Fresenius J Anal Chem (1999) 364 : 590–594 

ORIGINAL PAPER

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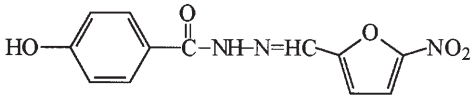
# Voltammetric study of nifuroxazide at unmodified and Sephadex-modified carbon paste electrodes

Received: 2 June 1998 / Revised: 29 January 1999 / Accepted: 2 February 1999

**Abstract** The electrooxidation of nifuroxazide was investigated by cyclic and differential-pulse voltammetry at carbon paste and Sephadex-modified carbon paste electrodes. Nifuroxazide is irreversibly oxidized at all pH values and gives rise to a well-defined oxidation peak. The modification of the carbon paste surface with Sephadex allowed a preconcentration process to take place for nifuroxazide such that higher sensitivity was achieved compared with the bare surface. The influence of the scan rate, time of accumulation, modifier loading, solution conditions and pH on the adsorptive step at the modified carbon paste electrodes was investigated. The direct determination of the drug in urine is also discussed.

## 1 Introduction



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Nifuroxazide, 4-hydroxybenzoic acid [(5-nitro-2-(furanyl) methylene] hydrazide (I) is used for the treatment of acute and chronic diarrhea, gastroenteritis, and colitis. Analytical methods used for the determination of nifuroxazide in dosage form include titrimetric [1], complexometric [2], ultraviolet spectrophotometric [3], and high-performance liquid chromatography (HPLC) [3]. Plasma and urinary concentrations of nifuroxazide after the absorption of the drug from the gastrointestinal tract were determined by HPLC with UV detection [4, 5]. The polarographic behavior of nifuroxazide and its analytical utility has been reported [6–9].

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The chemical modifications of the electrode open wide opportunities for improving the selectivity and the sensitivity of electroanalytical determination [10–13]. Most often, the modifying agents are introduced into the composition of the carbon paste electrode [14–16]. Modification of carbon paste electrodes with a typical liquid chromatographic stationary phase such as $C_{18}$ for direct determination of tifluadom [17] and ephedrine [18] in urine has been reported. The analyte was selectively retained and accumulated at the modifier on the electrode surface at the stage of its preconcentration, whereupon the concentration of the accumulated substance was determined by cyclic voltammetry.

As no work dealing with the electrooxidation of nifuroxazide has been reported, it was of interest to pursue the anodic behavior of the compound with view to the presence of an electrooxidizable phenolic hydroxyl group. A highly selective and sensitive stripping voltammetric procedure was developed for the determination of the drug with a carbon paste electrode modified with Sephadex. Sephadex is cross-linked dextran and is frequently used as a stationary phase in gel permeation chromatography [19]. The easy collection of nifuroxazide by the polydextran chain was exploited for designing novel modified electrodes for preconcentration/voltammetry.

## 2 Experimental

### 2.1 Reagents

Stock solution ($1 \times 10^{-3}$ M) of nifuroxazide was prepared daily in pure methanol and stored at 4 °C in the dark. Britton-Robinson buffers (acetic, phosphoric and boric acids, all at 0.04 M; pH adjusted with NaOH) were used as supporting electrolytes. All solutions were prepared from AnalaR-grade reagents in doubly distilled water.

### 2.2 Apparatus

Cyclic voltammetry measurements were performed with an Oxford PVSU potentiostat connected to Philips PM 8043 X-Y recorder. Differential pulse polarography measurements were made with a

SARGENT-WELCH model 4001 voltammetric analyzer. A three-electrode system was used; the working electrode was a carbon paste (CPE) or Sephadex-modified carbon paste (CME) electrode; the reference electrode was an Ag/AgCl/(3 M KCl) electrode separated by a salt bridge and all potentials reported here are referred to this electrode and a glassy carbon as the counter electrode.

Sephadex-modified carbon paste (CMEs) electrodes were prepared by grinding the Sephadex (Phamacia, Uppsala, Sweden) with mortar and pestle, then adding the spectroscopic graphite powder (Aldrich 1–2 micron); after additional grinding of the mixture, Nujol oil (Sigma) was added and the whole mixture was thoroughly hand-mixed. Carbon-paste electrodes containing 0.06, 0.24 and 0.48 g of Sephadex, together with 1.2 g of graphite powder and 0.8 g of Nujol oil, were employed. Plain (unmodified) carbon paste was prepared in the usual manner, and contained 40% Nujol oil. The body of the working electrode was a Teflon sleeve (3.5 mm i.d.) filled with carbon paste. A platinum wire embedded in the paste provided electrical connection. The electrode surface was manually smoothed on a deck of weighing paper.

