

## MACROCYCLIC POLYAMINE-MODIFIED GOLD ELECTRODES FOR AMPEROMETRIC DETECTION OF ACRYLIC ACID

Agnieszka Krajewska<sup>1</sup>, Izabela Grzybowska<sup>1</sup>, Jerzy Radecki<sup>1</sup>, Hanna Radecka<sup>1</sup>, Marek Pietraszkiewicz<sup>2</sup>,  
Oksana Pietraszkiewicz<sup>2</sup>

<sup>1</sup>Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn;

<sup>2</sup>Institute of Physical Chemistry of the Polish Academy of Sciences, Warsaw

Key words: acrylic acid, acrylamide, asparagine, polyamine, Osteryoung square wave voltammetry (OSWV)

The presence of highly toxic acrylamide in food products such as fried potatoes and chips was confirmed by Swedish scientists from the Stockholm University. Neurotoxicity of this compound and its metabolites imposes a duty to control it by qualitative and quantitative assays. Recent findings showed that acrylamide is formed in heat-treated foods rich in asparagine and reducing sugars such as glucose. Exposing acrylamide to pH extremes results in its hydrolysis to acrylic acid and ammonia.

The main objective of the work presented is the development of a new electrochemical sensor for the determination of acrylic acid in the presence of asparagine and acrylamide. We report on an intramolecular ion-channel sensor using self-assembled monolayers deposited onto gold electrodes. Macrocylic polyamine molecules with long alkyl chains were adsorbed into the monolayer of 1-dodecanethiol on the gold surface.

The signal generated due to the formation of a supramolecular complex between host and acrylic acid guest at the electrode interface was measured by Osteryoung square wave voltammetry (OSWV) with  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  as an electroactive marker.

### INTRODUCTION

The control of food quality and freshness is of growing interest for both consumer and food industry and because of that faster, more powerful, cleaner, and cheaper analytical procedures are required by food chemists. These analytical techniques must address an important number of problems providing information about food processing quality control, ensuring compliance with food and food legislation, contamination, and chemical composition of foods. In 2002 the Swedish National Food Authority reported the finding of elevated levels of acrylamide in heat-treated potato products and other baked goods [Tareke *et al.*, 2002]. Following this discovery, a world-wide monitoring of this substance in various food products was started immediately.

Generally, an analysis of acrylamide in food products focused primarily on chromatographic methods such as LC-MS or LC-MS/MS [Ono *et al.*, 2003; Rosén *et al.*, 2002] and GC-MS [Castle & Eriksson, 2005]. All of them are rather expensive and time consuming, especially sample preparation is for most of them complex.

A number of approaches to chemical sensing have been reported by mimicking with artificial receptors impressive selectivity and sensitivity of ligand gated ion-channel receptor protein [Aoki *et al.*, 1999, 2003; Aoki & Umezawa, 2003]. Because the operating principles of these sensors are similar to that of ligand gated ion-channel proteins in biomembranes, they are referred to as "ion-channel" or "ion-channel-mimetic" sensors. These types

of electrochemical sensors have been introduced by Umezawa and coworkers in 1987 [Sugawara *et al.*, 1987], and of which essence was recently reviewed again [Umezawa & Aoki, 2004].

Binding of analytes to receptors immobilized on electrodes facilitates or suppresses the access of an anionic (cationic) marker ion to the monolayer surface due to electrostatic attraction or repulsion of marker ions and/or distortion of self-assembled monolayer (SAM) arrangement.

In the present paper, we report on the use of macrocyclic polyamines (Figure 1b) for chemical modification of gold electrodes to prepare an ion-channel-mimetic sensor for detection of acrylic acid in food samples. Our previous investigations showed that this ionophore interacts with  $\alpha,\beta$ -unsaturated dicarboxylic acids such as maleic and fumaric acid and adenine nucleotides [Radecka *et al.*, 2005; Radecki *et al.*, 2006]. The recognition process between the ligand and analyte in this case was realized *via* the creation of hydrogen bonds and electrostatic interaction. Modification of the electrode was done by means of the embedment methods. The scheme of modification and structure of the investigated macrocyclic amine are introduced in Figure 1.

