DETERMINATION OF CHLORAMPHENICOL BY DIFFERENTIAL PULSE VOLTAMMETRY AT CARBON PASTE ELECTRODES – THE USE OF SODIUM SULFITE FOR REMOVAL OF OXYGEN FROM ELECTRODE SURFACE

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Dedicated to Professor Robert Kalvoda on the ocassion of his 85 birthday.

The possibility of determination of chloramphenicol by differential pulse voltammetry at four different carbon paste electrodes, in the full pH range (2–12) of Britton–Robinson (BR) buffer was investigated. Electrodes were prepared by mixing spectroscopic graphite powder or glassy carbon microbeads with mineral oil (Nujol) or tricresyl phosphate. Under optimal conditions (BR buffer pH 12, the electrode prepared from glassy carbon microbeads and tricresyl phosphate), linear calibration graph was obtained only in 10^{-5} M chloramphenicol concentration range. Determination of lower concentrations of chloramphenicol was complicated by irreproducible peak of oxygen from the carbon paste which overlapped with peak of chloramphenicol. Addition of sodium sulfite removed the oxygen peak without influence on the peak of chloramphenicol. Under optimal conditions (electrode paste made from glassy carbon microbeads, BR buffer pH 10 and 0.5 M sodium sulfite), straight calibration line was obtained in the 10^{-6} and 10^{-5} M chloramphenicol concentration range. Limit of determination was 5×10^{-7} mol/l.

Keywords: Chloramphenicol; Carbon paste electrodes; Oxygen peak; Oxygen removal; Sodium sulfite; Differential pulse voltammetry.

Chloramphenicol (CAP; Fig. 1) is a broad-spectrum bacteriostatic antibiotic effective against a wide variety of Gram-positive and Gram-negative bacteria including most of anaerobes. It binds to the 50s subunit of bacterial ribosome and act as inhibitor of protein synthesis[1]. Recommended peak levels in plasma, 1 h after the dose is given, is 15–25 mg/l ($5–8 \times 10^{-5}$ mol/l).

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In the veterinary practice in USA usage of chloramphenicol is restricted to non-food-producing animals. Isolation of bacteria, which are resistant to chloramphenicol from food-producing animals, indicates probable application of commercially available chloramphenicol, as an oral preparation for treatment of canine infection[2]. The usual therapeutic concentration range in animal serum is 5–15 mg/l ($1.7–5 \times 10^{-5}$ mol/l)[3].

There is a great number of papers devoted to chloramphenicol determination. Most of them are based on the use of HPLC, recently mostly with MS-MS detection[4–6] but UV-Vis detection is still in use[7,8]. Gas chromatography was also used mostly with MS detection[9,10]. Direct UV spectrophotometric[11,12] as well as spectrophotometric and colorimetric methods for CAP determination after chemical derivatization[13] and derivative spectrophotometric techniques[14] were also developed. About one quarter of published methods for chloramphenicol determination are electroanalytical methods. They are based on cathodic reduction of the present nitro group (there is no functional group in the molecule of CAP which can be anodically oxidized). Therefore, electroanalytical methods vary from DC polarography at a dropping mercury electrode[15] over differential pulse polarography[16], differential pulse and square wave voltammetries at different types of electrodes, e.g. carbon fiber microelectrodes[17], glassy carbon electrodes[18], up to the single-wall carbon nanotube-gold nanoparticle-ionic liquid composite film modified glassy carbon electrode[19]. Among those papers, to the best of our knowledge, there is no one concerning application of carbon paste electrodes (CPE) for determination of CAP, probably because chloramphenicol can only be cathodically reduced at negative potentials while CPE are usually applied in the positive potential range for anodic oxidations. Reason for that is the presence of oxygen in the paste (probably adsorbed on the carbon particles)[20]. Because of that, the removal of oxygen from solution by purging of nitrogen did not completely eliminate the peak(s) corresponding to the reduction of oxygen. Another problem is low reproducibility of the height and position of oxygen peak(s), which reduce reproducibility of determinations in negative potential range and cause increase of limits of de-

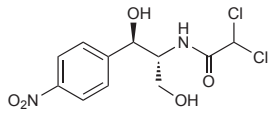

FIG. 1
Structural formula of Chloramphenicol

tection and determination. Therefore, in this paper attention is paid to the removal of negative influence of oxygen on voltammetric determination of CAP. Several types of carbon particles and pasting liquids were compared from this point of view. The carbon/binder ratio, which can be important in optimization process[24], was not investigated in this pilot study.

