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VOLTAMMETRIC DETERMINATION OF THE DRUG METRONIDAZOLE USING A SOLID BISMUTH DROP ELECTRODE

This paper is dedicated to Prof. Jiří Barek, CSc. on the occasion of his 75th birthday and is published in the series New Perspectives on Analytical Chemistry*.

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Solid bismuth drop electrode (SBiDE) is a new working electrode commercially available on the Czech market since 2020 by the company Metrohm. The aim of this work was to verify the applicability of SBiDE for the voltammetric determination of a model organic substance representing electrochemically reducible biologically active compounds, namely, the drug metronidazole (an antibiotic used to treat diseases caused by both Gram-positive and Gram-negative anaerobic bacteria). To the best of our knowledge, this is the very first published research work using SBiDE. Under optimum conditions (Britton-Robinson buffer of pH 12.0 was used as the supporting electrolyte and the working electrode surface was not electrochemically regenerated), a linear calibration dependence of metronidazole was obtained using differential pulse voltammetry (DPV) in the concentration range from 1 to 600 μmol L^{-1}, with the limits of detection (*LOD*) and quantification (*LOQ*) of 0.41 μmol L^{-1} and 1.4 μmol L^{-1}, respectively. Cyclic voltammetry (CV) on SBiDE was used to characterize the electrode process of the irreversible reduction of metronidazole. The newly developed DPV method was also successfully applied for the determination of metronidazole in authentic drinking water samples (*LOD* = 1.8 μmol L^{-1} and *LOQ* = 5.8 μmol L^{-1}) and in various dosage forms (UV-Vis spectrophotometry was used as a comparative analytical method).

Keywords: metronidazole, antibiotics, drug analysis, electrochemistry, differential pulse voltammetry, cyclic voltammetry, solid bismuth drop electrode, UV-Vis spectrophotometry

Introduction

Bismuth electrodes (BiEs) have been used as working electrodes in electroanalytical chemistry since 2000. Bismuth is very environmentally friendly and thus becomes a suitable electrode material that can very well replace metallic mercury[1,2]. The metal ions of Zn, Cd, Pb, Tl, In, Sn, Sb, Cu, Mn, Cr, and Mo (ref.[3]), also of Co and Ni (ref.[3,4]), but also some organic compounds such as pesticides or pharmaceuticals have been previously determined using these electrodes[1,2]. The basic types of bismuth working electrodes are bismuth film electrodes (BiFEs) and bismuth solid (bulk) electrodes (BiBEs), which include our newly investigated solid bismuth drop electrode (SBiDE).

BiFE is most commonly produced by electrochemical deposition of a thin bismuth film on the surface of a working (substrate) electrode at a constant temperature using an *in situ* or *ex situ* method[1,3,5]. The *in situ* method is feasible in two variants. The first variant is the formation

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of the bismuth coating directly in the solution to be analysed. The most commonly used source of bismuth ions is their well dissociated salt, bismuth nitrate[3,5]. The concentrations of the added Bi(III) salt are 10–20 times higher than the expected concentrations of the ions to be determined. The reduction of the salt is carried out at a constant potential (–1.0 to –1.4 V *vs.* Ag|AgCl), which allows the formation of a film of metallic bismuth. The bismuth film thus formed lasts for only one measurement and eventually its residues are electrochemically removed[3]. The second option for performing the *in situ* method is to reduce the metallic bismuth during the actual measurement using solid bismuth oxide which is mixed into carbon pastes or inks. The metal film is formed at potentials of –0.8 to –1.0 V *vs.* Ag|AgCl (ref.[3]). The *ex situ* method, on the other hand, consists in the formation of the bismuth film from special plating solutions. The bismuth films formed by the *ex situ* method are more stable than those of the *in situ* method and thus last, as long as no oxidation occurs during the dissolution step, for the entire measurement period[3,6–8].

BiBE is most commonly prepared from bismuth wire[5] or by sucking molten bismuth into a glass capillary, the end of which is then polished into a disc shape[1,3]. More details on the preparation and use of BiBEs can be found, for example, in papers[9,10].

SBiDE (Fig. 1) is a new working electrode commercially available on the Czech market since 2020 by the company Metrohm (ref.[11]). It is made up of a drop of solidified metallic bismuth with a diameter of approximately 2 mm at the end of a glass capillary. According to the manufacturer, this electrode can be used without polishing or further modification of its surface for the determination of low concentrations of heavy metals in lakes, rivers, groundwater, and especially in contaminated drinking water. It can be used to determine low concentrations from units of μg L^{-1}, but sometimes it is possible to determine concentrations as low as ng L^{-1}. The electrode is suitable for trace analysis of heavy metal ions such as Cd, Pb, Ni, Co, and Fe and can therefore completely replace the hanging mercury drop electrode in these determinations[11,12].

Fig. 1. **Solid bismuth drop electrode (SBiDE);** taken from materials provided by the manufacturer[11]

Metronidazole (Fig. 2), or 2-(2-methyl-5-nitro-1--imidazolyl)ethanol, is a white or yellowish crystalline powder, sparingly soluble in water, freely soluble in acetone, ethanol, dichloromethane, and ether. It is an antibiotic developed in 1960, used to treat diseases caused by Gram-positive and Gram-negative anaerobic bacteria (primarily *Bacteroides fragilis*)[13–16].

