Determination of Acrylamide in Food Using Adsorption Stripping Voltammetry

Helena VESELÁ and Emanuel ŠUCMAN

Institute of Biochemistry, Chemistry and Biophysics, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

Abstract

Veselá H., Šucman E. (2013): **Determination of acrylamide in food using adsorption stripping voltammetry.** Czech J. Food Sci., **31**: 401–406.

A new electroanalytical method for the determination of acrylamide in food has been developed. It was found that a complex of acrylamide and Ni^{2+} is suitable for the electrochemical determination of acrylamide. Ammonia buffer of pH = 9.5 was found to provide convenient conditions for the determination. Optimal concentration of Ni^{2+} was 500 μmol/l. The sample preparation procedure was optimised. The best results were found for an ethanol/water mixture (1:2) and pH = 1.4. The samples were extracted in an ultrasound bath, and after centrifugation 0.2 ml of the extract was taken for the measurement. Voltammetric measurements were done using the hanging mercury drop electrode. The peak height was a function of acrylamide concentration and deposition time. The accuracy of the method was verified by the use of standard reference materials.

Keywords: food contaminant; electroanalysis; sample preparation

Acrylamide (AA) is a commercially produced chemical substance which can be naturally formed in foods rich in starch at high temperatures. The major pathway of AA formation is the reaction between an amino acid and a carbonyl group of a reducing sugar, known as Maillard's reaction. AA is present in many types of food, especially in potato chips, biscuits, bread, cereals, and coffee (Andrzejewski *et al*. 2004; Taeymans *et al*. 2005; Claus *et al*. 2008). The non-dietary sources of acrylamide exposure are known, especially cigarette smoke and cosmetics (Ötles & Ötles 2004).

Acrylamide can be absorbed by animals and humans via ingestion, inhalation, or through the skin. Regardless of the absorption route, AA is distributed relatively rapidly to all tissues. It is found mainly in the thymus, liver, heart, brain, kidneys, placenta, and in breast milk. The major metabolite, glycidamide, is formed due to the oxidation of AA by cytochrome P450. AA and glycidamide can bind haemoglobin, serum albumins, DNA, and enzymes *in vivo*. AA produces neurotoxic effects in humans and in animals. Typical symptoms of acrylamide exposure include ataxia and skeletal muscle weakness (Capuano & Fogliano 2011).The International Agency for the Research on Cancer (IARC) has classified acrylamide in Group 2A (probable carcinogen to humans). It has been confirmed that AA causes cancer in mice and rats. It is a multi-organ carcinogen, causing tumours of lungs, uterus, mammary gland etc. (Törnquist 2005). The genetic toxicity and the reproductive toxicity have been proven in a variety of biological assays (Von Mühlendahl & Otto 2003; Törnquist 2005; Capuano & Fogliano 2011).

Analytical methods suitable for the determination of acrylamide in food have been explored.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6215712402), and by the IGA VFU Brno, Project No. 72/2011/FVHE.

Gas chromatography (GC), high performance liquid chromatography (HPLC), and capillary electrophoresis (CE) have been used for the determination of acrylamide. HPLC is used most often. Mass spectrometry (MS) is the standard detection technique (Keramat 2001; Zhang *et al*. 2005; Tekkeli *et al*. 2012).

AA is not formed uniformly on the food surface, therefore differences occur in the AA content in individual samples. For this reason, thorough homogenisation of the sample is a very important step (Castle & Eriksson 2005). With chromatographic methods, a representative sample is taken and an internal standard is added after homogenisation. The extraction and derivatisation of acrylamide are the next steps. Water or organic solvents are used as extraction reagents (Castle & Eriksson 2005). The extraction is carried out at ambient temperatures of about 20–25°C. Higher temperatures have not proved to be more effective (Castle & Eriksson 2005).

The GC methods are based on the derivatisation of the analyte with potassium bromate or potassium bromide. A method without derivatisation was also reported for various heat-processed foodstuffs (Dunovská *et al*. 2006). Clean-up after derivatisation is used to remove excess bromine. The analyte separation and quantification is the last phase of the determination process. The detection limit (LOD) for the GC method is 1–2 μg/kg (Wenzl *et al.* 2003). Other techniques for GC detection have also been reported. GS coupled with electron capture detector (GC-ECD) was developed and applied for the rapid determination of acrylamide in fried foods (Zhang *et al*. 2006). Gas chromatography with positive chemical ionisation tandem mass spectrometry (GC-PCI-MS-MS) was used to measure AA in aqueous matrices from French fries and potato crisps (Lee *et al*. 2007). In this study, a solid phase microextraction (SPME) was used to extract AA that was finally measured without derivatisation. AA was analysed also by GC with flame ionisation detection (GC-FID) in potato chips (Pedersen & Olsson 2003).

