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Voltammetric analysis of N-containing drugs using the hanging galinstan drop electrode (HGDE)

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The electrochemical behaviour of several N-containing voltammetric active drugs such as 1,4-benzodiazepines (chlordiazepoxide, nitrazepam and diazepam) as well as one nitro-compound (nitrofurantoin) and one azo-compound (phenazopyridine) is described using a new kind of liquid electrode, the hanging galinstan drop electrode. Concentrations of 10^{-5} – 10^{-8} mol L^{-1} are generally measurable. Differential pulse and adsorptive stripping voltammograms are recorded in different supporting electrolytes, like 0.1 M KNO_3, acetate buffer solution pH = 4.6 and phosphate buffer solution pH = 7.0. The effects of varying the starting potentials, U_{start} for DPV and accumulation times, t_{acc} for AdSV are considered. Briefly, it is shown that the novel galinstan electrode is suitable for reducing several functional groups in organic substances, here presented for N-oxide-, azomethine-, nitro- and azo-groups.

1. Introduction

1,4-Benzodiazepines are widely known as anti-anxiety drugs and sedatives with hypnotic and anticonvulsive character (Scultz 1982). To study the electrochemical reduction and oxidation behaviour of these substances is of high interest because this is fundamental for their relatively easy and fast quantitative electrochemical determination (Brooks et al. 1975; Brooks 1983; Smyth 1992; Kalvoda and Kopanic 1989). Usually, conventional mercury electrodes or solid electrodes, like glassy carbon or noble metal electrodes are used for the voltammetric determination of these compounds. In this paper we present the first study using the novel galinstan electrode for voltammetric analysis of selected 1,4-benzodiazepines and other N-containing drugs. Former studies with galinstan dealt with the reduction of metal cations using DPV (Surmann and Zeyat 2005) and anodic stripping voltammetry (Channaa and Surmann 2008).

For the benzodiazepine drugs the azomethine functional group in position four of the ring is characteristic. This group can be easily reduced by two-electron reduction at a potential of about −0.6 V at mercury electrodes. Besides, these substances have surface activity owing to the presence of phenyl rings. So they can be readily adsorbed on the electrode surface at potentials more positive than the reduction potentials and the determination step made by, for example, adsorptive stripping voltammetry at a static mercury drop electrode (SMDE) or hanging mercury drop electrode (HMDE) (Kalvoda 1984; Smyth and Yarnitzky 1988). Chlordiazepoxide, 7-chloro-2-methylamino-5-phenyl-3*H*-1, 4-benzodiazepine-4-oxide, is the only 1,4-benzodiazepine with a N-oxide functional group in position four, easily reducible in a two-electron reduction step. Three reduction steps have been reported for the electrochemical determination of chlordiazepoxide: the first one due to the two-electron reduction of the N-oxide at about −370 mV, the second step corresponding to a two-electron reduction of the 4–5-azomethine group at about −730 mV and the third one by reducing the second azomethine group in position one at about −1.17 V, all peak potentials at pH = 4.0 and by working with mercury electrodes (Jacobsen and Jacobsen 1971). Several studies have dealt with the determination of this drug and its N-desmethyl and lactam metabolites by differential pulse polarography (Hackman et al. 1974), adsorptive stripping voltammetry (Lorenzo and Hernandez 1987) and square wave voltammetry (Yarnitzky and Smyth 1991) preferring mercury as working electrode.

Nitrazepam, 1,3-dihydro-7-nitro-5-phenyl-2*H*-1, 4-benzodiazepine-2-one, has two reduction steps. First, the nitro-group in position seven is primarily reduced to the hydroxylamine- and then to the amine-derivative based on a four-electron reduction step. Second, like all the other 1,4-benzodiazepines, the 4–5-azomethine group is reduced by two-electron reduction (Smyth 1992).