In the literature, the most common analytical methods for the determination of metronidazole are electrochemical methods (DC voltammetry, differential pulse voltammetry (DPV), or square-wave voltammetry)[16–18], the separation method HPLC–UV[16], or the optical methods UV-Vis absorption spectrophotometry[16] and fluorescence spectrophotometry[19]. In Tab. I, selected examples of metronidazole determination by DPV on different working electrodes are shown, and the results of the present study are also presented for comparison. The presence of metronidazole can also be demonstrated using a pharmacopoeial method[20] consisting of two main steps. The first step is detection based on the melting point, which ranges from 159 to 163 °C. The second step is the recording of the infrared absorption spectrum, which is compared with the spectrum of the metronidazole standard. The determination of metronidazole by the pharmacopoeial method[20] consists of dissolving 0.1500 g of a sample of metronidazole in 50 mL of anhydrous acetic acid and titrating the resulting solution with 0.1 M $HClO_4$. The equivalence point is determined potentiometrically with a platinum indicator electrode and a calomel or argentochloride reference electrode.

Metronidazole was chosen in this research as a model drug representing electrochemically reducible biologically active compounds. The aim of this work was to verify the applicability of a new type of commercially available bismuth working electrode (SBiDE) in the voltammetric (DPV) determination of a model organic substance. According to the available information, this is the very first published work on this topic worldwide. Cyclic voltammetry (CV) on SBiDE was used in this study to characterize the cathodic reduction of metronidazole, and the newly developed DPV method was successfully applied in the determination of metronidazole in authentic drinking water samples and in various dosage forms, using UV-Vis spectrophotometry as a comparative analytical method. A part of this research was previously presented

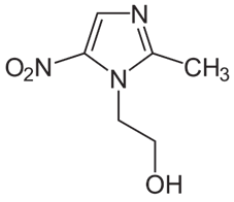

Fig. 2. **Structural formula of metronidazole**

Table I
Examples of DPV determinations of metronidazole with the limits of detection (*LOD*) and limits of quantification (*LOQ*) achieved

Electrode [a]	Medium	Linear range [μmol L^{-1}]	*LOD* [μmol L^{-1}]	*LOQ* [μmol L^{-1}]	Ref.
AgSE	BR buffer pH 10.0	1–400	0.55	1.8	17
BiF-PLE	BR buffer pH 4.0	0.2–2 and 2–30	0.039	0.13	18
DMIP/CPE	BR buffer pH 5.0	0.4–200	0.091	0.30	21
DNA/GCE	0.1 M acetate buffer pH 4.5	1–100	1.7	5.7	22
GCE	0.1 M acetate buffer pH 4.5	2.9–100	3.4	11	22
HMDE	BR buffer pH 9.0	0.23–1.8	0.036	0.12	23
m-AgSAE	BR buffer pH 8.0	2–100	0.057	0.19	24
p-AgSA-CE	BR buffer pH 4.0	2–100	1.2	4.0	25
SBiDE	BR buffer pH 12.0	1–600	0.41	1.4	this work

[a] AgSE – silver solid electrode, BiF-PLE – bismuth film modified pencil-lead electrode, DMIP/CPE – carbon paste electrode modified with duplex molecularly imprinted polymer hybrid film, DNA/GCE – glassy carbon electrode modified with DNA, GCE – glassy carbon electrode, HMDE – hanging mercury drop electrode, m-AgSAE – mercury meniscus modified silver solid amalgam electrode, p-AgSA-CE – polished silver solid amalgam composite electrode, SBiDE – solid bismuth drop electrode

at the Karel Štulík Prize 2024 student conference (ref.[26]), and this paper represents a significant extension of the competition contribution, including also a description of other experiments and their results.

Experimental part

Chemicals and samples

A stock solution of metronidazole (p.a., Sigma-Aldrich, Taufkirchen, Germany) at a concentration of 1×10^{-2} mol L^{-1} was prepared in deionized water. The supporting electrolyte was Britton-Robinson (BR) buffer prepared by mixing an alkaline component (0.2 M NaOH (p.a, Penta, Prague, Czech Republic)) with an acidic component (85% H_3PO_4, H_3BO_3 (p.a., Lach:Ner, Neratovice, Czech Republic), and 99.8% CH_3COOH (p.a., Penta, Prague, Czech Republic)) with a concentration of all acids of 0.04 mol L^{-1}. To optimize the medium of the authentic drinking water samples, an aqueous solution of chelaton III (Na_2EDTA, p.a., Penta, Prague, Czech Republic) was used with a concentration of 0.1 mol L^{-1}. Drinking water was taken from the water supply line in the building of the Institute of Chemistry (Hlavova 8, Prague 2) of the Faculty of Science of Charles University.

The drugs, infusion solution Efloran 500 mg/100 mL (KRKA, Slovenia), tablet Entizol 250 mg (Polpharma, Poland), vaginal tablet Entizol 500 mg (Polpharma, Poland), and infusion solution Noridem 500 mg/100 mL (Noridem Enterprises Limited, Cyprus), were used for voltammetric and comparative UV-Vis spectrophotometric determination of metronidazole content in dosage forms.

