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Sensors and Biosensors for Determination of Acrylamide and Acrylic Acid in Potato Food Products

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ABSTRACT

The presence of toxic acrylamide in a wide range of food products such as potato crisps, French fries or bread was confirmed by Swedish scientists from Stockholm University. The neurotoxity, and possible carcinogenicity, of this compound and its metabolites imposes a duty to control them by quantitative and qualitative assays. Acrylamide forms an adduct with hemoglobin (Hb) as a result of the reaction with the α-NH_2 group of the N-terminal valine of Hb. This interaction is the basis of a new voltammetric biosensor to detect acrylamide. The biosensor was constructed using a carbon-paste electrode modified with hemoglobin (Hb), which contains four prosthetic heme-Fe(III) groups. Such an electrode displays a reversible reduction/oxidation process of Hb-Fe(III)/Hb-Fe(II). Interaction between Hb and acrylamide was observed through a decrease of the Hb-Fe(III) reduction peak current. Exposing acrylamide to pH extremes results in its hydrolysis to acrylic acid. Apart from natural host molecules, synthetic receptors such as tetralactam or macrocyclic polyamine derivatives were applied as active elements of sensors for voltammetric detection of acrylic acid. The synthetic host molecules were immobilized on an electrode surface by covalent Au-S bond or by an embedment method into the thiol layer via hydrophobic and van der Waals interactions. The applicability of sensors was proved by a validation procedure made in the matrix obtained by water extraction of potato chips. The proposed sensor parameters such as sensitivity, selectivity, wide dynamic range, simplicity of sample preparation, in comparison to those presented by others in already reported methods, will be discussed.

Keywords: acrylamide, acrylic acid, carbon paste electrodes, gold electrodes, hemoglobin, tetralactam, voltammetry, potato chips
Abbreviations: AA, acrylamide; **AAc**, acrylic acid; **CPE**, carbon paste electrode; **CPK**, Corey-Pauling-Koltun atomic model; **CV**, cyclic voltammetry; **DDAB**, dimethyldioctadecyl-ammonium bromie; **DNA**, deoxyribonucleic acid; **GA**, glycidamide; **GC**, gas chromatography; **GC-MS**, gas chromatography-mass spectrometry; **GC-MS/MS**, gas chromatography-tandem mass spectrometry; **Hb**, hemoglobin; **HPLC**, high-performance liquid chromatography; **IARC**, the International Agency on Research on Cancer; **LC/MS**, liquid chromatography-mass spectrometry; **MS**, mass spectrometry; **OSWV**, Osteryoung square wave voltammetry; **QCM**, quartz crystal microbalance; **SAMs**, self-assembled monolayers; **VOCs**, volatile organic compounds

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INTRODUCTION

Acrylamide (AA) is a well known neurotoxin and potential carcinogen (Tareke *et al.* 2002; Friedman 2003; Ruden 2004). High levels of this compound have been found in potato chips, French fries and several other common foods (Tareke *et al.* 2002; Becalski *et al.* 2003; Gökmen *et al.* 2005). The first such report was announced by scientists from Stockholm University in 2002 (Tareke *et al.* 2002).

It is known that AA can create adducts with hemoglobin (Hb) (Tareke *et al.* 2002; Friedman 2003; Stobiecka *et al*. 2007). Therefore AA-Hb adducts can serve as a useful biomarker of human exposure to AA. The Hb-adduct method for *in vivo* monitoring of reactive compounds like AA was developed by Margareta Törnqvist of Stockholm University (Tareke *et al.* 2002). This method was used to determine adduct levels in blood of workers, who in 1997 constructing a railway tunnel in Hallandsas, Sweden, when AA was used as a monomer in the grouting agent. Swedish scientists discovered the adduct level in their blood as high as 4000 pmol/g Hb. Reference samples taken from people who were not exposed to AA contained 30-40 pmol/g Hb (Erickson 2004). This result suggested that the general population may be constantly exposed to AA and induced scientist to search for a possible source of this exposition. Research showed a higher Hb-AA adduct level in smokers than non-

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smokers blood (Schettgen *et al*. 2002), suggesting that burning or high temperature may be involved. Analysis of diets led to the conclusion that heating food can be responsible for the elevated background levels in the general population. Many studies have supported this theory (Tareke *et al.* 2002; Becalski *et al*. 2003; Friedman 2003; Svensson *et al*. 2003; Hoenicke *et al*. 2004; Gökmen *et al.* 2005; Kim *et al*. 2007; Geng *et al*. 2008).

TOXICICITY OF AA

AA has been classified as "probably carcinogenic to humans" by the International Agency on Research on Cancer (group 2A, IARC, 1994). In volume 60 of IARC Monographs on the Evaluation of Carcinogenic Risks to Humans it was stated that results of experiments on animals gave sufficient evidence for the carcinogenicity of AA (Raport IARC). Epidemiologic studies of possible health effects from exposures to AA have not produced consistent evidence of increased cancer risk, in either occupationally exposed workers (Granath *et al*. 2001) (except for a statistically significant doubling of risk for pancreatic cancer which was found for workers with highest cumulative exposure (Marsh *et al*. 1999) or in the general populations of several countries in which AA is present in certain foods and beverages (Dybing and Sanner 2003; Mucci *et al.* 2003; Pelucchi *et al*. 2003; Rice 2005). Nevertheless, positive bioassays of AA for carcinogenicity in experimental animals established that AA is a multi-organ (e.g. lung, skin, thyroid, brain) carcinogen in both rats and mice, when given in drinking water or by other means (Ruden 2004; Rice 2005; Besaratinia and Pfeifer 2007). These data strongly imply that AA presents a potential carcinogenic hazard to humans.

