Full Paper

Electroactive Dipyrromethene-Cu(II) Monolayers Deposited onto Gold Electrodes for Voltammetric Determination of Paracetamol

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Abstract
Dipyrromethene-Cu(II) derivatives possessing two dodecane alkyl chains have been used for the modification of gold electrodes. Electroactive host molecules have been incorporated into a lipophilic dodecanethiol SAM deposited onto gold electrodes through hydrophobic and van der Waals interactions (embedment technique). The presence of dipyrromethene-Cu(II) redox centers on the electrode surface was proved by cyclic voltammetry and Osteryoung square-wave voltammetry. The Au electrodes incorporating redox active Cu(II)-dipyrromethene SAMs were used for the direct voltammetric determination of paracetamol in human plasma.

Keywords: Electroactive monolayer, Cu(II)-dipyrromethene, Embedment modification, Voltammetric sensor, Paracetamol

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1. Introduction

Although typically techniques employed in clinical laboratories encompass titrimetry, chromatography, spectrophotometry and immunoassay, the use of electroanalytical interfacial techniques have attracted attention as accurate, sensitive and cost-effective methods of analysis [1].

Much progress has been made during the last 20 years in attaining electrodes with controllable surface properties. One of the most popular techniques to create well-defined functional surfaces is the formation of self-assembled monolayers (SAMs) [2, 3].

A major class of SAMs is based on the covalent bond formation between noble metal atoms and sulfur atoms of thiols, disulfides, sulfides, and other related molecules.

This phenomenon has been discovered by Allara and Nuzzo [4] in 1983 and since then a vast amount of research has been carried out in this field, e.g., by Whitesides and coworkers [5].

The advantages of SAMs include simplicity of preparation, versatility, stability, reproducibility and the possibility of introducing different chemical functionalities with high level of order at the molecular dimension. Several reports on the use of SAMs to improve selectivity and sensitivity of gold electrodes in a broad range of electroanalytical applications have been published [6, 7]. Despite of the promising properties of SAMs, they also have some weak points. The most important is the presence of pinholes and defects, which effect on the faradaic response of blocking monolayers [8–10].

Attaching a redox center to SAMs is a possible solution of the above problem.

The electroactive monolayers display many advantages in comparison to monolayers without a redox center: close packing prevents motion of the redox centers towards the electrode, towards a pinhole or a defect. Therefore, faradaic current due to redox centers at, or near, pinholes and defects becomes a negligible component of the total faradaic current.

The double-layer correction for the surface concentration versus the bulk concentration is no longer necessary. In addition, the same electron transfer theory concerning blocking SAMs might be applied to this system [9, 10]. The most popular electroactive SAMs contain ferrocenes [11, 12], ruthenium complexes [13, 14], phthalocyanines [15–17], metalloporphyrins [18, 19], cytochromes [20] and atrazine [21], just to name a few.

Here, we present a new electroactive SAM with Cu(II)-dipyrromethene redox centers. The Cu(II)-dipyrromethene molecules (Fig. 1) were immobilized on gold electrode surfaces, previously modified with a dodecanethiol monolayer via hydrophobic and van der Waals interactions (embedment technique) [22, 23]. In this modification technique, the host molecules should only posses an alkyl

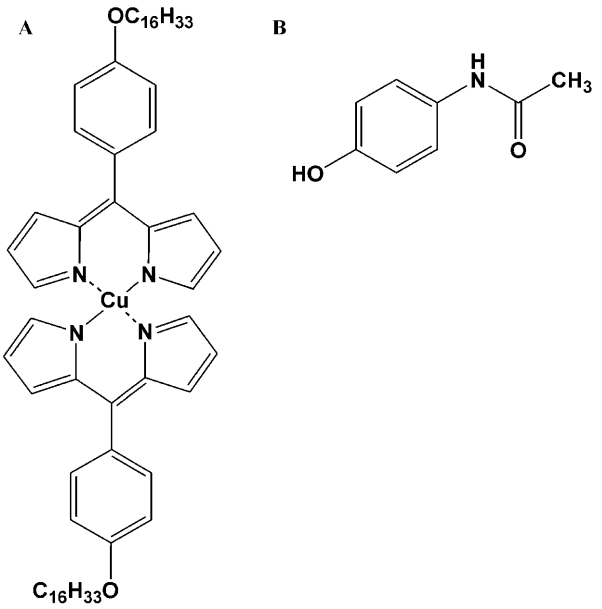

Fig. 1. Chemical structure of A) Cu(II)-dipyrromethene (Cu(II)-DPM) and B) paracetamol (PCT).

chain. The presence in their structures of an SH or S–S function is not necessary.

