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Electrochemical investigations into host–guest interactions of a natural antioxidant compound with β-cyclodextrin

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ABSTRACT

The electrochemical behavior of mangiferin (MGN), a natural antioxidant compound, is examined using cyclic and differential pulse voltammetry in a protic medium on a glassy carbon electrode. The voltammograms exhibit a single irreversible pH-dependent anodic wave with current controlled by adsorption.

Complexes of MGN with β-cyclodextrin (β-CD) were prepared and their formation was confirmed by UV–vis spectroscopy and electrochemical experiments, using a self-assembled monolayer of cyclodextrin on a gold electrode. The association constant of MGN:β-CD complexes was estimated by the Benesi–Hildebrand method, based on the spectrophotometric quantification of free β-CD and by the direct method using cyclic voltammetry and the Langmuir isotherm.

PM IRRAS experiments corroborated the inclusion process based on the observation of the corresponding peaks in the spectra of the samples.

MGN was quantified using a simple electrochemical method based on a β-CD incorporated carbon nanotube (CNT)-modified electrode (β-CDCNT). The presence of β-CD led to a 10-fold lower detection limit than that obtained with a CNT-modified electrode.

1. Introduction

Mangiferin (1,3,6,7-tetrahydroxyxanthone-C-2-β-D-glucoside) (MGN) (Fig. 1) is a natural glucoside xanthone obtained from traditional medicinal plants and several vegetables, among them *Mangifera indica* L. (common name: mango) [1,2], one of the most popular tropical fruit-bearing trees in Brazil and worldwide [3,4]. The interest in mangiferin stems from its wide variety of pharmacological activities, which include antibacterial, immunomodulatory, anticancer, antidiabetic, gastroprotective, anti-inflammatory, analgesic, anti-HIV, cardio- and radioprotective activities, as well as its antioxidant capacity [5–15]. In the case of polyphenols, this activity is related to free radical captodative functions, especially protection against reactive oxygen species and consequent prevention of tissue damage [8,15]. An aqueous decoction of mango (*M. indica* L.) stem bark for use as a nutritional supplement and phytomedicine was recently developed in Cuba and registered as Vimang® [15].

The use of MGN is limited in aqueous media due to its low solubility in water [2]. This problem can be solved by using an inclusion complex with cyclodextrin. Cyclodextrins are cyclic oligosaccharides consisting of (α-1,4)-linked glucopyranose units with a somewhat lipophilic central cavity and a hydrophilic outer surface [16]. Cyclodextrins have been used extensively due to their special ability to complex with a variety of guest molecules, which enables their solubility, stability, bioavailability and protection against oxidation to increase and undesirable tastes/odors and microbiological contaminations to be eliminated [17]. Although there are studies of MGN with cyclodextrins [18], to the best of our knowledge, no reports are available about electrochemical investigations and determination of the formation constant of the complex. Indeed, apart from a reduction study performed in an aprotic medium [19], no electrochemical oxidation investigation of xanthones has been reported to date. Self-assembled monolayers (SAMs) of thiolated CD derivatives have been prepared as model systems for membrane receptors and chemical sensors [20], and inclusion complexations of surface-confined CD on gold electrodes with various compounds have been investigated by cyclic voltammetry [20–26]. This paper reports on an investigation into the inclusion complex of MGN with β-CD by cyclic voltammetry and PM IRRAS, using SAMs of β-CD and MUA, and compares the results with those obtained by spectrophotometry, as yet not reported in the literature.

Several techniques are widely employed for determining the quality and quantity of mangiferin in pharmaceutical, pharma-

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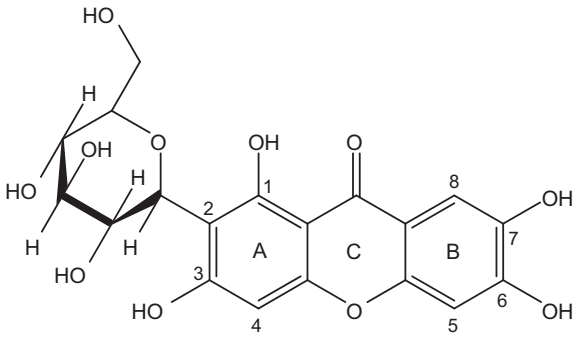

Fig. 1. Mangiferin (MGN) structure.

cokinetic or pharmacological studies. These techniques include high performance liquid chromatography (HPLC) alone or HPLC combined with mass spectrometry or UV–vis spectrophotometry, on-line coupling of high-speed counter-current chromatography (HSCCC) with high performance liquid chromatography–diode array detection (HPLC–DAD), ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS), capillary electrophoresis (CE), micellar electrokinetic capillary chromatography (MEKC) and capillary zone electrophoresis (CZE), LC electrospray ionization (ESI), tandem mass spectrometry (MS–MS), fluorescence, FT-IR and circular dichroism (CD) [27–32].

Several authors have discussed the use of cyclodextrin in carbon nanotube-modified electrodes for the analytical determination of some drugs [33–37]. For instance, Alarcón-Angeles et al. [33] demonstrated an improvement in the analytical performance of the catalytic oxidation of dopamine compared with the unmodified electrode. MGN is the main constituent of several medicinal phytoformulations; therefore, its determination and the use of the CNT modified electrode are relevant objects of investigation.

2. Experimental

2.1. Reagents and solutions

β-CD, 11-mercaptoundecanoic acid [$HS(CH_2)_{10}COOH$, i.e., MUA] and ferrocenecarboxylic acid (Fc-CO_2H) were purchased from Sigma–Aldrich and used without further purification. Mangiferin was isolated from dried bark of *M. indica* using ethanol, and after evaporation of the solvent, it was recrystallized in aqueous ethyl acetate, yielding a product of 95% purity [4]. All the other reagents (supporting electrolytes) used here were of the highest purity. The multiwall-CNT was purchased from Sigma–Aldrich and pretreated in HNO_3 before being used [33]. All the buffer solutions, whose ionic strength was 0.2 mol L^{-1} were prepared using analytical grade reagents and water purified in a Millipore Milli-Q system (conductivity <0.1 μS cm^{-1}). All the experiments were performed at room temperature (24 ± 2 °C).