The mechanism of AA carcinogenicity is still unknown. It is supposed that AA-induced DNA adduction and mutagenesis can play a significant role in this process (Besaratinia and Pfeifer 2007). AA showed low reactivity towards DNA *in vitro* and in AA-treated rats only DNA adducts from the AA metabolite - glycidamide (GA) were detected (Maniere *et al*. 2005). GA is the product of the biotransformation of AA to its epoxide (**Fig. 1**), which is chemically more reactive. This process occurs *in vivo* catalyzed by cytochrome P450 enzymes in the liver (Raport IARC).

Therefore, GA is assumed to be the genotoxic agent in AA exposure (Dearfield *et al*. 1995; Paulsson *et al*. 2001, 2003). The linearity of the dose-response suggests that AA and GA are DNA-reactive clastogens and induce lethal mutations in spermatids of mice and rats and are considered to be a mammalian germ cell mutagen (Waters *et al*. 1993; Ghanayem *et al*. 2005).

Exposure to high levels of AA cause damage to the human nervous system proving that this compound is a human neurotoxin. Workers exposed to AA exhibited symptoms of peripheral neuropathy. High Hb-AA adducts level in their blood was correlated with neurologic symptoms such as tingling or numbness in their hands or feet (Erickson 2004). Two mechanistic hypotheses of AA neurotoxincity were considered: inhibition of kinesin-based fast axonal transport (Sickles *et al*. 2002) and direct inhibition of neurotransmission (LoPachin 2002). AA acts directly at nerve terminal sites to cause primary presynaptic dysfunction (impairs neurotransmitter release) and eventual degeneration. The mechanism of toxic injury might involve an inhibition of membrane fusion processes (LoPachin 2004, 2005). Mechanism of adverse effects may include also alteration of the expression of genes governing the synthesis of brain proteins (neurofilament gene expression) (Lin *et al*. 2000).

FORMATION IN FOOD

A number of potential mechanisms for the formation of AA in food have been published. Preliminary investigations showed that carbohydrate-rich food is involved in AA formation. Analyses of fried potato revealed 10-100 times higher AA content than in protein-rich food (for example fried beef). Frying of another type of carbohydrate-rich food (e.g. beetroot) gave similar results (Tareke *et al.* 2002).

It was proved that AA can be generated from food components during heat treatment (above 100°C) as a result of the reaction between reducing sugars such as glucose and amino acid asparagine (**Fig. 2**). The Millard reaction mechanism has been proposed to account for its formation (Mottram *et al*. 2002; Stadler *et al*. 2002; Zyzak *et al*. 2003). Products of this reaction are responsible for flavour and colour generating during baking and roasting. During this reaction reducing sugars react with amino acids initiating a cascade of events leading to the browning of food. An important associated reaction is the Strecker degradation of amino acid by these intermediates, in which the amino acid is decarboxylated and deaminated to form an aldehyde.

As a possible substrate in the AA formation, few amino acids were tested: glycine, cysteine, methionine, glutamine, aspartic acid and asparagine. Asparagine was a particularly good suitable reactant as it already has an amide group attached to a chain of two carbon atoms (Mottram *et al*. 2002). Higher yield of AA from asparagine, a major amino acid in potato and cereals, demonstrated that it is a predo-

Fig. 1 AA metabolized *in vivo* by CYP 2E1 enzymes to form GA.

