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Adsorptive Stripping Voltammetric Determination of Mangiferin Using an Activated Chitosan Modified Carbon Paste Electrode

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Abstract

A medium molecular weight powdered chitosan modified carbon paste electrode was used to investigate the electrochemical behaviour by cyclic voltammetry of the pharmacologically-active ingredient mangiferin (MG). An irreversible system was observed, with a peak at +0.55 V (vs Ag/AgCl). The peak current increases about fourfold, at the modified electrode in comparison with that recorded at the chitosan free carbon paste electrode. This allowed the use of adsorptive stripping voltammetry to develop a simple and sensitive electroanalytical method for the determination of MG. The influence of key parameters was investigated, including the electrolysis potential, the preconcentration time, the pH of supporting electrolyte and MG concentration. Upon optimisation of these parameters, the electrode response was found to be directly proportional to the concentration of MG in the range from 2.06×10^{-6} M to 6.74×10^{-5} M, leading to a detection limit of 1.84 μM for 240 s preconcentration at −0.1 V. A mechanism was also proposed for the electrochemical oxidation of MG.

Keywords

Mangiferin (*Mangifera indica* L.), Xanthones, Carbon Paste Electrode, Chitosan, Stripping Voltammetry

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1. Introduction

The drug industry is experiencing a boom as a consequence of the advances in ethnopharmacology [1]. The identification, description and experimental investigation of the ingredients of indigenous drugs are becoming more important owing to the need to understand their therapeutic and toxic effects [1] [2]. One of such ingredients that have undergone close scrutiny in recent years is mangiferin (MG), a C-glucosylxanthone (**Scheme 1**). MG belongs to the class of yellow oxygenated heterocyclics called xanthones, whose role is well-known in medicinal chemistry [3] [4]. The extraction of this promising pharmacological ingredient from various parts of the tropical based *Mangifera indica* L. by different authors has been reviewed by [5]. One could also mention a related work by [6] who comprehensively reviewed literature data on phytochemical and pharmacological investigations conducted on MG. In fact, this product displays numerous therapeutic and bioactive activities. It has been shown to be among others an antiallergic and antioxidant [7], antidiabetic [8], antiviral [9], antitumoral and anti-HIV [10] molecule. To date, huge data are available which are focused on extraction, isolation and purification techniques leading to MG from leaves, fruits, roots and heartwood of *Mangifera indica* L. These techniques include mostly mass spectrometry [11], nuclear magnetic resonance [12] [13], microextraction [14]-[16] and chromatographic methods [17]-[19].

On the other hand, it is quite well-known that the quantification of bioactive molecules in body fluids can improve pharmacotherapy and patient care [20]. This aspect appears to be of key importance since biological matrices are often complex due to the presence of coexisting components, with chemical properties similar to those of the target analyte. In addition, sample pretreatment is usually necessary, and in most cases the analyte of interest is present at low concentrations. Therefore, the monitoring and the measurement of concentrations of drugs in biological media remain a daily and challenging question in both medicinal chemistry and analytical science. Following these lines, the development of methods based on electrochemical sensors is of great interest in biomedical analysis. Electrochemical procedures are increasingly under investigation and could advantageously serve as alternatives to chromatographic and spectral methods for their miniature size, rapid analysis time and low cost [21] [22]. Moreover, they can give rise to excellent sensitivity with a large useful concentration range for both inorganic and organic species [23]. Of great interest in this area are devices based on modified electrodes which can be easily designed for the sensitive and selective detection of a given analyte. Specifically, chemically modified carbon paste electrodes (CMCPEs) have been largely investigated and proved to be versatile tools for the detection of electroactive compounds. The continuing resort to these electrodes resides in their flexibility, as they can be conveniently modified by a compound and/or a material displaying a great affinity towards a precise analyte. Typical works dealing with the electroanalysis of organic and biologically important molecules using CMCPEs were reported [24]-[26]. Thus, the simultaneous determination of rutin and quercetin (flavonoids and antioxydants) was achieved at a multi-walled carbon nanotube paste electrode by reversing differential pulse voltammetry [27]. Similarly, using nanotube modified carbon paste electrodes, some pharmaceuticals were successfully determined which include oxytetracycline [28], urapidyl [29], piroxicam [30,] and isoniazid [31]. One could also mention some relevant works where the analytical performance of such electrodes towards the electrocatalytic properties of biomolecules involving important enzymatic processes has been demonstrated [32]-[34]. A review covering the exploitation of electrochemical tools for the determination of phenolic and MG-like compounds was released recently by [35]. However, to the best of our knowledge, no electroanalytical investigation has been reported for the quantification of MG using a CMCPE. In the present study, the electrochemical oxidation mechanism of MG was first investigated by cyclic voltammetry. Using differential pulse voltammetry, a quite simple and sensitive method was developed for its quantitative detection in aqueous buffer solution, by a powdered chitosan carbon paste composite electrode.
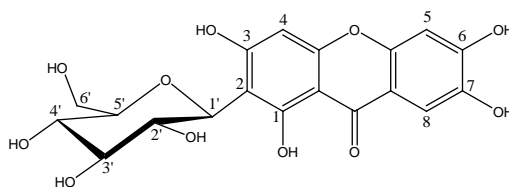
Scheme 1. Molecular structure of mangiferin.