2.2. Synthesis of the thiolated β-cyclodextrin derivative

Per-7-thio-β-cyclodextrin (β-CDSH) was synthesized from β-cyclodextrin in two steps, following the procedure described in the literature [22].

2.3. Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were performed with a conventional three-electrode cell in an Autolab PGSTAT-30 potentiostat (Echo Chemie, Utrecht, the Netherlands) coupled to a PC microcomputer, using GPES 4.9 software. The working electrode was a glassy carbon (GC) BAS (d = 3 mm), gold (d = 3 mm), gold bead (Au), β-CD-SAM or carbon nanotube-modified (β-CDCNT) electrode, while the counter electrode was a Pt wire and the reference electrode an Ag|AgCl, Cl^- (saturated), all contained in a one-compartment electrochemical cell with a volumetric capacity of 10 mL. The GC and gold electrodes were polished with alumina on a polishing felt (BAS polishing kit). After mechanical cleaning, the electrochemical pretreatment of the GC or gold electrodes involved a sequence of 10 cyclic potential scans from −1.2 to +1.4 V (100 mV s^{-1}) in a 0.1 mol L^{-1} H_2SO_4 solution. The gold bead working electrode was prepared by annealing the tip of a gold wire (99.999%, 0.5 mm diameter) in a gas–oxygen flame and the voltammetric response of this electrode was checked in 0.2 mol L^{-1} Na_2SO_4. Argon was used to degas the solution, and the solution was covered with an argon blanket during the experiments. The pH was measured with a MARCONI MAPA200 series 0113992 pH-meter with a combined glass electrode.

2.4. Spectrophotometric measurements

An UV–vis spectrophotometer (Shimadzu) was used to measure and analyze the inclusion of MGN in the cavity of free β-CD.

An aqueous solution of MGN (1.0×10^{-4} mol L^{-1}) was prepared with 1.5% (v/v) ethanol. Seven aliquots of 25 mL of this solution were removed and mixed with appropriate amounts of β-CD to obtain concentrations of 5.0×10^{-5}–7.0×10^{-4} mol L^{-1} of β-CD. The mixture was stirred (170 rpm) for 2 h at 25 °C. Absorbance values were measured in the wavelength range of 240–368 nm (240, 257, 318 and 368 nm). The constant of formation was determined based on the average value.

PM IRRAS experiments: The Polarization Modulation InfraRed Reflection Absorption Spectroscopy (PM IRRAS) experiments were performed at the University of Buenos Aires, on a Thermo Nicolet 8700 (Nicolet, Madison, WI) spectrometer equipped with a custom-made external tabletop optical mount, a MCT-A detector (Nicolet), a photoelastic modulator, PEM (PM-90 with II/Zs50 ZnSe 50 kHz optical head, Hinds Instrument, Hillsboro, OR), and a Synchronous Sampling Demodulator, SSD (GWC Instruments, Madison, WI).

The IR spectra were recorded with the PEM set for a half-wave retardation at 3000 cm^{-1} for the CH stretching region and at 1500 cm^{-1} for the CH, CO and C=O bending and aromatic regions. The angle of incidence was set at 80°, which gives the maximum mean square electric field strength for the air/gold interface. The demodulation technique developed in Corn's laboratory was used in this work. The signal was corrected by the PEM response, using a method described by Frey et al. [38]. 1500 scans were performed and the resolution was set at 4 cm^{-1}. For the PM IRRAS analysis, the pure SAMs of β-CD on Au substrate were constructed by the standard immersion process. The Au substrate was immersed in the prepared β-CDSH solutions for 20 h. After completing the SAM formation, the substrate was immersed in the MGN solution for 2 h and then washed with ethanol and water to remove physically adsorbed species (not encapsulated), leaving only encapsulated MGM on the electrode surface.

An additional experiment to confirm the ability of the SAM to include MGN in its cavity was performed using cyclic voltammetry, by competition, using increasing amounts of MGN (from 10 up to 100 μmol L^{-1}) in a solution containing Fc-CO_2H 1.0×10^{-4} mol L^{-1} in 10% EtOH in Na_2SO_4 0.2 mol L^{-1}, using ν = 0.010 V s^{-1}.

For ^1H NMR, stock solutions of isolated MGN and of CD were prepared in D_2O. Then, a complex obtained from both compounds with a molar ratio of 1:1 was also dissolved in D_2O. ^1H NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz proton frequency at room temperature and at 35 °C.

2.5. Preparation of the modified electrode

A gold surface modified only with β-CDSH cannot be used directly to build size-selective films due to the presence of defects whose guest molecules can permeate, reaching the electrode surface and impairing the electrode's selectivity. To cover the accessible electrode surface not blocked with cyclodextrin molecules, 11-mercaptoundecanoic acid (MUA) molecules were used, which have been shown to promote full blockage of electron transfer of electrochemical probes [25]. Thus, the monolayer of β-CDSH (β-CD SAM electrode) was built on the gold bead electrode by immersing it for 20 h in a solution containing a suitable ratio of β-CDSH:Fc-CO_2H:MUA (2:2:1 m/m/m) in the mixed solvent (DMSO:EtOH:H_2O = 5:3:2, v/v/v). After completing the SAM formation, the substrate was washed with copious amounts of ethanol and water to remove physically adsorbed species. Unlike most β-CD applications, the cavity of β-CD was filled with Fc-CO_2H prior to forming a mixed SAM with MUA molecules on the Au substrate. Formation of the inclusion complex of β-CD with Fc-CO_2H has two purposes. The first is to arrange MUA only in the voids generated between β-CD molecules in contact, but not in the β-CD cavity. This requires a regularly spaced arrangement of β-CD in the SAM as well as filling of cavities prior to the MUA coming into contact with β-CD, for instance, as a hexagonal packing of β-CD and cavity filling with Fc-CO_2H. Once it is confirmed that β-CD molecules are arranged with a constant spacing containing Fc-CO_2H in the cavities, β-CD acts as a spacer for arranging MUA on the substrate. The second purpose is to use the Fc-CO_2H included in the cavities to estimate the surface coverage of β-CD by electrochemical detection [22].

