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