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QUERCETIN OR GLYCINE REACTION PRODUCTS WITH ACRYLAMIDE: A QUANTATIVE APPROACH TO ACRYLAMIDE DETECTION USING A PENCIL GRAPHITE ELECTRODE

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In this article, the equimolar mixtures of acrylamide (AAm) and quercetin or glycine in aqueous solution at different pH, and after heating (100 °C for 60 min) have been studied. (Bare) pencil graphite electrode (PGE) in combination with square wave voltammetry were applied to the qualitative identification of the products. An interaction of AAm and quercetin at pH 2.0 resulted in the increase of reduction current at +128 mV, and an additional shift to +140 mV before and after heat treatment, respectively. PGE gave rise to only response for heat-treated AA/glycine mixture at pH 2.0, showing a higher oxidation current at positive potential and a higher reduction current at negative potential. In neutral media, two oxidation peaks were observed for heated AAm/glycine mixture. Theoretical considerations have been proposed.

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Introduction

Acrylamide (AAm) is formed in foods by heating as a consequence of asparagine degradation in the presence of the reducing sugars and other carbonyl compounds [1]. Since the substance is consensually genotoxic and carcinogenic, AAm was classified as a Category 2 carcinogen and Category 2 mutagen by the European Union legislative [2]. Therefore, the quantification of AAm in various food matrices is of continuing interest among researches; liquid or gas chromatography with MS-detection having prevailed among the methods of choice for the AAm quantification [3]. Separation techniques represent selective and sensitive tools for its determination; however, they require expensive laboratory equipment, special environment (air conditioning), and highly pure solvents. In contrast, electrochemical methods are capable to determine AAm with high accuracy and sensitivity at low costs and minimal consumption of solvents with a possibility of miniaturization toward portable lab-on-chip units.

Acrylamide is capable of reduction at relatively high negative potentials, which limits the direct electrochemical determination of AAm to the use of mercury-based working electrodes. A polarographic method was successfully applied to direct determination of AAm in water sample [4], and the formation of AAm-cobalt [5] or AAm-nickel [6] adducts was exploited for quantification of native substance in food samples using the hanging mercury drop electrode. Due to the health and environmental consequences of using mercury in industry and research, novel electrode materials are sought and the respective attempts examined. Fot instance, it has been shown that AAm forms an adduct with haemoglobin (Hb) — as a result of reaction with the α-NH_2 group of N-terminal valine in the Hb molecule — when experimenting with a carbon paste electrode [7]. This chemical reaction could also be used by other researchers for the determination of AAm by employing a sensor with gold nanoparticles [8] or a multiwalled carbon nanotubes / copper nanoparticles / polyaniline hybrid film deposited onto a pencil graphite electrode [9]. The strong affinity of the AAm molecule toward the nucleophilic groups, such as thiol [10] or carboxyl [11] has then been utilized in measurements with carbon-based electrodes, as well as by using the sol-gel electrodes with molecularly imprinted polymer membranes [12, 13]. Also, an enzymatic biosensor based on the detection of NH_4^+ ion released by the activity of microbial amidase was developed [14]. Finally, a carbon paste electrode modified with RuO_2 for indirect determination of AAm in a LiCl-based supporting electrolyte was described by the collective of authors [15]. This has confirmed that various organic molecules with nucleophiles group can react with AAm, including polyphenolic compounds [16], and amino acids [17-20].

In this research, an attempt to find the proper conditions for the determination of AAm via its reaction with nucleophiles at the bare pencil graphite electrode (PGE) is described. Since AAm did not give rise to any response at the

surface of PGE, we have at least tried to qualitatively detect the products of such a reaction at various pH accelerated by the increased temperature.

Materials and Methods

Reagents and Equipment

All the chemicals were purchased from Sigma-Aldrich and deionised water ($G \leq 0.055$ μS) used throughout this study. All the measurements were performed in the presence of dissolved oxygen. Britton–Robinson buffer solutions (B-R, 0.04 mol l^{-1}) were used as a supporting electrolyte. Stock solution of acrylamide (1.0 M AAm) was prepared in B-R buffer solutions at various pH (2.0, 5.0, 7.0 and 10.0) and stored in laboratory temperature for one month. Similarly, stock solutions of glycine and quercetin (both 1.0 M in concentration) were prepared by dissolving the appropriate amount in B-R buffer solutions. Since quercetin is poorly soluble in water, mixed ethanol/B-R buffer solutions (1:1) were used.

