Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1062 (2005) 95–101
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Comparison of voltammetric detection assisted by multivariate curve resolution with amperometric detection in liquid chromatographic analysis of cysteine-containing compounds

Olga González-García, Cristina Ariño*, José Manuel Díaz-Cruz, Miquel Esteban

Departament de Química Analítica, Universitat de Barcelona, Av. Diagonal 647, E-08028 Barcelona, Spain

Received 30 July 2004; received in revised form 21 October 2004; accepted 2 November 2004
Available online 28 November 2004

Abstract

A voltammetric detection mode (VD) in conjunction with multivariate curve resolution with alternating least squares (MCR-ALS) method is applied to the analysis of cysteine-containing compounds and compared with a well established amperometric detection (AD) mode in a thin-layer dual Hg/Au cell. VD-MCR-ALS provides an increase in selectivity for cases where satisfactory separation of electroactive compounds is not allowed. However, concentrations needed for a good quantification in VD are higher than in AD due to much large contribution of background in VD.

Keywords: Thiols; Amperometric detection; Voltammetric detection; HPLC; Multivariate curve resolution

1. Introduction

Low-molecular-mass thiols, such as cysteine (Cys), glutathione (GSH), and their respective disulfides are ubiquitous in nature and, based upon the many known functions of these compounds in a great variety of biochemical processes, their identification and accurate measurement is essential [1,2]. GSH is considered an essential constituent of all living cells and it is usually the most abundant non protein thiol [1]. GSH plays an important role in the complexation and elimination of toxic heavy metals from the organisms [2], shared with more complex peptides and proteins such as metallothioneins (MT) in mammals or phytochelatins (PCs) in plants [3]. The structure of GSH (γ-Glu–Cys–Gly) is strongly related to that of PC [(γ-Glu–Cys)$_n$–Gly]. For this reason, in media where PCs are synthesized their constituent peptides can be also present.

Many methods have been developed for the determination of thiols and disulfides. Several analytical approaches use a derivatization procedure in order to obtain compounds suitable for detection by UV or fluorometry [4–6]. In recent years, high-performance liquid chromatography with electrochemical detection (HPLC–ED) has been widely explored [7–23] as an alternative to such derivatization methods. Also capillary electrophoresis with electrochemical detection (CE–ED) has been used for the determination of RSH/RSSR analytes [24–26]. Among the different possible HPLC–ED approaches, amperometric detection (AD) in thin-layer dual Hg/Au electrodes in series is practically the only one used. In some few cases, coulometric detection is also applied [12,22].

In general terms, AD at a single potential lacks enough selectivity when two or more analytes coelute or when large background contributions are present in the measured signal. The change from AD to the voltammetric detection (VD) mode improves the selectivity of the technique. This change is equivalent to the band pass, in the spectroscopic domain, from a single wavelength to a diode-array multiwavelength detection (DAD UV–vis). However, this jump requires of:

* Corresponding author. Tel.: +34 93 402 15 45; fax: +34 93 402 12 33.
E-mail address: cristina@apolo.qui.ub.es (C. Ariño).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.11.018

(i) the use of a fast potential scanning technique, and (ii) a powerful chemometric technique for the large amount of information from every VD run.

Chemometrics has been very scarcely applied to electrochemistry in general [27], and to electrochemical detection in particular. This can be explained because of the lack of linearity between currents and concentrations in many electrochemical processes, and because of the complex relationship between concentrations in the electrode diffusion layer and in bulk solution. Application of chemometrics to HPLC–ED is mainly focused to optimization and prediction tasks [28–30]. At the best of our knowledge, only one previous work has been devoted to the use of multivariate analysis methods for analyte mixtures determination from HPLC–VD data [31]. In particular, multivariate curve resolution by alternating least squares (MCR-ALS) has been used. VD-MCR-ALS has allowed the determination of a mixture of pyrocathecol, ascorbic acid and epinephrine, compounds that have satisfactory oxidation processes in a glassy carbon electrode and clearly different voltammetric behaviours [31]. Voltammetric signals, as compared to amperometric measurements, give extra information allowing their better resolution. This implies that it is not necessary to achieve a complete chromatographic separation of the different eluting substances provided that their voltammetric responses are not completely equal.

In the present work, a comparison between AD and VD modes assisted by MCR-ALS has been made, showing the advantages of VD in cases with overlapping peaks. This has been applied to the separation of thiol compounds as PC and smaller cysteine-containing compounds.

