

Full Paper

Design of a Flow-through Polarographic Sensor Based on Metal Films for Determining N-nitrosodiethanolamine Levels in Rabbit Biological Fluids

Lai-Hao Wang *, Hung-Chang Hsia and Yuan-Zhi Lan

Department of Applied Chemistry, Chia Nan University of Pharmacy and Science, 60 Erh-Jen Road, Section 1, Jen Te, Tainan 71743, Taiwan

E-mails: vivideric678@hotmail.com (Hung-Chang Hsia); ryan-0702@yahoo.com.tw (Yuan-Zhi Lan)

* Author to whom correspondence should be addressed. Fax: 886-6-266-7319; E-mail: e201466.wang@msa.hinet.net

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Abstract: The construction and characterization of a flow-through polarographic detector for catalyzing the electroreduction of N-nitrosodiethanolamine (NDELA), is discussed. The flow-through cell is equipped with a gold wire electrode (a thin mercury film deposited on a gold substance). The response is evaluated with respect to substance diameter, length, concentration of modifying film, operating potential, supporting electrolyte and pH, and flow rate in the DC mode. The system allows the determination of N-nitrosodiethanolamine in rabbit biological fluids with relatively inexpensive equipment.

Keywords: Flow-through polarographic detector; thin-film modified metal electrode; N-nitrosodiethanolamine; rabbit biological fluids.

1. Introduction

During the last ten years, evidence has been collected indicating that N-nitrosamines are one of the most important classes of chemical carcinogens [1]. Secondary amines are common constituents of foodstuffs and can react with naturally occurring or added nitrite in acidic conditions or in the stomach to form N-nitrosamines [2-3]. N-nitrosodiethanolamine (NDELA) is a widespread and potent liver and nasal-cavity carcinogen in several species of rodents [4-6]. NDELA easily penetrates human skin and has been found in the urine of exposed metal workers [7]. Hazard characterizations and exposure-

response analyses of N-nitrosamines have been investigated in environmental water and the rubber industry [8-10].

Methods for determining N-nitroso compounds in foods and biological fluids system include a gas chromatography (GC) method coupled either with a mass spectrometer (MS) [11-14], and GC coupled with a thermal energy analyzer (TEA) [15-16]. Chemiluminescence detection after reducing nitrite to nitric oxide using suitable reductants has also been adopted [17-19]. High-performance liquid chromatography with either an ultraviolet (UV) [20] or a fluorescence detector [21] has been also used. A polarographic detector using the dropping mercury electrode (DME) has been used to study the pulse polarographic behaviour of N-Nitrosamines [22-26]. Electrochemical reduction of p-nitrosodiphenylamine was investigated at solid electrodes (Ag, Au, Pt, GCE, and Cu) using cyclic voltammetry and rotating disc voltammetry [27]. Adsorptive stripping voltammetry at resorcinarenetetra-thiol modified gold electrodes has been used for the determination of N-nitroso-n-butyl-n-propylamine [28]. However, the GC sample-preparation procedure, with derivatization and chemiluminescence detection before GC-TEA analysis, was very time-consuming. Liquid chromatography mass spectrometry (LC-MS), which has now become a routine technique, has also been used to analyze N-nitrosamines in various compounds. The drawbacks of LC-MS are that the equipment is expensive and complex to operate. We previously [29] developed a reverse phase liquid chromatography (RP-LC) technique that uses a photodiode array detector to detect the N-nitrosamines in cosmetic products and rabbit biological fluids. A liquid chromatography electrochemical detector (LC-ECD) was recently developed. There are two main types of LC-ECD: (i) voltammetric detectors using solid electrodes [23, 24] and (ii) polarographic detectors using dropping mercury electrodes [25, 26]. NDELA, however, is not easily reduced at a solid electrode. In addition, mercury is toxic and causes environmental problems. The aim of the present study was to design a solid electrode, such as a gold or a glassy-carbon electrode, modified by a film containing a metal ion as the working electrode. Metal film-based electrode materials were evaluated for reductive LC-ECD during the present study.

2. Results and Discussion

2.1. Choice of analytical method

A thin film of mercury was deposited on a gold electrode, a glassy carbon electrode (GCE), and a carbon fiber electrode (CFE), and a thin film of lead was deposited on a GCE. To compare modified electrode substances, pictures of the four electrodes were taken using a scanning electron microscope (SEM) (JXA-840; JEOL Co., Tokyo, Japan). Mercury nanoparticles were distributed more uniformly on the gold electrode (Fig. 1a) than on the other three (Fig. 1, b-d). The mercury particles were dispersed with very slight aggregation (Fig. 1, b and c), because it was weakly absorbed on the GCE and CFE surfaces. However, the particles of lead on the GCE surface (Fig. 1d) were dispersed with more aggregation than the mercury on the surface GCE (Fig. 1b).

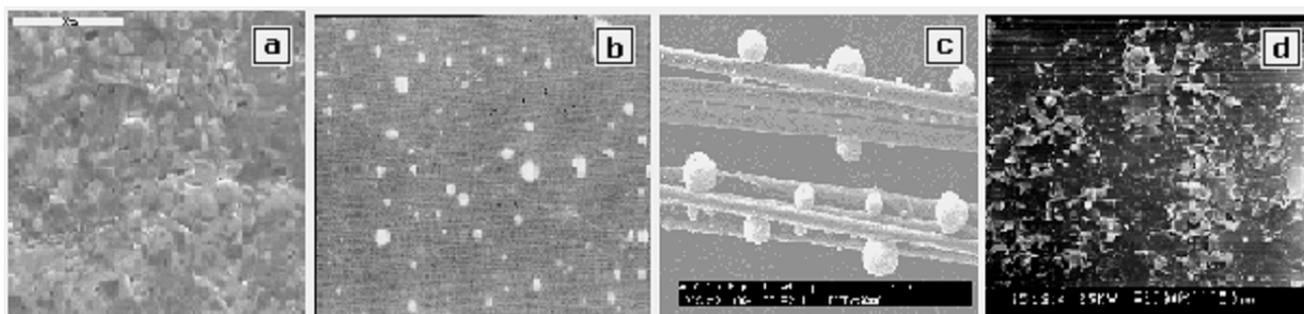


Figure 1. Scanning Electron Micrographs (at 8 kV) of Hg and Pb particle distribution on the different deposit surfaces: (a) Hg (4mM/gold), (b) Hg (4mM/GCE), (c) Hg (4mM/CFE), (d) Pb (6 mM)/GCE.

