Monatsh Chem (2015) 146:1211–1215
DOI 10.1007/s00706-015-1469-9

ORIGINAL PAPER

Determination of bromhexine at a glassy carbon paste electrode using differential pulse voltammetry and flow injection analysis with amperometric detection

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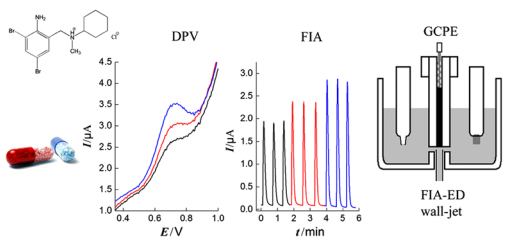
Received: 12 December 2014 / Accepted: 22 March 2015 / Published online: 8 April 2015

Abstract New electrochemical methods for the determination of bromhexine based on differential pulse voltammetry (DPV) and flow injection analysis with amperometric detection (FIA-ED) were developed using a glassy carbon paste electrode. Optimal supporting electrolyte for DPV measurement was methanol/Britton–Robinson buffer pH 9 (80:20, v/v). In the case of FIA-ED, optimal conditions were as follows: detection potential +1.1 V, flow rate 1.0 cm^3 min^{-1}, injected volume 0.1 cm^3, and carrier solution methanol–ten times diluted B-R buffer of pH 9 (80:20, v/v). Detection limit was 2.0×10^{-6} mol dm^{-3} for DPV and 3.1×10^{-7} mol dm^{-3} for FIA-ED. The applicability of the newly developed methods was verified by the determination of bromhexine in pharmaceutical preparations (tablets of Bromhexin-EGIS and Bromhexin 8 BC).

Graphical abstract



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Keywords Voltammetry · Electrochemistry · Amperometry · Pharmaceuticals

Introduction

Bromhexine is commonly used mucolytic agent. It is a frequent component of pharmaceutical preparations, which are used against diseases of the respiratory tract, to relieve coughing, prevent the formation of mucus, etc. [1]. Bromhexine is sparely soluble in water and ethanol, but very well soluble in methanol [1]. It is an easily oxidizable substance, thanks to its chemical structure (Fig. 1). Two electrons and one proton reaction leads to unstable intermediate, which disintegrates in the presence of aqueous solution with release of second proton to *N*-methylcyclohexanamine and 2-amino-3,5-dibromobenzaldehyde. Another product, 2,4,8,10-tetrabromodibenzo[*b*,*f*][1,5]-diazocine, probably results from dehydration of a dimeric structure [2, 3].

As mentioned above, bromhexine is commonly used in medicine, and thus there is a need for its fast and inexpensive determination. Most frequent techniques are HPLC with spectrophotometric and mass spectrometric detection [4–6] and flow injection analysis (FIA) with spectrophotometric detection [7]. Its chemical structure, namely the presence of NH_2 group on aromatic ring, enables its easy electrochemical determination on glassy carbon electrode [3].

Carbon paste electrodes (CPE) are well-known sensors, which are used for more than 50 years [8]. Their electrochemical properties are mainly based on carbonaceous working material, and their arrangement as a mixture of carbon with inert nonpolar liquid brings others advantages, such as low background current, wide potential window,

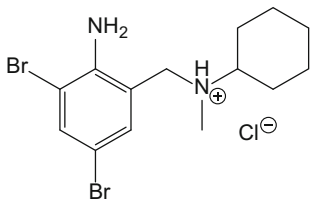

Fig. 1 Chemical structure of bromhexine hydrochloride

possibility of analyte accumulation, easy electrode modification, and last but not least easy surface renewal. CPE can be modified by admixing of other substance [9, 10] or simply by the change in basic components itself, due to diversity of electrode materials and pasting liquids. CPE of course have some disadvantages: they are unstable in organic solvents, which limits their application in such media. This problem can be diminished by replacing graphite powder by spherical microparticles of glassy carbon [11]; this type of CPE is called glassy carbon paste electrode (GCPE) [12, 13], and its electrochemical behavior is closer to glassy carbon electrode in comparison with graphite-based CPE [14].

In this paper, new fast and inexpensive methods for bromhexine determination by differential pulse voltammetry (DPV) and FIA with amperometric detection using GCPE as the working electrode were developed and applied for its determination in commercial pharmaceutical preparations.