Diazepam, 7-chloro-1-methyl-2-oxo-5-phenyl-3*H*-1, 4-benzodiazepine has only one moiety to be reduced, namely at the azomethine group in position four. There have been reports determining therapeutic levels of this drug in body fluids and in pharmaceutical formulations using DPP or other voltammetric methods (Dos Santos et al. 2002; Jacobsen et al. 1973; Berry 1971).

Nitrofurantoin, 1-[(5-nitro-2-furyl) 3-methylideneamino]-imidazolidine-2, 4-dione, represents the nitrofuran antibiotics. Like many other nitro-compounds nitrofurans have

been widely used to treat infections caused by anaerobic bacteria or protozarium like *Escherichia coli* and *Salmonella* (Leitner et al. 2001). After the prohibition from the European Union (Commission Regulation 1995; Commission Decision 2002) to use these substances as food additives for food-producing animals there a great demand exists for sensitive methods to determine low concentrations, for example in food samples. The prohibition was undertaken to avoid harmful effects on human health because of the toxicity of nitrofurans and their metabolites and due to their mutagenicity and carcinogenicity (Angelini et al. 1997). Voltammetric methods involving a rotating platinum electrode (Mason and Sandmann 1976) and a glassy carbon electrode (Morales et al. 1987) to detect this drug were developed.

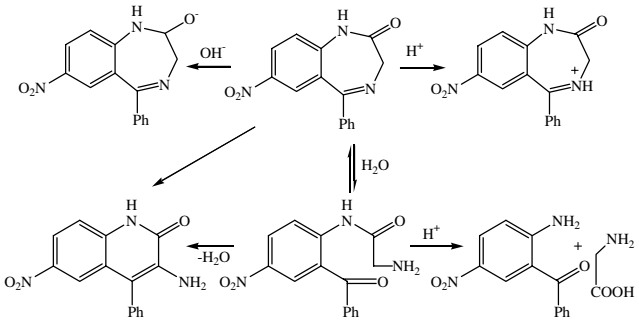
Phenazopyridine, 3-phenyldiazenylpyridine-2, 6-diaminemonohydrochloride, was also used as analgesic in the urinary tract (Wolff 1980). The azo-group of this substance is the only electroactive functional group; this can be reduced in one step to 2,3,6-triaminopyridine and aniline via a four-electron reduction. Recent research investigates voltammetric determination by complexation with copper (Çakir and Biçer 1997).

2. Investigations, results and discussion

For reducing the azomethine group, 1,4-bezodiazepines were the substances of choice in the history of polarography and voltammetry. Not only the electrochemical reduction but also the oxidation behaviour of these drugs was investigated (Smyth and Ivasaka 1985; Arenaza et al. 1995). Our investigations focus on the reduction behaviour of 1,4-benzodiazepines and other N-containing drugs, shown in Scheme 1.

Reducing the C=N functional group at the surface of the electrode is an irreversible process. This is the first time using a galinstan electrode surface to reduce organic functional groups. The studies were performed in 0.1 mol L^{-1} KNO_3, in acetate buffer solution at pH = 4.6 and in phosphate buffer solution at pH = 7.0. These supporting electrolytes were chosen due to the acidity constants of the azomethine group, and because the peak height in this pH range is constant. Besides, some 1,4-benzodiazepines like nitrazepam decompose in acidic or alkaline media as illustrated in Scheme 2 (Halvorsen and Jacobsen 1972). So a determination of this drug is only suggestive in neutral media.

Scheme 2: Decomposition of nitrazepam in acidic and alkaline media and its hydrolysis



In DPV and for our experimental conditions, chlordiazepoxide shows two reduction peaks, the first close to –700 mV (vs. Ag/AgCl) and the second at about –900 mV (vs. Ag/AgCl) (Fig. 1). We suggest the first reduction peak corresponding to the reduction of the N-oxide, whereas the second one results from the reduction of the 4–5-azomethine group.