2. Experimental

2.1. Reagents

L-Cysteine, γ-Glu–Cys (80% of purity as trifluoroacetate salt) and Cys–Gly (85% of purity) peptides were provided by Sigma (St. Louis, MO, USA). Glutathione, potassium hydroxide, octanosulfonic acid (OSA), *N*,*N*-dimethylformamide (DMF) and methanol (MeOH) were obtained from Merck (Darmstadt, Germany). Monochlororacetic acid (MCA) was provided by Aldrich (Milwaukee, WI, USA). Metal-free phytochelatins PC_2 and PC_3 were synthesized by Diverdrugs (Barcelona, Spain), both with a purity of 91.8%.

All solutions were prepared in ultrapure filtered water obtained from Milli-Q plus 185 system (Millipore, Bedford, USA).

2.2. Instruments and experimental conditions

An Agilent (Palo Alto, USA) 1100 chromatographic system, with a quaternary pump, a 20 μL-loop manual injector, a vacuum degasser and a handheld control module were used. Analytical and guard columns, Inertsil ODS C18 250 mm × 4.6 mm and 10 mm × 4.6 mm, respectively, were provided by Supelco (Bellefonte, PA, USA).

The electrochemical detector system was a CC-5C BAS flow cell (BAS, West Lafayette, IN, USA), with a three electrode system with a 0.2 in. gasket, connected to an Autolab PSTAT 10 (Ecochemie, NL). The GPES 4.4 software (Ecochemie) allowed potentiostatic control and data acquisition.

The working electrode was a dual Au/Hg amalgam thin layer electrode BAS (MF-1002) for which every electrode surface was disposed parallel to the flow direction. The amalgam was prepared by placing double-distilled Hg onto the polished Au surface and by removing, after 2 min, the excess Hg. A stainless steel auxiliary electrode and an Ag/AgCl (KCl sat.) reference electrode were used in all experiments.

Optimal mobile phase was: 93.25% (v/v) 0.1 M MCA aqueous solution, 5% MeOH, 1.75% DMF and 2.25 mM OSA, adjusted to pH 2.8 with KOH. The same mobile phase, but with 45 μM OSA, produces coelution of γ-Glu–Cys and GSH. All solutions were filtered through a 0.22 μm membrane filter and degassed. Samples were run isocratically at a flow-rate of 1.0 mL/min at controlled room temperature.

GPES 4.4 software records I versus t at E constant (AD mode), and a voltammogram at each elution time (thus obtaining I versus E versus t data matrix) in VD mode. For thiol detection in AD mode the applied potential was 0.15 V. At this potential Hg from the electrode is oxidized to form a very stable complex with sulphur groups [32] following the reaction:

$$2\mathrm{RSH} + \mathrm{Hg} \rightarrow (\mathrm{RS})_2\mathrm{Hg} + 2\mathrm{H}^+ + 2\mathrm{e}^-$$

The reaction is highly specific for the sulfhydryl group and proceeds rapidly and stoichiometrically.

2.3. Data treatment

In VD-MCR-ALS approach data are arranged in a current data matrix (**I**) with so many rows as recorded voltammograms and so many columns as potentials scanned during the measurements. The columns of **I** (I versus t when E is held constant) correspond to that we can call amperometric chromatograms. The rows of **I** (I versus E when t is held constant) correspond to that we can call hydrodynamic voltammograms.

The basis of MCR-ALS is to decompose mathematically **I** into a product of two orthogonal matrices, represented as **C** (containing the calculated concentration elution profiles of resolved electroactive species at the detector) and $\mathbf{V}^{\mathbf{T}}$ (corresponding to calculated pure voltammograms), plus an error matrix **X** (including the variations not explained by **C** and $\mathbf{V}^{\mathbf{T}}$) [31,33]:

$$\mathbf{I} = \mathbf{C}\mathbf{V}^{\mathrm{T}} + \mathbf{X} \quad (1)$$

The iterative decomposition of **I** needs an initial estimation of **C** and/or of $\mathbf{V}^{\mathbf{T}}$ for each component. For data obtained from VD the best initial estimation of $\mathbf{V}^{\mathbf{T}}$ is obtained by a

procedure based on the peak-shape nature of the chromatographic signals [31] through Gaussian functions described by the general equation:

$$\text{conc} = a \exp\left[\left(\frac{-(t-b)^2}{c}\right)\right] \quad (2)$$

where conc is the magnitude of the signal, t is the time and a, b, c are adjustable parameters related to the height, position and width of the peak, respectively. Due to the special characteristics of the electrochemical background signal in VD, and previous experience [31], it was explicitly modeled as an independent mathematical component, having its own concentration and voltammogram profiles. The initial estimation of the background elution profile was modelled by means of an exponential function describing the charging process of a capacitor [34]:

$$\text{conc} = a \exp(-bt) \quad (3)$$

where t is the time, a and b are adjustable parameters and conc is the magnitude of the background elution signal.