The redox active surface obtained in the above way was characterized by cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV). The Cu(II)-dipyrromethene/dodecane thiol-Au electrode was applied for the direct voltammetric determination of paracetamol (PCT), a common analgesic and antipyretic drug, in human plasma.

The majority of electroanalytical methods for determination of paracetamol relay on its ability for oxidation at: a gold nanoparticles-attached indium tin oxide [24], carbon film resistor [25], glassy carbon electrodes modified with C_{60} [26], gold electrodes modified with a 3-mercaptopropionic acid monolayer [27], carbon paste electrodes [28] or PANI-MWCNTs composite modified electrodes [29]. However, these analytical procedures are nonselective, since the potential involved in this process ranges from 0.6 to 0.8 V and various substances are electroactive in this potential interval [28, 30].

The main aim of the study presented is the development of voltammetric sensors for direct determination of paracetamol (PCT) free of the above interferences.

Supramolecular interactions between the Cu(II)-DPM host and the paracetamol guest induced changes of the redox properties of the Cu(II) centers and were detected by CV or OSWV techniques. The usability of the proposed sensor has been checked by the recovery test performed in the presence of human plasma.

2. Experimental

2.1. Materials

Cu(II)-dipyrromethene (Fig. 1) was synthesized according to an already published procedure [31]. 4-Acetamidophenol (paracetamol), 1-dodecanethiol and chloroform were used as received from Sigma-Aldrich (Germany). KCl, NaCl, KOH, ethanol and tetrahydrofuran (THF) were obtained from POCh (Gliwice, Poland). Human plasma was obtained from the Regional of Blood Donating Centre in Olsztyn, Poland.

All the solutions were prepared with deionized water (with the resistivity 18.2 MΩ cm) purified with a Milli-Q reagent grade water system (Millipore, Bedford, MA).

2.2. Electrode Modification

Gold disk electrodes 2 mm^2 area (Bioanalytical Systems BAS, West Lafayette, IN) were used for the experiments.

The gold disk electrodes were polished with wet 0.3 and 0.05 μm alumina slurry (Alpha and Gamma Micropolish, Buehler, USA) on a flat pad for at last 10 min and rinsed repeatedly with water and sonicated in water for 30 s. Finally the polished electrodes were dipped in 0.5 M KOH solution deoxygenated by purging with argon for 10 min, and the potential was cycled between −0.4 and −1.2 V versus a Ag/AgCl reference electrode with scan rate of 0.1 $V\,s^{-1}$ till cyclic voltammograms did not show any further change.

Cleaned electrodes were soaked in 1×10^{-6} M 1-dodecanethiol solution in ethanol at room temperature for 30 min. Then, after washing with ethanol and chloroform, the electrodes were dipped in 1×10^{-3} M Cu(II)-dipyrromethene solution in chloroform for 18 h. After Cu(II)-dipyrromethene immobilization, the electrodes were washed and stored 24 h in 0.1 M KCl until use.

2.3. Electrochemical Measurements

All electrochemical measurements were performed with a potentiostat-galvanostat AutoLab (Eco Chemie, Ultrecht, Netherlands) with three-electrode configuration with gold electrodes as working electrodes. Potentials were measured versus the Ag/AgCl electrode, and a platinum wire as the auxiliary electrode. Cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV) were performed in 0.1 M KCl or 0.9% NaCl solutions. In CV, the potential was cycled from +0.7 V to −0.2 V with a scan rate of 0.1 $V\,s^{-1}$.

Cyclic voltammetry (CV) while using the electrodes modified with Cu(II)-dipyrromethene/DDT was also performed with different scan rates: 10, 50, 100, 200, 300, 400, 500, 700, 800 $mV\,s^{-1}$.

The OSWV measurements were recorded within +0.6 to −0.3 V, with a step potential of 5 mV, a square wave frequency of 100 Hz and potential amplitude of 25 mV.

2.4. Paracetamol Determination in Human Plasma

Gold electrodes modified with Cu(II)-DPM/DDT SAM were used for voltammetric determination of PCT in human

plasma. The natural human plasma was diluted 10 times with a 0.09% NaCl solution and the samples were spiked for the known amount of PCT.

