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Voltammetric and amperometric determination of metoclopramide on boron-doped diamond film electrode

Research Article

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Abstract: New methods for the determination of metoclopramide, antiemetic and gastroprokinetic pharmaceutical, were developed, using differential pulse voltammetry (DPV) and flow injection analysis (FIA) with amperometric detection on a boron-doped diamond film electrode. Electrode pretreatment necessary to ensure the stable results was investigated and it was found, that while DPV requires frequent electrode cleaning, FIA with a sufficiently high flow rate can maintain a stable signal with no signs of electrode passivation. The calculated quantification limits of the DPV and FIA with amperometric detection were 0.13 μmol L^{-1} and 0.015 μmol L^{-1}, respectively. The applicability of the new methods was verified by the determination of metoclopramide in a pharmaceutical preparation. FIA with amperometic detection proved to be sensitive, accurate and, due to the resistance of the electrode to the passivation, also simple to handle.

Keywords: *Metoclopramide • Differential pulse voltammetry • Flow injection analysis • Boron-doped diamond film electrode • Passivation*

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1. Introduction

Boron-doped diamond film electrodes (BDDFE) are nowadays frequently used due to their advantageous electrochemical properties: low background current, wide potential window, and high current density [1,2]. They also exhibit excellent physical and chemical robustness [3]. Moreover, chemical inertness of the electrode surface and connected low passivation of the electrode [4] offers great advantage from the point of quick and reliable analysis. Nevertheless, not even this material is completely resistant to the surface fouling by certain analytes [2,5]. In such a case, conditions for the determination must be optimized with respect to diminishing the impact of fouling and a suitable electrode cleaning treatment must be found.

The applicability of BDDFE in pharmaceutical analysis was proved many times, both for the determination in pharmaceutical formulations and in biological samples, such as recent works [6,7]. In our case, BDDFE was employed for the determination of metoclopramide, antiemetic and gastroprokinetic agent. Application of this pharmaceutical is mainly connected with the support of the digestive process, either in the case of diseases connected with slow digestion problems or after some surgical procedures or in combination with some antineoplastic drugs, where it prevents some of their adverse effects. Usual dose is three tablets per 10 mg of metoclopramide daily [8]. Positive effects on migraine treatment [9] are also described. It is also used in veterinary medicine [10]. Most determination methods of metoclopramide are based on RP-HPLC with spectrophotometric [11,12] or MS detection [13-15], capillary electrophoresis [16] and gas chromatography [17] were also employed. High selectivity of the separation methods serves particularly for the determination in complicated matrices such as body fluids or tissues. Spectrometric methods utilize

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both native properties of the compound [18,19] and their enhancement by means of a suitable chemical reaction [20-22]. An interesting determination method based on the combination of spectrometric and electrochemical methods was described [23]. As a special type of determination method, particularly for the determination in pharmaceutical formulations, we can name flow injection analysis (FIA) [24,25] and sequential injection analysis [26,27].

Electrochemical methods include potentiometric sensors [28-30] as well as voltammetric methods; these methods employ a carbon paste electrode [31], a nafion-modified glassy carbon electrode [32] and a mercury-film modified carbon nanotube paste electrode [33]. The glassy carbon electrode was also used as a sensor for the amperometric detection following the HPLC separation [34-36].

The present work deals with the optimization of the determination of metoclopramide using DPV and FIA with amperometric detection on BDDFE with particular emphasis on the resistance of the electrode to the passivation, aiming to develop a method, which is sensitive, fast, reliable and inexpensive.

2. Experimental procedure

2.1. Chemicals

The stock solution of metoclopramide hydrochloride (1 mmol L^{-1}) was prepared by dissolving the exact amount of the substance (Sigma-Aldrich) in deionized water and it was kept at a laboratory temperature.

Britton-Robinson (BR) buffers served as supporting electrolytes and carrier solutions. All chemicals used for buffer preparation were of analytical grade purity and obtained from Lachema Brno, Czech Republic. Other chemicals used were nitric acid (65%, p.a., Lach-Ner, Czech Republic), methanol (p.a., Merck, Germany) and deionized water (Millipore, USA).