The surface morphology of a Hg/Au and Pb/GCE nanoparticle hybrid film was investigated using atomic force microscopy (AFM) with a scanning probe microscope (SPM) (NanoMan NS4+D3100; Digital Instrument Company, Taipei, Taiwan) that provided molecular imaging. Figures 2 and 3 show AFM images of a Hg/Au and Pb/GCE, respectively. Because the xy data are affected by tip-sample convolution, the observed diameters of nanoparticles appear larger than their actual sizes. However, the corresponding z -scale data (height) are closer to actual size. The mercury diameter (~ 500 nm) on the gold surface (Fig. 2, a and b) is smaller than lead diameter (~ 1000 nm) on the GCE surface (Fig. 3, a and b).

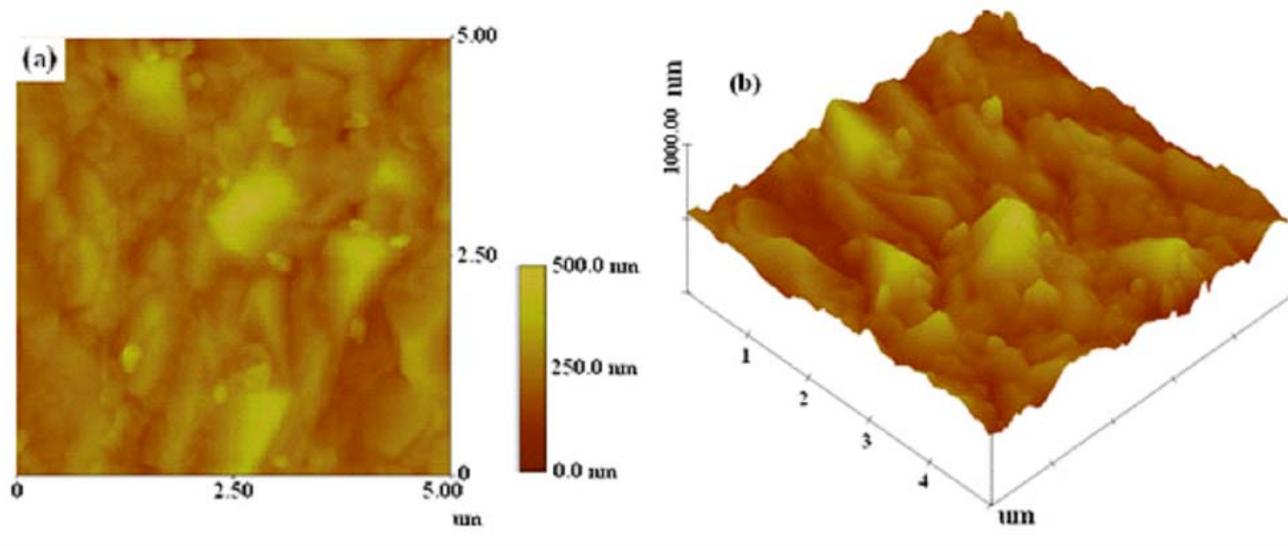


Figure 2. Atomic force microscopy images of a mercury (2 mM)/gold film.

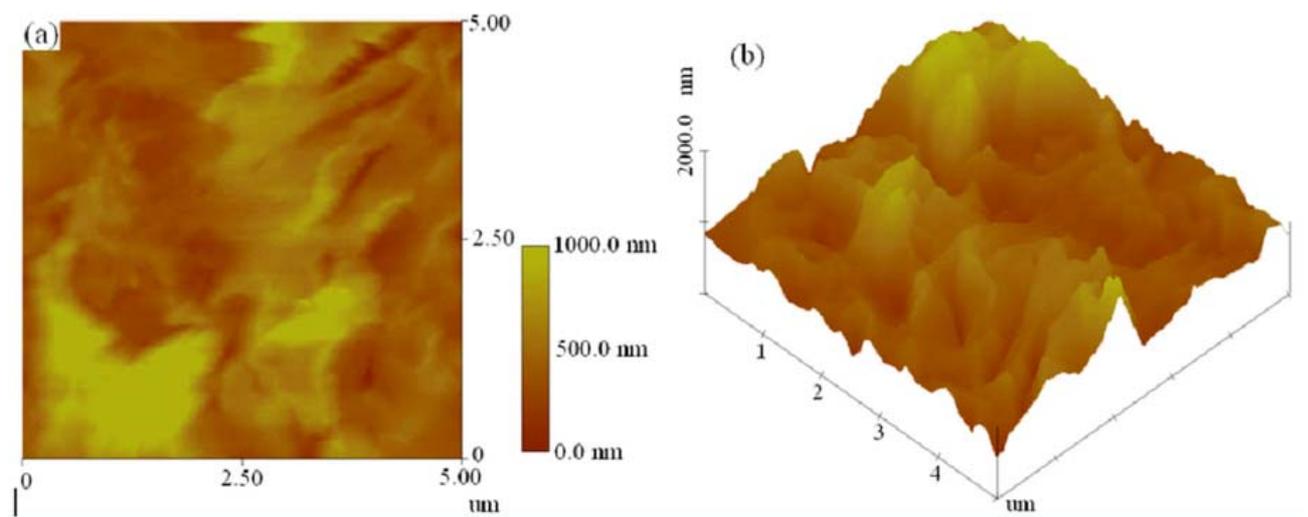


Figure 3. Atomic force microscopy images of a lead (6 mM)/glassy carbon film.

To confirm the electroanalytical utility of Hg/Au nano-composite electrodes, we performed electrochemical experiments in which the mercury molecules on the gold electrodes had diameters between 340 and 500 nm. In differential pulse voltammograms (DPV, Fig. 4), Hg/Au film gave a better performance than Pb/GCE did; therefore, the Hg/Au nano-composite electrode was used to determine NDELA levels in rabbit biological fluids.

