VOLTAMMETRIC AND AMPEROMETRIC DETERMINATION OF DOXORUBICIN USING CARBON PASTE ELECTRODES

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Dedicated to the memory of Professor Jaroslav Heyrovský on the occasion of 50th anniversary of the Nobel Prize for polarography.

Direct current voltammetric (DCV) and differential pulse voltammetric (DPV) determination of antineoplastic agent doxorubicin (DOX) at a carbon paste electrode (CPE) was developed. Britton–Robinson buffer (pH 7.0) was used as a supporting electrolyte. The limits of detection are 8×10^{-7} mol l^{-1} (DCV) and 6×10^{-8} mol l^{-1} (DPV). The accumulation of DOX at the electrode surface was used to decrease the limits of detection down to 2.2×10^{-7} mol l^{-1} for adsorptive stripping DC voltammetry (DCAdSV) and 2.8×10^{-9} mol l^{-1} for adsorptive stripping differential pulse voltammetry (DPAdSV) at CPE. The results of the voltammetric methods were utilized for the development of a new determination of doxorubicin using HPLC with amperometric detection on CPE based on spherical microparticles of glassy carbon in a wall-jet configuration. A column with chemically bonded C18 stationary phase and a mobile phase containing 0.01 M phosphate buffer (pH 5.0)–methanol 25:75 (v/v) were used. The limit of detection is 4×10^{-7} mol l^{-1} (HPLC with electrochemical detection (ED)).

Keywords: Doxorubicin; Carbon paste electrode; Direct current voltammetry; Differential pulse voltammetry; HPLC with amperometric detection.

Doxorubicin (DOX) (Fig. 1) is an anthracycline glycoside used in treatment of a variety of cancer diseases. It was isolated in 1969 by Arcamone et al.[1] from the *Streptomyces peucetius* bacterial strain. However, DOX exhibits a major cardiotoxicity that limited its use. Early observations indicated that the anti-tumour activity of the anthracyclines required the presence of the anthracycline ring system and the basic amino group of the daunosamine sugar. The cardiotoxicity was dependent on the daunosamine sugar, which enabled uptake by cardiac muscle cells[2]. DOX contains two electroactive sites: a reducible quinone ring and an oxidizable hydroquinone ring. Very

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often, the methods used for determination of DOX in human plasma or urine are liquid chromatography with mass spectrometric detection[3–5], HPLC with fluorimetric detection[6–8], and capillary zone electrophoresis with laser-induced fluorescence detection[9–11]. Only a few papers using electrochemical methods of DOX determination were found in literature, e.g. differential pulse polarography at a classical dropping mercury electrode[12], voltammetric determination at carbon paste electrode (CPE)[13,14] or flow injection analysis with electrochemical detection[15]. Kano et al.[16–18] studied electrochemical properties of DOX adsorbed on a mercury electrode surface or on a pyrolytic-graphite electrode. Ni-ion implanted glassy carbon electrode[19], modified mercury electrode [20], in situ mercury film electrode[21,22] and various DNA species modified gold electrode[23] were also employed for determination of DOX. The originally used carbon pastes based on classic spectrographic graphites[13–15] enabled reaching quite low limits of detection but their disadvantage was low stability in mixed water–organic solvents with higher contents of organic modifiers or the necessity of the exchange of the supporting electrolyte. Voltammetric methods for the determination of DOX are very sensitive but suffer from lower selectivity, which could limit their use for more complex real matrices. In addition, FIA could be used mainly for simple matrices, which usually contain only one oxidizable or reducible substance (at least at the chosen detection potential). Therefore, our aim was to evaluate the use of new carbon materials as paste components and to check their performance in HPLC.

The carbon paste electrodes (CPEs) could be used for analysis of drugs and their residues[24]. In organic analysis, CPEs are employed mainly for the direct determination of oxidizable pharmaceuticals in various pharmaceutical formulations or for the direct determination of some easily oxidizable phenols, aromatic amines or thiols[25]. CPEs are suitable for checking drug contents in tablets, injections or other formulations.

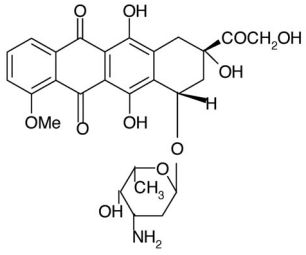

FIG. 1
Structural formula of doxorubicin

Bare CPEs are well suited also for the determination of drugs or their metabolites in urine because the matrix is not complex so that an electrochemically active substance is easily identifiable at an appropriate potential interval. Numerous pharmaceuticals with large and lipophilic molecules are easily accumulated on the surface of CPE: some of them by adsorption, others due to extracting capabilities of the carbon paste[26,27].