EXPERIMENTAL

Apparatus

Potentiostat Eco-Tribo-Polarograph (Eco-Trend Plus, Prague, Czech Republic) was used. The carbon paste working electrode body was made of Teflon with a 2-mm inner diameter. The contact was established via a stainless steel screw which also served to extrude the new paste from the electrode body[21]. Reference Ag|AgCl (3 M KCl) electrode and auxiliary platinum plate electrode were used.

Chemicals and Solutions

All reagents were of analytical grade. Chloramphenicol (≥99% (HPLC)) was supplied by Fluka. Before preparation of the stock solution (1.00×10^{-3} M CAP in deionized water (Mili-Q_{plus} system, Milipore, USA)), it was dried over silica gel for 30 days. Britton–Robinson (BR) buffers were prepared in a usual way by titration of acidic solution (0.040 M phosphoric, acetic and boric acids) with 0.2 M sodium hydroxide. Anhydrous sodium sulfite was supplied by Reanal (A.R., 14101, Budapest, Hungary). Spectroscopic graphite powder (2 μm, CR 2, Maziva, Týn nad Vltavou, Czech Republic), glassy carbon microbeads (0.4–12 microns, type 2, 038008 Alfa Aesar, Germany), mineral oil (Nujol, Ultra, for molecular biology, 69794 Fluka, USA), and tricresyl phosphate (pract., Fluka, Switzerland) were used for the preparation of carbon paste electrodes. For all experiments, deionized water was used.

Procedures

All four used carbon pastes were produced in the same way by thorough mixing of 500 mg of spectroscopic graphite powder or glassy carbon spherical microbeads with 200 μl of mineral oil (Nujol) or tricresyl phosphate by the porcelain mortar and pestle.

For differential pulse voltammetry (DPV), the scan rate 20 mV/s, pulse amplitude –50 mV, pulse duration 100 ms, pause between pulses 50 ms, and current sampling during last 20 ms of pulse and pause were used.

DPV measurements were done in solutions obtained by mixing of 8 ml of BR buffer of chosen pH, required volume of 1×10^{-3} M stock solution of CAP and water up to the final volume of 10 ml. When the measurements were done at renewed surface of carbon paste electrode, renewing was done immediately before measurement by wiping the surface on regular printing paper, after extrusion of small amount of paste by screw rotation. After that, electrode was immersed into solution, moved through it for a few seconds to remove air bubbles from its surface, and then a measurement was started. When sodium sulfite was not used, oxygen was removed from solution by purging with nitrogen for 5 min. For final concentration of sodium sulfite of 0.1 mol/l, 1 ml of 1.0 M stock solution was used. For

higher final concentrations (0.25–1.0 mol/l) required amount of solid Na_2SO_3 was added (0.32–1.26 g) and the solution was stirred by glass rod until all salt was dissolved (between 1 and 2 min, depending on the amount of Na_2SO_3). Saturated solution was prepared by addition of excess of salt (3.5 g in 10 ml of solution) and stirring for 2 min. Excess of sodium sulfite remained at the bottom of voltammetric vessel.

For higher concentrations of CAP ((2–10) × 10^{-5} mol/l) peak heights were measured in a common way, i.e. from tangent to the voltammogram joining beginning and the end of the peak, which was ca. 150 mV before and after the peak maximum. For lower concentrations (micromolar), peak heights were measured from the straight line connecting the points at the voltammograms 150 mV before and after the peak maximum. All voltammograms were recorded four times. For limits of detection (LOD), the standard deviation of intercept *s* and slope *b*, obtained from the linear regression analysis of calibration graph calculated by OriginPro 7.5 program (OriginLab Corporation, USA), were used. LOD was calculated as $3s/b$ [22].

RESULTS AND DISCUSSION

According to Švancara and Vytřas[20], position and intensity of oxygen peak are strongly dependent on the composition of carbon paste and may be practically absent at the paste of graphite powder and tricresyl phosphate.

In order to find the optimal conditions for determination of CAP at the carbon paste electrodes by DPV, we compared voltammograms of 1 × 10^{-4} M CAP at four different carbon pastes. The pastes were prepared by mixing spectroscopic graphite powder (S) or glassy carbon spherical microbeads (G) with mineral oil (N; Nujol) or tricresyl phosphate (T). Britton–Robinson buffers were used as supporting electrolytes. Measurements were done at renewed electrode surface.

Obtained results are given in Table I. Background current is measured at the mean value of the potentials of the maxima of the CAP peak. It can be seen that at all four investigated carbon paste electrodes, peak of the CAP moves to the lower potentials with increasing pH of BR buffer. At the ST-CPE that changes stop at pH 10 and at the GT-CPE at pH 4. At all four pastes, current of the peak maximum and also background current at the potential of the peak maximum increase with increasing pH. The shape and position of oxygen peak can be seen at the voltammograms of supporting electrolyte (Figs 2 and 3). Signal of oxygen has a shape of distorted peak, which sometimes resembles a wave, the position of which moves towards more positive potentials with increasing pH.