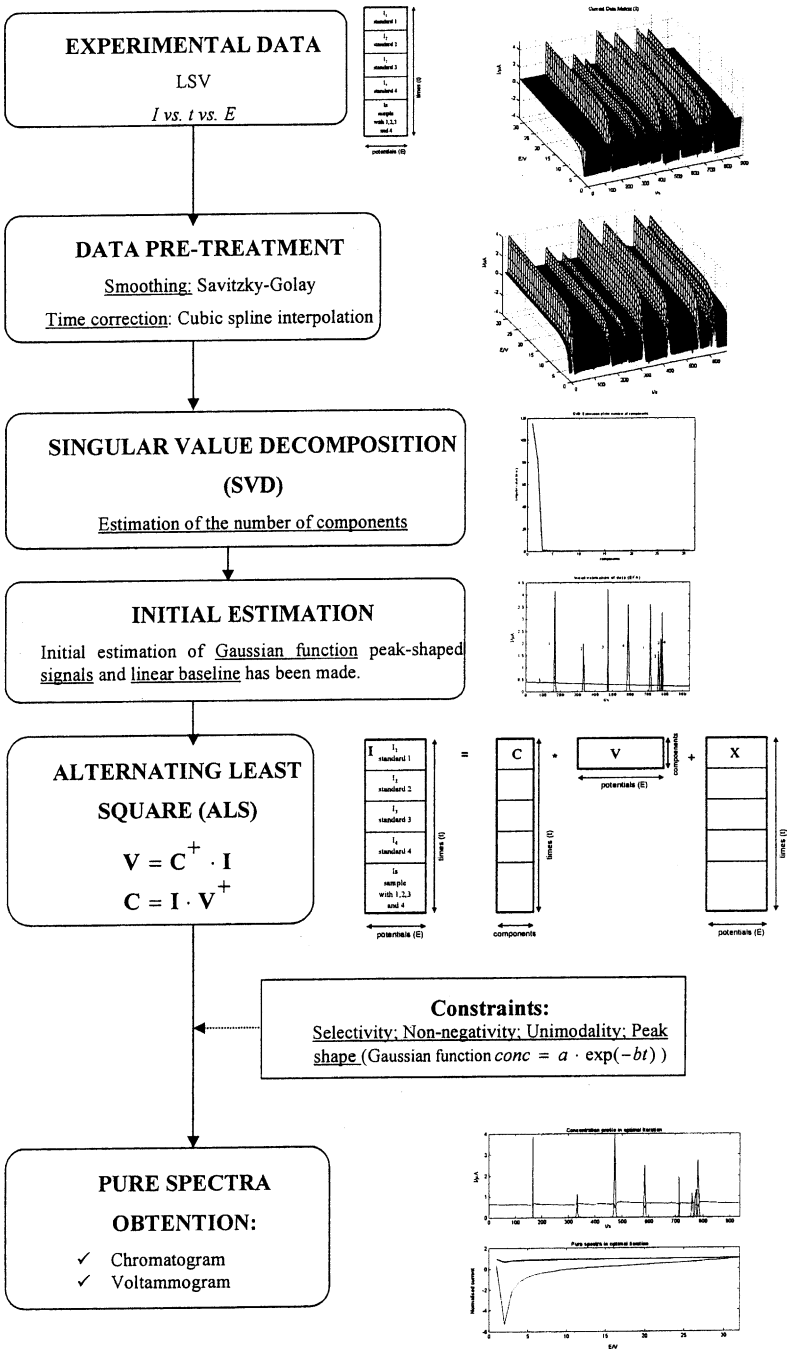

Fig. 1. Schematic flow-chart of multivariate curve resolution by alternating least squares as applied to voltammetric detection in HPLC.

Table 1
Figures of merit for thiol determination by HPLC–ED (amperometric mode at 0.15 V)

Compound	Linearity range (ng)	Calibration functions	Detection limits (ng injected)	R.S.D. (%)
Cys	8.77–780.00	$y = 0.175 + 0.549x$, $r^2 = 0.9995$	3.32	1.0
Cys–Gly	15.26–1069.00	$y = 0.226 + 0.177x$, $r^2 = 0.9994$	8.27	1.2
GSH	32.40–1113.99	$y = 4.697 + 0.146x$, $r^2 = 0.9932$	7.05	1.0
γ-Glu–Cys	22.02–699.99	$y = 5.955 + 0.273x$, $r^2 = 0.9952$	15.55	0.6
PC_2	45.53–1231.91	$y = -3.044 + 0.138x$, $r^2 = 0.9972$	12.49	3.4
PC_3	169.43–1881.00	$y = -0.825 + 0.038x$, $r^2 = 0.9965$	42.96	7.3

Calibration functions: y (peak area, as nA/min) and x (quantity injected, as ng). Detection limits calculated at a signal-to-noise ratio of 3.