CPEs based on glassy carbon microparticles can be employed as electrochemical sensors in HPLC with amperometric detection. They can be used for both oxidation and reduction methods[28]. Although it is possible to perform direct determination of pharmacologically active compounds even in complex media without previous separation, the application of separation techniques leads to a desirable increase in selectivity. Even though CPEs are not often used in combination with HPLC [29] or FIA [30,31], they have many advantages. They are inexpensive, selective, sensitive (typical limits of detection are around 1×10^{-7} mol l^{-1}), they show a broad potential window and low background currents. In flowing systems, the danger of passivation of CPEs is greatly diminished[32]. Moreover, the carbon paste made from glassy carbon microparticles is highly resistant to mobile phases containing high proportions of organic solvents[33], e.g. methanol or acetonitrile. Thus, the goal of this study was to evaluate the suitability of pastes based on glassy carbon microparticles for the determination of DOX using HPLC with mobile phases including methanol as organic modifier of the mobile phase.

EXPERIMENTAL

Reagents

Doxorubicin Ebewe (50 mg) containing doxorubicin hydrochloride (50 mg/25 ml) in aqueous solution of 0.9% w/v NaCl was obtained from Ebewe Arzneimittel, Austria. 1×10^{-4} M stock solution was prepared by diluting Doxorubicin Ebewe formulation with deionized water. It was kept at low temperature in the dark (refrigerator). Voltammetric experiments were carried out in Britton–Robinson (BR) buffer solutions prepared by mixing 0.2 M sodium hydroxide with acid solution consisting of 0.04 M boric acid, 0.04 M phosphoric acid and 0.04 M acetic acid (all p.a., Lachema Brno, Czech Republic). The carbon paste for voltammetric measurements was prepared by mixing 250 mg of carbon powder CR-2 (Tesla Lanškroun, Czech Republic) and 0.1 ml of mineral oil Nujol (Fluka Buchs, Switzerland), the paste for HPLC with electrochemical detection was prepared from 250 mg of glassy carbon microparticles 0.4–12 μm type 2 (Alfa Aesar, USA) and 0.1 ml of mineral oil Nujol. The paste was carefully hand-mixed for about 30 min in a mortar.

The mobile phase used for HPLC of DOX contained methanol (p.a., Lach-Ner Ltd., Czech Republic) and 0.01 M phosphate buffer. A 0.01 M phosphate buffer (pH 5.0) consisted of sodium hydrogenphosphate whose pH was adjusted with concentrated phosphoric acid

(both Lachema Brno, Czech Republic). All solutions were prepared using deionized water (Millipore Q-plus System, Millipore, USA).

Apparatus

Voltammograms were obtained with an Eco-Tribo polarograph controlled by PolarPro version 4 software (Polaro-Sensors, Prague, Czech Republic) working under Windows 95 (Microsoft). A three-electrode system consisting of working carbon paste electrode (Development workshop, University of Pardubice, Czech Republic), Ag|AgCl, 1 M KCl reference electrode and a platinum wire auxiliary electrode (both Monokrystaly Turnov, Czech Republic) was used. The instrumental parameters were set as follows: applied potential range from 0 to +1 200 mV; scan rate 10 mV s^{-1}; pulse amplitude +50 mV, pulse width 80 ms.

The HPLC system consisted of a high-pressure pump HPP 5001, injector valve CI-30 (both Laboratorní přístroje Praha, Czech Republic) with a 0.01 ml loop and Kromasil 100-7 μm C18 column, 250 × 4.6 mm ID (Prochrome, India). UV/Vis detector LCD 2040, amperometric detector ADLC 1 (both Laboratorní přístroje Praha, Czech Republic) and software CSW 32 (DataApex Ltd.) working under Windows 98 (Microsoft) were used.

A Model 4330 Conductivity/pH Meter (Jenway Ltd., UK) fitted with the combined glass electrode (Ag|AgCl|3 M KCl) (type 924 005) was employed to measure pH of the solutions.

Procedures

The techniques used for voltammetric determination of DOX were direct current voltammetry (DCV) differential pulse voltammetry (DPV) and their adsorptive stripping versions (DCAdSV, DPAdSV). DCV and DPV measurements were performed in an unstirred and not de-aerated BR buffer at a laboratory temperature. Unless otherwise stated, to an appropriate amount of the DOX stock solution in a volumetric flask, deionized water was added to the total volume 1 ml and the solution was filled up to 10 ml with BR buffer. The resulting solution was transferred into the voltammetric cell. For optimization of the accumulation conditions, a 2×10^{-7} M DOX was used in DCAdSV and a 2×10^{-8} M DOX in DPAdSV measurements. The accumulations were performed in stirred BR buffer solutions at pH 7.0.