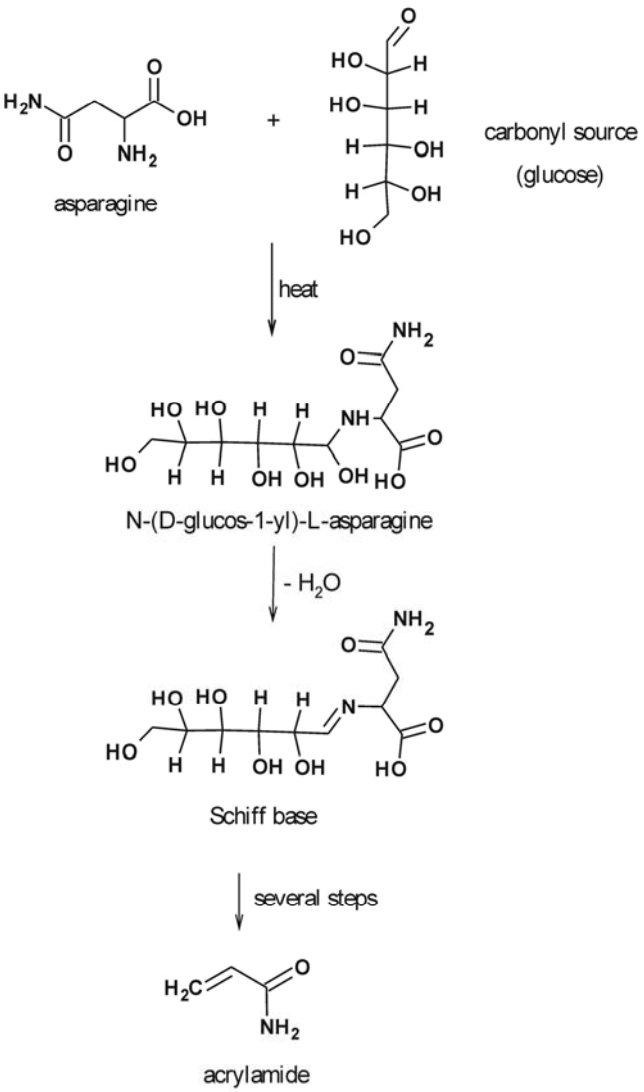


Fig. 2 Route of AA formation from asparagine and glucose.

Table 1 Acrylamide levels in processed foods.

Food	Acrylamide level (mean value) [μg/kg]	Method of analysis
potato chips (crisps)	620	HPLC-MS-MS (Hoenicke *et al*. 2004)
	1739	LC-MS/MS (Tareke *et al*. 2002)
	1305.4, 1814.8 (in two different commercial samples)	LC–DAD (liquid chromatography coupled with diode array detection) (Geng *et al*. 2008)
	980	LC–MS–MS (Svensson *et al*. 2003)
	1377	LC-MS/MS (Kim *et al*. 2007)
French fries	424	LC-MS/MS (Tareke *et al*. 2002)
	410	LC–MS–MS (Svensson *et al*. 2003)
bread	40	LC–MS–MS (Svensson *et al*. 2003)
	33	LC-MS/MS (Kim *et al*. 2007)
crisp bread	439	HPLC-MS-MS (Hoenicke *et al*. 2004)
	208	LC-MS/MS (Tareke *et al*. 2002)
	135	LC–MS–MS (Svensson *et al*. 2003)
biscuits	546	HPLC-MS-MS (Hoenicke *et al*. 2004)
	119.7, 182.8 (in two different commercial samples)	LC–DAD (Geng *et al*. 2008)
	230	LC–MS–MS (Svensson *et al*. 2003)
	714.2	LC-MS/MS (Kim *et al*. 2007)
coffee, roasted	282	HPLC-MS-MS (Hoenicke *et al*. 2004)
	25	LC–MS–MS (Svensson *et al*. 2003)
coffee, soluble	816	HPLC-MS-MS (Hoenicke *et al*. 2004)
hamburger	18	LC-MS/MS (Tareke *et al*. 2002)

minant amino acid responsible for the formation of AA (Mottram *et al*. 2002; Becalski *et al*. 2003; Zyzak *et al*. 2003). The results of series of stable isotope substitution experiments done by LC/MS technique with using isotope-substituted asparagine shown that three AA carbon as well as one nitrogen atom came from the amide side chain of asparagine (Stadler *et al*. 2002; Zyzak *et al*. 2003).

Heating asparagine on its own did not produce AA. This confirmed that Strecker degradation and dicarbonyl reactant (as glucose) was involved in the production of AA (Mottram *et al*. 2002). Further investigations showed the necessity of carbonyls in the formation of AA, but that dicarbonyls were not essential for the formation of AA from asparagine (Becalski *et al*. 2003; Zyzak *et al*. 2003; Knol *et al*. 2005).

Reaction of reducing sugars with amino acids lead to the formation of N-glycosides (Schiff bases when viewed in their open chain tautomeric form). Stadler *et al*. (2002) reported that N-glycosides formed by the reaction of asparagine, when heated, result in significant levels of AA, while reactions of methionine and glutamine result in minor amounts of AA (Stadler *et al*. 2002). This also confirms the role of asparagine in the process of AA formation.

Investigations on the kinetic model of AA formation showed that it is formed in the temperature range of 120-200°C. Higher temperatures resulted in higher AA content (Mottram *et al*. 2002; Knol *et al*. 2005). At 180 and 200°C an increase and subsequent decrease of AA concentration was observed. This suggests that AA is an intermediate of the Maillard reaction rather than an end product, and it can undergo a degradation reaction at higher temperatures (Knol *et al*. 2005).

ANALYSIS OF AA

The presence of toxic AA in a wide range of food products has been confirmed by Swedish scientists from Stockholm University. The neurotoxicity and possible carcinogenicity of this compound and its metabolites compel scientists to control them by quantitative and qualitative assays.

AA is formed from compounds intrinsically present in foods. Among analytical methods used to determine AA levels in food predominate chromatographic techniques based on gas chromatography (GC), high-performance liquid chromatography (HPLC), mass spectrometry (MS) and combinations of these (**Table 1**). Only a few examples of using different techniques for detection of AA exist.

Published data indicated that potato food products contain the highest amount of AA after frying (**Table 1**).

Preparation of samples from food involves extraction using water or methanol and the clean-up step typically consists of a combination of several solid-phase extractions. GC-MS often needs the additionally bromination step to form more volatile AA derivative and increase selectivity of determination (Wenzl *et al*. 2003).

