行政院國家科學委員會專題研究計畫　成果報告

化妝品中 N-亞硝基二乙基醇胺測定方法之研究

計畫類別：個別型計畫
計畫編號：NSC92-2113-M-041-003-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：嘉南藥理科技大學醫藥化學系

計畫主持人：王來好

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中　華　民　國 93 年 10 月 12 日

行政院國家科學委員會補助專題研究計畫ˇ□成果報告
□期中進度報告

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中文摘要

設計液相層析-極譜檢測器以測定 N-亞硝基二乙基醇胺及亞硝基混合物。並探討極譜檢測器中修飾薄膜金屬電極的種類、厚度、電解液組成及流速等效應。分析的偵測極限可達毫微克.同時以液相層析配合電化學與紫化線方法，偵測市售含 N-亞硝基二乙基醇胺之化妝品。

關鍵詞:液相層析-極譜檢測器,薄膜金屬電極,奈米粒子催化劑 ,N-亞硝基二乙基醇胺,化妝品.

Abstract

The polarographic detector for high performance liquid chromatography has been designed with respect to a model compound, N-nitrosodiethanolamine, and mixtures of nitrosamines. The effect of modifying kind of metal ion, the thickness of modifying film, supporting electrolyte and pH composition and flow rate has been investigated in the pulsed and DC modes. Analysis at nanogram levels is demonstrated. These electroreduction process are applied for the detection of N-nitrosodiethanolamine in various cosmetic products.

Keywords: Liquid chromatography-polarographic detector; Thin- film modified metal electrode; nanoparticles catalysts; N-nitrosodiethanolamine; cosmetics.

報告內容

前言

Di- and triethanolamine or certain derivatives there of, such as diethanolamines fatty acids are used extensively as cosmetic ingredients [1-3]. The high reactivity of secondary amines such as diethanolamine with nitrosating agents such as nitrite, can result in the formation of N-nitrosodiethanolamine (NDELA), a potent carcinogen. Diethanolamine is the major amine precursor of NDELA in cosmetic.

研究目的

The European Economic Community (EC) Cosmetics Directive which states that fatty acids diethanolamines raw materials should contain less than 5 % diethanolamine and less than 50 ppb NDELA and that cosmetic products should contain less than 0.5 % dialkanolamine [4-5]. NDELA can easily penetrate human skin and has been found in the urine of exposed metal workers [6-9]. It is therefore considers necessary to establish a method which would detect the sub- μ g/l level in the urine samples.

文獻探討

Methods for determining N-nitroso compounds in foods, biological fluids and cosmetic products include a gas chromatography method coupled either with a thermal energy analyzer (TEA) or with a mass spectrometer (MS) [10-12], a high-performance liquid chromatography method combined with either a UV detector or MS [13-15], chemiluminescence detection

after reduction of nitrite to nitric oxide by suitable reductants [16-17], and a polarographic method [18-22]. The present study was to design solid electrode such as gold, glassy carbon electrode, which was modified by a film that contained metal ion as the working electrode.

研究方法

Preparation of thin-film metal electrode surface

The thin-film metal electrode was produced by the following method, prior to analysis, the glassy carbon (3 mm diameter), carbon fiber (6 μm diameter) and gold (3 mm diameter) electrode was mirror polished sequentially with aqueous suspension of 1.0, 0.5 and 0.05 μm alumina, respectively. The electrode was rinsed with deionized water and electrolytically plated with lead and mercury metal ion from 25 ml of perchloric acid and acetate buffer (pH 4.5) that was 5.0×10^{-4} to 2.0×10^{-3} M, respectively. Plating time was 4 min. with a potential scan from –0.8 to 0.0 V.

Sample preparation

Taking into account about the N-nitrosamines content of the hair shampoo, foam bath and shower gels, washing creams, samples (approx. 1.0 – 3.0g) of the latter were weighed accurately in a 15 ml beaker, dissolved in 8 ml of ethyl acetate and mixed by vertex treatment for 15 min. A sufficient amount of sodium chloride was added to the mixture to break up the emulsion and the addition of ammonium sulphamate (500 mg) prevented the formation of N-nitrosodiethanolamine. The mixture is placed on a 9 cm length of glass column (1.5 cm i.d.) packed with 3.0 g silica gel 230 - 400 mesh). The ethyl acetate fraction was discarded, and the column was then washed with 20 ml of acetone-dichloromethane (60:40, v/v) and subsequently connected to a stream of nitrogen to force all solvent. After clean-up, the dried extracts were solubilized in 2 ml of methanol-water (1:1, v/v) for HPLC and differential pulse voltammetry (DPV) analysis.