Chitosan was chosen as efficient modifier for organophilic character and porous morphology that were previously reported to favour the accumulation of phenolic compounds at nanoparticles modified carbon paste electrodes [36]-[38].

2. Experimental

2.1. Reagents and Chemicals

The MG sample used was extracted from *Bersama engleriana* stem bark following a procedure previously described [39]. Prior to use, a stock solution of MG (1.04×10^{-3} M) was prepared in MeOH and then diluted to the desired concentrations by mixing with supporting electrolyte. The MG stock solution was stored at room temperature (about 23°C). Buffer supporting electrolyte solutions were prepared by mixing either solutions of KH_2PO_4 and Na_2HPO_4 (both from BDH), HCl and KCl (from Fischer scientific international) or CH_3COOH and CH_3COONa from Fischer scientific international and BDH respectively. The buffer solutions were used immediately after preparation. The pH of buffer solutions was adjusted with molar basic or acidic solutions prepared from pellets of NaOH (Prolabo) and commercial HCl solution. The medium molecular weight chitosan ($C_{12}H_{24}N_2O_9$) was purchased from Sigma-Aldrich. Ascorbic acid was purchased from Aldrich, uric acid from BDH-Prolabo, dopamine hydrochloride and D-(+)-glucose from Sigma-Aldrich, L-aspartic acid from Fischer Scientific International and citric acid monohydrate from J.T. Baker. Ascorbic acid, dopamine, L-aspartic acid, citric acid and D-(+)-glucose solutions were prepared in water while uric acid solutions were prepared in NaOH solutions, and used immediately thereafter. The human urine sample was collected from healthy laboratory staff, diluted 300 times with supporting electrolyte solution and used immediately thereafter.

2.2. Apparatus

Electrochemical measurements were carried out using a μ-Autolab potentiostat-galvanostat (type III) running with GPES software, supplied by EcoChemie, Utrecht (The Netherlands). A standard single compartment three electrode cell was used. Electrode potentials were measured with respect to an Ag/AgCl/3M KCl reference electrode (Metrohm, Ref 6.0726.100); a stainless steel rod was used as counter electrode. The working electrode was a chitosan modified carbon paste electrode prepared as described below. Electrochemical experiments were carried out without degassing the supporting electrolyte solution.

2.3. Preparation of the Chitosan/Carbon Paste Composite Electrode

The chitosan/carbon paste (100 mg) was made up of 67% graphite powder (Alfa), 3% chitosan powder and 30% silicone oil (Aldrich). The three components were mechanically mixed to obtain a homogenous paste. This paste was then firmly packed into the cavity (2.5 mm inner diameter) of a teflon tube and through which moves a screwed stainless steel rod. The electrode obtained is hereafter designated CPE-CHI (3%).

2.4. Electroanalytical Procedures

The modified electrode's active surface area was electrically activated by immersion in 0.5 M HCl solution, followed by five cyclic voltammetric scans from –0.2 V to +1 V. This electrical activation led to the protonation of the amino (-NH$_2$) groups of chitosan to form -NH$_3^+$ groups [40]. Cyclic voltammograms were recorded by transferring the chitosan-CPE either in a phosphate buffer (PB), HCl/KCl or in an acetate buffer (AB) solution containing MG at a given concentration. The potential scan rate was 75 mV·s^{-1} and the scanning was performed from –0.2 V to +1 V. MG stripping analysis was carried out following a 2 step procedure: 1) a preconcentration step at open-circuit, during which the working electrode was immersed for a defined period of time in an aqueous solution of MG under mild mechanical stirring; 2) a detection step where the electrode was rapidly removed from the preconcentration solution, rapidly rinsed with double distilled water and transferred to the buffer detection solution in the electrochemical cell. The electrode response was immediately recorded using differential pulse voltammetry (DPV) with the following conditions: pulse amplitude 50 mV, step potential 5 mV, initial potential +0.2 V, final potential +0.8 V and equilibrium time 5 s. Electrolysis potential and accumulation time were optimised. Prior to each measurement, the electrode surface was renewed by polishing with a soft and smooth piece of paper.