The mobile phase for HPLC measurement was prepared by mixing an appropriate amount of phosphate buffer and methanol. Solutions of DOX to be measured were prepared by diluting the original Doxorubicin formulation Ebewe with the mobile phase. The flow rate was 1 ml min^{-1}. The UV detection wavelength was 240 nm, 10 μl of the sample was injected. Calibration dependences were evaluated by linear regression. The detection limits were calculated as the concentration of an analyte which gave a signal three times higher than the background noise (S/N = 3). This approach was used also for voltammetric methods, as the noise at high sensitivities was easy to evaluate (see the figures, no smoothing function was used).

RESULTS AND DISCUSSION

Voltammetric Determination

First, the influence of pH on DOX behavior in BR buffer was studied. The dependence of the peak potential on pH was described using linear regression as E_p (mV) = –68.5 pH + 925.5 (mV) (correlation coefficient 0.99) for DCV and E_p (mV) = –72.1 pH + 893.5 (mV) (correlation coefficient 0.99) for DPV. The position of the oxidation peak moved to lower potentials and the peak height increased with increasing pH of the supporting electrolyte. BR buffer (pH 7.0) was chosen for the measurement of concentration dependences. In this medium, the peak was the highest and the peak shape was the most symmetric and easy to evaluate. Because of the high degree of DOX extraction into the bulk of the carbon paste, it was always necessary to remove more carbon paste than usually when mechanically resurfacing the CPE. Therefore, the carbon paste inside the electrode body was soon consumed and each concentration range was measured with newly prepared carbon paste.

Between the measurements of different concentrations of DOX, a voltammogram of the supporting electrolyte was measured to prove the absence of DOX in the bulk of the carbon paste. When a voltammogram of DOX was recorded, the CPE was resurfaced again. The limit of detection obtained with DCV technique was 8×10^{-7} mol l^{-1} (Fig. 2) and the calibration line was linear within the concentration range 1×10^{-4}–8×10^{-7} mol l^{-1}. At concentrations lower than 6×10^{-8} mol l^{-1}, it was difficult to evaluate the DP voltammograms (Fig. 3). They were evaluated as a peak by linking the minima on both sides of the peak by a straight line and measuring the peak height from this straight line. The slopes of calibration curves were different for different orders of magnitudes of concentrations. Subsequently, the non-linear shape of calibration curve (in DPV) in the whole concentration range was obtained. This shape was obviously obtained due to a very strong adsorption of DOX on the electrode surface and its extraction into the bulk of the carbon paste. Thereby, it was necessary to thoroughly renew the electrode surface between measurements.

The adsorptive properties of DOX were utilized in adsorptive stripping technique. The influence of the accumulation time of DOX on the CPE surface was investigated (Fig. 4). This dependence was measured for 2×10^{-6} M (DCAdSV) and 2×10^{-7} M (DPAdSV) stirred solutions of DOX in BR buffer (pH 7.0). For DCAdSV measurements, the accumulation time 120 s was chosen and the limit of detection 2.2×10^{-7} mol l^{-1} was obtained. The accu-

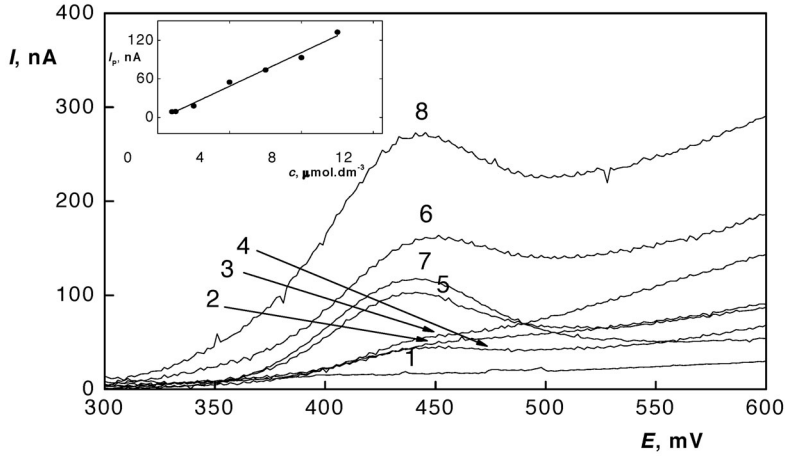

FIG. 2

DC voltammograms of DOX at CPE in BR buffer (pH 7.0); c(DOX) = 0 (1), 0.8×10^{-6} (2), 1×10^{-6} (3), 2×10^{-6} (4), 4×10^{-6} (5), 6×10^{-6} (6), 8×10^{-6} (7), 10×10^{-6} (8) mol l^{-1}. The calibration line is in the inset

