科技部補助專題研究計畫成果報告
期末報告

電化學方法及物理吸附修飾網版印刷碳電極在生物感測器的探討與應用

計 畫 類 別 ： 個別型計畫
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報 告 附 件 ： 出席國際學術會議心得報告

中 華 民 國 106 年 03 月 22 日

中 文 摘 要 ： 本研究有系統的探討網版印刷碳電極的預處理方法，利用物裡吸附修飾幾丁質、及電化學聚合三聚氰胺後之電極穩定性探討。我們也以低成本網版印刷碳電極（SPCE）和三聚氰胺作為基礎基質製備電化學生物傳感器。在電化學聚合三聚氰胺之後，三聚氰胺聚合沉積在SPCE表面上以得到具有-NH2官能基，可透過戊二醛與免疫球蛋白E抗體(anti-IgE)交聯鍵結。將所得SPCE-p(ME)-anti-IgE與各種濃度的IgE溶液一起反應，洗去未反應之其他物質然後再使用Ru(NH3)63+作為電化學介質，以計時安培法分析。在5.3和530fM之間觀察到計時電流和IgE濃度之間的對數關係。加入16fM IgE於胎牛血清樣品的回收率為114±14%，檢測極限為0.64fM。此方法，僅需要少量的樣品用於分析，且複雜血清基質並未造成干擾。因此，我們期望這種新穎的系統將有用於監測在過敏性哮喘和鼻炎的臨床治療期間血液IgE水平的變化。

中 文 關 鍵 詞 ： 網版印刷碳電極，電聚合三聚氰胺，免疫分析，電化學生物感測

英 文 摘 要 ： In this study, a systematic pretreatment of screen printing carbon electrode was discussed, and the stability of electrode after physical adsorption of chitosan and electrochemical polymerization of melamine was studied. We also report the use of a low-cost screen-printed carbon electrode (SPCE) and melamine as the base matrices for the preparation of an electrochemical biosensor. Following the electrochemical polymerization of melamine, the resulting polymelamine was deposited on the SPCE surface to give layers bearing –NH2 functional groups, which allowed the attachment of anti-IgE (immunoglobulin E) antibodies. The resulting anti-IgE-labeled SPCEs were then incubated with IgE solutions of various concentrations prior to analysis by chronoamperometry using Ru(NH3)63+ as an electrochemical mediator. A logarithmic relationship was observed between the chronoamperometric current and the IgE concentration between 5.3 and 530 fM (i.e. over 2 orders of magnitude). In addition, a detection limit of 0.64 fM was achieved in addition to a recovery of 114 ± 14% for a fetal bovine serum sample spiked with 16 fM IgE. Furthermore, only a small quantity of sample was required for analysis, and the IgE assay was suitable for use in a complex serum matrix without interference. We therefore expect that this novel system will be useful for monitoring the changes in blood IgE levels during the clinical treatment of allergic asthma and rhinitis.

英 文 關 鍵 詞 ： Polymelamine; Screen-printed carbon electrode; Electrochemistry; Immunoassays

科技部補助專題研究計畫成果報告

（□期中進度報告/■期末報告）

電化學方法及物理吸附修飾網版印刷碳電極在生物感測器的探討與應用

計畫類別：■個別型計畫　□整合型計畫

計畫編號：MOST 104 －2113－M－040－002－

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計畫主持人：蔡惠燕

共同主持人：

計畫參與人員：丘明達、吳羅賢、謝誌中

本計畫除繳交成果報告外，另含下列出國報告，共 _1 份：

□執行國際合作與移地研究心得報告

■出席國際學術會議心得報告

□出國參訪及考察心得報告

中　華　民　國 106 年 3 月 20 日

一、前言(研究目的、文獻探討)

網版印刷電極是開發簡單、快速和廉價的生物傳感器最具潛力的方法之一。可拋棄式的生物感測印刷電極可透過修飾電極表面，使其帶有具選擇性的生物探針(bioprobes)，和檢測生物分子，殺蟲劑，抗原，DNA，微生物和酶作用。其發展可分為 enzyme-labeled immunosensors 及 label-free immunosensors。Enzyme-labeled immunosensors 的應用，bioprobes 是利用吸附(adsorption)方式附著在電極上[1, 2], 然後利用 BSA 做 blocking，再將電極浸泡(incubate)在分析溶液中，最後與標有酵素的二抗反應(如 alkaline phosphatase (ALP) or horseradish peroxidae (HRP) -labeled second antibodies)，利用 p-aminophenyl phosphate 與 ALP 反應，透過電化學方法偵測生成之 p-aminophenol，此方法需要二級抗體、酵素等昂貴的藥品，且偵測較為麻煩，因此近幾年以發展 label-free immunosensors 為主流，將 bioprobes 以共價鍵方式與網版印刷電極結合，再和待檢測分子結合， 因蛋白質分子導電性不佳， 因此電極阻抗改變， 或利用 $Fe(CN)_6^{3-/4-}$ 當測量介質，蛋白質改變電子傳遞速度， 而改變電流信號。此類網版印刷電極一般使用金電極或於電極上加上金奈米粒子(AuNP)[3-5]，因為金與硫醇的反應性佳， 如 hexanedithiol or 6-mecapto-1-hexanol 可在金的表面形成自組裝單層(self-assembled monolayer)，方便做 cross-linking 反應接上具選擇性的 bioprobes (antibody or antigen)，而網版印刷碳電極因為缺乏官能，須與其他物質如 AuNP[6] 或功能化的 carbon nanotubes[7] 結合，不只可以增加電極表面積，亦可使電極表面適合進行 bioconjugation。另一發趨勢則是利用電氧化聚合 diamine 在電極表面上， 如 hexamethyldiamine [8]。近年有來文獻報導三聚氰胺 (Melamine)可透過電化學聚合方式在碳電極表面形成一層聚合物[9-13]，poly(melamine) 具導電性，且表面帶有 $–NH_2$ 官能基。以成本考量三聚氰胺較 hexamethyldiamine 便宜許多，為了降低生物感測印刷電極的生產成本， 本研究擬開發以電化學方法聚合三聚氰胺表面修飾網版印刷碳電極做為生物感測器， 並與物理吸附幾丁質表面修飾網版印刷碳電極做比較。

生物感測器是否具備商業化的潛能，與電極的製備是否具再現性及製備好的電極是否可以穩定保存有密切關係。而一般文獻僅報告用於該報導的方

法，前處理對電極信號及穩定度的影響並無系統性的報導，所以本研究分兩部份，第一部份針對所購買的網版印刷碳電極做一系列的前處理探討。第二部份則為生物感測之應用。以下分兩單元敘述結果。

第一單元: 網版印刷碳電極前處理系列的探討

研究方法、進行步驟

1.本計畫採用之研究方法與原因。

Melamine(ME)為一種常見的塑料原料，有安定、價格便宜和容易取得等優點。Fig 1 為 melanine 的結構可以看到其表面有三個-NH_2 基團，在過去的文獻中報導將 melamine 溶解於酸性環境，並施加高電位使 melamine 產生帶有自由基陽離子後修飾於電極表面[14], [15], [16] ,[17]。當施加足夠的時間於電極表面時，melamine 會於電極表面形成一層 poly(melamine)膜；這層膜因為是由 melamine 聚合而成，所以表面帶有相當多 NH_2 基團，可以用於後續使用 EDC/NHC 或 Glutaraldehyde 兩種交聯劑來和抗體進行交聯反應，進而固定抗體於電極表面。