A three-electrode system, consisting of pencil graphite working electrode, Ag/AgCl/3M KCl as the reference, and a platinum wire as the counter electrode, was connected to a potentiostat (PalmSens, Ivium Technologies, Utrecht, The Netherlands) was used for all electrochemical measurements. Hard and black (HB) pencil graphite lead with a diameter of 0.5 mm was obtained from Koh-i-noor Hardtmuth a. s. (České Budějovice, the Czech Republic). In this study, a common pencil was used as a holder for the graphite lead. Electrical contact was accomplished by wrapping a wire around the metallic part of the holder. For each individual measurement, a new graphite lead (with fresh surface) was used with a 10-mm long column immersed into the working solution.

Measurement Conditions

Square wave voltammetry (SWV) technique in the anodic and cathodic regime was used for measurements with the mixture of AAm (1×10^{-3} mol l^{-1}) and the testing substance (10^{-3} mol l^{-1}) at pH 2.0, 5.0, 7.0, and 10.0. The SWV mode was applied to measure the voltammetric characteristics of reaction mixtures before and after the temperature treatment in a block heater (100 °C for 60 min). The conditions for the SWV-potential ramp were as follows: potential range: –1.0 to +1.3 V vs. ref. and *vice versa* (i.e., applied either as anodic or cathodic scan), potential step increment: 0.004 V, pulse amplitude: 0.05 V, and SW-frequency: 25 Hz. An accumulation step was carried out in an open-circuit arrangement for 5 min at regular stirring (350 rpm), followed by the SWV measurement. These experiments were done doubly, and performed at laboratory temperature.

Results and Discussion

Polyphenolic compounds are known to prevent the formation of acrylamide upon heating by various reactions. On the contrary, once acrylamide is formed, its elimination is blocked by the polyphenolic free radicals leading to the polymer products [16]. Quercetin was chosen as a representative compound of a kind in this research belonging to the class of flavonoids with known beneficial effects on the health [21]. Reportedly, quercetin is readily oxidized on the surface of PGE, giving rise to a pair of oxidation peaks occurred at positive potentials in acidic media [22].

In this study, the measurement with PGE did not exhibit a distinct response in the fresh solution of quercetin at pH 7.0

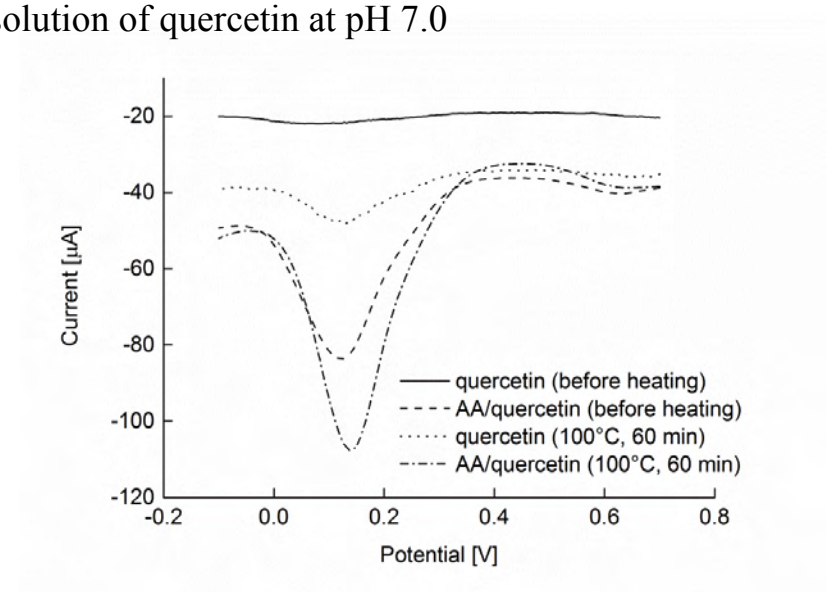

Fig. 1 Square wave voltammograms of quercetin and acrylamide / quercetin mixture (1×10^{-3} mol l^{-1}) in Britton–Robinson buffer (pH 7.0) using the bare pencil graphite electrode after 5 min accumulation (at 350 rpm) in open circuit. Experimental conditions: scan from +1.3 V to –1.0 V, potential step increment 0.004 V, amplitude 0.05 V, frequency 25 Hz