### MATERIALS AND METHODS

#### Reagents

The macrocyclic polyamine has been synthesized in the Institute of Physical Chemistry of the Polish Academy of Sci-

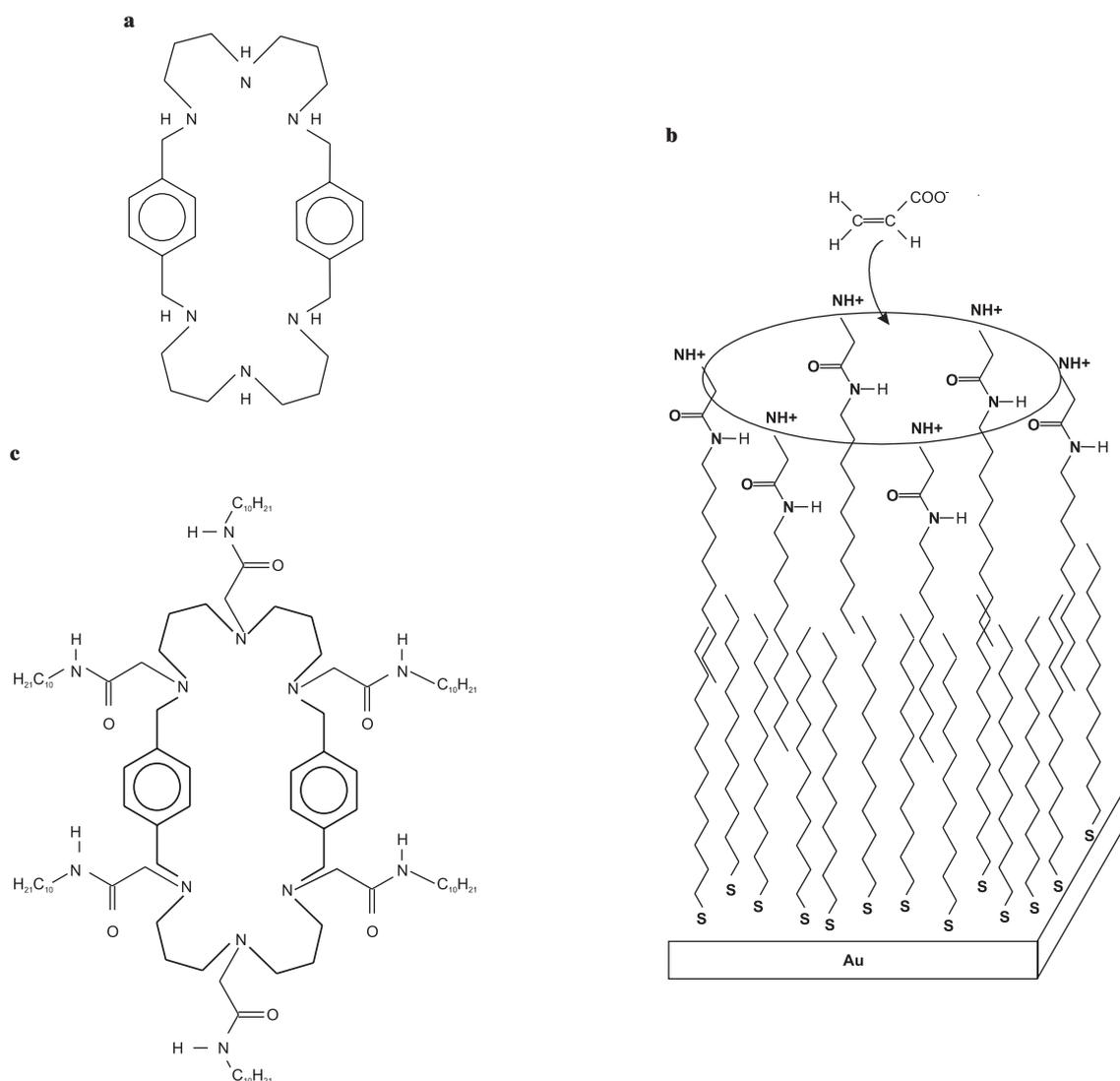


FIGURE 1. Structural formula of macrocyclic polyamine used for spectroscopic (a) and amperometric (b) measurements and schematic illustration of embedment modification of gold electrodes (c).

ences by the already reported procedure [Słowińska *et al.*, 1999].

1-Dodecanethiol was purchased from Dojindo (Kumamoto, Japan),  $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ , acrylamide, acrylic acid, asparagine were purchased from Sigma-Aldrich (Poznań, Poland). Boric acid was purchased from Standard (Lublin, Poland). Phosphoric acid, sodium dihydrogen phosphate, potassium nitrate and sodium hydroxide were purchased from POCh (Gliwice, Poland).

All aqueous solutions were prepared with freshly deionized water (resistivity of  $18.2 \text{ M}\Omega\text{cm}$ ) purified with a Simplicity® 185 Water System (Millipore, Molsheim, France).