Determination of N-nitrosamines by DPV

Differential pulse voltammograms were taken for N-nitrosamines in a phosphate buffer (pH 2.6 and 6.2), water containing various supporting electrolytes such as sodium perchlorate, lithium perchlorate, tetraethylammonium perchlorate, tetraethylammonium tetrafluoroborate, tetrabutylammonium perchlorate and tetrabutylammonium hydroxide solution. In order to obtain calibration graphs for the N-nitrosamines, 10 ml of supporting electrolyte were pipetted into a voltammetric cell and de-aerated with nitrogen for 4 min. before voltammetric measurement. By micropipette, aliquots of 1000 mg l^{-1} N-nitrosamines solution were added. After each addition voltammograms were obtained; the solution de-aerated for 2 min. after each addition before obtaining the voltammogram. Quantative analyses were performed in the differential pulse mode. The potential was set at 0.0 to - 1.0 V *versus* Ag/AgCl electrode (SCE) for reduction. The pulse height was 50 mV and the scan rate 10 mV s^{-1} with a drop time of 1.0 s. For sample solution analysis, 1 ml of the solution was pipetted into a 10 ml calibrated flask and diluted to volume with phosphate buffer solution. This solution was analysed by DPV using the same condition as for calibration graph.

Determination of N-nitrosamines by HPLC

A Phenomenex Luna CN column (particle size 5 μm , 250 mm x 4.6 mm i.d.) was used for reverse-phase HPLC. The mobile phase was methanol – 0.8 mM phosphate(5 : 95, v/v)

at a flow rate of 0.4 ml / min. The EC detector was operated at - 0.4 V. Detection after separation on the column was carried out using ultraviolet detector set at 234 nm. By means of the injection value , 25 μl 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.

結果與討論

Thin film of mercury and lead were deposited on the gold and glassy carbon electrode (GCE), respectively. Fig. 1 shows a typical SEM image of mercury. As shown in Fig. 1c and 1d, mercury nanoparticles were distributed more uniformly in gold than GCE. The surface morphology of a Hg/Au and Pb/GCE nanoparticle hybrid film were investigated by atomic force microscopy (AFM). The mercury diameter (～500 nm) on the gold surface (Fig. 2) is smaller than lead (～1000 nm) on the GCE (Fig. 3). It was found that Hg/Au film gave a better performance than Pb/GCE.To confirm the electroanalytical utility of For our electrochemical experiments the mercury had diameters in the range 340～500 nm, and were chosen for use in the determination of NDELA in cosmetic products. To confirm the electroanalytical utility of Hg/Au nano-composite electrode, we studied the electrochemical detector for high performance liquid chromatography in the determination of NDELA.

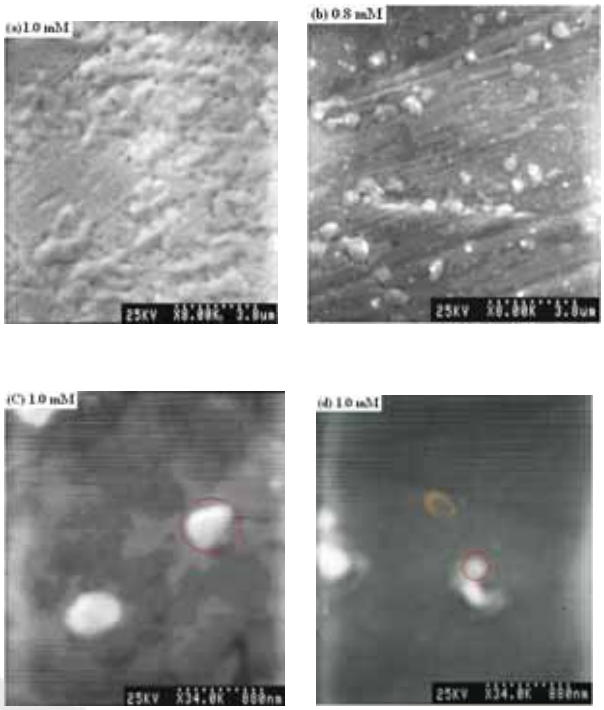
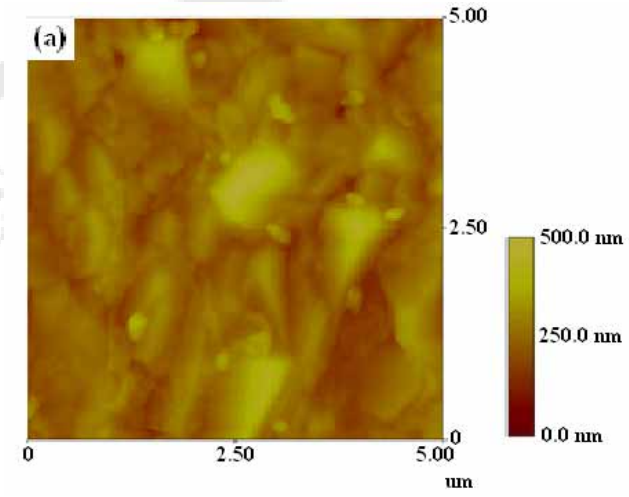