2.2. Apparatus

Voltammetric measurements were carried out using Eco-Tribo-Polarograph, controlled by software Polar Pro 5.1 (both PolaroSensors, Prague, Czech Republic). For DPV, three-electrode arrangements were used with working BDDFE (active area 12.4 mm^2, Adamant Technologies, Switzerland), a platinum wire auxiliary electrode, and an Ag/AgCl (3 M KCl) reference electrode RAE 113 (Monokrystaly Turnov, Czech Republic), to which all the potential values are referred.

FIA measurements were performed using a high pressure pump HPP 5001, injector valve with 20 µL loop, UV/VIS detector LCD 2083 and amperometric detector ADLC 2 connected in series (all Laboratorni pristroje Praha, Czech Republic). The system was controlled via Clarity 2.3 software (DataApex, Czech Republic). The three-electrode wall-jet system was used for amperometric detection with BDDFE working electrode adjusted against the outlet capillary at a controlled distance [37]. An auxiliary electrode and reference electrode were the same as in voltammetric measurements. Flow rate was set to 5 mL min^{-1}, unless stated otherwise.

The pH of the solutions was measured with a pH meter Jenway 3510 (Jenway, UK) with a combined glass electrode.

2.3. Procedures

Differential pulse voltammetry was performed with the following parameters: pulse width of 100 ms, pulse height of 50 mV, scan rate of 20 mV s^{-1}, and potential range from – 0.2 V to +1.3 V. A detection wavelength of 316 nm was selected from the UV spectra of the analyte. All measurements were made in triplicate. 100 µmol L^{-1} metoclopramide was used during optimization measurements, unless stated otherwise. BDDFE was cleaned and activated by application of selected reduction and oxidation potential in 1M nitric acid for a chosen time period, as described in chapter 3.1.

For the determination of metoclopramide in tablets, one tablet of Cerucal (AWD.pharma, Germany) was dissolved in 200 mL of deionized water and 0.1 mL of this solution was diluted by buffer of the selected pH to the volume of 10 mL.

Calibration dependences were evaluated by least squares linear regression method. The quantification limits were calculated as the concentration of the analyte which gave a signal ten times the standard deviation of the lowest evaluable concentration [38].

3. Results and discussion

3.1. Voltammetric determination

At first, the behavior of metoclopramide in media of different pH was investigated by differential pulse voltammetry (DPV). The response of the electrode was not stable even for a small number of repeated measurements, suggesting some degree of passivation. The activation step based on our previous results [39] and consisting of application of pre-selected reductive and oxidative potentials for certain period of time in stirred solution of 1M HNO_3 was repeated after each

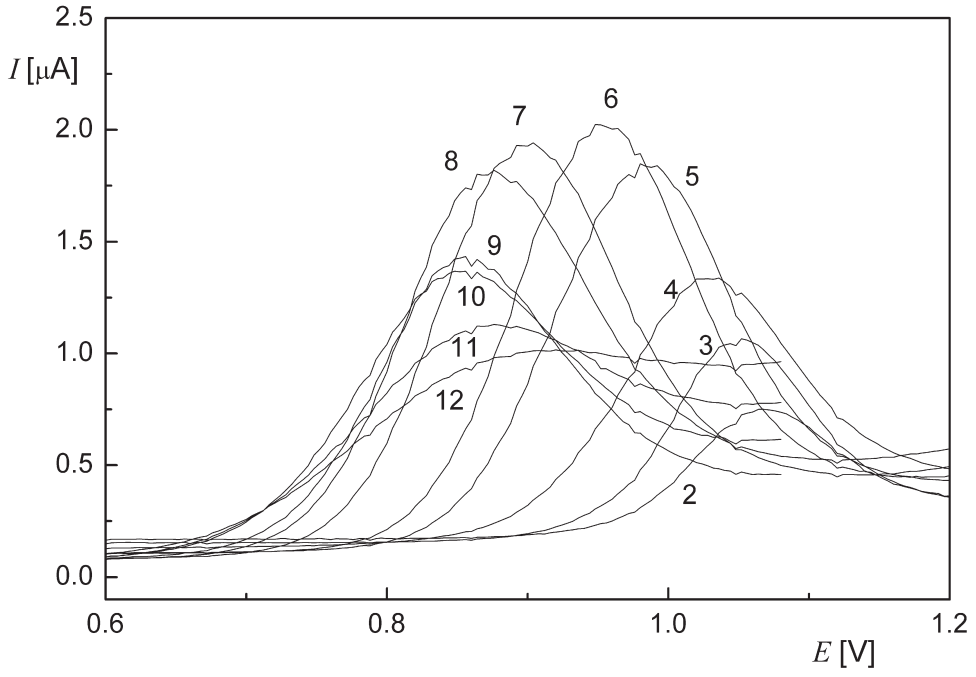

Figure 1. DP voltammograms of metoclopramide (100 μmol L^{-1}) at BDDFE in various BR buffers (pH value noted above the curve).