On the base of the responses of the gold electrodes modified with Cu(II)-DPM/DDT SAM, the calibration curve was prepared.

3. Results and Discussion

3.1. Characterization of Gold Electrodes Modified with a Cu(II)-Dipyrromethene-Dodecanethiol Layer

The deposition of Cu(II)-DPM on the surface of gold electrodes involved two steps. Firstly, the electrodes were modified with dodecanethiol to form a homogenous lipophilic monolayer on the surface. In the second step, Cu(II)-DPM with two lipophilic alkyl chains (Fig. 1) was immobilized via hydrophobic and van der Waals interactions. Such technique of modification is called embedment, and was already applied for immobilization of lipophilic macrocyclic polyamines [22, 23].

The presence of Cu(II)-DPM molecules on the electrode surface has been proved by CV and OSWV performed in 0.1 M KCl solution. Well defined anodic and cathodic peaks were visible at $E_{pa} = 332 \pm 22$ mV and $E_{pc} = 236 \pm 21$ mV, respectively (Fig. 2A, curve a). The peak separation of $\Delta E = E_{pa} - E_{pc} = 96$ mV indicated that the redox processes of Cu(II)-DPM immobilized on the DDT SAM displayed the reversible behavior. All potentials reported here are average values from nine measurements done with new electrode modification.

A well defined peak was observed at a potential of 313 ± 17 mV (Fig. 2B, curve a).

As the reference modification, an electrode covered only with DDT SAM was explored. This type of electrode presented good blocking behavior (Fig. 2A, B, curve b).

Figure 3A presents the current behavior of the Cu(II)-DPM/DDT-Au electrode as a function of the scan rate in the range between 10 and 800 mV s^{-1}. The plot of the anodic (I_{pa}) and cathodic (I_{pc}) peak current versus the scan rate shows a linear relation up to 800 mV s^{-1} (Fig. 3B) thus confirming the immobilized state of the Cu(II)-DPM complex.

3.2. Interaction Between Cu(II)-Dipyrromethene and Paracetamol

The peak current connected with the redox processes of Cu(II)-DPM immobilized on the electrode surface was observed at 313 ± 17 mV by OSWV (Fig. 2B). OSWV, as more sensitive than CV, was selected for exploring the interactions between Cu(II)-DPM and PCT.

In the presence of 15 mM of PCT in the 0.1 M KCl solution, the peak connected with Cu(II)-DPM redox processes was shifted to negative potential and was observed at -51 ± 12 mV (Fig. 4, curve a).

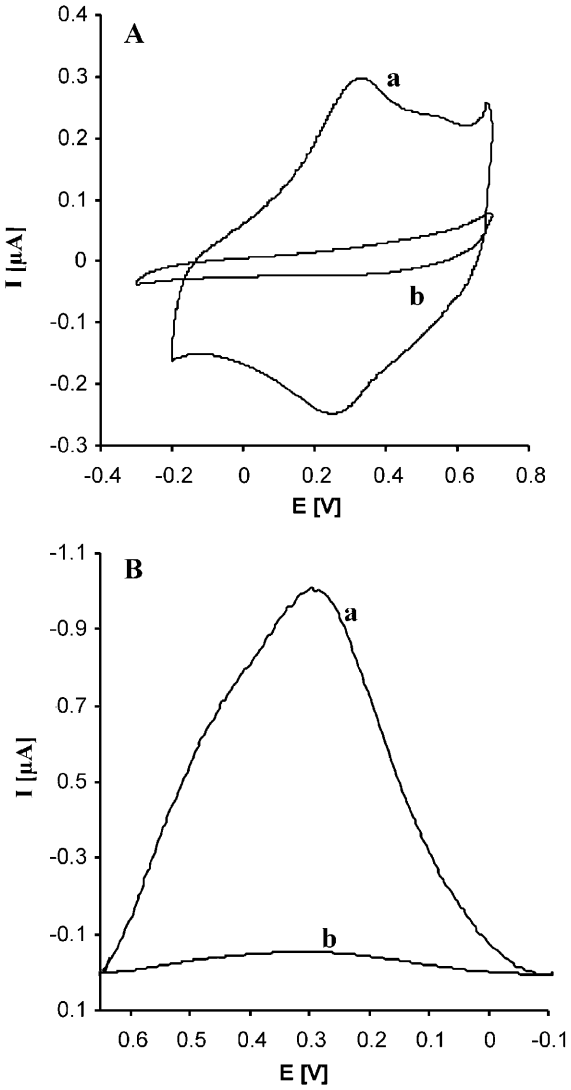

Fig. 2. A) CVs and B) OSWV of a) Cu(II)-DPM/DDT-Au electrode and b) DDT-Au electrode. Measuring conditions: 0.1 M KCl; scan rate 0.1 mV s^{-1}; Ag/AgCl as reference electrode.