3. Results and Discussion

3.1. Preliminary Investigations

Some preliminary studies were undertaken to evaluate the contribution of chitosan modification towards the electrochemical signal of MG. **Figure 1** compares the cyclic voltammograms recorded in 0.1 M PB (pH 5) using a bare CPE and an activated CPE-CHI (3%). The oxidation of MG occurred at +0.55 V, giving rise to a peak with intensity more than 4 times greater at the chitosan modified electrode in comparison with the bare CPE.

This enhancement could be reasonably explained by electrostatic interactions between the -NH$_3^+$ groups on the protonated chitosan and lone pairs of electrons on the -OH groups of MG. Since the current response is shown to be dependent on chitosan, the amount of the later was varied within the CPE from 1% to 10% by mass: the highest peak current was obtained when the proportion of chitosan in the paste was equal to 3%. Such an electrode was used throughout the work and is referred to as CPE-CHI (3%).

It is noteworthy to mention here that the activation of chitosan-modified CPEs was achieved in 0.5 M HCl solution, but performing electrochemical experiments in the same medium lead to the electrode surface collapse as a consequence of a stronger protonation of chitosan which render the paste less stable. Thus, buffer solutions were used as supporting electrolyte for investigations.

3.2. Optimisation of Experimental Conditions for Analytical Application

In order to establish an electroanalytical method for the quantitative determination of MG, differential pulse voltammetry, one of the most sensitive electrochemical techniques was further exploited in adsorptive mode.

The feasibility of the two step procedures described in the experimental section was beforehand checked for the analysis of MG by recording in 0.1 M PB (pH 5) the response of CPE-CHI (3%) after 4 minutes preconcentration in an aqueous solution of 1.03×10^{-5} M MG. A well defined anodic peak was then obtained at +0.43 V, with a current of 7 μA. In comparison with the experiment described in **Figure 1**, this revealed the sensitivity of the chitosan modified electrode when exploited through adsorptive stripping voltammetry.

The key parameters likely to influence the analytical performance of the sensor proposed herein were then evaluated. These include the immersion time of the electrode in MG solution (preconcentration step), the electrolysis potential, the pH of the supporting electrolyte and MG concentration in the preconcentration medium.

As shown on **Figure 2(a)**, the oxidation peak current of MG increased remarkably with accumulation time from 0 s to 240 s, and then reached a plateau. This fact probably indicates that the adsorptive equilibrium was reached after 4 minutes accumulation. A preconcentration time of 4 minutes was therefore taken as optimum accumulation time and used in subsequent analyses. The evolution of peak current as a function of electrolysis potential is shown on **Figure 2(b)**. The peak intensity increased with electrolysis potential from −0.6 V to attain a maximum at −0.1 V, then dropped beyond this value which was taken as the best compromise for the electrolysis of MG at CPE-CHI (3%).

MG is an organic molecule with hydroxyl and catechol-like groups in its structure, and the redox behaviour of such a compound is expected to involve protons. **Figure 3(a)** presents the evolution of the anodic stripping voltammograms of MG when the pH of buffer solutions used as detection media was varied from 1 to 6. The oxidation peak potential (E_p) of MG was found to shift negatively with the increase of pH. A good linear relationship was observed between E_p and the pH (**Figure 3(b)**), with the equation $E_p = -0.0618$ pH $+ 0.7577$ (R = 0.9978). A value of −0.0618 V per pH unit is close to the theoretical Nernstian value of −0.059 V [41] and indicated that equal number of electrons and protons are involved in the electrooxidation of MG. At pH ≥ 8, MG is easily and rapidly oxidised by the dissolved oxygen in the solution and a green coloration probably of the quinonic form appears. No peak was obtained for this solution. It is therefore recommended, as for all hydroquinone-like compounds in the presence of oxygen to work at pH ≤ 8.

