Multivariate Optimization Strategies in the Development of an Electroanalytical Method for the Assay of Metoclopramide Using Mercury-Film-Modified Carbon Nanotube Paste Electrode

Camila Bitencourt Mendes,[1] **Felipe Nascimento Andrade,**[1] **Mariana Gava Segatelli,**[2] **Arnaldo César Pereira,**[3] **Douglas Cardoso Dragunski,**[4] **and César Ricardo Teixeira Tarley***[2]

[1]Departamento de Ciências Exatas, Universidade Federal de Alfenas, Unifal-MG, Alfenas, MG, 37130-000, Brazil

[2]Departamento de Química, Universidade Estadual de Londrina (UEL), Rod. Celso Garcia PR 445 Km 380, 86051-990, Londrina, Brazil

[3]Departamento de Ciências Naturais, Universidade Federal de São João Del Rei, UFSJ, São João Del Rei, MG, 36300-000, Brazil

[4]Universidade Paranaense, Unipar, Praça Mascarenhas de Moraes, Umuarama-PR 87502-210, Brazil

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E-mail: ctarleyquim@yahoo.com.br

The present work describes an electroanalytical method based on adsorptive preconcentration of metoclopramide (MCP) onto mercury-film-modified carbon nanotube paste electrode followed by anodic stripping measures. The method presented a good linear range from 2.0 up 100 $\mu mol\,L^{-1}$ ($r = 0.998$) and a limit of detection of 0.51 $\mu mol\,L^{-1}$. The determination of MCP was successfully carried out in solid and liquid pharmaceutical formulations, as well as in urine samples without intense sample treatment.

Metoclopramide (MCP) has been confirmed as an effective antiemetic drug in treating and preventing some stomachal disorders, such as nauseas and vomiting and in a variety of gastrointestinal disorders.[1,2] Some works have reported the use of adsorptive stripping voltammetry methods for assay of the following drugs: omeprazole,[3] indomethacin and acemethacin,[4] and nimesulide,[5] by using hanging mercury drop electrode (HMDE). Nevertheless, the disadvantages of HMDE still persist and efforts have been made to develop new electrodes, but electroanalytical methods available in the literature for quantification of MCP still is limited. The use of carbon paste electrode has been proposed for MCP determination in pharmaceutical dosages forms and in biological fluids using square wave anodic stripping method. However, it was necessary a mechanical renewal of the carbon paste surface after measurement was reached.[6] Such drawbacks can be circumvented by using metallic film supported onto carbon-based electrodes.[7] So, the aim of this work was to assess for the first time the use of mercury-film-modified carbon nanotube paste electrode for the electroanalytical assay of MCP in pharmaceutical formulation and urine samples.

Experimental

Apparatus. All the electrochemical measurements were performed on a Potentiostat/Galvanostat Autolab PGSTAT12 (Eco Chemie BV, Utrecht, The Netherlands) with a conventional three electrode cell comprising a platinum wire as an auxiliary electrode, an Ag/AgCl ($3\,mol\,L^{-1}$ KCl) reference electrode, and the carbon nanotube paste electrode as working electrode. A glassy carbon electrode (Metrohm, 3.0 mm in diameter) was employed as a comparative electrode. For the same purpose, a graphite paste electrode with the same dimensions of the carbon nanotube paste electrode was used. The pH measurements were made with a Metrohm (Herisau, Switzerland) model 827 pH Lab.

Reagents and Solutions. MCP (99.99%, Sigma-Aldrich, Germany) standard solutions ($30\,mg\,L^{-1}$) were prepared with water obtained from a Milli-Q® purification system (MILLIPORE, Bedford, MA, USA). Multiwalled carbon nanotubes (MWCNTs), without further purification, were supplied by CNTs Co., Ltd. (Yeonsu-Gu, Incheon, Korea) with > 95% purity, diameters between 10–40 nm and lengths of 5–20 μm, paraffin oil and mineral oil were obtained from Sigma-Aldrich (USA). Graphite powder (purity 99.9%) was supplied by Sigma-Aldrich (USA). Mercury solutions were prepared from $Hg(NO_3)_2$ salt (Merck, Germany).

Preparation of Working Electrode. The working electrode was prepared by mixing MWCNTs and paraffin oil (30:70, w/w) for about 30 min until a paste consistence was obtained. After this step, the paste was carefully forced into a cavity (4.0 mm diameter; 1 mm depth) at the end of a glass tube. Next, the surface of the paste electrode was smoothed and rinsed carefully with deionized water. Graphite paste electrode (30:70, w/w) was prepared in a similar way to carbon nanotube paste electrode.

Analytical Procedure. The deposition of MCP on working electrode surface was carried out by applying a potential of −0.3 V vs. Ag/AgCl during 90 s. After this step, the stirrer was switched off and the potential was scanned toward the anodic region from 0.45 up to 1.14 V using differential pulse anodic stripping voltammetry with pulse amplitude of 25 mV, scan rate of $62\,mV\,s^{-1}$ and modulation time of 3 ms. The anodic peak potential for MCP was observed at 0.85V vs. Ag/AgCl. All experiments were carried out without oxygen removal. The electrochemical renewal of the electrode surface between measurements was not necessary, except for some electrode physical damage.