SENSORS AND BIOSENSORS

Sensor towards AA based on hemoglobin

As was mentioned above, AA can create adducts with Hb (Tareke *et al.* 2002; Friedman 2003; Stobiecka *et al*. 2007). It is known that AA, and related conjugated vinyl compounds, undergo the Michael-type nucleophilic addition reaction of amino (NH_2) and sulfhydryl (SH) groups of amino acids, peptides and proteins to its double bound (Friedman 2003). Investigations showed that AA-Hb adducts form as a result of reaction between the α-NH_2 group of N-terminal valine of Hb with AA (Tareke *et al.* 2002; Friedman 2003). Because of that, Hb can serve as useful biomarker of human exposure to AA. The tracing of background exposure to AA through biomarker measurements were conducted by GC-MS/MS method in the negative ion/chemical ionization mode (Hagmar *et al*. 2005) or with GC-MS (Paulsson *et al*. 2002).

Stobiecka and co-workers introduced a voltammetric sensor based on the reaction of Hb with AA (Stobiecka *et al*. 2007). The authors introduced a novel electrochemical biosensor designed for the direct determination of AA in food samples. The reversible conversion of Fe(III) to Fe(II) of heme (prosthetic group of Hb) was responsible for its electroactivity (Chan 2000; Scheller *et al*. 2005).

The rate of electron transfer from the protein to the surface of the electrodes modified directly by Hb is slow. It is connected with a large, three-dimensional structure of Hb, which make the direct electron transfer between the Hb and electrode difficult (Scheller *et al*. 2005). Intensification of electron transfer rate between Hb and electrode surface can be achieved using electromediators (Rusling *et al*. 1993; Gu *et al*. 2001; Ma *et al*. 2005; Zhang *et al*. 2005) like carbon nanotubes and gold nanoparticles, or surfactant like dimethyldioctadecyl-ammonium bromide (DDAB) as in the case of described biosensor.

Stobiecka and co-workers reported electrodes filled with carbon paste prepared by mixing graphite powder and paraffin oil. Than DDAB-Hb liposomes resulted from dispersion of Hb and DDAB in aqueous buffer solution were dropped on smooth surfaces of carbon paste electrodes

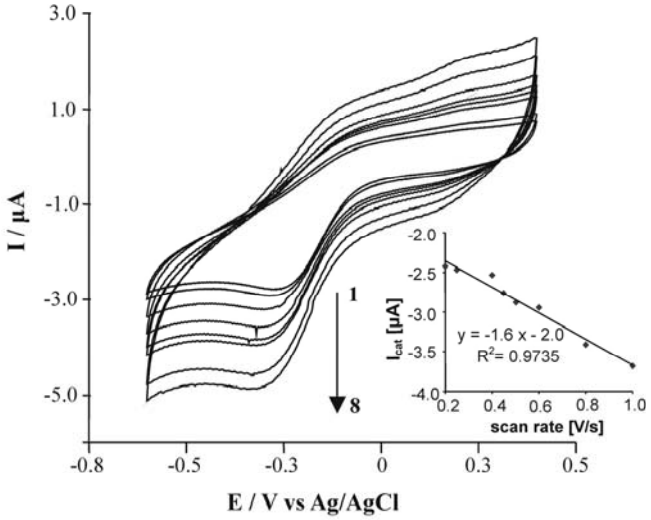


Fig. 3 The cyclic voltammetry (CV) curves for carbon-paste electrodes modified by Hb-DDAB liposomes measured vs. scan rates: (1) 0.2, (2) 0.25, (3) 0.4, (4) 0.45, (5) 0.5, (6) 0.6, (7) 0.8, (8) 1.0 V/s. The electrolyte composition: 0.05 mol/L NaBr, acetate buffer 0.2 mol/L, pH 4.8. Inset: linear relationship between cathodic peak current vs. scan rate. Based on and modified from data in Stobiecka *et al*. (2007).

(CPE).

Investigated electrodes modified with Hb-DDAB displayed a quasi-reversible electrochemical reaction of Hb-Fe^{2+}/Hb-Fe^{3+} (**Fig. 3**). The cathodic peak potential was located at E_{cat} =−234mV, anodic at E_{an} =−102mV and the formal potential was about $E^{0'}$ =−168mV. Cathodic peak currents I_p were linearly dependent on scan rate v (**Fig. 3**). This indicates that redox reaction is not a diffusion-controlled process, but a surface-controlled one, as expected for an immobilized system (Bard and Faulkner 2001).

The applicability of the proposed sensor was tested using the Osteryoung square wave voltammetry (OSWV) technique, which was more sensitive than cyclic voltammetry (CV). The responses of the carbon-paste electrodes modified with Hb-DDAB towards AA concentrations were measured in the presence of sample solutions based on water extract from potato crisps. The extraction procedure consisted on few steps: first crisps were homogenized in a mortar, then after addition of water left for swelling and incubation. The next step was centrifugation. Then the resulting supernatant was deffated by extraction with *n*-hexane and cleaned up with Carrez I and Carrez II solutions. It is a procedure typically used in chromatographic analysis.

The representative OSWV curves are illustrated in **Fig. 4A**. The presence of the matrix obtained from potato crisps influenced electrode sensitivity towards AA very little. These results allow to state that the sensor under study was very resistant to interference coming from the matrix obtained by extraction of potato crisps. **Fig. 4B** illustrates the relationship between the concentration of AA in sample solution and a decrease of peak current values. The limit detection was 1.2×10^{-10} mol/L. The linear range of this response was from 1.3×10^{-11} to 4.8×10^{-5} mol/L.