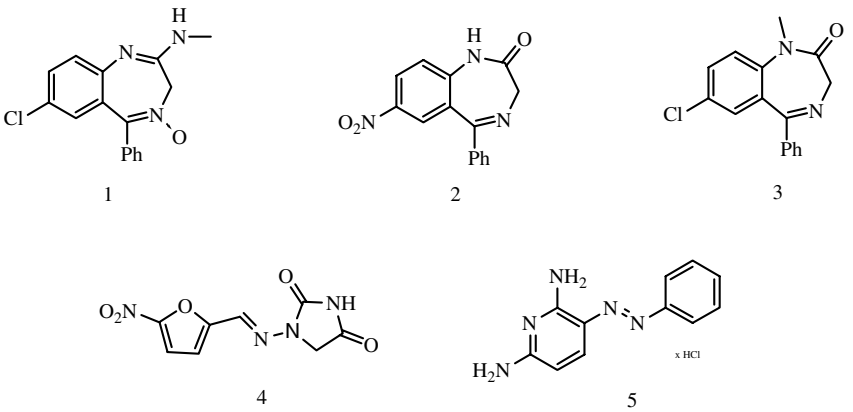
We suggest that the third reduction peak is not visible because of the limit on the cathodic site of the potential window of our acetate buffer solution. Each reduction peak, compared with the peak potential obtained with mercury electrodes, goes through a cathodic shift, which is different for every single peak potential. While the first reduction peak is shifted at about 330 mV in negative direction by applying differential pulse voltammetry, the second one is only shifted half the distance. So the third reduction peak would lie at approximately −1.25 V and subsequently outside the potential window.

For nitrazepam only one peak was obtained. In neutral phosphate buffer solution pH = 7.0 the reduction peak lies at about −0.83 V (Fig. 2). The second reduction peak is here also not visible in the range of the potential window.

By varying the initial potential we observe an anodic shift of the peak, whereas the peak current reaches a maximum at about $U_{Start} = -300$ to −400 mV (Fig. 3).

Because of the enhancement of the baseline it is possible to distinguish between the two concentrations (1×10^{-4} mol L^{-1} and 2×10^{-4} mol L^{-1}). We suggest that the peak intensity does not change drastically because the electrode surface is saturated with nitrazepam at these con-

Scheme 1: Test substances: 1) chlordiazepoxide, 2) nitrazepam, 3) diazepam, 4) nitrofurantoin, 5) phenazopyridine hydrochloride



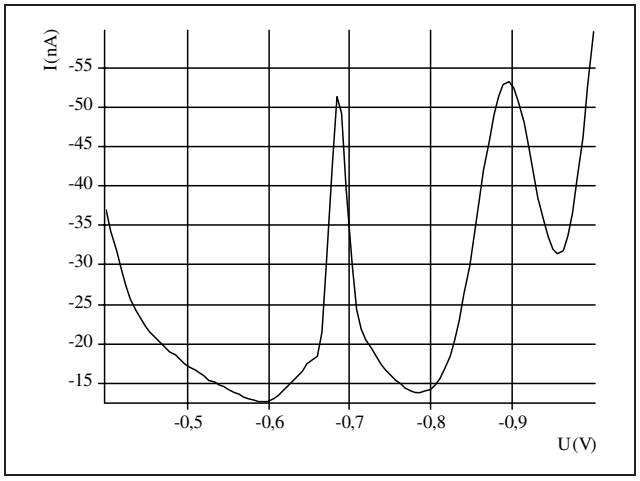


Fig. 1: Differential pulse voltammogram of 10^{-4} mol L^{-1} chlordiazepoxide in acetate buffer solution pH = 4.6. The first peak lies at $U_{Peak,1} = -686$ mV, $I_{Peak,1} = 33.26$ nA, the second one at $U_{Peak,2} = -892$ mV, $I_{Peak,2} = 29.01$ nA.

