Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 967–974
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Voltammetric and electrochemical quartz crystal microbalance studies of sulforaphane and its Zn(II) complexes

Zbigniew Fijałek [a,c,*], Katarzyna Sarna [a], Teresa Kasprzycka-Guttman [a,b]

[a] *Department of Pharmaceutical Chemistry, National Institute of Public Health, 30/34 Chełmska Str., 00-725 Warsaw, Poland*
[b] *Faculty of Chemistry, Warsaw University, 1 Pasteura Str., Warsaw, Poland*
[c] *Department of Drug Analysis, Warsaw Medical University, 1 Banacha Str., 02-097 Warsaw, Poland*

Received 24 April 2002; received in revised form 28 February 2003; accepted 3 March 2003

Abstract

Cyclic voltammetry (CV) and electrochemical quartz crystal microbalance (EQCM) methods were used to study the behavior of sulforaphane (SFR). To established electrochemical properties of SFR methyl-, ethyl-, 2-chloroethyl-, propyl-, butyl- and tert-butyl-isothiocyanates were examined at gold electrode. Optimal measurement parameters were established and stock solutions developed. The electrode activity of the studied compounds (cathodic peak $E_p = -0.6$ V) was found to be primarily due to the oxidation of the isothiocyanate group. By the EQCM method it was found that during the reduction of SFR the mass of the electrode systematically increased in successive scans by ca. 120 ng per scan and in the mixture of SFR and Zn^{2+} ions ca. 350 ng. Formation of SFR complexes with Zn^{2+} ions was confirmed by EQCM and UV methods. Cyclic voltammetic method has been elaborated for SFR determination and compared with UV method.

Keywords: Sulforaphane; Isothiocyanates; Cyclic voltammetry; Electrochemical quartz crystal microbalance; UV spectrophotometry; Determination; Zn(II) complexes

1. Introduction

Sulforaphane (1-isothiocyanato-4-(methylsulfinyl)butane, SFR) belongs to the group of compounds generally named isothiocyanates. These compounds, also called charlock essentials, occur naturally in plants as glycoside combinations of sulfur formed as a result of their enzymatic hydrolysis. The richest source of isothiocyanates is the plant from Cruciferae family, in which more than 20 species are occurring. The largest quantities of isothiocyanate occur in water-cress, broccolis, cucurbits and cauliflowers. Charlock essentials liberated from glycoside combinations demonstrate a strong irritating action on mucous membranes and skin. All of them have more or less strongly marked bacteriostatic, fungistatic and antioxidative properties. Some of these combinations act on gram-positive and gram-negative bacteria more strongly than the known antibiotics.

* Corresponding author. Fax: +48-22-841-0652.
E-mail address: z.e.fijalek@il.waw.pl (Z. Fijałek).

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(03)00198-5

Growing interest on therapeutic properties of isothiocyanates in various kinds of infections is recently observed. For the first time SFR was isolated from broccoli sprouts which are particularly rich in this substance. Synthesis of SFR has been performed in the forties [1] and next by Schenk et al. [2] in the 19th of the last century.

Isothiocyanates have been determined by UV [3–7] and IR spectroscopy [3], colorimetry [8], potentiometry [9] and by gas chromatographic [10] methods. However, in the literature only one information can be found on analytical studies of SFR itself. Chiang et al. applied gas chromatography coupled with mass spectrometry to determine SFR in natural extracts [11]. Much more reports concerned biological and pharmacological studies of SFR and its derivatives can be found. For example, the influence of the compounds isolated from broccolis on the course of enzymatic induction [12].

In the Department of Chemical Technology of Warsaw University studies on the elaboration of a new, efficient method of synthesis of SFR, determination of intermediates and effective purification of the final product in order to prepare a standard substance were started. The project forecasted also the development of new, sensitive analytical methods enabling identification and determination of SFR and selected isothiocyanates. An attempt was undertaken to develop suitable electrochemical methods, which would allow to solve some problems and to compare oxidation–reduction properties of various isothiocyanates. One of the main problems to be solved was the stability determination of SFR solutions.