The most important advantage of this biosensor is very good sensitivity in the 10^{-10} mol/L range. Also, the application of the proposed sensor for AA determination does not require sophisticated sample preparations.

It was proved that heme from hemoglobin molecules is not directly involved in the process of AA recognition (Stobiecka *et al*. 2007). This process relies on the interaction between AA and the hemoglobin valine, which leads to the formation of Hb-AA adduct. Formation of this adduct is associated with Hb structure change (Friedman 2003), what is probably responsible for the decrease of accessibility of redox-active centers of Hb immobilized on the surface of the electrode, which causes a decrease of current values of Hb redox reaction.

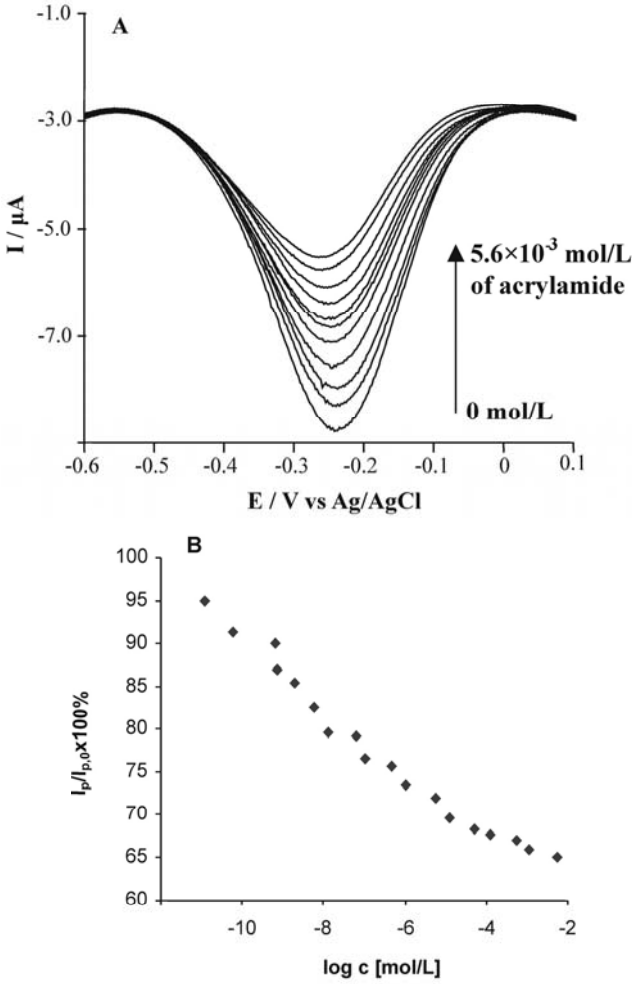


Fig. 4 (**A**) The response of carbon paste electrodes modified with Hb-DDAB liposomes towards acrylamide in the presence of water extract from the potato crisp. Measuring conditions: for electrolyte composition see **Fig. 3**; step potential of 0.0024 V; square-wave frequency 100 Hz; square-wave amplitude 0.025 V. (**B**) The ratio of OSWV peak current in the presence of a given concentration of AA (I_p) to that in absence of analytes ($I_{p,0}$) as a function of concentration of AA in water extract from the potato crisp. The currents were measured at the peak potential in OSWV curves in the solution with no analyte ($E_{p,0}$ =−242 mV); (n = 3; 3.1 < S.D. < 9.1). Based on and modified from data in Stobiecka *et al*. (2007).

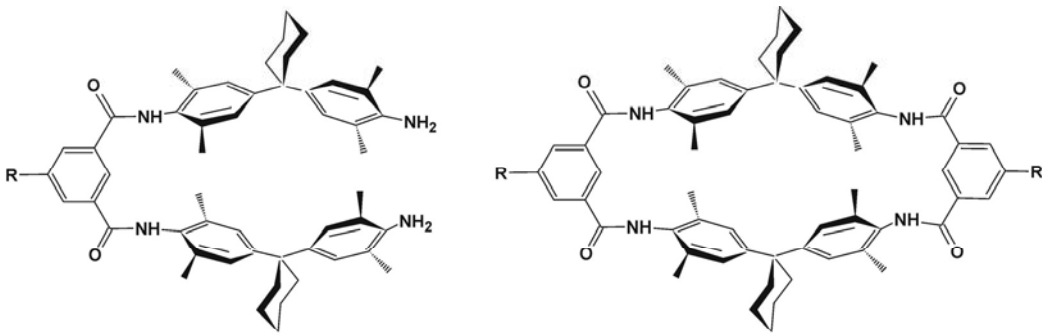
The carbon paste electrodes modified with Hb were very stable after cycling of the potential. After preparation, they could be stored in the buffer (at 4°C) *ca*. 1 month, but the interaction between Hb and AA is irreversible. Therefore, after contact with AA solution electrodes should be prepared again.

Sensor towards AA based on tetralactam

Kleefisch and co-workers reported a sensor in which AA and acrylic acid (AAc) were detected at a gas–solid interface using an 'electronic nose'-type quartz crystal microbalance (QCM) sensor covered with a tetralactam active layer (Kleefish *et al*. 2004).

