Rev Anal Chem 30 (2011): 177–185 © 2011 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/REVAC.2011.105

Covalent grafting of three flavonoids onto the glassy carbon electrode surface by cyclic voltammetry

İbrahim Ender Mülazımoğlu[1,*], Zafer Üstündağ[2], Erdal Özkan[1] and Ali Osman Solak[3,4]

[1] Department of Chemistry, Ahmet Keleşoğlu Education Faculty, Selçuk University, 42090 Konya, Turkey, e-mail: iemulazimoglu@gmail.com
[2] Department of Chemistry, Faculty of Arts and Sciences, Dumlupınar University, Kütahya, Turkey
[3] Department of Chemistry, Faculty of Science, Ankara University, 06100 Tandoğan, Ankara, Turkey
[4] Department of Chemical Engineering, Faculty of Engineering, Kyrgyz-Turk Manas University, Bishkek, Kyrgyzstan

*Corresponding author

Abstract

Electrochemical oxidation of quercetin (QR), 3-hydroxyflavone (3HF), and baicalein (BN) and their grafting onto the glassy carbon (GC) electrode have been studied using the cyclic voltammetric (CV) technique. Electrochemical studies were performed within a one-compartment three-electrode cell at room temperature. The modification was carried out only in nonaqueous media, while the electrochemical characterization was done in both aqueous and nonaqueous media. In the nonaqueous experiments, 0.1 M tetrabutylammonium tetrafluoroborate in acetonitrile was used, whereas in the aqueous experiments, Britton-Robinson buffer solutions (pH=2) and 0.1 M KCl solutions were. Surface modifications were performed by CV for all compounds. The presence of QR, 3HF, and BN at the GC electrode surface was characterized by CV, electrochemical impedance spectroscopy, contact angle measurement, ellipsometry, and atomic force microscopy.

Keywords: flavonoid; electrochemical modification; surface characterization; contact angle measurements; ellipsometric thickness.

Introduction

The term *flavonoid* refers to a class of aromatic, oxygen-containing heterocyclic pigments widely distributed among higher plants as secondary metabolites. Flavonoids constitute one of the most characteristic classes of compounds containing hydroxyl groups attached to ring structures (Kuhnau 1976). Many flavonoids are easily recognized as flower pigments in most angiosperm families. However, their occurrence is not restricted to flowers but includes all parts of the plants. They constitute most of the yellow, red, and blue colors in flowers and fruits (Nematollahi and Malakzadeh 2003). Flavonoids are broken down into categories of isoflavones, anthocyanidins, flavans, flavonols, flavones, and flavanones (Peterson and Dwyer 1998). The chemical structure of flavonoids is based on a C_{15} skeleton, which consists of two phenyl rings and a heterocyclic ring (Robards and Antolovich 1997, Bohm 1998).

Several studies concerning the solution electrochemistry of flavonoids have been reported in literature (Zhu et al. 2002, Nematollahi and Malakzadeh 2003, Janeiro et al. 2005, Chen et al. 2009, He et al. 2009, Zare et al. 2009, Zielinska and Pierozynski 2009, Jin et al. 2010, Pierozynski and Zielinska 2010). Electrochemistry of the interaction of quercetin (QR) with DNA was investigated by Zhu et al. (2002) to infer the active part of QR that interacts with DNA and to explain the possible DNA-damaging activity of QR (Brett and Diculescu 2004). Nematollahi and Malakzadeh (2003) described the electrooxidation of QR in the absence and presence of benzensulfonic acid and 4-toluenesulfonic acid as nucleophiles to synthesize new sulfonyl derivatives of QR. Liu (2006) and Liu and Guo (2006) studied the interaction of QR with organized molecular assemblies of anionic surfactant, sodium dodecyl sulfate, and cationic surfactant, cetyltrimethyl ammonium bromide. Zare et al. (2005) studied the electrochemical behavior of QR and proposed a mechanism for the oxidation electrode reaction. Timbola et al. (2006) discussed the electrochemical oxidation of QR in hydroalcoholic solution on glassy carbon (GC) electrode. They proposed an electrode reaction scheme and analyzed the controlled potential electrolysis products.

The above-mentioned pertinent literature suggests very different electrochemical oxidation mechanisms and electrochemical behaviors of QR and other flavonoids. Most of the authors tried to imply a relationship between electrochemical behavior and antioxidant capacity of flavonoids in aqueous and nonaqueous media. Some physicochemical parameters, which are relevant for explaining the antioxidant capacity of flavonoids, can be determined by electrochemical measurements in solution. For example, half-wave potential ($E_{1/2}$) may be a useful parameter for inferring information on the scavenging activity of the flavonoids. The ease of electrochemical oxidation of a flavonoid is of importance for its antioxidant capacity. The rationale behind this idea is the assumption of the similar mechanism of electrochemical oxidation and free radical scavenging (Rice-Evans et al. 1997, Janeiro and Brett 2005).

Although some correlation can be found between solution electrochemical behavior and antioxidant capacity, it is more realistic to find such a relation in solid state of these

compounds. The study of solid-state electrochemistry of flavonoids offers many advantages such as strong H-bonding and different isomerism. Solid-state electroanalysis of flavonoids can be investigated either by mechanical immobilization or by covalent grafting at the surface of carbon or metal electrodes. The former methodology, the so-called voltammetry of microparticles, presents some disadvantages such as dissolution of immobilized molecules during electrochemical measurements and unknown amount of deposited microparticles at the electrode surface (Bond 1997, Janeiro and Brett 2005). Covalent grafting methods based on amine oxidation (Downard 2000), aryl diazonium salt reduction (Isbir et al. 2005), and oxidation in the presence of alcohols (Maeda et al. 1997, Downard 2000) have been employed to derivative the surfaces of carbon and metals.

To date, no report has been published on the covalent immobilization of flavonoids to the solid surfaces and on the electrochemical behavior of the solid state of flavonoids in nonaqueous media. Janeiro and Brett (2005) studied the electrochemistry of solid-state morin mechanically attached to the GC electrode. In a recent article, He et al. (2007) reported a QR-modified wax-impregnated graphite electrode for the detection of uric acid in the presence of ascorbic acid.