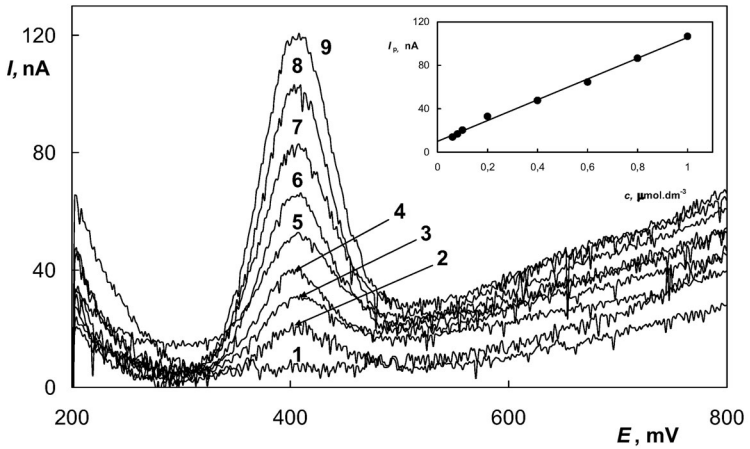

FIG. 3

DP voltammograms of DOX at CPE in BR buffer (pH 7.0); c(DOX) = 0 (1), 0.6×10^{-7} (2), 0.8×10^{-7} (3), 1×10^{-7} (4), 2×10^{-7} (5), 4×10^{-7} (6), 6×10^{-7} (7), 8×10^{-7} (8), 10×10^{-7} (9) mol l^{-1}. The calibration line is in the inset

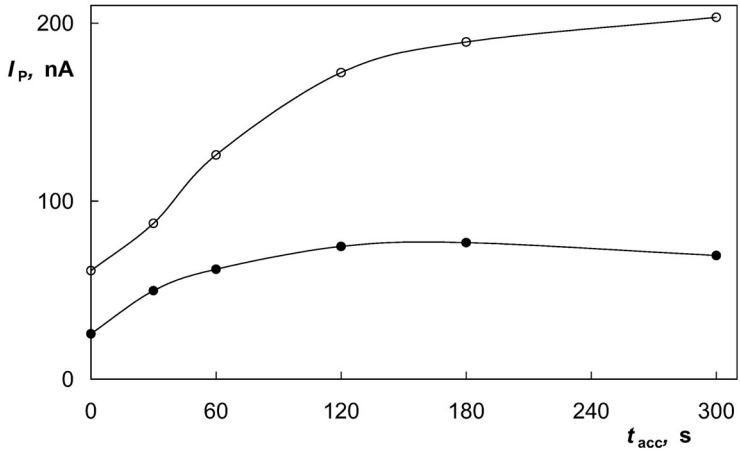

FIG. 4
The dependence of DCV (●) and DPV (○) peak heights (I_p) of DOX on accumulation time in stirred BR buffer (pH 7.0). Concentrations of DOX 2×10^{-6} mol l^{-1} (DCAdSV) and 2×10^{-7} mol l^{-1} (DPAdSV)

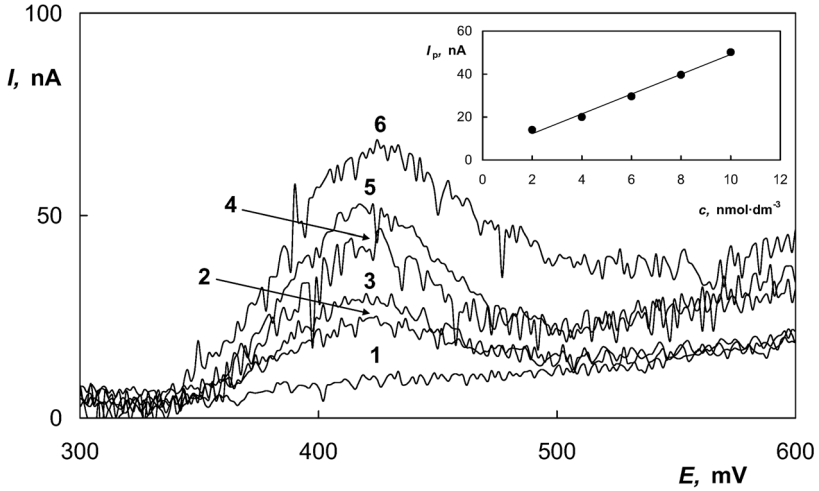

FIG. 5
DPAdS voltammograms of DOX at CPE in BR buffer (pH 7.0); c(DOX) = 0 (1), 2×10^{-9} (2), 4×10^{-9} (3), 6×10^{-9} (4), 8×10^{-9} (5), 10×10^{-9} (6) mol l^{-1}. Accumulation time 120 s in stirred solution. The calibration line is in the inset

