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Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 871–878
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Effects of EDTA on signal stability during electrochemical detection of acetaminophen

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Received 1 July 2003; received in revised form 24 October 2003; accepted 25 November 2003

Abstract

The use of EDTA in the medium to avoid the passivation of a solid electrode during electrochemical analysis of acetaminophen is presented in this work. The performance of this system was investigated with respect to pH, applied potential and supporting electrolyte concentration. The major advantage in using EDTA in the supporting electrolyte is the significant increase in sensitivity, precision and stability of the measurements, when compared to the system in absence of the chelating agent. The sensitivity increases 5.5 times (21.5 and 3.9 mA l mol^{-1} in the presence and the absence of EDTA, respectively), the repeatability ($n = 20$) is 3.5 times better, expressed by within-run-precision of 4.0% for 6.0×10^{-5} mol l^{-1} acetaminophen in the presence of EDTA while, in its absence, the within-run-precision was higher than 14%. Moreover, the system showed excellent stability, allowing more than 120 measurements with no significant changes.

Keywords: EDTA; Acetaminophen; Solid electrodes; Electroanalytical; High performance method

1. Introduction

Acetaminophen (paracetamol or *N*-acetyl-*p*-aminophenol) is a common over-the-counter drug that is present in many medications, without the secondary effects of the salicylates on the gastric mucose, although it may cause liver damage in some instances [1]. Acetaminophen (AC) is an effective and safe analgesic agent used worldwide for the relief of mild to moderate pain associated with headache, backache, arthritis and postoperative pain. It is also used for reduction of fevers of bacterial or viral origin [2,3].

Several analytical methods have been proposed for the determination of acetaminophen: spectrophotometric [2–6], spectrofluorimetric [7], reversed-phase HPLC [8–13], ion-pairing HPLC [12], capillary electrophoresis [13] and electrochemical [4,14,15] as well as in flow-injection analysis (FIA) coupled to various detection systems [15]. Although simple UV-spectrometric methods are frequently employed, the direct determination of analytes based on intrinsic absorbance measurements in the UV region

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doi:10.1016/j.jpba.2003.11.005

usually shows problems arising the non-specific absorption that limit its application with real samples [4].

To avoid this drawback, the spectrometric determination of acetaminophen has been carried out after prior chemical derivatization based upon nitration, oxidation and diazo coupling [16]. Several papers based on the chemical derivatization of acetaminophen have been published [8,17–19]. However, this methodology requires complex steps and a long preparation time.

Electrochemical methods have been widely exploited for analysis of pharmaceutical samples containing acetaminophen [4,14,15]. However, this drug is a phenolic compound and it is well known that these compounds have the ability to foul solid state electrodes. The tarry deposits formed on the electrodes during phenol oxidation are attributed to polymerization products, which are formed when phenoxy radicals attack an unreacted substrate. The accumulation of reaction products, which leads to the loss of electrode activity, is commonly referred as "poisoning" or "fouling" [20–22]. Thus, phenolic compounds determination by electrochemical sensors usually presents poor signal stability. Some procedures have been described attempting to diminish the problems caused by these films; e.g. the use of voltammetric sensors based on chemically modified carbon electrodes, improving the rate of the heterogeneous charge-transfer and the selectivity of the working potentials [23–25]. Although the modification of electrode surfaces avoids the inactivation of the electrodes due to the deposition of oxidized phenolic compounds, the steps involved in the procedures are laborious and, generally, not very reproducible.

In an earlier work [26], the EDTA addition to the supporting electrolyte showed a very simple and efficient strategy to avoid electrode passivation for the electrochemical determination of dopamine, as well as, of other phenolic compounds. Based on this premise, the present paper describes the ability of EDTA to avoid the passivation of a pyrolytic graphite electrode during acetaminophen determination by flow-injection analysis with electrochemical detection. Optimization of operational conditions and the performance of this system are reported. In addition, the method was compared with results obtained in the absence of EDTA and validated, for the determination of acetaminophen in pharmaceutical samples, comparing it with the reference method.

