嘉南藥理科技大學專題研究計畫成果報告

化妝品與養髮製品中維生素 B 群測定方法之研究

計畫類別：□個別型計畫　　□整合型計畫

計畫編號：CNAC-94-05

執行期間：94 年 1 月 1 日至 94 年 12 月 31 日

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中華民國　95　年　　2 月　　28　日

一、中文摘要

研究維生素B群在玻璃碳與電沉積汞電極之電化學行為;包括Britton-Robinson (pH 4.86 - 10.03)、磷酸(pH 2.12 and 6.06)、醋酸等緩衝溶液及不同支持電解質等對電化學行為之影響。探討各種因素如沉積材料、時間與汞濃度對汞膜金屬結構電極之特性影響。比較玻璃碳電極與電沉積汞電極之偵測極限，同時測定市售具有抗老化及增白效果之化妝品或養髮製品維生素B群之含量。

關鍵詞：

汞膜金屬結構電極，電化學測定，維生素B群，
化妝品。

Abstract

The electrochemical behaviors of vitamin B groups on glassy carbon, and electrodeposited mercury electrodes were investigated in a Britton-Robinson buffer (pH 4.86 - 10.03), phosphate buffer (pH 2.12 and 6.06), acetate buffer and an aqueous medium containing supporting electrolyte. The various factors, such as deposition material, time, and concentration of mercury on the precision of the analysis have been explored. A comparision in made between the detection limit of glassy carbon, thin-film modified on glassy carbon and gold electrodes. The electroreduction process is applied for the quantitative determination of vitamin B groups in commercial cosmetic products.

Keywords:

Mercury film metal electrodes; electrochemical
detection; vitamin B groups; cosmetics.

二、緣由與目的

維生素B6(pyridoxine)及其衍生物，pyridoxal, pyridoxal-5-phosphate與pyridoxamine,可擴大低於正常大小的毛球。同時具有抗皮脂溢(anti-seborrhea)功能，促進毛髮生長。所以最適合當毛髮製品成分，特別是毛髮需經過氧化或還原處理過之染髮及燙髮製品[1-5]。維生素B6可增加皮膚膠原(collagen)表現，常與氨基酸調製成乳液，典型應用於皮膚外觀的改善並激勵頭皮毛髮再生 [2, 6]。維生素B3 (nicotinamide) 為內在的代謝物，它可抑制由反應氧化物種

(reactive oxygen species)所產生的氧化破壞。其抗氧化效果比維生素 C 與維生素 E 好，可當有效的抗氧化劑。且維生素 B3 會增加角質層脂質(ceramide)之生物合成，並促進表皮滲透性障礙(epidermal permeability barrier)，降低變異性皮膚炎(atopicdermatitis)及老化[7]。維生素 B5 (D-panthenol)又稱泛醇，它以兩種旋光形式存在於自然界中，但只有右旋形式的才具有生物活性。它對碳水化合物、脂肪和含氮化合物的代謝具有重要的作用。使用 5％D-panthenol 三個月，可消除肝斑、脂漏性及色素沉澱。用於護髮劑可增進毛幹水份之保持，防止毛髮斷裂及分叉。用於護膚劑可使皮膚柔軟有彈性，且不油膩，有溫和的抗炎作用。因此被廣用於化妝品製品[8]。抗發炎的乳液及敷面濕布製品也常有 B2 及 B6 衍生物。　市售具有抗老化及增白效果之化妝品或養髮製品，通常含有維生素 B1、B2、B3、B5 及 B6 等 B 群。而 B 群在配方含量 B1 0.1- 0.3％、B2 0.04-0.06％，B3 0.3-0.5％，B5 0.25-0.3％及 B6 0.1-0.5％。國內衛生署之允許濃度需 0.1％，B5 為 0.3 ％，B6 0.01 ％。 文獻研究 B 群大都在人體代謝及生物效率方面[9-11]，僅一篇菸酸(nicotinate)維生素的人體皮膚浸入研究[12]。且使用 14C 當標幟偵測系統，但放射性分析不適合一般實驗室測定。　文獻分析 B 群之方法，包括紫外光[13-15]、螢光[16]、傅立葉轉換紅外線光譜法[17-18]、離子選擇電極[19]與液相層析法連接紫外光檢測器[20-22]。文獻檢測 B5 是在界面活性劑中與 p-sulfanilic acid 偶合反應，衍生物在 450 nm 測定。檢測 B3 需利用微生物 Lactobacillus plantarun 形成衍生物，經酸水解、萃取，再以螢光測定。或以液相層析連接傅立葉轉換紅外線檢測水溶性維生素，但 LC-FTIR 儀器並不普遍，且 FTIR 當檢測器，靈敏度比 UV 差，只能檢測 ppm。波峰會重疊，需更高的解析力，才可分離高濃度成份。或利用 tetra(2-chlorophenyl)borate 溶解在 o-nitrophenyloctyl ether 中，沉積固定在 PVC 上，製成對 B1 及 B6 具有特殊選擇功能的電極。所以本實驗用電沉積汞電極當工作電極，研究電沉積汞電極對 B 群之偵測極限、靈敏度 、解析度。並應用於市售化妝品中 B 群之測定。

實驗條件

(1). 儀器

a. 電化學偵測器: EG&G 公司極譜儀 (Model 394)連接於 EG&G325 Faraday cage with Smart stir 及 KO269 A Faraday cage。參考電極(Ag /AgCl / 0.1M KCl) 及白金輔助電極。

b. 儀器部份包括使用 Hitachi 公司高效能液相層析儀系統(Model L-7110 pump)；連接 20-μl 的注入樣品圈(Rheodine 7125)；及紫外線偵測器(Shimadzu SPD 10 A).