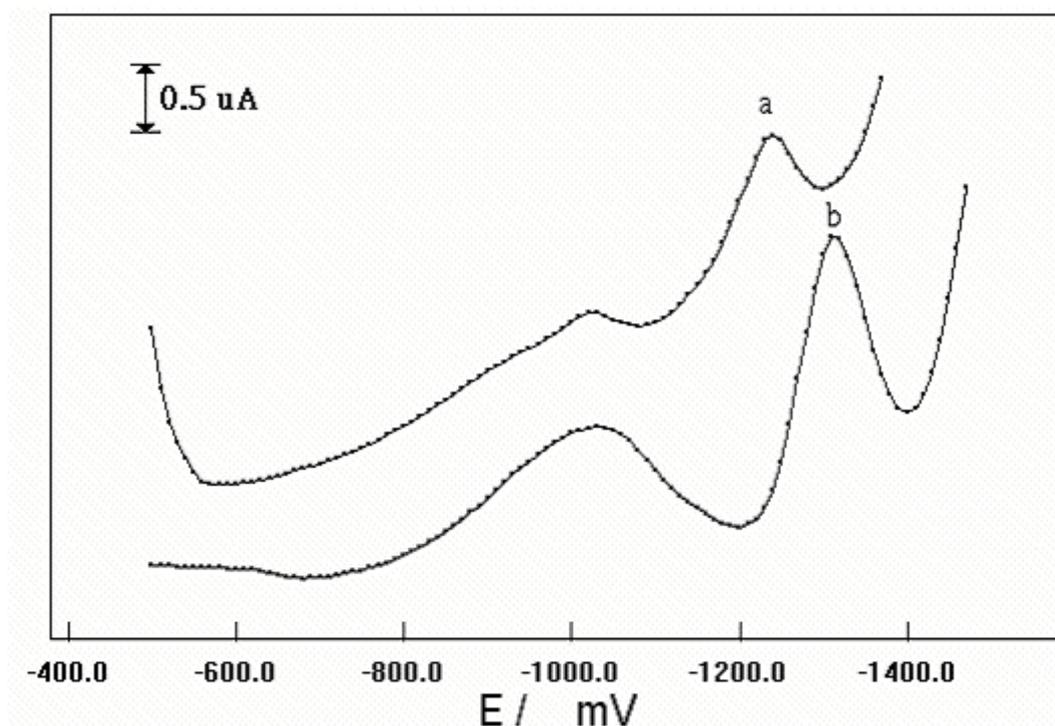


Figure 4. Differential pulse voltammograms of NDELA (16 mg/l) for different modified electrodes in LiClO_4 : (a) Pb (4 mM)/GCE; and (b) Hg (4 mM)/Au, Scan rate 10 mV/s; pulse height 50 mV.

The electroreduction of N-nitroso compounds is reported to be irreversible and its diffusion is controlled, both in strong acid and in alkaline media [30]. In acid solution, there is a 4-electron reduction to the hydrazine, and in alkaline solution there is a 2-electron reduction to the secondary amine as well as the formation of nitrous oxide. In general, the = N-NO group is reduced in a 4-electron step in acidic media, which is most suitable for differential pulse polarographic analysis at the trace level. Comparative tests were conducted of supporting electrolytes and pH-levels, such as Britton-Robinson buffer (pH 2.50-8.42), phosphate buffer (pH 2.12 and 6.06), acetate buffer (pH 4.50), and an aqueous solution containing 0.1 M tetrabutylammonium hydroxide (pH 12.01) and 0.1 M lithium perchlorate (pH 6.59) supporting electrolyte. Cyclic voltammograms of NDELA in Britton-Robinson buffered solution with a mercury-modified gold electrode showed one well-defined reduction. In the reverse scan, no oxidation peak is observed (Fig. 5). Good linearity was observed between the peak height (current) and the square root of the scan rate (Fig. 6a). The relation between the peak potential and the logarithm of the scan rate (Fig. 6b) can be used to roughly estimate the number of electrons involved in the catalytic oxidation. For analytical purposes, the best supporting electrolytes for determining NDELA is lithium perchlorate (pH 6.59). Differential pulse voltammograms (DPV) obtained using the standard (NDELA) addition method on an Hg/Au electrode (Fig. 7, the regression equation used was $y = 0.18x - 0.298$; the correlation coefficient was $r = 0.9998$) showed one well-defined reduction peak.