The aim of the present article is concerned with the covalent modification of GC electrode with QR, 3-hydroxyflavone (3HF), and baicalein (BN) in nonaqueous media by oxidation of these structurally related flavonoids at a GC electrode. In this study, we also investigate the electrochemical oxidation of flavonoids in nonaqueous media and propose a grafting mechanism of flavonoids onto the GC electrode surface.

Experimental

Reagents, chemicals, electrodes, and apparatus

All chemicals were of analytical reagent grade from Merck, Fluka, Riedel, and Sigma-Aldrich. Water of ultrapure quality with a resistance of 18.3 MΩ cm (Millipore Milli-Q purification system; Millipore, Bedford, MA, USA) was used in the preparation of aqueous solutions, cleaning of glassware, and polishing of electrodes. The modification and characterization experiments were performed in both aqueous and nonaqueous media. All flavonoid solutions used in the modification were 1 mM concentration in acetonitrile (MeCN) containing 0.1 M tetrabutylammonium tetrafluoroborate (TBATFB) as supporting electrolyte. Solutions were thoroughly deoxygenated by purging with purified argon gas (99.999%) for 10 min before the electrochemical experiments. Argon blanket was maintained over the solutions to supply an inert atmosphere during voltammetric measurements. All electrochemical experiments were performed at room temperature (25°C±1°C). Electrochemical surface modification experiments were performed in the 300- and 2800-mV potential range at a scan rate of 100 mV s^{-1} with 10 cycles. A traditional three-electrode cell system was used in all electrochemical experiments. In our experiments, Gamry Reference PCI4/750 series Potentiostat/Galvanostat/ZRA (Gamry Instruments, Warminster, PA, USA) and electrochemical analyzer with BAS Model MF-2012 (Bioanalytical Systems, West Lafayette, IN, USA) and Tokai GC-20 GC electrodes were used. Ag/Ag^+ (10 mM $AgNO_3$ in 0.1 M TBATFB) (BAS Model MF-2042) for nonaqueous media and an Ag/AgCl/3 M KCl (BAS Model MF-2063) for aqueous media were used as reference electrodes. A Pt wire counter electrode (BAS Model MW-1032) was used. As a buffering media, Britton-Robinson (BR) solution was used for adjusting the pH of the solutions between 1.81 and 11.98 using Jenway 3010 pH meter.

Cyclic voltammetric (CV) and electrochemical impedance spectroscopy (EIS) techniques were performed using a Gamry Reference PCI4/750 Potentiostat/Galvanostat/ZRA (Gamry Instruments) equipped with a BAS model C3 cell stand. Electrochemical characterization was performed with the same electrochemical devices and software used for the modification. Characterizations of the modified surfaces were recorded by EIS, contact angle measurement (CAM), ellipsometry, and atomic force microscopy (AFM) techniques. EIS experiments were carried out with a Gamry Reference PCI4/750 potentiostat in conjunction with EIS 300 software. EIS surface data were obtained in the 1-mM $Fe(CN)_6^{3-/4-}$ redox couple at the frequency range of 100.000–0.05 Hz at 10-mV wave amplitude.

The CAMs were carried out with a contact angle measuring system: G-III model Contact Angle Meter (Kernco Instrument, El Paso, TX, USA) at 25°C. The sessile drop method was used to measure the contact angle, and the drop was formed by depositing the liquid from above using a manual microsyringe on the GC electrode surface. Both the left and the right contact angles and drop dimension parameters were automatically calculated from the digitalized image. The reproducibility of contact angle results is in the range of ±0.5°.

Ellipsometer measurements were performed with a Nanofilm EP3 (Germany) imaging ellipsometer with a 532-nm laser (green light) reflection with 73°. The results of these measurements were recorded as an average of four computations in a 50×50-μm area. Four-zone autonulling procedure was used in thickness measurements. The pattern formed 1.0000, 1.4600, and 3.0841 values for *n* and 0.0000, 0.0000, and 1.7820 values for *k* to the air, organic layer, and graphite, respectively.

Preparation of the electrodes

GC electrodes were prepared for the modification experiments by polishing to gain a mirror-like appearance, first with fine wet emery papers (grain size 4000) and then with 1.0- and 0.3-μm alumina slurry on microcloth pads (Buehler, USA). After the initial polishing, GC electrodes were resurfaced using 0.05-μm alumina slurry. First, GC electrodes were sonicated in water and then in 1:1 (v/v) isopropyl alcohol and MeCN (Aldrich) mixture for 10 min. All of these steps were performed before the electrochemical experiments to avoid contamination of oxidation products and to obtain a clean renewed electrode surface.

Modification of GC electrodes

The electrochemical modification was performed in a conventional three-electrode system at room temperature. After cleaning and polishing, the GC electrode surfaces were dried with argon gas prior to modification. QR, 3HF, and BN solutions (1 mM) were prepared in 0.1 M TBATFB in MeCN. The electrodes were then treated by CV in the potential range from 300 to 2800 mV at a 100-mV s^{-1} scan rate at 10 cycles for all molecules.

Results and discussion

Electrochemical grafting of flavonoids onto the GC electrode surface

Three structurally different but related flavonoids, QR, 3HF, and BN (Table 1) were investigated electrochemically both in solution and in solid state attached to the GC electrode surface via the covalent bonds.

From Figure 1, it is very apparent that the overall voltammogram profiles are different for the three kinds of flavonoids in MeCN. This shows that the electrochemical oxidation of flavonoids is strongly related to the structure, especially to the number and position of the OH groups. Since there is no systematic study on the electrochemical oxidation mechanism of flavonoids in both aqueous and nonaqueous media, the flavonoids under investigation are purposely chosen regarding their structural differences to elucidate their electrochemical oxidation mechanism.