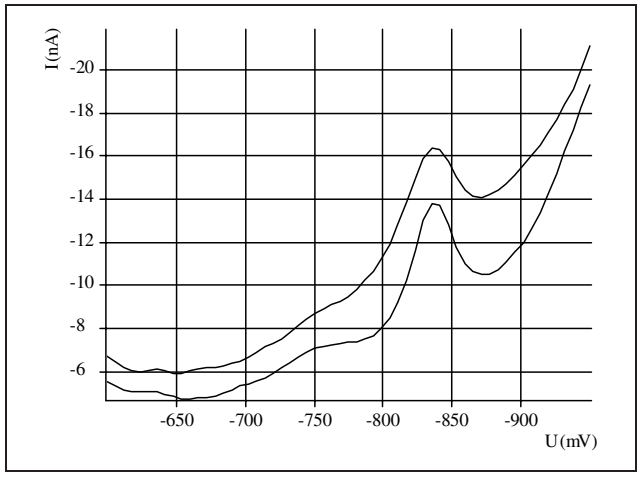


Fig. 2: DP voltammograms of nitrazepam at the HGDE in phosphate buffer solution pH = 7.0 at two different concentrations; initial potential $U_{Start} = -400$ mV; DPV of 10^{-4} mol L^{-1} nitrazepam, lower line, $U_{Peak} = -836$ mV, $I_{Peak} = 4.68$ nA; DPV of 2×10^{-4} mol L^{-1} nitrazepam, higher line, $U_{Peak} = -835$ mV, $I_{Peak} = 4.16$ nA

centrations. Nitrazepam has been fully adsorbed onto the HGDE surface.

Diazepam is slightly soluble in water. It was first solved in 96% ethanol and then pipetted into the electrolyte, so that the ethanolic concentration in the investigated solution was at least 1%. Diazepam gives only one reduction response at about −0.85 V due to the two-electron reduction of the imine group. In Fig. 4 the baseline of the ethanolic acetate buffer solution pH = 4.6 is shown in comparison with the DPV response by adding 5.5×10^{-5} mol L^{-1} of diazepam and adsorbing for t = 90 s. So we have again adsorption onto the electrode surface, but surprisingly no significant adsorptive accumulation for different accumulation times (see peak heights in the Table).

Aromatic nitro groups like in nitrofurantoin can be reduced in two steps in acidic solution. First the nitro group is reduced via a four-electron transfer to the phenylhydroxamine and then to the corresponding amine. The azomethine group in nitrofurantoin is also electro active and able to react to the amine (Scheme 3). Burmicz et al. (1976) have considered a reduction mechanism for this drug and showed that nitrofurantoin splits irreversibly in two smaller molecules by reduction, namely in imidazolidine-2,4-dione and 5-aminomethyl-furan-2-ylamine.

Prior to the determinations, nitrofurantoin was solved in 1% dimethylformamide to enhance the solubility in water. Figure 5 shows the linear dependence of the concentration of nitrofurantoin on the peak height. With increasing concentration the peak height naturally increases, too. The peak potential lies here in the range of −1.06 V, which is visible in a 1% DMF/ 0.1 M KNO_3 electrolyte system.

It is remarkable, that with nitrofurantoin we have a sensitive drug system which can be determined at concentrations down to 10^{-7} mol L^{-1}, respectively concentrations in the range of microgram per litre with normal differential pulse voltammetry, giving a good signal response (Fig. 6) and indicating a strong adsorption onto the electrode surface. The first four-electron reduction step to the phenylhydroxamine derivative is the current intensive step, visible in our potential window. All the following steps are strongly shifted in the negative direction, lying at potentials, which are 800 mV more negative than the main peak (by analogous inspection with the potentials obtained with mercury electrodes), that means outside the usable potential window.

Azo groups, like in phenazopyridine hydrochloride, can also be reduced to the corresponding amine with our galinstan electrode. The reduction is because of the splitting of the azo bridge an irreversible and fast process, which takes place by potentials of about −1 V at the HGDE, depending of the pH of the supporting electrolyte. Figure 7 presents the differential pulse voltammogram of 5×10^{-4} mol L^{-1} phenazopyridine hydrochloride in 0.1 M KNO_3.