2. Experimental

2.1. Chemicals and solutions

Acetaminophen, potassium nitrate (Synth, Brazil), and ethylenediaminetetraacetic acid disodium salt dihydrate ($Na_2H_2EDTA{\cdot}2H_2O$) (Nuclear, SP, Brazil), were analytical grade and used without previous purification.

The working standard solutions (10^{-4} mol l^{-1}) were prepared by dissolving acetaminophen and KNO_3 in ultra pure (Milli-Q) water in the presence or absence of EDTA at the concentrations described in the text.

2.2. Electrochemical studies

All voltammetric measurements were carried out using a potentiostat-galvanostat–Autolab® PGSAT-30 (Eco Chemie B.V.; The Netherlands). A platinum wire was used as counter electrode and all potentials were recorded against a saturated calomel reference electrode (SCE) to measurements in bath. A pyrolytic graphite electrode (Metrohm, 2 mm of diameter) was used as working electrode.

In the voltammetric experiments carried in bath, the potential was scanned over the range from –200 to 600 mV using 5 ml of standard solution containing the mentioned analytes. Pure N_2 was bubbled through the sample solutions for 10 min before the voltammetric measurements.

2.3. Electrodes cleaning

The pyrolitic graphite electrode was previously polished with 0.5 μm alumina. After rinsing, the electrode was introduced into an ultrasonic cleaner for 3 min immersed first in ethanol and then in distilled water and, to eliminate any trace of alumina.

2.4. Flow injection system

The basic manifold used for the flow injection system is illustrated in Fig. 1A. For flow measurements,

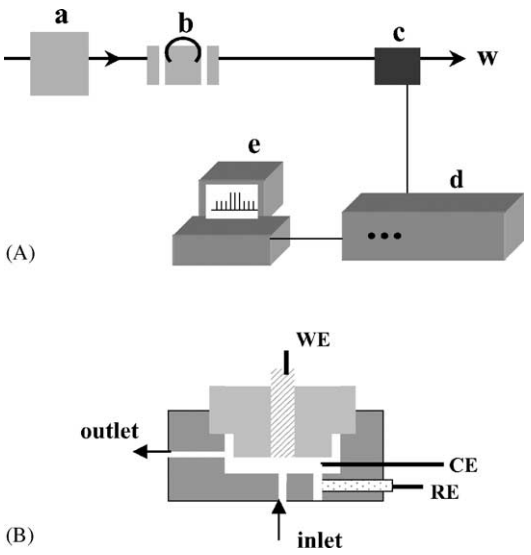

Fig. 1. Schematic diagram of the analytical system. Flow injection (A): pump (a); injector (b); electrochemical cell (c); potentiostat (d); PC microcomputer (e); waste (W). (B) Electrochemical cell: pyrolytic graphite as working electrode (WE); counter electrode (CE), and Ag/AgCl reference electrode (RE).

the pyrolytic graphite electrode was inserted into a confined wall-jet flow through the amperometric cell as described in Fig. 1B Platinum wire was used as a counter electrode and all potentials were recorded against a saturated Ag/AgCl reference electrode. The electrodes were connected to a potentiostat (Autolab® model PGSTAT 30, EcoChemie) interfaced to a PC microcomputer for potential control and data acquisition.

A mixture of EDTA and KNO_3 was used as carrier, pumped by a peristaltic pump (Ismatec). The sample loop comprises a volume of 100 μl of dilute standard solution and/or samples of the acetaminophen prepared in a 1.2×10^{-3} mol l^{-1} EDTA solution, which were inserted into the carrier, by using a sliding central bar sampling valve [27].

2.5. Sample preparation

Two tablets of each commercial sample from the same batch were weighed, ground and homogenized in a mortar. One hundred and fifty milligram of this powdered sample was used to prepare the sample solution. This solution was filtered and diluted with a 1.2×10^{-3} mol l^{-1} EDTA solution.