Using the previous optimised parameters, increasing the concentration of MG in the supporting electrolyte in the range from 2.06×10^{-6} M to 6.74×10^{-5} M led to the curve in **Figure 4(a)**. The results obtained revealed a linear relationship between MG concentration (C_{MG}) and peak current (I_p), with the following equation: I_p (μA) $= 0.676\ C_{MG}$ (μM) $- 1.524$. A correlation coefficient of 0.997 was obtained for the calibration graph, thus indicating a good linearity between peak current and MG concentration. For the elaborated analytical method, the limit of detection defined as 3 s/m (where s is the blank standard deviation (N = 16) and m the slope of the calibration curve) was calculated and found to be 1.84 μM MG. For practical analysis, the developed analytical
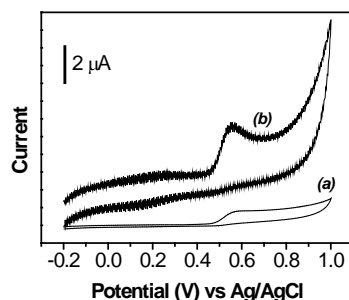
Figure 1. Cyclic voltammetric response of 2.04×10^{-5} M MG in PB (pH 5) on (a) bare CPE and (b) activated CPE-CHI (3%). Potential scanrate 75 m·Vs^{-1}.
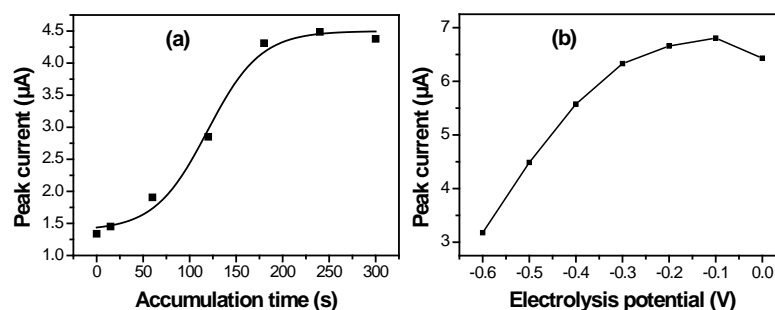
Figure 2. Dependence of current reponse at CPE-CHI (3%) for 1.03×10^{-5} M MG in 0.1 M PB (pH 5) on (a) accumulation time and (b) electrolysis potential.

method was applied to the quantification of MG in human urine. The urine sample was collected, diluted 300 times with HCl/KCl buffer to reduce interferences and MG of different concentrations added. In the absence of MG, a current of about 5.03 µA was measured. This was principally due to the presence of uric acid in the human urine sample. Upon successive addition of MG, the latter value was subtracted from the current responses and the currents due to the addition of MG alone are represented in **Figure 4(b)** against concentration. In this biological medium, a linear relation was obtained between peak current and MG concentration in the range 2.04×10^{-6} M to 5.95×10^{-5} M with a correlation coefficient of 0.996. The calibration equation was I_p (µA) = 0.325 C_{MG} (µM) + 0.308 and a limit of detection (3 s/m) of 2.98 µM was calculated for MG quantification in the presence of human urine.

To test the accuracy of the developed method and of the calibration equation, recovery experiments were carried out. An average percent recovery of 96.70% was found. A relative standard deviation of 4.10% (N = 4) was calculated, which is indicative of a good reproducibility of measurements. To validate the proposed MG analysis method, the influence of six potential compounds that usually coexist in biological media, namely uric acid (UA), ascorbic acid (AA), dopamine (DA), L-aspartic acid (AsA), citric acid (CA) and D-(+)-glucose, was evaluated. As shown in **Table 1**, all the species interfered with MG signal: their oxidation occurred at almost the same potential, and their presence increased or decreased the signal of MG. It was noted that for the same concentration, DA interfered most with the oxidation peak of MG while CA and AsA interfered least.

3.3. Electrochemical Oxidation Mechanism of Mangiferin

This work reported the first electrochemical investigation of MG, and we were interested in the mechanism involved in its electrochemical behavior. To better understand the electrochemical oxidation mechanism of MG, its electrochemical behavior was compared to that of compounds with similar moieties to those present in MG. The dependence of the oxidation peak potential of MG on pH (**Figure 3(b)**) indicates that protons are involved in the oxidation mechanism [42]. The slope of this graph (−0.0618 V per pH unit) equally indicated a mechanism with equal number of protons and electrons [41]. At pH ≥ 8, a chemical reaction took place upon addition of MG to the supporting electrolyte. This is typical hydroquinonic behaviour at pH ≥ 8. Previous studies report hydroquinonic and catechol-like oxidations as 2 electrons and 2 protons transfer processes at electrode surface in

acidic media [43]-[46]. Both electrons and protons are therefore removed from the MG molecule during oxidation to produce a stable electroinactive quinonic form. Based on the above, we proposed the mechanism shown in **Scheme 2** for the electrochemical oxidation of MG.