The pH of buffer solution was measured with a SCHOTT Geräte digital pH-meter with glass combination electrode.

### 2.3 Procedure

The voltammetric procedure consisted of accumulation of the analyte at the electrode surface from a solution stirred at ~400 rpm, by magnetic stirrer and stirring bar 1 cm long, for a given period of time at open circuit potential. After a delay period of 15 s at +0.3 V to settle the solution and decrease the background current, a linear sweep or a differential-pulse voltammogram was recorded between +0.3 V and +1.2 V.

For medium exchange experiments, two 20 mL cells were used, a preconcentration cell containing the analyte solution and a measurement cell containing a blank solution. After preconcentration, the electrode was washed with water, then transferred to the measurement cell to record the voltammograms in the positive direction. Quantitative measurement was done by the standard additions method. For electrode regeneration, the working electrode was transferred to a blank electrolyte solution and the series of cyclic scan was continued until a voltammogram corresponding to the residual current was obtained. The electrode was then ready for use in a next measurement cycle.

*2.3.1 Analysis of nifuroxazide in urine.* Urine (1 mL) containing the drug was mixed with 18 mL of Britton-Robinson buffer, pH 4.0, and diluted to 20 mL with methanol. The solution was stirred at 400 rpm at open circuit conditions, and the Sephadex-modified electrode was immersed for 30 s (preconcentration step). The electrode was then washed with water, dried, and placed in the measurement cell containing Britton-Robinson buffer, pH 2.0 (20 mL) and the differential-pulse voltammogram was recorded with a pulse amplitude of 25 mV and sweep rate of 2 V $min^{-1}$.

## 3 Results and discussion

### 3.1 Electrochemical behavior at the bare carbon paste electrode

Figure 1 shows a cyclic voltammogram (CV) for the oxidation of nifuroxazide in Britton-Robinson buffer pH 4.0. The potential was cycled between 0.3 V and 1.2 V (vs. Ag/AgCl) at a scan rate of 100 $mVs^{-1}$. One oxidation peak at 1.174 V was observed for the oxidation of the phenolic hydroxyl group. No corresponding reduction peak was observed on scan reversal, indicating irreversibility of the process. As the scan rate was increased from 10 to 200 $mVs^{-1}$, the peak potential shifted towards more positive potential as expected for an irreversible oxidation

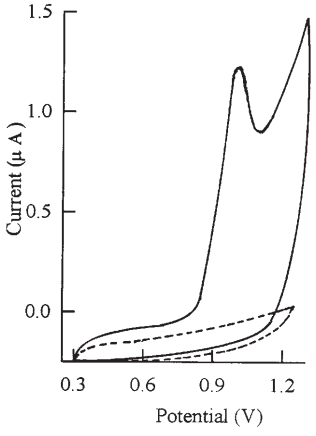


**Fig. 1** Cyclic voltammogram for $5 \times 10^{-6}$ M nifuroxazide in Britton-Robinson buffer pH 4.0 at carbon paste electrode; scan rate: 100 mV $s^{-1}$. Broken lines represent the background current

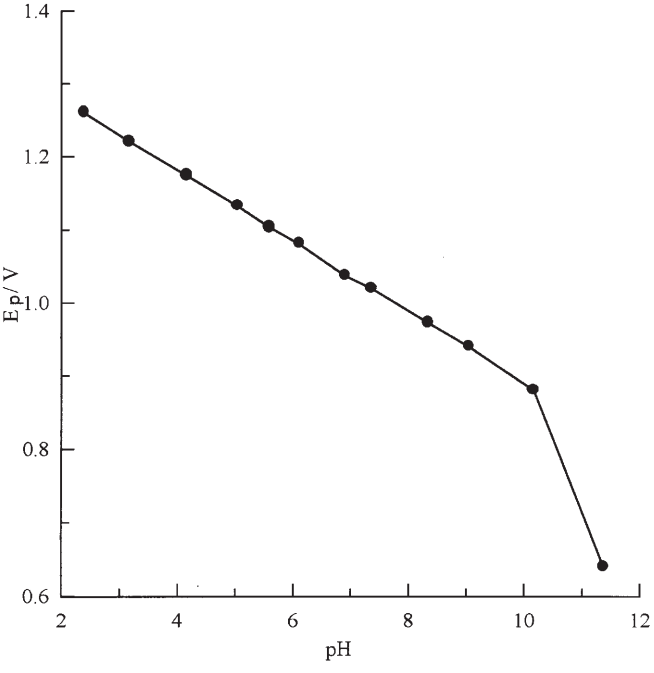


**Fig. 2** Effect of pH on the nifuroxazide peak potential; conditions as in Fig. 1

[20]. The correlation of the peak current against $v^{1/2}$ is linear, indicating a diffusion-controlled process.