Apparatus

Voltammetric measurements were performed using an Eco-Tribo Polarograph analyser controlled by Polar Pro software, version 5.1 (Eco-Trend Plus, Prague, Czech Republic). A three-electrode arrangement was used for the measurements. SBiDE (type 6.0346.000, bismuth drop diameter of 2 mm, Metrohm, Herisau, Switzerland) was used as a working electrode, an argentochloride electrode (type 10-20+polaro Ag 10 10-2014-3, 3 M KCl, Elektrochemické Detektory, Turnov, Czech Republic) was used as a reference electrode, and a platinum wire electrode (ETP CZ P01306, Elektrochemické Detktory, Turnov, Czech Republic) was used as an auxiliary electrode.

The DPV technique was used with the following parameters: a polarization rate of 20 mV s^{-1}, a potential step of 3 mV, a pulse width of 100 ms (current sampled for the last 20 ms), a pulse height of –50 mV, and a pulse period of 150 ms. CV used polarization rates of 10–1000 mV s^{-1} and a potential step of 3 mV. Samples with a total volume of 20.0 mL were analysed in the voltammetric vessel.

Spectrophotometric measurements were performed on an Agilent 8453 instrument using UV-Visible ChemStation software, version 9.01 (both Agilent Technologies, Santa Clara, CA, USA). Measurements were made in quartz cuvettes with a specific thickness of 1.0 cm against aqueous BR buffer solution of a given pH. The absorbance of the prepared solutions was measured in the wavelength range of 200–1100 nm.

The pH was measured using a Jenway 3510 digital pH meter (Jenway, Felsted, UK) with a combined glass electrode.

Results and discussion

Voltammetric behaviour of metronidazole on a solid bismuth drop electrode

The first step was to find a suitable onset of the potential window (E_{in}) so that the measured analyte peak was as well developed and evaluable as possible and the voltammograms of the supporting electrolyte showed the lowest background current (Fig. 3a). SBiDE is very sensitive to the application of potentials that result in the formation of bismuth oxides and/or dissolution of bismuth itself. When the bismuth electrode was left in the buffer solution for 5 s or longer at open circuit potential or in air, the bismuth began to oxidize, and the oxides formed were subsequently reduced to provide signals with a large peak height during the first voltammetric measurement (Fig. 3b).

The next step in the development of the DPV method was to find a suitable supporting electrolyte. The voltammetric behaviour of metronidazole at a concentration of 1×10^{-3} mol L^{-1} was investigated in aqueous BR buffers at pH 2.0–12.0. From Fig. 4a, it can be seen that metronidazole in aqueous BR buffer solution in the pH range of 3.0–12.0 provides one well-developed peak which corresponds to the four-electron irreversible reduction of the nitro group to the corresponding hydroxylamino group[15]. It is certainly noteworthy that the peak potential (E_p) of metronidazole shifts to more negative values with increasing pH in a somewhat sigmoidal dependence (Fig. 4b), whereas the common trend observed for this analyte on electrodes of other materials is for E_p to shift linearly with pH, with a slope value approaching the expected theoretical value of –59 mV pH^{-1} (ref.[21,24,25]). However, we currently have no explanation for this phenomenon, and it will be

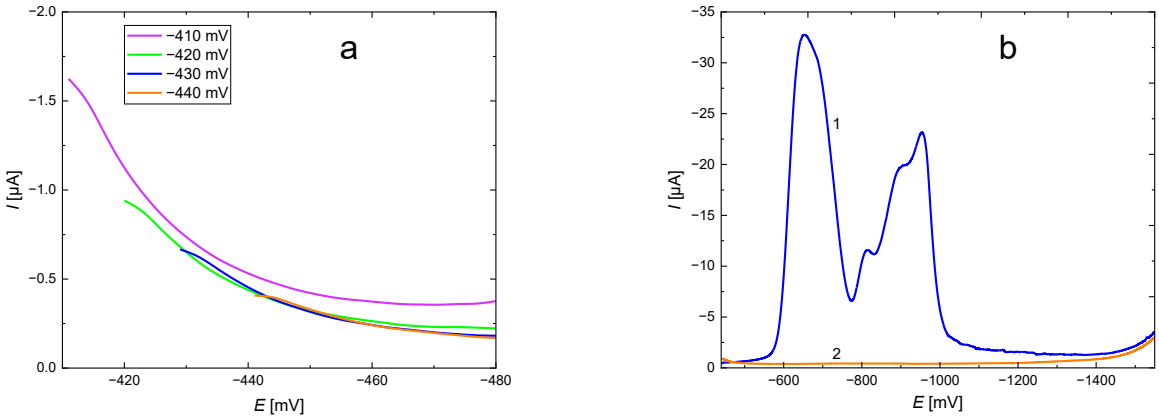

Fig. 3. **(a) Finding the onset of the potential window of aqueous BR buffer solution at pH 12.0 by DPV on SBiDE (ref.[26]). (b) Example of the first (1) and second (2) measurements in aqueous BR buffer solution at pH 12.0 by DPV on SBiDE at E_{in} = –440 mV (ref.[26]).** Colour versions of all images are available on the website of the journal Chemicke Listy