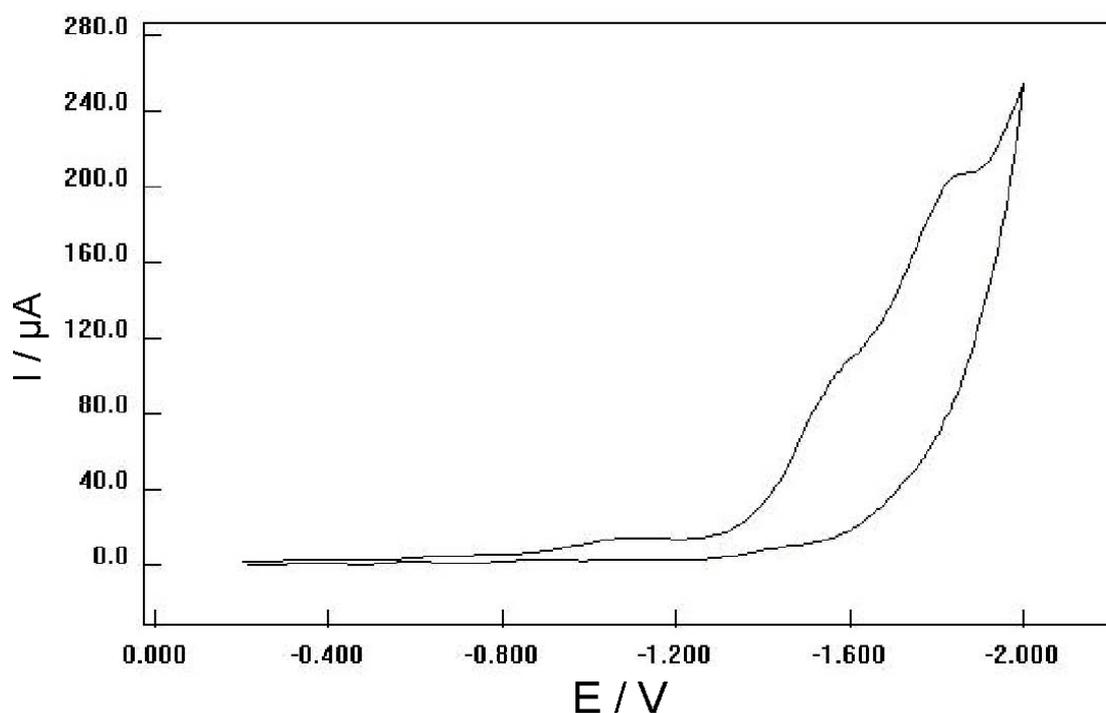


Figure 5. Cyclic voltammogram of NDELA (1×10^{-3} M) on an Hg/Au electrode in Britton-Robinson buffer (pH 6.19) at a scan rate of 25 mV/s.

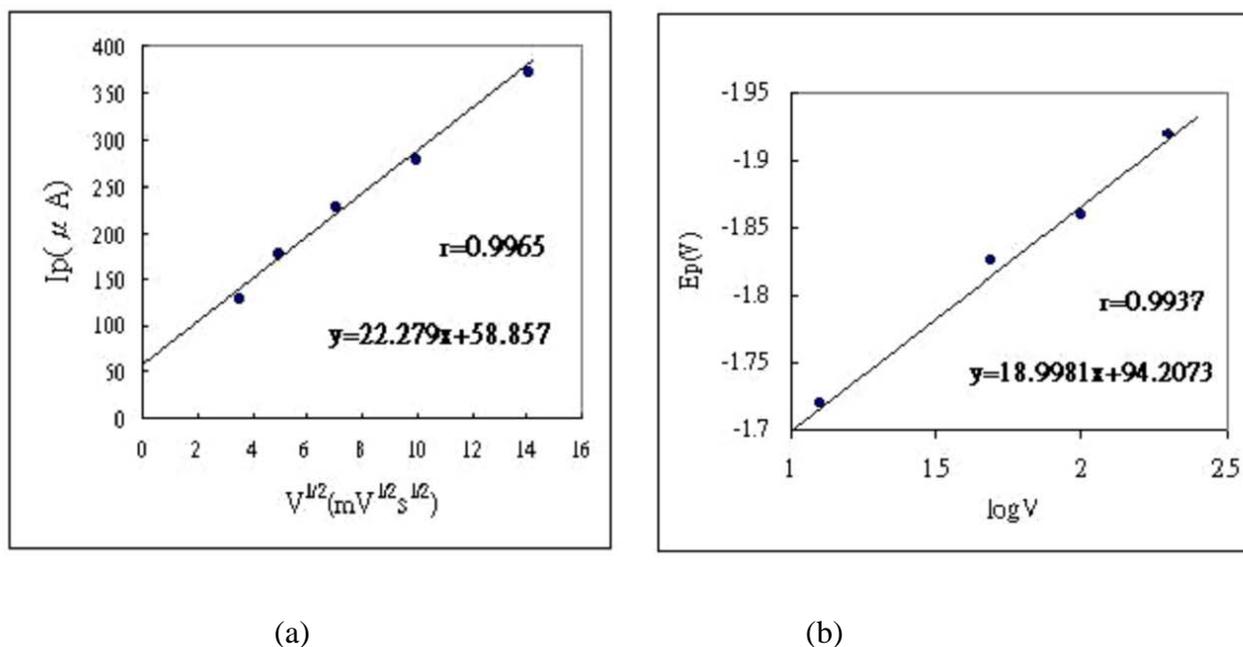


Figure 6. (a) Magnitude of the peak current, i_p , as a function of the square root of the scan rate for NDELA reduction, and (b) peak potentials as a function of the logarithm of the scan rates for NDELA reduction.