After acid treatment, 2 mg of CNT [33] was dispersed by ultrasonic stirring in 1 mL of β-CD aqueous solution (2% m/v) to yield a 2 mg mL^{-1} black solution. The β-CDCNT electrode was prepared by dropping this solution (5 μL, three times) on the GC electrode and then heating it at 60 °C to remove the solvent. For purposes of comparison, a CNT-DMF dispersion was prepared and added to the GC electrode surface, as described above.

2.6. Stock solution of MGN

The stock solution of the MGN (c = 1.0 mmol L^{-1}) was prepared by dissolving the compound in ethanol.

2.7. Electrochemical studies

The electrochemical studies of the compounds were performed in a protic medium by cyclic and pulse differential voltammetries on glassy carbon and gold electrodes, applying scan rates in the range of 0.02–1.00 V s^{-1}.

2.8. Electrolysis

For electrolysis, 10 mg (0.79 mmol L^{-1}) of MGN was dissolved in 30 mL of phosphate buffer + 20% ethanol, using carbon felt (3.36 cm^2) as the working electrode. After 6 h of electrolysis, the solution turned cloudy, although no decrease was observed in the cell current related to the redox process of MGN. Because the electrode surface was blocked, the charge (number of electrons transferred in the oxidation) was determined by an alternative method. MGN (1.0×10^{-3} mol L^{-1}) was dissolved in phosphate buffer pH 7.5 using 30% of EtOH and was subjected to cyclic voltammetry. The number of electrons was determined from these voltammograms, which were obtained at different scan rates (35–300 mV s^{-1}) (see Section 3).

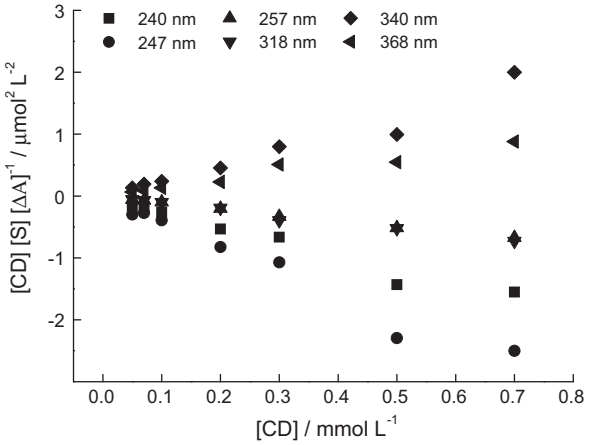

Fig. 2. Determination of the equilibrium constant of the MGN:β-CD complex according to the Benesi–Hildebrand equation at different wavelengths. [MGN] = 1.0×10^{-4} mol L^{-1} (1.5% v/v EtOH); [β-CD] = 5.0×10^{-5}–7.0×10^{-4} mol L^{-1}. Contact time 2 h.

2.9. Analytical curves

After optimizing the experimental parameters for the CNT-DMF and β-CDCNT electrodes, analytical curves were built by adding aliquots of the stock solution of MGN to the cell containing 0.2 mol L^{-1} phosphate buffer solution, at pH 7.0.

3. Results and discussion

3.1. Spectrophotometric determination of the apparent formation constant of the β-CD/MGN complex

A spectrophotometric investigation was made of the interaction between MGN with β-CD. To determine the apparent formation constant (K_F) of the MGN:β-CD complex, experimental data were analyzed according to the method proposed by Benesi–Hildebrand [39]. Based on this method, the dissociation constant of the complex can be calculated by Eq. (1):

$$\frac{[C][S]_0}{\Delta A} = \frac{K_D}{\Delta\varepsilon} + \frac{[C]}{\Delta\varepsilon} \quad (1)$$

where [C] and $[S]_0$ are the β-CD and MGN concentrations, respectively, K_D is the dissociation constant, $K_D = 1/K_F$; ΔA is the change in absorbance, and $\Delta\varepsilon$ is the change in the molar absorption coefficient. Plotting the values of [C] $[S]_0/\Delta A$ *vs.* [C] yielded a straight line. The slope was $1/\Delta\varepsilon$.

Fig. 2 displays the fit to the Benesi–Hildebrand equation at several wavelengths from 240 to 368 nm at a contact equilibrium time of 2 h. The formation equilibrium of MGN:β-CD complex can be written as Eq. (2):

$$\text{MGN} + \beta\text{-CD} \leftrightarrows \text{MGN} : \beta\text{-CD} \quad (2)$$

The apparent dissociation and formation constants were obtained from these curves, as shown in Table 1. The average value of $K_F = 1.6 \pm 0.7 \times 10^5$ L mol^{-1} was then calculated.

Table 1
The dissociation and formation constants of MGN:β-CD complexes after 2 h of the contact obtained from Benesi–Hildebrand equation.

λ/nm	2 h		
	K_D/mol L^{-1}	K_F/L mol^{-1}	R
240	9.03×10^{-6}	$1.1 \pm 0.4 \times 10^5$	0.9979
257	4.30×10^{-6}	$1.2 \pm 0.3 \times 10^5$	0.9992
318	4.10×10^{-6}	$2.4 \pm 1.2 \times 10^5$	0.9936
368	5.88×10^{-6}	$1.7 \pm 0.2 \times 10^5$	0.9999