The LC method coupled with MS-MS detection is the most preferred for the determination of AA in laboratories. Many laboratories use HPLC methods (Tekkeli *et al*. 2012). The analytical procedure involves the extraction, clean-up, LC separation and detection. Tandem mass spectrometry (MS-MS) is a commonly used method of detection (Wenzl *et al*. 2003). The method has been successfully used for the determination of AA in many types of high-risk foods such as potato and cereal products, cocoa, coffee, and tea, as well as in traditional foods of many countries (Andrzejewski *et al*. 2004; Bermudo *et al*. 2008; Liu *et al*. 2008). HPLC coupled to diode array detector (LC-DAD) was used for the determination of acrylamide in potato-based foods at low levels (Gökmen *et al*. 2005). HPLC procedure with an ultraviolet detector has also been described for the determination of AA in foodstuff (Paleologos & Kontominas 2005).

Electrophoretic techniques have been also used for the analysis of AA in foods (Tekkeli *et al*. 2012). Microemulsion electrokinetic chromatography with UV detection has been applied to measuring AA levels in samples of homemade French fries (Bermudo *et al*. 2004). Methodology for the determination of AA by capillary zone electrophoresis (CZE) has also been introduced. Homemade French fries, breakfast cereals, and biscuits were measured (Bermudo *et al*. 2006). Non-aqueous capillary electrophoresis method (NACE) with diode array detection was applied to measure AA in processed food. These methods are simple, rapid, and inexpensive as compared with the chromatographic techniques (Baskan & Erim 2007; Tezcan & Erim 2008).

Electrochemical methods are widely used in the environmental assessment. They are commonly used for the determination of inorganic ions and molecules (heavy metals, noble metals), organic substances (e.g. nitrogen and oxygen derivatives, pesticides, chlorinated polyaromatic hydrocarbons). The advantages of the electrochemical determinations are the low cost of equipment and materials. The greatest advantage is unquestionably the relatively short measuring time including the sample preparation (Wang 2001).

The possibilities for voltammetric determination of AA have been already described. The differential pulse polarographic method (DPP) was used for the determination of AA in aqueous solutions. The method, however, was not used for the analysis of food (Niaz *et al*. 2008). The amounts of acrylamide in potato chips were measured by square-wave voltammetry (Zargar *et al*. 2009).

In our study, we propose the use of adsorption differential pulse voltammetry for the determination and quantification of AA content in food samples. The sample preparation procedure is showed and the reference material is measured.

MATERIAL AND METHODS

The measurements were performed by an Autolab measuring unit (Eco Chemie B.V., Eindhoven, the Netherlands) and the polarographic analyser VA Stand 663 (Metrohm, Herisau, Switzerland) equipped with the software Nova 1.5 (Eco Chemie B.V., Eindhoven, the Netherlands) for the measurement evaluations. The polarographic equipment was used in the following arrangement. The hanging mercury drop electrode as the working electrode, the Ag/AgCl reference electrode (3 mol/l KCl), and the glassy carbon electrode as the auxiliary electrode. Mercury (polarographic grade) and acrylamide (electrophoresis grade) were supplied by Merck (Darmstadt, Germany). As the standard of nickel, the solution Astasol (Analytika, Prague, Czech Republic) was used (1.000 ± 0.005 g/l). Argon (Linde Technoplyn a.s., Prague, Czech Republic) was used to remove the dissolved oxygen from the solutions. All other chemicals were of analytical grade (Merck, Darmstadt, Germany). Ultrapure water (GenPure; TKA GmbH, Niederelbert, Germany) of specific conductivity < 1 µS/m was used to prepare all solutions.

The most suitable conditions for the sample preparation were investigated after optimisation of the methods. Rusk (European Reference Material ERM®-BD274, Germany) and crispbread (European Reference Material ERM®-BD272, Germany) were used as the standard reference materials. Samples of bake rolls (Bake rolls salt, 7 Days), potato chips (Bohemia Chips salt), and crackers (Tuc mini original) were purchased from a local supermarket. Five grams of each food sample were used for the extraction. All samples were extracted in 50 ml of ethanol/water (1:2) mixtures. The pH range was adjusted by the addition of 0.55 ml of 30% hydrochloric acid. The samples were extracted in an ultrasonic bath (Sonic 10; Polsonic, Poznań, Poland) at 60°C for 1 h and finally centrifuged at 4000 rpm for 20 min (Table Top Centrifuge Z 300; HERMLE Labortechnik GmbH, Wehingen, Germany). The extracts were immediately taken for the voltammetric measurements.