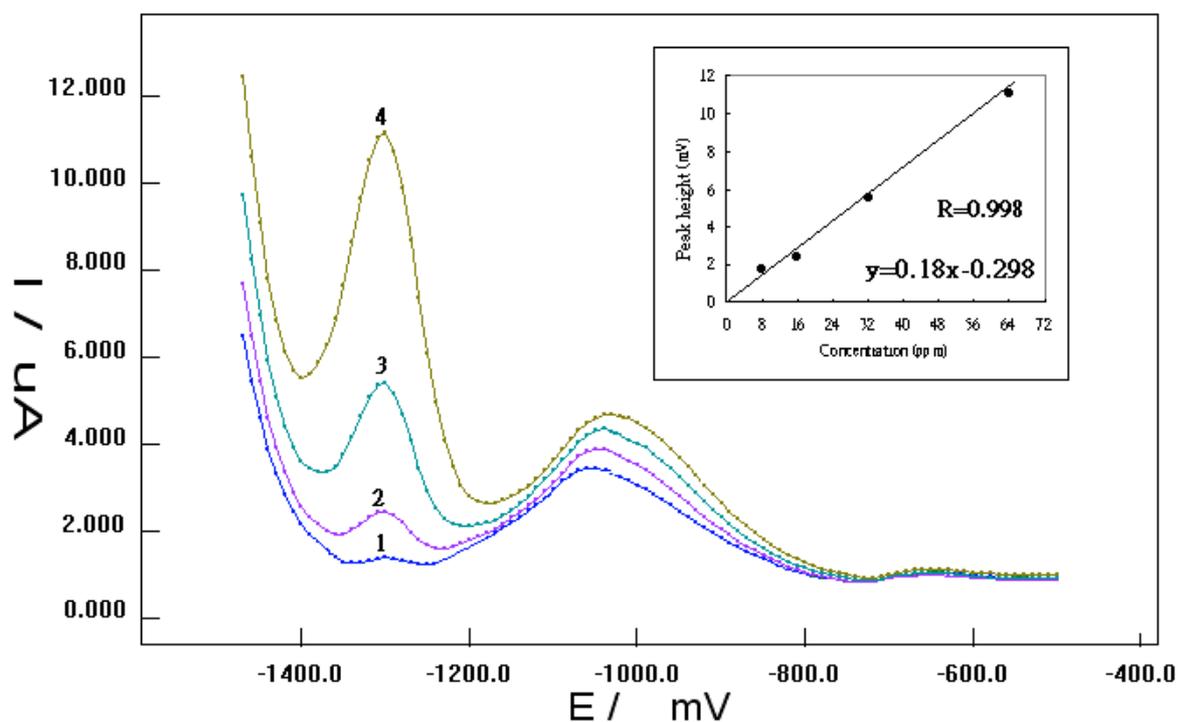


Figure 7. DPV obtained to produce a calibration plot for NDELA at a Hg (4 mM)/Au. The current peak value was at -1.32 V (1) with 8 mg/l of NDELA, (2) with 16 mg/l of NDELA, (3) with 32 mg/l of NDELA, and (4) with 64 mg/l of NDELA. Deposition time, 240 sec; scan rate, 10 mV/s; pulse height, 50 mV.

In order to determine the optimum applied potential for electrochemical detection, after the LC analysis, hydrodynamic voltammograms were constructed (Fig. 8) for NDELA. The maximum current, measured as peak height, was achieved at a potential of -1.5 V. The peak height is dependent on the working diameter (i.d., 0.05-0.5 mm) and length (20-100 mm) of the gold wire, and on the mobile phase flow-rate; it varied from 0.2 ml/min to 0.7 ml/min. It is apparent that the 0.3-mm diameter and 80-mm long gold wire and the flow rate of 0.2 ml/min are most suitable, because the peak height of NDELA is at its highest with those values. Retention time is independent of detector length. An improvement in the sensitivity at low flow-rates (0.2-0.3 ml/min) was observed for NDELA. However, the retention time at 0.2 ml/min (12.5 min) was longer than at 0.3 ml/min (8.3 min). Therefore, the suitable conditions of a polarographic detector for flow-through liquid chromatography when determining NDELA are a gold wire 0.3 mm in diameter and 8-cm long, and a flow rate of 0.3 ml/min.

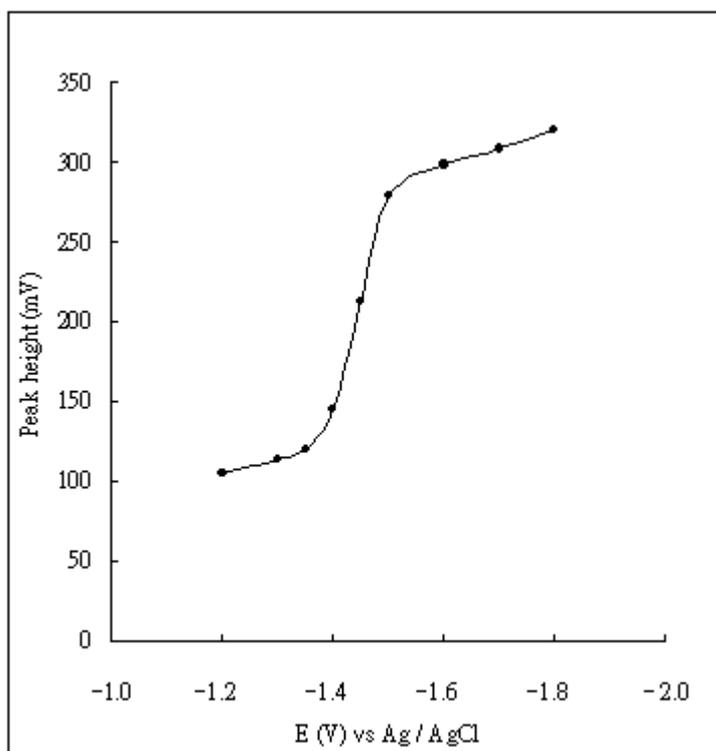


Figure 8. Hydrodynamic voltammogram obtained for NDELA (20 μ g) using an Hg/Au detector. Stationary phase, Phenomenex Luna CN column (particle size, 5 μ m; 250 mm \times 4.6 mm i.d.); Mobile phase, methanol:water (10:90, v/v) containing 0.2 mM lithium perchlorate; flow rate, 0.3 ml/min.

2.2. Stability of the sensor

The operational stability of the sensors was studied by continuous exposure to the flow stream. **Figure 9** shows the stability of the sensor over 10 h of repetitive injections. Cracks were observed on the SEM images after 15 h (Fig. 9b). This is due to the leaching out effect of mercury from the gold surface.

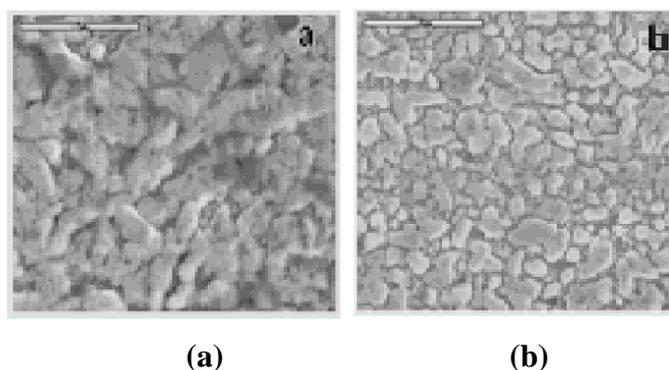


Figure 9. Scanning electron micrographs (at 8 kV) of Hg-coated (4 mM) gold wire as the working electrode in the flow cell after (a) 0 h and (b) 15 h.

2.3. Application to rabbit serum and urine

The proposed LC-ECD method was used to determine N-nitrosodiethanolamine (NDELA) in rabbit serum and urine. The representative LC-ECD chromatograms for the NDELA in a rabbit serum and urine extract after intake drug and are shown in Figure 10a and 10b, respectively, and compare with a chromatogram of pure standard (Fig. 10c). Sample constituent with retention characteristics identical to this of NDELA was identified and measured.