To impress chemical sense to the mathematical MCR-ALS solution, the iterative ALS procedure allows several restrictions (constraints) to be imposed: selectivity (restrict the presence of one species to some zone of the matrix), non-negativity (applied to concentration profiles and/or voltammograms), unimodality (single peak shape of concentration elution profiles and/or unit voltammograms) and signal-shape (fitting of the signals to Gaussian functions owing to the peak-shaped nature of chromatographic signals).

This VD-MCR-ALS treatment can be applied to a single matrix obtained in the injection of one sample, or to a column-wise augmented data matrix obtained in successive injections of several standards and the unknown sample. This last procedure has been applied in this work due to its usefulness for quantitative purposes.

Fig. 1 shows a flow chart that summarizes the steps of this VD-MCR-ALS approach applied to the quantification of different analytes by HPLC–ED, using the VD mode. It will be described further in more detail.

All data treatment, including data smoothing, base line correction, data matrices preparation, and the own VD-MCR-ALS analysis were performed by means of home-made programs implemented in MATLAB version 5.3 [35], some of them freely available at http://www.ub.es/gesp/mcr/mcr.htm.

3. Results and discussion

Using AD, separation of Cys, GSH, its fragments and the phytochelatins PC_2 and PC_3 is allowed in similar conditions (see Section 2) to those recommended for thiol and disulphur separations [20,23]. In those conditions PC_n need more than 20 min to be eluted, while GSH and its fragments can be separated in less than 15 min (Fig. 2a). Table 1 summarizes the figures of merit using these experimental conditions. The highest detector response was for Cys and the lowest one for PC_3, the compound eluted at last. Table 1 also shows the intra-day repeatability that was evaluated injecting 10 replicates of 20 μL of a 2 10^{-5} M solution of each compound. Relative standard deviation (R.S.D.) values are lower than 1.2% for Cys, GSH and its fragments; R.S.D. for PC_2 and PC_3 are higher because they elute later and yield wider peaks. Inter-day assays were not evaluated due to the important baseline fluctuations, which strongly depend on both Hg film electrode and the stability of the power supply line.

This methodology was tested in the separation and quantification of thiol compounds in three synthetic samples with recoveries ranging from 89 to 111%, showing that it is appropriate for separation and quantification of PC_n and related compounds.