Many organic compounds containing in the molecule sulfhydryl, thiocarbonyl or isothiocyanates group forms complexes with metal ions. Pharmacological mechanism of their action is connected precisely with the formation of zinc complexes. In cyclic voltammetry (CV) and electrochemical quartz crystal microbalance (EQCM) studies we have revealed the formation of complexes of thiosalicylic and dithiodibenzoic acids with Zn ions [13], therefore, it was decided to check whether SFR forms such complexes.

2. Experimental

2.1. Apparatus

A UV–VIS spectrometer, type U-2000 (Hitachi) with quartz cuvettes Hellma (Optic GmbH, Jena) of 10 mm path length, a microcomputer-controlled μ-Autolab (Eco Chemie, Utrecht) voltammeter equipped with a General Purpose Electrochemical System software (GPES, Version 4.8) and an (EQCM, type M-105, Uelko, Warsaw) were used. In voltammetric studies disk electrodes were used (Bioanalytical Systems, West Lafayette) gold electrode ($A = 0.02$ cm^2). The saturated calomel electrode (SCE) or Ag/AgCl electrode was used as the reference electrode; a spirally wound Pt wire was used as a third electrode.

2.2. Materials

SFR (98%) was synthesized in the Laboratory of Physicochemical Principles of Chemical Technology of Warsaw University and purified by double distillation under a pressure of 0.01 mmHg. The distillate was collected at a temperature of 130–135 °C. The structure of the compound was confirmed by NMR, MS and elemental analysis (results of these studies will be published separately). The obtained SFR is an oily substance, soluble in methanol, acetonitrile, methylene chloride, water and ethanol. It is optically active, its specific rotation is: $[a]^{22} = -79.3^\circ$ ($c = 1.233$; $CHCl_3$); $[a]^{25} = -78.6^\circ$ ($c = 1.19$; $CHCl_3$). The IR spectrum exhibits a strong absorption at 2179, 2108 and also at 1350 cm^{-1}. The last value is characteristic for the NCS group. The UV spectrum of SFR aqueous solution is characterized by a maximum absorption at a wavelength of 238 nm.

Methyl-isothiocyanate (97%), ethyl-isothiocyanate (97%), 2-dichloroethyl-isothiocyanate (98%), propyl-isothiocyanate (98%), butyl-isothiocyanate (99%) and tert-butyl-isothiocyanate were from Aldrich. The remaining reagents were suprapure quality from Merck. Water used in experiments was double-distilled and additionally repurified in a Nanopure Deionization System (Barnstead).

2.3. Procedures

2.3.1. Spectrophotometric studies

Aqueous and methanolic solutions of 40–200 μg cm^{-3} SFR were prepared. Then absorbance of the particular solutions was measured at a maximum of 238 nm. In 100 ml volumetric flasks aqueous solutions of ca. 72 μg cm^{-3} SFR were prepared. The solutions were kept in various conditions: at 20 °C in daylight, at 20 and at 6 °C in darkness. Absorbance of the particular solutions was measured after 0, 1, 2, 3, 4, 6, 7, 8 and 12 days at a wavelength of 238 nm.

Ten of each aqueous and methanolic solutions of 148 and 175 μg cm^{-3} SFR were prepared. Then absorbance of the particular solutions was measured at 238 nm. Statistical analysis of the obtained results is presented in Table 1.

2.3.2. Voltammetric studies

Measuring solutions were prepared in 10 ml volumetric flasks and aliquots were transferred to measuring cells. Before each measurement the disk electrodes were polished with a 0.05 μm alumina (Union Carbide) on a Buehler's polishing cloth and then washed with water and with aqueous 95% ethanol. Argon (99.995% pure) was bubbled for 10 min to deaerate the solutions. Experimental temperature was ca. 20 °C.