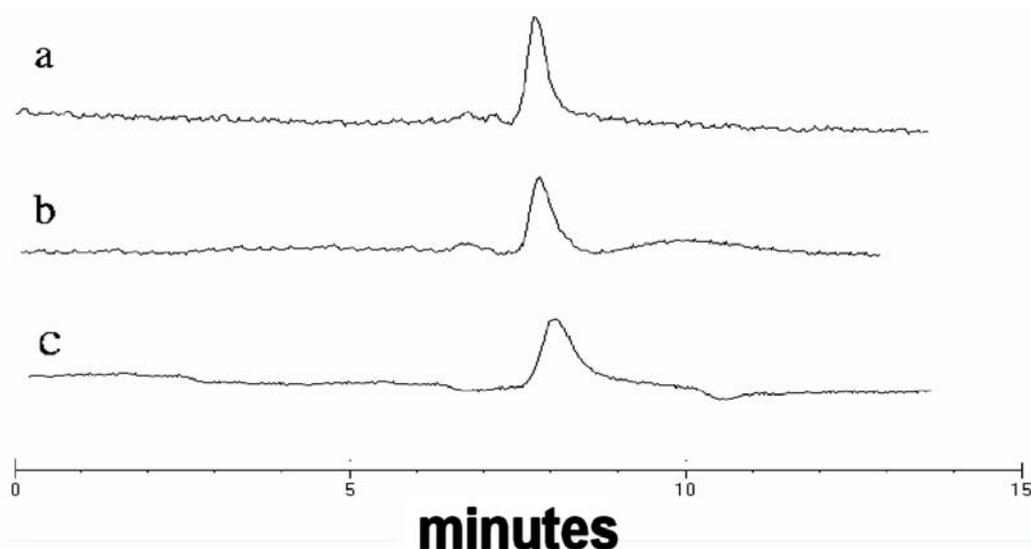


Figure 10. LC-ECD chromatograms obtained from rabbit biological fluids after oral administration of NDELA (60 mg): (a) serum (b) urine (c) NDELA standard. Stationary phase, Phenomenex Luna CN column (particle size 5 μm , 250 mm \times 4.6 mm i.d.). Mobile phase, methanol:water (10:90, v/v) containing 0.2 mM lithium perchlorate; flow rate, 0.3 ml/min.

3. Experiment Section

3.1. Apparatus and materials

The HPLC system used consisted of a Hitachi Model L-7110 pump with a Rheodine 7125 injection valve with 20- μ l sample loop and coupled with an EG&G (Princeton, NJ, USA) PARC 400 controlled potentiostat. The flow-through electrolysis cell was designed with the following electrodes: [Ag/AgCl]/[0.1 M] KCl reference electrode (BAS), platinum auxiliary electrode, and mercury-modified gold electrode as working electrode for detecting NDELA. DPV experiments were done using an EG&G Model 394 connected to an EG&G 325 Faraday cage with Smart Stir and a KO269 A Faraday cage. All solvents and analytes were filtered through 0.45- μ m cellulose acetate and polyvinylidene fluoride syringe (PVDF) membrane filters, respectively. A chromatogram of NDELA was acquired and peak height calculated using an SISC Chromatogram Data Integrator.

3.2. Preparing a thin-film metal electrode surface for DPV

Thin-film metal electrodes were produced using the following method. Before the analysis, the glassy carbon (3 mm in diameter) and gold (3 mm in diameter) electrodes were mirror-polished sequentially with aqueous suspensions of 1.0, 0.5, and 0.05 μ m alumina. The electrode was rinsed with deionized water and electrolytically plated with lead (1.0×10^{-3} to 6.0×10^{-3} M) and mercury (8.0×10^{-4} to 4.0×10^{-3} M) metal ions from 25 ml of perchloric acid and acetate buffer (pH 4.5), respectively. Plating times were 2, 4, 6, and 8 min, respectively, according to a potential scan between -0.8 and 0 V (*versus* Ag/AgCl) (at 10 mV/s).

3.3. Constructing a flow-through polarographic detector

A flow-through electrolysis cell was used for DC-mode electrochemical detection. The detection cell (Fig. 11) was constructed in the laboratory.

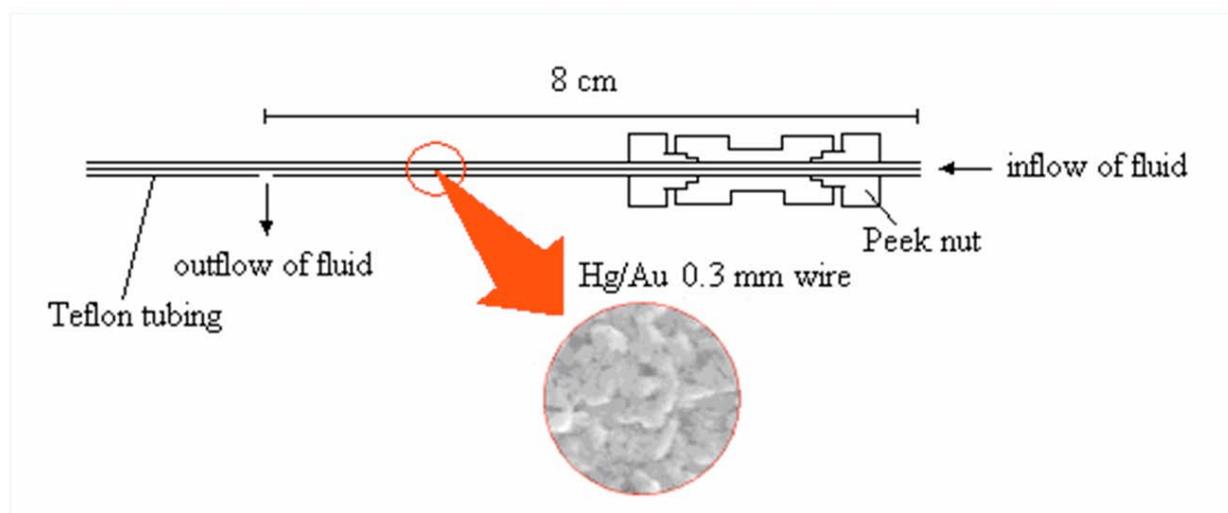


Figure 11. Diagram of a mercury-modified gold-wire flow-through electrolysis cell.