#### Preparation of electrodes

Gold disk electrodes ( $2 \text{ mm}^2$  area, Bioanalytical Systems (BAS), West Lafayette, IN, USA) were used for the experiments. Before modifications all electrodes were cleaned physically by polishing with wet  $0.3$  and  $0.05 \text{ }\mu\text{m}$  alumina slurry

(Alpha and Gamma Micropolish; Buehler, Lake Bluff, IL, USA) and in a sonicator (1 min), then electrochemically by dipping in  $0.5 \text{ mol/L}$  KOH solution and sweeping the potential between  $-400 \text{ mV}$  and  $-1200 \text{ mV}$  (*versus* a Ag/AgCl reference electrode) with a scan rate of  $100 \text{ mV/s}$ .

The electrodes were soaked into  $1 \text{ }\mu\text{mol/L}$  1-dodecanethiol solution in ethanol for 30 min. After washing with ethanol, the electrodes were dipped into  $1 \text{ mmol/L}$  polyamine (Figure 1b) solution in ethanol for 3 h. All electrodes were stored in water at room temperature until use.

#### Electrochemical measurements

All electrochemical measurements were performed with a potentiostat-galvanostat AutoLab (Eco Chemie, Utrecht, Netherlands) with a three-electrode configuration. Potentials were measured *versus* the Ag/AgCl reference electrode, and a platinum wire was used as the auxiliary electrode. Osteryoung square-wave voltammetry (OSWV) was performed

and potential was cycled from +100 mV to 500 mV for  $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ , with step potential of 5 mV, a square-wave frequency of 100 Hz, and amplitude of 25 mV. The dependence of the sensor responses on the concentration of analytes was expressed as the currents at the peak potential in Osteryoung square-wave voltammograms for  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  measured in a solution containing 0.01 mol/L  $\text{KNO}_3$  and 0.01 mol/L borate buffer.

The dependence of Osteryoung square-wave voltammograms on pH was measured in a solution containing 0.01 mol/L  $\text{KNO}_3$  and pH was adjusted with 0.1 mol/L KOH or  $\text{HNO}_3$ .

### Spectroscopic measurements

The complex formation of macrocyclic hexaamine (Figure 1a) with acrylic acid, acrylamide and asparagine was observed on UV spectra recorded with an UV mini-1240 spectrophotometer (Shimadzu, Kyoto, Japan).

The concentration of analysed compounds was changed from 0 to  $9.0 \times 10^{-3}$  mol/L by adding a stock solution of 0.1 mol/L. Spectroscopic measurements for polyamine and investigated analytes were performed in the presence of 0.01 mol/L phosphate buffer, pH = 2.5.

## RESULTS AND DISCUSSION

### Spectroscopic measurements

Polyamine is a 34-membered macrocycle characterised by the presence of six amine groups. This compound was chosen as an ionophore capable for complexation of  $\alpha,\beta$ -unsaturated acrylic acid in the presence of acrylamide and asparagine. To check if any recognition process proceeds between the proposed ligand and analytes under consideration, the spectroscopic measurement was done. Results reported in Figure 2a showed that in the UV spectra of the mixture of acrylic acids and polyamine upon increasing concentration of acrylic acid, the absorption band in the range of 220–225 nm decreased and shifted towards short wavelength. This confirmed the formation of a complex polyamine – acrylic acid.

In the case of arylamide and asparagine spectra (Figure 2b, c) we have observed only negligible changes in absorbance. It indicated that there was no supramolecular complex formed between them and the investigated ligand.

Thus, the obtained results strongly suggest that the investigated sensor should be selective towards acrylic acid in the presence of acrylamide and asparagine.

### Formation and characterization of polyamine incorporating SAMs

Polyamine with six side chains  $-\text{CH}_2\text{COC}_{10}\text{H}_{21}$  was used for chemical modification of gold electrodes by the embedment method. The modification consists in the following steps: (i) immobilization of 1-dodecanethiol on the surface of the gold electrode with covalent Au-S bonds, and (ii) immobilization of polyamine with physical hydrophobic and van der Waals interactions.