4. Conclusion

In this work, a simple method was developed for the quantitative determination of mangiferin, a compound of
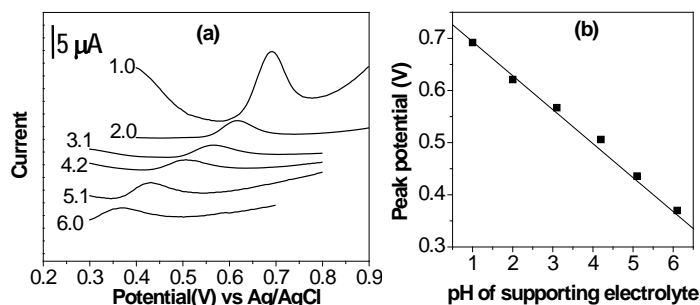
Figure 3. (a) Effect of detection medium pH on the anodic peak current of MG, at CPE-CHI (3%). (b) Variation of the peak potential as a function of pH of the detection medium.
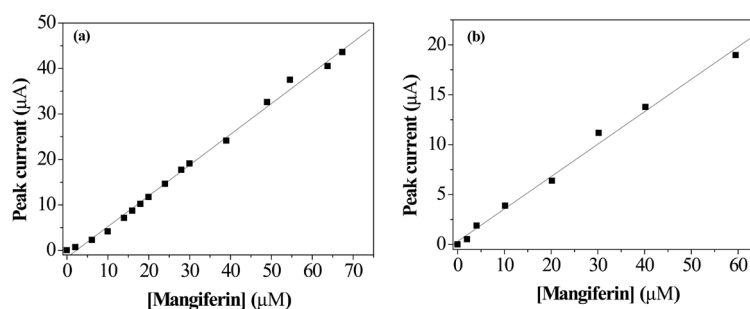
Figure 4. (a) Calibration graph of MG obtained on CPE-CHI (3%) in HCl/KCl buffer solution (pH 1) under optimised conditions: 0, 0.206, 0.614, 1.0, 1.4, 1.6, 1.8, 2.0, 2.4, 2.8, 3.0, 3.96, 4.9, 5.46, 6.38, 6.74 × 10^{-5} M MG. (b) Calibration graph of MG obtained on CPE-CHI (3%) in HCl/KCl buffer (pH 1)/human urine mixture: 0, 2.04, 4.08, 10.2, 20.2, 30.2, 40.2, 59.5 µM MG.
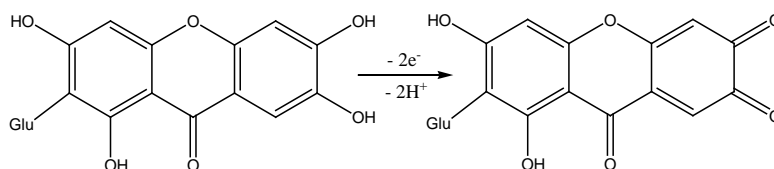
Scheme 2. Proposed electrochemical oxidation mechanism of mangiferin.

Table 1. Influence of some organic compounds on the determination of 1.01×10^{-5} M MG.

Interfering organic species	Concentration (µM)	Increase in peak current of MG
Ascorbic acid	51.1	≈19%
Uric acid	51.1	≈30%
Dopamine	51.1	≈63%
L-aspartic acid	51.1	≈−2%
Citric acid	51.1	≈1%
D-(+)-glucose	51.1	≈18%

great interest because of its potential benefits in medicine. This new study reveals that the electrochemical oxidation of mangiferin is an irreversible, two-electron two-proton transfer and pH dependent process. The oxidation product of mangiferin adhered at the electrode surface, resulting in a decrease of peak currents in successive scans. The medium exchange method reduces the effect of interfering electroactive species such as ascorbic acid and uric acid. Discrimination of the signals of mangiferin, ascorbic acid and uric acid is an ongoing project. The deprotonation of hydroxyl groups in the course of the electrochemical oxidation of mangiferin confirms the fact that its radical scavenging (antioxidant) activity, as well as that of other polyphenols, is related to its electron/proton donating capacity.

Acknowledgements

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