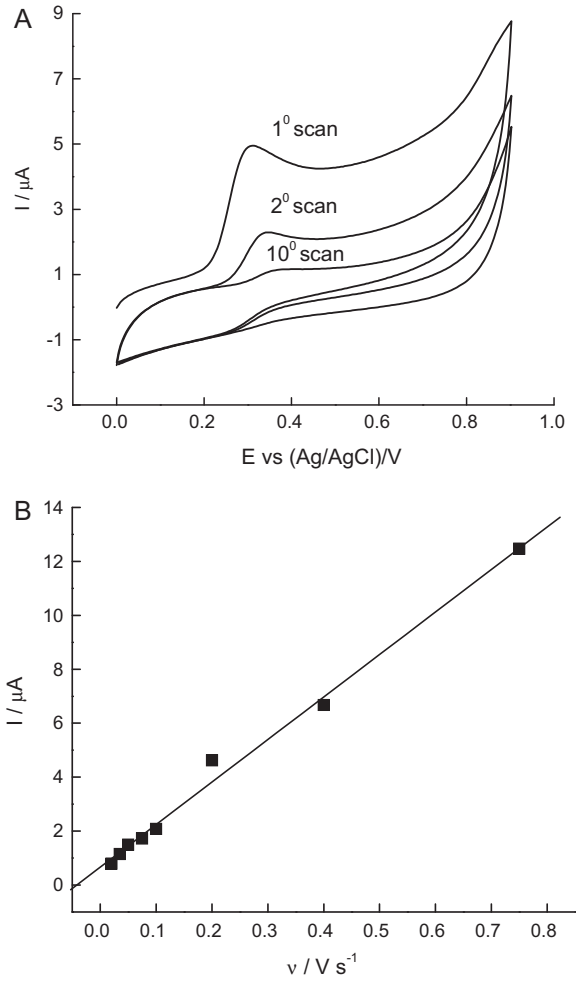

Fig. 3. Cyclic voltammetry of 0.1 mmol L^{-1} MGN in phosphate buffer pH 7.0 on GC electrode. Scan rate 100 mV s^{-1}.

Alvarez-Parrilla et al. [40] studied the complexation of quercetin with β-CD with a stoichiometry 1:1 and found an average value of $K_F = 1.3 \times 10^3$ L mol^{-1}.

3.2. Electrochemistry

3.2.1. Cyclic voltammetry study

The electrochemical behavior of MGN was studied in phosphate buffer 0.2 mol L^{-1}, pH 7.0, on a GC electrode. The cyclic voltammograms for this compound exhibited a single irreversible anodic wave at $E_{p_a} = +0.300$ and 100 mV s^{-1} (Fig. 3A), which shifted to more positive potentials upon increasing the scan rate (υ). The peak current (I_{p_a}) varied linearly with υ (Fig. 3B), which is the characteristic behavior of an adsorption process. Blockage of the electrode surface (Fig. 3A) was found to occur after successive scans.

The pK_a values of mangiferin are associated with the four hydroxyl groups in the xanthone structure, namely C1–OH, C3–OH, C6–OH and C7–OH. The hydroxyl groups directly linked to the glycopyranosyl system are not acid. In the literature [2], the acid constants of MGN in aqueous solution were determined in an UV–vis spectroscopic study using the SQUAD program as a computational tool, coupled with NMR studies. The pK_a values determined by this procedure [2] were as follows: $pK_{a_1} = 6.52 \pm 0.06$; $pK_{a_2} = 7.97 \pm 0.06$; $pK_{a_3} = 9.44 \pm 0.04$; $pK_{a_4} = 12.10 \pm 0.01$. The authors were able to associate pK_a values to the hydroxyl groups, such as pK_{a_1} = C6–OH; pK_{a_2} = C3–OH; pK_{a_3} = C7–OH and pK_{a_4} = C1–OH. The deprotonation of C6–OH (B-ring) would give a more stable conjugated base due to the presence of a resonant structure, a quinone methide. The same holds true for C3–OH, also conjugated to the carbonyl C-ring, which is influenced by the bulky glycopyranosyl system. C7–OH is less acid due to its conjugation with the electron donating oxygen of the xanthone system, while C1–OH is the most stable due to hydrogen bonding established with the carbonyl in the C-ring.

The oxidation peak at pH 7.5 is attributed to oxidation of the more acidic phenol group present in MGN, i.e., the C6–OH that is electron-rich and not sterically confined. For an irreversible adsorption peak according to Laviron's theory [41], a linear relationship between the peak currents I_p and the scan rate ν is described as follows (Eq. (3)):

$$I_p = n^2F^2\frac{A\nu\Gamma}{4RT} \quad (3)$$

However, $\Gamma = Q/nFA$ and Q is the area of the peak in the voltammogram. ν^{-1}. Therefore, Eq. (4) is used, instead:

$$I_p = \frac{nFQ\nu}{4RT} \quad (4)$$

where I_p is expressed in the unit of amperes and Q is the peak area of the voltammogram (in coulombs). The above equation shows that n can be calculated provided Q is obtained at a certain scan rate. Based on this finding, the number of transferred electrons (n) was calculated as 0.97.

Unfortunately, we are unable to describe in detail the mechanism of oxidation of mangiferin since, during its electrolysis, which was attempted several times, the oxidation product(s) was (were) strongly adsorbed on the electrode surface, causing blockage of the surface. As a result, we were unable to isolate any product from the electrolysis, although we were able to infer the possible oxidation site. The blockage of the electrode surface is also revealed in the cyclic voltammograms. The peak current decreased sharply after the first scan (Fig. 3A). This is a common occurrence in the electrolysis of phenols, as has been reported for rutin and catechin [42–44]. However, the absence of a reversible system and the almost immediate passivation of the electrode as early as in the second scan points to the oxidation of the B-ring catechol system (C7–OH, C6–OH), especially due to the higher acidity of C6–OH. Provided the formation of the phenolate is fast enough, it is the phenolate that oxidizes first. Oxidation would be far more positive in the A-ring due to the presence of the pyranosyl group, as has been stated before by several researchers, such as Volikakis and Efstathiou [44]. The free *ortho* position (C5–H, C6–H) is adequate for a dimerization/polymerization reaction, as reported earlier for 2,3-dihydroxynaphthalene [45].