Results and discussion

Optimization of supporting electrolyte for DPV measurements

Bromhexine is not soluble in aqueous media, particularly in alkaline range of pH. Therefore, the measurements were taken in the presence of 20 % (v/v) of methanol in the acidic pH range and in the presence of 80 % (v/v) of methanol in the whole pH range. The optimum pH of B-R buffer for bromhexine determination was found from dependence of DP voltammograms on pH in the range from pH 2 to 12 (pH of aqueous buffer before mixing with methanol) (Fig. 2). The peak potential shifted to the less positive potentials, and the peak height decreased slightly with the increase in pH in medium containing 20 % (v/v) of methanol. In the presence of 80 % (v/v) of methanol, however, the behavior changed markedly. The peak height increased generally with pH, reaching its maximum at pH 9.0, and the peak position was less dependent on pH. Optimum composition (highlighted by bold curve in Fig. 2b) of the supporting electrolyte was selected on the basis of peak height as mixture of B-R buffer pH 9.0 and methanol (20:80, v/v).

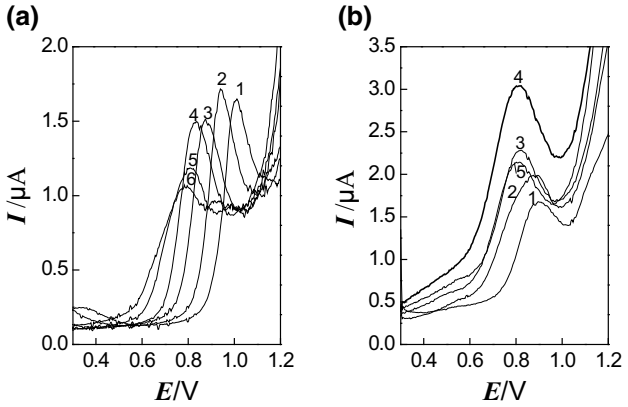

Fig. 2 DP voltammograms of bromhexine (1×10^{-4} mol dm^{-3}) at GCPE in B-R buffer pH 2 (*1*), 3 (*2*), 4 (*3*), 5 (*4*), 6 (*5*), and 7 (*6*): MeOH (80:20, v/v) mixture (**a**) and in B-R buffer pH 4 (*2*), 6 (*2*), 8 (*3*), 9 (*4*), and 10 (*5*): MeOH (20:80, v/v) mixture (**b**)

Optimization of FIA measurements

FIA with amperometric detection at GCPE was used as the second technique for bromhexine determination. The first step of optimization procedure was measurement of hydrodynamic voltammograms in carrier solutions consisting of methanol and ten times diluted B-R buffer pH in the range from 2.5 to 11 (80:20, v/v) (Fig. 3). Optimal pH of B-R buffer was found to be 9 (bold curve in Fig. 3), and the optimal detection potential of +1.1 V was chosen from hydrodynamic voltammogram measured at this pH. Under these conditions, different values of flow rate were tested in the range from 0.5 to 5.0 cm^3 min^{-1}. A value of 1.5 cm^3 min^{-1} was selected as optimal, because at this flow rate, time of one determination was short, the peak height reached its maximum, and, at the same time, the decrease in the peak area was not too big. Afterwards, injected volume was optimized. The highest signal was observed at injected volume of 0.1 cm^3, which was therefore selected as optimal. Further increase in injected volume leads only to the widening of the peaks.

Repeatability of measurements

After finding optimal conditions, stability of GCPE signal was tested, because products of electrochemical reaction of bromhexine could passivate working electrode. This effect was obvious, especially with DPV. When the surface of working electrode was not renewed, the signal of GCPE decreased about 30 % after ten measurements (under the optimum conditions). Thus, the surface of GCPE had to be renewed periodically after each measurement. The repeatability of the measurements with renewal, expressed by relative standard deviation (RSD) of 10 measurements, was 4.0 %.

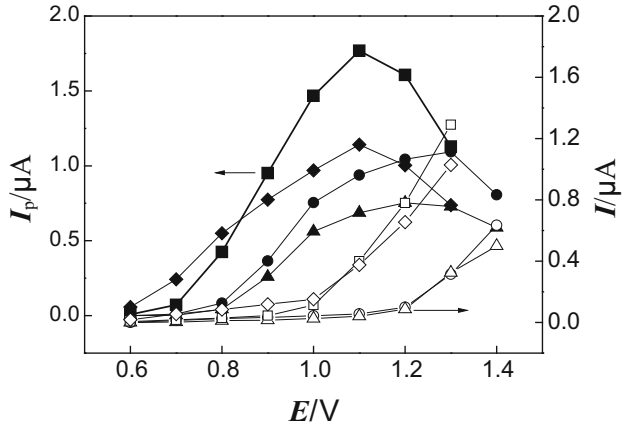

Fig. 3 Hydrodynamic voltammograms of bromhexine (1×10^{-4} mol dm^{-3}) at GCPE in mixture (80:20, v/v) of methanol and B-R buffer pH 2.5 (*filled circle*), 5 (*filled triangle*), 9 (*filled square*), and 11 (*filled diamond*) and their corresponding background currents (analogical *empty symbols*); flow rate 1.5 cm^3 min^{-1} and injected volume 0.1 cm^3