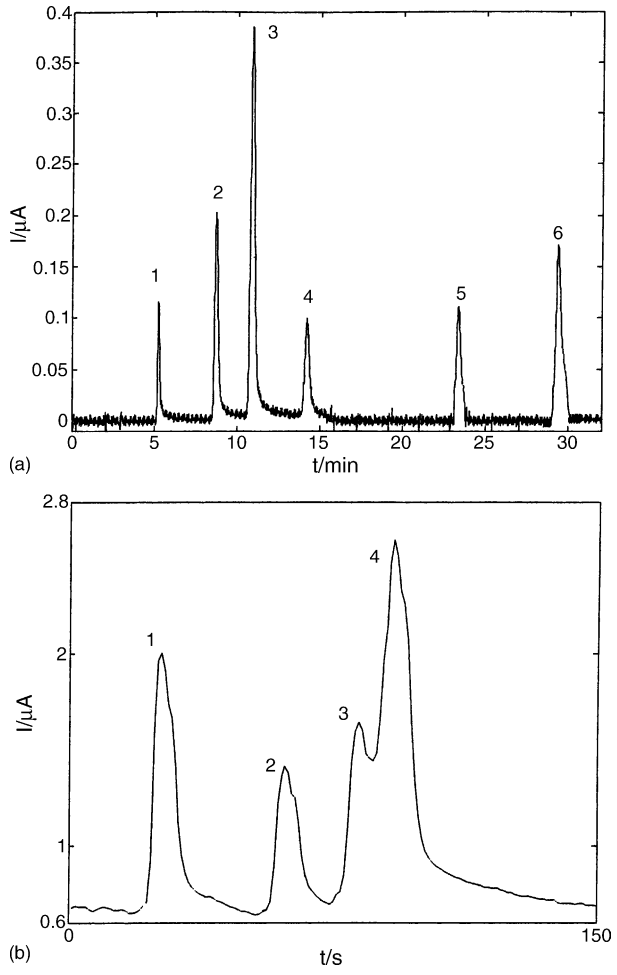

Fig. 2. Chromatograms of a synthetic mixture of thiol compounds: (a) cysteine (1), Cys–Gly (2), GSH (3), γ-Glu–Cys (4), PC_2 (5) and PC_3 (6), amperometric detection at +0.15 V (vs. Ag/AgCl). Mobile phase: 93.25% (v/v) 0.1 M MCA aqueous solution, 5% MeOH, 1.75% DMF and 2.25 mM OSA adjusted to pH 2.8; (b) cysteine (1), Cys–Gly (2), GSH (3) and γ-Glu–Cys (4), single amperometric detection at +0.15 V (vs. Ag/AgCl). Mobile phase: 93.25% (v/v) 0.1 M MCA aqueous solution, 5% MeOH, 1.75% DMF and 45 μM OSA adjusted to pH 2.8.

Although separation of those thiol compounds is easily allowed, sometimes chromatographic separation can not be satisfactorily achieved. In those cases the use of a more selective detection method plays a crucial role.

The VD-MCR-ALS detection mode has been applied, as a first approach and for the sake of simplicity, to the analysis of PC fragments Cys, Cys–Gly, GSH and γ-Glu–Cys. In the case of complex thiol mixtures, such as those from algae extracts, presence of overlapping peaks is quite probable because of small size PC fragments. On the contrary, it does not seem very likely to find overlapping of the signals from PC_2, PC_3, . . ., PC_n molecules, whose molecular weights are quite different.

Linear sweep voltammetry (LSV) scan mode has been used for VD-MCR-ALS. It requires the optimization of some experimental parameters as the scan rate (v) and the potential range. The influence of v was investigated in order to achieve an optimal compromise value. Thus, low v values imply a few numbers of scans per minute, i.e., a poor time resolution on chromatograms. High v values involve an increase of the background current and of the noise. The window of applied potentials was selected to allow better differentiation of signals. At low potentials, no oxidation signals were detected. At high potentials, all substances quickly attained the same plateau corresponding to limiting current conditions (i.e., producing the same voltammetric behaviour in all species and, thus, hindering electrochemical selectivity). Additionally, high potential values increase dramatically the background currents. Finally, a potential range of 150 mV (from 0.00 to 0.15 V) and $v = 0.15$ V/s (one scan per second) were selected.

In the application of VD-MCR-ALS to the determination of compounds with very similar electrochemical responses, the selection of the potential range to be analyzed plays a key role. Then, it has been necessary to consider the part of the experimental **I** matrix that shows a more important change in the baseline, because in this zone the differences among the Faradaic responses of the compounds are more accused. However, the need to include this potential range on the treatment introduces more error in the mathematical solution, as lack of fit values show. But, if this part is avoided, convergence is not achieved or non realistic results are obtained. On the other hand, this fact would explain why in VD the concentrations required for a satisfactory quantitation were higher than in AD.

As mentioned, in order to check the possibilities of the VD-MCR-ALS approach, chromatographic conditions for uncompleted thiols separation were selected. This can be achieved by using the same conditions as in AD but modifying the concentration of ion pair compound (45 μM of OSA) in the mobile phase. Fig. 2b shows that by HPLC–ED in AD GSH was not completely separated from γ-Glu–Cys.

In order to apply VD-MCR-ALS, injections of the four standards and the synthetic mixture sample were done consecutively during the same chromatographic run, and voltammograms were recorded at any time of the elution (Fig. 3).