2.6. Reference method for acetaminophen

The electrochemical flow injection results were compared with the results from the official procedure recommended by the British Pharmacopoeia [28]. An appropriate amount of the powdered sample (Section 2.5) of acetaminophen was treated with 50 ml of 0.1 mol l^{-1} NaOH and made up to 100 ml with deionized water. After shaking for 15 min, 50 ml of deionized water was added and insoluble excipients were separated by filtration. An aliquot of 10 ml of the filtered solution was made up to 100 ml with deionized water and 10 ml of the resulting solution was mixed with 10 ml of 0.1 mol l^{-1} NaOH. The absorbance of the solutions obtained were measured at 257 nm, using a spectrophotometer (Pharmacia Biotech-Ultrospec 2000) interfaced a PC microcomputer for data acquisition.

3. Results and discussion

In a previous work [26], we have reported that EDTA addition to the supporting electrolyte is a simple and efficient strategy to avoid the passivation of solid electrodes by phenolic compounds during their voltammetric determination. Based on these results, the effects of EDTA on the stability of the electrochemical detection signal in the flow injection analysis of acetaminophen were studied. The preliminary experiments were made in a bath to learn about the system behavior. The following parameters were evaluated: effect of EDTA on the redox behavior of acetaminophen the influence of the EDTA concentration on the signal and the influence of the supporting electrolyte KNO_3.

3.1. Electrochemical behavior of acetaminophen in presence of EDTA

Fig. 2 shows cyclic voltammogram of acetaminophen obtained with the pyrolytic graphite electrode. The scans were performed in 0.1 mol l^{-1} KNO_3, pH 6.5 in the absence (curve B) and presence (curve C) of EDTA. In the absence of EDTA, acetaminophen is oxidized at the graphite pyrolytic electrode at 492 mV versus SCE and the corresponding cyclic voltammogram presents well defined anodic and cathodic

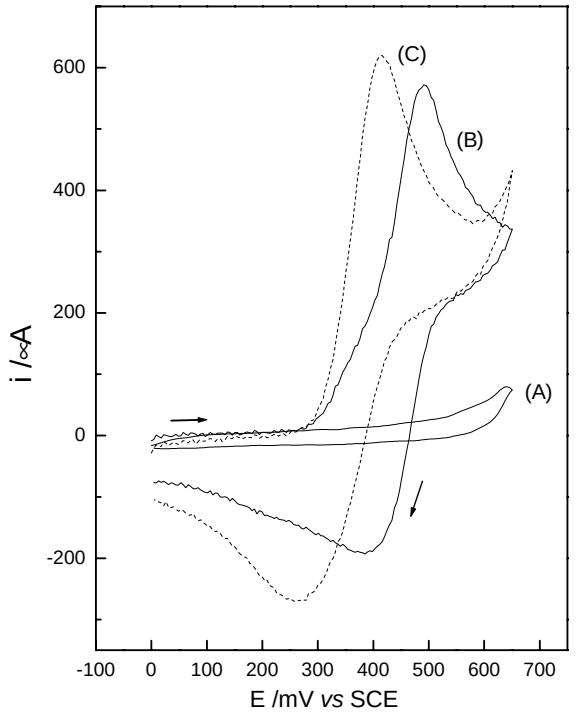

Fig. 2. Cyclic voltammograms obtained for acetaminophen with a pyrolytic graphite electrode. The scans were performed in 0.1 mol l^{-1} KNO_3, pH 6.5, at a scan rate of 20 mV s^{-1}. Curve A: 0.1 mol l^{-1} KNO_3; curve B: (A) plus 1.0×10^{-4} mol l^{-1} acetaminophen and curve C: (B) plus 2.0×10^{-3} mol l^{-1} EDTA.

peaks with an formal potential $E^{0'}$ of 438 mV versus SCE. In the presence of EDTA the shape of the cyclic voltammograms does not change in a significant way, however, the $E^{0'}$ shifts to 337 mV. This behavior suggests that EDTA improves the redox process of acetaminophen and stabilizes the oxidized form. According to the previous studies [26], the mechanism involved for the electrochemical behavior of phenols in the presence of EDTA can be explained by two assumptions: (i) the process of electron transfer of phenolic compound/electrode in the presence of EDTA could be similar to that observed for surfactants with an amphiphilic character, which are able to change the electrical properties of the electrode/solution interface, making the electron transfer easier or (ii) a coupled chemical reaction after the electrochemical process (EC mechanism) takes place.