Cyclic voltammograms of 1 mM QR, 3HF, and BN solutions in MeCN show several oxidation peaks at the GC electrode surface. Anodic peaks are observed at 1078, 1433, 2038, and 2428 mV for QR; 944, 1746, and 2042 mV for 3HF; and 690, 1345, and 2093 mV for BN vs. Ag/Ag^+ electrode. Cyclic voltammogram of 1 mM QR solution is very similar to that of 3HF with the exception of the second anodic peak observed in QR but not in 3HF. QR and 3HF have one hydroxyl group in the C ring and show similar electrochemical behavior. Although QR and BN both have two hydroxyl groups of the same position in the A ring, BN has additional hydroxyl group at the position 6 in the A ring but has no OH groups in the B and C rings. It is electrochemical behavior of QR that has been most extensively studied in aqueous media (Brett and Diculescu 2004, Timbola et al. 2006). Although the number

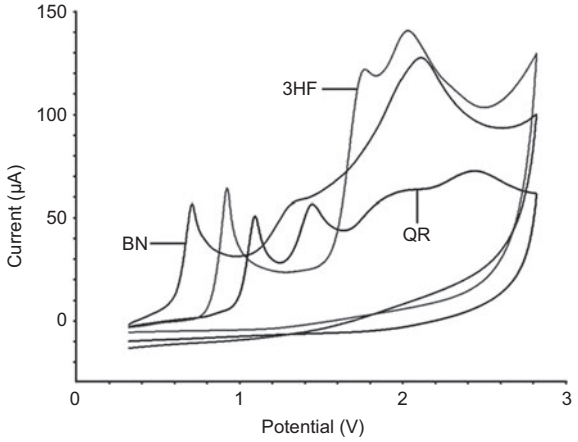


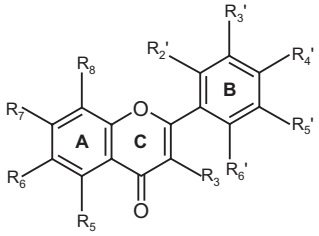
Figure 1 First scan cyclic voltammograms of QR, 3HF, and BN in MeCN containing 0.1 M TBATFB and 1 mM flavonoid on GC electrode. Scan rate is 100 mV s^{-1} and vs. Ag/Ag^+ (10 mM).

of peaks observed are different and depend on the experimental conditions, most of the authors have reported three peaks for the oxidation of QR. The first (the less positive potential) one has been attributed to the relatively reversible oxidation of hydroxyl groups in the B-ring-forming quinone species. The second one has been attributed to the irreversible oxidation of hydroxyl group in the C ring and the third one (most positive potential) to the irreversible oxidation of 5,7-dihydroxy substituents in the A ring (Brett and Diculescu 2004). Timbola et al. (2006) proposed a very different mechanism for the second and third oxidation peaks of QR in hydroxy-alcoholic media at the wax impregnated graphite electrode. As Figure 1 shows, the overall CV profiles of the flavonoids under investigation in MeCN are similar to each other. Our purpose here is not only to investigate the mechanism of oxidation of flavonoids in solution but rather to investigate the mechanism of deposition of flavonoids to the GC surface. Deposition mechanism will be discussed later in mechanistic studies section.

Figure 2 shows the multiscan cyclic voltammograms of QR, 3HF, and BN in MeCN containing 0.1 M TBATFB at the GC electrode surface. The potential was cycled anodically by 10 scans from 300 to 2800 mV. During the first scan, a few of the flavonoids exhibited a number of oxidation peaks depending on the structure of the compound at the bare GC electrode. The currents of these peaks decreased as the cycle

Table 1 Chemical structures of flavonoids.

Flavonoids	R_3	R_5	R_6	R_7	$R_{3'}$	$R_{4'}$
QR	OH	OH	H	OH	OH	OH
3HF	OH	H	H	H	H	H
BN	H	OH	OH	OH	H	H



$R_2'=R_8=R_5'=R_6'=H$.

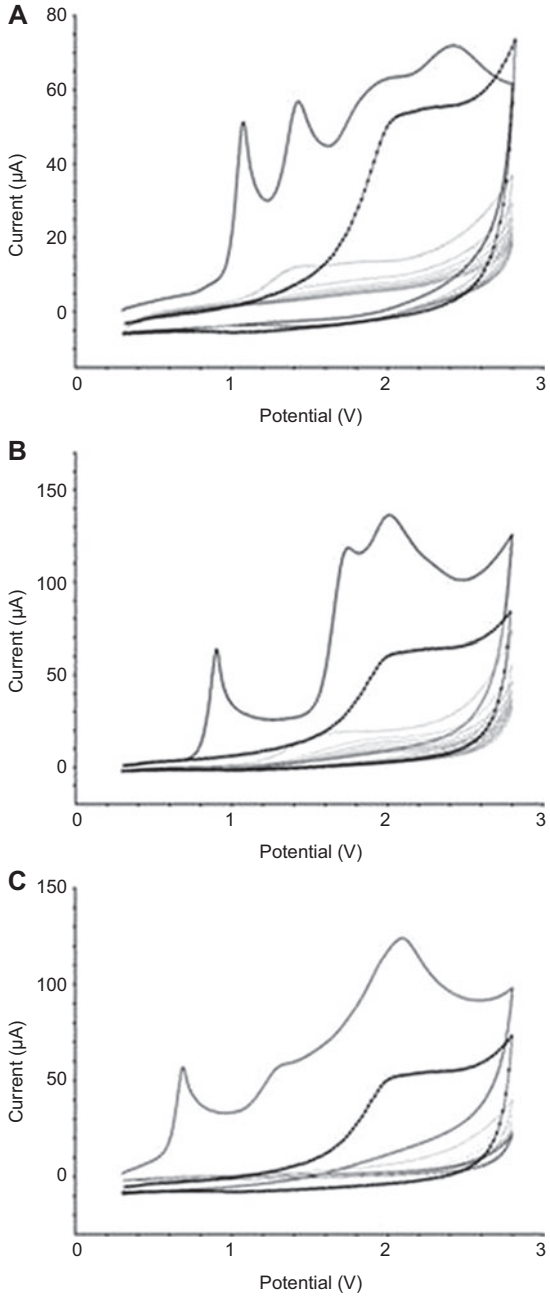


Figure 2 Repetitive cyclic voltammograms of (A) GC-QR, (B) GC-3HF, and (C) GC-BN in MeCN containing 0.1 M TBATFB and 1 mM flavonoid on GC electrode. Scan rate is 100 mV s^{-1} and vs. Ag/Ag^{+} (10 mM) electrode. Grafting was accomplished by 10 sequential scans. Dotted voltammogram is for the bare GC electrode in solution without any QR, 3HF, and BN.