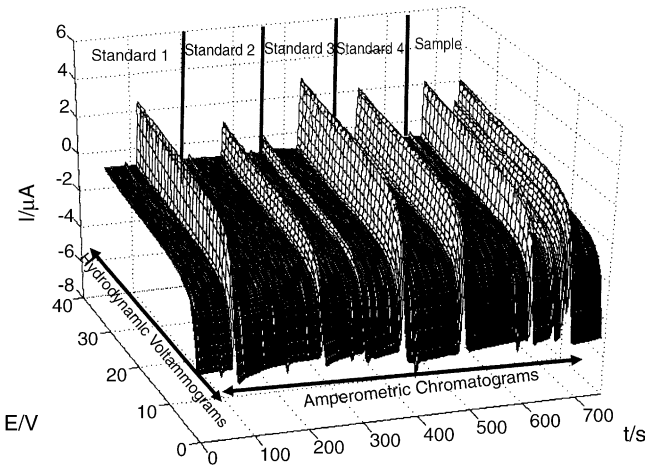

Fig. 3. Current measured as a function of potential and time in HPLC analysis (voltammetric detection). Injections of four standards and mixture were done consecutively during the same chromatographic run. Standard 1 corresponding to Cys standard of 34.65 ng/μL, standard 2 to Cys–Gly (44.91 ng/μL), standard 3 to GSH (87.90 ng/μL) and standard 4 to γ-Glu–Cys (74.59 ng/μL) and the mixture (sample) which contains the 4 thiols under peak coelution conditions, cysteine with a concentration of 27.75 ng/μL, Cys–Gly with 36.00 ng/μL, GSH with 70.38 ng/μL and γ-Glu–Cys with a 59.57 ng/μL concentration. The composition of the mobile phase was: 93.25% (v/v) 0.1 M MCA aqueous solution, 5% MeOH, 1.75% DMF and 45 μM OSA adjusted to a final pH of 2.8.

In previous experiments (not shown) standards and mixtures were injected separately, but differences among background baseline contributions of the different chromatographic runs compelled accurate quantitative determinations of the analyte components in the mixtures. VD-MCR-ALS simultaneous analysis of these consecutively injected samples using the column-wise augmented data matrix were done following the procedure summarized in Fig. 1. In this figure, the experimental data matrix and the calculated matrices (results) are associated to every main step in MCR-ALS.

Fig. 4 shows a set of experimental data and the corresponding results obtained from the application of this methodology to the determination of a mixture of Cys, GSH, and its fragments Cys–Gly and γ-Glu–Cys. Thus, the augmented data matrix (sample plus standards) is shown as a function of time (Fig. 4a) and of potential (Fig. 4b). In other words, Fig. 4a shows the column view of the experimental augmented **I** matrix that is constituted by the chromatograms with amperometric detection at different potentials for the four standards and the sample. Complementarily, Fig. 4b shows the row view of the same experimental matrix **I** that corresponds to the hydrodynamic voltammograms, i.e., the I versus E plots at different elution times. After the MCR-ALS treatment of these data according to the scheme shown in Fig. 1, what we call concentrations profiles (Fig. 4c), by analogy to the usual nomenclature in MCR-ALS [31,33], are obtained (see also Fig. 1). Simultaneously, the normalized unitary pure voltammograms are also reached (Fig. 4d and e). In this particular case the lack of fit is of 12.7% that from the mathematical point of view is quite satisfactory. It must be mentioned that