Tetralactams (**Fig. 5**) belong to a wide group of neutral compounds able to complex anions by hydrogen bond formation (Sigel and Martin 1982; Choi and Hamilton 2003). These macrocycles have been used recently as the macrocyclic host for detection of carbonyl compounds like *p*-benzoquinone (Hunter 1991), and anions (Br^-, Cl^-) (Hubner *et al*. 1999).

The measuring chamber accommodates up to 24 quartz sensors (QCM) at one time. Each sensor consists of a thin quartz plate with gold electrodes deposited onto both sides. The top electrode was coated with the sensor-active mate-



1. R = H
2. R = OAc
3. R = *t*-Bu

4. R = R' = H
5. R = H, R' = OMe
6. R = R' = OAc
7. R = H, R' = *t*-Bu
8. R = R' = *t*-Bu

Fig. 5 Tetralactam derivatives synthesized by Kleefisch and co-workers and used as active element of electronic nose'-type quartz crystal microbalance (QCM) sensor for detection of acrylamide and AAc. Based on and modified from Kleefish *et al*. (2004).

rial using the electrostatic spray method. The coated quartz plates are then transferred into the measuring chamber and exposed to the different analytes, i.e. the volatile organic compounds (VOCs). The sensor response was monitored by a computer-based data system.

Proposed sensor characterized very good sensitivity. Even at concentrations of AA as low as 10 ppb the sensor yields a well detectable signal.

To analyze the characteristic responses toward AA, a series of tetralactam macrocycles and its derivatives were used (**Fig. 5**). Receptor marked as 5 in the **Fig. 5** gave the best response. Results showed that the closed macrocycles, were favored over the open-chain analogues, which likely exists in an extended conformation. These findings lead to the conclusion that an interaction of the macrocycles with AA takes place within the cavity.

The QCM electrodes coated with tetralactam exposing to propionic amide, AAc, and propionic acid, showed lower response as compared with response for AA. The difference between AA and propionic amide and that between AAc and propionic acid are of the same order of magnitude. This effect could reflect the influence of the additional double bond of AA and AAc on the recognition process by tetralactam. One might speculate that π-π interactions of double bond present in target molecules with the aromatic rings of the macrocycle hosts play some role.

Sensor towards AAc based on tetralactam

Exposing AA to pH extremes results in its hydrolysis to acrylic acid (AAc), or its salt. The most frequently used methods of AAc determination similarly as in the case of AA include GC (Steward *et al*. 1995) and HPLC (Casella *et al*. 2006).

One example of a biosensor for detection of AA and AAc was prepared based on respiratory activity of microbial cells (Ignatov *et al*. 1996).

The gold electrodes coated with self-assembled monolayers (SAMs) containing tetralactam macrocycle and its acyclic derivative (**Fig. 5**) as active elements were used for voltammetric detection of AAc in water solution (Krajewska *et al*. 2009). The receptor molecules have been immobilized on an electrode surface by covalent Au-S bonds (receptor 1 with disulphide group) (**Fig. 6**) or by embedment method into the thiol layer via hydrophobic and van der Waals interactions (macrocyclic and open-chain recaptors 2 and 3 possessing long lipophilic side chains) to create a ion-channel type sensors (Sugawara *et al*. 1987; Umezawa and Aoki 2004).

The determination of AAc by tetralactam incorporated gold electrodes was examined using Osteryoung square wave voltammetry (OSWV) in 0.01 M KNO_3 with redox marker $[Ru(NH_3)_6]Cl_3$,. This marker was the most suitable for the investigated system. The measurements were performed in a pH = 5.0 solution. To evaluate electrodes responses, the parameter of relative decreasing of peak current was used. The ratio of OSWV peak current in the presence of different concentrations of AAc (I_p) to that in the absence of AAc ($I_{p,0}$) ($I_p/I_{p,0}$ x 100%) was plotted *versus* the AAc concentration. Comparison of responses showed by electrodes modified by covalent method with macrocyclic **receptor 1** and by embedment technique with macrocyclic **receptor 2** allowed to state that in both cases detection limit was similar (1.0×10^{-5} mol/L), but linearity region was slightly different (**Fig 6**). In the case of covalent modification it was from 1.0×10^{-5} to 2.5×10^{-4} mol/L, with slope -42.8 %/logC and regression coefficient 0.980. In the case of embedment it was from 1.0×10^{-5} to 1.0×10^{-3} mol/L with a -10.6 %/logC slope and a regression coefficient of 0.930.

A stronger response toward AAc showed electrodes modified by covalent method in relation to those modified with embedment (Krajewska *et al*. 2009). The highest AAc concentration studied (1.0×10^{-3} mol/L) caused 67.5% current decrease in OSWV in the case of covalent modification and 21.3% for electrodes modified by embedment (**Fig. 6**)

Electrodes modified with acyclic derivative (**receptor 3**) were also investigated. These electrodes prepared by embedment and measured in the same conditions of pH, showed inconsiderable response towards AAc. This suggested that only interaction between macrocyclic tetralactam and AAc was sufficient for the generation of an analytical signal. As a control experiment electrodes modified only with 1-dodecanethiol were used. These electrodes showed only negligible response toward the analyte in the solution pH = 5.0. This proved that only the tetralactam host presence was responsible for the recognition process. At pH=5.0 analyte existed in the solution in 85% as anionic form and in 15% as neutral form, whereas tetralactam existed in the layer on the electrode surface as a neutral compound.