numbers increased and almost became zero in a few subsequent scans. This type of multiscan voltammogram clearly demonstrates that a film immediately forms after the oxidation of flavonoids at the GC electrode on which flavonoid cannot be oxidized. This electrochemical behavior is analogous to the ones obtained in modification of surfaces with various organic compounds (Downard 2000, Pinson and Podvorica 2005, Isbir et al. 2006, Üstündağ and Solak 2009). The immediate decrease of the peak current implies that a monolayer is grafted and the electrode surface is passivated just after the generation of the oxidation products of the flavonoid. Multilayer formation is also possible, but the disappearance of the voltammograms clearly indicates the existence of at least a monolayer at the surface. From Figure 2, it is interesting to note that an irreversible surface peak appears and survives from beginning of the second scan for all flavonoid derivatives but especially apparent for QR and 3HF. This peak can be attributed to the oxidation of surface confined diphenols to quinones in the anodic scan and vice versa in the cathodic scan due to the existence of large amount of residual water in the media when compared with the small surface concentration of the grafted flavonoid (Sawyer et al. 1995, Isbir et al. 2006). The flavonoid-modified surfaces prepared by the anodic oxidation at the GC electrode surfaces are depicted as GC-QR, GC-3HF, and GC-BN for QR, 3HF, and BN, respectively.

Characterization of flavonoid-modified GC electrode surfaces by CV and EIS

To confirm the existence of flavonoids at the GC electrode surface, cyclic voltammograms of ferrocene in MeCN (Figure 3) and hexacyanoferrate III, $(Fe(CN)_6^{3-})$ in BR buffer (Figure 4) were acquired on the bare and modified GC electrode. As Figure 3 shows, although the bare GC electrode allows the electron transfer for the ferrocene oxidation, the modified surface does not.

Figure 4 shows the voltammograms of $Fe(CN)_6^{3-}$ in pH 2 of BR buffer solution on the bare GC electrode and after modification of GC electrode with QR, 3HF, and BN. As can be seen from Figure 4, electrochemical response of $Fe(CN)_6^{3-}$ on the flavonoid-modified GC has been strongly suppressed by the layers of all kinds of flavonoids. Oxidation-reduction of redox probe species occurs with rapid redox kinetics at the bare electrodes as indicated by the peak shapes of the voltammograms. The inhibition and suppression of the rapid electron transfer for this redox system was observed for pH values between 1.81 and 11.98 in BR buffer solution, indicating that electron transfer was not affected by the surface charge density due to the protonation or deprotonation of polyphenol-type flavonoids.

The potential scan range is an important parameter in the grafting process of the flavonoids to the GC electrode surface. To investigate the potential scan range, the initial potential was fixed at 300 mV, and the applied potential was changed to more positive values than the potential of the starting point. When the positive applied potential was at a value between the first and the second peaks of QR, no modification was observed due to the fact that the generated product cannot interact with the surface because no oxygen-containing groups form before the third peak potential. The third peaks at about 2 V are attributed to the oxidation of the GC surface, facilitated by the presence of the *o*-quinone forms of flavonoids. Figure 2 shows the oxidation voltammogram of the bare GC electrode in 0.1 M TBATFB (in MeCN) without any flavonoid derivatives.

Figure 5 shows the Nyquist graphs for redox couple of $Fe(CN)_6^{3-/4-}$ solution in 0.1 M KCl at the bare GC electrode and after modification of GC electrode with QR, 3HF, and BN

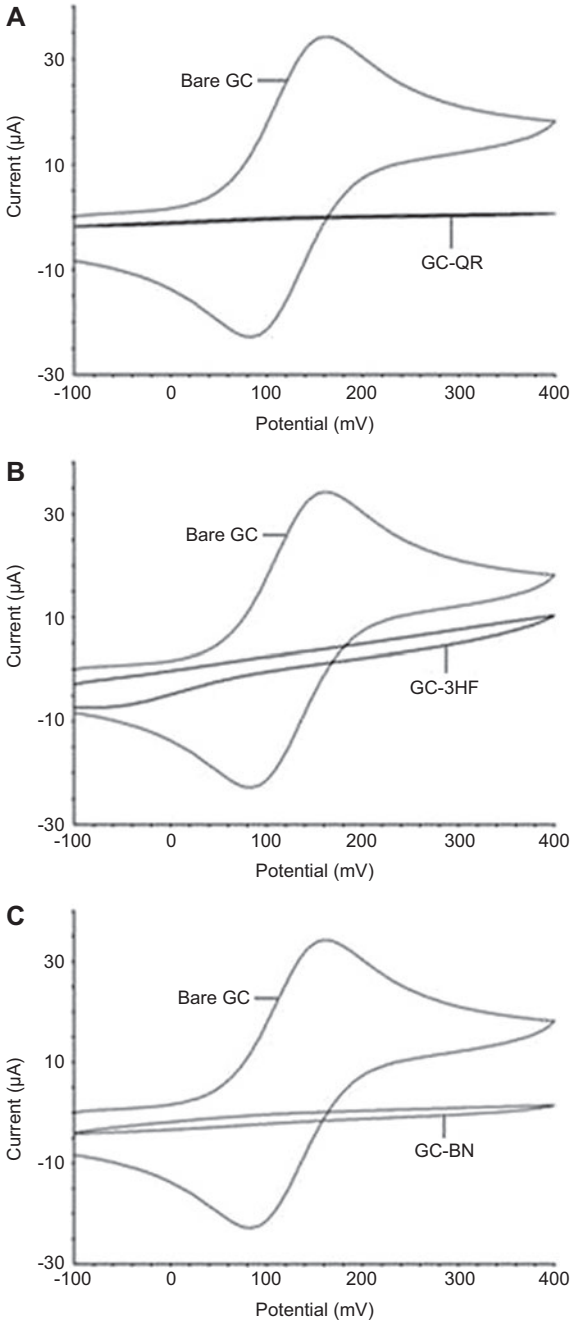


Figure 3 Cyclic voltammograms of 1 mM ferrocene vs. Ag/Ag^{+} (10 mM). (A) bare GC electrode and QR-modified GC electrode, (B) bare GC electrode and 3HF-modified GC electrode, and (C) bare GC electrode and BN-modified GC electrode in MeCN+0.1 M TBATFB. Scan rate is 100 mV s^{-1}.

