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Application of hot platinum microelectrodes for determination of flavonoids in flow injection analysis and capillary electrophoresis

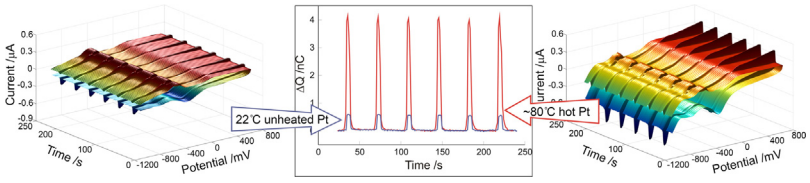
Jolanta Magnuszewska*, Tadeusz Krogulec

Institute of Chemistry, University of Bialystok, ul. Hurtowa 1, 15-399 Bialystok, Poland

HIGHLIGHTS

- The unheated and hot platinum microelectrodes were used for flavonoids detection.
- Cyclic voltammetry detection of flavonoids under FIA and CE condition was achieved.
- Increase of analytical signal in FIA and CE at hot Pt microelectrode is observed.
- Flavonoids were determined in natural plant and in pharmaceutical.
- The LOD are better than obtained under spectrophotometric or amperometric detection.

GRAPHICAL ABSTRACT



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ABSTRACT

The determination of quercetin and rutin by flow injection analysis (FIA) and capillary electrophoresis (CE) using electrochemical detection was described. These flavonoids were determined at normal (unheated) and hot platinum microelectrodes using cyclic voltammetry. When quercetin or rutin is reaching the platinum electrode, a change of the current in the region of the platinum oxide formation is observed. Integration of the current changes in this in this region creates analytical signals in the form of peaks. An increase of temperature to about 76 °C in a small zone adjacent to the microelectrode causes an increase of the analytical signal by more than 6 times under FIA conditions. This method enables the use of hot microelectrodes as detectors in HPLC or CE. In CE the improvement of the analytical signal at hot microelectrodes is smaller than in FIA and increase only 1.3–3.4 times. Heated microelectrodes were used for analysis of the flavonoids in natural samples of the plant (extract of sea buckthorn) and a pharmaceutical preparation (Cerutin).

1. Introduction

First attempts at using hot electrodes were carried out by Ducret and Cornet [1], and later by Harima and Aoyagui [2] and Gabrielli et al. [3]. In these experiments, a resistance heating of the electrodes made of thin plates or wires was employed. Those studies gave the basis for an original design of heating electrodes introduced and developed by Gründler at al. [4]. Recently, this technique was used by Wachholz et al. [5] for analytical purpose.

Gründler proposed two techniques of heating up electrodes. The first one required a continuous heating of the electrode in order to keep its temperature constant at all time [6]. The time of the heating as well as that of the experiment itself were very short and lasted only a few seconds. According to Gründler, the heating process was easy to carry out, as it required only a slight adaptation of commercially available electronic devices. This technique, however, allowed performing experiments exclusively below the boiling

* Corresponding author. Tel.: +48 85 7457829; fax: +48 85 7470113.
E-mail addresses: jolmag@uwb.edu.pl (J. Magnuszewska), krogulec@uwb.edu.pl (T. Krogulec).

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point of the solution. The other method proposed by Gründler called Temperature Pulse Voltammetry (TPV) [7] is similar to difference pulse voltammetry (DPV). Gründler's TPV made use of short pulses of heat at frequency of 100 kHz. In this technique a temperature slightly above the boiling point of the electrolyte can be reached. Wachholz at al. [5] proposed another model of heating called Temperature Pulse Amperometry (TPA). It is based on amperometric measurements conducted during a short pulse of applying heat. This method of heating was used to determine picric acid [8]. Heating methods similar to those used by Gründler are now used by other researchers [9].

Hot-wire electrodes have been used in the analytical methods to determine metals and semimetals [10], anions [11], ascorbic acids [12] and glucose [13]. Furthermore, Korbut at al. made attempts to employ hot-wire electrodes to determine flavonoids [14]. He applied the electrodes in flow conditions using amperometry as a detection technique.

Baranski [15] proposes another method of obtaining hot microelectrodes. His method, instead of heating up electrode material, entails heating a small aqueous zone close to the surface of the disk microelectrode by means of alternating current of high amplitude up to 2 V and high frequency of 0.1–2 GHz. In water solutions, the highest temperature of the zone in the vicinity of the surface of the electrode disk (25 μm in diameter) can significantly exceed the boiling point and be maintained for a long time (over 15 min). In the process, it is essential to determine the temperature at the surface of the microelectrode. Baranski [15] proposes to determine of the temperature from the slope of the steady-state current-potential curves. In another work, Boika and Baranski [16] reported that more reliable values are obtained from a change in the half-wave potential of the voltammetric curve.

It is worth to emphasize, that Baranski's method is partly similar to microwave electrochemistry initiated and developed by Compton and coworkers [17–21]. In both techniques a solution spot close to electrode, not electrode itself, is heated.

