Adsorptive stripping voltammetric detection of daunomycin at mercury and bismuth alloy electrodes

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We investigated the analytical response of daunomycin, an effective drug used for cancer chemotherapy, at different types of electrodes. The following five different materials for the electrodes have been compared: hanging mercury drop, gold, gold/bismuth, gold/mercury and silver mercury. Especially at the hanging mercury drop electrode and also at the silver/mercury and gold/mercury amalgam electrode, nicely shaped cyclic voltammograms with nearly no peak separation could be found whereas both the gold and the gold/bismuth electrode showed a significant peak separation. The linear dependency to the scan rate of the signal of all the electrodes is a hint for adsorption rather than diffusion control. In adsorptive stripping voltammetry it could be shown that especially the gold/bismuth alloy (4 % Bi) has a great linear calibration range of daunomycin, whereas the linear range at the hanging mercury drop electrode is surprisingly limited. The reason for the advantageous properties of the gold/bismuth alloy in adsorptive stripping analysis can probably be addressed to sufficiently strong and particularly constant adsorption at low daunomycin concentrations at the electrode surface. Hence, the gold/bismuth electrode can be recommended for adsorptive stripping voltammetry of trace amounts of daunomycin.

1. INTRODUCTION

Electrochemical stripping analysis is a very important and widely used tool for the trace determination of various elements and compounds. Major advantages include low detection limits coupled with good selectivity and reproducibility due to the in situ pre-concentration step. This is especially true for adsorptive stripping voltammetry (AdSV), where the analyte is deposited on the working electrode by adsorption. However, an electrode material is needed providing not only good adsorption of the analyte but also a stable and reproducible electrode surface. The most commonly used working electrode material in this method is mercury. The advantages of the hanging mercury

drop electrode (HMDE) include renewable surface and high hydrogen overpotential. Thus, it is possible to study reactions of compounds that otherwise would be hidden by reduction of water. Due to toxicity of mercury, several approaches were supposed to replace the mercury by other materials such as carbon, gold etc., but none of these materials was found to be an acceptable replacement for common mercury electrodes. Nevertheless, there is high interest in new alternative electrode materials with similar properties especially for on-site environmental monitoring.

Bismuth provides several advantageous properties in electrochemical analysis. Compounds of Bi(III) adsorb on platinum electrodes and can catalyze the oxidation of glucose [1]. Wang et al. [2] introduced the bismuth film electrode (BiFE) representing an attractive alternative to mercury film electrodes. They demonstrated that bismuth films, deposited on various electrode substrates, offer well-defined, undistorted and highly reproducible signals of heavy metals. High hydrogen overvoltage, favourable signal-to-background ratio, and excellent resolution and reproducibility are sometimes superior to those of common mercury electrodes. Besides, bismuth is an environmentally unconcerned non-toxic element. All bismuth electrodes provide a wide potential window of -1.2 to -0.2 V vs. SCE. It allows for stripping detection of most metals that can be determined also at mercury electrodes. Examples include anodic stripping of zinc, cadmium, lead, and tin [2,3,4,5,6,7,8,9,10], as well as adsorptive stripping of nickel [11], uranium [12], aluminium [13], and cobalt [14,15]. Organic compounds such as organic nitro compounds have been determined by means of differential pulse voltammetry and amperometry [16]. The bismuth electrode can also be modified with a Nafion film to minimize the interferences of surfactants during the voltammetric determination of metal ions like lead and cadmium in some real samples [17]. Bismuth electrodes are less prone to interferences by dissolved oxygen in non-deaerated solutions, often still yielding a flat baseline [2,11].

In case of the preparation of a BiFE for AdSV application, external plating is recommended. However, non-complexed Bi(III) ions very easily form insoluble hydroxo-compounds. Due to this risk of hydrolysis, only acidic media can be considered as plating solutions for the bismuth film preparation. The application of bromide containing plating solution and the use of strongly acidic media (1 mol/L HCl) allows using higher concentrated Bi(III) solution (0.02 mol/L) and minimizing the risk of their hydrolysis [15].

