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Short communication

Voltammetric determination of amoxicillin using a poly (*N*-vinyl imidazole) modified carbon paste electrode☆

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1. Introduction

The penicillins are one of the most important group of antibiotics. Since the first penicillin became available numerous other antimicrobial agents have been produced, but penicillins are still widely used, major antibiotics and they are presently the drugs used for a number of infectious diseases.

A group of penicillins covering amoxicillin and ampicillin have antimicrobial activity which can be extended to include such gram-negative microorganisms as *Haemophilus influenzae*, *Escherichia coli* and *Proteus mirabilis*. Even though this group of antibiotics is widely used the incidence of oversensitive reactions may be as high as 10% and as they are used for fighting bacterial infections in various domestic animals [1], this can cause the transfer of penicillins to milk and meat products by and from the animals. So the methods for the determination of this group of compounds must have very low determination limits.

Numerous methods are available for the determination of penicillins. The iodometric method has been officially recognised [2]. HPLC is the most widely used technique for the determination of this group of drugs [3–6]. In addition, a number of penicillin-sensitive sensors were described in the literature [7–16]. Polarographic investigation of penicillins is generally based on the reduction of the chemically oxidised compounds [17] or degradation products formed by acidic or alkaline hydrolysis [18–20] or their derivatives [21]. Although the chemical oxidation of penicillins was studied extensively [22,23], papers about the electro-oxidation of these compounds are very few [24,25]. Koprowski et al. investigated anodic oxidation of some penicillins using gold and platinum electrodes [24] and they reported that electro-oxidation of some penicillins had been catalysed by the surface oxide of the gold electrode.

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Some papers have been published related to the polymer modified electrodes recently [26,27].

In our recent studies using poly (*N*-vinyl imidazole) (PVI) modified electrode, which was planned in our laboratory, satisfactory results were obtained for the determination of some drugs [28]. According to our knowledge no examination has appeared related to the voltammetric determination of amoxicillin [D-(−)-α-amino-*p*-hydroxybenzyl-penicillin trihydrate] in the literature. The aim of this study was to investigate the electro-oxidation of this substance with various solid electrodes and to test the performance of PVI modified carbon paste electrode for the electroanalysis of amoxicillin and to develop a rapid, simple and sensitive method for its determination.

2. Experimental

Voltammetric measurements were carried out using a PRG-3 polarograph (Tacussel) associated to an EPL-2 recorder (Tacussel). Reference and counter electrodes were a saturated calomel (SCE) (Tacussel XR 100) and a platinum wire respectively. All the potentials in the text were given versus SCE.

Modified carbon paste (9.8 mm in diameter) was prepared by mixing 1.25 g of graphite powder (Aldrich) and 1 ml of mineral oil (Sigma) and 60 mg poly (*N*-vinyl imidazole) was added to this mixture.

Poly (*N*-vinyl imidazole) was prepared in Middle East Technical University Laboratories according to process given in the literature [29]. Amoxicillin was of drug standard grade and kindly supplied by FAKO-Turkey. All other reagents were of analytical grade. Doubly distilled water was used to prepare the solutions.

3. Results and discussion

Electro-oxidation of amoxicillin can take place via the phenol group at the side chain of the molecule. In the present study ruthenium, platinum, glassy carbon and carbon paste electrodes were tested in the electro-oxidation of amoxicillin but no response could be obtained with ruthenium, platinum and glassy carbon electrode. Using carbon paste electrode, the voltammograms indicated oxidation of amoxicillin had taken place but the results were not good enough for analytical purposes. Tests were performed also in the reductive direction with glassy carbon and carbon paste electrodes but this compound behaved as an electroinactive substance under these conditions.

When the carbon paste electrode was modified by the addition of PVI, oxidation of amoxicillin took place on this electrode. The composition of the electrode was changed and best results, regarding to the reproducibility and lowest detection limit, could be obtained with modified carbon paste electrode having the composition mentioned in the experimental section. Voltammograms were obtained with various scan rates such as 100, 50, 25 and 10 mV s^{-1}, 100 mV s^{-1} was found most suitable.

Modified carbon paste electrode was tested in 0.1 M H_2SO_4, in acetate buffer of pH 4.7, phosphate buffer of pH 7.4 and Britton–Robinson buffer solutions having the pH values of 2.0, 4.0, 6.0, 7.0, 8.0 and 12.0.