Results and Discussion

Preparation of Mercury-Film-Modified Carbon Nanotube Paste Electrode. The first study was carried out to establish the best condition for mercury film deposition onto

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carbon nanotube paste electrode. For this task, a carbon nanotube electrode containing 50% (w/w) of mineral oil was employed. The range of deposition potential varied from −0.9 up to −1.3 V by setting the time deposition at 180 s. This assay was carried out in stirred solution in an electrochemical cell consisting of 15 mL of 0.1 mol L^{-1} acetate buffer solution and containing 500 mg L^{-1} Hg^{2+} ions. Afterwards, the mercury-modified carbon nanotube electrode was inserted in another electrochemical cell containing 100 μmol L^{-1} MCP and 0.1 mol L^{-1} acetate buffer at pH 3.75. The anodic stripping voltammetric measures of MCP at mercury-modified carbon nanotubes electrode were carried out as follows: pH 3.70, pulse amplitude of 25 mV, scan rate of 50 mV s^{-1}, modulation time of 3 ms, deposition potential of −0.3 V, and deposition time of 60 s. It is important to emphasize that during anodic stripping measures, the oxidation of mercury film was not observed. The levels evaluated for potential deposition were −0.9, −1.0, and −1.3 V. A slight increase was observed in the MCP anodic peak current by applying −1.0 V for mercury film deposition. After this value, no significant difference was observed. Therefore, −1.0 V was adopted as the best condition for mercury film deposition. The deposition time ranged from 60 up to 240 s. According to results, an improvement on MCP anodic peak current was verified with decreasing the deposition time to 60 s. It seems that by using high deposition time, the thickness of mercury film is increased, and as a consequence, it difficults the mass transport of MCP toward the electrode surface. Thus, 60 s for film deposition was adopted in this work.

After the conditions for film deposition were established, two binder materials (mineral oil and paraffin oil) at different proportions were evaluated for electrode preparation. The assays were performed at a medium containing 0.1 mol L^{-1} of acetate buffer solution (pH 3.75) and 100 μmol L^{-1} MPC. The anodic stripping voltammetric measures of MCP were carried out by setting the following conditions: pH 3.70, pulse amplitude of 25 mV, scan rate of 50 mV s^{-1}, modulation time of 3 ms, deposition potential of −0.3 V, and deposition time of 60 s. Pastes containing 70% (w/w) of paraffin oil presented the best results. Therefore, the proportion 30:70 (w/w) for MWCNT and paraffin oil was used for further experiments.

Optimization Procedure Based on Factorial Design. The investigated factors and their levels are shown in Table 1. In order to assess the significance of the factors, analysis of variance (ANOVA) represented by Pareto chart, was employed with a confidence interval of 95%.

As expected, the positive effect (3.20) of the scan rate indicates that high scan rate favors the electron transfer on the electrode surface, suggesting an adsorption-controlled process of the MCP.[8] Sample pH presented a negative effect (−3.08), thus higher anodic peak current is observed in acid medium (pH 3.7). The effect (3.02) of deposition time on the MCP retention onto mercury-film-modified carbon nanotube paste shows that the anodic peak current is increased when deposition time is changed from 25 to 60 s. So, the three significant factors were further optimized from Doehlert matrix. Within the experimental domain, pulse amplitude, deposition potential, and modulation time were not significant, being maintained for subsequent studies at 25 mV, −0.3 V, and 3 ms, respectively. These lower levels were chosen since very well resolved analytical signals, as well as reduced oscillations on the base line were observed.

Table 1. Factors and Their Respective Tested Levels on the 2^{6-1} Fractional Factorial Design

Factors	Levels	
	Low (−)	High (+)
pH[a)]	3.7	5.7
Deposition potential (*DP*)/V	−0.30	0.45
Deposition time (*DT*)/s	25	60
Scan rate (*SR*)/mV s^{-1}	15	45
Pulse amplitude (*PA*)/mV	25	50
Modulation time (*MT*)/ms	3	6

a) Acetate buffer solutions at 0.1 mol L^{-1} concentration were used for pH adjustment.

Table 2. Doehlert Matrix Used in the Optimization of Factors *SR*[a)], Sample pH, and *DT*[b)]

Assay	*SR* /mV s^{-1}	pH	*DT* /s	Anodic peak current /μA
1	75	2.7	90	4.5
2	75	1.7	90	2.2
3	70	3.7	150	5.1
4	65	2.7	30	4.3
5	65	4.7	30	4.45
6	60	1.7	90	2.3
7	60	3.7	90	6.4/6.7/6.2/6.2
8	60	5.7	90	2.0
9	55	3.7	150	4.2
10	55	4.7	150	4.3
11	50	3.7	30	4.9
12	45	2.7	90	4.1
13	45	4.7	90	3.75

a) *SR*: scan rate. b) *DT*: deposition time.