At the SN-CPE, there is no overlapping of the peak of CAP with oxygen wave at all used pHs except most acidic one. Unfortunately, the background current is very high and the reproducibility of the peak height in

TABLE I
Peak heights (I_p), background currents (I_B) and peak potentials (E_p) for DPV of 1×10^{-4} M CAP in BR buffer of various pH at four investigated CPEs. Oxygen was removed by nitrogen bubbling for 5 min

pH of BR buffer	$I_p \pm SD^a$ (nA)	$I_B \pm SD^{a,b}$ (nA)	$E_p \pm SD^a$ (mV)
		SN-CPE	
1.9	–1810 ± 260	–810 ±280	–363 ± 10
7.3	–2940 ± 1310	–12100 ± 400	–641 ± 0
10.5	–1360 ± 110	–15800 ± 1300	–678 ± 4
12.4	–1536 ± 92	–16400 ± 300	–706 ± 12
		GN-CPE	
1.9	–1395 ± 46	–420 ± 120	–457 ± 10
4	–1950 ± 220	–716 ± 75	–528 ± 19
6	–3200 ± 230	–1120 ± 72	–563 ± 7
8	–2110 ± 300	–1102 ± 88	–609 ± 2
10	–1260 ± 140	–1300 ± 220	–636 ± 6
12	–866 ± 90	–1490 ± 400	–655 ± 5
		ST-CPE	
1.9	–1940 ± 170	–2980 ± 210	–441 ± 8
4	–2320 ± 260	–1560 ± 150	–536 ± 9
6	–2145 ± 70	–2140 ± 180	–610 ± 4
8	–1830 ± 180	–3530 ± 560	–656 ± 3
10	–1320 ± 150	–3890 ± 470	–664 ± 0
12	–1590 ± 160	–6690 ± 500	–664 ± 0
		GT-CPE	
1.9	–362 ± 75	–381 ± 24	–625 ± 7
4	–465 ± 75	–416 ± 55	–681 ± 16
6	–630 ± 270	–600 ± 120	–719 ± 27
8	–480 ± 210	–728 ± 58	–734 ± 29
10	–652 ± 68	–900 ± 120	–687 ± 7
12	–730 ± 38	–1135 ± 85	–682 ± 4
0.5 M HCl[c]	–671 ± 89	–570 ± 120	–473 ± 6
2 M HCl[c]	–409 ± 39	–620 ± 150	–478 ± 0

[a] Mean values and standard deviations from four determinations. [b] Background current measured at the mean value of the potentials of E_p. [c] HCl was used instead of BR buffer

BR buffer of pH 7.3, where the height of the CAP peak is maximal, is very low. This paste is thus not suitable for the determination of CAP.

At ST-CPE, peak of CAP almost does not coincide with oxygen signal at pH 6, and the two peaks are completely separated at pH 8 and higher. From the point of view of peak height and its standard deviation, BR buffer at pH 7 seems to be the best choice for CAP determination at ST-CPE.

GN-CPE has the best ratio between the peak height and background current. The CAP peak is the highest at pH 6 but at pH 10 there is no coincidence with the oxygen peak. Therefore, we have further investigated the possibility of the determination of CAP at both pH values.

Residual current is the smallest at GT-CPE but CAP gives here the smallest peaks. Here the CAP peak and oxygen wave overlap to some extent at all