Using CV the electrochemical properties of SFR and methyl-, ethyl-, 2-chloroethyl-, propyl-, butyl- and tert-butyl-isothiocyanates were studied at gold electrode (AuE) in various buffered and non-buffered solutions in the pH range 1–10. To 10 ml volumetric flasks 1.0 cm^{-3} of ethanolic solution of 0.01 mol dm^{-3} isothiocyanates and 1.0 cm^{-3} of anhydrous ethanol (99.8%) v/v were added, and then variable quantities of supporting electrolytes: 0.1 mol dm^{-3} H_2SO_4; 1.0 mol dm^{-3} CH_3COOH and 1.0 mol dm^{-3} CH_3COONa; 0.5 mol dm^{-3} Na_2HPO_4 and 0.5 mol dm^{-3} Na_2HPO_4 and 0.1 mol dm^{-3} NaOH. The solutions were made up to 10 cm^{-3} with water and cyclic voltammograms were recorded at a scan rate of 50 mV s^{-1} (Fig. 1).

Then the relation between the current intensity and the content of ethanol in the investigated solution was followed. To 10 ml volumetric flasks 0.5 ml of 0.01 mol dm^{-3} SFR and 1 cm^{-3} of 1.0 mol dm^{-3} H_2SO_4 were added and next variable quantities of anhydrous ethanol (99.8%) v/v (10, 20, 30, 40 and 60%). The flasks were made up to volume with water and cyclic voltammograms were recorded at scan rate 50 mV s^{-1}. As the best supporting electrolyte 0.1 mol dm^{-3} acetate buffer pH 4.7 containing 10% v/v of ethanol was chosen.

By the CV method the relation between the isothiocyanates limiting current intensities and

Table 1
Quantitative parameters for SFR determination by UV spectrophotometry (aqueous solution) and cyclic voltammetry (0.1 mol dm^{-3} H_2SO_4) methods

Parameter	Method	
	UV	CV
Concentration range (μg cm^{-3})	40–200	(1) 8–35 (2) 35–180
$Y = aX + b$	$0.0052X + 0.2133$	(1) $-0.1012X - 0.3132$ (2) $-0.0238X - 0.5078$
Correlation coefficient	0.9998	(1) 0.9985 (2) 0.9968
Standard error of the slope	0.001	(1) 0.001 (2) 0.002
Standard error of the intercept	0.001	(1) 0.002 (2) 0.002
Detection limit (μg cm^{-3})	14.0	3.0
Quantitation limit (μg cm^{-3})	44.5	10.5
Between-day R.S.D. (%)	0.85	2.10
Within-day R.S.D. (%)	0.60	1.60
Precision of determination, mean $\pm tS/\sqrt{N}$ (taken 100 μg cm^{-3}, $N = 10$)	100.34 ± 0.50	99.43 ± 1.31

peak potentials (Table 2), the scan rate (10, 50, 200, 1000 and 3000 mV s^{-1}) and SFR concentration (concentration range: 0.5×10^{-5}–1×10^{-3} mol dm^{-3}) were studied at a gold electrode.

Then solutions of $CuSO_4$ with isothiocyanates (in different molar concentration ratio) in 0.1 mol dm^{-3} acetate buffer (10% of ethanol) were prepared and cyclic voltammograms were recorded at gold electrode (scan rate 50 mV s^{-1}). The above experiments were repeated using solution of 5×10^{-4} mol dm^{-3} $ZnCl_2$ instead of $CuSO_4$. For SFR voltamperometric curves were recorded also at the scan rates 10, 200, 1000 and 3000 mV s^{-1}.

Complexes of SFR with Zn^{2+} ions in various buffer solutions in the pH range 2.7–7.8 were studied by CV. To electrochemical cells containing solution of 5×10^{-4} mol dm^{-3} SFR, 1×10^{-3} mol dm^{-3} $ZnCl_2$ variable quantities of 1 mol dm^{-3} CH_3COOH and CH_3COONa solutions were added. The cells were completed to 5 ml with water and cyclic voltammograms were recorded at gold electrode with 50 mV s^{-1} scan rate. Then formation of the SFR complexes as a function of the molar concentration ratio of SFR to Zn^{2+} ions in the solution were investigated (Table 2, Fig. 2).