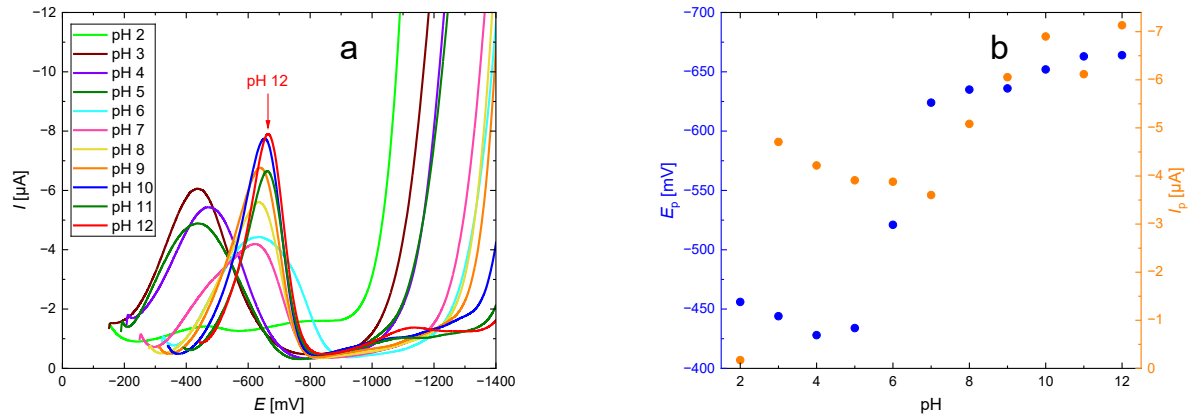

Fig. 4. **(a) DP voltammograms of metronidazole ($c = 1\times10^{-3}$ mol L^{-1}) recorded on SBiDE in aqueous BR buffer solutions at pH 2.0–12.0 (ref.[26]). (b) Corresponding dependence of the peak potential (E_p) and the peak height (I_p) of metronidazole ($c = 1\times10^{-3}$ mol L^{-1}) on the pH**

investigated in future research, primarily through the study of other structurally similar electrochemically active compounds on SBiDE. An aqueous BR buffer solution at pH 12.0 was chosen as the optimum medium for further measurements, as this medium provided a well-developed peak and the highest current response (I_p) of the analyte (Fig. 4b).

The voltammetric behaviour of metronidazole on SBiDE was also investigated by CV, with the main emphasis on determining the reversibility of the electrode reaction during reduction of the nitro group present in the metronidazole structure and on determining by which step this reaction, or the whole electrode process, on SBiDE is controlled. The effect of different polarization rates (10, 20, 50, 100, 200, 500, and 1000 mV s^{-1}) on the CV behaviour of metronidazole (c = 1×10^{-4} mol L^{-1}) was investigated in three different media – acidic (BR buffer pH 3.0), neutral (BR buffer pH 7.0), and alkaline (BR buffer pH 12.0). In the acidic medium, the response of metronidazole could not be evaluated on the voltammograms obtained. The CVs of metronidazole in neutral and alkaline media at a polarization rate of 200 mV s^{-1} are shown in Fig. 5a, and it can be seen that the electrode reaction corresponding to the four-electron reduction of the nitro group to the corresponding hydroxylamino group is also irreversible on SBiDE (or can be also considered quasi-reversible in a pH 12.0 medium). In both investigated media, the peak heights (I_p) of metronidazole were evaluated at each polarization rate (v), and the dependences of log $|I_p|$ *vs.* log v were plotted (Fig. 5b), from which the absolute values of the slopes ($|k|$) can be used to determine which step (most commonly

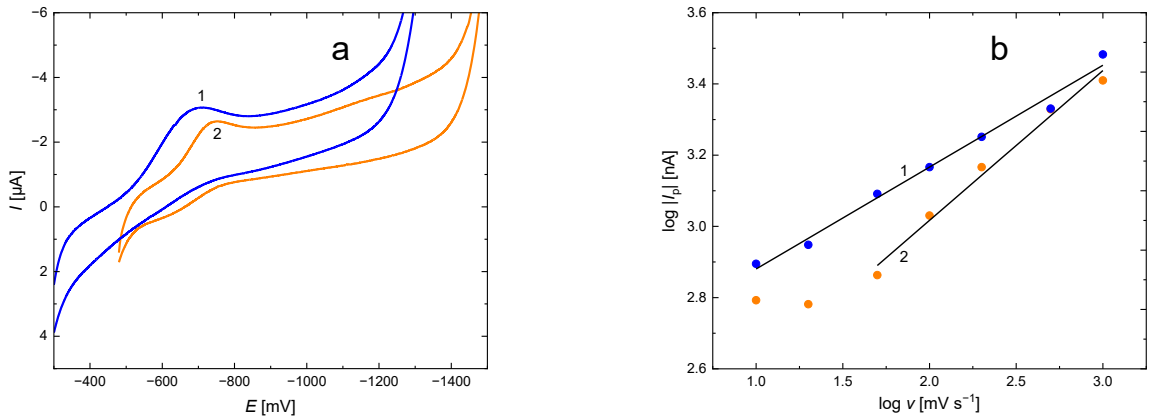

Fig. 5. **(a) Cyclic voltammograms of metronidazole (c = 1×10^{-4} mol L^{-1}) recorded on SBiDE at a polarization rate (v) of 200 mV s^{-1} in aqueous BR buffer solutions at pH 7.0 (1) and 12.0 (2). (b) Dependence of the log $|I_p|$ of metronidazole (c = 1×10^{-4} mol L^{-1}) on the log v measured by cyclic voltammetry on SBiDE over a range of polarization rates of 10–1000 mV s^{-1} in BR buffer at pH 7.0 (1) and 12.0 (2)**