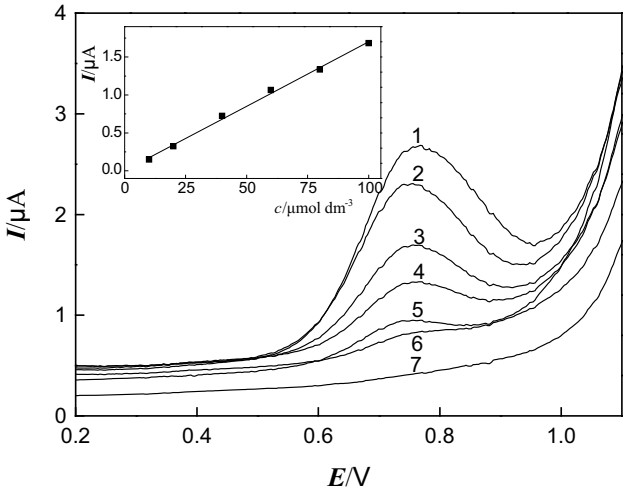

Fig. 4 DP voltammograms of 100 (*1*), 80 (*2*), 60 (*3*), 40 (*4*), 20 (*5*), 10 (*6*), and 0 μmol dm^{-3} (*7*, base electrolyte) of bromhexine in B-R buffer pH 9.0 and methanol mixture (20:80, v/v) at GCPE and corresponding concentration dependence (in *inset*)

In the case of FIA with electrochemical detection, the signal corresponding to 20 consecutive injections of bromhexine ($c = 1 \times 10^{-4}$ mol dm^{-3}) was stable enough without the need of electrode surface renewal, and the repeatability expressed by RSD of 20 consecutive measurements was 1.8 %. Better stability of signal was probably caused by washing away products of electrochemical reaction from the working electrode surface by flow of carrier solution, and therefore, the GCPE passivation was lower. Renewing of GCPE surface after each measurement has led to the increase in RSD of 10 measurements to 4.6 %. The surface of GCPE was renewed after 20 injections of sample.

Calibration

Dynamic linear range and other figures of merit of newly developed methods were calculated from concentration dependences measured under the optimal conditions. Obtained DPV voltammograms and corresponding concentration dependence are shown in Fig. 4. FIA-ED records are shown in Fig. 5. Measured dependences are linear in the concentration range from 2.0×10^{-6} for DPV and 3.1×10^{-7} for FIA to 1×10^{-4} mol dm^{-3}, and their parameters are given in Table 1. The limit of detection obtained by FIA with electrochemical detection was almost one order of magnitude lower than in the case of DPV, which can be associated with the stable baseline of the FIA measurements and the corresponding easier evaluation of the peaks at low analyte concentrations.

The possibility to increase the sensitivity of DPV by a preliminary adsorption step was tested, but significant decrease in detection limit was not obtained. It was probably due to the high content of methanol in the supporting electrolyte which prevents adsorption of bromhexine on the working electrolyte surface.

Real samples of pharmaceutical preparations

Newly developed methods were applied for the determination of bromhexine in real samples of commercial pharmaceutical formulas in the form of tablets. Concentrations of bromhexine were measured also by FIA with spectrophotometric detection as a comparative method with the electrochemical ones. Obtained results are given in Table 2, and selected voltammograms are shown in Fig. 6. Results from electrochemical methods are generally in good agreement with producers' declared quantities and with comparative method (FIA-UV). FIA-ED achieved more accurate results in comparison with DPV measurements.

Conclusions

New electrochemical methods of bromhexine determination in pharmaceutical preparations were developed, using DPV and FIA with amperometric detection at GCPE. Optimum supporting electrolyte for the DPV determination was the mixture of methanol and B-R buffer pH 9.0 (80:20, v/v). Optimum conditions of the FIA determination were: detection potential +1.1 V, flow rate 1.5 cm^3 min^{-1}, the volume of injection 0.1 cm^3, and the carrier solution consisting of methanol and ten times diluted B-R buffer pH 9.0 (80:20, v/v).