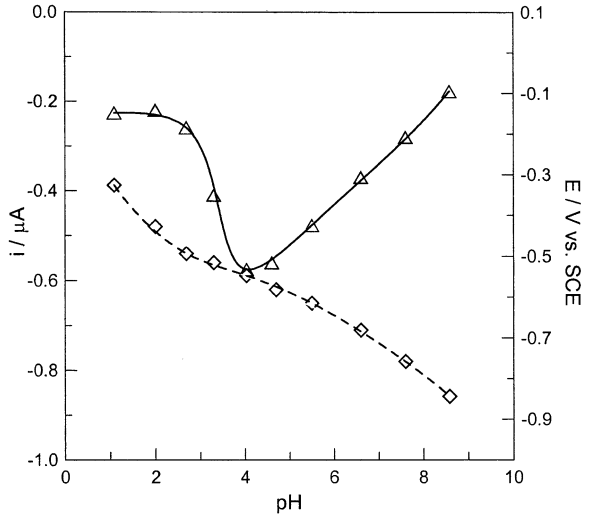

Fig. 1. The limiting current intensities (solid line) and anodic peak potential (dotted line) in relation to pH of the solution, obtained in 5×10^{-4} mol dm^{-3} SFR solution (20% of ethanol) at gold electrode. Scan rate 50 mV s^{-1}.

Table 2
Electrochemical characteristic of isothiocyanates and Zn(II) complexes (in molar concentrations ratio) obtained at gold electrode

Isothiocyanates	Ratio	Anodic peaks				Cathodic peaks	
		i_{pa} (μA)	E_{pa} (V)	i_{pa} (μA)	E_{pa} (V)	i_{pc} (μA)	E_{pc} (V)
Methyl	–	0.212	−0.508	0.715	0.610	−1.265	−0.662
	1:3	0.321	−0.237	–	–	−0.926	−0.490
Ethyl	–	0.287	−0.369	0.682	0.624	−0.509	−0.652
	1:3	0.319	−0.229	–	–	−0.718	−0.229
2-Chloroethyl	–	0.367	−0.267	0.687	0.624	−0.963	−0.631
	1:3	0.197	−0.117	–	–	−0.566	−0.454
Propyl	–	0.135	−0.185	0.849	0.624	−0.359	−0.628
	1:3	0.189	−0.249	–	–	−0.593	−0.562
Butyl	–	0.306	−0.434	–	–	−0.583	−0.626
	1:3	0.261	−0.225	–	–	−0.486	−0.458
Tert-butyl	–	0.096	−0.194	0.236	0.449	–	–
4-(Methylsulfinyl)-butane (SFR)	–	0.352	−0.305	0.615	0.581	−1.208	−0.630
	1:1	0.671	−0.031	0.740	0.096	−1.555	−0.470
	1:3	1.194	−0.052	0.857	0.069	−1.735	−0.439
	1:5	0.956	−0.071	0.473	0.072	−1.484	−0.426
	2:1	0.637	−0.063	0.590	0.104	−1.412	−0.462
	5:1	0.232	−0.112	0.081	0.169	−0.717	−0.485

Scan rate 50 mV s^{-1}.

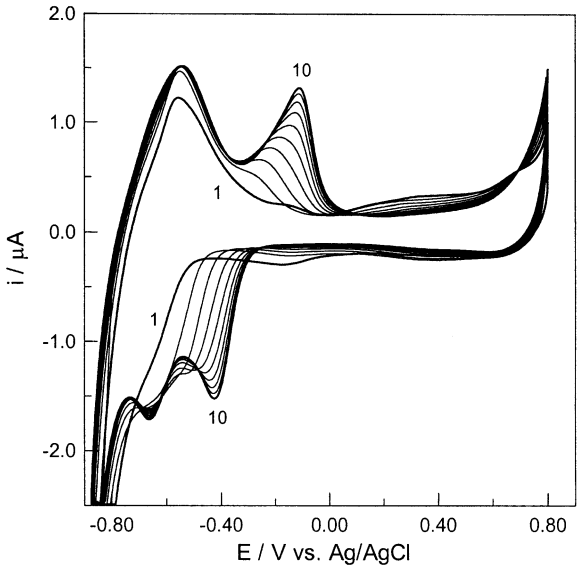

Fig. 2. Cyclic voltammograms of 2.4×10^{-4} mol dm^{-3} SFR and 8×10^{-5} mol dm^{-3} $ZnCl_2$ solutions obtained at gold electrode in 0.1 mol dm^{-3} acetate buffer pH 4.7 (20% of ethanol). Scan rate 50 mV s^{-1}. Ten scans.

