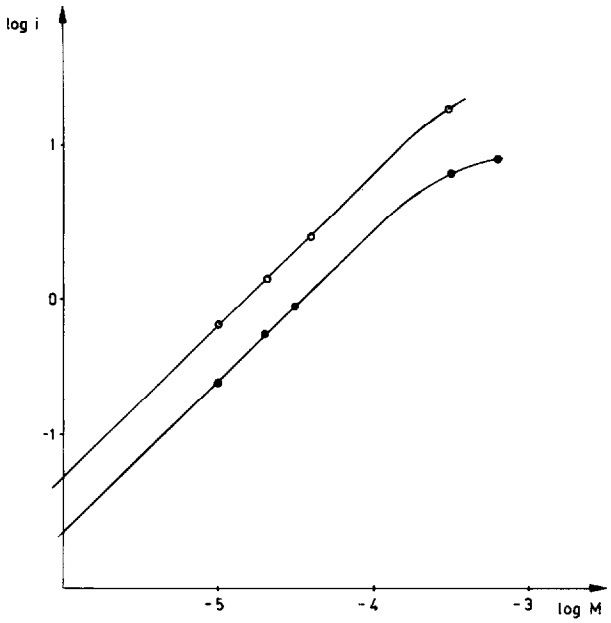


Fig. 6. Plots of logarithm of current intensity (nA) as a function of cysteine (●) and cystine (○) concentration (M) in a solution of 0.03 M phosphate buffer (pH 2.1) and $2 \cdot 10^{-2}$ M CSA.

by simple d.c. polarography. With cysteine and cystine, the DC-TAST polarographic results are comparable to those attained with chemical derivatization and the fluorescence method[4].

The developed method, which is sufficiently specific, can be used for the determination of cysteine and cystine in various complex mixtures. Substances that are polarographically active, such as ascorbic acid, glutathione, chlorides and bromides, do not interfere because we have found that they are eluted separately.

REFERENCES

1 M. Brezina and P. Zuman, *Die Polarographie in der Medizin, Biochemie und Pharmazie*, Akademische Verlagsgesellschaft Geest and Portig K.-G., Leipzig, 1956.
2 M. T. Stankovich and A. J. Bard, *J. Electroanal. Chem.*, 75 (1977) 487.
3 K. Kužel, *Clin. Chim. Acta*, 48 (1973) 377.
4 J. D. H. Cooper and D. C. Turnell, *J. Chromatogr.*, 227 (1982) 158.
5 R. Eggli and R. Asper, *Anal. Chim. Acta*, 101 (1978) 253.
6 M. Przasnyski, A. Bylina and J. Pięcek, *7th Int. Symp. on Column Liquid Chromatography, Baden-Baden, May 3–6, 1983.*
7 J. B. F. Lloyd, *J. Chromatogr.*, 256 (1983) 323.