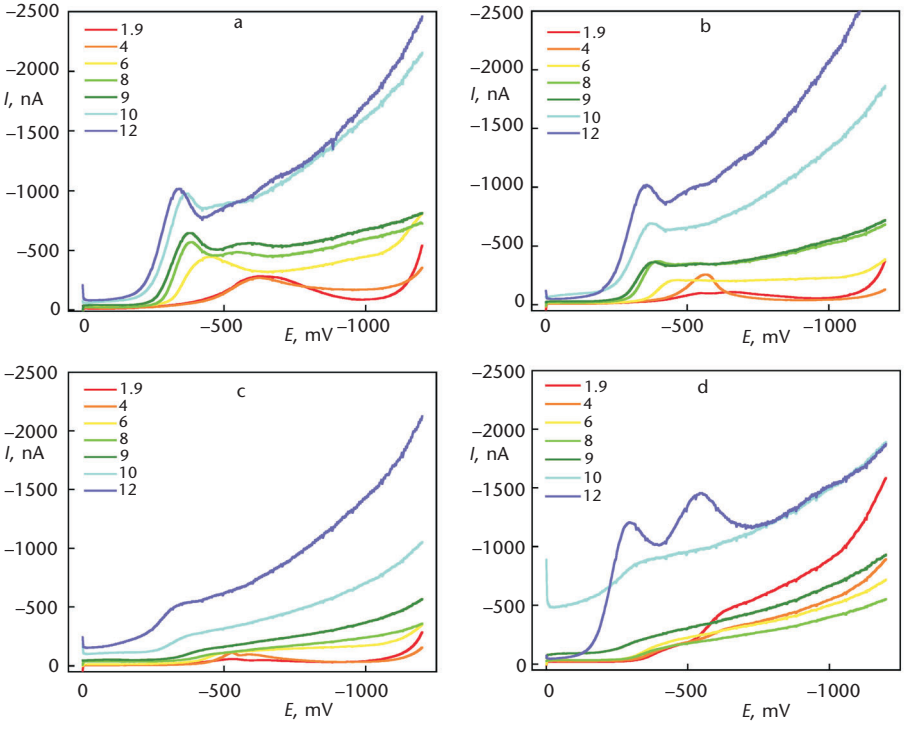

FIG. 2

Peak of oxygen (a) and its reduction by bubbling the solution with nitrogen for 5 min (b), by keeping the electrode surface in a stream of nitrogen bubbles for 5 min (c) and by addition of 1 M Na_2SO_3 (d). Voltammograms recorded in various pHs of BR buffer (assigned by line colors) by DPV at SN-CPE at scan rate 20 mV/s

examined pH. At other three investigated pastes that overlapping is absent at high pH values because of shifting of the oxygen peak to higher and CAP peak to the lower potential. Attempt to avoid that overlapping in highly acidic media, 0.5 and 2.0 M hydrochloric acid, was not successful. In 2 M HCl, oxygen wave is very small but the peak of CAP broadened and for 1×10^{-4} M CAP it is more than 550 mV wide. At this electrode, the CAP peak is highest at pH 12 where reproducibility is also best.

Calibration straight line for DPV determination of CAP at ST-CPE in BR buffer at pH 7 in 10^{-5} mol/l concentration range gives acceptable correlation coefficient (R = –0.982), but the variation of peak heights was high and similar at smallest and highest concentrations and because of that limits of detection and determination are too high.

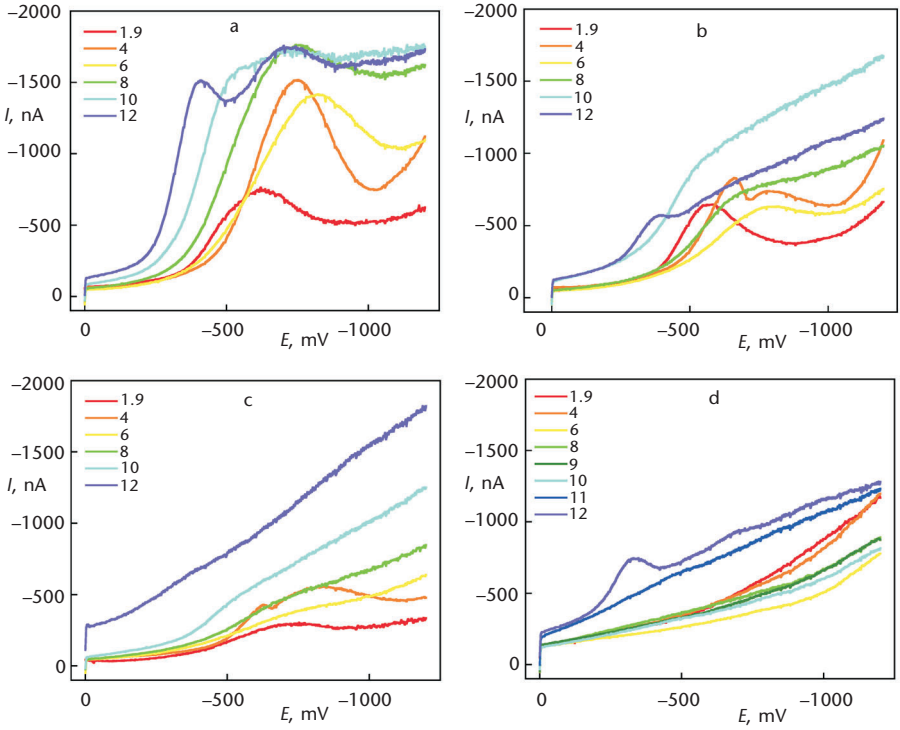

FIG. 3

Peak of oxygen (a) and its reduction by bubbling the solution with nitrogen for 5 min (b), by keeping the electrode surface in a stream of nitrogen bubbles for 5 min (c) and by addition of 1 M Na_2SO_3 (d). Voltammograms recorded in various pHs of BR buffer (assigned by line colors) by DPV at GT-CPE at scan rate 20 mV/s