(2) 分析方法

a. 化妝品與藥品樣品前處理: 精稱 0.1 克市售的抗老化及增白效果之化妝品或綜合維生素製品，置入 15 mL 的燒杯中，加入 10 mL 的甲醇攪拌 30 分鐘。離心 30 分鐘，取澄清液製備成檢液。

b. 汞薄膜修飾電極的製備:利用差式脈動極譜法 (differential pulse stripping，DPS)將玻碳(GCE)、金絲電極，分別放入含 0.8- 4 mM 汞溶液之 0.1M 醋酸緩衝溶液(pH4.0)。電位範圍在 -1 到 0 V，掃描速率為 10 mV/s，沉積時間分別 120, 240, 360, 480 秒，製備備 Hg /GCE 和 Hg/Au 電極。

c. 逆相分配層析系統利用 Phenomenex (Luna 5 μ, C 18 , 250 x 4.6 mm) 分析管柱，甲醇-水(含 30 mM 磷酸, pH 2.69) 52 : 48 (v/v)做為移動相。移動相通過紫外線偵測器(波長設定為 265 nm 與 280 nm)。

三、結果與討論

表一 列出維生素 B 群在各種 pH 緩衝溶液中的波峰電位。由表一顯示在 0.1M KCl 溶液最適合同時測定 B1, B2 和葉酸 B 9。由循環伏安法(cyclic voltammetry)技術，Fig. 1, 2 顯示 B1 有不可逆還原行為，Fig. 3,4 顯示 B2 有可逆還原行為，Fig. 5,6 顯示葉酸有不可逆還原行為。

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Table 1 Peak potentials (vs Ag/AgCl) of the vitamin B factors at various pH values.

Substance	pH 5.21	pH 6.68	pH 8.08	pH 10.03	0.1M KCl
Thiamine (B1)	-1.56	-1.62	-1.66	-1.96	-1.26
Riboflavin (B2)	-0.36	-0.39			

-0.47

-0.54

-0.56

Folic acid (B9)

-0.59

-0.91

-1.04

-1.28

-0.91

Nicotinic acid

-1.54

-1.60

-1.97

-1.92

-1.33

Pyridoxine (B6)

-1.62

-1.67

ND

ND

-1.39

Calcium pantothenat

ND

ND

-1.43

-1.44

-1.56

Nicotinamide

-1.77

-1.68

-1.90

-2.0

-1.69

Fig. 1 Reductive cyclic voltammograms for 1.0 x 10-3 M of thiamine HCl recorded in Britton-Robinson buffer (pH 6.68) on Hg/Au modified electrode at different scan rates (a) 25 mV/s,
(b) 50 mV/s, (c) 100 mV/s.

(A) (B)

Fig.2 (A) peak potentials of thiamine HCl reduction show in Fig.1 as a function of logarithm of
scan rates, and (B) magnitude of the peak current, ip, for thiamine HCl reduction From Fig.1 as a
function of square root of scan rate.

Fig. 3 Reductive cyclic voltammograms for 1.0 x 10-3 M of riboflavin recorded in Britton-Robinson
buffer (pH4.86) on Hg/Au modified electrode at different scan rates (a) 25 mV/s, (b) 50 mV/s, (c)
100 mV/s,. (d) 200 mV/s, (e) 400 mV/s.

(A) (B)

00.5111.522.5LogV(mV/S)
Ep(V)

Fig.4. (A) peak potentials of riboflavin reduction show in Fig.3 as an independent of logarithm of
scan rates. (B) magnitude of the peak current, ip, for riboflavin reduction From Fig .3 as a function
of square root of scan rate

Fig. 5 Reductive cyclic voltammograms for 1.0 x 10-3 M of folic acid recorded in Britton-Robinson
buffer (pH 4.86) on Hg/Au modified electrode at different scan rates (a) 25 mV/s, (b) 50 mV/s, (c)
100 mV/s, (d) 200 mV/s, (e) 400 mV/s, (f) 800 mV/s.

(A) (B)

00.30.60.911.522.53LogV (mV/S)
Ep (V)

Fig.6 (A) peak potentials of folic acid reduction show in Fig.5 as an independent of logarithm at

low scan rates. (B) magnitude of the peak current, ip, for folic acid reduction From Fig 5 as a
function of square root of scan rate.

Fig.7 Chromatograms of vitamin standards on a Phenomenex (Luna 5 μ, C 18 , 250 x 4.6 mm)
column. Peaks:1= thiamine ; 2 = folic acid; 3 =. riboflavin; Mobile phase: methanol-water(52：4
8,v/v) containing 30 mM phosphoric acid (pH =2.69); flow rate 0.8 mL/min; detection UV at 265
nm.