The potential-pH behavior of nifuroxazide was investigated in Britton-Robinson buffers over a pH range of 2.0–12.0. As shown in Fig. 2, the potential-pH graph yielded a slope of –48.0 mV/pH. A break in the $E_p$ versus pH appears at pH 10.5, which may be due to the removal of phenolic proton. For an irreversible system the slope involves the transfer coefficient $\alpha$, and is equal to $-59.2\ P/\alpha\ n$ (mV/pH), where P is the number of protons and n the number of electrons. The peak current decreases with increasing pH.

### 3.2 Electrochemical behavior at Sephadex-modified electrodes

The incorporation of Sephadex into the carbon-paste matrix results in effective accumulation of nifuroxazide from

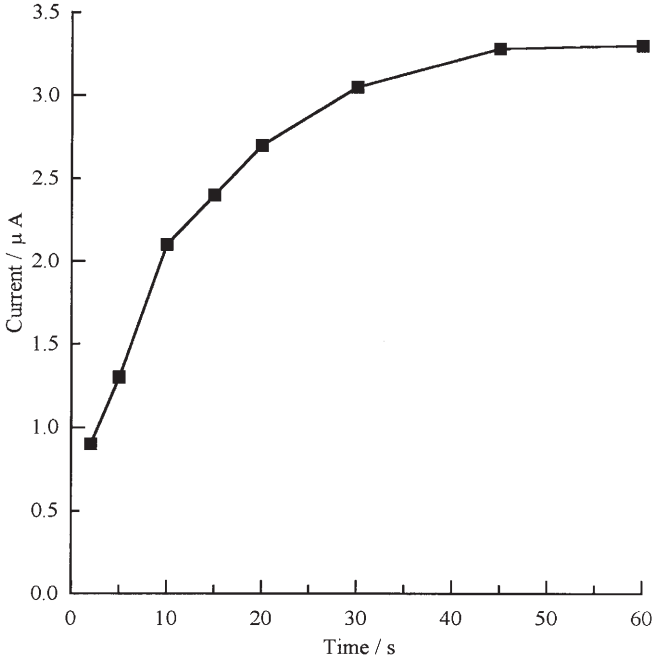


**Fig. 3** Effect of the preconcentration period on the stripping voltammogram for $5 \times 10^{-6}$ M nifuroxazide in Britton-Robinson buffer pH 4.0 at the Sephadex-modified carbon paste electrode containing 0.24 g Sephadex

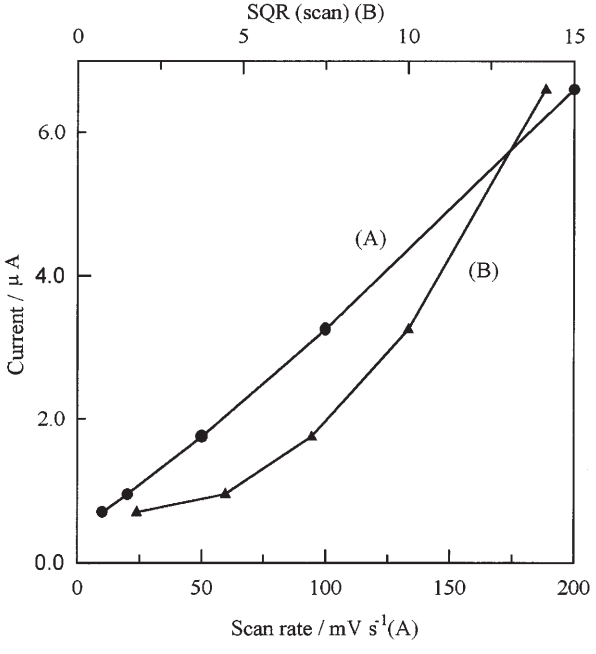


**Fig. 4** Dependence of the LSV on the scan rate (A) and on the square root of the scan rate (B), obtained at the modified electrode; concentration of nifuroxazide: $5 \times 10^{-6}$ M; preconcentration period: 60 s at open circuit condition

the solution prior to voltammetric analysis. It can be expected that an increase in the accumulation period should enhance the voltammetric response. Figure 3 shows the dependence of the current in the LSV mode on the accumulation time for $5 \times 10^{-6}$ M nifuroxazide in Britton-Robinson buffer pH 4.0 at the Sephadex-modified carbon paste electrode containing 0.24 g Sephadex. The current increases enormously within only a few seconds. After about 60 s, the peak current reaches its maximum indicating that the electrode surface becomes saturated with the analyte molecules. This accumulation time is very short and is doubtlessly advantageous for the practical use of this electrode.