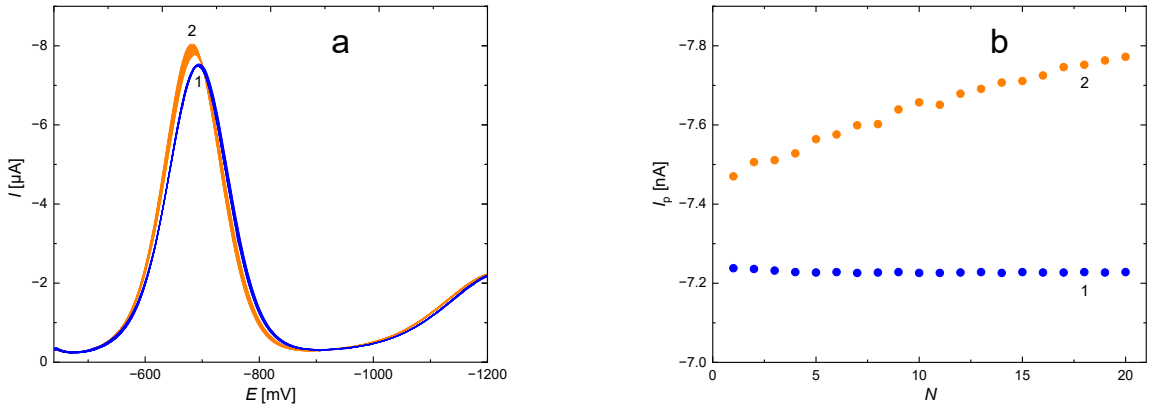

Fig. 6. **(a) DP voltammograms of metronidazole (c = 1×10^{-3} mol L^{-1}) (n = 20) recorded on SBiDE in aqueous BR buffer solution at pH 12.0 without using regeneration potentials (1) and with regeneration potentials E_{in_r} = –490 mV, E_{fin_r} = –1500 mV (2). (b) Corresponding dependence of the current response (I_p) of metronidazole (c = 1×10^{-3} mol L^{-1}) on the serial number of measurement (N) (n = 20), measured by DPV on SBiDE in aqueous BR buffer solution at pH 12.0 without using regeneration potentials (1) and with regeneration potentials E_{in_r} = –490 mV, E_{fin_r} = –1500 mV (2) (ref.[26])**

analyte diffusion at $|k| = 0.5$, analyte adsorption at $|k| = 1.0$, or electrode reaction kinetics at $0 < |k| < 0.5$) controls the electrode processes on SBiDE[28]. In a neutral medium, the dependence of the logarithm of the absolute value of the metronidazole peak height on the logarithm of the polarization rate was obtained with an absolute slope value of 0.29 and $r^2 = 0.9891$ (Fig. 5b, dependence 1). This indicates that in this medium, the electrode process is controlled purely by the kinetics of the electrode reaction, *i.e.*, by its low rate. In an alkaline medium (Fig. 5b, dependence 2), the obtained dependence cannot be reliably fitted with a straight line over the entire studied interval of polarization rates, but it can be fitted in the range of higher polarization rates of 50–1000 mV s^{-1}, and an absolute slope value of 0.42 and $r^2 = 0.9871$ can be obtained, suggesting a process controlled more by diffusion; in the polarization rate range of 50–200 mV s^{-1}, the influence of diffusion is already completely dominant ($|k| = 0.50$ and $r^2 = 0.9965$).

Furthermore, it was investigated whether electrochemical regeneration is necessary during repeated DPV determinations of metronidazole on SBiDE to avoid passivation of the working electrode and to ensure good repeatability of the determinations. Regeneration potentials[27] $E_{in\_r} = -490$ mV and $E_{fin\_r} = -1500$ mV were inserted for 30 s before each measurement (each alternating for 100 ms every time for a total of 150 cycles). The applied regeneration potentials slightly increased the current response of metronidazole (Fig. 6a) but impaired the repeatability (there was a gradual increase in peak height; Fig. 6b, point set 2 with *RSD* = 1.2%, $n = 20$); therefore, subsequent measurements by the DPV technique were performed without inserting these regeneration potentials (Fig. 6b, point set 1 with *RSD* = 0.045%, $n = 20$).