mulation times 60 and 120 s were chosen as optimal for DPAdSV measurements. The accumulation time 60 s was used, because the peak height was already twice as high in comparison with 0 s accumulation measurements. The accumulation time of 120 s was tested just for the comparison to DCAdSV method. The limit of detection 2×10^{-8} mol l^{-1} was obtained using the accumulation time 60 s. The accumulation time 120 s provided the limit of detection 2×10^{-9} mol l^{-1} (Fig. 5). A comparison of concentration ranges and limits of detection for the methods developed in this study are shown in Table I.

HPLC with UV and Amperometric Detection

To optimize the separation conditions of HPLC determination of DOX, the mobile phase described in literature[34] was used at first. To speed up the separation, the content of methanol was increased from 65 to 85% (v/v). The retention times and retention factors are shown in Fig. 6. The mobile phase containing 75% of methanol was chosen for the measurements. In the mobile phase containing more than 75% of methanol, the retention time of DOX was too close to column dead-time. Carbon paste made from glassy carbon microparticles and Nujol was used because of the high content of methanol in the mobile phase (methanol–0.01 M phosphate buffer (pH 5.0) 75:35 (v/v))[33]. The carbon paste based on carbon powder CR-2 and Nujol degraded in the presence of organic solvents (e.g. methanol, acetonitrile) very fast which resulted in a high noise of the baseline. Hence, carbon

TABLE I
The comparison of concentration ranges and limits of detection for the developed methods of DOX determination

Method	Concentration range mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	Correlation coefficient	L_D mol l^{-1}
DCV	1×10^{-4}–8×10^{-7}	13.09	–3.9	0.995	8×10^{-7}
DPV	1×10^{-4}–6×10^{-8}	n.l.[a]	n.l.	n.l.	6×10^{-8}
DCAdSV	2×10^{-6}–2×10^{-7}	68.26	7.5	0.997	2.2×10^{-7}
DPAdSV (t_{acc} 60 s)	2×10^{-7}–2×10^{-8}	6.72×10^{2}	5.5	0.996	2.7×10^{-8}
DPAdSV (t_{acc} 120 s)	2×10^{-8}–2×10^{-9}	4.59×10^{3}	3.1	0.996	2.8×10^{-9}

[a] n.l., non-linear calibration dependence.

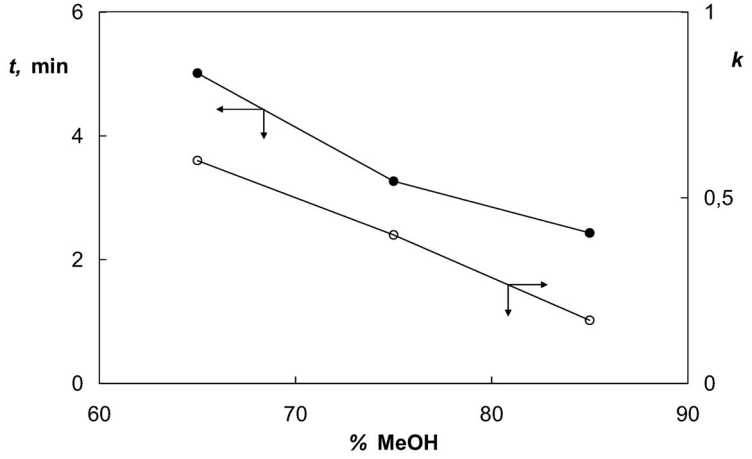

FIG. 6

The dependence of retention time t_r (●) and retention factor k (○) of DOX on methanol content in the mobile phase methanol–0.01 M phosphate buffer (pH 5.0). UV detection at 240 nm; Kromasil 100-7 μm C18 column, 250 × 4.6 mm ID; flow rate 1 ml min^{-1}; injected 0.01 ml of 1×10^{-4} M solution of DOX in mobile phase