Scan rate studies were made to ascertain whether the processes on carbon paste electrodes were under diffusion or adsorption control. Figure 4 shows the dependence of the current response on the scan rate and its square root value obtained at the Sephadex-modified electrode. The current is linearly proportional to the scan rate, which illustrates that the peak current is adsorption-controlled. This may be the adsorption of drug molecules at the polymer chain in the electrode surface.

Voltammetric responses have been tested for different types of Sephadex (G10, G25, G50 and G100) under identical conditions. It was observed that the sensitivity of voltammetric response (based on the voltammetric peak current) decreases in the order G100 > G50 > G 25 > G10 and the maximum sensitivity was obtained for G100. Hence, Sephadex G100 was used in the subsequent experiments. The stripping response increases as the Sephadex loading is increased as expected from the increased binding capacity of the electrode. However, at higher modifier loading, an increase in the background current was also observed. This could be attributed to an increase in the electrode resistance. The electrode containing 0.24 g Sephadex was employed for the subsequent work because of its better signal-to-background characteristics and its mechanical stability.

It has been noted that after more than 35 measurements with the same paste filling, the electrode material starts to get swollen at the surface due to the repetitive collection/measurement/cleaning cycles, whereupon the background current increased.

*3.2.1 Medium exchanges.* Medium exchange experiments were then carried out on unmodified and Sephadex-modified carbon paste electrodes. Figure 5 illustrates cyclic voltammograms for nifuroxazide recorded after stirring for 30 s at open circuit potential on the modified electrode. A large and defined anodic peak, due to the oxidation of the accumulated drug, is observed in the anodic direction. A medium exchange experiment resulted in a peak that was 60% of the height of the original peak. No peak was recorded after medium exchange using an unmodified electrode. These experiments demonstrate clearly that a strong binding exists between nifuroxazide molecules and the Sephadex polymer.

The oxidative and accumulation behaviors of nifuroxazide and hence the adsorptive stripping response, are affected by the solution conditions. The effect of pH of the preconcentration solution on the current signal was studied for $5 \times 10^{-6}$ M nifuroxazide at the Sephadex-modified carbon paste electrode containing 0.24 g Sephadex. The peak current increased gradually with increasing pH of

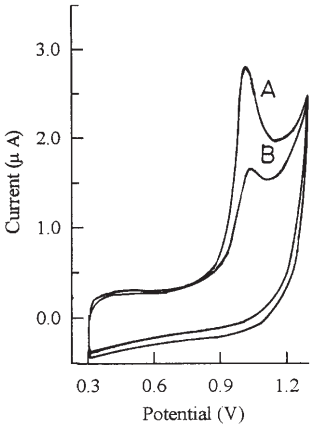


**Fig. 5** (*A*) Cyclic voltammogram for $5 \times 10^{-6}$ M nifuroxazide in Britton-Robinson buffer pH 4.0 at the Sephadex-modified carbon paste electrode containing 0.24 g Sephadex after a preconcentration period of 30 s at open circuit condition; scan rate: 100 $mVs^{-1}$. (*B*) represents the response after medium exchange

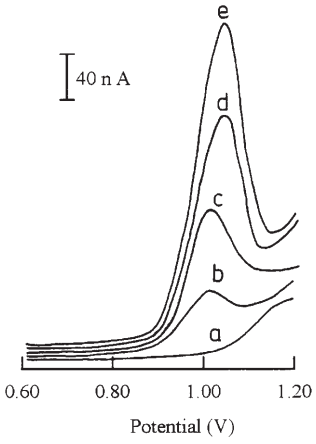


**Fig. 6** Differential pulse voltammograms at the Sephadex-modified carbon paste electrode containing 0.24 g Sephadex in a standard additions experiment with 30 s accumulation from a solution of pH 4.0 spiked with (*a*) 0.0 M, (*b*) $5.0 \times 10^{-8}$ M, (*c*) $3.0 \times 10^{-7}$ M, (*d*) $5.5 \times 10^{-7}$ M and (*e*) $8.0 \times 10^{-7}$ M nifuroxazide, stirred at 400 rpm at open circuit potential; Pulse amplitude: 25 mV and sweep rate: 2 V $min^{-1}$