Table 2 shows the Doehlert matrix built for three factors and, as verified, within experimental domain, the assay performed at the central point showed greatest sensitivity. It indicates the presence of curvature of the statistical model that represents the experimental data. Therefore, the quadratic model validation was carried out by using ANOVA. According to ANOVA, the lack of fit of model was not significant. Hence, in order to find the maximum values for the studied factors, response surfaces (data not shown) were built. The response surfaces led us to select the following experimental conditions: 3.75, 62 mV s^{-1}, and 90 s, for sample pH, scan rate, and deposition time, respectively.

Comparative Study of Mercury-Film-Modified Carbon Nanotube Paste Electrode with Other Electrodes. The mercury-film-modified carbon nanotube paste electrode, under optimized conditions, was compared with graphite paste and glassy carbon electrodes, both submitted to loading with mercury film in a similar way to carbon nanotube electrode. A 3.14- and 5.60-fold increase on anodic peak current by using carbon nanotube in detriment of graphite paste and glassy carbon electrodes was observed, respectively; thus confirming the benefits of nanostructured material as support for mercury film. In addition, in order to emphasize the advantages of

mercury-film-modified carbon nanotube paste electrode in relation to bare carbon nanotube electrode (unloaded carbon nanotube electrode), stripping voltammograms were recorded under optimized conditions. It was observed that the modification of the carbon nanotube surface with mercury film improves significantly the anodic peak current.

Interference Studies. The method was submitted to MCP determination at 50 $\mu mol\,L^{-1}$ concentration in the presence of commonly coexistent species in pharmaceutical samples, such as glucose, sucrose, lactose, and fructose. A tolerable limit that causes an interference (±10% as regards the anodic peak current of MCP) was only verified to high analyte:interferent proportion, i.e., 1:10000 (w/w). Furthermore, the metallic ions Cd^{2+}, Co^{2+}, Sn^{2+}, Ni^{2+}, Mn^{2+}, and Zn^{2+} at 1000-fold excess as regards MCP, did not influence the MCP determination. Moreover, the effect of uric and ascorbic acids usually present in urine samples, was checked. Voltammograms showed two well-distinguished anodic peaks at potentials 0.85 and 0.20 V, corresponding to the oxidation of MCP and uric acid, respectively. If necessary, the overlapped anodic peaks of ascorbic and uric acids can be separated by using measurements in micelle medium with cationic surfactant cetylpyridinium chloride, in accordance to literature data.[9] Thus, it seems that MCP and the uric and ascorbic acids can be determined simultaneously.

Analytical Features and Analysis of Pharmaceutical Formulation and Urine Samples. The electroanalytical method provided, under optimum conditions, a calibration graph ranging from 2.0 up to 100.0 $\mu mol\,L^{-1}$ with a good linear correlation coefficient ($r = 0.998$). The limits of detection ($LD = 0.51\,\mu mol\,L^{-1}$) and quantification ($LQ = 1.70\,\mu mol\,L^{-1}$) were calculated according to IUPAC recommendation.[10] The precision assessed as relative standard deviation ($n = 10$) were 0.89% and 0.70%, respectively, for the concentration of 4.0 and 27.0 $\mu mol\,L^{-1}$. The sample treatment of pharmaceutical formulation was carried out by dissolving a known amount of sample in deionized water, filtered through qualitative paper and buffered with acetate buffer at 0.1 $mol\,L^{-1}$, while the liquid pharmaceutical formulation was diluted with deionized water and buffered with acetate buffer at 0.1 $mol\,L^{-1}$. As observed from Table 3, the method can successfully be applied for MCP determination in pharmaceutical samples. Urine samples were collected from a healthy volunteer who received an oral dose of 10 mg of Sanofi-Aventis® tablet. The samples were collected at two different times after administration, being 1 h and 45 min and 3 h and 45 min and determined from analyte addition method. The amounts of MCP determined in human urine were, respectively, 16.1 and 24.6 $\mu mol\,L^{-1}$, for samples collected 1 h and 45 min and 3 h and 45 min after drug administration. These samples were also spiked with a known amount of MCP and quantitative recovery values (108.9 ± 2.0%) were achieved, thus confirming that the proposed method is suitable for the MCP in urine samples.

Table 3. Determination of Metoclopramide in Pharmaceutical Samples

Sample	Label amount	Found amount[a)]	Recovery /%[b)]
Solid sample (Sanofi-Aventis®) (mg/tablets)	10.0	10.1 ± 0.1	101
Solid sample (Medley®) (mg/tablets)	10.0	10.6 ± 0.2	106
Liquid sample (Sanofi-Aventis®) /mg L^{-1}	4.0	4.4 ± 0.2	110

a) Mean of three measurements. b) Recovery as regard those labeled amounts.

Conclusion

The use of MWCNT as substrate (support) for film deposition has shown a reliable advantage in detriment of glassy carbon and graphite carbon methods, in which a 3.14- and 5.60-fold increase on anodic peak current of metoclopramide was found, respectively. The multivariate optimization using fractional factorial design and Doehlert matrix allowed the rapid identification of significant factors as well as their optimization using a reduced number of experiments. The method can be an alternative for metoclopramide determination in pharmaceutical formulation and urine samples.

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