In order to prove that within the potential window used in the present study (-300 – 600 mV), the paracetamol is not electrochemically active, OSWV was performed using gold electrodes modified only with a DDT SAM. In this case no current was observed (Fig. 4, curve b).

In the next step, the voltammetric response of Cu(II)-DPM/DDT-Au electrode was checked in the presence of different concentrations of PCT. The electrodes modified by embedding of Cu(II)-dipyrromethene into DDT SAM, display different current of redox process, within the range from 1.5 µA to 1.2 µA (mean value obtained from 8 electrodes: 1.4 µA $\pm$ 0.2 *sd*). Therefore, in order to eliminate the influence of current value observed in the supporting electrolyte solution on the changes observed in the presence of paracetamol, the relative current change $(I_p/I_{p0}) \times 100\%$ vs. log(PCT concentration) was selected for getting the calibration curves. Enhancement current responses (at potential -51 mV) were observed from 3.5×10^{-5} M to

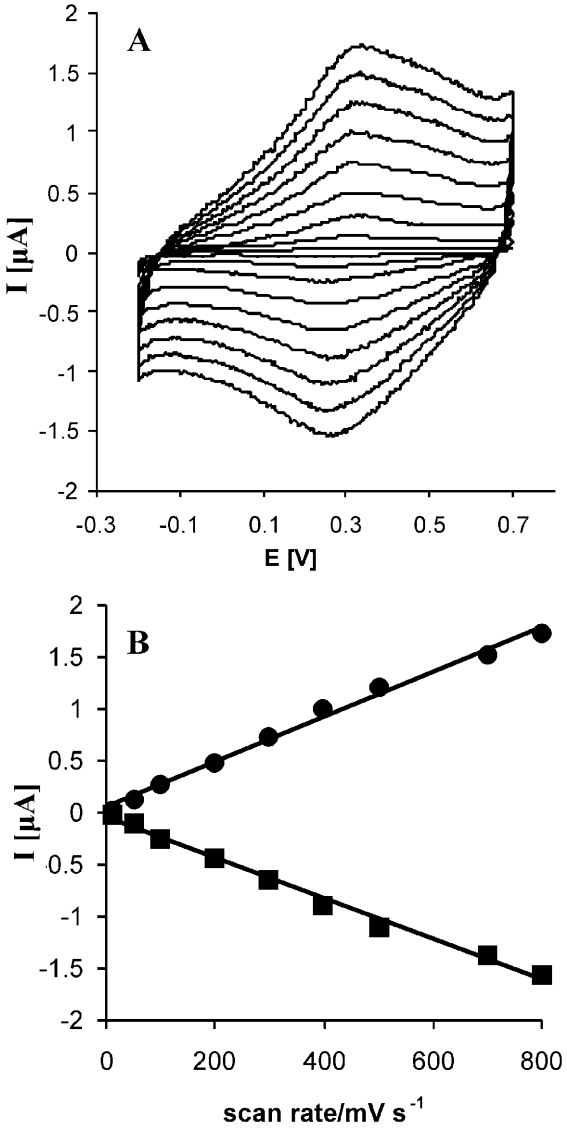

Fig. 3. A) Cyclic voltammograms of Cu(II)-DPM/DDT-Au electrode measured at different scan rate. B) Plot of anodic (●, I_{pa}) and cathodic (■, I_{pc}) peak current against potential scan rates. Measuring conditions: see Figure 2.

1.5×10^{-3} M PCT concentration. A linear calibration curve was obtained over the range 2.0×10^{-4} M – 1.5×10^{-3} M of paracetamol (Fig. 5). The detection limit estimated based on the linearization method recommended by IUPAC [32] was $1.2 \pm 0.07 \times 10^{-4}$ M.