At GN-CPE the highest peak for 1×10^{-4} M CAP was obtained in BR buffer of pH 6, but peak at pH 8 overlaps with peak of oxygen to a smaller extent, so calibration curve was constructed for 10^{-5} mol/l concentration range at pH 7. The correlation coefficient was not too good (R = –0.942) and reproducibility of five successive measurements at every concentration was rather low (RSD up to 18.5% in 8×10^{-5} M CAP).

At determined optimal condition for GT-CPE (BR buffer at pH 12), the correlation coefficient of calibration straight line for 10^{-6} mol/l concentration range was R = –0.877 and for 10^{-5} mol/l range R = –0.988.

Because of not too good results obtained, especially low reproducibility, whose probable cause was presence of irreproducible wave of oxygen from the electrode paste in the potential area where reduction of CAP occurs, we started to search for a convenient method to eliminate oxygen wave.

Sodium sulfite is well known means for removal of oxygen dissolved in water solutions. It was introduced in polarographic practice 80 years ago by Varasova[23]. Nowadays Na_2SO_3 is practically out of use in voltammetry for oxygen removal, it can not be used in acidic pH range, hydrolysis of sodium sulfite can change the pH of solution, it is relatively a strong reductant and can reduce determined substances, etc. However, if that disadvantages does not prevent voltammetric determination, the usage of sodium sulfite can have some advantages over nitrogen bubbling because it can easily be used for field measurements where nitrogen bubbling is difficult.

Therefore, we investigated the possibility of sodium sulfite to remove not only oxygen dissolved in water but also oxygen from the carbon paste present at the electrode surface. We have used GN-CPE whose surface was freshly renewed before each measurement. Figure 4 shows influence of the presence of 1 M sodium sulfite in BR buffer of pH 10 on the height of peak of oxygen at GN-CPE. It can be seen that in the presence of sulfite, background current is close to the base line of the peak. (Peaks around –1 V on curves *5* and *3* can be connected with a two-step reduction of nitro group in CAP molecule.)

In order to examine stability of chloramphenicol in the presence of high concentrations of sodium sulfite, we were following the peak height of 2×10^{-5} M CAP in 1 M Na_2SO_3 in BR of pH 10 buffer for 40 min. No change of height was observed.

At the GT-CPE calibration straight lines for 10^{-5} and 10^{-4} mol/l concentration range in 1 M Na_2SO_3 in BR buffer of pH 8 and 10 show low reproducibility and low correlation coefficients (–0.963 and –0.927, respectively).

At SN-CPE DP voltammograms in 1 M Na_2SO_3 in BR buffer in absence and presence of 5×10^{-5} M CAP were not reproducible both at renewed and at unrenewed electrode surface. The same is valid for ST-CPE where residual current and noise were also high.

The best reproducibility of CAP peak heights was obtained at the GN-CPE in the BR buffer of pH 10. Therefore, calibration graphs at that electrode, with and without surface renewing, in different concentrations of Na_2SO_3 were measured. Obtained results are summarized in Table II. It can be seen that when GN-CPE electrode surface is not renewed between measurements, all used concentrations of sulfite allow determination of CAP in the concentration range 10^{-5} and 10^{-4} mol/l, respectively. In 10^{-6} mol/l concentration range, best results were obtained in 0.5 and 1.0 M sodium sulfite, the saturated solution gave worse results.

With renewing of electrode surface before each measurement, calibration graphs for 1×10^{-5} M CAP concentration range in 0.25, 0.5, 1.0 M and saturated Na_2SO_3 were determined and are linear and results are well reproducible. For 1×10^{-6} M CAP concentration range and in 0.5, 1.0 M and

