

CONFERENCE CONTRIBUTION

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Determination of nifuroxazide with polarography and adsorptive stripping voltammetry at mercury and carbon paste electrodes

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Abstract A simple method for the determination of the urinary tract antibiotic nifuroxazide has been developed. The electrochemical reduction of the nitro group at mercury and carbon paste electrodes can be used for the determination with and without adsorptive preconcentration. The influence of parameters like pH of the background electrolyte, preconcentration potential and preconcentration time on the sensitivity of the method has been studied. Modification of the carbon paste by addition of non-polar polystyrene/divinylbenzene particles has been investigated to enhance the adsorption properties of the surface. Concentrations as low as 10 ng/mL could be determined in urine without interferences.

1 Introduction

Nifuroxazide {4-hydroxybenzoic acid [(5-nitro-2-furanyl)methylene]hydrazide} possesses antimicrobial and antibacterial activity and is therefore used for the treatment of diseases of the skin and various kinds of urinary tract infections. Nifuroxazide is not absorbed from the alimentary tract and is therefore very rapidly removed from the body without metabolism. The substance has been determined in pharmaceutical preparations, urine and plasma with methods such as spectrophotometry [1, 2], indirect complexometry [3], high performance liquid chromatography [4, 5] and potentiometric titration [6].

The electrochemical behavior of nifuroxazide has not been studied in detail. Cisak and Szuminska [1, 2] found that nifuroxazide could be reduced in two steps by dc-polarography at a dropping mercury electrode. But no further studies have been made on the nature of the electrode processes. The electrochemical behavior of other derivatives of nitrofurans has been studied more thoroughly.

These compounds, containing the same reducible functional groups, namely the nitro and the imino group, were shown to give two or three reduction waves, depending on pH and composition of the background electrolyte and on the material of the electrode. Determination was done by dc-polarography and differential pulse polarography at mercury and glassy carbon electrodes. Among the compounds studied were nitrofurazone [7–10], furazolidone [7, 10], nitrofurantoin [7, 11, 12], and nitrofuraxim [13]. Their nitro group is reduced in a 4 electron process to the hydroxylamine which can be reduced to the primary amine at lower potentials [8, 9, 11–13]. This behavior agrees with the general polarographic reduction scheme for aromatic nitro compounds described by Zuman et al. [15, 16].

The possibility of a six-electron reduction to the amine in one step is mentioned for a solvent-buffer system of pH 4.5, containing pyridine and formic acid in conjunction with tetramethylammonium chloride solution [7, 10].

The second electroactive group, the $>C=N-N<$ moiety, is reduced at more negative potentials and coincides with the reduction wave of the electrochemically generated hydroxylamine at certain pH values. Therefore, either two or three waves can be found for most of these compounds depending on the background electrolyte. A fourth reduction signal was mentioned for nitrofurantoin which was attributed to the reductive fission of the $>N-N<$ bond [11, 12]. A similar reduction behavior was to be expected for nifuroxazide.

So far voltammetric analyses of these compounds have been done by dc-polarography or differential pulse polarography without any preconcentration. Liquid-liquid extraction has been used only as a cleanup step for the determination in urine to separate the analyte from interfering matrix components [4, 12]. Therefore the determination limits reported are in the range of 10^{-6} to 10^{-7} mol/L.

The aim of this work was the development of a simple differential pulse voltammetric procedure with enhanced sensitivity for the determination of nifuroxazide in various matrices. For this purpose, adsorptive preconcentration techniques at a hanging mercury drop electrode as

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well as at carbon paste electrodes with and without surface modification were investigated.

2 Experimental

2.1 Reagents

Nifuroxazide was obtained from Sigma, Germany. Carbon paste was obtained from Metrohm, Switzerland. Polystyrene/divinylbenzene particles were produced in our laboratory by emulsion polymerization and had a diameter of 1 μm . All other chemicals were of analytical reagent grade.

2.2 Apparatus

Polarographic measurements were performed using a Metrohm 646 VA Processor with a 647 VA Stand and a Metrohm multi-mode mercury electrode or a home-made carbon paste electrode of 3.5 mm diameter. The reference electrode was a silver/silver chloride/3 M KCl electrode. Carbon paste modified with polystyrene/divinylbenzene particles was prepared by homogenizing a mixture of both components in a mortar for at least 10 min. Particle concentrations up to 20% (mass) were used, the nujol content of the paste was kept at 30%.