Furthermore, the carbon paste electrode (CPE) can be used as a substrate for the preparation of BiFE [5]. However, the electrochemical activity of the BiFE depends on the structure of the electrode surface, which is not ideal with CPEs for external plating of a film. AFM studies of a bismuth-modified carbon paste electrodes have recently been published [18]. The bulk phase modification of carbon paste with Bi_2O_3 is very efficient and useful for the stripping voltammetric determination of Cd and Pb [19].

Only a few contributions report about the bismuth bulk electrode. Pauliukaite et al. [20] investigated the cathodic behaviour of polycrystalline metal bismuth electrodes during the voltammetric detection of cadmium(II) and 2-nitrophenol. The characteristics of the BiBE under anodic conditions, i.e., at bismuth surface coated with a thin conductive Bi_2O_3 film, were examined by testing potassium hexacyanoferrate(III) and hexaammineruthenium(III) chloride. Daunomycin has been analyzed by means of adsorptive stripping voltammetry on a bismuth bulk electrode [21]. We recently reported about self-assembled monolayers of alkanethiols on bismuth [22]. A general problem

arising during the application of massive bismuth electrodes is corrosion and formation of oxide films on the surface. Hence, the electrode surface has often to be renewed by polishing. Electroanalysis on bismuth electrodes has been reviewed [23,24,25].

Mikkelsen and Schrøder found that small addition of bismuth (about 4 %) can improve the analytical applicability of electrode materials with low hydrogen overvoltage such as gold [26,27] and silver [28,29]. Such electrode materials combine advantages of both bismuth and noble metal electrodes. They are less prone to corrosion. The alloy made of gold and 4 % bismuth for example has been found to be applicable in both the negative (detection of cadmium and iron) and positive (detection of mercury) potential range [30].

Amalgam electrodes have been introduced by Mikkelsen and Schrøder [28,31], and by Yosypchuk and Novotny [32], independently. Solid amalgams provide most of the positive properties found on mercury. However, they still contain considerable amounts of the toxic element mercury. Organic [33,34] and inorganic [31,35,36] substances have been analyzed on amalgam electrodes. Nucleic acids have also been detected on amalgam electrodes [37]. Analytical methods basing on amalgam electrodes have been reviewed [38].

Daunomycin (DM), an anthracycline antibiotic, is an effective drug used for cancer chemotherapy. In electroanalytical chemistry it is also widely used as an indicator of DNA [39]. Cytostatic activity of anthracyclines like daunomycin has been known for over 20 years, but it has not been resolved, by which mechanism they exert the cytostatic action. Apparently the DM molecule intercalates into GC base pairs of DNA or binds to nucleotides and subsequently the synthesis of DNA and RNA is inhibited. Ultrafast electron-transfer reactions between DM and base guanine were found. The reduced DM radicals can catalyze super oxide formation from dioxygen which then triggers redox cycling. Thus, molecular oxygen also plays a partial role in the drug action. The most important side effect of anthracycline antibiotics is their cardio toxicity at high doses. Patients taking these drugs are at increased risk of arrhythmias, irreversible cardio myopathy and the risk of congestive heart failure. For that reason it is very important to monitor the drug level. A traditional assay for the detection of these compounds is HPLC (High Performance Liquid Chromatography) with fluorescence detection [40]. We found that daunomycin strongly adsorbs on bismuth electrodes, which can be used for trace detection of this compound by means of AdSV [21].

This contribution reports for the first time about the application of gold-bismuth alloys for adsorptive stripping voltammetry. As a model analyte, daunomycin has been determined at different electrode materials including Au/Bi (4 % Bi), Ag/Hg, Au/Hg, and the hanging mercury drop electrode (HMDE).

2. EXPERIMENTAL PART

2.1. Materials

All measurements were carried out in 50 mM phosphate buffer solution (pH 7) or 200 mM acetate buffer solution (pH 5). During the measurements with the Autolab, we applied an Ag/AgCl (3 M KCl) reference electrode (from Meinsberg Sensortechnik GmbH, Meinsberg, Germany) and a

laboratory-made platinum counter electrode. For the measurements with the polarograph, we used an Ag/AgCl (3 M KCl) reference electrode und a platinum counter electrode (delivered by Metrohm AG, Filderstadt, Germany).