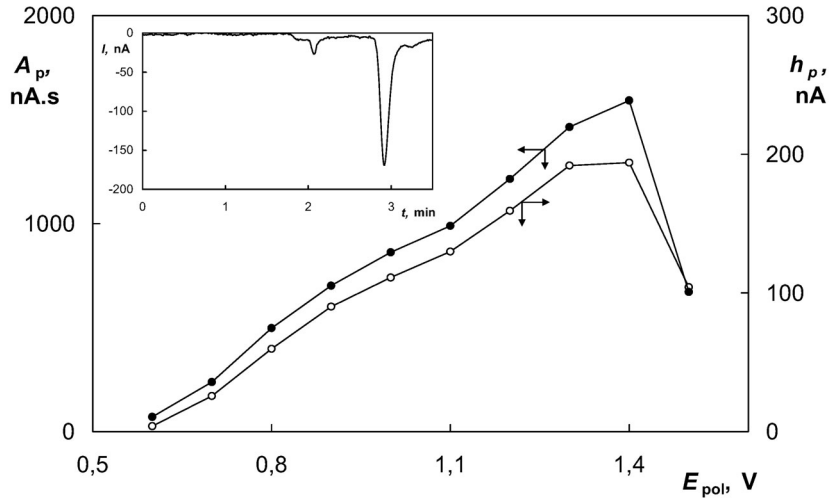

FIG. 7

Hydrodynamic voltammograms of DOX and the chromatogram of DOX using detection potential E_{det} = +1.2 V (inset); mobile phase 0.01 M phosphate buffer (pH 5.0)–methanol 25:75 (v/v); Kromasil 100-7 μm C18 column, 250 × 4.6 mm ID; flow rate 1 ml min^{-1}; injected 0.01 ml of 1×10^{-4} M solution of DOX in mobile phase

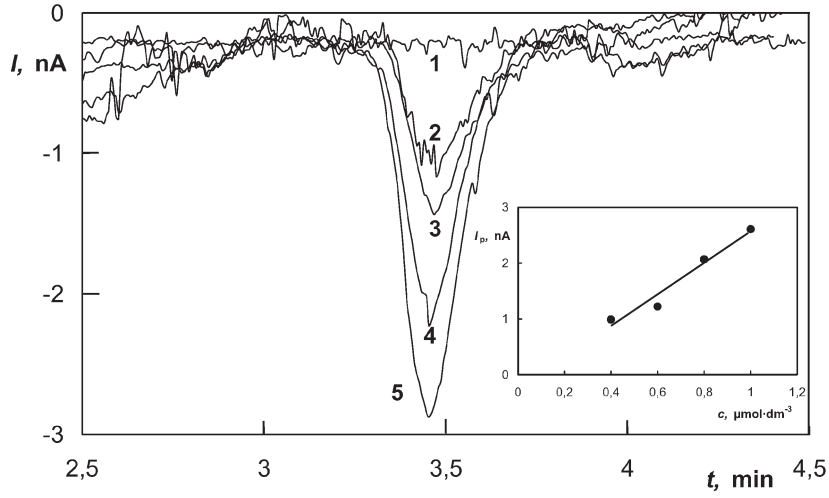

FIG. 8

HPLC-ED chromatograms of DOX. Kromasil 100-7 μm C18 column, 250 × 4.6 mm ID; mobile phase methanol–0.01 M phosphate buffer (pH 5.0) 75:25 (v/v); flow rate 1 ml min^{-1}; E_{det} = +1.2 V; 0.01 ml sample; c(DOX) = 0 (1), 4 × 10^{-7} (2), 6 × 10^{-7} (3), 8 × 10^{-7} (4), 10 × 10^{-7} (5) mol l^{-1}. Glassy carbon microparticles based paste. The calibration line is in the inset