Our previous results demonstrated that SAM prepared using this method showed better sensitivity in comparison with

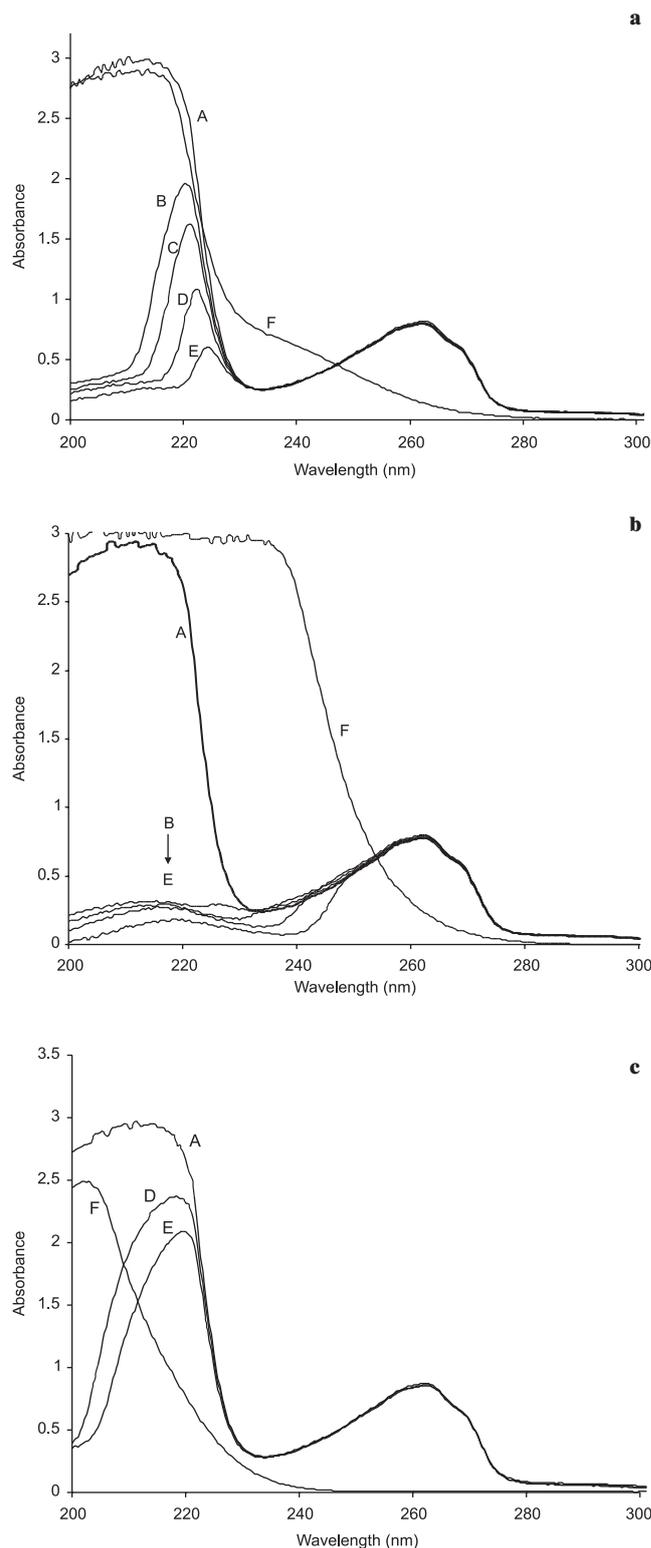


FIGURE 2. The absorption spectra of polyamine ( $5.0 \times 10^{-3}$  mol/L) upon addition of: (a) acrylic acid, (b) acrylamide and (c) asparagine. Analyte concentration: (A) 0, (B)  $1.5 \times 10^{-3}$ , (C)  $2.5 \times 10^{-3}$ , (D)  $5.0 \times 10^{-3}$  and (E)  $9.0 \times 10^{-3}$  mol/L. (F)  $5.0 \times 10^{-3}$  mol/L analyte without addition of polyamine. Measurement conditions: phosphate buffer 0.01 mol/L, pH = 2.5.

that modified by means of the covalent method [Radecka *et al.*, 2005; Radecki *et al.*, 2006]. What is more, such modification is stable and reproducible.

### Dependence of Osteryoung square-wave voltammograms on pH of electrodes modified with polyamine SAMs

Macrocylic polyamine hosts have strong affinity towards protons. This property makes them very useful for sensing anionic species. These neutral compounds, incorporated into PVC-supported liquid membrane electrodes, after conditioning in the aqueous solution from alkaline to acidic ( $\text{pH} = 10^{-3}$ ), acquire a strong anion receptor property due to multiple protonation [Umezawa *et al.*, 1988]. This phenomena we have observed when the investigated gold electrodes were coated with macrocylic polyamine SAM films formed by embedment modification [Radecka *et al.*, 2005; Radecki *et al.*, 2006].