The differential pulse voltammetric procedures developed with the hanging galinstan drop electrode enables the determination of electro active organic drugs, like benzodiazepines, nitro-compounds and azo-compounds with concentrations that might be found in pharmaceutical formulations. Organic compounds are in direct contact with the electrode, that means they are adsorbed; another principle as by the determination of ions. It was shown that by work with the HGDE it is also able to determine organic compounds by further adsorption onto the electrode surface and by following reduction of the functional groups, like the N-containing groups applied here. The characteristics of galinstan electrodes similar to those of

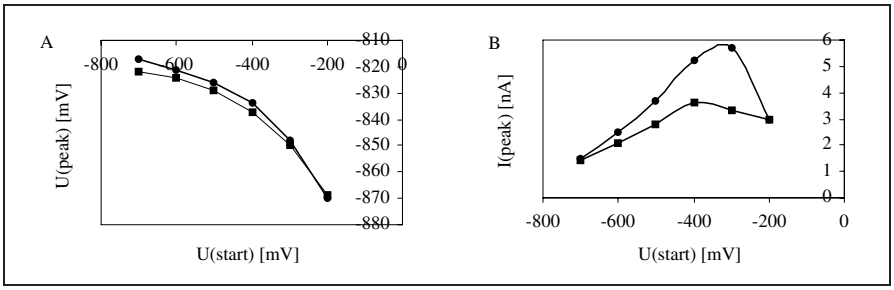


Fig. 3:
Dependence of a) the peak potentials, b) the peak heights on the initial potential U (Start) for different concentrations. Nitrazepam concentrations (mol L^{-1}) = 1×10^{-4} (circles), 2×10^{-4} (squares), conditions: DPV, phosphate buffer solution pH = 7.0

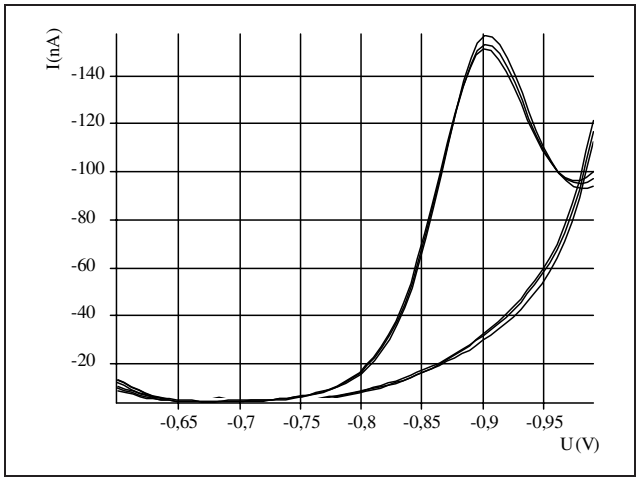


Fig. 4: DP voltammograms of 5.5×10^{-5} mol L^{-1} diazepam for t = 90 s with belonging ethanolic acetate buffer solution baseline (pH = 4.6), $U_{Peak,1} = -900$ mV, $I_{Peak,1} = 93.85$ nA; $U_{Peak,2} = -899$ mV, $I_{Peak,2} = 89.16$ nA; $U_{Peak,3} = -898$ mV, $I_{Peak,3} = 86.47$ nA

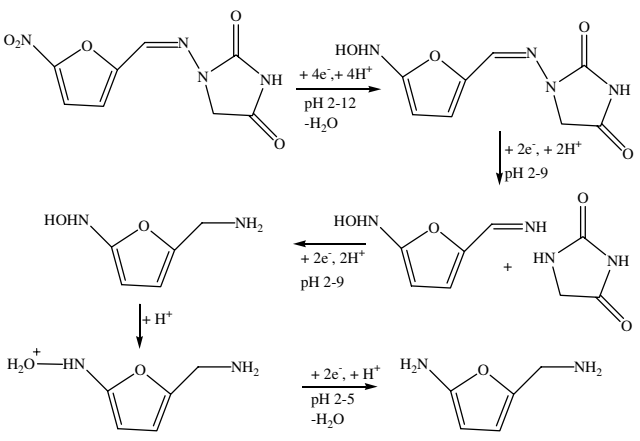
Table: Differential pulse adsorptive stripping voltammetric response (AdSV) of 2×10^{-5} mol L^{-1} diazepam at different accumulation times and same accumulation potential