With normal carbon paste electrode (having no polymer) in 0.1 M H_2SO_4 solution (Fig. 1) oxidation of amoxicillin began at about 150 mV and three small peaks at about 300, 400 and 500 mV were observed. A step beginning from about 550 mV and reaching a limiting current region at 600 mV also appeared. Another step, in the shape of a broad peak, the peak potential of which is 1100 mV, was observed on the oxidation branch. In the reduction branch, peaks at 1100, 1000, 550 and 450 mV and a step beginning from 350 mV were observed. As some of these steps were also seen on the voltammogram taken in 0.1 M H_2SO_4 supporting electrolyte, they must be related to the structure of the electrode. This means that oxidation of amoxicillin and reduction of these oxidation products take place at the same potentials with the functional groups on the electrode. With normal carbon paste electrode the increase in current with the increase in amoxicillin concentration and reproducibility was not enough for quantitative determination of this compound. Addition of the PVI to carbon paste electrode increased the

ratio of the faradaic current to background current but the degree of this increment was dependent on the composition of the electrode. Best results were obtained by the addition of 60 mg of PVI. The voltammograms obtained in 0.1 M H_2SO_4 solution with modified carbon paste electrode were given in Fig. 2. On the oxidation branch three well defined steps were obtained at about 400, 500 and 1050 mV. On the reverse scan the steps became ill defined.

When pH increased (Fig. 3), the ratio of faradaic current to background current decreased and above pH 2.3 faradaic current even became smaller than background current. The same results were obtained in acetate and phosphate buffer solutions (Fig. 4a and 4b) revealing that role of the pH of the solution was more important than the nature of supporting electrolyte in the electro-oxidation of amoxicillin. Koprouwst et al. [25] observed only one oxidation peak at about 1230 mV on a cyclic voltammogram obtained with gold electrode in acetate buffer of pH 4.7.

Comparison of the results obtained with modified carbon paste electrode in our laboratory with gold electrode by Koprouwst et al. [25] reveals that electro-oxidation of amoxicillin can be possible by the action of gold oxides or PVI with the substance. Possibly the electron rich nitrogen atoms on the polymer surface facilitates the oxidation of the substance.

A linear relationship was observed between the square root of the scan rate and the peak current