2.3.3. Electrochemical quartz crystal microbalance studies

In the EQCM procedure, the International Crystal Mfg. Co. (Oklahoma) AT-cut 10 MHz quartz crystals with gold electrodes sprayed ca. 90 nm thick on either side, each 0.25 cm^2 in active surface area, served as the working electrode. The mass sensitivity of the gold electrodes was 0.226 Hz cm^2 ng^{-1}, or 0.9 Hz ng^{-1} per unit active surface area. The electrolytic cells were cup-shaped with the bottom of the cup replaced by a quartz crystal cemented with a silicone rubber adhesive (General Electric). During the voltammetric measurements, the solution-facing side functioned as the working electrode, and the quartz crystal was incorporated into the microbalance generator circuit. The signal produced in this generator was delivered to the central processor of the microbalance with an accuracy of 0.1 Hz. This unit, controlled by the microprocessor, received information from the generator and displayed the difference between the initial frequency of 10 MHz and the actual frequency varying during the electrochemical measurement. The central unit received also two analog signals from the potentiostat which, after having been digitalized, were delivered together with the information about frequency changes collected at 50 ms time intervals through a standard interface RS232 to a PC computer. The EQCM measurements and data analyses were carried out with the aid of the GPES software.

Using the EQCM method current-potential and mass-potential characteristics of 1×10^{-3} mol dm^{-3} isothiocyanates solution in 0.1 mol dm^{-3} acetate buffer of pH 4.7 (10% of ethanol) and in SFR solutions with Zn^{2+} ions at the different molar concentration ratio ($ZnCl_2$:SFR = 1:1, 1:2, 1:3, 1:4, 1:5, 1:10 and 2:1) was studied at the EQCM gold electrode at 5, 10 and 50 mV s^{-1} scan rates (Fig. 3). From the chosen solutions also chronovoltamperometric experiments during potential changes (+0.60 and −0.45 V) were performed (Fig. 4).

3. Results and discussion

The spectrophotometric experiments with UV detection has shown one maximum at a wave-

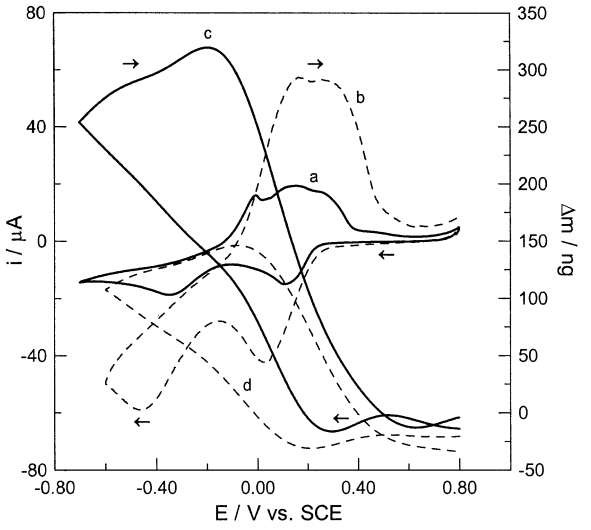

Fig. 3. Current–potential and mass–potential characteristics of 2.4×10^{-3} mol dm^{-3} SFR with 8×10^{-4} mol dm^{-3} $ZnCl_2$ solution obtained in 0.1 mol dm^{-3} acetate buffer pH 4.7 (20% of ethanol) at EQCM gold electrode. Scan rate 10 (solid line) and 50 mV s^{-1} (dotted line).