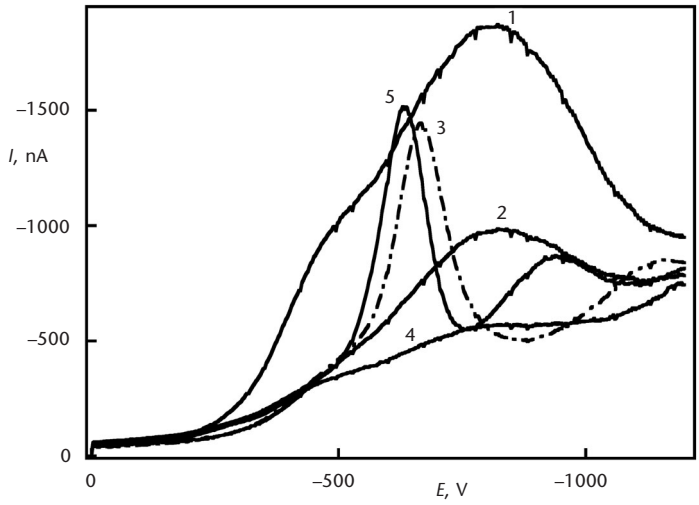

FIG. 4

DP voltammograms of BR buffer of pH 10 before oxygen removal (*1*), after oxygen removal by purging with nitrogen for 5 min (*2*), 1×10^{-4} M CAP in BR buffer of pH 10 purged with nitrogen for 5 min (*3*), 1 M Na_2SO_3 in BR buffer of pH 10 (*4*) and 1×10^{-4} M CAP in this solution (*5*). All voltammograms were recorded at freshly renewed GN-CPE immediately after its immersion in the solution

saturated solutions of sulfite only semiquantitative determination is possible because of relatively high RSD.

The best results were obtained in 0.25 and 0.5 M solutions of Na_2SO_3 in BR buffer of pH 10 without renewing GN-CPE surface. Voltammograms of CAP in 0.5 M Na_2SO_3 in BR of pH 10 recorded with and without renewing of GCP electrode surface are given in Figs 5–10. Because of relatively high noise, voltammograms corresponding to lower concentrations were smoothed using OriginPor 7.5 program, function Smoothing-Adjacent Averaging.

TABLE II
Parameters of calibration straight line for DPV determination of CAP in the presence of various concentrations of sodium sulfite in BR buffer of pH 10 at GN-CPE with and without renewing its surface

$[Na_2SO_3]$ mol l^{-1}	Slope nA l mol^{-1}	Intercept nA	R	LOD mol l^{-1}	RSD %	RSD %
Without GN-CPE surface renewing – (2–10) × 10^{-5} M CAP						
0.1	-1.21×10^7	–11.1	–0.9996	7.9×10^{-7}	4.86[a]	0.93[b]
0.25	-1.24×10^7	–29.3	–0.9999	2.4×10^{-7}	0.95[a]	0.40[b]
0.5	-1.27×10^7	10.0	–0.9982	1.5×10^{-6}	3.81[a]	1.01[b]
1.0	-1.16×10^7	–16.6	–0.9993	1.0×10^{-6}	6.27[a]	0.45[b]
Saturated	-1.16×10^7	–27.3	–0.9966	2.1×10^{-6}	10.40[a]	2.71[b]
Without GN-CPE surface renewing – (2–10) × 10^{-6} M CAP						
0.5	-1.07×10^7	–6.3	–0.9918	4.5×10^{-7}	10.58[c]	2.37[d]
1.0	-1.05×10^7	–27.8	–0.9892	5.1×10^{-7}	13.93[c]	7.47[d]
Saturated	-9.46×10^6	–33.6	–0.9439	1.2×10^{-6}	15.13[c]	1.27[d]
With GN-CPE surface renewing – (2–10) × 10^{-5} M CAP						
0.25	-1.34×10^7	–23.7	–0.9974	2.0×10^{-6}	5.23[a]	2.48[b]
0.5	-1.33×10^7	–47.2	–0.9985	1.5×10^{-6}	6.53[a]	3.11[b]
1.0	-1.24×10^7	–8.2	–0.9970	2.7×10^{-6}	14.27[a]	8.78[b]
Saturated	-1.46×10^7	–19.3	–0.9961	2.7×10^{-6}	12.94[a]	0.60[b]
With GN-CPE surface renewing – (2–10) × 10^{-6} M CAP						
0.5	-1.66×10^7	7.0	–0.9845	4.7×10^{-7}	20.97[c]	10.05[d]
1.0	-1.33×10^7	–3.6	–0.9500	1.1×10^{-6}	29.04[c]	10.61[d]
Saturated	-1.70×10^7	7.1	–0.9721	7.4×10^{-7}	9.83[c]	10.52[d]

[a] For 2 × 10^{-5} M CAP. [b] For 10 × 10^{-5} M CAP. [c] For 2 × 10^{-6} M CAP. [d] For 10 × 10^{-6} M CAP.