As can be seen from Fig. 1, SWV of AAm/quercetin mixture recorded before heating has resulted in a reduction peak at +128 mV (with a magnitude of 35.0-40.0 µA). After a treatment at 100˚C for 60 min, a small reduction peak at + 140 mV (with 4.8-6.4 µA) appeared for quercetin alone (at pH 7.0). The heated mixture of AAm/quercetin gave significantly higher reduction current (56.0-65.0 µA) at the same potential. In this experiment, a positive shift of the potential for the reduction current in the case of heated AAm/quercetin sample was observed compared to the response for non-heated AAm/quercetin sample. The presence of reduction current at +128 mV and an additional increase (and shifting) at +140 mV for non-heated and heated AAm/quercetin solution, respectively, might indicate a formation of the electroactive species; this process being pronounced by elevated temperature. In literature, it has been described in detail that the thermal

degradation of quercetin at physiological pH (in the presence of oxygen) is initiated by the formation of chalcone structure with the subsequent attack of nucleophilic species [23]. The similar reaction pathways have been documented for UV-light induced degradation of quercetin in solution [24]. Since the respective products seemed to be consumers of nucleophiles, the reaction of double C-C bond of AAm with any of known degradation products of quercetin can unlikely occur [23]. The presence of reduction peak for both heat untreated and heat treated mixtures of AAm/quercetin may imply the electrochemical reduction of polymer-like structure [16]. Acrylamide is well known to behave like a monomer unit for the preparation of AAm polymers using electrochemical initiation [25], and polyphenolic compounds easily form polymer-like structure on the electrode surface. Therefore, we suppose that the formation of polymer-like structure between AAm and quercetin is the case in our study and responsible for electroactivity of the samples chosen. No significant response of PGE was observed in acidic (pH 2.0) and alkaline (pH 10.0) environment.

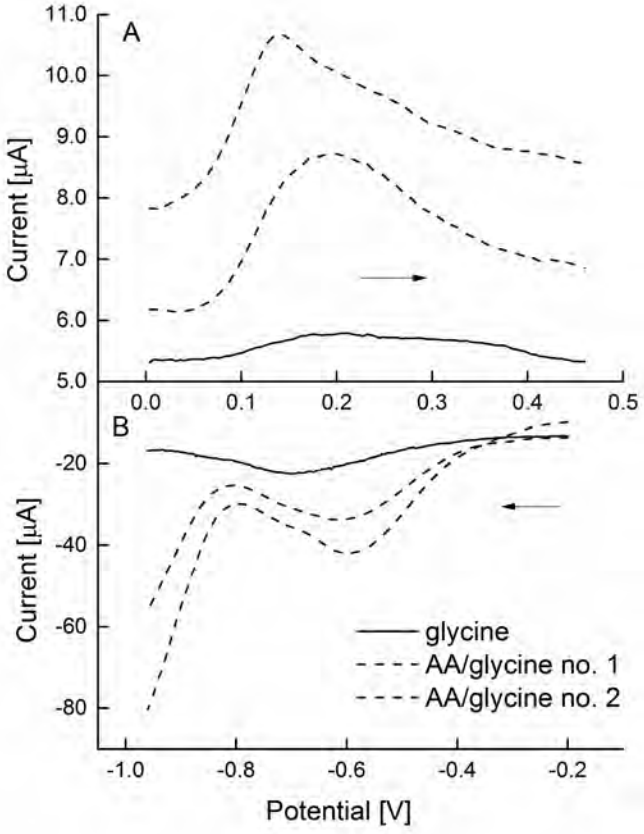

Fig. 2 Square wave voltammograms of heated (100 °C, 60 min) glycine and acrylamide / glycine mixture (1×10^{-3} mol l^{-1}) in Britton–Robinson buffer (pH 2.0) using the bare pencil graphite electrode after 5 min accumulation step (350 rpm) in open circuit. Experimental condition: potential range from +1.3 to –1.0 V, potential step increment 0.004 V, amplitude 0.05 V, frequency 25 Hz. Dash curves indicate AAm/glycine voltammograms in duplicate

As previously documented, the heating of AAm with various nucleophiles has resulted in a decrease of acrylamide content in the solutions tested [17-20]. The reactivity varied with temperature, the structure of nucleophile, time, and pH value of the working solution. In our research, the glycine neither alone nor in mixture with AAm gave response in PGE, when measured in solutions at various pH before temperature treatment. However, SW-voltammogram of heated mixture of AAm/glycine (at pH 2.0) showed the oxidation current at a potential ranging from +140 to +180 mV in comparison with the heated glycine solution (Fig. 2A). In the reverse scan, glycine alone gave a small reduction current at –680 mV, whereas the mixture of AAm/glycine was reducible at –590 mV (with 12.0-19.0 μA) on the surface of PGE (see Fig. 2B).

At extreme acidic conditions — namely, in 1M H_2SO_4 —, glycine was found to decompose to various intermediates that subsequently adsorbed on the surface of Pt working electrode, and then involved in the electrooxidation process [26]. Two alternative pathways were found in the study of reaction mechanisms after heating AAm and glycine: the first one was (i) Michael addition and oxidation followed by (another) Michael addition [20]. Secondly, Zamora et al. [17] have revealed that 3-(alkylamino)propionamides (Fig. 3A) are formed in the reaction between AAm and glycine in the acidic environment, which may be theoretically attributed to the proper electrochemical process at the PGE.