In the present study, the dipyrromethene complex with Cu(II) was selected as a host molecule for voltammetric sensor for paracetamol determination. This molecule shows the reduction/oxidation peaks within the potential windows in which the PCT molecules remain electrochemically inactive. To our knowledge, this is the first report on PCT voltammetric sensors based on host – guest recognition. The interaction between Cu(II)-DPM and PCT changed the redox properties of Cu(II) centers and the peak current observed with OSWV was shifted into negative direction for ca. 360 mV.

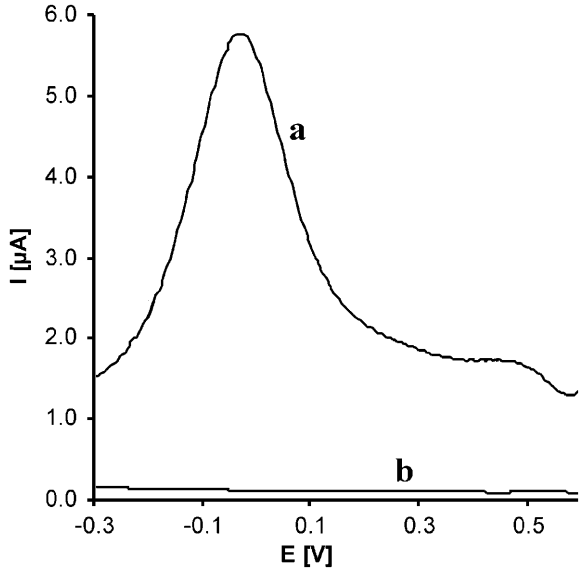

Fig. 4. OSWV curves of: a) DDT-Cu(II)-DMP/DDT-Au electrode and b) DDT-Au electrode measured in the presence of 1.5×10^{-3} M PCT concentration. Measuring conditions: see Figure 2.

The recognition process occurred between Cu(II)-dipyrromethene host and paracetamol guest was irreversible. Therefore, with using one electrode, only one set of voltammetric measurements in the presence of different concentrations of PCT was performed. On the other hand, the modification of electrodes by embedding of Cu(II)-dipyrromethene into DDT SAMs was very stable. No changes in the sensitivity towards PCT was observed after storing during one month (longer time was not explored) in the supporting electrolyte solution.

3.3. Determination of Paracetamol in Human Plasma

Paracetamol is a safe antipyretic and analgesic agent. However, when taken in very large doses, this drug causes severe liver injury [33 – 35]. The hepatic toxicity begins with plasma levels of paracetamol in the 120 µg mL^{-1} (0.8 Mmol) [30].

Thus, the sensitivity of gold electrodes modified with Cu(II)-DPM/DDT is sufficient for controlling the safe concentration level of PCT or its overdose in human plasma.

The voltammetric responses of the electrode studied towards PCT were explored in the presence of human serum 10 times diluted with 0.09% NaCl. In the absence of PCT, the peak current connected with the redox processes of Cu(II)-DPM was observed at 290 ± 16 mV (Fig. 6). The position of this peak was shifted a little into the negative direction in comparison to OSWV measurements performed in the 0.01 M KCl solution (Fig. 2B). With increasing concentration of PCT in the plasma solution, a new peak current was created at -230 ± 24 mV (Fig. 6). The new peak created upon interaction between Cu(II)-dipyrromethene host and paracetamol guest in the presence of human plasma was