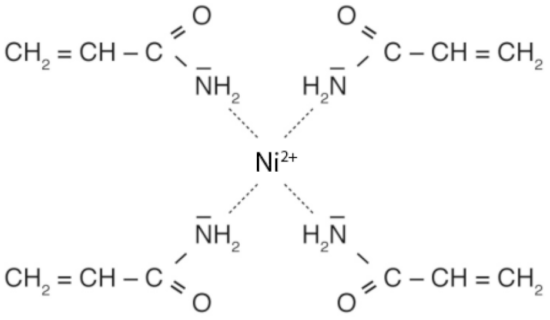

Figure 1. Expected form of the acrylamide complex with Ni^{2+}

The analysis of AA was done by the technique of adsorptive stripping voltammetry. The principle of the method is based on the formation of AA-Ni^{2+} complex. Its possible structure can be seen in Figure 1. Such a complex seems to be suitable for adsorptive voltammetric measurements. A similar approach was used in AA analysis by the method of square-wave voltammetry, in which the transitional metal cobalt was used (Zargar *et al*. 2009).

All samples were measured in ammonia buffer (pH = 9.5) containing 500 µmol/l of Ni^{2+}. Oxygen was removed by high purity argon 4.6 UN 1006, GA260 (Linde Gas, Prague, Czech Republic). Voltammograms were recorded from the initial/deposition potential E_d = –0.72 V to the final potential E_f = –0.05 V. The scan rate (SR) was 5 mV/s, deposition

Table 1. Concentrations of acrylamide in standard reference materials and real samples

Sample	Certified (µg/kg)	*n*	95% CI (µg/kg)	SD (µg/kg)	RSD (%)
Standard reference materials					
Rusk BD-272	72 ± 4	3	77 ± 10	4.7	6.1
Crispbread BD-274	980 ± 90	4	992 ± 113	92.8	9.3
Real samples					
Bake rolls		3	318 ± 14	6.5	2.0
Potato chips		3	709 ± 5	2.4	3.3
Crackers		3	250 ± 4	1.8	0.7

n – number of samples; CI – 95% confidence interval; SD – standard deviation; RSD – relative standard deviation

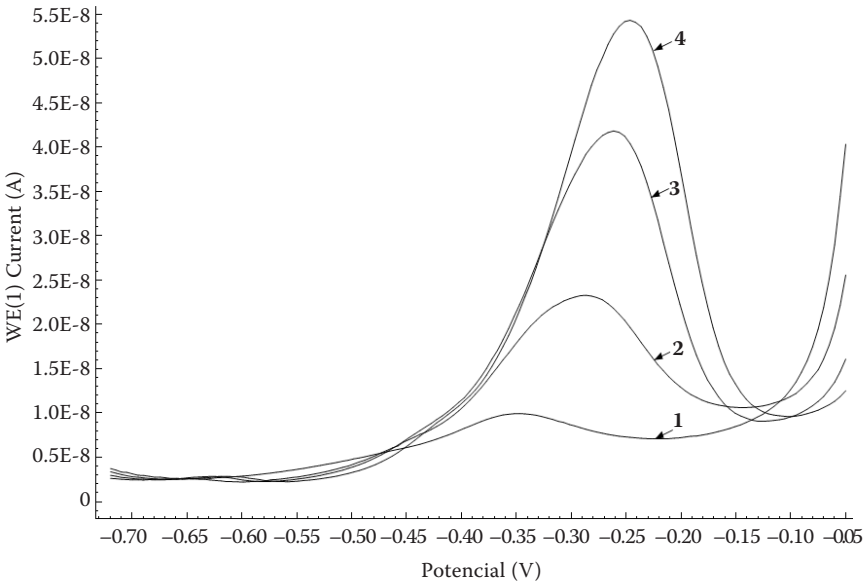

Figure 2. Voltammetric curves of AA in rusk

E_d (deposition potential) = –0.72 V; E_f (final potential) = –0.05 V; SR (scan rate) = 5 mV/s; PH (puls height) = 50 mV; T_d (deposition time) = 300 s; curves: **1** – sample, 200 µl; **2** – standard addition 1.56 ng AA; **3** – standard addition 3.13 ng AA

time (T_d) 300 s, puls height (PH) 50 mV, and drop size 3 (instrumental setting). The method of standard additions for the analyte determination was used: 15 ml of the ammonia buffer were pipetted into the voltammetric cell together with 0.2 ml of the sample extract and the first measurement was done. Thereafter two additions of acrylamide standard solution (c = 0.001 mmol/l) containing Ni^{2+} (c = 0.001 mmol/l) were measured. The electrodes were after each measuring procedure thoroughly rinsed with ultra pure water and dried with filter paper. All measurements were performed at room temperature.