3.2.2. Electrochemistry of mangiferin:β-CD inclusion complex

The electrochemical behavior of MGN in aqueous solution is shown in Fig. 3. The cyclic voltammograms obtained for MGN at 0.2 mol L^{-1} Na_2SO_4 solution in the absence and presence of β-CD are shown in Fig. 4. An initial increase was observed in the peak current (Fig. 4) up to a molar ratio of [β-CD]/[MGN] = 1.0. On the other hand, a further increase of β-CD concentration caused a decrease in the oxidation peak current and a positive shift in the peak potential. As soon as β-CD is added (first addition), there is a punctual increase in the current, due to de-aggregation of the MGN into monomers (cleavage of hydrogen bonding), leading to a temporary increase of solubility, and this causes the increase in current. A further decrease and anodic shift of potential can be explained by the formation of an inclusion complex with β-CD. As Doriguetto and co-workers [46] demonstrated, the single-crystal X-ray diffraction experiment unambiguously determined the crystal structure and molecular geometry of MGN, with identification of intra- and intermolecular H-bonds. Albeit in solid phase, it is possible to infer

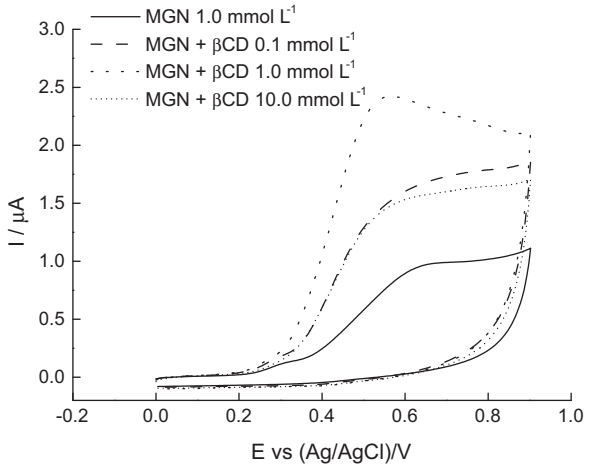

Fig. 4. Cyclic voltammograms recorded in Na_2SO_4 0.2 mol L^{-1} of MGN (1.0 mmol L^{-1}), pH 7.0, in the presence of free β-CD concentration.

this behavior in solution. The addition of CD would disrupt these hydrogen bonds, establishing new bonds within and outside the CD cavity.

Once an electroactive guest forms a stable inclusion complex with a β-CD host, the corresponding electrochemical reaction is suppressed in the complex, which is the case in the oxidation of MGN.

To obtain quantitative parameters, the study was performed using a SAM of β-CD. Previously, the formation of the SAM of β-CD was confirmed by the absence of the signal of the redox pair of $Fe(CN)_6^{3-}$ and the presence of peaks corresponding to the oxidation of ferrocenecarboxylic acid (Fc-CO_2H), which was used as an electroactive marker in cyclic voltammetry since it undergoes encapsulation in β-CD (Fig. 5). This behavior is expected for $Fe(CN)_6^{3-}$ because its size, which is larger than the cavity of the β-CD, precludes its arrival on the surface, and hence, its detection. On the other hand, the β-CD monolayer does not suppress Fc-CO_2H redox processes, indicating that it is possible to build selective systems based on this mixed monolayer film [β-CDSH and MUA] [24] (see Section 2). Cyclic voltammetry of MGN in solution, on this modified electrode showed an irreversible voltammetric response, suggesting an inclusion process. The charge under the

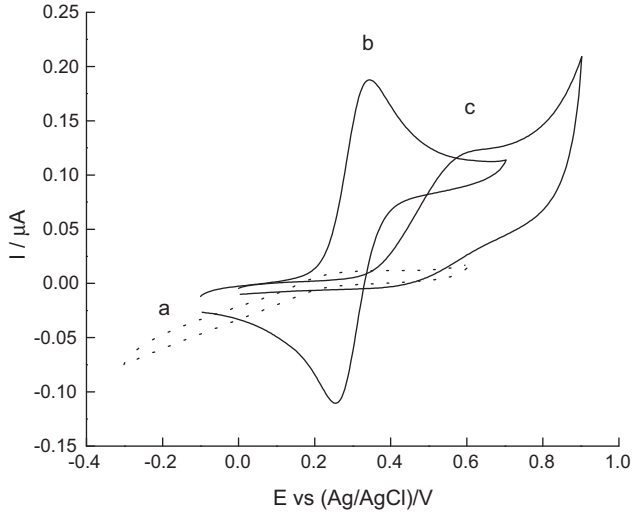

Fig. 5. Voltammetric response of 1.0×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ (curve a), 1.0×10^{-4} mol L^{-1} Fc-CO_2H (curve b), 6.0×10^{-5} mol L^{-1} MGN (curve c) on SAM β-CD modified electrode in 0.2 mol L^{-1} Na_2SO_4. Scan rate = 10 mV s^{-1}.

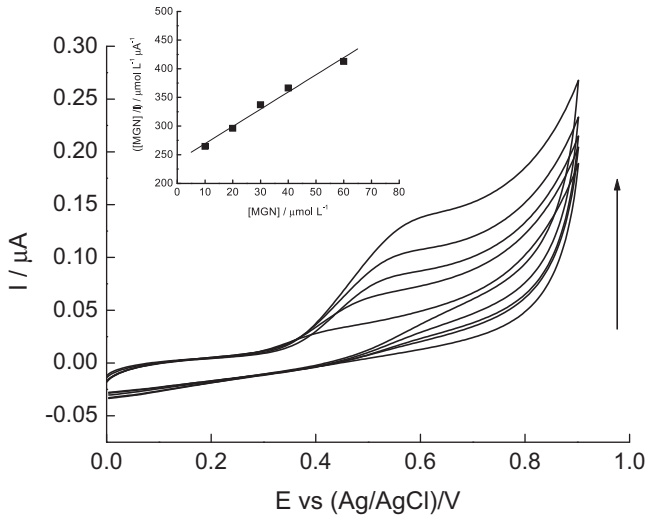

Fig. 6. Cyclic voltammograms recorded in 0.2 mol L^{-1} Na_2SO_4 solution at different concentrations of MGN (5 μmol L^{-1} up to 60 μmol L^{-1}). Inset: Determination of the equilibrium constant of the MGN:β-CD.

desorption wave provides a measure of the surface coverage, Γ, of the thiolated cyclodextrin β-CDSH). Γ was determined to be 3.43×10^{-9} mol cm^{-2}, which is a good surface coverage similar to reported values [20–26] and consistent with that expected for a densely packed monolayer [47,48].