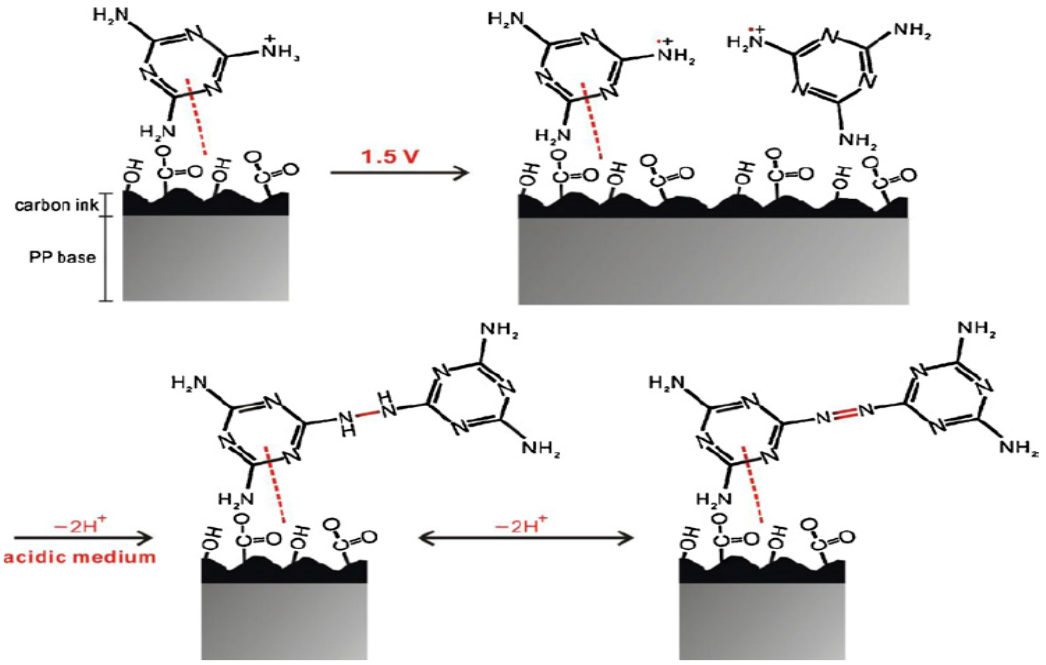


Fig 1 melamine(ME)氧化修飾於 SPCE*表面示意圖

Chitosan(CS)則是由糖苷鍵組合而成為長鏈狀分子，由約 8000 個單體組成的多醣，是昆蟲類和甲殼類生物殼中主要成分。最早在 1811 年由法國科學家 Braconnot 利用鹼性溶液處理洋菇時發現，有不溶物而命名為〝Funggine〞。經過 20 年後 Odier 科學家又於昆蟲表皮較為堅硬部分發現類似的物質。幾丁質(chitin)此名稱是來自於法文，〝chitine〞是衍生自希臘字 chiton，意思是覆蓋(Covering)；1859 年 Rouget 發現經由鹼性溶液共煮加熱後幾丁質可以轉變為可溶於有機酸的物質。1894 年 Hoppe-seyler 將此物質命名為幾丁聚醣(chitosan)如 Fig 2 所示。Chitosan 的 Isoelectric point(pI)約為 pH=6.5 所以當 chitosan 於酸性溶液下表面的 NH_2 會質子化形成 NH_3^+使得 chitosan 成為帶有正電的聚合物，所以 chitosan 可溶於醋酸、甲酸、乳酸和蘋果酸等有機酸，鹽酸、磷酸等無機酸亦可溶解，但 chitosan 並不溶於中性或鹼性溶液。chitosan 表面帶有大量的 NH_2，可利用交聯劑與蛋白質結合，所以本研究中利用物理性吸附方式將 chitosan 修飾於電極表面。

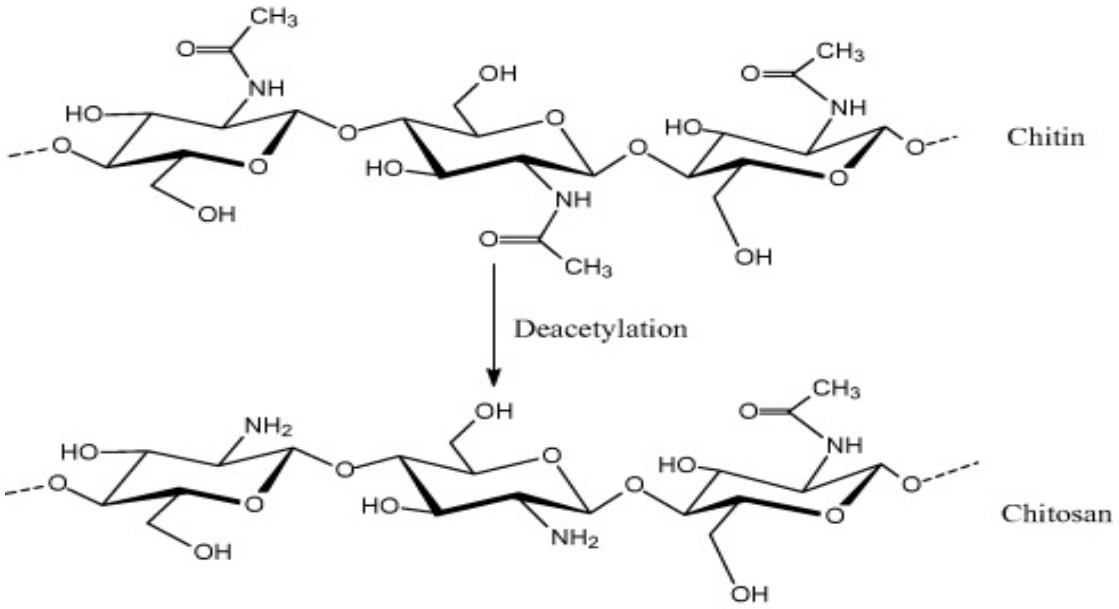
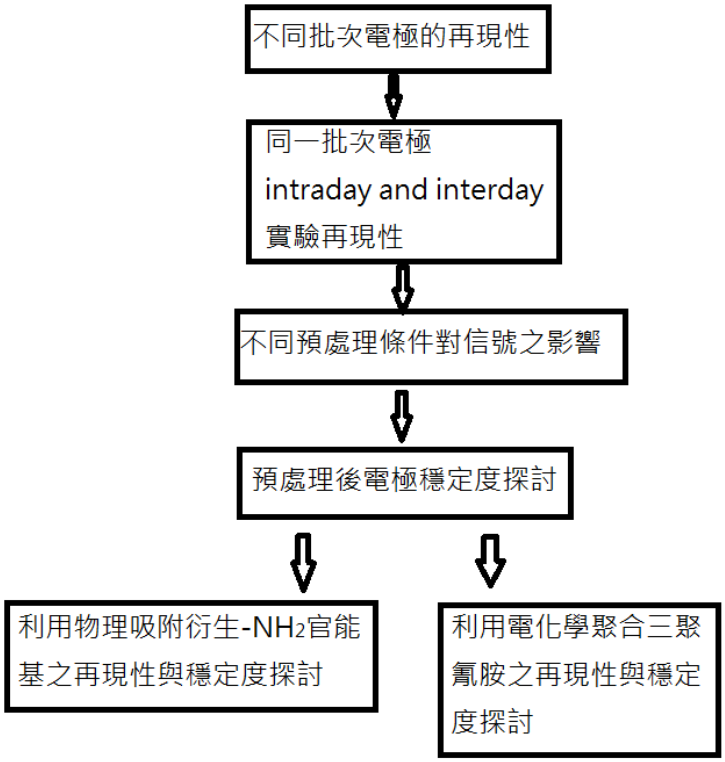


Fig 2 幾丁質(chitin)去乙醯基後轉變為幾丁聚醣[18]

探討流程如下，所有條件主要透過測量電化學介質 (mediator) $Fe(CN)_6^{3-}$ 訊號變化做比較。



2. 實驗步驟

2.1 SPCE*-chitosan 電極修飾：預處理及物理性吸附 chitosan

(1.) 預處理

將單碳電極浸入 pH 7.4 之 0.1M 磷酸緩衝溶液(phosphate buffer solution, PB)，以環伏安法(Cyclic Voltammetry，CV)施加電位-0.6~1.8V(vs. Ag/AgCl)掃描 20 圈，掃描速率 0.3 V/s。已預氧化的電極，標示為 SPCE*。