Geometry optimization of a number of different conformations of the macrocycle in vacuum using CaChe Workspace programme (CaChe Workststem Pro Version 7.5.0.85) and AM1 geometry procedure (MOPAC 2002 Version 2.5.3, J. J. P. Stewart, Fujitsu Ltd., Tokyo, Japan) as well as using Corey-Pauling-Koltun (CPK) atomic model (**Fig. 7**) showed that most favorable conformation of the host molecule is when all four amide-NH group (acceptors of H-bond) are oriented inside the macrocycle cavity. AAc in dissociated

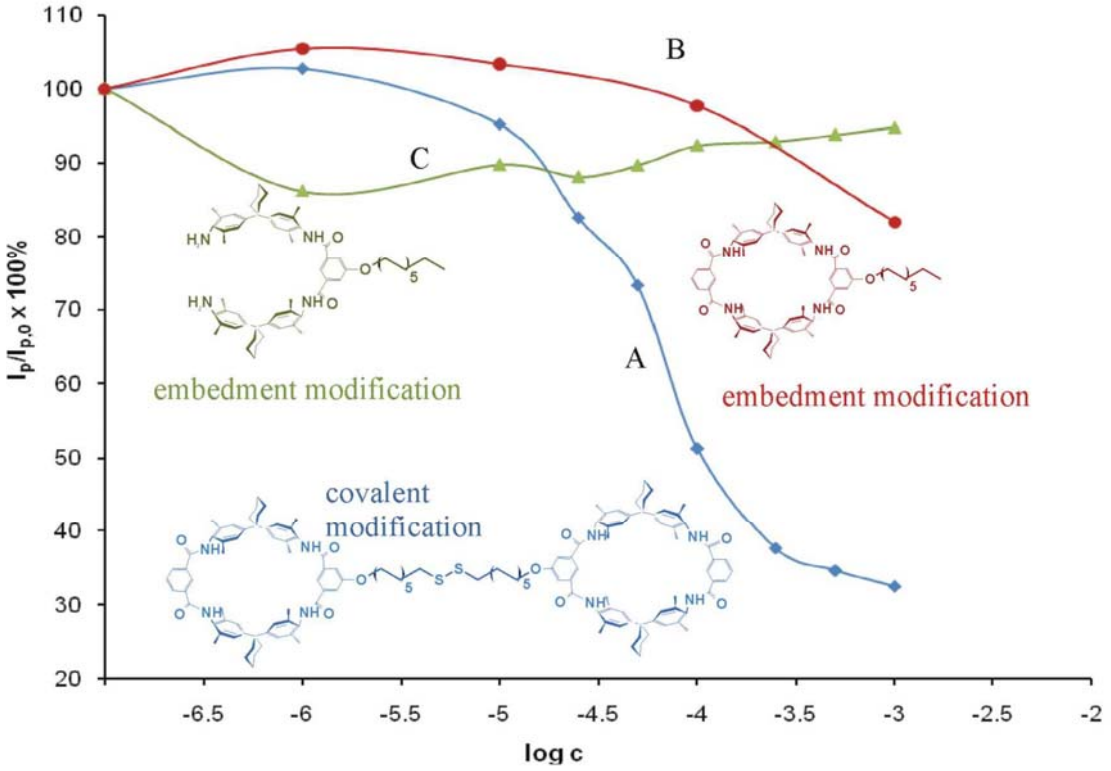


Fig. 6 The ratio of OSWV peak current for electrodes modified with receptor 1(A), receptor 2 (B) and receptor 3 (C) in the presence of different concentrations of AAc (I_p) to that in the absence of AAc ($I_{p,0}$) as a function of the AAc concentration. The electrolyte composition: 0.01 mol/L KNO_3, pH=5.0, 0.1 mmol/L $[Ru(NH_3)_6]^{3+}$. Step potential: 5 mV, square-wave frequency 100 Hz, and square-wave amplitude 25 mV. Based on and modified from data in Krajewska *et al*. (2009).

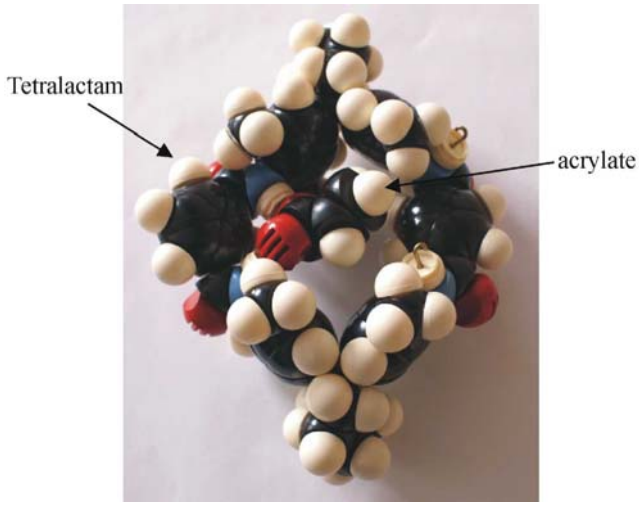


Fig. 7 Corey-Pauling-Koltun atomic model of complex that forms between tetralactam and acrylate.

form possess stronger electron donor characters and, because of that, stronger affinity to the tetralactam cavity in comparison with neutral form, which suggests that the observed response was caused more by anionic than by neutral form of analyte.