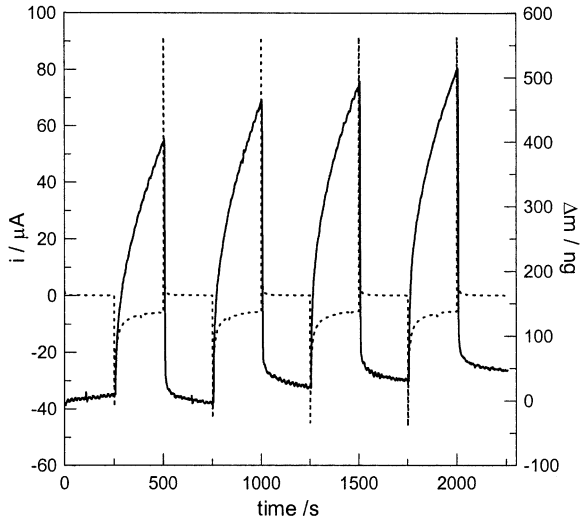

Fig. 4. Chronovoltammograms (current–potential dotted line and mass–potential solid line) of 2.4×10^{-3} mol dm^{-3} SFR with 8×10^{-4} mol dm^{-3} $ZnCl_2$ solution obtained during potential changes (+0.60 and −0.45 V) at EQCM gold electrode in 0.1 mol dm^{-3} acetate buffer pH 4.7 (20% of ethanol).

length of $\lambda = 238$ nm in SFR solution. In the temperature 20 °C SFR underwent hydrolysis more quickly when kept in the day light than in the darkness. During the first 72 h the absorbance of solutions changed almost linearly (in the daylight $Y = 0.0057X + 0.5832$, $R = 0.9823$; in the darkness $Y = 0.0037X + 0.5788$, $R = 0.9952$). On the other hand, the solutions kept at 6 °C in the darkness did not show any changes in the absorbance during 12 days. Examination of the relation between the absorbance and concentration of aqueous and methanolic SFR solutions revealed a linear dependence in the concentration range from 40 to 200 μg cm^{-3} (Table 1).

The CV method was used to study peak current intensities and peak potentials in relation to pH of the solution, to scan rate and to the concentration of the analyte in various buffered and non-buffered solutions over the pH range from 1 to 10 at gold electrode. In SFR solutions at the gold electrode the limiting current intensities and the anodic peak potentials varied as the pH of the solution was raised (ca. 63 mV per pH) and the best-shaped voltammograms were recorded in 0.1 mol dm^{-3} H_2SO_4, in which one cathodic peak ($E_p = -0.40$ V) and two poorly shaped anodic peaks ($E_p = +0.11$ and $+0.58$ V) occurred and 0.1 mol dm^{-3} acetate buffer at pH 4.7 (10% v/v of ethanol). Studies of the cathodic peak limiting current intensities on the SFR concentration on the gold electrode has shown a linear dependence in the two concentration ranges from 8 to 35 and from 35 to 180 μg cm^{-3}. For the analytical purposes 0.1 mol dm^{-3} H_2SO_4 was selected and a cyclic voltammetric method for the determination of SFR in the substance has been elaborated. Statistical estimation of the obtained results (Table 1) has demonstrated that determinations could be performed with a good accuracy (1.60% R.S.D.) and precision.

For the selectivity determination methyl-, ethyl-, 2-chloroethyl-, propyl-, butyl- and tert-butyl-isothiocyanates were studied by the cyclic voltammetry method at gold disk electrode. The dependence of peak current intensities and peak potentials in relation to pH of the solution, to scan rate and to the concentration of the analyte in various buffered and non-buffered solutions over the pH range from 1 to 10 at gold electrode were studied. It was found that for all the investigated isothiocyanates and SFR only a cathodic peak at a potential of about −0.6 V is being repeated. The highest value of the cathodic current intensity was observed for methyl-isothiocyanate ($i_p = -1.295$ μA) and the lowest for propyl-isothiocyanate ($i_p = -0.359$ μA); for SFR $i_p = -1.208$ μA. On the other hand, the position of anodic peaks of the studied isothiocyanates was already not as close as the cathodic peaks. The specific for isothiocyanates and SFR reduction process occurring at ca. $E_p = -0.6$ V corresponds to the anodic oxidation at a potential of about $E_p = 0.6$ V. A significant increase in the values of recorded currents with increasing polarization rate of the electrode was observed.