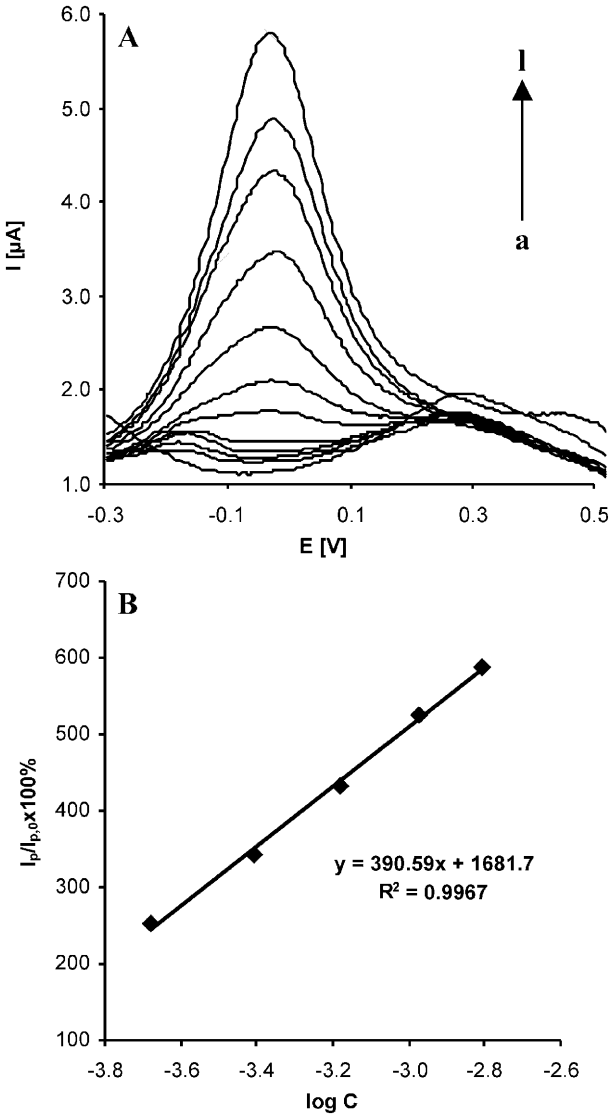

Fig. 5. A) OSWV curves of Cu(II)-DPM/DDT-Au electrodes measured in the presence of various concentrations of PCT: a) 0, b) 1.6×10^{-7} M, c) 1.6×10^{-6} M, d) 6.5×10^{-6} M, e) 1.6×10^{-5} M, f) 3.6×10^{-5} M, g) 1.1×10^{-4} M, h) 2.1×10^{-4} M, i) 3.9×10^{-4} M, j) 6.6×10^{-4} M, k) 1.1×10^{-3} M, l) 1.5×10^{-3} M. Measuring conditions: see Figure 2. B) The ratio of OSWV maximum peak current for electrodes modified with Cu(II)-DPM/DDT in the presence of different concentration of PCT (I_p) to that in the absence of PCT (I_{p0}) as a function of PCT concentration ($n = 3$; $5.1\% < sd < 13.3\%$).

shifted to a more negative potential in comparison to measurements carried out in 0.1 M KCl. Thus, it might be concluded that the voltammetric signals observed were generated based on changes of redox properties of Cu(II)-dipyrromethene occurring upon interaction with paracetamol molecules. In the potential window (−0.6 to 0.6 V) applied for the research presented, the oxidation of paracetamols is not possible. The OSWV measurement carried out in the presence of 15 mM PCT using an Au electrode modified only with dodecane/thiol SAM, showed no voltammetric response (Fig. 4, curve b).

The linear relationship between log C of PCT in the presence of human plasma versus the relative peak current increase was observed within the concentration range of 2.4×10^{-4} M to 3.16×10^{-3} M. Although the values of the peak current observed in the presence of plasma were lower than those observed in 0.1 M KCl solution, the electrode was quite sensitive towards PCT. The detection limit estimated based on the linearization method recommended by IUPAC [32] was $1.3 \pm 0.07 \times 10^{-4}$ M, very similar to the value observed in 0.1 M KCl solution.

In order to show the applicability of the sensor proposed, a recovery test based on the calibration curve made in the presence of human plasma was done. 5 mL of human plasma, 10 times diluted with 0.09% NaCl, was spiked with a known amount of PCT in order to get three concentrations: 0.3, 1.0 and 1.3 mM. Next, known concentrations of PCT were determined, voltammetrically using the calibration curve obtained in the presence of 10 times diluted human plasma (Fig. 6B).

The results obtained were collected in Table 1. Recoveries were in the range 93.7% to 105.4%. This indicated that the sensor proposed could be applied for the direct amperometric determination of a toxic level of PCT in human plasma.

4. Conclusions

The electrochemically active Cu(II)-dipyrromethene complex was immobilized on the surface of gold electrodes previously modified with a dodecane/thiol monolayer via hydrophobic and van der Waals forces. The linear relationship between the cathodic and anodic current vs. the scan rate proved that the redox centers are immobilized on the electrode surface.