In measuring conditions the acrylate anion can interact with neutral tetralactam molecules to form a negatively charged complex. Even though the positive charged electroactive marker $[Ru(NH_3)_6]^{+3}$ was used, the sensor reported showed the decreasing of redox peak current upon increasing concentration of acrylate anion in the sample solution. Probably the physical blocking of intermolecular spaces prevented access of marker ions to the electrode and played a decisive role in the generation of the investigated sensor response. This can be explained by the idea of an intermolecular ion-channel mechanism (Sugawara *et al*. 1987; Umezawa and Aoki 2004).

Sensor towards AAc based on polyamine

Polyamine is a macrocycle characterized by the presence of a six amine group (**Fig. 8**). Macrocyclic polyamine hosts have strong affinity towards protons. This property makes them very useful for sensing anionic species. Therefore, lipophilic macrocyclic polyamine derivative was chosen as a ionophore capable to complexation of α,β-unsaturated

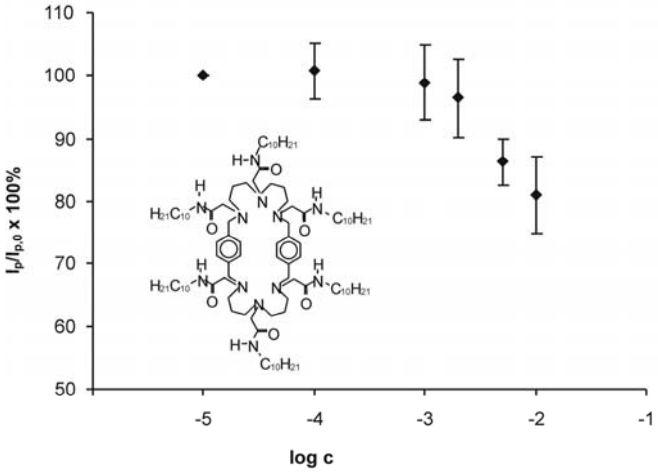


Fig. 8 The ratio of OSWV peak current in the presence of a given concentration of AAc (i_p) to that in the absence of analyte ($i_{p,0}$) as a function of the concentration of CH_2=CHCOOH. The currents were measured at the peak potential in OSWV curves in the solution with no analyte ($E_{p,0}$ = -166.0 mV), n=4. The electrolyte composition: 0.01 mol/L KNO_3, pH = 6.2, 0.1 mmol/L $[Ru(NH_3)_6]^{3+}$. Step potential: 5 mV, square-wave frequency 100 Hz, and square-wave amplitude 25 mV. Based on and modified from data in Krajewska *et al*. (2008).

AAc (Krajewska *et al*. 2008). Polyamine with six side chains – $CH_2COC_{10}H_{21}$ was used for chemical modification of gold electrodes by embedment method.

Our previous results demonstrate SAM prepared using this method showed better sensitivity in comparison with covalent method of modification (Radecka *et al.* 2005; Radecki *et al.* 2006). What is more, such modification is stable and reproducible.

The sensing of AAc molecules by gold electrodes modified with macrocyclic polyamine host was examined with OSWV technique. The measurements were performed in the presence of borate buffer, because components of this buffer showed no influence on the voltammetric behavior of gold electrodes coated with macrocyclic polyamine film.

The polyamine incorporated electrodes showed the current decrease of reduction/oxidation processes of $[Ru(NH_3)_6]^{3+}$ with increasing concentrations of anionic analyte (**Fig. 8**).

The OSWV measurements were performed in the presence of a borate buffer of pH = 6.0. At this pH value the AAc is dissociated and the macrocyclic polyamine SAM films immobilized on the gold electrodes were protonated. The partial neutralization of the positive charge of the macrocyclic polyamine SAM films upon interaction with anionic guests may decrease the repulsion between the electrode and positively charged redox marker $[Ru(NH_3)_6]^{3+}$, as could be expected according to general idea of ion-channel mimetic sensors (Sugawara *et al*. 1987; Umezawa and Aoki 2004). These results indicated that sensitivity and selectivity of sensor under discussion is related with intramolecular recognition between polyamine (host molecule) and AAc (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 govern the analytical signal generation (Radecka *et al.* 2005; Radecki *et al.* 2006).

SUMMARY

The formation, occurrence and determination of AA have been extensively studied in the last few years. Toxicity of AA compels us to control its level in food, especially in potato products, where the highest occurrence of AA was observed.

Chromatographic techniques are most frequently used to determine AA and AAc. These methods are time consuming and expensive, therefore using of electrochemical sensors and biosensors seems to be good alternative. In this paper we introduce sensors based on synthetic ionophores like tetralactam or polyamine and biosensors based on neutral receptor hemoglobin. Interactions between receptor and analyte can be observed using OSWV technique, which provides good sensitivity of measurements.

Taking into account the parameters such as: good sensitivity and selectivity, lack of interferences form natural matrix components, the relatively easy and inexpensive way of preparation, the proposed sensors and biosensors could be recommended for the direct determination of acrylamide and acrylic acid in food samples.

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