The decrease of the oxidation potential of acetaminophen in the presence of EDTA is very desirable for analytical purposes, besides avoiding the passivation of solid electrodes. Thus, an improvement in the stability, repeatability and sensitivity, is expected. Additionally, a decrease in the applied potential can minimize interference problems.

Table 1
Electrochemical parameters evaluated by cyclic voltammetry, $E^{0'}$, ΔE_p for acetaminophen in solutions with different EDTA concentrations

EDTA (10^{-4} M)	$E^{0'}$ mV vs. SCE	ΔE_p mV vs. SCE
0	478	253
1	426	185
2	361	165
4	340	152
6	336	149
8	326	169
10	321	158
12	321	158
14	321	158
16	321	158
18	321	158
20	321	158

Acetaminophen: 1.0×10^{-4} mol l^{-1}; KNO_3: 0.1 mol l^{-1}; scan rate: 20 mV s^{-1}.

3.2. Effect of EDTA and KNO_3 concentration

The influence of the EDTA concentration over the formal potential ($E^{0'}$) for acetaminophen is presented in Table 1. For a solution of 1.0×10^{-4} mol l^{-1} acetaminophen in 0.1 mol l^{-1} KNO_3 (pH 6, scan rate of 20 mV s^{-1}) $E^{0'}$ is shifted to lower values as the EDTA concentration is increased. This behavior suggests that EDTA improves the electrochemical redox process. When the EDTA/acetaminophen ratio is higher than 10, the $E^{0'}$ becomes independent on the EDTA concentration.

It was verified that the E^0 and anodic current of acetaminophen are independent of the KNO_3 concentrations between 0.025 and 0.200 mol l^{-1}.

3.3. Flow Injection experiments

The influence of experimental variables such as applied potential and pH were optimized using the conditions described in Section 2, as well as the variables; such as carrier flow-rate and injection volume. It is important to indicate that these experiments were

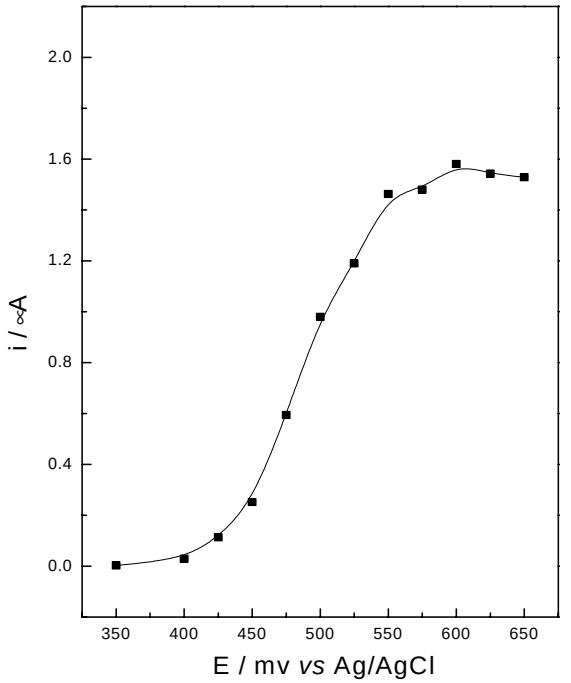

Fig. 3. Dependence of the anodic peak current on the applied potential, obtained for a 4.0×10^{-5} mol l^{-1} acetaminophen solution in the presence of 1.2×10^{-3} mol l^{-1} EDTA and 0.1 mol l^{-1} KNO_3 at pH 6.5. Injected volume: 100 μl of acetaminophen; flow rate: 3.89 ml min^{-1}.