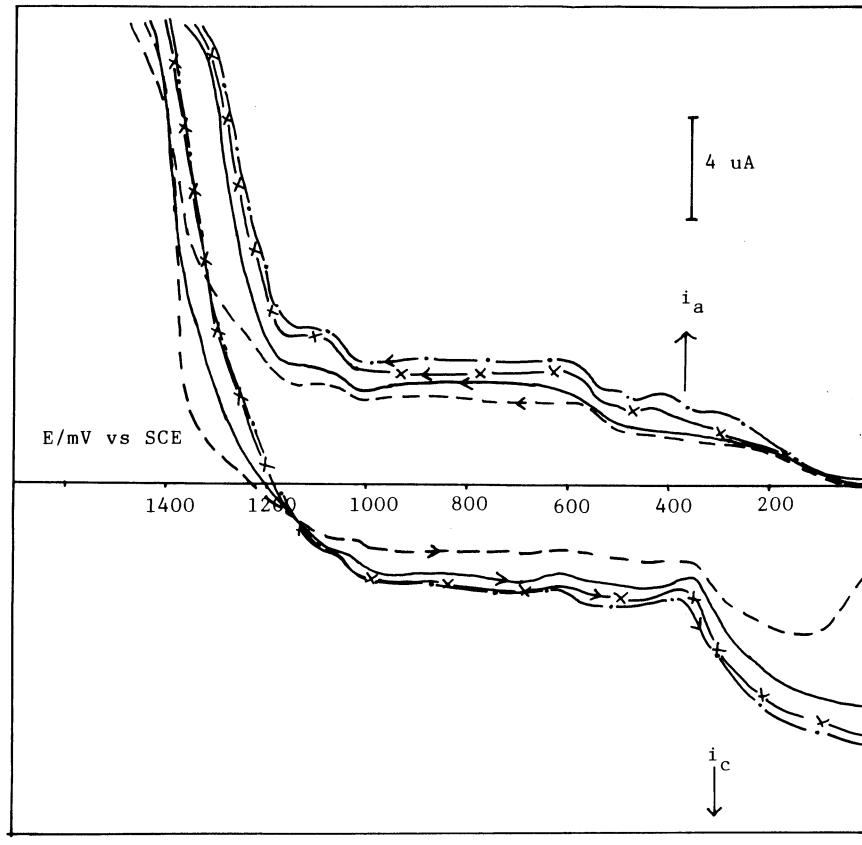

Fig. 1. Voltammograms of amoxicillin recorded in 0.1 M sulphuric acid solution with normal carbon paste electrode. Scan rate, 100 mV s^{-1}. Solutions: (----), 0.1 M H_2SO_4; (—), 4×10^{-6} M amoxicillin; (- × - × -), 4×10^{-5} M amoxicillin; (-·-·-·), 6×10^{-5} M amoxicillin.