The flavonoids are one of the most important classes of bioactive polyphenols [22]. Their basic structure is 2-phenyl chromane. They are divided in different groups (flavonols, flavones, anthocyanidins etc.) and they either occur as aglycons (i.e. rutin) or as C- or O-glycosides (i.e. quercetin). The flavonoids are ubiquitous in plant kingdom and are also known as components (natural pigments) in fruits, vegetables, flowers, green tea, wine, seeds and berries. The flavonoids have been widely used in medicine due to their capability of sealing up and strengthening blood vessels as well as anti-inflammatory, antibacterial, antiviral and anti-oxidant activities [23].

Both carbon [24–26] and polycrystalline platinum [27,28] electrodes have been used to investigate electrochemical properties of quercetin and rutin as well as to identify their oxidation products. Furthermore, electrodes made from a variety of materials, such as carbon paste [29], carbon nanotubes [30–32], glassy carbon electrode coated with graphene nanosheets, chitosan and a poly (amido amine) dendrimer [33,34], DNA-modified glassy carbon [14], carbon paste modified by enzymes [35], mercury electrode [36] or gold electrode modified with CeO_2 [37] have been used as sensors.

In order to determine flavonoids, flow injection analysis (FIA) has been occasionally used in combination with chemiluminescent [38] or electrochemical detection on carbon electrodes [39].

A large number of works can be found on the chromatographic separation and identification of flavonoids using spectrophotometric [40] and electrochemical detection techniques [41]. Among them, a considerable number of papers have been devoted to determining flavonoids separated using capillary electrophoresis (CE) with amperometric detection on carbon electrodes [42] and spectrophotometric detection in various types of wine [43], fruit juices [44], vegetable extracts [45], grains [46] and pharmaceuticals [47].

So far platinum electrodes have not been used for determination of quercetin or rutin by CE using electrochemical detection. This work presents FIA and CE experiments carried out on normal (unheated) and hot platinum microelectrode with voltammetric detection. Quercetin and rutin have served merely as an example to show the possibility of the use of Baranski's method in analytical practice.

2. Experimental

2.1. Reagents and chemicals

Quercetin, rutin, ascorbic acid (vitamin C), sodium tetraborate (borax) were purchased from Sigma, potassium heksacyanoferrate (II) and (III) from Chempur (Piekary Śląskie, Poland), ethanol (96%) and hydrochloric acid (36%) from POCh (Gliwice, Poland), sodium hydroxide from Aldrich. Rutin tablets (Cerutin, Polfarmex S.A. Kutno, Poland) labeled to contain 25 mg of rutin and 100 mg of ascorbic acid. All chemicals were of ACS grade.

Stock solutions of the flavonoids standards (0.01 mole L^{-1}) were prepared in 0.04 mole L^{-1} sodium tetraborate (pH 9.2). The studied solutions of analytes were prepared each day, just before use, by diluting the appropriate stock solutions with a sodium tetraborate solution.

All solutions were prepared with deionised water (KB-5522 DW from Cobrabid – AQUA, Warsaw, Poland). All electrochemical measurements were carried out at room temperature (about 22 °C), without removal of oxygen dissolved in the solutions.

2.2. Preparation of pharmaceutical sample

1 tablet of Cerutin (Polfarmex S.A. Kutno, Poland) was added into 25 mL solution of ethanol:sodium tetraborate (1:1, v/v) followed by 30 min ultrasonication for extracting rutin. Then, the solution was filtered through the filter paper (POCh Gliwice, Poland) and stored 30 day in refrigerator at 4 °C.

2.3. Preparation of sea-buckthorn Fructus hippophaës rhamnoides extract

2 g dry fruit sea-buckthorn *Hippophaës rhamnoides L.* ("Flos" Mokrsko, Poland) without seed was accurately weighted and added into 25 mL solution of ethanol:sodium tetraborate (1:1 *v*/*v*) followed by 30 min ultrasonication for extraction of rutin. The extraction was repeated three times, and liquid phase was collected in a 100 mL volumetric flask. Finally, the extract was diluted to volume with solution of ethanol: sodium tetraborate for further analysis and stored 30 day in refrigerator at 4 °C.

2.4. Electrodes and the experimental setup

In all experiments, a standard three-electrode electrochemical cell was used. The disk working electrodes were made by sealing platinum (10 μm in radius) microwires (Mennica Panstwowa Warsaw, Poland) into Corning Kovar Sealing glass tubing #7052 (World Precision Instruments). A lead was made by inserting the end of the micro-wire and a thicker copper wire into a stainless steel tubing ~1 cm long (cut off from a syringe needle, gauge 23) and squeezing the tubing with pliers. The glass tubing was then cut perpendicular to its length, and the electrode was polished with a 1000-grade and 2500-grade carborundum paper, and finally mirror like polishing was accomplished using 0.3 μm aluminum oxide finishing films (True View Products Inc.). The electrode surface was cleaned by replicate scans (for about 30 min at 20 V s^{-1}) between potentials of hydrogen and oxygen evolution in 0.05 mole L^{-1} H_2SO_4 and