The dependence of the peak currents on the concentration of MGN in solution was investigated. The peak current was saturated at high concentrations and the graph exhibits a shape similar to that expected for a Langmuir adsorption isotherm (Fig. 6). The stability or association constant of the complex was calculated according to the following equation (Eq. (5)) [23]:

$$\frac{[MGN]_0}{I} = \frac{1}{KI_{max}} + \frac{[MGN]_0}{I_{max}} \quad (5)$$

where I is the peak current at an initial concentration of MGN of $[MGN]_0$, I_{max} is the maximum peak current, and K is the association constant of inclusion with surface-confined CD. The K value was calculated at $1.3 \pm 0.3 \times 10^4$ L mol^{-1}, which is 10-fold lower than the value obtained spectrophotometrically.

An additional experiment to confirm the ability of SAMs to include MGN in their cavity was performed by competition, using increasing amounts of MGN in a solution containing Fc-CO_2H. The K_F for Fc-CO_2H in SAM was determined previously as 1.3×10^3 L mol^{-1} according to Eq. (5) [24]. The peak current reached saturation at high concentrations of Fc-CO_2H and the graph (Fig. 7) shows a profile similar to the Langmuir adsorption isotherm. As expected for inhibition competition, the difference between peak currents in the redox reaction of Fc-CO_2H, in the absence and presence of MGN, (ΔI_{p_a}), increased with increasing concentrations of MGN, according to Eq. (6) [24]:

$$\frac{[GUEST]_0}{\Delta I} = \left\{ \frac{(1 + K_{Fc\text{-}CO_2H}[Fc\text{-}CO_2H])}{c[CD]_0} \right\} \times \left\{ \frac{1 + K_{Fc\text{-}CO_2H}[Fc\text{-}CO_2H]}{KGUEST} + [GUEST] \right\} \quad (6)$$

where K_{GUEST} represents the K_F of the formation constant of MGN into β-CD on the electrode surface, $[CD]_0$ is the concentration of β-CD on the electrode surface, and c is the current/mol of Fc-CO_2H. Based on the above results, the value of $K_{GUEST} = K_{FMGN}$ was estimated at $1.7 \pm 0.8 \times 10^4$ L mol^{-1}, which is similar to the one obtained by the direct method based on Eq. (5), just described (Fig. 7).

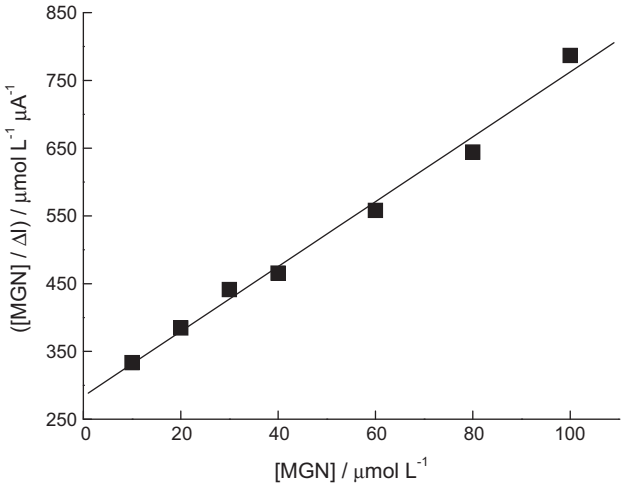

Fig. 7. Plot of [MGN]/ΔI (difference between the peak current of the redox processes of Fc-CO_2H in the presence and absence of MGN) *vs.* [MGN] on CDSH/MUA modified electrode at Na_2SO_4 0.1 mol L^{-1}.

The differences in the values obtained by spectrophotometry and electrochemistry are likely explained by differences in experimental conditions and by the steric hindrance for the solutes to penetrate the CD cavity on the gold electrode [26].

3.2.3. Calibration curve and determination of the limit of detection

Several articles in the literature have reported the interaction between MWCNTs and β-CD [33,49]. The analytical performance of the electroanalytical method developed for MGN determination was evaluated on CNT and β-CDCNT working electrodes. On the β-CDCNT electrode, the MGN oxidation peak appeared at more negative potential (ΔE_p = 60 mV) than on the unmodified electrode (the CNT-DMF electrode) and the current showed a significant increase (45%). The response mechanism of the β-CDCNT modified electrode for MGN is based on the combination of electrostatic and inclusion interaction of β-CD with MGN which is distinguished from the response mechanism of non-modified electrode. From the differential pulse voltammograms, which were recorded with increasing amounts of MGN using the optimized parameters, the peak current was found to increase with the analyte concentration within an adequate range. A good linear relationship was obtained between the anodic peak current and concentrations from 1×10^{-6} to 7.4×10^{-5} mol L^{-1}. The regression equation was (I_{p_a} (mA), c (mM), R = 0.996), and the detection and quantification limits are described in Table 2. No blockage of the electrode surface was observed up to this point, which is a major advantage of this procedure.

3.3. PM-IRRAS spectroscopy studies

PM IRRAS on a gold surface was used to confirm the presence of the inclusion complex. Fig. 8 shows spectra of the gold surface recorded before and after the inclusion of mangiferin. The IR spectrum of MGN/β-CD exhibits an intense band at 1265 cm^{-1}

Table 2
Analytical parameters: limit of detection (LOD) (mol L^{-1}) and limit of quantification (LOQ) (mol L^{-1}), obtained by using CNT-DMF and β-CDCNT.

Electrode	CNT (mol L^{-1})	β-CDCNT (mol L^{-1})
LOD	3.28×10^{-6}	8.64×10^{-7}
LOQ	1.09×10^{-5}	2.88×10^{-6}

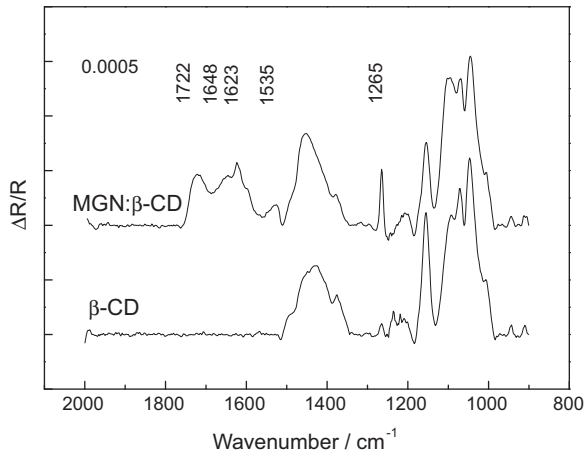

Fig. 8. PM IRRAS spectra of β-CD and MGN:β-CD complex.

and several others in the range of 1800–1600 cm^{-}, which can be assigned to C–O and C=O stretching vibrations from MGN.