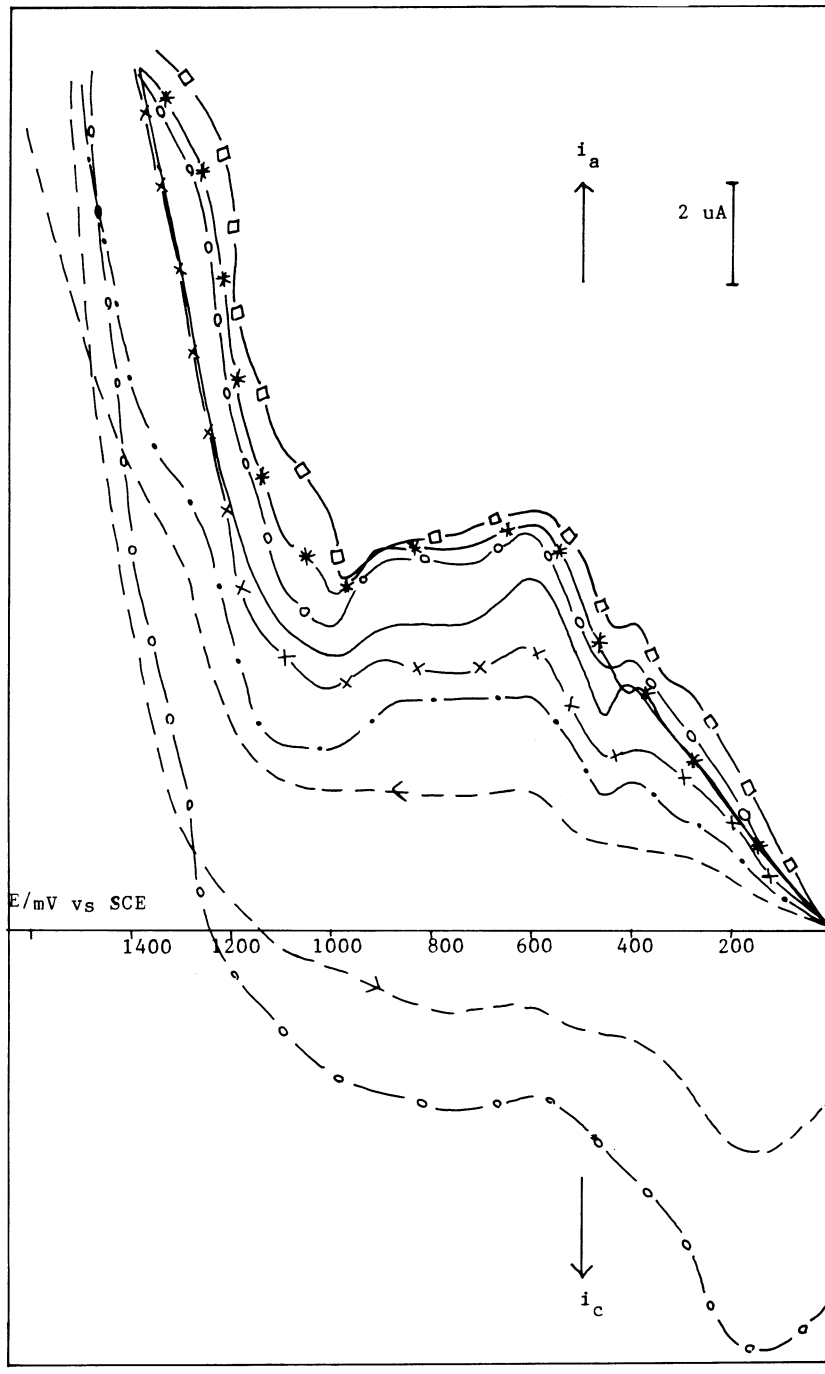

Fig. 2. Voltammograms of amoxicillin recorded in 0.1 M sulphuric acid solution with modified carbon paste electrode. Scan rate, 100 mV s^{-1}. Solutions: (----), 0.1 M H_2SO_4; (-·-·-·), 1×10^{-6} M amoxicillin; (– × – × –), 1×10^{-5} M amoxicillin; (—), 2×10^{-5} M amoxicillin; (-○-○-), 4×10^{-5} M amoxicillin; (-*-*), 6×10^{-5} M amoxicillin; (-□-□-), 8×10^{-5} M amoxicillin.

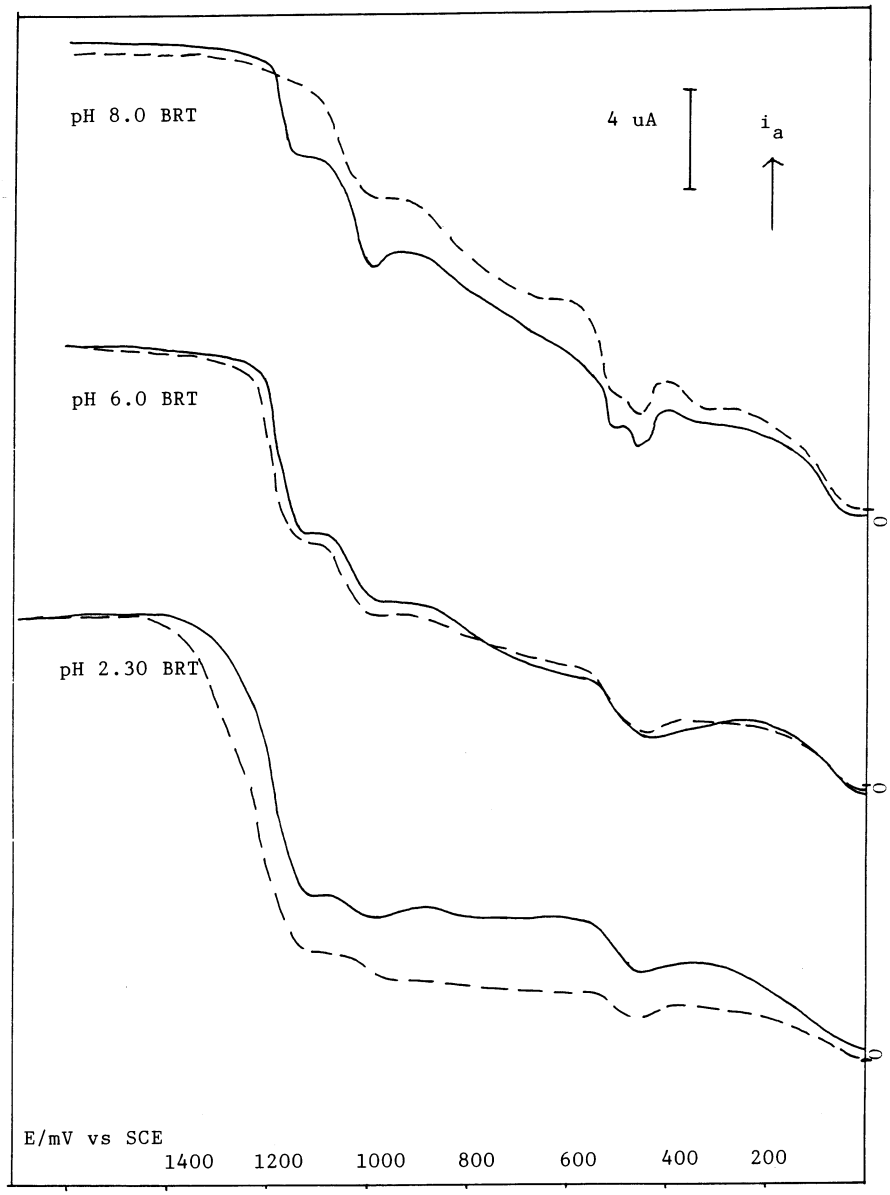

Fig. 3. Voltammograms of amoxicillin recorded in Britton–Robinson buffer of different pH with modified carbon paste electrode. Scan rate, 100 m Vs^{-1} (----), supporting electrolyte; (—), 4×10^{-5} M amoxicillin.

of the peak at 1050 mV with modified carbon paste electrode in 0.1 M H_2SO_4 the solution having 8×10^{-5} M amoxicillin ($r = 0.9989$, $m = 0.38$, $n = -1.06$) but this line did not pass through the origin indicating that the reaction was not a simple diffusion controlled one.