Voltammetric determination of metronidazole on a solid bismuth drop electrode

The calibration dependences of metronidazole were measured in the concentration range of 1×10^{-6} – 1×10^{-3} mol L^{-1}. The calibration was also measured in media with pH 3.0 and 7.0 in addition to the optimum aqueous BR buffer solution at pH 12.0. It was of interest to see what the concentration dependence of metronidazole would be in acidic or neutral medium, but it was confirmed that both acidic and neutral media were unsuitable for the determination of metronidazole. The calibration dependence in BR buffer at pH 12.0 is linear over the concentration range of metronidazole of 1×10^{-6} – 6×10^{-4} mol L^{-1}. The limit of quantification (*LOQ*; $10\sigma/k$) was 1.4 μmol L^{-1}, and the limit of detection (*LOD*; $3\sigma/k$) was 0.41 μmol L^{-1}. Fig. 7a shows the DP voltammograms of metronidazole ($c = 1\times10^{-6}$ – 1×10^{-3} mol L^{-1}) in BR buffer at pH 12.0. Fig. 7b shows the corresponding calibration dependence of the peak height of metronidazole on its

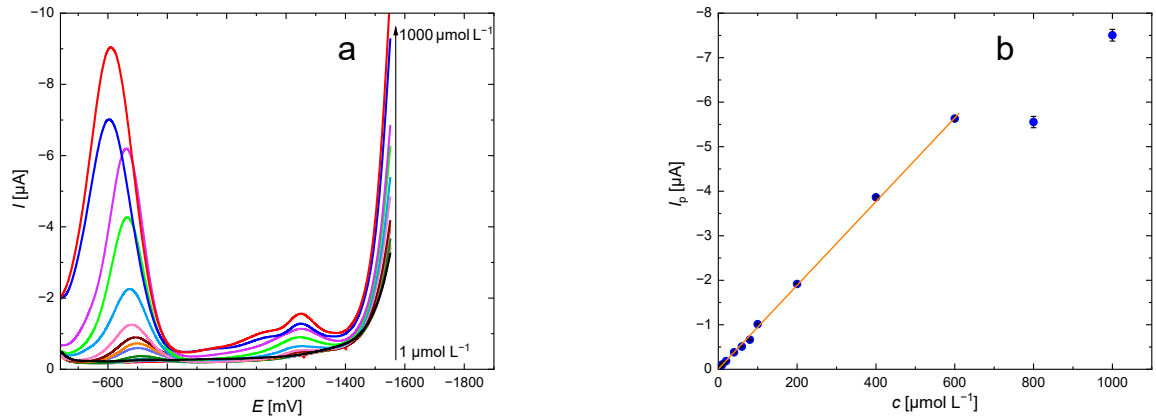

Fig. 7. **(a) DP voltammograms of metronidazole (c = 1–1000 μmol L^{-1}) recorded on SBiDE in aqueous BR buffer solution at pH 12.0;** black colour indicates the supporting electrolyte (ref.[26]). **(b) Calibration dependence of the peak height on the concentration of metronidazole (c = 1–1000 μmol L^{-1});** error bars for $n = 5$; regression equation: I_p [nA] = –9.47c [μmol L^{-1}] – 0.585; $r^2 = 0.9993$ (ref.[26])

Table II
Parameters of metronidazole calibration straight lines for DPV on SBiDE in aqueous BR buffer solution at pH 12.0

c [μmol L^{-1}]	Slope [mA L mol^{-1}]	Intercept [nA]	r^2
1–10	−10.2	−2.62	0.9963
10–100	−9.43	10.2	0.9723
100–600	−9.30	−84.8	0.9976

concentration (c = 1×10^{-6} – 1×10^{-3} mol L^{-1}). In Tab. II, the parameters of the individual calibration straight lines are listed.

For a number of organic electrochemically active compounds, it is possible to use potential-stimulated adsorptive accumulation of the analyte on the working electrode surface to further increase the sensitivity of their determination (and thus reduce the *LOD* and *LOQ* values)[29,30]. However, in the case of the metronidazole determination on SBiDE, the differential pulse adsorptive stripping voltammetric technique could not be used. Between the beginning of the usable potential window bounded by the electrochemical oxidation of the bismuth droplet surface (–440 mV, Fig. 3a) and between the peak potential of metronidazole (*ca.* –700 mV, Fig. 7a), there are no suitable accumulation potentials for this purpose at which either degradation of the working electrode surface or unwanted electrolysis of metronidazole would not occur.

The applicability of the newly developed DPV method was verified in the determination of metronidazole in authentic drinking water samples. Drinking water was taken from the water supply network in the building of the Institute of Chemistry, Faculty of Science, Charles University, Prague. Authentic samples with a volume of 20.0 mL consisted of 18.0 mL of drinking water containing an added metronidazole standard of a certain concentration and 2.0 mL of BR buffer at pH 12.0, which additionally contained the necessary addition of 100 µL of chelaton III solution at a concentration of 0.1 mol L^{-1} to suppress the interfering effect of cations present in the drinking water[6,7]. The calibration dependence of metronidazole in authentic drinking water samples was measured in the concentration range of 1×10^{-6} – 1×10^{-4} mol L^{-1} with the regression equation of the overall linear dependence: I_p [nA] = –6.47c [µmol L^{-1}] – 132; r^2 = 0.9586 (*LOD* = 1.8 µmol L^{-1} and *LOQ* = 5.8 µmol L^{-1}). It can be seen from the lower slope value of the obtained dependence (compared to the dependences from Fig. 7b and Tab. II) that the drinking water matrix slightly reduces the sensitivity of the determination, and the dilution of the authentic drinking water samples with the supporting electrolyte in a volume ratio of 9:1 also plays a role.