The pharmacological activity of compounds containing in the molecule thiocarbonyl or sulfhydryl group is connected, among others, with the formation of active compexes with metals ions (first of all with zinc ions). In the literature there are reports concerning studies of such complexes with Cu(II) [14,15], Zn(II) [16] or Pd(II) ions [17]. Captopril belongs to a group of drugs lowering

arterial blood pressure and diminishing peripheral vascular resistance. It is the first specifically competitive inhibitor of the enzyme that catalyzes a transformation reaction of angiotensine I into angiotensine II, designed for oral applications. Its mechanism of action involves binding the enzyme in its five active sites. In particular, the sulphydryl group binds zinc ion, present in the enzyme. In our earlier studies [13,18], we have proven that the formed equimolar complexes of thiosalicylic acid with Cu(II) increase almost twice the sensitivity of voltammetric determinations. To carry on these studies, an attempt was undertaken to recognize electrochemical properties of SFR complexes with Cu(II) and Zn(II) ions. It was found that SFR forms such complexes only with Zn(II) ions at various molar ratios depending on the pH of the solution. The best for the complex formation was pH 4.7. In such solution Zn(II) ions were reduced at the gold EQCM electrode at a potential of −0.66 V, and in anodic cycle a badly shaped peak were appeared at −0.55 V. In the presence of SFR, during successive scans (from second to tenth) two additional peaks appeared, a cathodic at −0.44 V and an anodic at −0.11 V. The current intensities of these peaks were the highest at 1:3 molar concentrations ratio (Zn(II) and SFR).

The EQCM method is based on a linear proportionality between a possible mass change of the quartz resonator electrode and the corresponding change of its resonance frequency and it can be used as a very sensitive, dynamic, piezoelectric sensor. It allows for the simultaneous in situ determination of both electrochemical parameters of oxidation and reduction of compounds and the effective mass of the electrode. With careful circuit design and signal averaging it is, therefore, possible to measure very small mass changes in the low nanogram range. While applying this method, a completely different current–potential and mass–potential characteristics for SFR alone and for mixture with Zn(II) ions was observed. During the reduction of SFR, the electrode mass increased in successive scans by ca. 120 ng per scan. These changes were persistent and pointing out the strong adsorption of the reaction products on the electrode surface. However, in the solutions containing mixture of SFR and Zn(II) ions the changes in the electrode mass were reversible, the adsorption of the products starting already at about +200 mV (maximally ca. 350 ng) and next underwent quick desorption. These properties were confirmed by chronovoltamperometric experiments (Fig. 4). In the latter studies it was found, that at potential of 0.00 V about 200 ng of the products was quickly (2 ng s^{-1}) adsorbed on the surface of gold electrode. On the other hand, after the potential jump to 0.45 V the electrode mass increased less considerably (ca. 0.7 ng s^{-1}).

During investigations of isothiocyanates and SFR solutions with copper ions it was found that the recorded voltammograms were not very characteristic. Their shape and course indicated that the studied compounds do not form complexes with Cu^{2+} ions.

Mixture of SFR and zinc ions were analyzed also by UV spectrophotometry. The spectra were recorded directly after preparation of the solution and up to 24 h. The obtained spectra indicate that the complex formation is a slow reaction and the amount of the complex increased with time (Fig. 5).