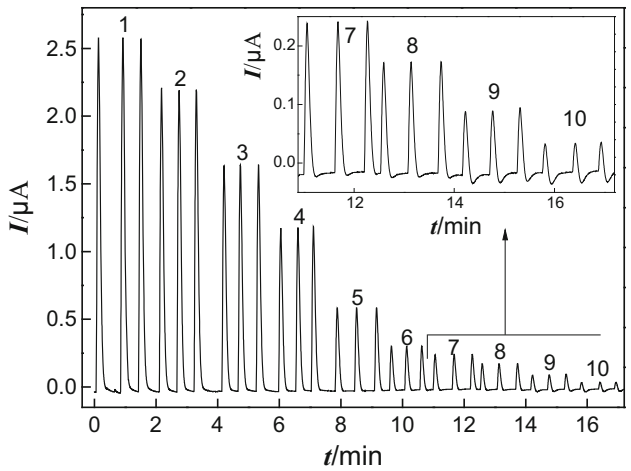

Fig. 5 FIA records of 100 (*1*), 80 (*2*), 60 (*3*), 40 (*4*), 20 (*5*), 10 (*6*), 8 (*7*), 6 (*8*), 4 (*9*), and 2 (*10*) μmol dm^{-3} of bromhexine at GCPE in the carrier solution consisting of methanol and ten times diluted B-R buffer pH 9.0 (80:20, v/v) with detection potential +1.1 V, flow rate 1.5 cm^3 min^{-1}, and injection volume of 0.1 cm^3. *Inset* contains enlarged FIA records of solutions at lower concentrations

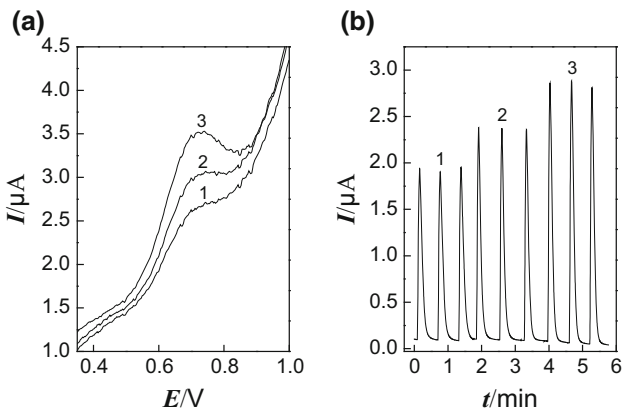

Fig. 6 DP voltammograms of Bromhexin-EGIS in the mixture of methanol and B-R buffer pH 9.0 (80:20, v/v) at GCPE (**a**) and FIA records of Bromhexin 8 BC at GCPE in the carrier solution consisting of methanol and ten times diluted B-R buffer pH 9.0 (80:20, v/v) with detection potential +1.1 V, flow rate 1.5 cm^3 min^{-1}, and injection volume of 0.1 cm^3 (**b**); real sample (*1*), first addition of 0.2 cm^3 of 1×10^{-4} mol dm^{-3} solution (*2*); second addition of 0.4 cm^3 of 1×10^{-4} mol dm^{-3} solution (*3*) of standard

Table 1 Parameters of calibration straight lines and values of detection limit of the developed methods of bromhexine determination

Method	Slope/mA mol^{-1} dm^3	Intercept/μA	Correlation coefficient	Limit of detection/mol dm^{-3}
DPV	17.2	−0.117	0.9989	2.0×10^{-6}
FIA	26.9	−0.047	0.9986	3.1×10^{-7}

Table 2 DPV and FIA-ED determination of tested analytes in real samples

Sample	Method of determination	Found/mg per tablet	Declared quantity/mg per tablet	Found by FIA-UV/mg per tablet
Bromhexin-EGIS	DPV	8.3 ± 0.5	8.0	8.1 ± 0.8
Bromhexin-EGIS	FIA	8.0 ± 0.5	8.0	8.1 ± 0.8
Bromhexin 8 BC	DPV	8.9 ± 0.6	8.0	8.0 ± 0.8
Bromhexin 8 BC	FIA	8.3 ± 0.6	8.0	8.0 ± 0.8

Uncertainty of results is presented as standard deviation

Concentration dependences were linear in the whole examined range, i.e., from determination limits, namely 2.0×10^{-6} mol dm^{-3} for DPV and 3.1×10^{-7} mol dm^{-3} for FIA-ED, to 1×10^{-4} mol dm^{-3}. FIA provides faster results and almost ten times lower detection limit than DPV. It also uses smaller volume of samples and assures much higher sample throughput.