3.1. Validation of the method

The most suitable supporting electrolyte was H_2SO_4 so the quantitative evaluation was made using the curves obtained in 0.1 M H_2SO_4.The relationship between peak current of the peak at

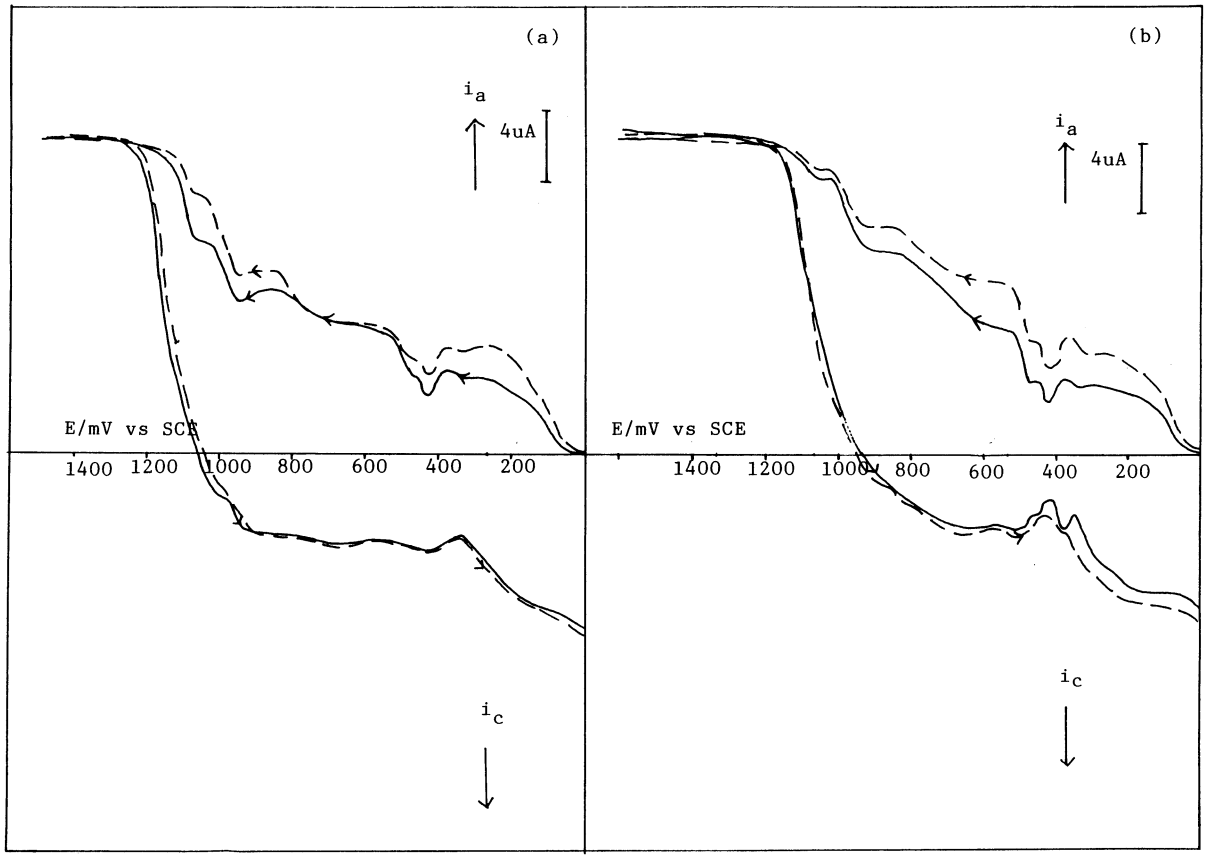

Fig. 4. Voltammograms of amoxicillin with modified carbon paste electrode. Scan rate, 100 mV s^{-1}; (----), supporting electrolyte; (—), 4×10^{-5} M amoxicillin. (a) pH 4.7 acetate buffer; (b) pH 7.4 phosphate buffer.