scan. In most cases, pretreatment conditions consisted of two steps with application of potential – 2.0 V and +2.0 V for 30 s each. In BR buffers with pH 2 and 3, milder pretreatment conditions were sufficient, *i.e.,* application of – 1.5 V and + 1.5 V potentials for a period of 20 s each. Resulting voltammograms are shown in Fig. 1; BR buffer pH 6 was selected as optimum due to the highest and well developed peak.

As the frequent cleaning of the electrode is somewhat demanding and time consuming, the degree of the passivation was monitored in lower concentration of analyte and in mixed aqueous-methanolic media. Resulting voltammograms are shown in Fig. 2. While with 100 μmol L^{-1} metoclopramide in BR buffer pH 6 the peak height decreased to 14% of its original value after 10 consecutive scans, the response of 10 μmol L^{-1} metoclopramide in BR buffer pH 6 decreased to 41%, and the height of 100 μmol L^{-1} metoclopramide peak in BR buffer pH 6 : methanol (1:1, v/v) decreased even to 8% of the value of the first scan of each sequence. It can be seen, that no significant improvement was achieved and that the cleaning step is necessary.

Concentration dependence was measured under the optimized conditions in the concentration range from 0.1 to 100 μmol L^{-1}. Obtained parameters are summarized in Table 1 and selected voltammograms are shown in Fig. 3. The concentration dependence is linear only below the concentration 10 μmol L^{-1} and the determination limit reaches value 0.13 μmol L^{-1}.

3.2. Flow injection analysis with electrochemical detection

Flow injection analysis (FIA) provides the combination of a sensing method with the transport of the analyte in a flow. The advantage of this approach in the case of electrochemical detection is shorter time of analysis and easy automation. Moreover, measurement in flowing systems also diminish problems with electrode passivation as the electrode is in contact with analyte for a shorter period of time and the reaction products are washed away from electrode surface by the carrier solution. In the case of metoclopramide, this feature suggested the possibility to eliminate the frequent cleaning step.

Dependence of the peak height on the detection potential was measured in the carrier solutions of pH from 2 to 12 with the flow rate of 5 mL min^{-1}. In media of pH 2 and 4, a low electrode response was obtained and in media of pH 12, the measurement suffered from high background current. Other media provided similar results; pH 6 was selected due to analogy with voltammetric measurement. The detection potential of +1.3 V was selected as the optimum value based on measured hydrodynamic voltammogram. Under these conditions, fifteen consecutive injections were made to test the stability of the response; resulting peak heights are stable with RSD 3.9%.

With increasing flow rate, FIA peaks are generally higher due to the reduced diffusion of the analyte.