The binding of protons by macrocylic polyamine receptors changes the surface charge from neutral to positive, thereby suppressing the access of a positively charged  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  and subsequent electron transfer between the electrode and the marker. The investigated electrode responded well towards pH. It indicated that the process of modification was successful and macrocylic polyamine was immobilized on the surface of electrode. The results obtained by OSWV are shown in Figure 3. The protonation of polyamine SAMs on the surface of gold was reproducible. The same responses were observed for one month. This suggests that macrocylic polyamine SAMs, modified by the embedment method, are stable and could be stored for one month in milli-Q water in the course of measurements. Thus, the OSWV performed at different pH values was used as a simple test for checking the formation quality of macrocylic polyamines SAMs. And only these electrodes which displayed good response towards protons have been used for further measurements for sensing acrylic acids molecules.

### Selectivity of macrocylic polyamine SAMs towards acrylic acid, acrylamide and asparagine molecules

The sensing of acrylic acid, acrylamide and asparagine molecules by gold electrodes modified with macrocylic polyamines have been examined with the OSWV technique.

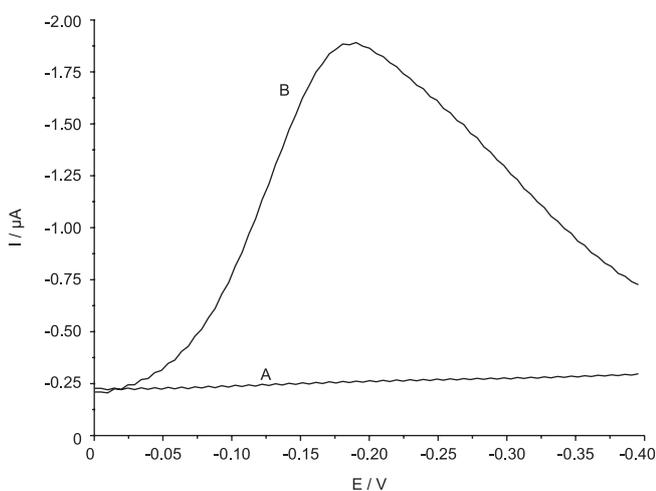


FIGURE 3. The OSWV curves for  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  measured at different pH values: (A) 4.1 and (B) 9.2. The electrolyte composition: 0.01 mol/L  $\text{KNO}_3$ , 0.1 mmol/L  $[\text{Ru}(\text{NH}_3)_6]^{3+}$ . Step potential: 5 mV, square-wave frequency 100 Hz, and square-wave amplitude 25 mV.

The measurements have been performed in the presence of borate buffer, because components of this buffer showed no influence on the voltammetric behaviour of gold electrodes coated with macrocylic polyamine films (results not shown).

Next, the OSWV measurements were performed using the investigated working electrode in order to choose the redox marker appropriate for the study of the interactions between protonated polyamine hosts immobilized on the gold electrode surface and our guests existing in the aqueous phase. Among the redox markers, the  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  was selected. It showed quasi-reversible CV curves in the absence of guests (results not showed). The interactions of interest analytes with the polyamine host were examined with this marker. The results obtained are shown in Figures 4-6.

In the case of acrylic acid, with increasing concentrations of anionic analyte reversibility of reduction/oxidation processes of  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  decreased (Figure 4). The peak