1050 mV and amoxicillin concentration of the curves in Fig. 2 manifested itself as two ines with two different slopes in the concentration ranges of 10^{-6}–10^{-5} M ($r = 0.9961$, $m = 1.54 \times 10^5$ μA M^{-1}, $n = 0.63$ μA) and 10^{-5}–2×10^{-4} M ($r = 0.9996$, $m = 3.34 \times 10^4$ μA M^{-1}, $n = 1.81$ μA) (Table 1). The reproducible voltammetric signals were obtained with relative standard deviations of 2.32 and 2.45% for four replicate measurements of 1×10^{-4} M amoxicillin in the same day and for different days, respectively. For analytical purposes the second concentration range (10^{-5}–2×10^{-4} M) was more convenient according to the statistical results. The detection limit was calculated as 8.12×10^{-7} M for five experiments.

3.2. Analytical application

3.2.1. Tablets

Ten tablets were accurately weighted and ground to fine powder. The required amount of sample corresponding to a stock solution of concentration of 10^{-3} M was weighted and diluted to the mark with 0.1 M H_2SO_4 in a calibration flask. The contents of the flask were stirred magnetically for 15 min and then filtered through a fine pore filter paper. This solution was used as stock.

3.2.2. Capsules

In the case of capsule form, the capsule contents were taken and by weighting the proper amount of this substance a 10^{-3} M stock solution

Table 1
Characteristic of amoxicillin calibration plots

Medium	Concentration range (M)	Slope (μA M^{-1})	Intercept (μA)	Correlation coefficient	SE of slope (μA M^{-1})	SE of intercept (μA)
0.1 M H_2SO_4	1×10^{-6}–1×10^{-5}	1.54×10^{5}	0.63	0.9961	7.92×10^{3}	5.22×10^{-2}
	1×10^{-5}–2×10^{-4}	3.34×10^{4}	1.81	0.9996	4.16×10^{2}	3.92×10^{-2}

Table 2
Results of alfoxil® formulations analysis for amoxicillin trihidrat

Formulations	Labelled claim (mg)	Amount found (mg)[a]	% RSD amount found	Official method (mg)[b]	% RSD of official method	Student's t-test
Tablet	500.00	504.28	2.58	498.73	0.99	0.318 ($t^{c}_{0.05}$) = 2.306
Capsule	250.00	253.38	2.13	256.09	0.96	1.011 ($t^{c}_{0.05}$) = 2.306
Oral suspension	125.00	126.69	2.13	121.55	1.55	3.459 ($t^{c}_{0.01}$) = 3.355

[a] Each value is the mean of five experiments.
[b] USP XXII.
[c] Tabulated (significant) level, at $P = 0.05$ and $P = 0.01$, in parentheses.

was prepared by dissolving and diluting it with 0.1 M H_2SO_4 in a volumetric flask.

3.2.3. Oral suspensions

A 10^{-3} M stock solution for oral suspension of amoxicillin was prepared by taking the proper volume of suspension and diluting it with 0.1 M H_2SO_4 in a volumetric flask.

By diluting these stocks, solutions which were equivalent to 8×10^{-5} M amoxicillin were prepared. Voltammograms were taken in these solutions and the amount of amoxicillin was calculated. In order to establish the reliability and suitability of the proposed method recovery experiments were performed. The voltammetric results were compared with USP XXII method by means of t-tests. The results are given in Tables 2 and 3. As one can see from these tables t values did not exceed the theoretical value for a significance level of 0.05 so the difference between voltammetric and official results is not important for tablet and capsule forms. But for suspension the difference between voltammetric and official results was found to be significant so direct voltammetric method is not suitable for suspension.

4. Conclusion

In the present study it was shown that anodic oxidation of amoxicillin was possible using a PVI added carbon paste electrode. Thus voltammetric determination of amoxicillin could be made in tablets and capsules directly without any separation step because the excipients present in the pharmaceutical forms did not interfere with the voltammetric analysis.

The PVI added carbon paste electrode can be easily prepared and has the advantage of chang-

Table 3
Recovery studies by proposed method at modified carbon paste electrode

Formulations	Recovery[a] (%)	RSD (%)
Tablet	98.15	2.47
Capsule	101.41	2.39
Oral suspension	101.41	2.39

[a] Each result is the average of five experiments.

ing the composition of the electrode and adding the correct amount of polymer. Additionally, the electrode was stable for nearly 120 experiments and for a month. To obtain a fresh carbon paste surface smoothing with a filter paper was enough.

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