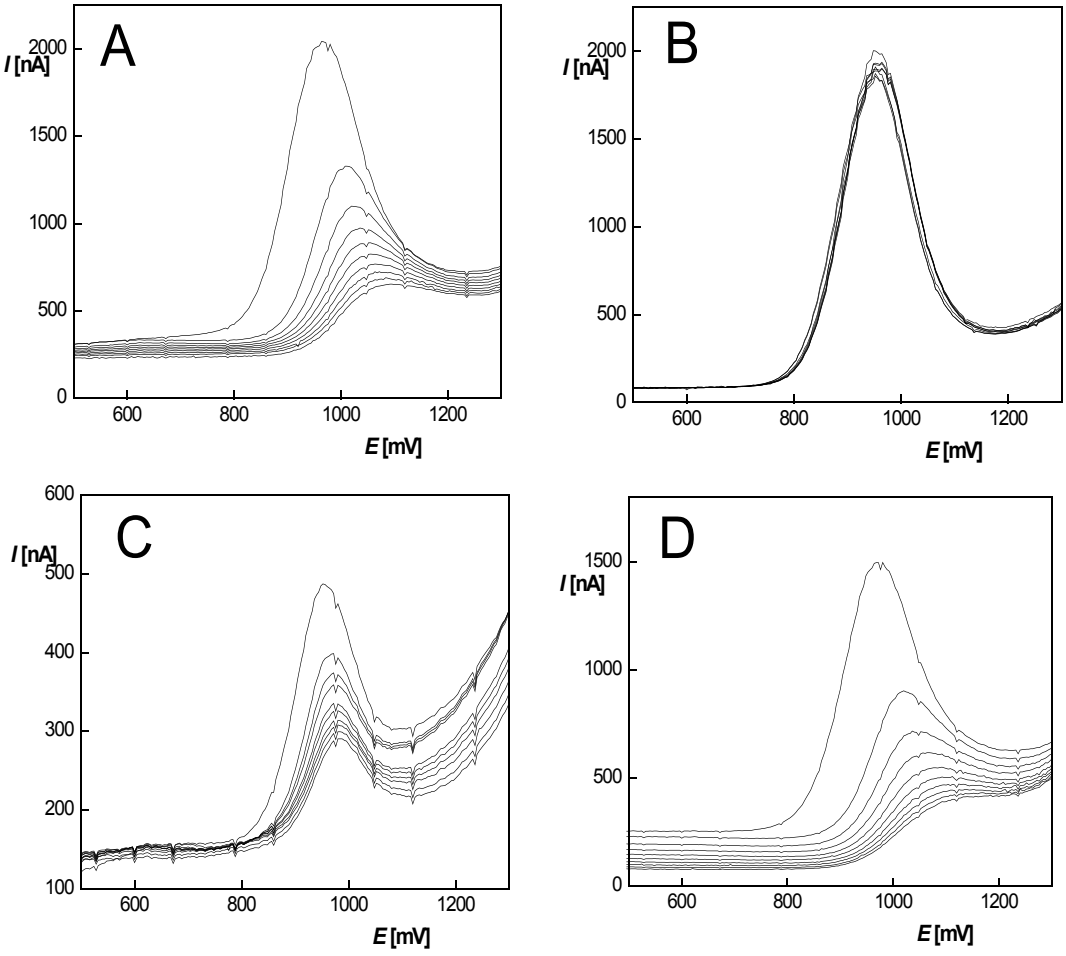

Figure 2. Ten repeated DP volammograms of metoclopramide at BDDFE (A) in BR buffer pH 6, c = 100 μmol L^{-1}; (B) in BR buffer pH 6, c = 100 μmol L^{-1}, activated between measurements; (C) in BR buffer pH 6, c = 10 μmol L^{-1}; (D) in BR buffer pH 6 : methanol (1:1, v/v), c = 100 μmol L^{-1}.

Lower flow rates and consecutive longer contact of the analyte zone with the electrode surface can, on the other hand, provide larger peak areas. In the case of metoclopramide determination on BDDFE, the increase of the peak area is only small, while decrease of peak height is quite rapid. Moreover, with decrease of the flow rate, the electrode tends to be passivated, apparently due to prolonged electrode exposition to the analyte. The decrease gradually advances and after 15 injections at flow rate 1 mL min^{-1}, the signal height decreases to 70% of the original value. It can be concluded, that FIA allows the determination of metoclopramide without frequent surface activation, if high flow rates are employed.

Under the optimized conditions, concentration dependence was measured; obtained parameters are summarized in Table 1 and selected part of FIA-ED records are shown in Fig. 4. As well as in the case of DPV, the dependence is linear only up to the concentration of 10 μmol L^{-1}, while spectrophotometric detection allows the determination at least to the concentration of 100 μmol L^{-1} and the precision of the electrochemical detection, expressed by the correlation coefficient, is lower than that of spectrophotometric detection. The sensitivity of the electrochemical detection is, on the other hand, more than one order of magnitude higher, reaching the quantification limit of 15 nmol L^{-1}. Moreover, electrochemical detection is more selective and is not influenced by the turbidity of measured solutions. Considering that metoclopramide tablets contain mainly insoluble excipients, this fact might simplify the sample preparation.