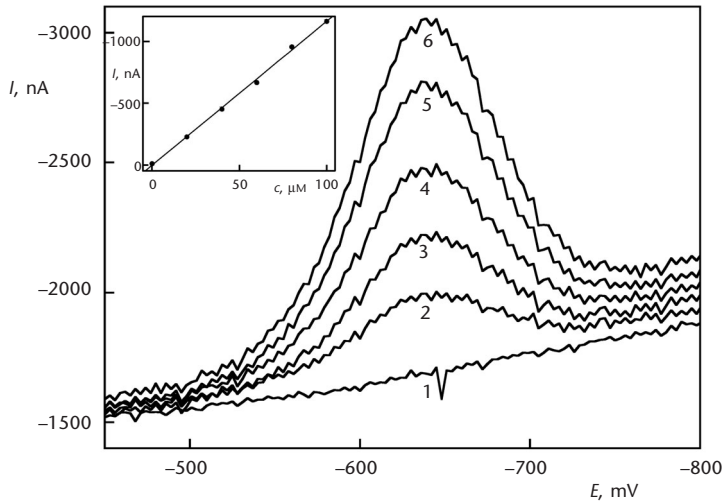

FIG. 5

DP voltammograms of CAP in 0.5 M Na_2SO_3 in BR buffer of pH 10 at unrenewed GN-CPE. CAP concentrations (μmol/l): *1* 0, *2* 20, *3* 40, *4* 60, *5* 80 and *6* 100. The calibration straight line is shown in the inset

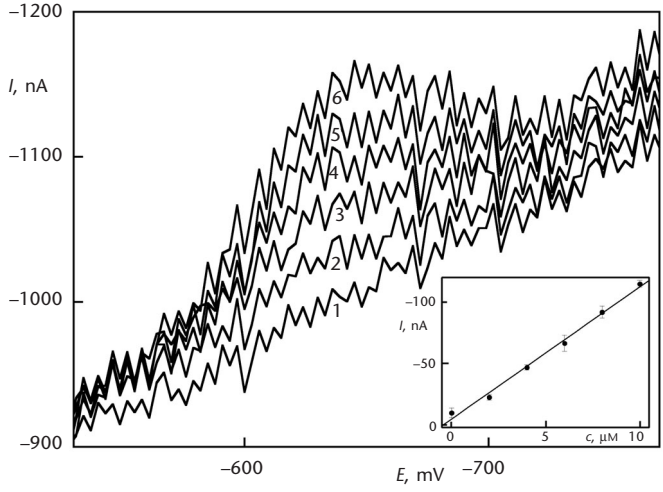

FIG. 6

Unsmoothed DP voltammograms of CAP in 0.5 M Na_2SO_3 in BR buffer of pH 10 at unrenewed GN-CPE. CAP concentrations (μmol/l): *1* 0, *2* 2, *3* 4, *4* 6, *5* 8 and *6* 10. The calibration straight line is shown in the inset

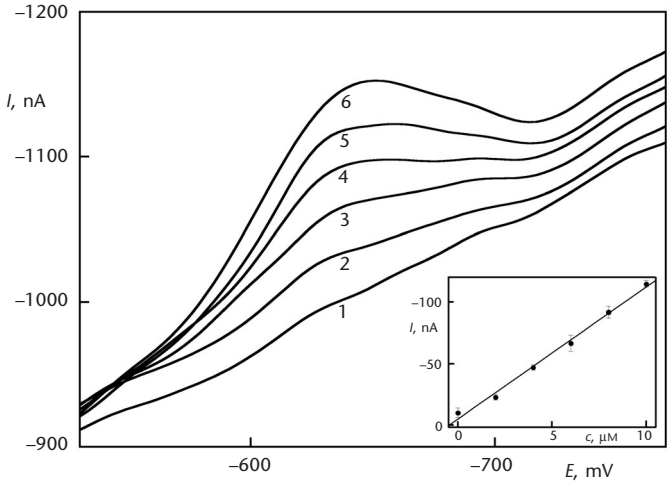

FIG. 7

Smoothed DP voltammograms of CAP in 0.5 M Na_2SO_3 in BR buffer of pH 10 at unrenewed GN-CPE. CAP concentrations (μmol/l): *1* 0, *2* 2, *3* 4, *4* 6, *5* 8 and *6* 10. The calibration straight line is shown in the inset