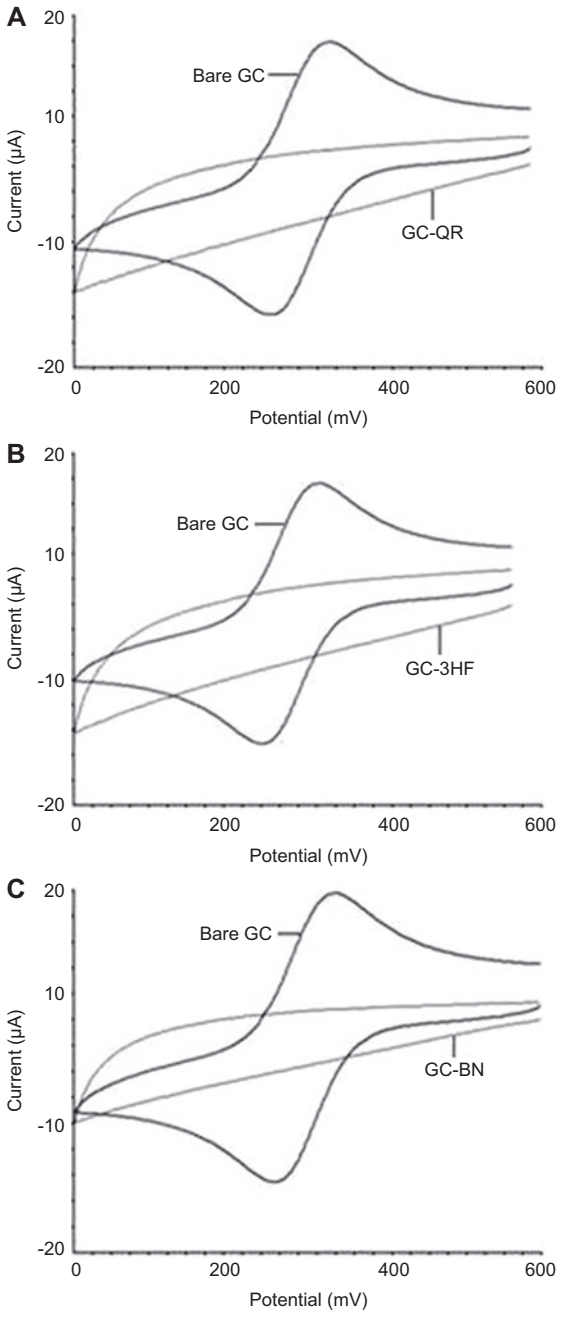


Figure 4 Cyclic voltammograms of 1 mM $Fe(CN)_6^{3-}$ vs. Ag/AgCl/3 M KCl reference electrode. (A) Bare GC electrode and QR-modified GC electrode, (B) bare GC electrode and 3HF-modified GC electrode, and (C) bare GC electrode and BN-modified GC electrode in BR buffer solution, pH=2. Scan rate is 100 mV s^{-1}.

at 300- and 2800-mV potential ranges. Modification begins only when the applied potential is more positive than the last peak potential.

The results of CV and EIS measurements suggest that strongly attached surface films are formed on the GC electrode by imposing anodic scan from 300 to 2800 mV in 0.1 M TBATFB+MeCN solution containing 1 mM of QR, 3HF, and BN. These films completely block the electrochemical response of $Fe(CN)_6^{3-/4-}$, which shows highly reversible electron transfer rate on the bare GC electrode.

CAM spectroscopic characterization of flavonoid-modified GC surface

In the CAM technique, if the dropped substance is water, the hydrophilicity of the modified surface can be investigated by

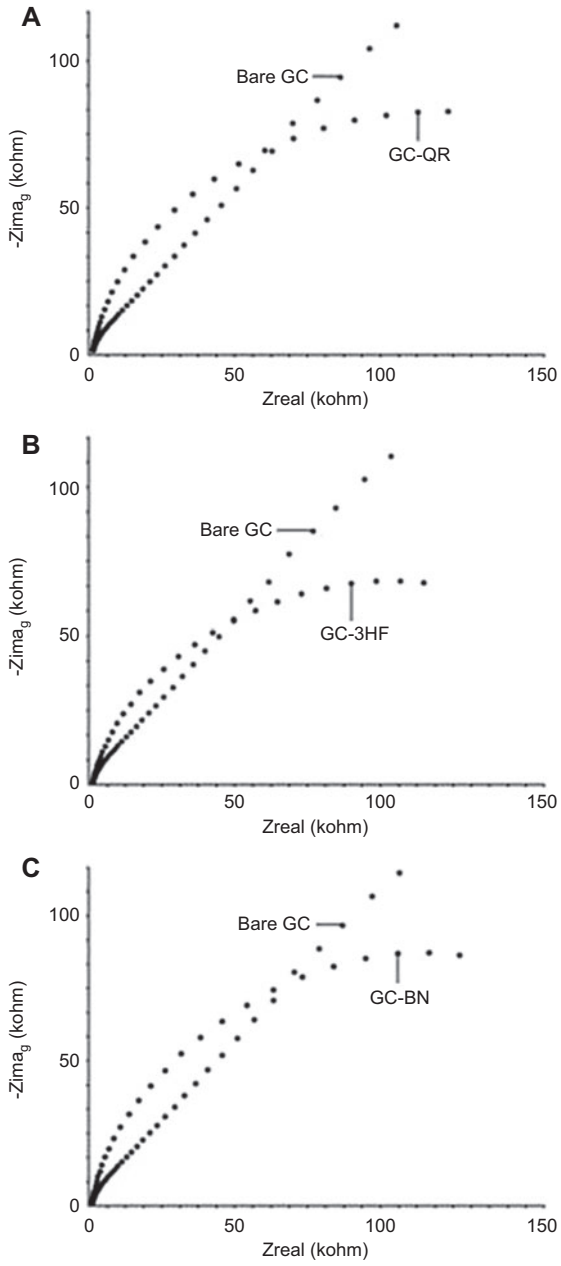


Figure 5 Nyquist plot for (A) bare GC electrode and QR-modified GC electrode, (B) bare GC electrode and 3HF-modified GC electrode, and (C) bare GC electrode and BN-modified GC electrode. Electrochemical impedance spectra of the redox couple $Fe(CN)_6^{3-/4-}$ solution in 0.1 M KCl at the frequency range of 100.000–0.05 Hz at 10-mV wave amplitude.

comparing the water drop contact angles on both bare GC electrode and flavonoid-modified GC electrode. In the current study, bare GC electrode surface was found to be quite hydrophobic, as revealed by a high contact angle of 86°, which is very close to 90°. As Table 2 shows, 3HF molecule bears only one OH group in the C ring, has a higher contact angle, and hence is more hydrophobic as compared with QR and BN. Besides, QR and BN molecules bear more OH functional groups than that of 3HF; therefore, GC-QR and GC-BN

Table 2 CAMs of unmodified and modified GC surfaces corresponding to water.