Determination of metronidazole in dosage forms

Voltammetric determination of metronidazole in drugs was performed by the standard addition method under previously obtained optimum conditions in infusion solution Efloran 500 mg/100 mL, tablet Entizol 250 mg,

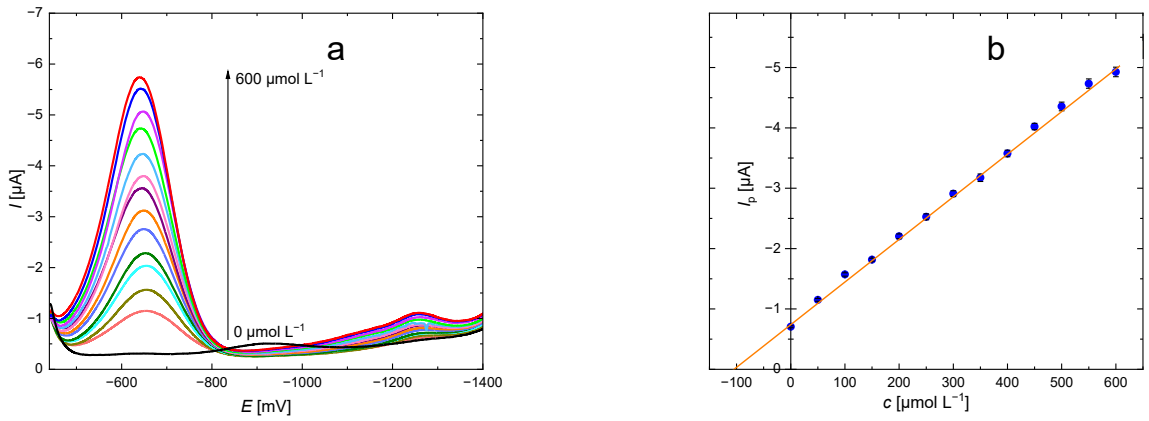

Fig. 8. **(a) DP voltammograms of metronidazole recorded on SBiDE in samples consisting of a portion of tablet Entizol 250 mg and an added analyte standard (c = 0–600 µmol L^{-1}) using the method of standard addition in aqueous BR buffer solution at pH 12.0.** The black colour indicates the supporting electrolyte. **(b) Dependence of the peak height on the concentration of added metronidazole standard in tablet Entizol 250 mg (c = 0–600 µmol L^{-1});** error bars for n = 5

Table III
Parameters of linear concentration dependences of the added metronidazole standard in each drug obtained by DPV on SBiDE in aqueous BR buffer solution at pH 12.0

Drug	$c_{\text{metronidazole standard}}$ [µmol L^{-1}]	Slope [mA L mol^{-1}]	Intercept [nA]	r^2
Infusion solution Efloran	0–400	−7.31	−132	0.9961
Tablet Entizol 250 mg	0–600	−7.08	−775	0.9982
Tablet Entizol 500 mg	0–600	−4.80	−466	0.9944
Infusion solution Noridem	0–600	−7.42	−580	0.9991

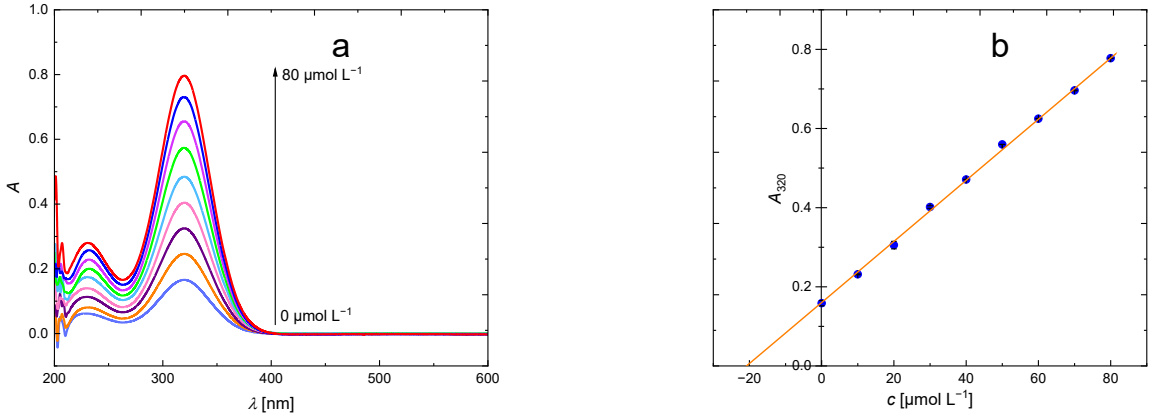

Fig. 9. **(a) Absorption spectra of metronidazole recorded in quartz cuvettes with a specific thickness of 1.0 cm in the wavelength range of 200–1100 nm in samples consisting of a portion of tablet Entizol 250 mg and an added analyte standard (c = 0–80 µmol L^{-1}) using the method of standard addition in aqueous BR buffer solution at pH 12.0. (b) Dependence of the absorbance at a wavelength of 320 nm (A_{320}) on the concentration of added metronidazole standard in tablet Entizol 250 mg (c = 0–80 µmol L^{-1});** error bars for n = 5