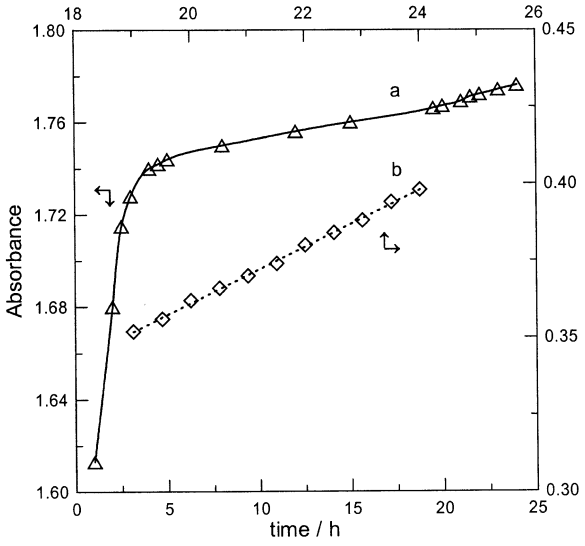

Fig. 5. The absorbance of 2.4×10^{-4} mol dm^{-3} SFR and 8×10^{-5} mol dm^{-3} $ZnCl_2$ solutions in relation to time, obtained in a maximum at $\lambda = 238$ nm (solid line) and $\lambda = 279$ nm (dotted line).

4. Conclusion

The novel voltammetric methods for SFR determination based on the oxidation of the isothiocyanate group (cathodic peak $E_p = -0.6$ V) has been developed. Statistical evaluation of the present results (Table 1) shows the procedures developed to enable SFR to be determined with a satisfactory accuracy (1.60% R.S.D.) and precision. Stability of the analyzed solutions was confirmed by UV spectrophotometry. Considering the satisfactory sensitivity, selectivity and rapidity as well as ease of performing determinations, the CV method with the use of gold electrode may be recommended to identification and determination of this compound.

EQCM studies demonstrate that oxidation products of suforaphane are adsorbed at gold electrode and it is possible to study changes in film formation during repetitive cyclic scans. In solutions containing mixture of SFR and Zn(II) ions complex formation was indicated by cyclic voltammetry, EQCM and chronovoltamperometric methods. This was confirmed by a gradually formed peak with an absorption maximum at $\lambda = 279$ nm. Thus the complex formation of SFR with Zn(II) ions was confirmed by two independent analytical methods.

References

[1] H. Schimid, P. Karrer, Helv. Chim. Acta 31 (1948) 1497–1505.
[2] W.A. Schenk, Chem. Eur. J. 3 (1997) 713–716.
[3] E. Svatek, R. Zahrodnik, A. Kjaer, Acta Chem. Scand. 13 (1959) 442–447.
[4] Y. Zhang, C.-G. Cho, G. Posner, P. Talalay, Anal. Biochem. 205 (1992) 100–107.
[5] Y. Zhang, K.L. Wada, T. Prestera, P. Talalay, Anal. Biochem. 239 (1996) 160–167.
[6] B.C. Verma, S. Chauhan, N. Sharma, U. Sharma, W.K. Sharma, A. Sood, Talanta 33 (1986) 703–704.
[7] A. Kjaer, J. Larsen, R. Gmelin, Acta Chem. Scand. 9 (1955) 1311–1316.
[8] Y.M. Chae, M.A. Tabatabai, Anal. Lett. 16 (1983) 1197–1206.
[9] S. Kumar, W.K. Sharma, A. Sud, Talanta 38 (1991) 217–221.
[10] P. Konieczka, E. Luback, J. Namieśnik, J. Biernat, Anal. Chim. Acta 265 (1992) 127–132.
[11] W. Chiang, W.J. Pusateri, R. Leitz, J. Agric. Food Chem. 46 (1998) 1018–1021.
[12] C. Huggins, L.C. Grand, F.P. Brillantes, Nature 189 (1961) 204–207.
[13] Z. Fijałek, K. Sarna, A. Piwońska, Anal. Lett. 33 (2000) 1293–1307.
[14] M.S. Abu-Bakr, Monatshefte Chem. 128 (1997) 563–571.
[15] E.G. Ferrer, P.A.M. Williams, Polyhedron 16 (1997) 3323–3328.
[16] G. Dalmata, J. Electroanal. Chem. 67 (1997) 431–438.
[17] Z. Koricanac, B. Stankovic, M. Dugandzic, L. Milovanovic, Acta Pharm. Jugosl. 27 (1997) 171–176.
[18] K. Sarna, Z. Fijałek, Chem. Anal. (Warsaw) 42 (1997) 863–872.