If the GCPE surface was renewed after each measurement, repeatability was almost the same for both methods (RSD 4.0 % for DPV and 4.6 % for FIA), but in the case of FIA, the signal is stable even without renewing of the surface for 20 injections of sample, and then, RSD is just 1.8 %. Thanks to this, FIA provided slightly more stable response than DPV.

Newly developed methods were used for the determination of bromhexine in samples of pharmaceutical preparations (Bromhexin 8 BC tablets and Bromhexin-EGIS tablets). The results were in agreement with producers' declared content and also with results from reference method based on FIA with spectrophotometric detection.

Experimental

Bromhexine hydrochloride (Fig. 1, ≥98 %, Sigma-Aldrich, USA) was used for the preparation of stock solutions (1×10^{-3} mol dm^{-3}) in methanol; solutions of lower

concentrations were prepared by their exact dilution with methanol (Merck, Germany, ≥99.9 %) or with Britton–Robinson (B-R) buffer. Alkaline component of B-R buffer was prepared from sodium hydroxide (98 %, Lach-Ner, Czech Republic) and acidic component from phosphoric acid (85 %, Lach-Ner, Czech Republic), boric acid (99.5 %, Lach-Ner, Czech Republic), and acetic acid (99 %, Lach-Ner, Czech Republic). Deionized water from Millipore Q-plus System (Millipore, USA) was used. Newly developed methods were tested on real pharmaceutical preparations: Bromhexin 8 BC (coated tablets, Berlin-Chemistry AG, Germany) and Bromhexin-EGIS (tablets, Egis Pharmaceuticals, Hungary).

Apparatus

Eco-Tribo-Polarograph (Polaro-Sensors, Czech Republic) was used for all voltammetric measurements with three-electrode arrangement, consisting of working GCPE, and platinum auxiliary electrode and Ag/AgCl (3 M KCl) reference electrode (both from Monokrystaly Turnov, Czech Republic), to which all the potential values are referred. Parameters of DPV were: scan rate 20 mV s^{-1}, width of pulse 100 ms, and height of pulse 50 mV.

FIA system consisted of HPP 5001 high-pressure pump with ADLC2 amperometric detector (both from Laboratorni pristroje Praha, Czech Republic) and six-port injection valve (Rheodyne, USA). The same electrode set as in the case of voltammetric measurements was employed. Working electrode was adjusted in wall-jet arrangement against the outlet capillary at a controlled distance [15]. FIA with spectrophotometric detector Sapphire (ECOM, Czech Republic) set on 210 nm in the same arrangement was used as a comparative method for the determination of real samples of bromhexine.

Working glassy carbon paste electrode was prepared from 250 mg of glassy carbon spherical microparticles (size 0.4–12 μm, Alfa Aesar, Germany) and 0.1 cm^3 of mineral oil (Fluka, Switzerland). The mixture was packed into a Teflon piston-driven holder with inner diameter of 2 mm [16].

Procedures

Acidic and basic components of B-R buffer were prepared separately and then mixed together to obtain required pH of the resulting buffer. The accurate pH was measured by pH meter. In the case of FIA, acidic and basic components were ten times diluted by deionized water to prevent the crystallization in the system before mixing. Thus, prepared B-R buffers of particular pH were mixed with methanol in a defined volume ratio to prepare supporting electrolytes and carrier solutions.

Real samples of pharmaceutical preparations were prepared as follows. One tablet of Bromhexin-EGIS or Bromhexin 8 BC was dissolved in 50 cm^3 of methanol, and 1 cm^3 of this solution was mixed with supporting electrolyte [methanol and Britton–Robinson buffer pH 9 (80:20, v/v)] for DPV or with carrier solution [methanol and ten times diluted B-R buffer pH 9 (80:20, v/v)] for FIA-ED to obtain solution with total volume of 10 cm^3. Samples of bromhexine were determined by standard addition method with 0.2 cm^3 and 0.4 cm^3 additions of 1×10^{-4} mol dm^{-3} solution.

Limits of detection were calculated as three times the standard deviation ($\alpha = 0.05$), calculated from ten repeated measurements of the lowest concentration of the determined analyte, divided by slope of calibration dependence [17]. All the measurements were taken in triplicate, unless stated otherwise.

Acknowledgments This work was performed under the framework of specific university research (SVV). Financial support from the Grant Agency of the Czech Republic (project P206/12/G151) is gratefully acknowledged.

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