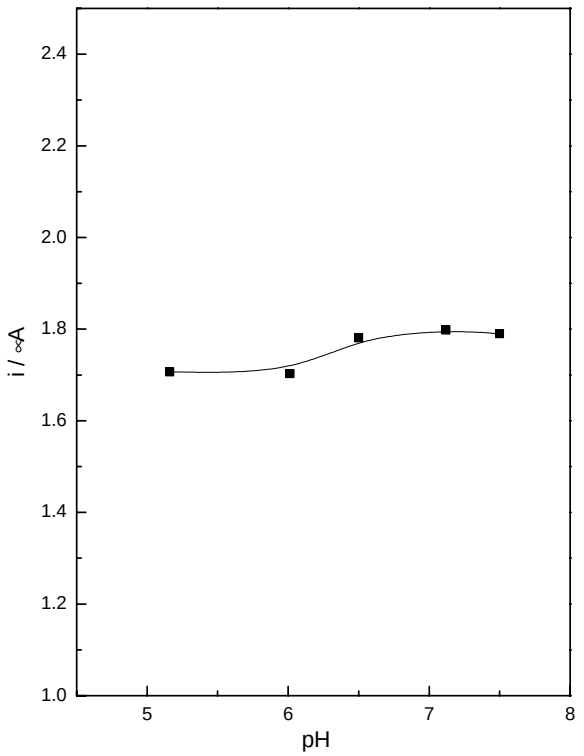

Fig. 4. Current dependence as a function of solution pH. Injected volume: 100 μl of 4.0×10^{-5} mol l^{-1} acetaminophen prepared in 1.2×10^{-3} mol l^{-1} EDTA and 0.1 mol l^{-1} KNO_3. Flow rate: 3.89 ml min^{-1}. Applied potential of 600 mV vs. Ag/AgCl.

performed using amperometric measurements and Ag/AgCl as reference electrode.

3.3.1. Optimization of the applied potential on the detection system

Its is well known that the potential can affect the sensitivity of the method. In spite of this, the effect of the applied potential was studied over the potential range between 350 and 650 mV. Peak height increased significantly by increasing the potential up to 600 mV. For higher potentials ($E > 600$ mV), the current becomes independent of the applied potential (Fig. 3). The most favorable signal was obtained at 600 mV.

3.3.2. Influence of the pH

The pH studies were performed with an applied potential of 600 mV, and concentrations of acetaminophen and EDTA of 4.0×10^{-5} and 1.2×10^{-3} mol l^{-1}, respectively. The results are shown in Fig. 4. The current intensity is not significantly changed with changes in the solution pH. For pH > 7.5 the system did not show a linear relationship between the current and the concentration due to the instability of acetaminophen in alkaline media. For pH values lower than 7.0 a good linear relationship to acetaminophen concentration was observed, over the same concentration range. Based on these results the optimum condition was defined as pH 6.5.

3.3.3. Effect of carrier flow-rate and sample injection volume

Fig. 5 shows the variation of the flow system response for acetaminophen as a function of the flow rate and sample volume. The signal response increases for flow rates between 0.50 and 3.89 ml min^{-1}, decreasing for higher flow rates. The decrease in the signal at higher flow rates is explained by the low residence time of the electroactive species in the amperometric