The chemicals potassium dihydrogenphosphate, potassium hydrogenphosphate, potassium acetate, silver powder and daunomycin were purchased from Fluka. The used gold/bismuth rod was delivered by MaTeck GmbH, Jülich, Germany. The used purified water (18 MOhm, TOC < 2 ppb) was prepared by means of an SG Ultra Clear water system (SG Water GmbH, Barsbüttel, Germany).

2.2. Instrumentation

The measurements with the gold/bismuth electrode (Au/Bi), the silver/mercury electrode (Ag/Hg) and the gold/mercury electrode were carried out with an Autolab PGSTAT 20 (Eco Chemie, Utrecht, Netherlands). All experiments at the hanging mercury drop electrode were performed by means of a 797 VA Computrace (Metrohm AG, Filderstadt, Germany).

2.3. Preparation of the electrodes

2.3.1. Gold-bismuth electrode

A gold rod containing 4 % bismuth was delivered by Mateck GmbH (Germany, Jülich). We machined a rod with 3 mm in diameter. A 10 mm diameter PTFE rod with a 3 mm hole was used as housing for the gold-bismuth rod. The connection has been realised by a copper bolt inside the PTFE rod. The detailed construction of this type of electrode has been described earlier [21].

2.3.2. Gold mercury electrode

The formerly described (indirectly heatable) LTCC (Low Temperature Cofired Ceramics) sensor, but here with a gold instead of a platinum electrode [41,42], was dipped for 30 s into mercury. With the thin mercury and gold amalgam film on it, the electrode was left for one hour to allow further amalgamation and then polished carefully with slurry consisting of alumina powder (first 0.3 micron, then 0.05 micron) and water.

2.3.3. Silver amalgam electrode

To build a silver amalgam electrode, we followed an earlier procedure [32], and firstly drilled a 3 mm hole into a 10 mm diameter PTFE rod. There has been a connection made of copper inside this rod forming the base of the hole. Secondly, we filled the hole with silver powder and pressed it. On the top of the compressed powder we put a drop of mercury and left the electrode for 24 h. Finally we removed the liquid mercury and ground the electrode with fine sandpaper.

Before each measurement, the electrodes were firstly ground on very fine (grit #1000) wet sandpaper. Secondly the electrodes were polished with alumina slurry in two steps (first 0.3 micron, then 0.05 micron, both from Bühler, Uzwil, Switzerland).

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetry

As depicted in Fig. 1, we applied cyclic voltammetry to study the affinity of daunomycin to adsorb on different electrode materials.
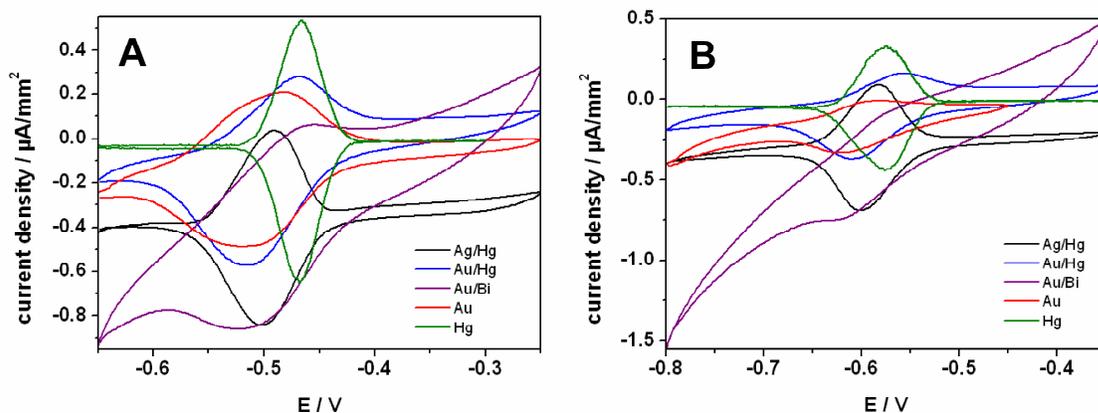

Figure 1. Cyclic voltammograms of 10 µM daunomycin on different electrode materials in A) 200 mM acetate buffer at pH 5.0 and B) 50 mM phosphate buffer at pH 7.0; scan rate 200 mV/s.