2.3 Electrochemical measurements

Measurements were performed using Britton-Robinson or Theorell-Stenhagen buffers with a pH between 2 and 12 as background electrolytes. Standard solutions were prepared by dissolving nifuroxazide in a buffer of appropriate pH with an ethanol content of 50%. All solutions were purged with nitrogen for at least 5 min prior to the measurement. The influence of the parameters pH, potential, and time on the efficiency of preconcentration was studied. Preconcentration times were varied from 0 s up to 5 min, preconcentration potentials from 0 to 1 V, and pH values between 2 and 12 were used. The scan rate was 25 mV/s and the pulse height was 50 mV.

2.4 Determination of nifuroxazide in urine

For the determination in urine samples 1 mL of buffer pH 5 was added to 2 mL of urine spiked with nifuroxazide, the solution was extracted with 5 mL ethyl acetate by shaking for 10 min. The organic phase was brought to dryness with a gentle stream of nitrogen, the residue redissolved in 2 mL of a mixture of ethanol and buffer (1/1 by volume) and sonicated. This mixture was transferred to the polarographic vessel and diluted to 20 mL with the background electrolyte.

3 Results and discussion

3.1 Electrochemical behavior at mercury electrodes

As expected, nifuroxazide yielded 2 or 3 reduction signals depending on the pH of the background electrolyte. Only the first of these peaks, resulting from the 4-electron reduction of the nitro group to the corresponding hydroxylamine, was suitable for quantification. A differential pulse polarogram of the first peak is shown in Fig. 1. The reduction potential as well as the height of the differential pulse peak were strongly pH-dependent. The reduction potential showed an almost linear behavior, being shifted

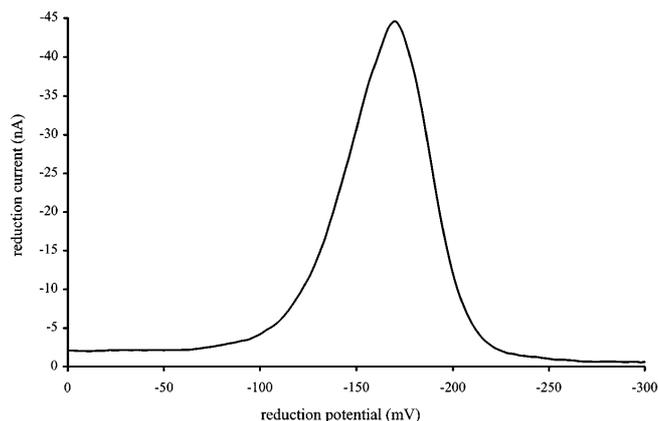


Fig. 1 Differential pulse polarogram of 1 $\mu\text{g/mL}$ nifuroxazide in a background electrolyte of pH 4, measurement at the hanging mercury drop electrode

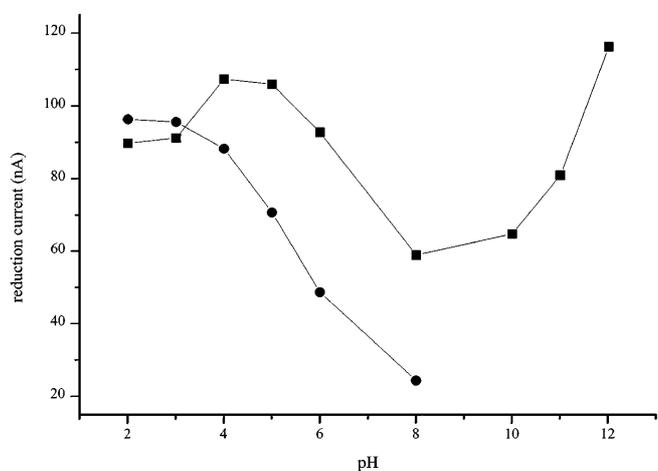


Fig. 2 Dependence of the reduction current for the first (■) and second (●) reduction signal of 100 ng/mL nifuroxazide on the pH of the background electrolyte, measurement with a hanging mercury drop electrode

from -20 mV at pH 2 to -470 mV at pH 12. The dependence of the reduction current on the pH is shown in Fig. 2. In the range from pH 4 to 5 the best sensitivity could be achieved; pH 4 was used for all further measurements. The peak height was linear in the concentration range from 0.1 to 10 $\mu\text{g/mL}$.