Surface	Contact angle (°)
Bare GC	86.00±0.50
GC-QR	30.59±2.56
GC-3HF	66.55±4.85
GC-BN	31.00±0.35

surfaces are more hydrophilic than the bare GC electrode surface and GC-3HF surface.

The hydrophilicity of the surface is based on the interaction of water with the oxygen or oxygen-containing groups

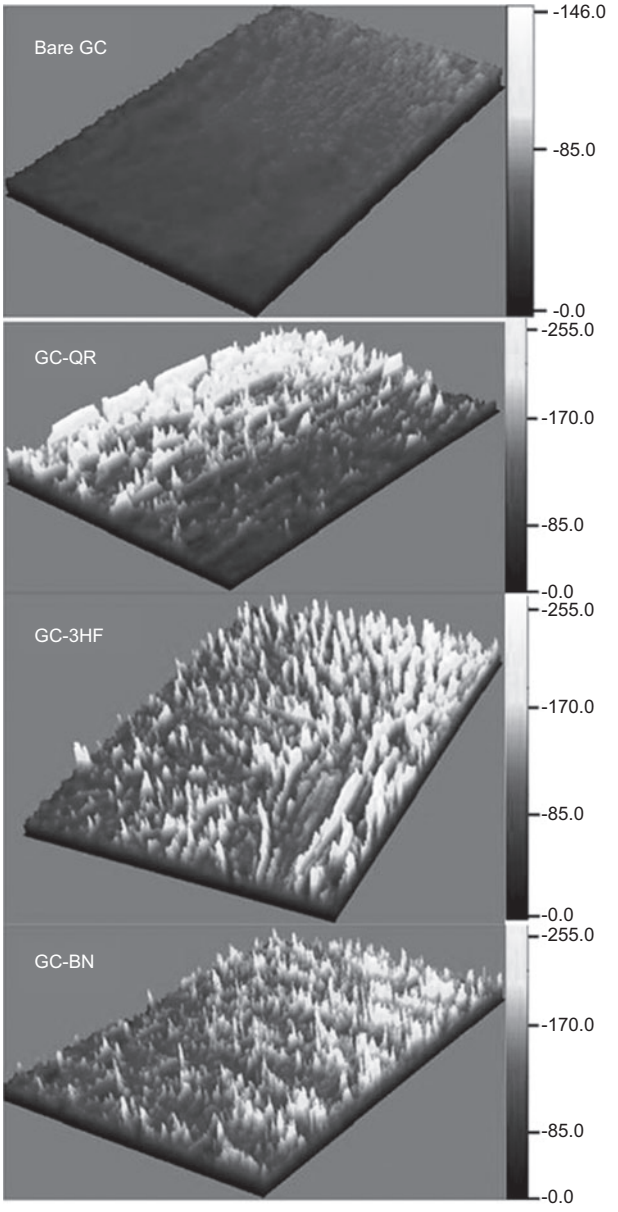
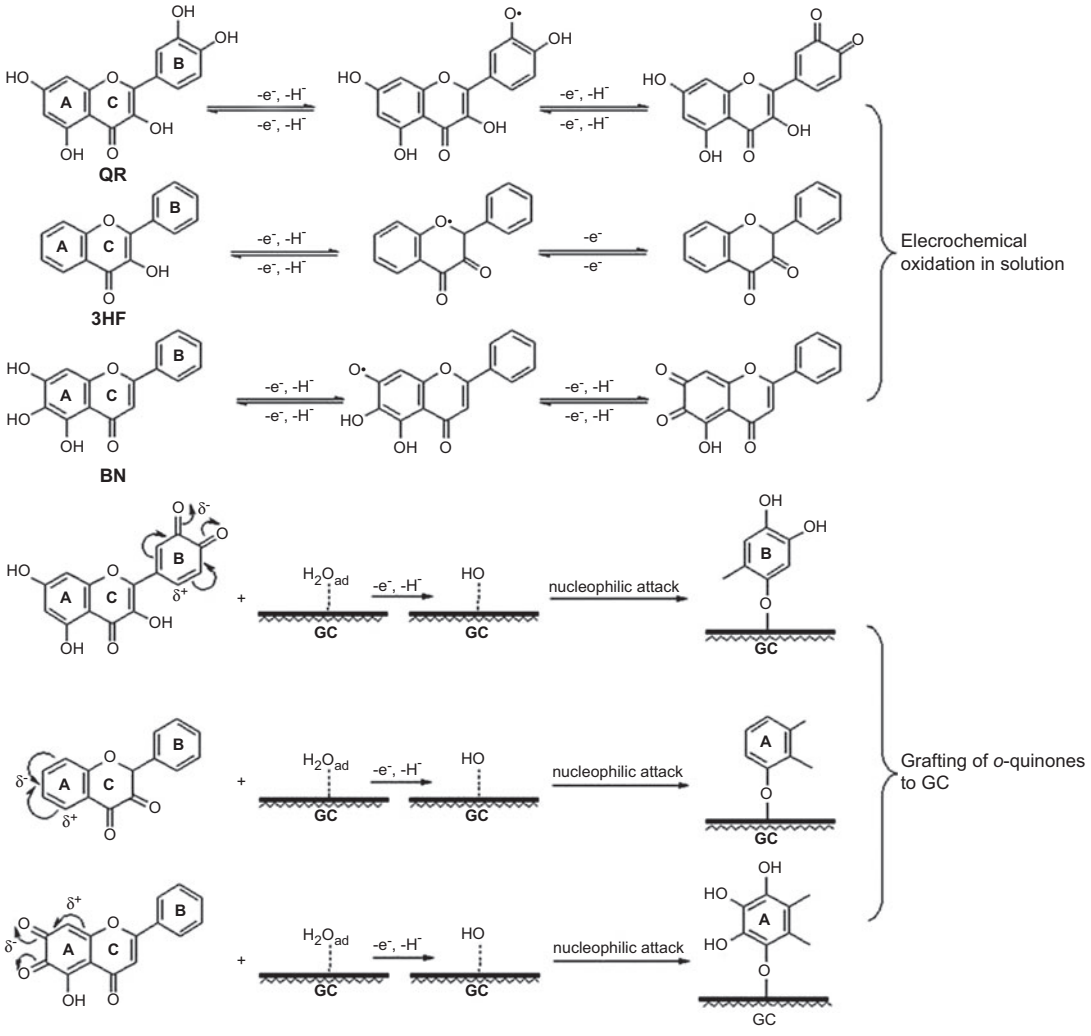