(2.) 物理性吸附 chitosan

將預處理後的 SPCE*，以浸鍍機進行電極修飾，將 SPCE*浸入含 1mg/ mL chitosan 之 2%醋酸溶液 3 秒後(上升、下降速率：200cm/min)，電極於室溫下乾燥。修飾 chitosan 後的電極標示為 SPCE*-CS。

2.2 SPCE*-poly(melamine)電極修飾

(1.) 電極清洗及預氧化

將單碳電極浸入 pH 7.4 之 0.1M 磷酸緩衝溶液(phosphate buffer solution, PB)，以環伏安法(Cyclic Voltammetry，CV)施加電位-0.6~1.8V(vs. Ag/AgCl)掃描 20 圈，掃描速率 0.3 V/s。以 Amperometry 的方式，施加 2V 的電位 300s，將電極預氧化。已預氧化的電極，標示為 SPCE*。

(2.) 電化學聚合沉積 melamine

將 SPCE*浸入含 1mM melamine 之 0.1 M HCl 溶液中，施加電位 0.2~1.5V(vs. Ag/AgCl)掃描 10 圈，掃描速率 0.1 V/s。經修飾後的電極，標示為 SPCE*-p(ME)。

結果與討論

1. 網版碳印刷電極(SPCE)以不同電化學方法預處理最適化探討

1.1 探討不同購買批次電極之間的差異

過去的經驗顯示網版印刷碳電極使用前均需做預處理以除去表面之雜質及活化碳。本實驗先用安培計時法(amperometry) 施加 1.8V (Ag/AgCl) 180s，預處理後馬上放入含 5mM 赤血鹽 之 PB 中掃 CV。圖 3 為不同批次電極的再線性及同批次電極不同天實驗之比較。CV 之相關數據整理於表 1。綜合各項參數表現， MD 批次電極再相同預處理條下， 其電極表面活性比 LG 批次好 (t-test 結果 $p<0.05$)。同一批電極以赤血鹽還原信號而言，intra-assay 再現性為 0.2%-2.0%, 三天平均值之再現性(interday)為 0.3%。若將不同批次各三重複數據平均，其再現性為 3.7% (LG and MD-1 同天不通批次電極)，所以要做條件探討應盡量用同一批次生產之電極，再現性比較好。

表 1　不同批次電極所得 CV 結果

電極批次	Epc	Ipc(μA)	Epa	Ipa(μA)	ΔE	Ipa/Ipc
MD (N=9)	0.0504	128.2(±1.2%)	0.3596	114.6 (±3.5%)	0.309	0.89
LG (N=3)	0.0123	120.6(±1.6%)	0.3883	95.86(±4.5%)	0.38	0.79

註: Epc: cathodic peak potential, Epa: anodic peak potential, Ipc: cathodic peak current, Ipa: anodic peak current, ΔE=Epa-Epc 表示氧化/還原電位之差值，其值越接近於 59mV，表示電極表面動力學越符合擴散控制； Ipa/Ipc 表示氧化/還原電位之比值，其值越接近 1，表示氧化還原為完全可逆反應(reversible redox)

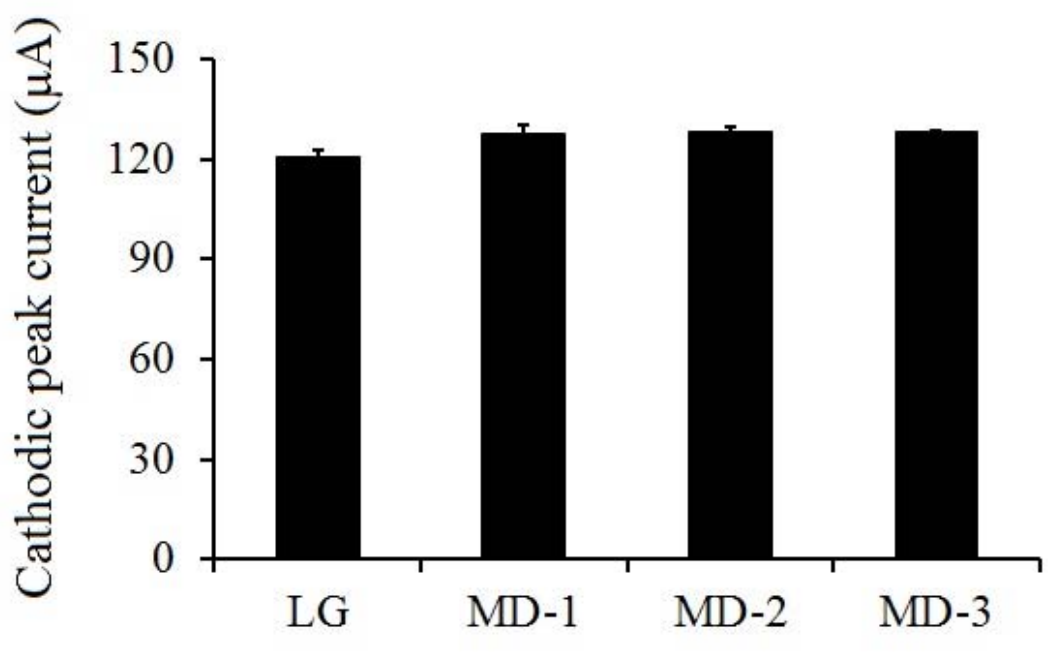


Fig 3　不同批次電極預處理後之再現性。LG、MD 為電極之批次編號，MD-1、MD-2、MD-3 分別為三天之實驗。每一次實驗均為三重複。

1.2 預處理條件探討

圖 4 為利用不同條件預處理 SPCE 的結果。從赤血鹽還原信號顯示，施加 2.0V 180 sec 可得最佳訊號根據文獻推測原因是碳表面氧化增加 C=O 或 C-OH 等親水性官能基， 2.0V 240sec 信號反而下降，可能是過度氧化造成碳膠剝落所致。不同預處理條件均可獲得良好再現性。

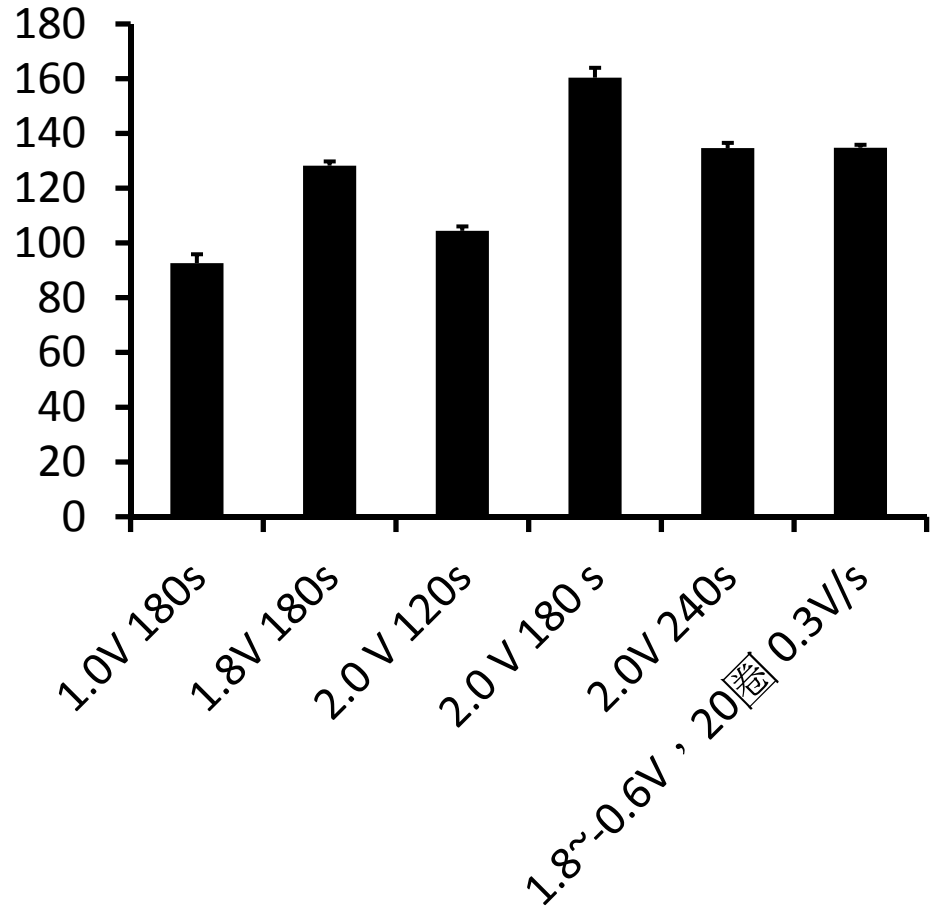


Fig 4 不同預處理條件對 SPCE 活性之影響。(N=3)