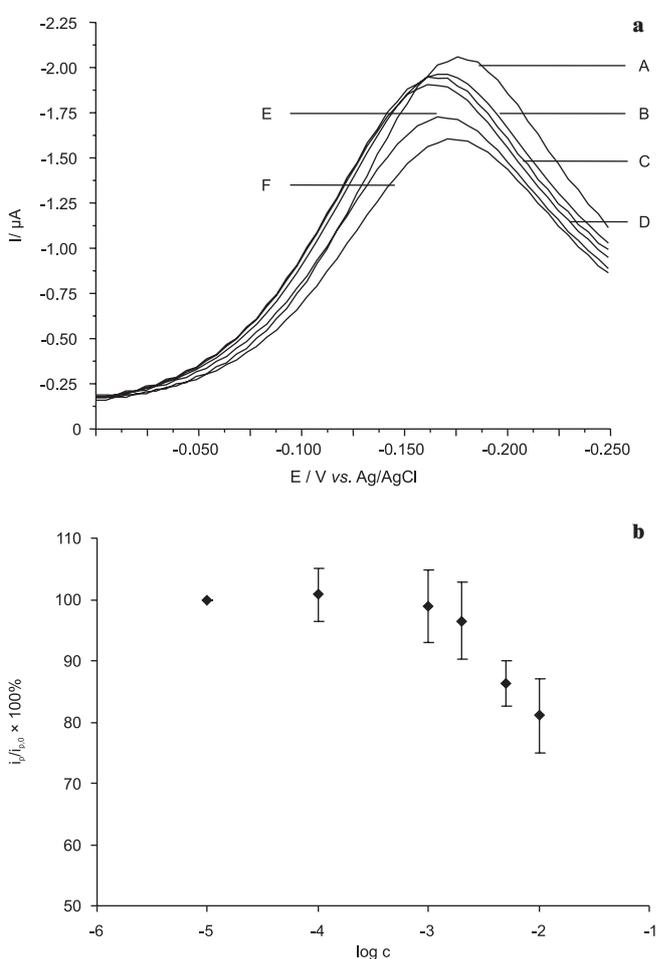


FIGURE 4. (a) The OSWV curves for  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  in borate buffer  $\text{pH}=6.2$  measured in the presence of various concentrations of acrylic acid. The concentration of analyte was as follows: (A) 0, (B)  $1.0 \times 10^{-4}$ , (C)  $1.0 \times 10^{-3}$ , (D)  $2.0 \times 10^{-3}$ , (E)  $5.0 \times 10^{-3}$ , (F)  $1.0 \times 10^{-2}$  mol/L. For electrolyte composition and measuring conditions see Figure 3. (b) The ratio of OSWV peak current in the absence of a given concentration of acrylic acid ( $i_p$ ) to that in the presence of analyte ( $i_{p,0}$ ) as a function of the concentration of  $\text{CH}_2=\text{CHCOOH}$ . The currents were measured at the peak potential in OSWV curves in the solution with no analyte ( $E_{p,0} = -166.0$  mV),  $n=4$ .

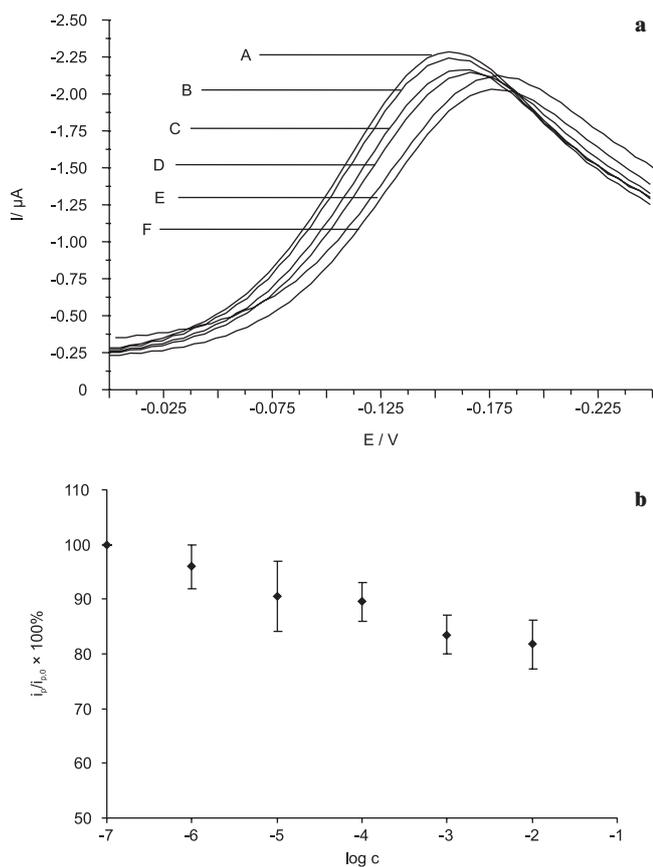


FIGURE 5. (a) The OSWV curves for  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  in borate buffer pH=6.0 measured in the presence of various concentrations of acrylamide. The concentration of analyte was: (A) 0, (B)  $1.0 \times 10^{-6}$ , (C)  $1.0 \times 10^{-5}$ , (D)  $1.0 \times 10^{-4}$ , (E)  $1.0 \times 10^{-3}$ , (F)  $1.0 \times 10^{-2}$  mol/L. For electrolyte composition and measuring conditions see Figure 3. (b) The ratio of OSWV peak current in the presence of different concentration of analyte ( $i_p$ ) to that in the absence of analytes ( $i_{p,0}$ ) as a function of the acrylamide concentration. The currents were measured at the peak potential in OSWV curves in the solution with no analyte ( $E_{p,0} = -157.0$  mV),  $n=4$ .