Due to the observation that nifuroxazide was readily adsorbed at the surface of a mercury electrode, adsorptive preconcentration was used to enhance the sensitivity of the method. The influence of parameters such as applied potential, adsorption time, and pH of the background electrolyte on the efficiency of the preconcentration procedure was investigated. No significant difference could be discerned when the potential applied to the electrode was varied from 0 to 1 V; adsorption with an open circuit worked quite as well. An adsorption behavior practically independent of the pH was observed in the pH range from 2 to 8; the best sensitivity was achieved in the region of pH 4 to 5. Saturation of the surface was reached after 1 min for a concentration of 200 ng/mL and after more

than 5 min for 20 ng/mL. A decrease of the signal could be observed for longer adsorption times for both concentrations. As was to be expected, the saturation level was reached faster when using higher stirring speeds, and a direct dependence of the maximum peak current upon the surface area of the mercury electrode could be observed. A concentration of 1 ng/mL in the measuring solution could be determined after a preconcentration time of 120 s with a signal to noise ratio higher than 10.

3.2 Electrochemical behavior at carbon paste electrodes

Only one reduction signal could be found when using the carbon paste electrode, arising from the reduction of the nitro group. Compared to the mercury electrode, the reduction potential was more negative and showed an almost linear dependence on the pH of the background electrolyte, being shifted from -220 mV at pH 2 to -510 mV at pH 8. The best sensitivity could be achieved at pH values between 2 and 3, mainly because at higher pH the reduction peak appeared in a region of increasing background current.

Several types of paper of different roughness were tested for the renewal of the electrode surface. The best results were achieved when the surface was not too smooth. This resulted in a better sensitivity and reproducibility compared to very smooth electrode surfaces. Good results were obtained by renewing the surface on a blue ribbon filter paper. It proved to be sufficient to renew the surface once every day, as the sensitivity decreased negligibly over the day. The standard deviation of the current was 3% for 6 measurements with fresh surfaces. The signal was linear for concentrations in a range of 0.1 to 10 $\mu\text{g/mL}$. The electrode used for these measurements had a diameter of 3.5 mm. When using a larger electrode with a diameter of 5 mm, the signal for nifuroxazide was higher, but this was compensated by a higher background current which resulted in the same signal to noise ratio.

Concerning preconcentration parameters like potential, time, and electrolyte pH, considerable differences in comparison with the behavior of the mercury electrode were observed. At a pH of 2 the best results were achieved when applying a potential of 800 mV, while adsorption occurred to a much smaller degree at 0 mV or with an open circuit. An increasing preconcentration efficiency was observed for potentials increasing from 200 to 800 mV, whereas for 1 V no adsorption could be discerned. When changing the pH to 4 and 6, the maximum of preconcentration efficiency shifted to 600 and then to 400 mV, while at pH 7 no adsorption was observed for a potential of 800 mV. The reason for this behavior was found in an oxidation signal (potentially due to the phenolic hydroxy group) which appeared at 1 V in an electrolyte of pH 2 and at about 750 mV in an electrolyte of pH 7. To sum up, adsorptive preconcentration worked better at higher potentials, but as soon as oxidation occurred preconcentration could no longer be achieved. Saturation of the surface was reached after 4 min and no decrease of the signal was found for longer adsorption times. After applying a poten-

tial of 800 mV in a buffer pH 2 for 2 min, 1 ng/mL of nifuroxazide could be quantitatively determined in a standard solution with a signal to noise ratio higher than 10. The method was found to be linear up to 200 ng/mL when using a preconcentration time of 120 s, the linear range could be shifted to higher concentrations by using shorter adsorption times.

Modification with polystyrene/divinylbenzene particles

To enhance the adsorption properties of the electrode surface for apolar compounds, the effect of the addition of polystyrene/divinylbenzene particles to the carbon paste was investigated. The addition of C18 particles to carbon paste electrodes has been investigated for the determination of bentazepam [16], methyl parathion and nitrophenol [17], and ioxynil and 2-methyl-3-nitroanilin [18] with varying results. As the adsorption behavior of polystyrene/divinylbenzene particles for nonpolar compounds often exceeds that of C18 particles [19], they were used in our work. The particle content of the paste was varied from 3.5 to 20%, the nujol content was kept at 30 mass %. Unfortunately the results were not satisfying. No change of the adsorption behavior was observed up to a particle content of 7%; at higher concentrations the baseline started to deteriorate while still no increase in preconcentration efficiency occurred. The current reproducibility of new surfaces was decreased even at a concentration of 3.5%, therefore no further experiments were conducted. The data indicate that in the present case hydrophobic adsorption is not the major mechanism for preconcentration.