1.3 SPCE*保存穩定性探討

如果要將 SPCE*應用於現場即時檢測(in-field or on-site)，最好是先預處理好電極，在現場檢測使用上比較方便，SPCE 經過預處理後，表面生成 C=O 或是 C-OH 於空氣可能不穩定，因此探討放置時間對於 SPCE*測量物質時所產生的影響。以上述所得最佳條件 2.0 V 180s 預處後之電極 SPCE*靜置於室溫並暴露於空氣中的時間對電極表面活性之影響，利用 CV 測量 5 mM $Fe(CN)_6^{3-}$ in 0.1 M PB，所得結果如圖 5(a)，結果顯示

高度氧化之 SPCE*是不穩定的， 1 h 後活性降低 28 %。圖 5b 為用 CV 1.8V ~ −0.6V , scan rate = 0.3 V/s, 20 cycles 預處理後 SPCE*靜置於室溫並暴露於空氣中的時間對電極表面活性之影響，結果顯示電極預處理後放置於室溫並暴露於空氣半小時內會造成信號變化較明顯， 但在 95% confidence level 下仍無顯著的差異 ($p>0.05$)，儲存(up to 22 h)期間各時段三支電極所得平均值並無顯著差異，但再現性較差(RSD% 增加到 7.4%)。此組實驗 0 小時為對應三個不同儲存時間點做一組(N=3)預處理完馬上測，共 9 個數據的平均值。實驗結果顯示除非有特殊需求，購買之 SPCE 電極使用前之活化處理以 CV 1.8V - −0.6V , scan rate = 0.3 V/s, 20 cycles 較佳。

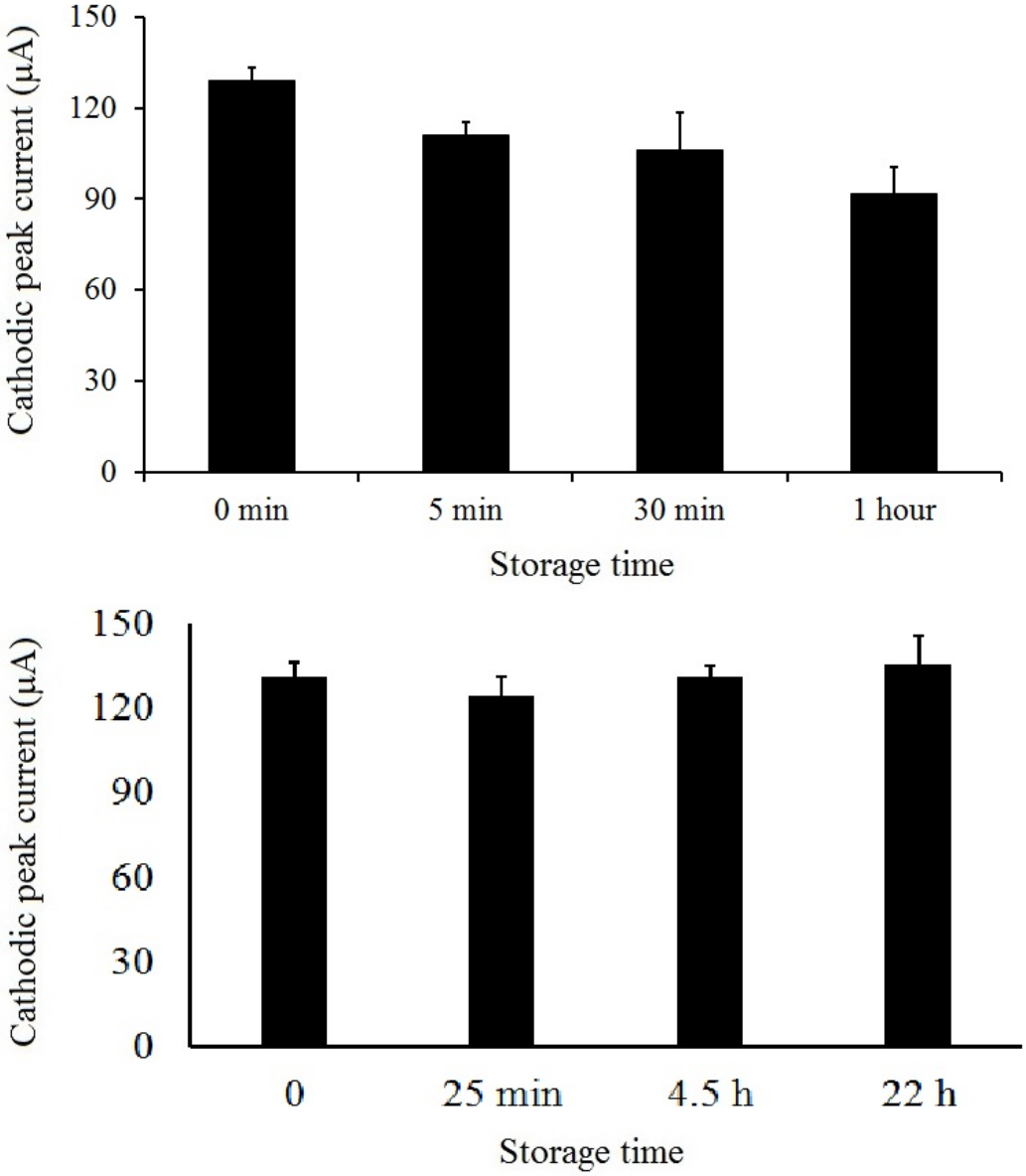


Fig 5　SPCE* 靜置時間對電極表面活性之影響。(a) 以 2.0 V 180s 預氧化 (b) 以 CV 1.8V~−0.6V , scan rate = 0.3 V/s, 20 cycles 預氧化，預處理後馬上測量，及靜置於室溫蓋上拭鏡紙防塵埃，於不同時段取出測量。cathodic peak current 為測量 5mM $Fe(CN)_6^{3-}$ in 0.1 M PB。

2. 物理吸附 chitosan 之探討

SPCE 預處理後需經過表面修飾(surface modification)使電極表面帶官能基才能與生物探針(bioprobe)鍵結，做為 biosensor 之應用。此處探討以物理吸附 chitosan 之可行性。圖 6 為 SPCE 不同階段處理後所測得赤血鹽之 CV 圖，SPCE*在修飾 chitosan 之後，初步推測因為 chitosan 表面擁有許多的親水性官能基，如-NH_2 及-OH，能使電極表面親水性增加，水溶液中的 $[Fe(CN)_6]^{3-}$ 更容易擴散至電極表面進行反應，因此阻抗下降，電流信號增加。圖 7(a)為 SPCE* 浸泡在 2% 醋酸溶液(acetic acid) 中及 coating chitosan 後其電極活性變化。結果顯示以 CV 1.8V - −0.6V , scan rate = 0.3 V/s, 20 cycles 預處理之 SPCE* 電化學活性不只在空氣中穩定，浸泡在醋酸溶液 25min 後，仍無顯著差異，與浸泡錯酸溶液之結果比較，SPCE*-CS 的信號有顯著增加，顯示赤血鹽還原信號增加確實是因為電極表面吸附 chitosan 所致。比較 SPCE* 浸泡在含 chitosan 的醋酸溶液 3s 及 25 min 後以 200 cm/min 的上升速度拉出液面，所形成之 SPCE*-CS 測量赤血鹽，還原信號並無顯著差異，顯示浸泡於溶液的時間並不影響塗布結果，所以進行 dip coating 時，每次 dipping 的時間並不需要特別去控制，只需控制拉起來的速度維持固定即可。圖 7(b)為 SPCE*-CS 電極保存穩定性之探討，結果顯示 SPCE*-CS 乾燥保存 6 天，此期間電化學活性無顯著差異。Intra-assay 再現性在 0.4% - 1.7%之間。 Inter-assay 再現性為 1.7% (N=5). 顯示本研究所用之製備方法可生產具有良好再現性的 SPCE*-CS 且至少可保存 6 天。