currents decreased and the peak potentials shifted more negatively. With increasing the concentration of acrylamide,  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  redox peak current decreased slightly and the peak potentials shifted slightly towards the cathodic side (Figure 5). The results we have obtained for the interaction of asparagine with polyamine showed no response towards changes of analytes concentration in the solution (Figure 6). The selectivity sequence of electrodes towards the investigated analytes was in order of:

arylic acid  $\gg$  acrylamide  $>$  asparagine

The detection limit for acrylic acid measured with the investigated sensor was  $\sim 1.0 \times 10^{-3}$  mol/L.

#### Mechanisms of amperometric responses of polyamine coated gold electrodes towards acrylic acid

The OSWV measurements were performed in the presence of a borate buffer of pH=6.0. At this pH value the acrylic acid is dissociated and the macrocyclic polyamine SAM films immobilized on the gold electrodes were still protonated. The partial neutralization of the positive charge of the macrocyclic polyamine

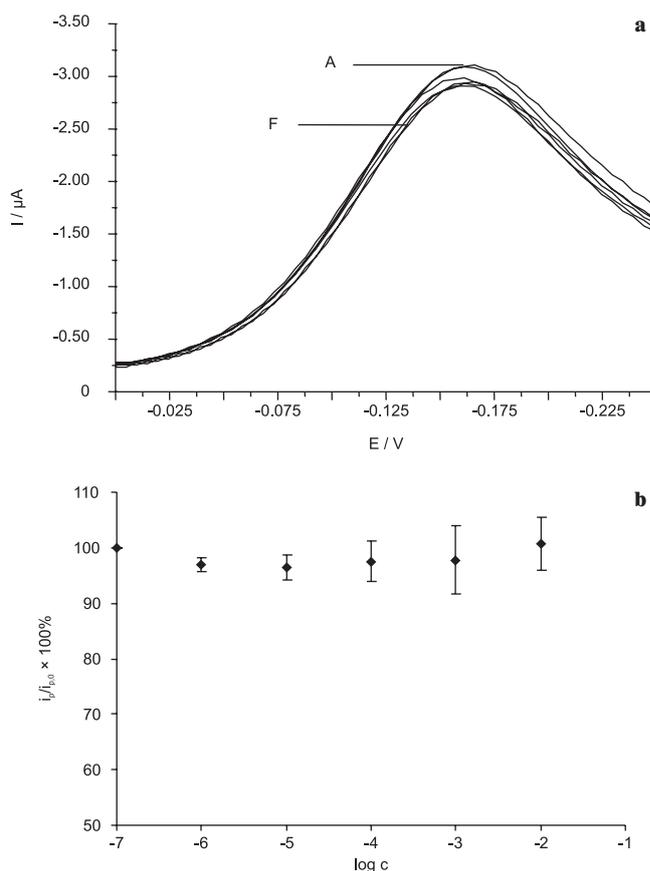


FIGURE 6. (a) The OSWV curves for  $[\text{Ru}(\text{NH}_3)_6]^{3+}$  in borate buffer pH = 6.0 measured in the presence of various concentrations of asparagine. The concentration of analyte was: (A) 0, (B)  $1.0 \times 10^{-6}$ , (C)  $1.0 \times 10^{-5}$ , (D)  $1.0 \times 10^{-4}$ , (E)  $1.0 \times 10^{-3}$ , (F)  $1.0 \times 10^{-2}$  mol/L. For electrolyte composition and measuring conditions see Figure 3. (b) The ratio of OSWV peak current in the presence of different concentrations of asparagine ( $i_p$ ) to that in the absence of analytes ( $i_{p,0}$ ) as a function of the asparagine concentration. The currents were measured at the peak potential in OSWV curves in the solution with no analyte ( $E_{p,0} = -161.0$  mV),  $n=4$ .

SAM films upon interaction with anionic guests may decrease the repulsion between the electrode and positively charged redox marker  $[\text{Ru}(\text{NH}_3)_6]^{3+}$ , as could be expected according to general idea of ion-channel mimetic sensors [Aoki *et al.*, 1999, 2003; Aoki & Umezawa, 2003; Umezawa & Aoki, 2004; Bühlmann *et al.*, 1998; Xiao *et al.*, 1999]. The creation of the supramolecular complex between the polyamine and acrylic acid was proved by spectroscopic methods. These results indicated that sensitivity and selectivity of the sensor under discussion were related to intramolecular recognition between polyamine (host molecule) and acrylic acid (guest molecule). In such type of ion-channel sensors, the decreasing of permeability of monolayer deposited onto electrode surface upon creation of host-guest complexes is the main factor which governs the analytical signal generation [Radecka *et al.*, 2005; Radecki *et al.*, 2006].