Figure 6 Ellipsometric 3D images of the bare GC electrode, GC-QR, GC-3HF, and GC-BN surfaces. Dimensions of the specimen is 50×50 μm.



Scheme 1 The proposed electrochemical oxidation and grafting mechanisms of QR, 3HF, and BN with two-electron process resulting *o*-quinone derivatives and then grafting to GC electrode surface.

to form hydrogen bonds. As will be discussed later, the interaction sites of flavonoids with the oxidized GC electrode surface are different for each compound. We propose that the oxidized forms of flavonoids in solution, i.e., *o*-quinone products, interacts with the oxidized GC electrode surface through the B ring for QR and through the A ring for 3HF and BN, making the surface-bound molecules more hydrophilic due to the regained hydroxyl groups. These functional groups give an enhanced contribution to the hydrophilic character of QR and BN. Therefore, QR and BN show lower contact angles with water drops due to the hydrogen bonds with abundant hydroxyl groups, when compared with 3HF.

Characterization of flavonoid-modified GC surfaces by ellipsometry

The surface thicknesses have been measured by ellipsometry, and three-dimensional (3D) images were obtained and shown in Figure 6. Ellipsometric measurements of QR-modified GC electrode surface were performed at two different regions and calculated as 26.10±0.17 and 53.20±0.51 Å, with an average of four measurements. As it is seen from the 3D image, QR was modified to the GC electrode surface in two different ways. The thickness of those regions differs from each other by half or twice. The difference in the thickness in the two different regions is not well understood but can be attributed to either the formation of dimerization before grafting or the deposition of the cation radicals to the grafted layer by intermolecular hydrogen bonds (Zhou et al. 2007).

The ellipsometric thickness of 3HF-modified GC electrode surface was calculated as 25.12±0.52 Å, with an average of four measurements from the 3D image, and one can conclude that 3HF exists on the surface with a smooth and homogeneous appearance. Accordingly, the thicknesses of QR and BN are almost the same. BN was also modified to the GC electrode surface either by hydrogen bonding or by dimerization of the BN itself. The thickness of BN was calculated as 61.62±0.87 Å.

Mechanistic studies of the attachment of flavonoids to the GC electrode surface

Although there are some discrepancies about the oxidation mechanism of flavonoids, almost all authors agree that the first oxidation occurs in the B ring hydroxyl groups to quinones, either in aqueous or nonaqueous solution (Pinson and Podvorica 2005). On the other hand, the mechanism of grafting of flavonoids to the solid surfaces is not extensively investigated and still amenable to many conflicts. Scheme 1 illustrates the oxidation mechanism of QR, 3HF, and BN in solution and the grafting the *o*-quinone products to the oxidized GC electrode surface, in accordance with the voltammograms of these compounds in Figure 1. All of the voltammograms display the similar properties with three irreversible oxidation peaks, the first one is for the one-electron oxidation of QR to phenolic radical in B ring, of 3HF to phenolic radical in C ring, and of BN to phenolic radical in A ring (Janeiro and Brett 2005, Timbola et al. 2006). Removal of the second electrons results on the conversion of phenolic radicals to *o*-quinone products, as shown in Scheme 1.

As stated above, modification occurs only beyond the third peak, which is attributed to the oxidation of GC electrode surface with the involvement of residual water in MeCN (Kessler et al. 2003, Isbir et al. 2006). As Scheme 1 shows, QR, 3HF, and BN are converted to *o*-quinone derivatives with two-electron oxidation of the B, C, and A rings, respectively. Anodically formed quinone derivatives interact with the functional groups of the oxidized GC electrode surface beyond ~2 V. Oxygen containing functionalities at the GC electrode surface interact as nucleophiles with *o*-quinone rings in positions 6′ of QR (B ring), position 5 of 3HF (A ring), and position 8 of BN (A ring) (Kessler et al. 2003, Isbir et al. 2006, Pineda et al. 2009). Attachment styles of the flavonoids are also depicted in Scheme 1. Deposition of the flavonoid derivatives follows electrochemical and chemical mechanisms during the electrochemical modification to GC electrode surface, forming etheric bonds between flavonoid moieties and GC electrode surface. In summary, all three molecules are electrochemically oxidized to *o*-quinones by the application of potential to the solution, and these *o*-quinone products are chemically bonded to the GC electrode surface through etheric covalent bonds.

Conclusions

The results obtained in this study show that the electrochemical behavior of QR, 3HF, and BN strongly depends on the nonaqueous medium provided by MeCN. The mechanism of electron transfer of QR, 3HF, and BN can be clarified using CV, EIS, CAM, AFM, and ellipsometry. As can be seen from the results, QR, 3HF, and BN are strongly grafted on the electrode surface, and thus the oxidation products block the whole electrode surface. The experiments showed that the oxidation of QR, 3HF, and BN on the electrode surface is an irreversible process.

Acknowledgements

We would like to thank the Research Foundation of Selçuk University, Konya, Turkey (BAP-06101020), for financial support of this work.

References

Bohm, B. A. Introduction to Flavonoids. Harwood Academic Publishers: Singapore, 1998; Chapter 2.

Bond, A. M. Voltammetric techniques for studying the redox chemistry of solids. *J. Solid State Electrochem.* **1997**, *1*, 185–186.