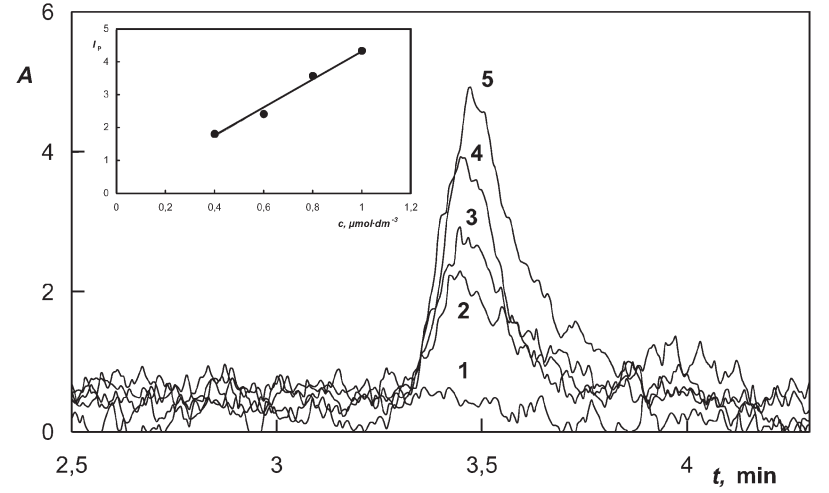

FIG. 9

HPLC-UV chromatograms of DOX. Kromasil 100-7 μm C18 column, 250 × 4.6 mm ID; mobile phase methanol–0.01 M phosphate buffer (pH 5.0) 75:25 (v/v); flow rate 1 ml min^{-1}; 0.01 ml sample; c(DOX) = 0 (1), 4 × 10^{-7} (2), 6 × 10^{-7} (3), 8 × 10^{-7} (4), 10 × 10^{-7} (5) mol l^{-1}. UV detection at 240 nm. The calibration line is in the inset

pastes based on glassy carbon microparticles were used for further measurements. The hydrodynamic voltammograms of DOX were measured in the potential range from +0.6 to +1.5 V. 0.01 ml of 1×10^{-4} M solution of DOX in mobile phase was injected. The potential of working electrode +1.2 V was chosen as optimal because of the highest signal to noise ratio (Fig. 7).

The concentration dependences were measured under optimized conditions (flow rate 1 ml min^{-1}, detection potential 1.2 V, mobile phase with 75% of methanol). DOX was determined in the concentration range 4×10^{-7}–1×10^{-4} mol l^{-1}. The chromatograms of DOX in the lowest concentration range measured are shown in Figs 8 and 9. The calibration curve was linear in the whole concentration range. The calibration line parameters were calculated by linear regression and are given in Table II. The results

TABLE II
Parameters of calibration lines of DOX; HPLC with electrochemical (EC) and spectrophotometric (UV) detection

Detection	Concentration range mol l^{-1}	Slope mA mol^{-1} l mAU mol^{-1} l	Correlation coefficient	L_D mol l^{-1}
EC	4×10^{-7}–1×10^{-4}	1.72 ± 0.03	0.997	2×10^{-7}
UV	4×10^{-7}–1×10^{-4}	4.35 ± 0.09	0.999	2.5×10^{-7}

TABLE III
Determination of DOX in the Ebewe drug by DPV, HPLC-ED and HPLC-UV. The values are means of five determinations

DOX taken µg	DOX found, DPV		DOX found, HPLC-ED		DOX found, HPLC-UV	
	µg	%	µg	%	µg	%
4	3.79	94.8	3.91	97.9	3.97	99.3
10	10.11	101.1	10.41	104.1	10.36	103.6
20	20.17	100.9	20.31	101.6	19.93	99.6
100	100.50	100.5	91.32	91.3	99.79	99.8
200	192.83	96.4	203.56	101.8	199.84	99.9
300	295.29	98.4	295.93	98.6	300.12	100.0
600	594.64	99.1	593.56	98.9	598.85	99.8

obtained by HPLC with electrochemical detection (HPLC-ED) of DOX were comparable with those obtained by HPLC with UV detection (HPLC-UV). The peaks of DOX concentrations lower than 1×10^{-6} mol l^{-1} measured by HPLC-UV suffered from lower *S*/*N* ratios compared with those in amperometric detection.

Finally, the newly developed DPV and HPLC-ED methods of DOX determination (and the classic HPLC-UV method) were tested using Ebewe drug samples and compared. The obtained results are shown in Table III. The relative standard deviations for DOX determination by DPV ranged from 0.5 to 4.5%, by HPLC-ED from 8.2 to 11.5%, and using HPLC-UV from 0.6 to 3.8%. There were no significant differences between the methods. Hence, the new methods present an independent alternative to determination of DOX in pharmaceutical drug forms. A high relative standard deviation for DPV is associated with a very high adsorbability of DOX on CPE. The possibility of utilizing an increased sensitivity and selectivity of the developed methods for DOX determination in biological samples (blood, urine) is under investigation.

CONCLUSION

Carbon paste electrodes are suitable for the determination of trace amounts of DOX. The limits of detection were improved by accumulation of the analyte on the surface (adsorption) and in the volume (extraction) of the carbon paste. HPLC with amperometric detection at carbon paste electrode can be used for the determination of submicromolar concentrations of DOX. It was shown that electrochemical detection of DOX with carbon paste electrode is more sensitive than common UV detection at 240 nm. The previously described method of electrochemical detection of DOX [35] by HPLC utilizing commercial detectors with glassy carbon electrodes report even slightly lower detection limits for DOX and these are in agreement with carbon paste electrode well below the detection limits of classical UV detection. However, the main advantage of CPE is its easy renewable surface, which eliminates most problems with working electrode passivation. The newly developed methods for DOX determination were tested on a DOX drugs form.