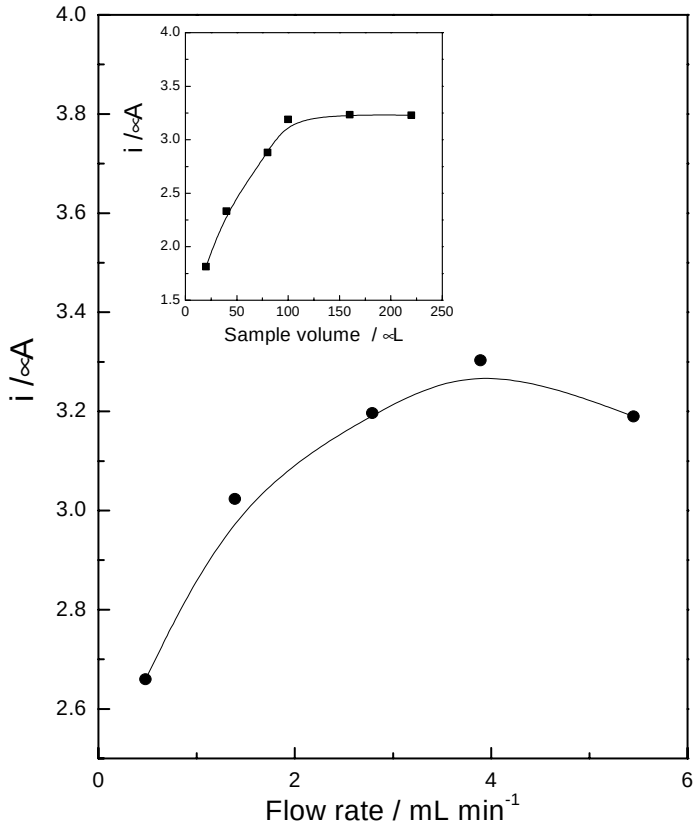

Fig. 5. Effect of the flow rate on the response. Applied potential of 600 mV vs. Ag/AgCl; injected volume = 100 μl of 6.0×10^{-5} mol l^{-1} acetaminophen with 1.2×10^{-3} mol l^{-1} *EDTA* + 0.1 mol l^{-1} KNO_3, pH 6.5, as carrier. Inset, the variation of the response with the injected sample volume (flow rate: 3.89 ml min^{-1}).

cell so that as, a consequence, only a small fraction of the compound is oxidized. Considering the sensitivity and the stability of the baseline, a flow-rate of 3.89 ml min^{-1} was chosen. The inset in Fig. 5 shows the electrode response as a function of the sample injection volume. It can be observed that, in the range between 20 and 100 μl, there is a linear dependence between these variables; for volumes higher than 100 μl the current values became independent. For the further experiments a volume of 100 μl was used.

3.3.4. Comparison of stability and repeatability of the flow injection system in the presence and absence of EDTA

In the presence of EDTA, the stability of the electrochemical signal for acetaminophen is very high (Fig. 6A), especially when compared with the amperometric response of the system in the absence of the chelating compound (Fig. 6B).

In the absence of EDTA, subsequent measurements lead to a notable decrease in the anodic current. The decrease in current, up to 25% after 100 determinations, could be explained by the surface fouling of the working electrode. On the other hand, no sensor passivation was observed in presence of EDTA in these experiments and throughout this study. Theses results show that the use of EDTA is a good approach to avoid passivation problems observed with carbon electrodes from phenol electrooxidation, in flow as well as in bath systems [26].

The sensitivity is amplified 5.5 times when EDTA is employed in the supporting electrolyte (21.5 mA l mol^{-1}) in comparison with the results

Table 2
Results obtained for acetaminophen determinations in pharmaceutical preparations

Pharmaceutical sample	Nominal value (mg unit^{-1})[a]	Determined (mg unit^{-1})[b]		Difference (%)
		Proposed	Reference method	
1	750	768 ± 10	731 ± 10	5.1
2	500	496 ± 9	465 ± 11	6.7
3	750	765 ± 7	773 ± 9	−1.0
4	500	502 ± 8	475 ± 10	5.6

[a] From the package.
[b] Average value with $n = 3$.

obtained in absence of EDTA (3.9 mA l mol^{-1}), for the acetaminophen concentration range of 2.0×10^{-5} *to* 10.0×10^{-5} mol l^{-1}. In addition, the repeatability ($n = 20$) is 3.5 times better, expressed by within-run-precision of 4.0% for a 6.0×10^{-5} mol l^{-1} acetaminophen solution in the presence of EDTA. In the absence of EDTA the within-run-precision was higher than 14%. These results show that the presence of EDTA is a good strategy to improve acetaminophen determination using a solid electrode.