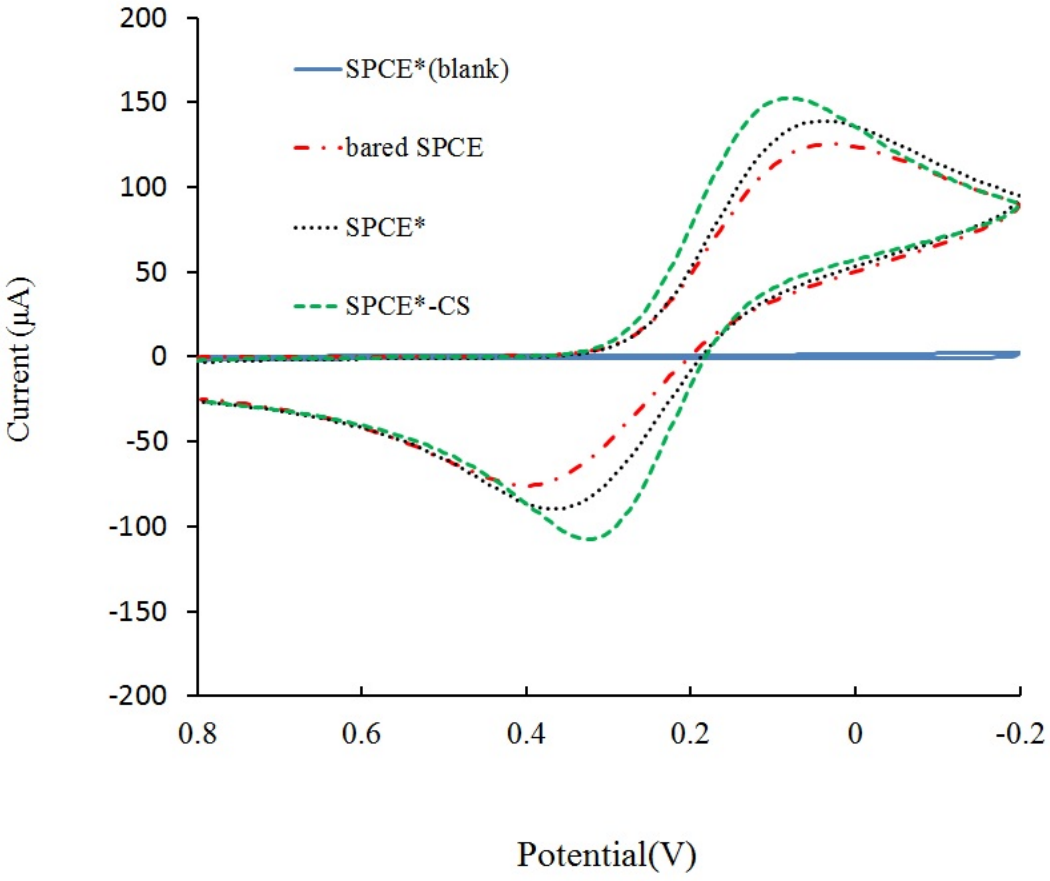


Fig 6　不同階段之 SPCE 所測得赤血鹽之 CV 圖，scan rate 0.1V/s 。blue line 為 SPCE* in pH7.4 0.1M PB(background signal)；red —·—、black ···· and green - - - 分別為 bared SPCE、SPCE*、and SPCE*-CS measured in pH7.4 0.1M PB with 5mM $Fe(CN)_6^{3-}$。

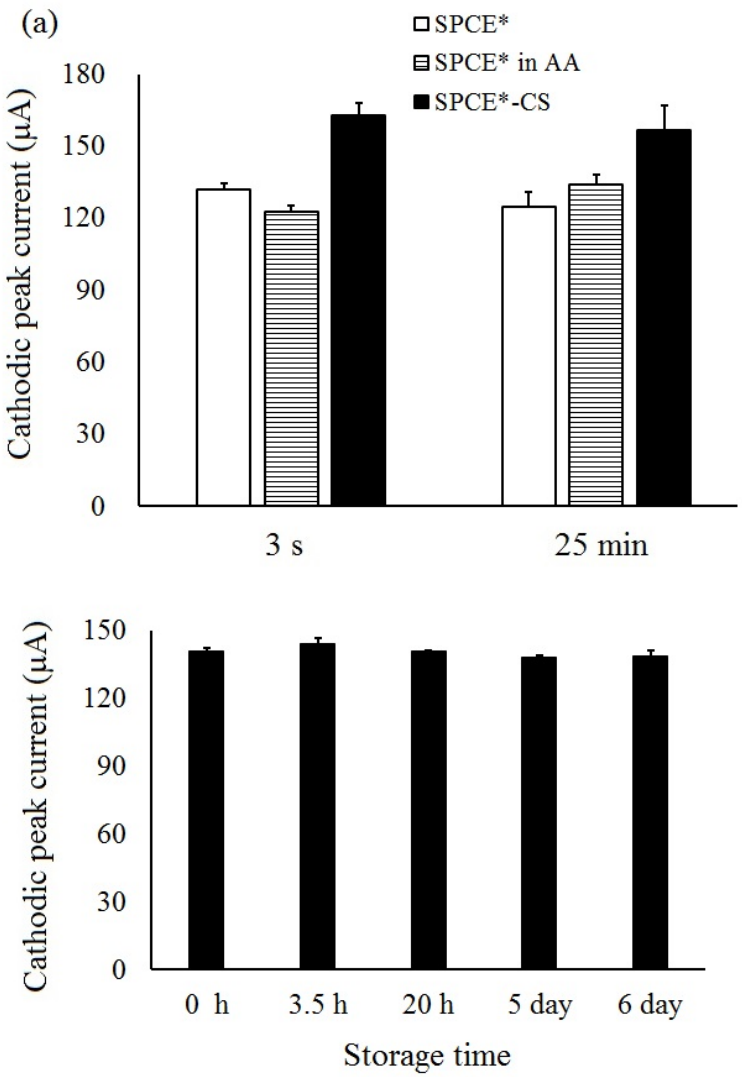


Fig 7. SPCE* coating chitosan (a) 浸泡溶液之影響，SPCE* 為電極預處理後直接使用，SPCE* in AA 為 SPCE*浸泡在 2% 醋酸溶液(acetic acid)中，SPCE*-CS 為 SPCE* 浸泡在含 1mg/mL chitosan 之 2%醋酸溶液中，然後利用 dipping coater 定速拉出於室溫下風乾。SPCE*靜置於空氣中或浸泡於溶液中的時間分別是 3s 及 25 min.。(b) SPCE*-CS 電極保存穩定性之探討。製備好的 SPCE*-CS 放置於室溫蓋上拭鏡紙防塵埃，於不同時間拿出來測 5mM $Fe(CN)_6^{3-}$ in 0.1 M PB。

Chitosan 的等電點為 pH=6.5，當溶液的 pH 低於 6.5 時 chitosan 表面的 NH_2 質子化轉變為 NH_3^+使電極表面呈現帶正電，為進一步驗證，chitosan 有吸附在電極表面，且因其親水性或靜電作用(electrostatic force)