#### CONCLUSIONS

Macrocyclic polyamines were permanently immobilized on the surface of gold electrodes by hydrophobic and van der Waals interactions as well. The polyamine-modified sensors were able to selectively recognize acrylic acid against acrylamide

and asparagine. The electrochemical signals were generated based on the supramolecular complex formation between the polyamine hosts and anionic guests *via* hydrogen bonds as well as electrostatic interactions.

#### ACKNOWLEDGEMENTS

The work presented was supported by grant No. 2PO6T08827 and the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, Poland

#### REFERENCES

1. Aoki H., Bühlmann P., Umezawa Y., Self-assembly of a tri-carboxylate receptor through thioamide groups and its use for electrochemical detection of protonated amines. *J. Electroanal. Chem.*, 1999, 473, 105–112.
2. Aoki H., Hasegawa K., Tohda K., Umezawa, Y., Voltammetric detection of inorganic phosphate using ion-channel sensing with self-assembled monolayer of a hydrogen bond-forming receptor. *Biosens. Bioelectron.*, 2003, 18, 261–267.
3. Aoki H., Umezawa Y., Trace analysis of an oligonucleotide with a specific sequence using PNA-based ion-channel sensors. *Analyst*, 2003, 128, 681–685.
4. Bühlmann P., Aoki H., Xiao K.P., Amemiya S., Tohda K., Umezawa Y., Chemical sensing with chemically modified electrodes that mimic gating at biomembranes incorporating ion-channel receptor. *Electroanalysis*, 1998, 10, 1149–1158.
5. Castle L., Eriksson S., Analytical methods used to measure acrylamide concentrations in foods. *J. AOAC Int.*, 2005, 88, 274–284.
6. Ono H., Chuda Y., Ohnishi-Kameyama M., Yada H., Ishizaka M., Kobayashi H., Yoshida M., Analysis of acrylamide by LC-MS/MS and GC-MS in processed Japanese foods. *Food Add. Cont.*, 2003, 20, 215–220.
7. Radecka H., Szymańska I., Pietraszkiewicz M., Pietraszkiewicz O., Aoki H., Umezawa Y., Intramolecular ion-channel sensors using gold electrodes immobilized with macrocyclic polyamines for voltammetric detection of adenine nucleotides. *Chem. Anal. (Warsaw)*, 2005, 50, 85–102.
8. Radecki J., Szymańska I., Bulgariu L., Pietraszkiewicz M., Covalent and embedment immobilization of macrocyclic polyamines on gold electrodes and their voltammetric responses towards ethene dicarboxylic acids. *Electrochim. Acta*, 2006, 51, 2289–2297.
9. Rosén J., Hellenäs K.E., Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst*, 2002, 127, 880–882.
10. Słowińska K.U., Słowiński K., Pietraszkiewicz M., Bilewicz R., Hexaazamacrocyclic ligand with long alkyl chains as functional units in monomolecular Langmuir-Blodgett films. *Supramol. Chem.*, 1999, 10, 201–211.
11. Sugawara M., Kojima K., Sazawa H., Umezawa, Y., Ion-channel sensors. *Anal. Chem.*, 1987, 59, 2842–2846.
12. Tareke E., Rydberg P., Karlsson S., Eriksson S., Törnqvist M., Analysis of acrylamide, a carcinogen formed in heated food-stuffs. *J. Agric. Food Chem.*, 2002, 50, 4998–5006.
13. Umezawa Y., Aoki H., Ion channel sensors based on artificial receptors. *Anal. Chem.*, 2004, 76, 320A–326A.
14. Umezawa Y., Kataoka M., Takami W., Kimura E., Koike T., Nada, H., Potentiometric adenosine triphosphate polyanion sensor using a lipophilic macrocyclic polyamine liquid membrane. *Anal. Chem.*, 1988, 60, 2392–2396.
15. Xiao K.P., Bühlmann P., Umezawa, Y., Ion-channel-mimetic sensing of hydrophilic anions based on monolayers of a hydrogen bond-forming receptor. *Anal. Chem.*, 1999, 71, 1183–1187.

Received January 2008. Revision received and accepted March 2008.