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REFERENCES

1. Arcamone F., Cassinelli G., Fantini G., Grein A., Orezzi P., Pol C., Spalla C.: *Biotechnol. Bioeng.* **1969**, *11*, 1101.
2. Murdock K. C., Child R. G., Fabio P. F., Angier R. B., Walace R. E.: *J. Med. Chem.* **1979**, *22*, 1024.
3. Abdel-Hamid M. E., Sharma D.: *J. Liq. Chromatogr., Relat. Tech.* **2004**, *27*, 641.
4. Arnold R. D., Slack J. E., Straubinger R. M.: *J. Chromatogr., B: Biomed. Appl.* **2004**, *808*, 141.
5. Sottani C., Tranfo G., Bettinelli M., Faranda P., Spagnoli M., Minoia C.: *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2426.
6. Pierce R. N.: *J. Chromatogr.* **1979**, *164*, 471.
7. Fraier D., Frigerio E., Pianezzola E., Benedetti M. S., Cassidy J., Vasey P.: *J. Pharm. Biomed. Anal.* **1995**, *13* , 625.
8. Liu Y., Danielsson B.: *Anal. Chim. Acta* **2007**, *587*, 4.
9. Sepaniak M. J.: *J. Chromatogr.* **1980**, *190*, 377.
10. Hempel G., Haberland S., Schulze-Westhoff P., Möhling N., Blaschke G., Boos J.: *J. Chromatogr., B: Biomed. Appl.* **1997**, 698, 287.
11. Hempel G., Schulze-Westhoff P., Flege S., Laubrock N., Boos J.: *Electrophoresis* **1998**, *19*, 2939.
12. Sternson L. A.: *Anal. Lett.* **1977**, *10*, 99.
13. Baldwin R. P., Packett D., Woodcock T. M.: *Anal. Chem.* **1981**, *53*, 540.
14. Chaney E. N., Baldwin R. P.: *Anal. Chem.* **1982**, *54*, 2556.
15. Chaney E. N., Baldwin R. P.: *Anal. Chim. Acta* **1985**, *176*, 105.
16. Kano K., Konse T., Nishimura N., Kubota T.: *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2383.
17. Kano K., Konse T., Kubota T.: *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1879.
18. Kano K., Konse T., Kubota T.: *Bull. Chem. Soc. Jpn.* **1985**, *58*, 424.
19. Hu J. B., Li Q. L.: *Anal. Sci.* **1999**, *15*, 1215.
20. Lindholm-Sethson B., Geladi P., Nelson A: *Anal. Chim. Acta* **2001**, *446*, 121.
21. Abd El-Hady D., Abdel-Hamid M. I., Seliem M. M., El-Maali N. A.: *Arch. Pharm. Res.* **2004**, *27*, 1161.
22. Abd El-Hady D., Abdel-Hamid M. I., Seliem M. M., El-Maali N. A.: *Talanta* **2005**, *66*, 1207.
23. Yau H. C. M., Chan H. L., Yang M. S.: *Biosens. Bioelectron.* **2003**, *18*, 873.
24. Uslu B., Ozkan S. A.: *Anal. Lett.* **2007**, *40*, 817.
25. Patriarche G. J., Viré J.-C.: *Anal. Chim. Acta* **1987**, *196*, 193.
26. Wang J., Freiha B. A., Deshmukh K. B.: *Biochem. Bioeng.* **1985**, *14*, 457.
27. Wang J., Freiha B. A.: *Anal. Chem.* **1983**, *55*, 1258.
28. Wang J.: *Analytical Electrochemistry*, 2nd ed. Wiley–VCH, New York 2000.
29. Zima J., Dejmkova H., Barek J.: *Electroanalysis* **2007**, *19*, 185.
30. Ibrahim H.: *J. Pharm. Biomed. Anal.* **2005**, *38*, 624.
31. Issa Y. M., Ibrahim H., Abu-Shawish H. M.: *Microchim. Acta* **2005**, *150*, 47.
32. Barek J., Fischer J., Navratil T., Peckova K., Yosypchuk B., Zima J.: *Electroanalysis* **2007**, *19*, 2003.
33. Barek J., Muck A., Wang J., Zima J.: *Sensors* **2004**, *4*, 47.
34. Alvarez-Cedron L., Sayalero M. L., Lanao J. M.: *J. Chromatogr., B: Biomed. Appl.* **1999**, *721*, 271.
35. Kotake A. N., Vogelzang N. J., Larson R. A., Choporis N.: *J. Chromatogr.* **1985**, *337*, 194.