Brett, A. M.; Diculescu, V. C. Electrochemical study of quercetin-DNA interactions: part II. In situ sensing with DNA biosensors. *Bioelectrochemistry* **2004**, *64*, 143–150.

Chen, P. Y.; Vittal, R.; Nien, P. C.; Ho, K. C. Enhancing dopamine detection using a glassy carbon electrode modified with MWCNTs, quercetin, and Nafion. *Biosens. Bioelectron.* **2009**, *24*, 3504–3509.

Downard, A. J. Electrochemically assisted covalent modification of carbon electrodes. *Electroanalysis* **2000**, *12*, 1085–1096.

He, J. B.; Jin, G. P.; Chen, Q. Z.; Wang, Y. A quercetin-modified biosensor for amperometric determination of uric acid in the presence of ascorbic acid. *Anal. Chim. Acta* **2007**, *585*, 337–343.

He, J. B.; Yuan, S. J.; Du, J. Q.; Hu, X. R.; Wang, Y. Voltammetric and spectral characterization of two flavonols for assay-dependent antioxidant capacity. *Bioelectrochemistry* **2009**, *75*, 110–116.

Isbir, A. A.; Solak, A. O.; Ustundag, Z.; Bilge, S.; Natsagdorj, A.; Kilic, E.; Kilic, Z. The electrochemical behavior of some podands at a benzo[*c*] cinnoline modified glassy carbon electrode. *Anal. Chim. Acta* **2005**, *547*, 59–63.

Isbir, A. A.; Solak, A. O.; Üstündağ, Z.; Bilge S.; Kılıç, Z. Preparation and characterization of diethylene glycol bis(2-aminophenyl) ether-modified glassy carbon electrode. *Anal. Chim. Acta* **2006**, *573–574*, 26–33.

Janeiro, P.; Brett, A. M. Solid state electrochemical oxidation mechanisms of morin in aqueous media. *Electroanalysis* **2005**, *17*, 733–738.

Janeiro, P.; Corduneanu, O.; Brett, A. M. Chrysin and (±)-taxifolin electrochemical oxidation mechanisms. *Electroanalysis* **2005**, *17*, 1059–1064.

Jin, J. H.; Cho, E.; Kwon C.; Jung, S. Selective monitoring of rutin and quercetin based on a novel multi-wall carbon nanotube-coated glassy carbon electrode modified with microbial carbohydrates α-cyclosophorohexadecaose and succinoglycan monomer M3. *Bull. Korean Chem. Soc.* **2010**, *31*, 1897–1901.

Kessler, M.; Ubeaud G.; Jung, L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J. Pharm. Pharmacol.* **2003**, *55*, 131–142.

Kuhnau, J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet.* **1976**, *24*, 117–191.

Liu, W.; Guo, R. Interaction between flavonoid, quercetin and surfactant aggregates with different charges. *J. Colloid Interface Sci.* **2006**, *302*, 625–632.

Liu, Y. Some consideration on the Langmuir isotherm equation. *Colloids Surf. A Physicochem. Eng. Aspects* **2006**, *274*, 34–36.

Maeda, H.; Itami, M.; Katayama, K.; Yamauchi, Y.; Ohmori, H. Anodization of glassy carbon electrodes in oligomers of ethylene

glycol and their monomethyl ethers as a tool for the elimination of protein adsorption. *Anal. Sci.* **1997**, *13*, 721–727.

Nematollahi, D.; Malakzadeh, M. Electrochemical oxidation of quercetin in the presence of benzenesulfinic acids. *J. Electroanal. Chem.* **2003**, *547*, 191–195.

Peterson, J.; Dwyer, J. Taxonomic classification helps identify flavonoid-containing foods on a semiquantitative food frequency questionnaire. *J. Am. Diet Assoc.* **1998**, *98*, 677–682.

Pierozynski, B.; Zielinska, D. The process of electrooxidation of quercetin 3,4′-di-*O*-β-glucopyranoside at glassy carbon electrode. *Croat. Chem. Acta* **2010**, *83*, 127–133.

Pineda, L. E.; Oropeza-Guzmán, M. T.; Pina-Luis, G. Identification of flavonoid oxidation potentials as a function of pH (24th Meeting of the Mexican Electrochemical Society and 2nd Meeting of the Mexican Section of the Electrochemical Society) **2009**, *20*, 141.

Pinson, J.; Podvorica, F. Attachment of organic layers to conductive or semiconductive surfaces by reduction of diazonium salts. *Chem. Soc. Rev.* **2005**, *34*, 429–439.

Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152–159.

Robards, K.; Antolovich, M. Analytical chemistry of fruit bioflavonoids. A review. *Analyst* **1997**, *122*, 11–34.

Sawyer, D. T.; Sobkowlak, A.; Roberts, J. L. Experimental Electrochemistry for Chemists; 2nd Ed., Wiley-Interscience: New York, 1995.

Timbola, A. K.; de Souza, C. D.; Giacomelli C.; Spinelly, A. Electrochemical oxidation of quercetin in hydro-alcoholic solution. *J. Braz. Chem. Soc.* **2006**, *17*, 139–148.

Üstündağ, Z.; Solak, A. O. EDTA modified glassy carbon electrode: preparation and characterization. *Electrochim. Acta* **2009**, *54*, 6426–6432.

Zare, H. R.; Namazian, M.; Nasirizadeh, N. Electrochemical behavior of quercetin: experimental and theoretical studies. *J. Electroanal. Chem.* **2005**, *584*, 77–83.

Zare, H. R.; Samimi, R.; Ardakani, M. M. A comparison of the electrochemical behavior of rutin at an inactivated, activated, and multi wall carbon nanotubes modified glassy carbon electrode. *Int. J. Electrochem. Sci.* **2009**, *4*, 730–739.

Zhou, A.; Kikandi, S.; Sadik, O. A. Electrochemical degradation of quercetin: isolation and structural elucidation of the degradation products. *Electrochem. Commun.* **2007**, *9*, 2246–2255.

Zhu, Z.; Li, C.; Li, N. Q. Electrochemical studies of quercetin interacting with DNA. *Microchem. J.* **2002**, *71*, 57–63.

Zielinska, D.; Pierozynski, B. Electrooxidation of quercetin at glassy carbon electrode studied by a.c. impedance spectroscopy. *J. Electroanal. Chem.* **2009**, *625*, 149–155.