3.4. ^1H NMR spectroscopy studies

^1H NMR spectroscopy is one of the most useful techniques for investigating the stability and stoichiometry of complexes, including host–guest systems. The NMR technique allows for direct and detailed observation of individual nuclei relevant to the structure and dynamics of the system. It should be emphasized that these experiments are difficult to perform due to the low solubility of MGN and the need for using D_2O. Fig. 9 depicts ^1H NMR spectra of MGN and MGN:β-CD complex. We were able to analyze only the downfield region of the spectrum (from 6 ppm). Due to the complexity of the signals related to the sugar moiety in the region of 5.0–1.0 ppm, it was not possible to analyze the chemical shifts. A comparison of A (without β-CD) and B (with β-CD) indicated that the NMR signals for H-5 and H-8 underwent downfield shifts from 7.4 to 7.6 ppm and from 6.8 to 6.9 ppm, while, due to the enhanced

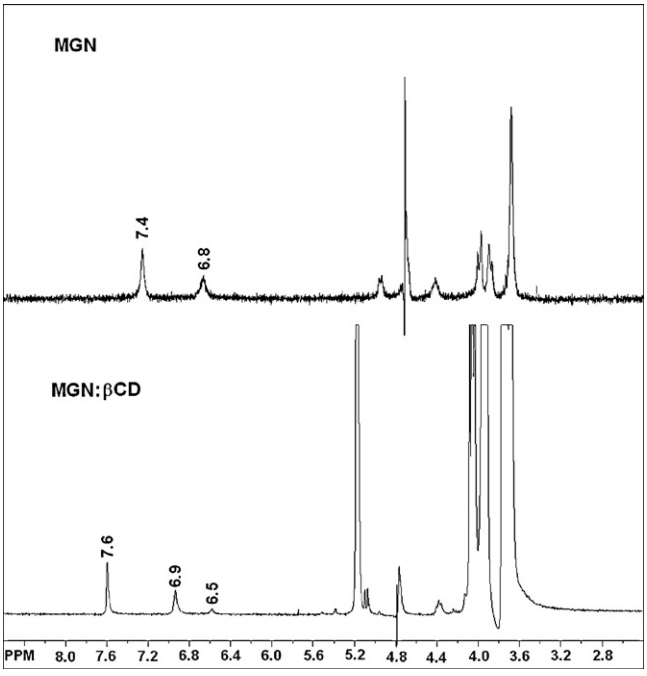

Fig. 9. ^1H NMR spectra of MGN (upper) and MGN:β-CD complex (lower) in D_2O.

concentration of MGN provided by the β-CD complexation, a signal appeared for H-4 at 6.5 ppm. These results indicate that the inclusion of MGN in the CD cavity occurs through its aromatic group.

4. Conclusions

This work involved an electrochemical study of MGN in the presence of β-CD, aiming to gain further insights on the solubility, bioavailability, and reactivity of this biologically active molecule.

The inclusion complex between β-CD and MGN was formed with a stoichiometry of 1:1 and an apparent formation constant K_F $1.6 \pm 0.7 \times 10^5\,L\,mol^{-1}$ was obtained by spectrophotometry. Electrochemical methods (direct and indirect) led to values of $1.3 \pm 0.3 \times 10^4\,L\,mol^{-1}$ and $1.7 \pm 0.8 \times 10^4\,L\,mol^{-1}$, respectively. The different values obtained by spectrophotometry (higher) and electrochemistry are likely attributable to differences in experimental conditions and to the steric hindrance for the solutes to penetrate the CD cavity on the gold electrode.

The use of the β-CDCNT electrode enhanced the response in voltammetric measurements of MGN. Therefore, the use of a combination of host–guest interactions may be an advantageous analytical strategy in the development of sensors. The inclusion process was corroborated by the ^{1}H NMR and PM IRRAS studies.