3.3. Determination in pharmaceutical sample

The developed methods were employed for the determination of metoclopramide in tablets Cerucal with declared content of active substance expressed as the

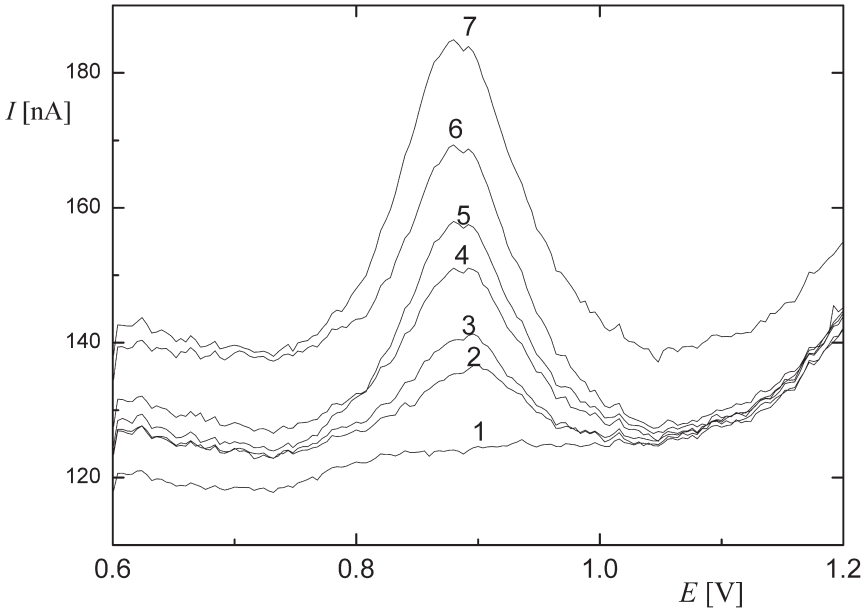

Figure 3. DP voltammograms of metoclopramide (0 (1); 1 (2); 2 (3); 4 (4); 6 (5); 8 (6); 10 (7) μmol L^{-1}) measured on BDDFE in BR buffer pH 6.

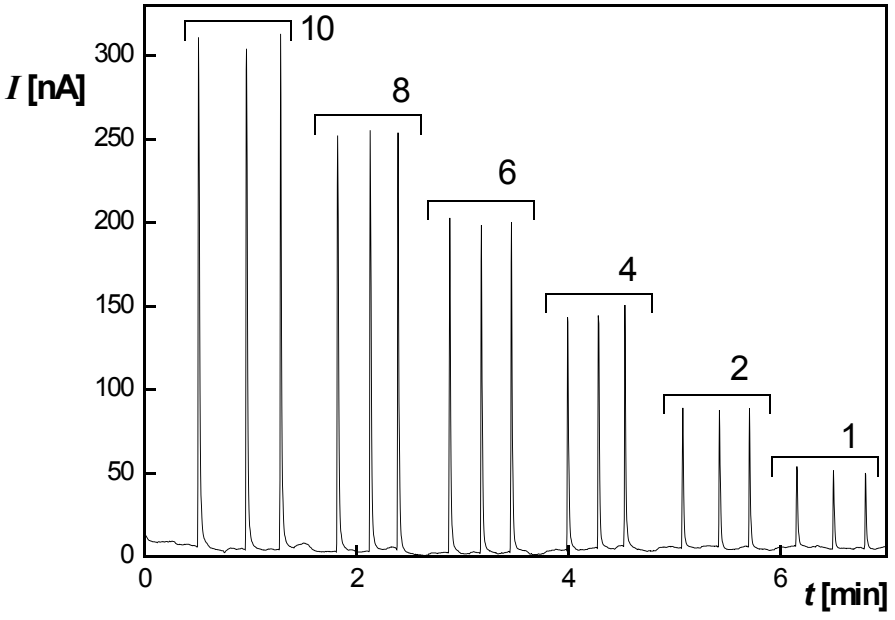

Figure 4. FIA-ED amperometric responses of metoclopramide with the concentration of 10; 8; 6; 4; 2; 1 μmol L^{-1} at BDDFE; concentration in μmol L^{-1} marked above the peaks. Carrier solution B-R buffer pH 6, flow rate 5.0 mL min^{-1}, E_{DET} = +1.3 V, injected 20 μL of metoclopramide solution.

Table 1. Selected parameters of the concentration dependences of metoclopramide, obtained by DPV and FIA with spectrophotometric and amperometric detection.