The mercury-modified gold wire (i.d., 0.05-0.5 mm) electrodes were constructed from a length (~10 cm) of Teflon tubing (1/32 in., i.d.; 1/16 in., o.d.). The mercury modified gold wire electrode was inserted into one end of the Teflon tube and sealed with acrylic resin (Struers). A small copper wire was placed at the other end of the Teflon tube to allow for an electrical connection to the mercury-modified gold wire electrode. The platinum wire, which served as a counter, and the Ag/AgCl wire, a reference electrode, were then attached in series with the Teflon tube. For stability, the cell compounds were secured to an insulated plastic box with tape. The polarographic detector, i.e., the eluate, was fed to the mercury-modified gold wire electrode, which had been placed in an overflow vessel containing counter- and reference electrodes.

3.4. Rabbits

Male and female rabbits weighing between 2836 and 4200 g were used. After they had fasted overnight, the rabbits were given oral NDELA (60 mg) with 10 ml of water. The blood and urine were separately collected at prescribed intervals and stored at -30°C until analyzed.

3.5. Extracting NDELA

Urine (1.0 ml) and blood (1.0 ml) samples from the rabbits were centrifuged at 6000 g for 30 min, after which 0.5 ml of serum and 0.9 ml of supernatant urine were added to 5 ml of ethanol and centrifuged for 30 min to sediment protein aggregates, respectively. The deproteinized samples were then extracted twice using 1-3 ml dichloromethane. The aqueous phase was collected and evaporated under nitrogen at a temperature less than 37°C . Samples were reconstituted with mobile phase (1 ml) and loaded onto a Sep-Pak[®] C₁₈ waters cartridge that had been conditioned with 2 ml of methanol and 2 ml of water before the samples were loaded. The sample on the C₁₈ cartridge was washed with 1.0 ml of acetonitrile:water (1:1, v/v) solution (the eluent collected contained NDELA), 1.0 ml of acetonitrile (the eluent collected contained NDELA). These two fractions were combined and dried under nitrogen at 45°C . The dry extract was reconstituted with 500 μl of pure methanol and filtered through 0.45- μm membrane filters before LC analysis.

3.6. Determining NDELA using DPV

Differential pulse voltammograms were taken for NDELA in Britton and Robinson buffer solutions (pH 2.50, 3.96, 4.96, 6.19, 7.11, and 8.42) and water that contained various supporting electrolytes: sodium perchlorate, lithium perchlorate, tetraethylammonium perchlorate, tetraethylammonium tetrafluoroborate, tetrabutylammonium perchlorate, and tetrabutylammonium hydroxide solution. In order to obtain calibration graphs for the NDELA, 10 ml of supporting electrolytes were pipetted into a voltammetric cell and de-aerated with nitrogen for 4 min before voltammetric measurement. Using a micropipette, aliquots of 1000 mg/l of NDELA solution were added and left to de-aerate for 2 min. Voltammograms were then taken. Quantitative analyses were performed in the differential pulse mode. The potential was set at 0.0 to -1.5 V versus the Ag/AgCl electrode for reduction. The pulse height was 50 mV with a scan rate of 10 mV/s with a drop time of 1.0 s. One milliliter of sample solution was pipetted into a 10-ml calibrated flask and diluted to volume with phosphate buffer solution. This solution was analyzed using DPV under the same conditions used for the calibration graph.

3.7. Determining NDELA using a flow-through polarographic detector

The polarographic detector was operated at -1.5 V. Using the injection value, $25\ \mu\text{l}$ of the prepared sample solution and standard solution were chromatographed under the operating conditions described above. Quantitation was based on the peak area of the sample.

Conclusions

In this work, we report on the construction of gold, glassy carbon, and carbon fiber electrodes with surface deposits of mercury and lead. These electrodes were used as electrocatalytic sensors in liquid chromatography-electrochemical detection (LCEC) or flow-injection analysis (FIA) to determine levels of NDELA in rabbit serum and urine. These electrodes not only catalyzed this analyte, but also provided stable, quantitatively reproducible performance in the chromatographic stream. Thus, the proposed analytical method offers an attractive alternative to UV, GC, and CL detection of NDELA where derivatization procedures are needed.

Acknowledgements

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References

References

1. Rustemeier, K.; Stabbert, R.; Haussmann, H-J.; Roemer, E.; Carmines, E.L. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. *Food Chem. Toxicol.* **2002**, *40*, 93-104.
2. Bavin, P.M.G; Darkin, D.W.; Viney, N.J. Total nitroso compounds in gastric juice. *IARC Sci. Publ.* **1984**, *41*, 337-344.
3. Pignatelli, B.; Richard, I.; Bourgade, M.C.; Bartsch, H. An improved method for analysis of total N-nitroso compounds in gastric juice. *IARC Sci. Publ.* **1987**, *84*, 209-215.
4. Loepky, R.N.; Fuchs, A.; Janzowski, C.; Humberd, C.; Goelzer, P.; Schneider, H.; Eisenbrand, G. Probing the mechanism of the carcinogenic activation of N-nitrosodiethanolamine with deuterium isotope effects: in vivo induction of DNA single-strand breaks and related in vitro assays. *Chem. Res. Toxicol.* **1998**, *11*, 1555-1566.
5. Mori, Y.; Koide, A.; Kobayashi, Y.; Morimura, K.; Kaneko, M.; Fukushima, S. Effect of ethanol treatment on metabolic activation and detoxification of esophagus carcinogenic N-nitrosamines in rat liver. *Mutagenesis* **2002**, *17*, 251-256.
6. Sakai, H.; Tsukamoto, T.; Yamamoto, M.; Hirata, A.; Inagami, A.; Shirai, N.; Iidaka, T.; Yanai, T.; Masegi, T.; Tatematsu, M. Summation of initiation activities in the liver after partial hepatectomy. *Cancer Lett.* **2002**, *176*, 1-5.