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References

[1] S. Prabhu, M. Jainu, K.E. Sabitha, C.S.S. Devi, J. Ethnopharmacol. 107 (2006) 126.
[2] B. Gómez-Zaleta, M.T. Ramírez-Silva, A. Gutiérrez, E. González-Vergara, M. Güizado-Rodríguez, A. Rojas-Hernández, Spectrochim. Acta A 64 (2006) 1002.
[3] S.M.R. Ribeiro, J.H. de Queiroz, M.E.L.R. de Queiroz, F.M. Campos, H.M.P. Sant'ana, Plant Foods Hum. Nutr. 62 (2007) 13.
[4] J.C. Barreto, M.T.S. Trevisan, W.E. Hull, G. Erben, E.S. de Brito, B. Pfundstein, G. Wurtele, B. Spiegelhalder, R.W. Owen, J. Agric. Food Chem. 56 (2008) 5599.
[5] T. Miura, N. Iwamoto, M. Kato, H. Ichiki, M. Kubo, Y. Komatsu, T. Ishida, M. Okada, K. Tanigawa, Biol. Pharm. Bull. 24 (2001) 1091.
[6] A.C.S. Carvalho, M.M. Guedes, A.L. de Souza, M.T.S. Trevisan, A.F. Lima, F.A. Santos, V.S.N. Rao, Planta Med. (Stuttgart) 73 (2007) 1.
[7] G. Garrido, D. González, Y. Lemus, D. García, L. Lodeiro, G. Quintero, C. Delporte, A.J. Núñez-Sellés, R. Delgado, Pharmacol. Res. 50 (2004) 143.
[8] A. Dar, S. Faizi, S. Naqvi, T. Roome, S. Zikr-Ur-Rehman, M. Ali, S. Firdous, S.T. Moin, Biol. Pharm. Bull. 28 (2005) 596.
[9] S. Guha, S. Ghosla, U Chattopadhyay, Chemotherapy 42 (1996) 443.
[10] J.M. Leiro, E. Álvarez, J.A. Arranz, I.G. Siso, F. Orallo, Biochem. Pharmacol. 65 (2003) 1361.
[11] N. Makare, S. Bodhankar, V. Rangari, J. Ethnopharmacol. 78 (2001) 133.
[12] S. Muruganandan, S. Gupta, M. Kataria, J. Lal, P.K. Gupta, Toxicology 176 (2002) 165.
[13] G.C. Jagetia, M.S. Baliga, Phytomedicine 12 (2005) 209.
[14] G. Pardo-Andreu, R. Delgado, J.A. Velho, C. Curti, A.E. Vercesi, Eur. J. Pharmacol. 513 (2005) 47.
[15] A.E. Beltrán, Y. Alvarez, F.E. Xavier, R. Hernanz, J. Rodrıguez, A.J. Núñez, M.J. Alonso, M. Salaices, Eur. J. Pharmacol. 499 (2004) 297.
[16] M.E. Brewster, T. Loftsson, Adv. Drug Deliv. Rev. 56 (2007) 645.
[17] L. Szente, J. Szejtli, Trends Food Sci. Technol. 15 (2004) 137.
[18] H. Teng, C. Yu, D. Zhai, Z. Li, J. Liu, Hainan Shengtian Artif. Pharm. (2008) (CN1977855-A).
[19] A.E. Carvalho, G.B. Alcantara, S.M. Oliveira, A.C. Micheletti, N.K. Honda, G. Maia, Electrochim. Acta 54 (2009) 2290.
[20] H. Endo, T. Nakaji-Hibarayashi, S. Morokoshi, M. Gemmei-Ide, H. Kitano, Langmuir 21 (2005) 1314.
[21] M.T. Rojas, R. Königer, J.F. Stoddart, A.E. Kaifer, J. Am. Chem. Soc. 117 (1995) 336.
[22] S.-W. Choi, J.-H. Jang, Y.-G. Kang, C.-J. Lee, J.-H. Kim, Colloids Surf. A 257–258 (2005) 31.
[23] Y. Maeda, T. Fukuda, H. Yamamoto, H. Kitano, Langmuir 13 (1997) 4187.
[24] F.S. Damos, R.C.S. Luz, L.T. Kubota, Electrochim. Acta 53 (2007) 1945.
[25] F.S. Damos, R.C.S. Luz, A.A. Sabino, M.N. Eberlin, R.A. Pilli, L.T. Kubota, J. Electroanal. Chem. 601 (2007) 181.
[26] T. Fukuda, Y. Maeda, H. Kitano, Langmuir 15 (1999) 1887.
[27] Y. Yue, X. Chen, J. Qin, X. Yao, J. Pharm. Biomed. Anal. 49 (2009) 753.
[28] D. Han, C. Chen, C. Zhang, Y. Zhang, X. Tang, J. Pharm. Biomed. Anal. 51 (2010) 260.
[29] C. Nong, W. He, D. Fleming, L. Pan, H. Huang, J. Chromatogr. B 826 (2005) 226.
[30] T. Zhou, Z. Zhu, C. Wang, G. Fan, J. Peng, Y. Chai, Y. Wu, J. Pharm. Biomed. Anal. 44 (2007) 96.
[31] L. Lai, L.-C. Lin, J.-H. Lin, T.-H. Tsai, J. Chromatogr. A 987 (2003) 367.
[32] T.-T. Jong, M.-R. Lee, Y.-C. Chiang, S.-T. Chiang, J. Pharm. Biom. Anal. 40 (2006) 472.
[33] G. Alarcón-Angeles, B. Pérez-López, M. Palomar-Pardave, M.T. Ramírez-Silva, S. Alegret, A. Merkoçi, Carbon 46 (2008) 898.
[34] G. Wang, X. Liu, B. Yu, G. Luo, J. Electroanal. Chem. 567 (2004) 227.
[35] Z. Wang, S. Xiao, Y. Chen, J. Electroanal. Chem. 589 (2006) 237.
[36] J.-L. He, Y. Yang, X. Yang, Y.-L. Liu, Z.-H. Liu, G.-L. Shen, R.-Q. Yu, Sens. Actuators B 114 (2006) 94.
[37] U. Yogeswaran, S. Thiagarajan, S.-M. Chen, Carbon 45 (2007) 2783.
[38] B.L. Frey, R.M. Corn, S.C. Weibel, Handbook of Vibrational Spectroscopy, John Wiley & Sons, 2001.
[39] J. Hernández-Benito, S. González-Mancebo, E. Calle, M.P. García-Santos, J. Casado, J. Chem. Educ. 76 (1999) 419.
[40] E. Alvarez-Parrilla, L.A. de La Rosa, F. Torres-Rivas, J. Rodrigo-Garcia, G.A. González-Aguilar, J. Incl. Phenom. Macrocycl. Chem. 53 (2005) 121.
[41] E. Laviron, J. Electroanal. Chem. 100 (1979) 263.
[42] P. Janeiro, A.M.O. Brett, Anal. Chim. Acta 518 (2004) 109.
[43] D. Zielinska, B. Pierozynski, W. Wiczkowski, J. Electroanal. Chem. 640 (2010) 23.
[44] G.J. Volikakis, C.E. Efstathiou, Talanta 51 (2000) 775.
[45] F. Amat-Guerri, M. Carrascoso, M. Luiz, A.T. Soltermann, A. Biasutti, N.A. García, J. Photochem. Photobiol. A: Chem. 113 (1998) 221.
[46] J.W. da Cruz Jr., L.R. de Moraes, M.H. dos Santos, G.A. da Silva, M.R.P.L. Brigagão, J. Ellena, A.C. Doriguetto, Helv. Chim. Acta 91 (2008) 144.
[47] D.E. Weissham, M.M. Walczak, M.D. Porter, Langmuir 9 (1993) 323.
[48] M.M. Walczak, C.A. Alves, B.D. Lamp, M.D. Porter, J. Electroanal. Chem. 396 (1995) 103.
[49] K. Liu, H. Fu, Y. Xie, L. Zhang, K. Pan, W. Zhou, J. Phys. Chem. C 112 (2008) 951.