3.3 Determination of nifuroxazide in urine

For both mercury and carbon paste electrodes the direct determination of nifuroxazide in a solution containing 2 mL of urine in 20 mL of background electrolyte was only possible at concentrations higher than 100 ng/mL. The surface of the carbon paste electrode had to be renewed after each measurement and an overlapping peak appeared in the polarogram when using the mercury electrode. To avoid these problems and to enhance the specificity of the method, an extraction step employing ethyl acetate was carried out prior to the determination. After this sample pretreatment step neither the determination with the mercury electrode nor with the carbon paste electrode gave any interfering signals as can be seen in Figs. 3 and 4. Solid phase extraction (SPE) with an RP-C18 material was tested as an alternative to liquid-liquid extraction and was found to yield satisfactory results; nevertheless, SPE showed no obvious advantages and the costs arising from the extraction cartridges were considerably higher than the chemicals necessary for liquid-liquid extraction. Therefore, the latter technique was used for all further measurements. In the region from 50 ng/mL to 8 $\mu\text{g/mL}$ of nifuroxazide in urine the recoveries were better than 97% with typical standard deviations of 1% for the mercury electrode and 3% for the carbon paste electrode. Concentrations down to 10 ng/mL of

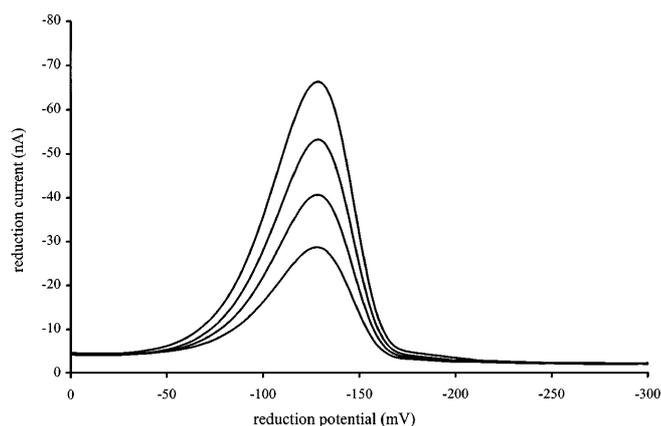


Fig. 3 Voltammogram of 50 ng/mL nifuroxazide in urine after liquid/liquid-extraction with ethyl acetate; measurement at the hanging mercury drop electrode with a preconcentration time of 120 s; standard additions 25 ng/mL each

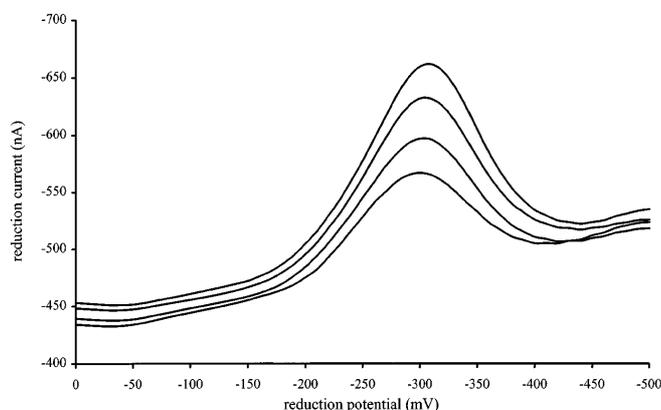


Fig. 4 Voltammogram of 50 ng/mL nifuroxazide in urine after liquid/liquid-extraction with ethyl acetate; measurement at the carbon paste electrode with a preconcentration time of 180 s; standard additions 20 ng/mL each

the compound in urine could be determined with a signal to noise ratio of larger than 10 to 1. The excellent recoveries for all concentrations proved the suitability of ethyl acetate as extraction solvent.

4 Conclusions

The electrochemical determination of nifuroxazide proved to be an attractive alternative to the existing methods us-

ing spectroscopy or high performance liquid chromatography. Concentrations as low as 1 ng/mL could be determined in a standard solution without any problems using the hanging mercury drop electrode or the carbon paste electrode. The influence of various preconcentration parameters was studied. The optimized parameters include the use of a background electrolyte with a pH of 2 and a preconcentration potential of 800 mV for the carbon paste electrode and the use of a background electrolyte with pH 4 and a preconcentration potential of 0 V for the hanging mercury drop electrode. The addition of polystyrene/divinylbenzene particles to the carbon paste did not improve the preconcentration behavior but led to an increased background noise. The determination in urine necessitated an extraction step prior to the electrochemical measurement. The use of ethyl acetate gave recoveries from 97 to 102%; no interfering coextractives were found. Concentrations as low as 10 ng/mL of nifuroxazide in urine could be determined.

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