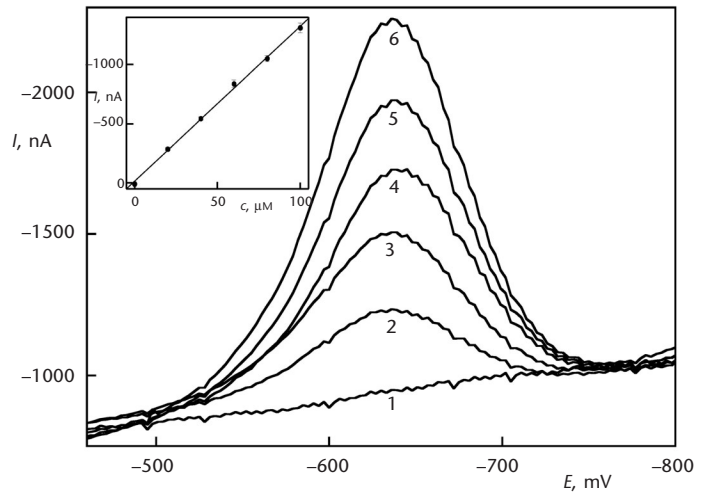

FIG. 8

DP voltammograms of CAP in 0.5 M Na_2SO_3 in BR buffer of pH 10 at renewed GN-CPE. CAP concentrations (μmol/l): *1* 0, *2* 20, *3* 40, *4* 60, *5* 80 and *6* 100. The calibration straight line is shown in the inset

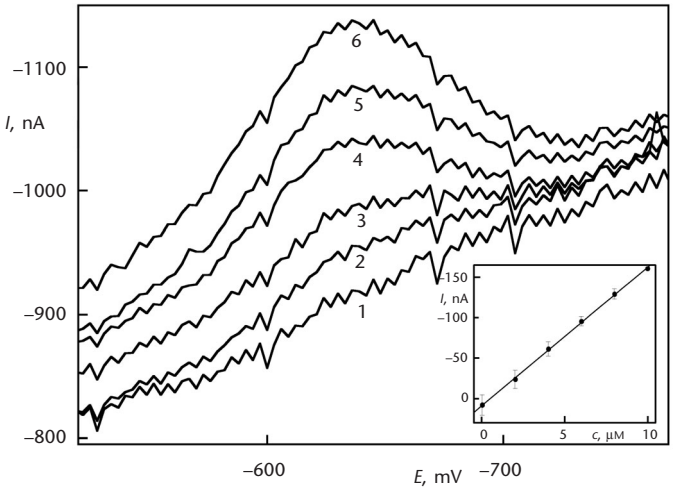

FIG. 9
Unsmoothed DP voltammograms of CAP in 0.5 M Na_2SO_3 in BR buffer of pH 10 at renewed GN-CPE. CAP concentrations (μmol/l): *1* 0, *2* 2, *3* 4, *4* 6, *5* 8 and *6* 10. The calibration straight line is shown in the inset

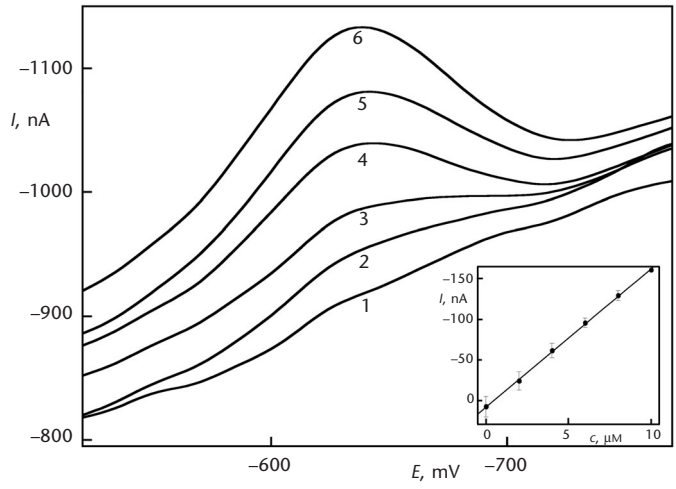

FIG. 10
Smoothed DP voltammograms of CAP in 0.5 M Na_2SO_3 in BR buffer of pH 10 at renewed GN-CPE. CAP concentrations (μmol/l): *1* 0, *2* 2, *3* 4, *4* 6, *5* 8 and *6* 10. The calibration straight line is shown in the inset

CONCLUSION

Because of the irreproducible oxygen peak from the electrode pastes, chloramphenicol can be determined by the differential pulse voltammetry at the carbon paste electrodes only in the 10^{-5} and 10^{-4} mol/l concentration range. Addition of 0.5 to 1 M sodium sulfite enables determination of chloramphenicol in a lower, i.e. 10^{-6} mol/l concentration range, too. Comparison of efficiencies of sodium sulfite and of keeping CPE surface in a stream of nitrogen bubbles during 5 min in reducing peak of oxygen from the electrode pastes shows comparable results in neutral and alkaline pH ranges. Obtained results show applicability of sodium sulfite in determinations of substances which can be reduced at CPEs in neutral and alkaline pH ranges, especially for in field analysis.

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