促使赤/黃血鹽($Fe(CN)_6^{3-}$及 $Fe(CN)_6^{4-}$)更易進入擴散層及促進電極表面與電化學介質間之電子傳遞，我們探討溶液 pH 值對電流信號之影響。圖 8 結果顯示當電極只有經過預氧化(SPCE*)，改變溶液的 pH 並不會造成赤/黃血鹽氧化訊號有明顯的變化，當 SPCE*修飾上 chitosan 後赤/黃血鹽氧化及還原電流均會隨著 pH 降低而增高，因為 chitosan 的等電點為 pH=6.5，當環境的 pH 低於 6.5 時 chitosan 表面電荷呈現帶正電性，赤/黃血鹽於水溶液中帶負電，兩者產生電荷吸引(electrostatic attraction)使赤/黃血鹽更容易靠近電極表面加速氧化還原反應，導致電流上升。此結果進一步證明 chitosan 吸附在電極表面，及信號增強的原因。

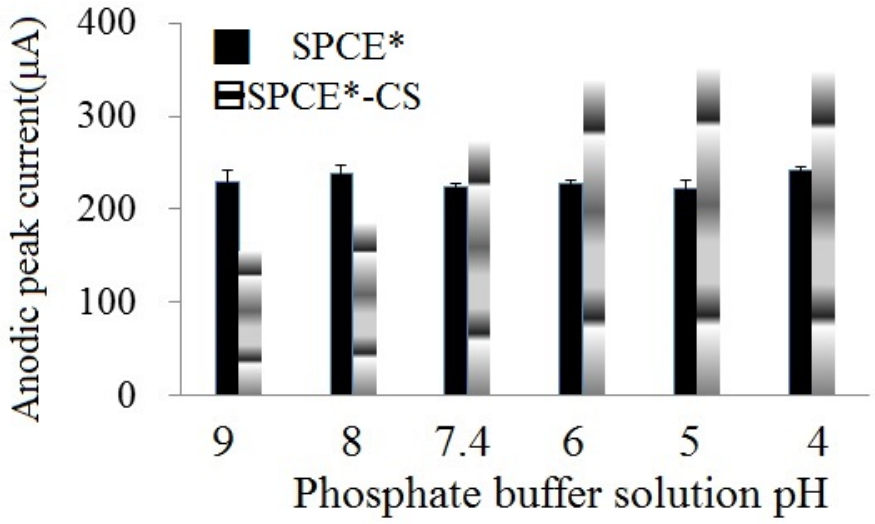


Fig 8　pH effects， SPCE*和 SPCE*-chitosan 浸泡於含赤/黃血鹽各 5 mM 之不同 pH 值的 PB 緩衝溶液中，CV -0.2 V ~0.8 V，scan rate=0.1V/s，所得之之氧化電流。

註: 本實驗為尚未購置 dip coater 時做的，利用手動方式做 coating。

Chitosan 並非良好導體如果電極表面 Chitosan layer 加厚則會阻礙電子傳遞。圖 9 (a) 比較 SPCE*及做一次至三次 dip-coating chitosan(即 I、II、III layers)之電極所得電流。圖 9 (b)則為其對應電極所得之阻抗值(at frequency= 1 Hz)。實驗結果顯示修飾兩層 chitosan 的 SPCE*CS 電極有最佳的電流信號表現，從阻抗的數據也得到相對應的結果。在 SPCE*修飾

兩層 chitosan 後，其親水性質達到最佳狀態，但是修飾到三層 chitosan 時，因為電極表面上所修飾 chitosan 的厚度已經對$[Fe(CN)_6]^{3-}$的電子傳遞造成阻礙，因此阻抗值再次上升，使電流信號下降。

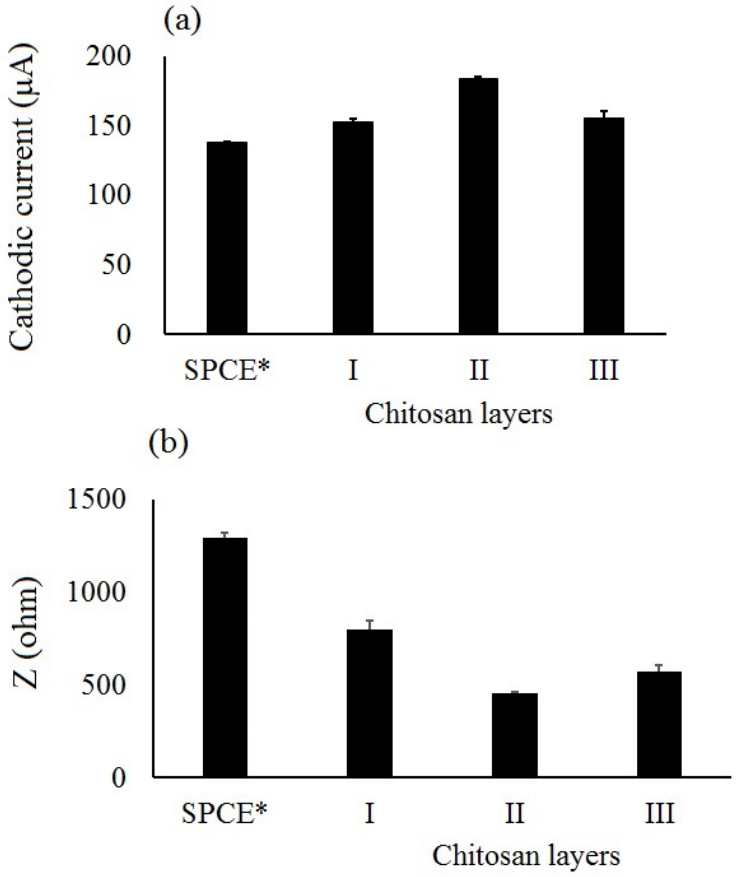


圖 9 (a) 比較 SPCE*及做一次至三次 dip-coating chitosan(I、II、III layers)之電極在 5mM 赤血鹽溶液中的 CV 0.8~-0.4V, scan rate=0.1V/s 所得之還原電流。 (b)為其對應電極所得之阻抗值(at frequency= 1 Hz)。在 5mM 赤血鹽溶液中，在 0.2 V (振幅± 5mV)之交流電位下的阻抗值。(n=3)

綜合上述結果，修飾兩層 chitosan 為 SPCE*-CS 的最佳化條件，因為 chitosan 的親水性使電流信號增強。且 SPCE*-CS 至少可穩定保存 6 天。

3. 電化學聚合三聚氰胺修飾電極 SPCE*-p(ME)探討

3.1 預氧化溶液最佳化條件

從過去的參考文獻[11]中得知網版碳印刷電極在修飾 melamine 之前需要將電極表面碳膠做 2V 300s 高度預氧化的處理，使電極表面達到高度氧化狀態，帶有許多 C=O 官能基，可以將 poly(melamine)吸附在電極表面。為了增加 C=O 官能基的數目，本研究探討利用具有 CO_3^{2-}的溶液及磷酸緩衝溶液來進行預氧化，圖 10 的結果顯示，在 1M Na_2CO_3 溶液中預氧化處理後的 SPCE*-p(ME)沉積在電極表面之 polymelamine 的自身養化還原信號遠小於那些在 0.1M pH 7.4 PB 溶液中預氧化處理後的 SPCE*-p(ME)所得之信號，表示以 1M Na_2CO_3 溶液進行預氧化處理並沒有預期增加 C=O 官能基數目的效果，所以之後選用 0.1M pH 7.4 PB 溶液進行預氧化處理。