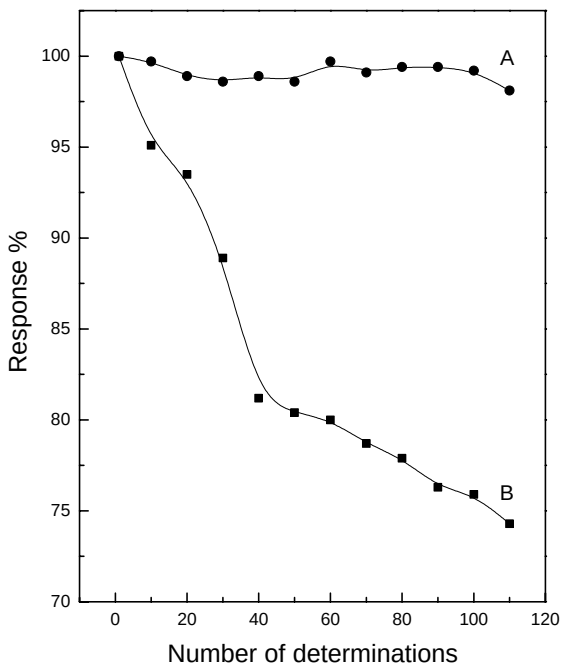

Fig. 6. Operational stability obtained using the FI system in 6.0×10^{-5} mol l^{-1} acetaminophen and 0.1 mol l^{-1} KNO_3, in absence (■) and presence (●) of 1.2×10^{-3} mol l^{-1} EDTA. Flow rate: 3.89 ml min^{-1}; injected sample volume: 100 μl, and pH 6.5.

3.4. System performance

The system presents two linear response ranges: 1.0×10^{-6} and 8.0×10^{-6} mol l^{-1}, fit by the equation $I/A = (-42.3 \pm 0.8) \times 10^{-8} + 0.0227 \pm 0.01$ (acetaminophen) in mol l^{-1}, for $n = 5$, with a correlation coefficient of 0.9987 and 2.0×10^{-5} *to* 10.0×10^{-5} mol; $I/A = (-11.4 \pm 0.8 \times 10^{-8}) + 0.0215$ (acetaminophen) in mol l^{-1}, for $n = 5$, with a correlation coefficient of 0.9988. The detection limit was 1.42×10^{-8} mol l^{-1}, calculated as three time the noise.

3.5. Applications of the flow injection system for acetaminophen determination in pharmaceuticals

Four pharmaceutical samples with two different acetaminophen contents were analyzed using the proposed flow injection method. Average concentrations were calculated from three determinations. In order to validate the method, the results was compared with those obtained using the procedure recommended by the British Pharmacopoeia. As can be seen in Table 2, good agreement was found between the standard and proposed method (statically proved according to the paired *t*-test), within the confidence level accepted by the pharmacopoeias.

4. Conclusions

The sensitivity and stability of the system in the presence of EDTA was excellent when compared with the results obtained in the absence of the chelating compound. In the absence of EDTA, the signal current decrease up to 30% after 100 measurements. This effect could be explained by the surface

fouling of the working electrode. In the presence of EDTA, no sensor passivation was observed. This result shows that the use of EDTA is a good approach to avoid the passivation problems observed for carbon electrodes by phenol electrooxidation, in flow and batch systems. These characteristics allow an improvement in sensitivity, precision and repeatability of the electrochemical determination of acetaminophen.

The proposed flow method with amperometric detection using a pyrolitic graphite electrode and EDTA in the carrier stream is suitable for determination of acetaminophen in pharmaceutical formulations. Furthermore, the system permits 100 determinations/h with a detection limit of 1.42×10^{-8} mol l^{-1}. The results obtained are in good agreement with others reported in the literature employing similar system configurations [14]. However, this methodology is a simple, rapid and efficient strategy for acetaminophen determination using a solid state electrode.

Acknowledgements

The authors thank FAPESP and CNPq for financial support.

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