The hanging mercury drop electrode is well known to provide a perfect and easily renewable surface but also to strongly adsorb many organic substances. We found a nearly perfect cyclic voltammogram of adsorbed daunomycin on mercury. Here, the peak potentials of the cathodic and the anodic scan have been equal in both acetate and phosphate buffer. As one would expect following a general rule, the peak potentials are more positive in the acetate buffer at lower pH. The silver amalgam electrode exhibits almost the same behaviour. This can be attributed to a thorough amalgamation of the silver powder which served as a base for the amalgam formation. In contrast, both the gold amalgam and the gold electrodes show a significant peak separation, which can be attributed to a considerable contribution of analyte diffusion. Amalgamation at the LTCC gold layer electrode is probably not as efficient as compared to the silver amalgam electrode. Therefore, adsorption is not as strong at these types of gold electrodes. The peak shapes are deformed to a significant degree. This is particularly true for the gold-bismuth alloy electrode in phosphate buffer. Another possibility for such peak separation is kinetic hindrance of the electrode reaction. In earlier studies [21], a bismuth bulk electrode exhibited a cyclic voltammetric behaviour of daunomycin that now can be compared with the silver amalgam electrode. Obviously, a small percentage of bismuth in an alloy with gold is not sufficient to meet all the positive properties of the pure bismuth electrode.

We also studied the effect of the scan rate. Dependence of the cyclic voltammetric peak currents upon the scan rate is displayed in Figure 2.

A linear relation between peak currents and scan rate is a hint for reactions of an adsorbed or otherwise deposited substance. Diffusion controlled electrode processes would, in contrast, yield a linear function to the square root of the scan rate. We found a linear dependence for all types of

electrodes studied, particularly for gold, gold-bismuth, silver amalgam, and gold amalgam; and a somewhat strange behaviour of the HMDE.
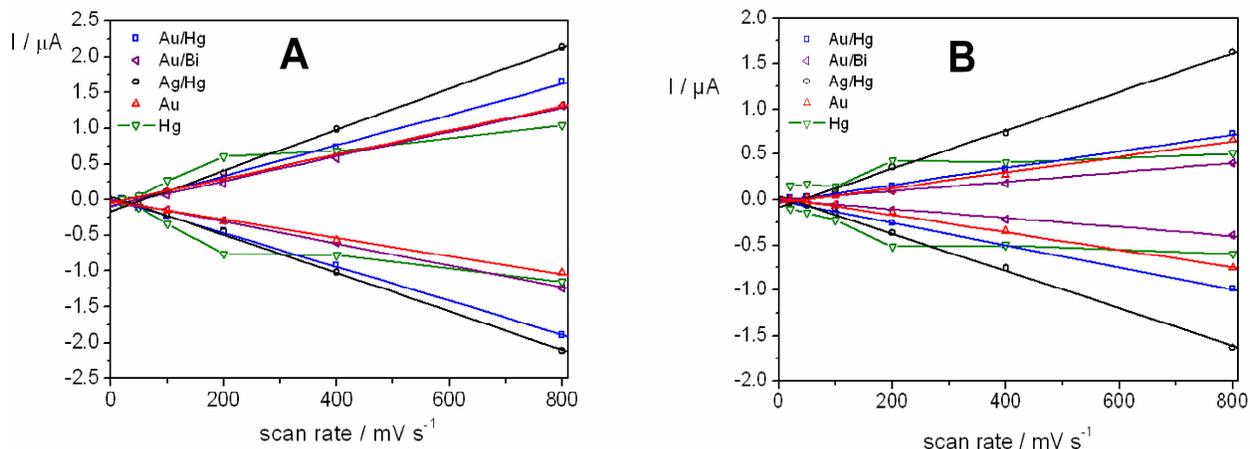
Figure 2. Effect of the scan rate upon cyclic voltammetric signals of daunomycin in A) acetate buffer and B) phosphate buffer. Other conditions as in Fig. 1.