RESULTS AND DISCUSSION

The peak heights were found to be a function of both acrylamide concentration and the deposition time. The measurement parameters were thoroughly checked and the above-mentioned values gave the best results. Linear relationship between the currents of peak maxima and AA concentrations was found. The detection limit of the method was found to be 12 µg/kg using the measuring procedure described above.

One of the aims of the work was to develop a sample preparation procedure. Chemical properties of acrylamide were an important factor for the approach in the sample preparation. AA is highly soluble in water, low alcohols, and other polar organic solvents. The extraction was performed in water/ethanol mixture (2:1 v/v). This composition was found to be the most suitable providing the best voltammograms as compared with the extraction solutions of different composition. The effect of pH was also evaluated and the pH = 1.4 was found to be the optimum. The extraction of the samples in alcohol/water mixture in the acidic medium proved to be adequate for the determination of acrylamide.

The samples of the standard reference materials (Rusk BD-272 and Crispbread BD-274) were analysed in order to confirm the accuracy of the method. Samples of bake rolls, potato chips and crackers were chosen for the analyses as they represent the

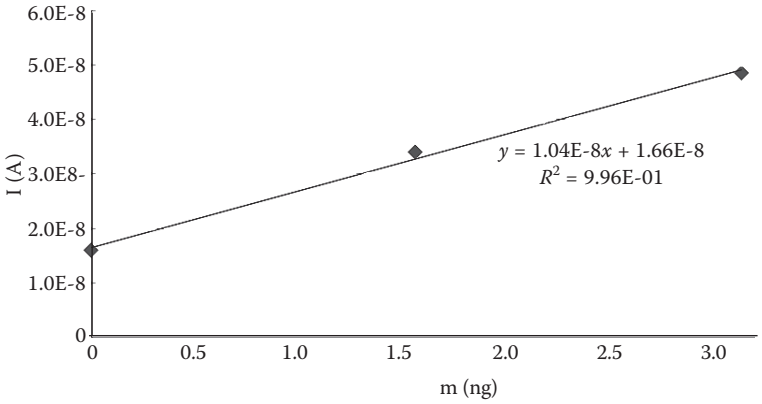

Figure 3. Dependence of peak heights on the concentration of AA in rusk

Table 2. Accuracy checking in real samples using acrylamide standard addition

Sample	*n*	95% CI (μg/kg)	Added AA (μg/kg)	Recovery (%)
Bake rolls	3	318 ± 14	318	98.7
Potato chips	3	709 ± 5	709	99.7
Crackers	3	250 ± 4	250	98.8

n – number of samples; AA – acrylamide; 95% CI – confidence interval

risk food containing higher amounts of acrylamide (see Taeymans *et al*. 2005). Additionally, in these samples the accuracy was also checked using the standard addition aproach.

The ammonia buffer of pH 9.5 of buffer and Ni^{2+} concentration of 500 μmol/l proved to be the most suitable for the voltammetric measurements. Under the above given conditions, both the peak heights and peak areas of acrylamide were well-defined and well-resolved.

An example of voltammogram and its evaluation is given in Figures 2 and 3. The linear dependence can be clearly seen. The measured values were 77 ± 10 μg/kg for rusk and 992 ± 113 μg/kg for crisp bread. The concentrations of acrylamide found in these materials are in a good agreement with the data specified in the certificate (Table 1). The peak heights were a linear function of both the acrylamide concentration and the deposition time.

The concentrations found in real samples of bake rolls, potato chips, and crackers are shown in Table 1. Well-defined peaks were observed in voltammograms measured for these samples. The calculated recoveries of AA in the samples with known standard additions of AA are close to the required 100% and give evidence for a good accuracy of the voltammetric method described above (Table 2).

The voltammetric procedure seems to be a reliable, sensitive, rapid, and low-cost analytical technique for the determination of acrylamide in food and feed. The usability of the proposed method for the analysis of some other kinds of samples important in view of their AA content needs to be further checked.

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Received for publication June 29, 2012
Accepted after corrections March 4, 2013

Corresponding author

Prof. RNDr. Emanuel Šucman, CSc., Veterinární a farmaceutická univerzita Brno, Fakulta veterinární hygieny a ekologie, Ústav biochemie, chemie a biofyziky, Palackého 1/3, 612 42 Brno, Česká republika; E-mail: sucmane@vfu.cz