Fig. 1 Scanning electron micrographs of different concentration and deposit surfaces. (a) mercury (1.0 mM) / gold (b) mercury (0.8) mM / gold (c) mercury (1.0 mM) / gold (d) mercury (1.0 mM) / glassy carbon



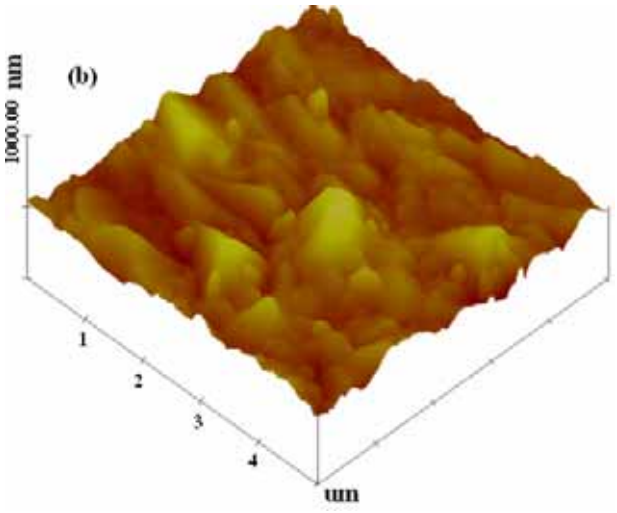
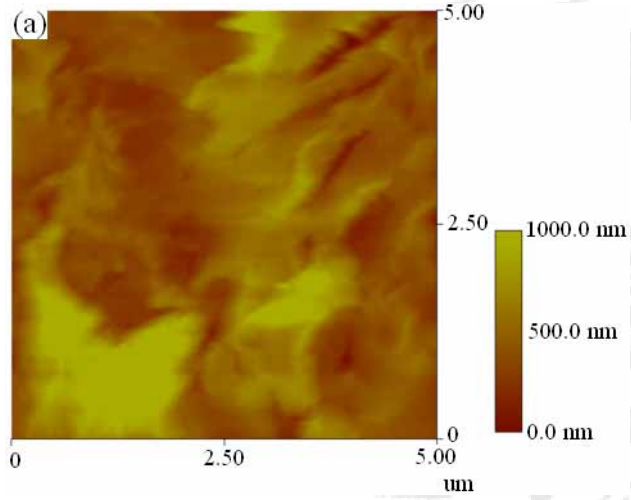


Fig. 2 Atomic force microscopy images of a mercury (2 mM) / gold film.



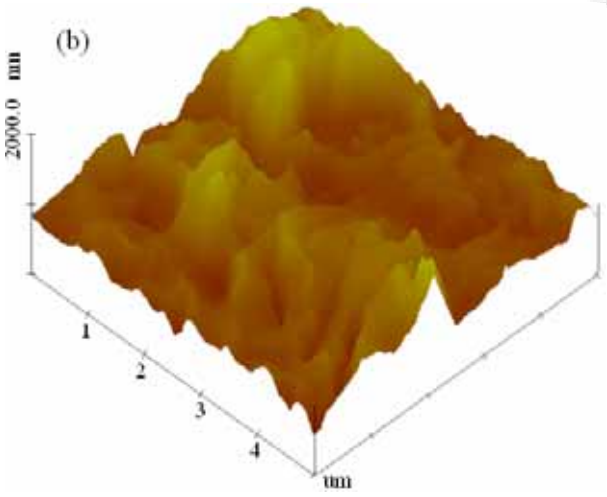


Fig. 3 Atomic force microscopy images of a lead (6 mM) / glassy carbon film.