3.2. Adsorptive stripping voltammetry

Figure 3 depicts effect of deposition time on a mercury drop electrode. The background has been very constant during all experiment, since the drop has been renewed every time. At 60 s deposition time and above, we observed nearly constant stripping signals indicating a saturated mercury surface.
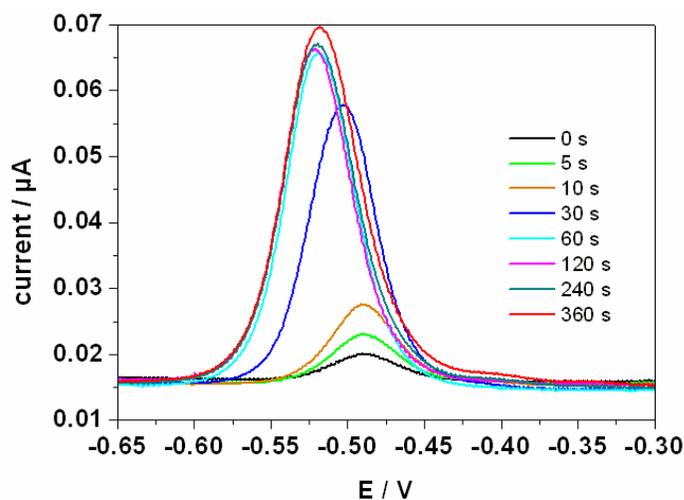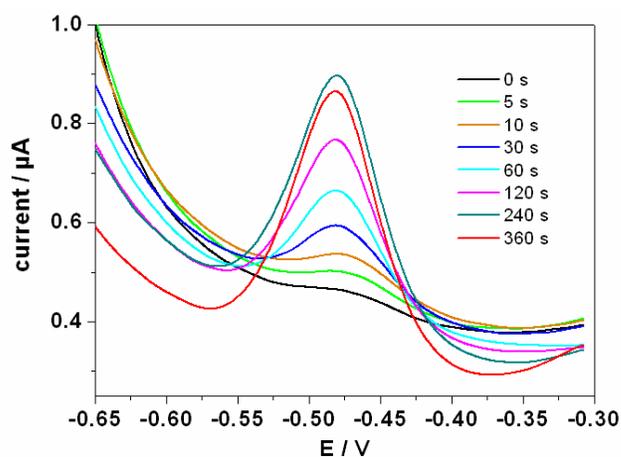

Figure 3. Effect of the deposition time upon DPV adsorptive stripping detection of 10 μM daunomycin in 200 mM acetate buffer at pH 5.0 on a HMDE; deposition potential -0.65 V, scan rate 10 mV/s, pulse amplitude 25 mV.

Figure 4 exhibits the influence of the deposition time upon the adsorptive stripping DPV signals of daunomycin at a silver amalgam electrode. Here, a slightly changing background current has been observed, a typical disadvantage of solid metal electrodes. We observed saturation beginning at 240 s deposition time. This is significantly longer than on mercury, probably due to significant lower adsorption of daunomycin compared to pure mercury.
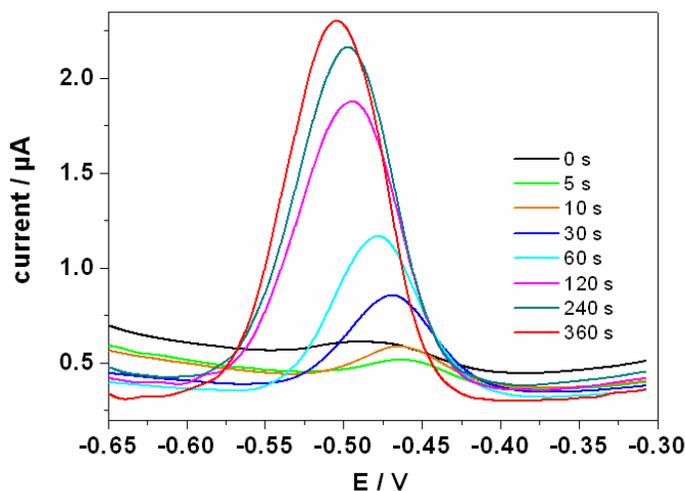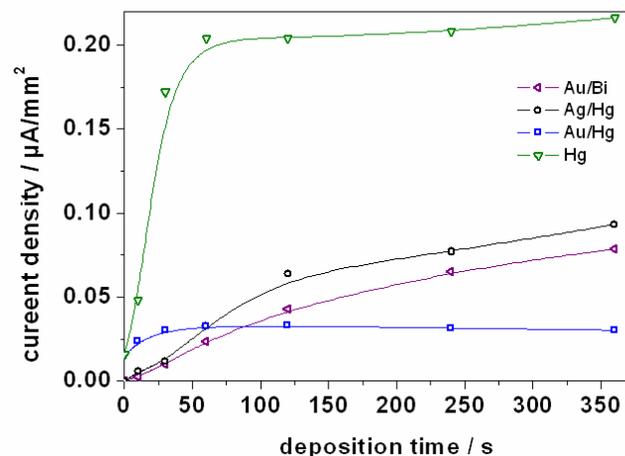