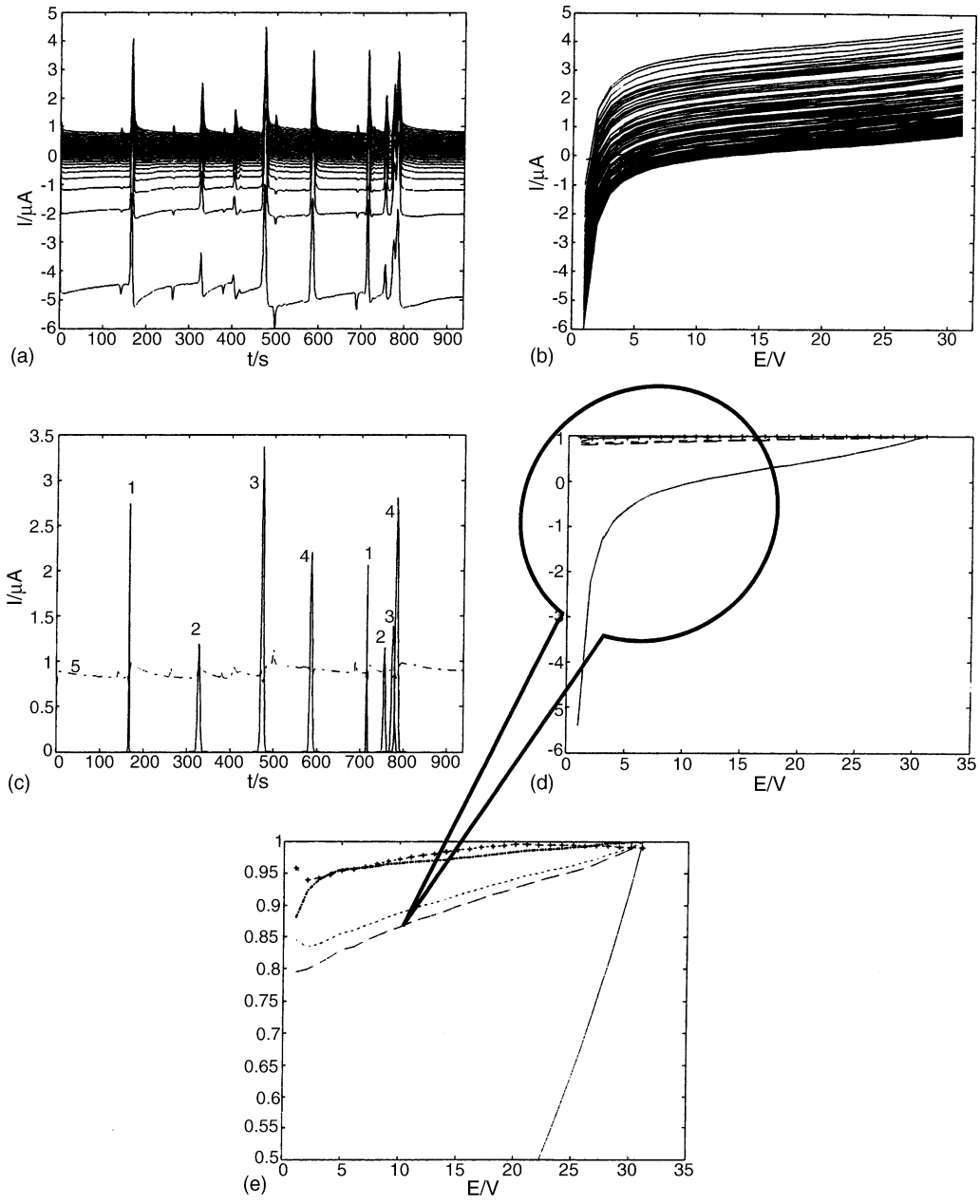

Fig. 4. Application of the VD-MCR-ALS approach to the analysis of Cys, GSH, Cys–Gly and γ-Glu–Cys to the data shown in Fig. 3. MCR-ALS constraints were selectivity, non-negativity, unimodality and Gaussian peak shape. The augmented data matrix (Fig. 3) is shown as function of time at different potentials (a) and as function of potential at different eluting time (b). MCR-ALS decomposition produces the concentration profiles (c) and the normalized unitary voltammograms (d and e).

Table 2
Determination of a mixture of thiol compounds by HPLC–ED and the VD-MCR-ALS approach

	$A_{standard}$[a]	$C_{standard}$[b]	A_{sample}[a]	$C_{sample\ (real)}$[b]	$C_{sample\ (determined)}$[b]	Percentage error
Cys	4.89	290.94	3.67	232.75	218.35	6.2
Cys–Gly	8.26	575.20	6.41	460.16	446.37	3.0
GSH	22.00	272.98	19.00	218.38	235.75	7.9
γ-Glu–Cys	13.90	683.18	10.30	546.54	506.24	7.4

$C_{sample(real)}$ and $C_{sample(determined)}$ are the real concentrations of the different components of the sample and the concentrations found after the application of HPLC–ED and VD-MCR-ALS analysis respectively.

[a] *A*: area of the peaks in nA min.

[b] *C*: concentrations in ng injected.

this solution has been reached assuming five mathematical components (from analysis by SVD; see Fig. 1). Four components correspond to the four analytes (Cys, GSH, Cys–Gly and γ-Glu–Cys), while the background current is modeled as the fifth component. Without this last assumption very poor mathematical resolutions are reached. The concentration profiles (Fig. 4c) are the basis for the quantitative analysis that is done by using the areas under the peaks of each analyte in the sample and in the corresponding standards. Table 2 shows the results obtained by the VD-MCR-ALS approach here proposed.

4. Conclusions

Experiments with synthetic thiol mixtures suggest that HPLC–ED, with AD mode, method [17] could be applied to the analysis of natural PC samples.

On the other hand, the proposed VD-MCR-ALS approach appears as a promising feature in the HPLC analysis of cases where satisfactory separation of electroactive compounds is not allowed. Furthermore, VD-MCR-ALS can provide a fast way to optimize the potential to be used in AD mode since a full set of hydrodynamic voltammograms can be obtained easily and quickly with a single sample injection.

Main advantage of VD-MCR-ALS approach lies on the selectivity increasing, while main drawback on the higher concentrations required for good quantifications (with respect to AD). This appears to be due to the much larger background contribution in VD because the fast potential change along the multiple scans induces greater perturbations in the electric double layer at the electrode surface than in the case of AD.

Acknowledgements

The authors gratefully acknowledge financial support from the Spanish Ministerio de Ciencia y Tecnología (project BQU2003-01525) and from the Generalitat of Catalonia (project 2001SGR-00056). O.G.-G. thanks the Spanish Ministerio de Ciencia y Tecnología for a Ph.D. grant.