	LDR[a] (μmol L^{-1})	Slope (mA L mol^{-1}) or (kAU L mol^{-1})	Intercept (nA) or (mAU)	Correlation coefficient	LoQ[b] μmol L^{-1}
DPV	0.10 - 10	25.2	8.6	0.9991	0.130
FIA-UV	0.04 - 100	10.1	5.7	0.9999	0.250
FIA-ED	0.01 - 10	31.0	13.3	0.9907	0.015

[a] *Linear dynamic range;* [b] *Limit of quantification*

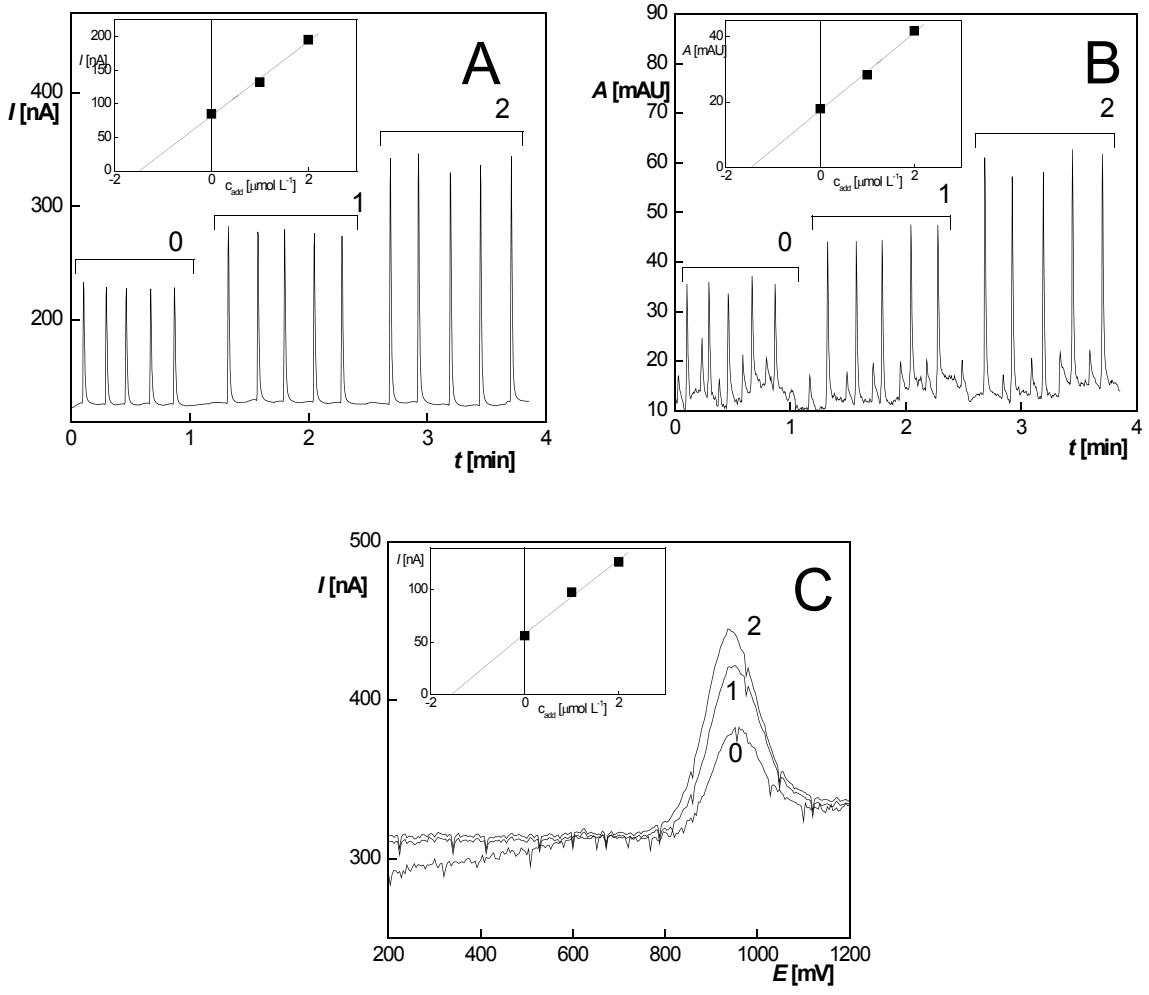

Figure 5. Records of the determination of metoclopramide in Cerucal tablets using standard addition method, obtained by FIA with amperometric detection (A), FIA with spectrophotometric detection (B) and DPV (C); number of additions shown above the curves, insets show the corresponding concentration dependences. Medium B-R buffer pH 6, flow rate 5.0 mL min^{-1}, E_{DET} = +1.3 V, λ_{DET} = 316 nm, injected volume 20 μL.