vaginal tablet Entizol 500 mg, and infusion solution Noridem 500 mg/100 mL. For DPV measurements, 20.0 mL solutions of BR buffer at pH 12.0 were prepared, containing 200 µL of the drug stock solution without addition and with the addition of 50 µL of the standard stock solution (c = 1×10^{-2} mol L^{-1}), the volume of which was increased by 50 µL in each subsequent sample. The determination of metronidazole in Efloran infusion solution was carried out in the concentration range of the added standard of 5×10^{-5} – 4×10^{-4} mol L^{-1}. The determination of metronidazole in tablets and Noridem infusion solution was carried out in the concentration range of the added standard of 5×10^{-5} – 6×10^{-4} mol L^{-1}. For illustration, Fig. 8a shows the DP voltammograms of metronidazole recorded during its determination in tablet Entizol 250 mg, and Fig. 8b shows the dependence of the peak height on the concentration of the added metronidazole standard in the same tablet. In Tab. III, the parameters of the linear concentration dependences of the added metronidazole standard in individual drugs are presented.

UV-Vis absorption spectrophotometry was used as a comparative method to the voltammetric determination of metronidazole in drugs using the standard addition

Table IV
Parameters of linear concentration dependences of the added metronidazole standard in each drug obtained by UV-Vis spectrophotometry at a wavelength of 320 nm in quartz cuvettes with a specific thickness of 1.0 cm in aqueous BR buffer solution at pH 12.0 in the wavelength range of 200–1100 nm

Drug	$c_{\text{metronidazole standard}}$ [µmol L^{-1}]	Slope [L mol^{-1}]	Intercept	r^2
Infusion solution Efloran	0–60	7.96×10^3	0.256	0.9999
Tablet Entizol 250 mg	0–80	7.76×10^3	0.167	0.9957
Tablet Entizol 500 mg	0–80	7.60×10^3	0.131	0.9940
Infusion solution Noridem	0–80	7.94×10^3	0.167	0.9995

Table V
Values of the calculated amount of metronidazole in drugs relative to the declared amount of metronidazole stated on the packaging of drugs obtained using a newly developed method ($w_{\text{voltammetry}}$) and a comparative analytical method ($w_{\text{spectrophotometry}}$). The ratio of the above $w_{\text{voltammetry}}$ / $w_{\text{spectrophotometry}}$ values then gives the actual recovery of the DPV method on SBiDE against the comparative analytical method

Drug	$w_{\text{voltammetry}}$ [%]	$w_{\text{spectrophotometry}}$ [%]	$w_{\text{voltammetry}}$ / $w_{\text{spectrophotometry}}$ [%]
Infusion solution Efloran	180	161	112
Tablet Entizol 250 mg	127	131	97
Tablet Entizol 500 mg	134	119	113
Infusion solution Noridem	78	106	74

method under previously obtained optimum conditions. For measurements by the standard addition method, 10.0 mL solutions of BR buffer at pH 12.0 were prepared, containing 200 μL of the drug stock solution without addition and with the addition of 100 μL of the standard stock solution (c = 1×10^{-3} mol L^{-1}), the volume of which was increased by 100 μL in each subsequent sample. The determination of metronidazole in Efloran infusion solution was carried out in the concentration range of the added standard of 1×10^{-5} – 6×10^{-5} mol L^{-1}. The determination of metronidazole in tablets and Noridem infusion solution was carried out in the concentration range of the added standard of 1×10^{-5} – 8×10^{-5} mol L^{-1}. The UV-Vis absorption spectra of metronidazole recorded during its determination in tablet Entizol 250 mg are shown in Fig. 9a for illustration, and Fig. 9b shows the corresponding dependence of the absorbance at a wavelength of 320 nm on the concentration of the added metronidazole standard. In Tab. IV, the parameters of the linear concentration dependences of the added metronidazole standard in individual drugs are presented. In Tab. V, the results obtained by DPV on SBiDE and by UV-Vis spectrophotometry are compared.

Conclusion

In this work, a new commercially available working electrode, the solid bismuth drop electrode (SBiDE), was studied, and its use for the voltammetric determination of the drug metronidazole using the differential pulse voltammetric (DPV) technique was investigated. Under optimum conditions, LOD = 0.41 μmol L^{-1} and LOQ = 1.4 μmol L^{-1} were achieved in a BR buffer medium at pH 12.0. The newly developed DPV method was also successfully applied to the determination of metronidazole in authentic drinking water samples (LOD = 1.8 μmol L^{-1} and LOQ = 5.8 μmol L^{-1}) and in different dosage forms – infusion solution Efloran 500 mg/100 mL, tablet Entizol 250 mg, vaginal tablet Entizol 500 mg, and infusion solution Noridem 500 mg/100 mL. UV-Vis spectrophotometry was used as a comparative analytical method for the dosage forms.

For infusion solution Efloran 500 mg/100 mL, tablet Entizol 250 mg, and vaginal tablet Entizol 500 mg, the values of the determined amount of metronidazole drug (reported as percentage recovery values) are comparable under the given optimum conditions using DPV on SBiDE and UV-Vis spectrophotometry. For infusion solution Noridem 500 mg/100 mL, 78% metronidazole was determined by the voltammetric method and 106% metronidazole was determined by the spectrophotometric method. This indicates that the DPV on SBiDE is suitable for the determination of metronidazole in the following dosage forms: infusion solution Efloran 500 mg/100 mL, tablet Entizol 250 mg, and vaginal tablet Entizol 500 mg.

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