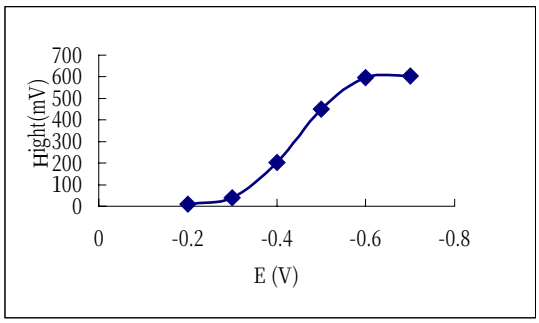


Fig. 4 Hydrodynamic voltammogram obtained for NDELA (0.4 ng) by use of Hg/Au detector. Stationary phase , Phenomenex Luna CN column (particle size 5 μm , 250 mm x 4.6 mm i.d.); mobile phase, methanol –water(5 : 95, v/v) containing 0.8 mM phosphate; flow rate 0.5 mL/min.

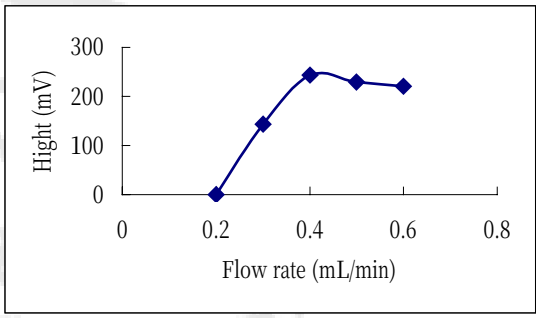


Fig. 5 Dependence of peak height (0.2 ng NDELA) on mobile phase flow rate for the Hg/Au detector. Analysis conditions are identical to those listed in Fig. 4.except that the detection was at – 0.4 V versus the Ag/AgCl reference electrode.

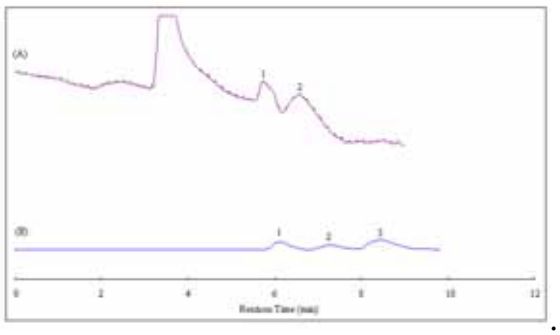


Fig 6 Chromatograms obtained by (A) LC-EC 5 ng/mL mixtures of nitrosamines; (B) (B) LC-UV 20 mg/mL mixtures of nitrosamines; (1) N-nitrosodiethanolamine (NDELA); (2) N-nitroso-bis-(2-hydroxypropyl)amine(NBHP

A); (3)N-nitrosodimethylamine(NDMEA). Analysis conditions are identical to those listed in Fig. 4.except that the flow rate was 0.6 mL/min and detection at – 0.4 V versus the Ag/AgCl reference electrode.

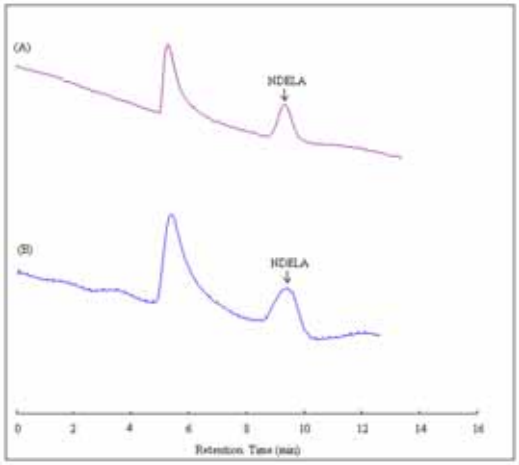


Fig. 7 Chromatograms obtained by LC-EC from (A) 5 ng/nL NDELA; (B) commercial shower gel. Analysis conditions are identical to those listed in Fig. 4.except that the flow rate was 0.4 mL/min and detection at – 0.4 V versus the Ag/AgCl reference electrode.

Table 1 Analytical results for the determination of
N-nitrosodiethanolamine (DNELA) in commercialhair shampoo and foam bath and shower gels

Samples	Concentration (ng mL^{-1})[a]
Hair shampoo	24.9 (5.3 %)[b]
Foam bath	274 (4.5%)
Shower gels	2.81 (5.7 %)

[a] Number of determination (n = 3)

[b] Relative standard deviation

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計畫成果自評

High sensitivity and low detection limit (3 nM) are achieved using gold modified with mercury. The sensitivity decreases when the NDELA concentration increased. However linear portions enough for performing quantitative determinations are easily individuated. The Hg/Au electrode shows to be powerful tools for the electroanalytical determination of trace NDELA in complex sample matrix such cosmetic products and biological fluids. It would be very difficult to analyze N-nitrosamines mixture by ordinary differential pulse voltammetry. However, the combination of the separation capability of HPLC with the sensitivity of the voltammetric detector results in a good chromatogram.