Well defined, electrochemically active Cu(II)-dipyrromethene/dodecanethiol-Au electrodes were applied for the direct voltammetric determination of paracetamol in 0.1 M KCl and in human plasma, 10 times diluted with 0.9% NaCl. The presence of human plasma decreased the electrode responses towards paracetamol, but in both measuring conditions a similar detection limit (ca. 0.1 mM) and linear dynamic range (0.2 – 3.2 mM) were observed. It has been proved that within the potential window applied (−0.3 to 0.6 V) paracetamol can not be oxidized. Therefore, the voltammetric signals were generated based on the change of redox properties of Cu(II)-dipyrromethene redox centers immobilized on the electrode surface caused by their interaction with paracetamol. The recognition process occurred between Cu(II)-dipyrromethene host and paracetamol guest was irreversible. Therefore, one electrode could be used for only one set of voltammetric measurements performed in the presence of different concentration of PCT. On the other hand, the modification of electrodes by embedding of Cu(II)-dipyrromethene into DDT SAMs was very stable. The storing in the supporting electrolyte solution have no influence on the electrode response towards paracetamol.

Table 1. Recovery test for PCT determination in human plasma with Cu(II)-DPM/DDT-Au electrode (measuring conditions see Fig. 6; $n=4$).

Paracetamol added (mM)	Paracetamol determined (mM)	Recovery (%)
0.32	0.30 (±0.02)	93.7 (±7.6)
1.00	0.96 (±0.05)	96.0 (±5.4)
1.29	1.36 (±0.07)	105.4 (±4.6)

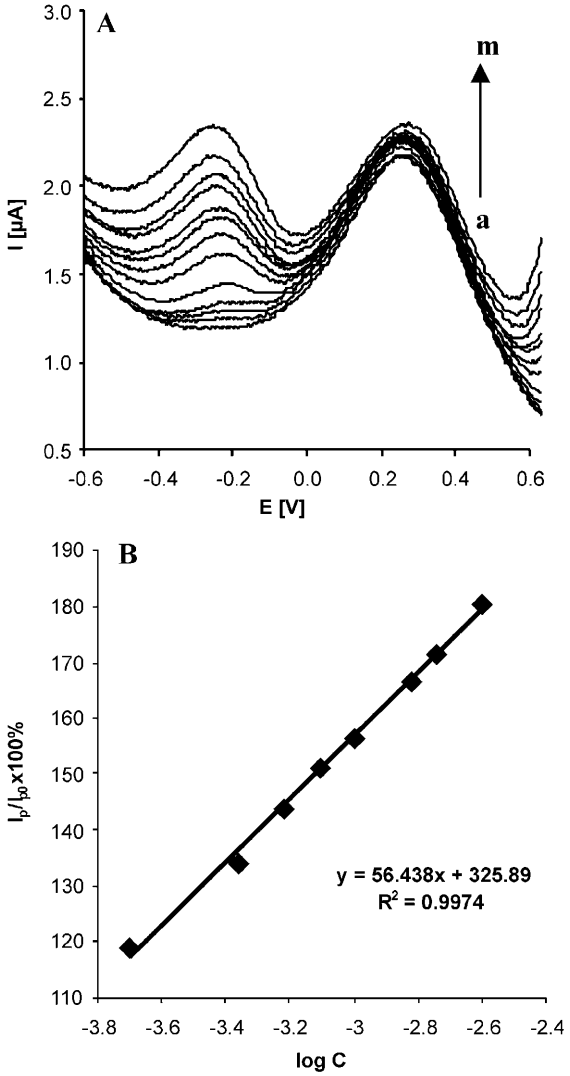

Fig. 6. A) OSWV curves for Cu(II)-DPM/DDT-Au electrodes measured in the presence of various concentrations of PCT in human plasma 10 times diluted with 0.09% NaCl: a) 0, b) 4.0×10^{-5} M, c) 6.0×10^{-5} M, d) 1.0×10^{-4} M, e) 2.0×10^{-4} M, f) 4.0×10^{-4} M, g) 6.0×10^{-4} M, h) 7.9×10^{-4} M, i) 1.0×10^{-3} M, j) 1.5×10^{-3} M, k) 1.8×10^{-3} M, l) 2.5×10^{-3} M, m) 3.2×10^{-3} M. Measuring conditions: see Figure 2. B) The ratio of OSWV maximum peak current for electrodes modified with Cu(II)-DPM/DDT in the presence of different concentration of PCT (I_p) to that in the absence of PCT (I_{p0}) as a function of PCT concentration ($n=6$; $4.7\% < sd < 13.2\%$).

The sensitivity of the present sensor is sufficient for controlling the toxic level of paracetamol in human plasma without previous treatment of the samples.

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