References

[1] A. Meister, M.E. Anderson, Annu. Rev. Biochem. 52 (1983) 711.
[2] J. Viña, Glutathione: Metabolism and Physiological Functions, CRC Press, Boca Raton, FL, 1990.
[3] M.J. Stillman, C.F. Shaw III, K.T. Suzuki, Metallothionein, Synthesis, Structure and Properties of Metallothioneins, Phytochelatins and Metal–Thiolate complexes, VCH, New York, 1992.
[4] D.J. Reed, J.R. Babson, P.W. Beatty, A.E. Brodie, W.W. Ellis, D.W. Potter, Anal. Biochem. 106 (1980) 55.
[5] R.A. Winters, J. Zukowski, N. Ercal, R.H. Matthews, D.R. Spitz, Anal. Biochem. 227 (1995) 14.
[6] A.M. Svardal, M.A. Mansoor, P.M. Veland, Anal. Biochem. 184 (1990) 338.
[7] L.A. Allison, R.E. Shoup, Anal. Chem. 55 (1983) 8.
[8] S.M. Lunte, P.T. Kissinger, J. Liq. Chromatogr. 8 (1985) 691.
[9] C.B. Jensen, S.J. Grossman, D.J. Jollow, Adv. Exp. Med. Biol. 197 (1986) 407.
[10] A.F. Stein, R.L. Dills, C.D. Klaassen, J. Chromatogr. 381 (1986) 259.
[11] J.P. Richie Jr., C.A. Lang, Anal. Biochem. 163 (1987) 9.
[12] K. Shimada, T. Oe, T. Nambara, J. Chromatogr. 419 (1987) 17.
[13] E.G. DeMaster, B. Redfern, Methods Enzymol. 143 (1987) 110.
[14] C.T. Garvie, K.M. Straub, R.K. Lynn, J. Chromatogr. 413 (1987) 43.
[15] R.E. Shoup, Curr. Sep. 8 (1987) 38.
[16] D.L. Rabenstein, G.T. Yamashita, Anal. Biochem. 180 (1989) 259.
[17] Y. Kuninori, J. Nishiyama, Anal. Biochem. 197 (1991) 19.
[18] Y. Sun, D.L. Smith, R.E. Shoup, Anal. Biochem. 197 (1991) 69.
[19] E.P. Gallagher, T.J. Kavanagh, D.L. Eaton, Methods Tox. 1B (1994) 349.
[20] W.A. Kleinman, J.P. Richie Jr., J. Chromatogr. B 672 (1995) 73.
[21] G.S. Owens, W.R. LaCourse, Curr. Sep. 14 (1996) 82.
[22] S. Melnyk, M. Pogribna, I. Pogribna, R.J. Hine, S.J. James, J. Nutr. Biochem. 10 (1999) 490.
[23] W.A. Kleinman, J.P. Richie Jr., Biochem. Pharmacol. 60 (2000) 19.
[24] S.M. Lunte, T.J. O'Shea, Electrophoresis 15 (1994) 79.
[25] G.S. Owens, W.R. LaCourse, J. Chromatogr. B 695 (1997) 15.
[26] M. Zhong, S.M. Lunte, Anal. Chem. 71 (1999) 251.
[27] S.D. Brown, R.S. Bear Jr., Crit. Rev. Anal. Chem. 24 (1993) 99.
[28] A. Kotani, Y. Hayashi, R. Matsuda, F. Kusu, J. Chromatogr. A 986 (2003) 239;
A. Kotani, Y. Hayashi, R. Matsuda, F. Kusu, Anal. Sci. 19 (2003) 865.
[29] T.A. Nguyen, S. Kokot, D.M. Ongarato, G.G. Wallace, Electroanalysis 11 (1999) 1327.
[30] W.J. Bachman, J.T. Stewart, J. Chromatogr. 481 (1989) 121.
[31] F. Berbel, E. Kapoya, J.M. Díaz-Cruz, C. Ariño, M. Esteban, R. Tauler, Electroanalysis 15 (2003) 499.
[32] J.M. Séquaris, in: M.R. Smith, J.G. Vos (Eds.), Comprehensive Analytical Chemistry: Analytical Voltammetry, vol. 27, Elsevier, Amsterdam, 1992.
[33] M. Esteban, C. Ariño, J.M. Díaz-Cruz, M.S. Díaz-Cruz, R. Tauler, Trends Anal. Chem. 19 (2000) 49.
[34] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and Applications, second ed., Wiley, New York, 2002.
[35] Matlab's User Guide, Mathworks, Cochituate Place, MA, 1990.