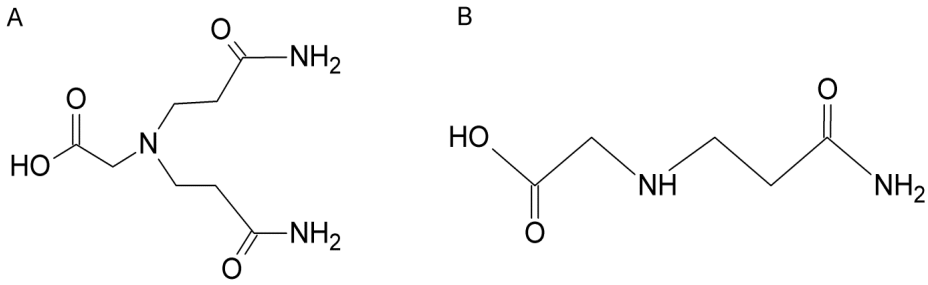

Fig. 3 The reaction product of acrylamide and glycine identified at higher temperature in A) acidic and B) neutral condition as described by Zamora *et al*. [17] and Zhu *et al*. [19], respectively

The voltammograms obtained from two separately prepared AAm/glycine solutions exhibited a behaviour similar to that observed at the PGE electrode. In the respective voltammograms, two oxidation peaks were detected for the adduct of glycine-AAm at 7.0 pH; see Fig. 4.

The first one appeared in the negative potential region, at –110 mV, the second at +88 mV vs. ref. Reportedly, the AAm molecule was able to react with nucleophilic compounds at a neutral pH and the reaction kinetics increased with elevated temperature [18]. Zhu *et al*. [19] identified 2-((3-amino-3-oxopropyl) amino)acetic acid as the major reaction product between glycine and AAm. Although further identification of the reaction product(s) has not been performed

in our study, the structure of the above-mentioned compound may imply its possible electroactivity (see Fig. 3B).

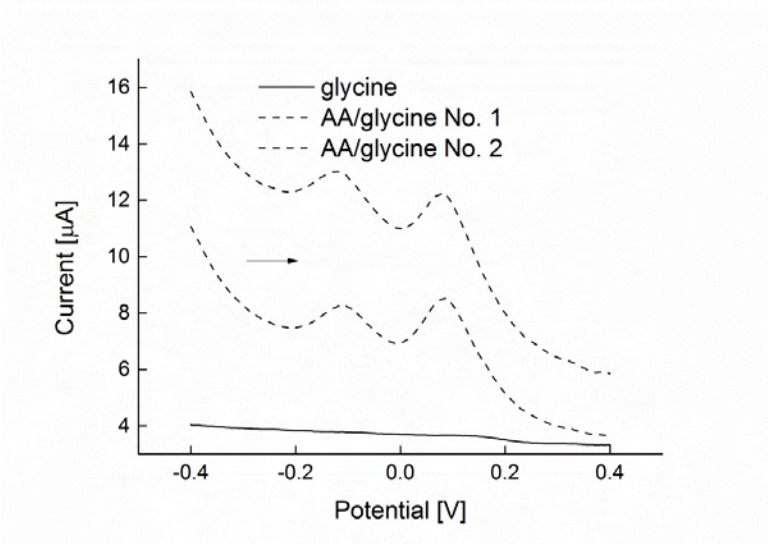

Fig. 4 Voltammogram of acrylamide / glycine mixture (1×10^{-3} mol l^{-1}) heated at 100 °C for 60 min in Britton–Robinson buffer (pH 7.0) using the bare pencil graphite electrode after 5 min accumulation (at 350 rpm) in open circuit. Experimental condition: potential range from –1.0 to +1.3 V, potential step increment 0.004 V, amplitude 0.05 V, frequency 25 Hz. Dash curves indicate AAm/glycine voltammograms in duplicate

Conclusion

In mixtures of acrylamide with quercetin or glycine, the reaction products observed at the bare pencil graphite electrode have exhibited noticeable electrochemical activity. Although the specific electroactive compound was not identified within the study presented herein, an attempt to explain the redox properties of such reaction mixtures has been made. It seems that the reaction mechanism between acrylamide and glycine corresponds to an addition to the double C-C bound of acrylamide according to the literature cited. Quercetin and acrylamide might probably be subjected to the polymerization-like reactions, although it was not confirmed by other electrochemical methods (e.g., cyclic voltammetry).

Our preliminary results have shown that, at particular conditions, the bare pencil graphite electrode is capable to detect electrochemically the active species after reactions of acrylamide with quercetin or glycine. A further research is needed to elucidate the yield of these reactions and to determine the sensitivity with respect to various acrylamide concentrations.

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