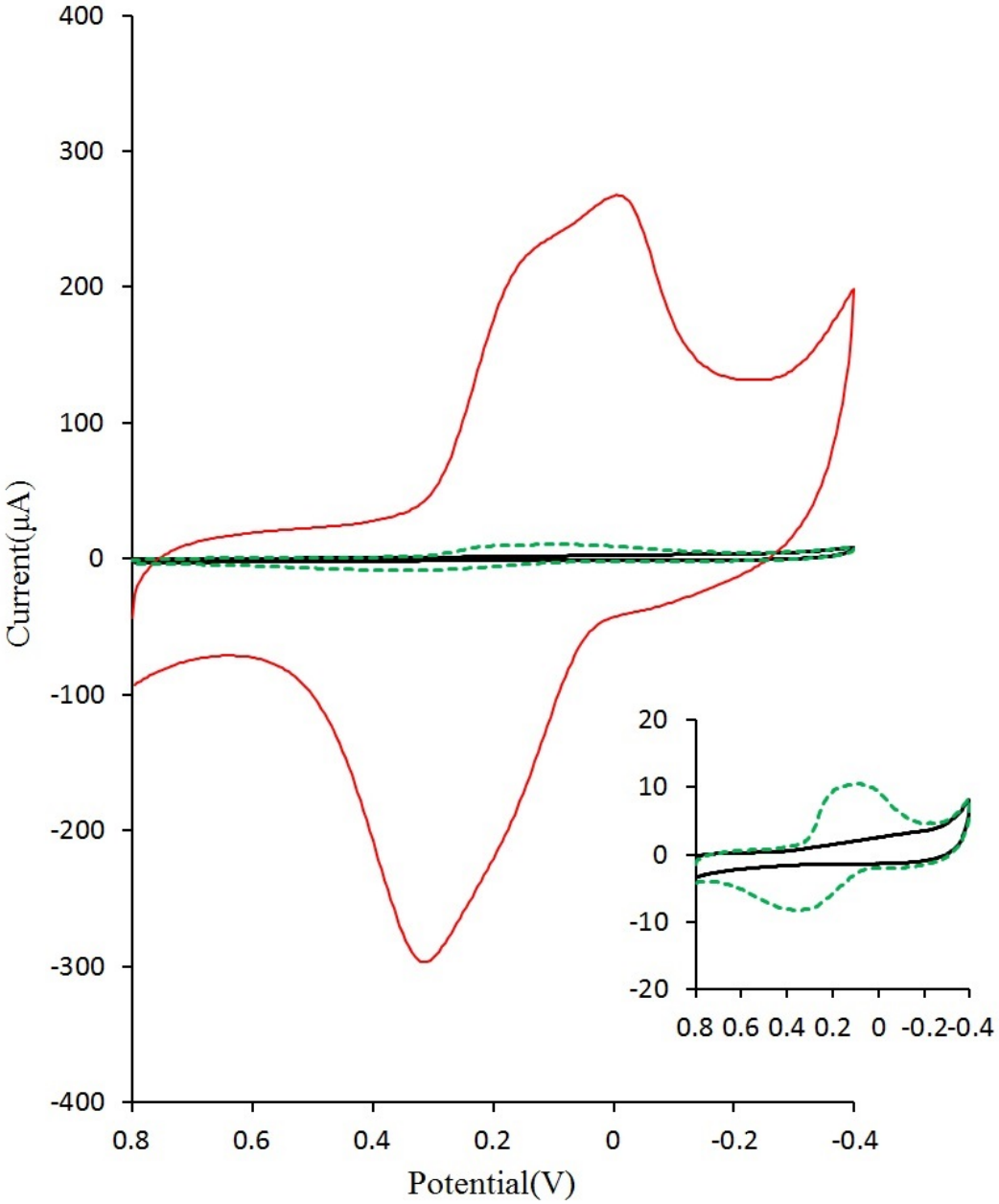


圖 10 電極在不同溶液預氧化對後續電化學聚合沉積 polymelamine 之影響。將 SPCE*-p(ME)置於 pH7.4 0.1M PB 中，CV 電位範圍 0.8 V~ -0.4V， scan rate=0.1V/s 所得之 poly(melamine)自身氧化還原信號。電極在電化學聚合 poly(melamine)之前的處理方法，分別為只用 CV 預氧化 SPCE 未進行電聚合沉積 polymelamine(black line)，在 1M Na_2CO_3 中預氧化(green dash line)，在 0.1M pH 7.4 PB 中預氧化(red line)。插圖(inserted fig)為預氧化 SPCE 未進行電聚合沉積 polymelamine(black line)，在 1M Na_2CO_3 中預氧化(green dash line)之放大圖。

3.2 清洗電極之最佳化電位範圍

在進行 2V 300s 預氧化的步驟前，會對網版碳印刷電極進行清洗的步驟，將電極表面上的殘膠以電化學方法清除乾淨，此步驟可以增加後續 polymelamine 的沉積，本研究測試了溫合的清洗電位範圍及之前用來活化電極之 CV 電位範圍，圖 11 為實驗結果，顯示網版碳印刷電極在經過 1.8~-0.6V 電位範圍的清洗後，其 SPCE*-p(ME)的沉積效果較好，可獲得較高的自我氧化還原信號且再現性也較好，推論此清洗電位除了清洗表面殘膠，也有助於提高電極表的氧化程度，提高後續 polymelamine 的聚合沉積的效果。

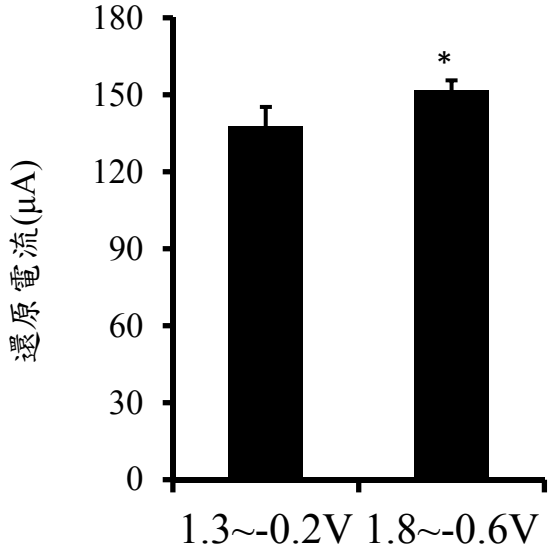


圖 11 清洗電極之電位範圍，對 polymelamine 沉積之影響。CV scan 清洗後電極，施加 2V 的電位 300s，將電極預氧化，然後將電極浸入含 1mM melamine 之 0.1 M HCl 溶液中，施加電位 0.2~1.5V(vs. Ag/AgCl)掃描 10 圈，掃描速率 0.1 V/s。SPCE*-p(ME)在 5mM 赤血鹽溶液中的 CV 0.8V~ -0.4V, scan rate=0.1V/s，還原電流信號 t-test 結果有顯著差異(*$p<0.05$) (N=3)

3.3 電化學聚合沉積 polymelamine 步驟最佳化

在電化學聚合沉積 melamine 步驟中，兩個關鍵實驗條件，其一是電化學聚合的速率，另一個為電化學聚合 CV 掃描電位範圍及圈數。根據

Baskar[11]的結果 CV 掃描電位最佳範圍為 0.2~1.5V(vs. Ag/AgCl)，掃描 20 圈後達 steady-state 狀態，但 Baskar et. al.並未探討 scan rate，所以本研究首先探討 scan rate 的影響。理論上放慢 scan rate 可以使 melamine 的聚合堆疊效果更完整，圖 12(a)的結果顯示兩個不同的 scan rate 修飾出的 SPCE*-p(ME)其電流信號表現並無顯著差異，而且較慢的 scan rate 其修飾再現反而比較差，所以 0.1V/s 的 scan rate 所修飾出的 SPCE*-p(ME)有較好的修飾表現。已知增加施加電位的時間或掃描圈數時，可以增加 melamine 在電極表面的聚合量[11]，本研究的目的是修飾上 polymelamine 後利用-NH_2 與 bioprobe 上的-NH_2，所以並不希望三聚氰胺之-NH_2 完全用來做聚合反應，因此只需有穩定的一層 polymelamine 在電極表面即可。圖 12(b)顯示掃描 10 圈所得的 SPCE*-p(ME)有較良好的再現性表現(RSD%=0.92%，N=3)，無論是 10 圈還是 20 圈的 CV 電聚合沉積，所得電極在 PB 中都有良好的穩定性 (圖 13)，SPCE*-p(ME)保存在 PBS 中 3hr 後， 20 圈的電極信號與 10 圈所得電極自身氧化還原的信號無顯著差異。所以選擇修飾 10 圈 melamine 作為最佳化條件。此表面處理方式，**在本研究中已利用來做 biosensor 的應用，相關結果已整理，目前在審稿中。所以有關 polymelamine 之探討將於第二單元中一並討論。**