t (s)	0	90	180
U_{Peak} (mV)	−872	−857	−861
I_{Peak} (nA)	21	18	16

$U_{acc} = -700$ mV; conditions: HGDE, ethanolic acetate buffer solution pH = 4.6

mercury electrodes, like the simply renewable surface and the resulted good reproducibility, the high hydrogen potential and the low background makes the HGDE a possible liquid electrode alternative to mercury even for the analysis of organic molecules. The detection range of differential pulse voltammetry is not only suitable for the determination of these drugs in pharmaceutical preparations but also for quantification of these drugs when body fluid levels are in microgram per litre level or higher, as in toxicological cases. Continuous research in the field of adsorptive accumulation of organic compounds with the galinstan electrode is required. Then, it would be possible to determine concentrations which are lower than 10^{-7} mol L^{-1}.

Scheme 3: Reduction mechanism of nitrofurantoin



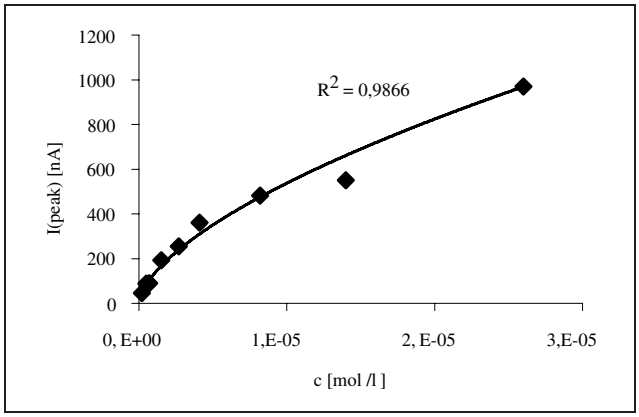


Fig. 5: Dependence of the concentration of nitrofurantoin on the peak height. Conditions: HGDE, DPV with $U_{ampl} = -50$ mV, t.step = 1.00 s, t.meas = 20 ms, t.pulse = 40 ms and sweep rate: 6 mV s^{-1}, supporting electrolyte: 0.1 M KNO_3 with 1% DMF

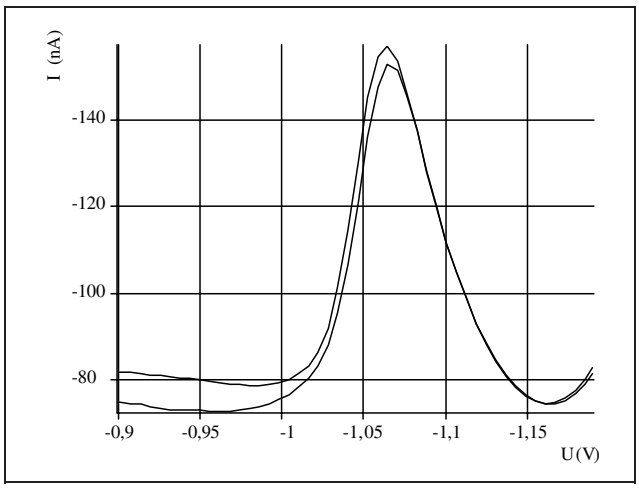


Fig. 6: DP voltammograms of 7×10^{-7} mol L^{-1} nitrofurantoin, obtained by applying the same experimental conditions as described in figure legend 5; $U_{Peak} = -1.064$ V, -1.062 V; $I_{Peak} = 78.12$ nA, 79.78 nA