Figure 4. Effect of the deposition time upon DPV adsorptive stripping detection of daunomycin on a silver amalgam electrode; conditions as in Fig. 3.

Figure 5 displays the effect of the deposition time upon the adsorptive stripping DPV signals of daunomycin at a gold-bismuth electrode. In contrast to cyclic voltammetry, DPV provides well shaped stripping peaks on this electrode material. Although the background changed even more during one series compared to the silver amalgam electrode, all signals could be easily processed. Saturation has not been observed even at 360 s deposition time.
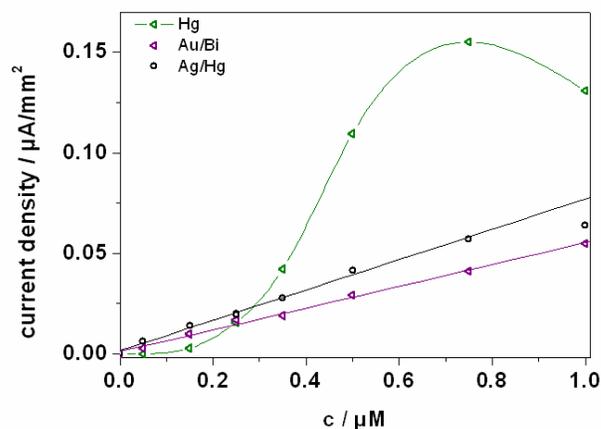

Figure 5. Effect of the deposition time upon DPV adsorptive stripping detection of 10 µM daunomycin in 200 mM acetate buffer on a gold-bismuth electrode; other conditions as in Fig. 3.

Figure 6 illustrates the effect of deposition time on the adsorptive stripping DPV signals of daunomycin on four different electrodes. Whereas gold amalgam and mercury electrodes showed saturation at 30 and 60 s, respectively, the stripping voltammetric signals on silver amalgam and gold-bismuth electrodes has been increasing in the whole range between 0 and 360 s deposition time.

Figure 6. Effect of the deposition time upon DPV adsorptive stripping detection of 10 µM daunomycin in 200 mM acetate buffer at pH 5.0 on several electrodes; other conditions as in Fig. 3.

Figure 7 depicts calibration plots of daunomycin on three different electrodes. The very strong adsorption on mercury limits the linear range significantly. The sigmoidal shape of the calibration curve obtained at the HMDE can probably be addressed to the formation of daunomycin multi layers during the deposition step. At the lowest concentrations, a relative weak adsorption has been visible, whereas the surface seemed saturated at concentration above 700 nmol/L. The silver amalgam electrode has exhibited a linear range up to 750 nmol/L. Only the gold-bismuth electrode yielded linearity in the whole range between 0 and 1000 nmol/L daunomycin.

Figure 7. Calibration plots of DPV adsorptive stripping detection of daunomycin in 200 mM acetate buffer at pH 5.0 on several electrodes; deposition time 120 s, other conditions as in Fig. 3.

4. CONCLUSIONS

We could demonstrate the applicability of all five electrode materials for the detection of daunomycin in phosphate and acetate buffer solution. Especially the gold/bismuth electrode is well applicable due to the great linear concentration range. The strongest adsorption has been found for the hanging mercury drop electrode, which causes a serious drawback in regard of lacking linearity of the calibration plot. Silver amalgam and gold amalgam electrodes perform better than mercury but still show effects of saturation due to strong adsorption. Despite the large background in the cyclic voltammograms, the gold/bismuth electrode seems to be a very promising electrode material for the routine analysis of daunomycin. This is supported by the easy handling of the Au/Bi electrodes and its mixture of nontoxic metals.

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