7. Ducos, P.; Gaudin, R.; Francin, J.M. Determination of N-nitrosodiethanolamine in urine by gas chromatography thermal energy analysis: application in workers exposed to aqueous metalworking fluids. *INT. Arch. Occup. Environ. Health* **1999**, *72*, 215-222.
8. Liteplo, R.G.; Meek, M.E. N-nitrosodimethylamine: hazard characterization and exposure-response analysis. *Environ. Carcinog. Ecotoxicol. Rev.* **2001**, *C19*, 281-304.
9. Watanabe, S.; Kamiguchi, Y. Chromosome analysis of human spermatozoa following in vitro exposure to cyclophosphamide, benzo(a)pyrene and N-nitrosodimethylamine in the presence of rat liver S9. *Mutat. Res.* **2001**, *491*, 57-63.
10. Monarca, S.; Feretti, D.; Zanardini, A.; Moretti, M.; Villarini, M.; Spiegelhalder, B.; Zerbini, I.; Gelatti U.; Lebbolo, E. Monitoring airborne genotoxicants in the rubber industry using genotoxicity tests and chemical analyses. *Mutat. Res.* **2001**, *490*, 159-169.
11. Jurtchenko, S.; Tenno, J.; Mölder, U.; Reinik, M. Determination of volatile N-nitrosamines by gas chromatography-mass spectrometry with positive-ion chemical ionization. *Chemistry [Proceedings of the Estonian Academy of Sciences]* **2002**, *51*, 169-184.
12. Kawata K.; Ibaraki, T.; Tanabe, A.; Yagoh, H.; Shinoda, A. Gas chromatographic-mass spectrometric determination of hydrophilic compounds in environmental water by solid-phase extraction with activated carbon fiber felt. *J. Chromatogr. A* **2001**, *911*, 75-83.
13. Yurchenko, S.; Mölder, U. N-nitrosodimethylamine analysis in Estonian beer using positive-ion chemical ionization with gas chromatography mass spectrometry. *Food Chem.* **2004**, *89*, 455-463.
14. Gang, D.; Luo, X.; Hinderberger, E.; Clevenger, T.E. An improved N-nitrosodimethylamine (NDMA) determination method using solid-phase extraction (SPE), GC/MS and chemical ionization. *American Chemical Society, Division of Environmental Chemistry. Preprints of Extended Abstracts* **2003**, *43*, 1093-1097.
15. Byun, M.W.; Ahn, H.J.; Kim, J.H.; Lee, J.W.; Yook, H.S.; Han, S.B. Determination of volatile N-nitrosamines in irradiated fermented sausage by gas chromatography coupled to a thermal energy analyzer. *J. Chromatogr. A* **2004**, *1054*, 403-407.
16. Andrade, R.; Reyes, F.G.R.; Rath, S. A method for the determination of volatile N-nitrosamines in food by HS-SPME-GC-TEA. *Food Chem.* **2004**, *91*, 173-179.
17. Muegge, B.D.; Richter, M.M. Electrochemiluminescent detection of metal cations using a ruthenium (II) bipyridyl complex containing a crown ether moiety. *Anal. Chem.* **2002**, *74*, 547-550.
18. Grebel, J.E.; Suffet, I.H. Dual column gas chromatographic method for analysis of eight nitrosamines with a nitrogen phosphorus detector or a nitrogen chemiluminescence detector. *American Chemical Society, Division of Environmental Chemistry. Preprints of Extended Abstracts* **2004**, *44*, 273-277.
19. Pérez-Ruiz, T.; Martínez-Lozano, C.; Tomás, V.; Martín, J. Flow injection chemiluminescent determination of N-nitrosodimethylamine using photogenerated tris(2,2'-bipyridyl) ruthenium (III). *Anal. Chim. Acta* **2005**, *541*, 69-72.
20. Luque-Pérez, E.; Rios, A.; Valcárcel, M. Automated flow-injection spectrophotometric determination of nitrosamines in solid food samples. *Fresenius J. Anal. Chem.* **2001**, *371*, 891-895.

21. Cardenes, L.; Ayala, J.H.; Gonzalez, V.; Afonso, A.M. Determination of N-nitrosodimethylamine by HPLC, with fluorescence detection. A survey of N-nitrosodimethylamine in commercial beers. *J. Liq. Chromatogr. Rel. Technol.* **2002**, *25*, 977-984.
22. Samulsson, R.; Sundstrom, O. Pulse polarographic behaviour of N-Nitrosamines in mixtures of water with methanol and acetonitrile. *Anal. Chim. Acta* **1982**, *138*, 375-380.
23. Gorski, W.; Cox, J.A. Amperometric determination of N-Nitrosamines in aqueous solution at an electrode coated with a ruthenium-based inorganic polymer. *Anal. Chem.* **1994**, *66*, 2771-2774.
24. Dobberpuhl, D.A.; Johnson, D.C. Pulsed electrochemical detection of alkanolamines separated by multimodal high performance liquid chromatography. *J. Chromatogr. A* **1995**, *694*, 391-398.
25. Vohra, S.K.; Harrington, G.W. The evaluation of a polarographic detector for high performance liquid chromatography in the determination of N-Nitrosamines. *J. Chromatogr. Sci.* **1980**, *18*, 379-383.
26. Vohra, S.K.; Harrington, G.W. Chromatopolarography of N-Nitrosamines including determination of N-Nitrosodiethanolamine in cosmetic products. *Food Cosmet. Toxicol.* **1981**, *19*, 485-487.
27. Davidovic, A.; Davidovic, D.; Tabakovic, I. Electrochemical reduction of p-nitrosodiphenylamine on solid electrodes in cationic micellar system. *J. Serb. Chem. Soc.* **1991**, *56*, 677-683.
28. Collyer, S.D.; Bradbury, S.; Davis, F.; Hatfield, J.V.; Stirling, C.J.M.; Higson, S.P.J. Calix[4]resorcinarenetetrathiol modified gold electrodes: applications to the adsorption and electrochemical determination of N-nitrosamines. *Electroanalysis* **2004**, *16*, 324-327.
29. Wang L.H.; Hsia H.C.; Wang C.C. Determination of N-Nitrosamines in cosmetic products, rabbit biological fluids by RP-LC with photodiode array detector. *J. Liq. Chromatogr. Rel. Tech.* **2006**, *29*, 1737-1751.
30. Lund H. Electroorganic preparation III. Polarography and reduction of N-Nitrosamines. *Acta Chem. Scand.* **1957**, *11*, 990-996.