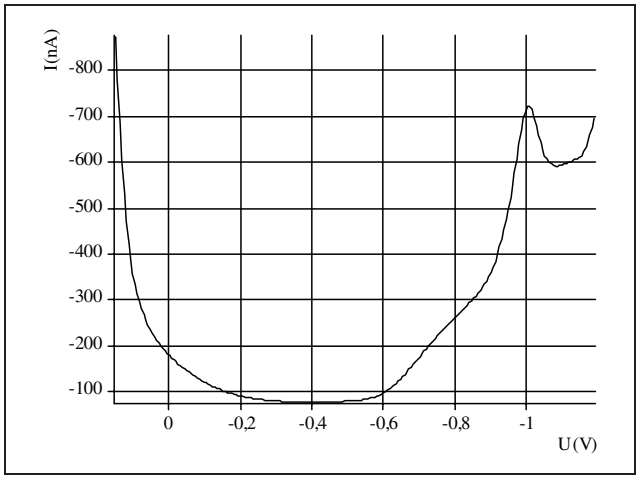


Fig. 7: DP voltammogram of 5×10^{-4} mol L^{-1} phenazopyridine hydrochloride in 0.1 M KNO_3, obtained with the above mentioned conditions (see figure legend 5), $U_{Peak} = -1.003$ V, $I_{Peak} = 238.2$ nA

3. Experimental

3.1. Apparatus

Voltammetric measurements were obtained by use of a VA Trace Analyzer 746 with pertinent VA Stand 747, driven by VA Database 2.00 software (Metrohm, Herisau, Suisse). pH values were measured with a WTW digital pH 525 pH meter (Weilheim, Germany) equipped with a combined glass-Ag/AgCl electrode.

3.2. Electrodes

Applying to the three-electrode technique, voltammetric determinations were equipped with the hanging galinstan drop working electrode (for details, see Surmann and Zeyat 2008), a double-junction silver/silver chloride reference electrode and a platinum coil auxiliary electrode.

3.3. Chemicals

Chlordiazepoxide and diazepam was obtained from Fagron (Barsbüttel, Germany). Nitrazepam, nitrofurantoin and phenazopyridine were supplied by Sigma Aldrich (Steinheim, Germany). All buffers and supporting electrolytes were prepared from analytical-grade reagents, obtained from Merck (Darmstadt, Germany) and Fluka (Seelze, Germany). Dilute Solutions were prepared by using high-purity doubly distilled water. Galinstan was received from Geratherm Medical (Geschwenda, Germany).

3.4. Recording the voltammograms

Before each voltammetric measurement the solutions were deoxygenated by purging with high-purity nitrogen (99.999 vol%, Praxair Berlin, Germany) for 10 min after having been purified by pyrogallol, dissolved in 3 M KOH. 20 ml of the blank supporting electrolyte were put into the electrochemical cell before each series of measurements.

We chose differential pulse voltammetry and adsorptive stripping voltammetry as measuring methods. For differential pulse voltammetry (DPV) the measurement conditions were as follows, unless otherwise described: pulse amplitude, −10 mV; pulse repetition time, 1 s; measuring time, 5 ms; pulse time, 60 ms; scan rate, 6 mV s^{-1}.

For adsorptive stripping voltammetry (AdSV) the instrumental settings were as for DPV, but with an accumulation time changing from 0 to 180 s. The accumulation potential (usually −0.7 V) was applied to the HGDE by simultaneous stirring of the solution. After the accumulation time the stirring was stopped and after 10 s for equilibrium forming the voltammogram was recorded by applying a negative-going potential scan. The scan was usually terminated at −1.05 V.

3.5. Preparation of the acetate buffer solution pH = 4.6

5.4 g of sodium acetate was dissolved in 50 ml of doubly distilled water. 2.4 g of acetic acid (98%) were added and the solution was diluted with water to 100 ml. If necessary, the pH was corrected.

3.6. Preparation of phosphate buffer solution pH = 7.0

82.4 ml of a solution of sodium monohydrogenphosphate (71.5 g L^{-1}) and 17.6 ml of a solution of citric acid (21 g L^{-1}) were mixed. The pH was corrected, if required.

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