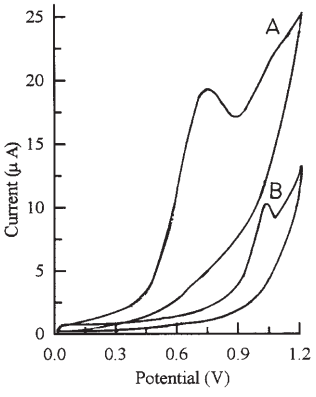


**Fig. 7** (*A*) Cyclic voltammogram for diluted (1:20) urine with Britton-Robinson buffer pH 4.0 spiked with $2.5 \times 10^{-5}$ M nifuroxazide after a preconcentration period of 30 s and at open circuit condition. (*B*) represents the response after medium exchange

the preconcentration solution and reached a maximum at about pH 4.0, then decreased sharply until pH 10.0. From pH 10.0 to 12.0 no change in the current was observed. The observations revealed that the chemical form of nifuroxazide in the preconcentration solution has a profound effect both on its adsorption into the modified electrode, and on the voltammetric response. The effect of pH of the measurement solution on the voltammetric response was investigated. The best current signal occurs at pH 2.0, and it decreases rapidly as the pH is increased. Hence, the best results with respect to the peak enhancement and shape, base line current and reproducibility were obtained for accumulation from a preconcentration solution of pH 4.0 with measurement in a solution of pH 2.0.

*3.2.2 Electroanalytical studies.* Figure 6 shows the differential pulse voltammograms of a working electrode containing 0.24 g of Sephadex in a standard additions experiment with 30 s accumulation from a solution of pH 4 spiked with (a) 0.0 M, (b) $5 \times 10^{-8}$ M, (c) $3.0 \times 10^{-7}$ M, (d) $5.5 \times 10^{-7}$ M and (e) $8.0 \times 10^{-7}$ M nifuroxazide, stirred at 400 rpm at open circuit potential. Measurements were made using a pulse amplitude of 25 mV and a sweep rate of 2 V $min^{-1}$. When calibration was performed for 60 s, a linear calibration graph was obtained from $2.0 \times 10^{-6}$ M to $1 \times 10^{-8}$ M (r = 0.988) with a slope of $1.48 \times 10^{8}$ nA $M^{-1}$. At a concentration of $5.0 \times 10^{-6}$ M, a curvature of the calibration graph was observed. This presumably indicates the saturation of the electrode surface. When accumulation was performed for 30 s, better sensitivity ($2.66 \times 10^{8}$ nA $M^{-1}$), a linear calibration range from $2 \times 10^{-6}$ M to $1.5 \times 10^{-8}$ M (r = 0.993) and a detection limit (calculated as S/N=3) of $8.0 \times 10^{-9}$ M were obtained. The reproducibility of the peak current at new surfaces as measured by the relative standard deviation (RSD) for a concentration of nifuroxazide of $1.0 \times 10^{-6}$ M ($n$ = 5) was 3.5% and at the same electrode after collection/measurement/cleaning cycles, it was 1.8%

As shown in Fig. 7 the direct determination of nifuroxazide in a diluted urine sample is not possible due to the large oxidation peak of blank urine. In contrast, after medium exchange, none of the urine components contributes to the response and the nifuroxazide can easily be measured. Assays of doped urine samples diluted with Britton-Robinson buffer pH 4.0 were performed at the Sephadex-modified electrode applying a medium-exchange procedure. In the differential pulse mode the electrode response measured in a blank solution of pH 2.0 was linearly related to the nifuroxazide concentration within the range of $5.0 \times 10^{-8}$ M to $4.0 \times 10^{-7}$ M. Recovery experiments were performed by repeated measurements ($n$ = 5) of urine samples containing different amounts of nifuroxazide in the range mentioned above. The mean recovery was 101.2% with a mean relative error of 4.2%. The relative standard deviation was 5.6%. A detection of $2 \times 10^{-8}$ M was estimated by taking the concentration that gave a signal of three times the standard deviation.

## 4 Conclusions

Application of the Sephadex-modified carbon paste electrode allows the preconcentration of nifuroxazide and increases the sensitivity as against the bare carbon paste electrode. The remarkable sensitivity of these modified electrodes, together with the ease of preparation and regeneration, mercury-free and low-cost make them very promising for drug determination. Compared with other techniques [5] the proposed method is cheap and is adequately accurate and precise.

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