Table 2. Parameters of the standard addition method determination and found content of metoclopramide in tablets Cerucal, determined by DPV and FIA with amperometric and spectrophotometric detection. Declared content of anhydrous metoclopramide hydrochloride 10 mg per tablet.

Method	Slope (mA L mol^{-1}) or (kAU L mol^{-1})	Concentration in the measured solution (μmol L^{-1})	Metoclopramide content (mg per tablet)
DPV	35.2	1.657	11.14 ± 0.71
FIA-ED	55.2	1.491	10.03 ± 0.63
FIA-UV	11.9	1.465	9.85 ± 0.85

amount of anhydrous metoclopramide hydrochloride corresponding to 10 mg per tablet. Simple sample handling was preferred, consisting only in the dissolution of the tablet in 200 mL of deionized water and diluting 0.1 mL of the resulting solution by BR buffer pH 6 to 10 mL. The content of metoclopramide in the sample was determined by the standard addition method, using two additions of 10 µL of 1 mmol L^{-1} metoclopramide. The results of metoclopramide determination by all developed methods are summarized in Table 2 and selected records are shown in Fig. 5. Flow injection analysis regardless of the detection technique provides corresponding results that are in agreement with the metoclopramide content declared by the manufacturer. On the other hand, lower sensitivity and selectivity of the spectrophotometric detection is apparent from baseline noise and system peaks observable in Fig. 5B in comparison with Fig. 5A. Results obtained by DPV

are slightly higher than both the declared content and the content found by other methods; the cause might be the influence of compounds present in the sample on the baseline of the measurement.

4. Conclusion

New methods for the determination of metoclopramide using BDDFE were optimized, employing DPV and FIA with amperometric detection. DPV measurement was performed in BR buffer pH 6, containing 10% of methanol (v/v). Due to the strong electrode passivation, cleaning and activation of the electrode surface was necessary in DPV after each measurement, consisting of application – 2.0 and +2.0 V potentials for 30 s each in 1M nitric acid. The same medium as for DPV was used as carrier solution in FIA. In this case, a high flow rate ensured the stable response of amperometric detection, a suppressed electrode passivation and eliminated the necessity of the demanding cleaning step; flow rate 5 mL min^{-1} was used as optimum. The BDDFE resistivity towards the passivation, declared in a number of papers, is apparently not sufficient to ensure the repeatability during batch voltammetric measurements of metoclopramide and needs to be supported by continuous washing of the electrode surface which is the case of FIA-ED.

Concentration dependences were measured using both the developed methods and by FIA with UV spectrophotometric detection. Both electrochemical methods provide linear concentration dependence up to 10 μmol L^{-1} metoclopramide. Quantification limit reaches 0.13 μmol L^{-1} for DPV and 0.015 μmol L^{-1} for FIA with amperometric detection, respectively.

Applicability of the newly developed methods was tested on the determination of metoclopramide content in tablets Cerucal. FIA with amperometric detection provides excellent agreement with both the manufacturer declared content and the results of FIA with spectrophotometric detection, proving that this method is a useful tool for the determination. Results obtained by DPV are slightly higher, which might be due to the baseline deformation by compounds present in the sample.

FIA with amperometric detection appears to be a useful method for the determination of metoclopramide in simple matrices, such as pharmaceutical formulations. Its sensitivity does not reach the values of highly advanced methods such as mass spectrometry, but is higher than less demanding methods used for the metoclopramide determination. The method is superior in simple and cheap instrumentation and quick and easy handling of the electrode; in comparison with FIA with UV spectrophotometric detection, it provides higher selectivity and sensitivity. The developed DPV method is less advantageous, not only because of its lower accuracy and sensitivity, but mainly due to the time-consuming electrode treatment.

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