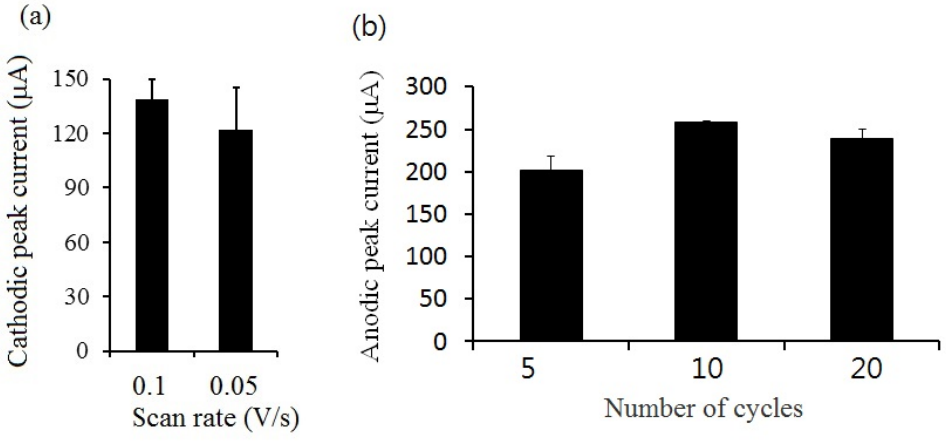


圖 12 不同聚合速率(a)與不同聚合圈數(b)對 SPCE*-p(ME)的影響， SPCE*放入含有 1mM melamine 的 0.1M HCl 中以 CV 掃描不同的圈數的後，再放入 0.1M HCl 中做 CV0.8~-0.4V, scan rate=0.1V/s 掃描測定三聚氰胺自身的還原

信號。(N=3)

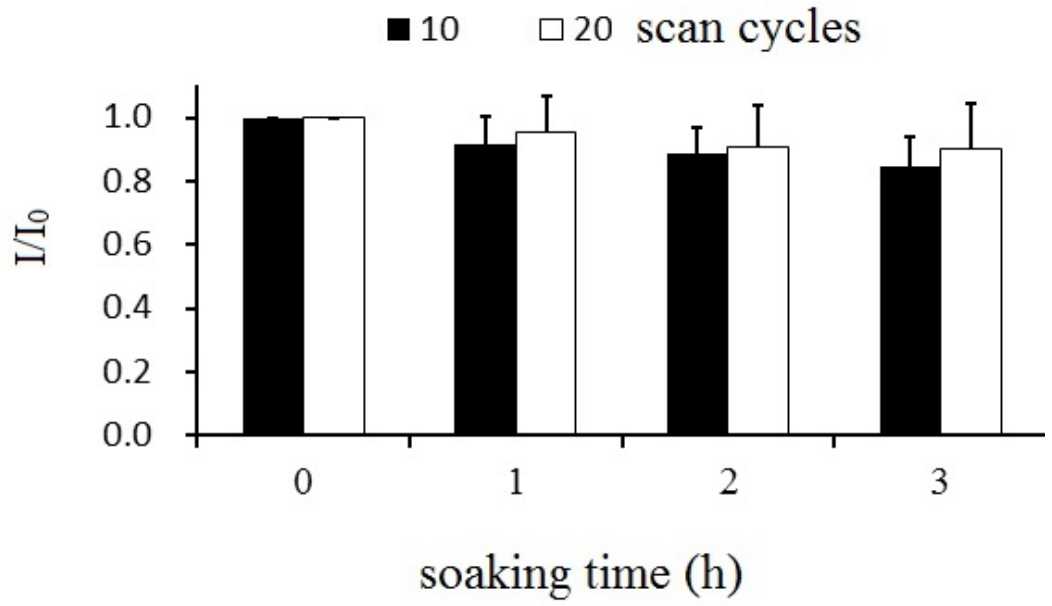


圖 13 SPCE*-p(ME)保存在 PB 中的穩定度，SPCE*-p(ME)取出以去離子水清洗後保存於 PBS(Phosphate buffered saline)溶液中，每隔 1hr，在 0.1M HCl 溶液中再掃描 poly(melamine)自身氧化還原信號。掃描電為範圍 1.0~0.2V, scan rate 0.1V/s) (n=3)， I_0 為製備好的電極馬上測之 polymelamine 自身還原電流，I 為浸泡後之電極所得電流。

第二單元利用 SPCE 做 biosensor 材開發生物感測器之可行性探討

初步結果顯示 SPCE*-CS 無論是用 EDC/NHS 還是 glutaraldehyde 做交聯， 所得 bioprobe 效果並不理想， 所以我們探討利用 polymelamine 的應用， 相關結果如下。

Feasibility study of biosensors based on polymelamine-modified screen-printed carbon electrodes

1. Introduction

The prevalence of allergic diseases and asthma is increasing worldwide, with both the complexity and the severity of such diseases being expected to continue to increase, particularly in children and in young adults. In children, the initial symptoms of allergies are similar to those of the common cold, and are therefore often misdiagnosed. In addition, patients suffering from allergies often have other comorbid symptoms, such as diabetes, obesity, or cardiovascular disease, resulting in complex clinical situations and poor outcomes [19]. Furthermore, patients suffering from atopic allergic diseases, such as hay fever, asthma, and allergic rhinitis, have been shown to have increased total immunoglobulin E (IgE) levels [20]. Indeed, Freeman and Olivier [21] recently reported that hyper-IgE syndromes could be associated with parasitic infections in addition to a range of pulmonary conditions. As such, the diagnosis of allergies can be achieved by screening methods, including quantitative serum IgE immunoassays, which can confirm that the observed asthma and rhinitis are associated with allergic reactions, and to facilitate the appropriate treatment decisions. Thus, although the monitoring of reductions in IgE content is helpful for the assessment of therapeutic responses [21], the process of withdrawing blood is challenging in children and in some adults. We therefore wished to develop a highly sensitive method for IgE detection. In this context, our first choice was the development of a suitable electrochemical method, as such techniques are facile, have low instrument costs, and could be similar to those employed in blood glucose meters. Thus, to develop a biosensor with good commercial potential, it must be low cost, and

exhibit both low reagent consumption and a rapid response.

To date, a number of studies have reported the electrochemical sensing of IgE [22-33], with the majority of these methods employing gold electrodes along with silver or gold nanoparticles as the base matrices for binding either an IgE-specific aptamer or anti-IgE antibodies. However, silver and gold are expensive materials, and so the use of screen-printed carbon electrodes (SPCEs) has been examined due to their design flexibility and reasonably priced process automation [3]. It was therefore considered that SPCEs may be a suitable material for the construction of disposable biosensors. However, as these materials bear no surface functional groups that can bind with biomaterials, surface modification is therefore required. To date, a number of electrochemical biosensors have been prepared through the immobilization of biomolecules onto conducting polymer films using polyaniline, polypyrrole, and poly(*p*-phenylene) moieties [34]; however, use of the melamine $–NH_2$ group as a functional group to link antibodies to the electrode surface has not yet been reported. Thus, melamine was of particular interest as it is relatively cheap, and because a number of studies have reported the use of a polymelamine film on SPCEs as a catalyst for electrochemical detection [35-41].

Thus, we herein report the electrochemical polymerization of melamine and use of the resulting polymelamine as a matrix for linking an anti-IgE probe, ultimately yielding a biosensor on an SPCE surface. This system will then be employed for the *chronoamperometric* detection of an electrochemical mediator to demonstrate the potential of this system for use as a highly sensitive and cost-effective biosensor.

2. Experimental

2.1 Chemicals and reagents

Disodium hydrogen phosphate (≥99%) was purchased from Showa (Tokyo, Japan) and sodium dihydrogen phosphate (assay purity 98.0-102.0%) was purchased from Fisher Scientific (Geel, Belgium). Melamine, glutaraldehyde (25%), and hexaammineruthenium(III) chloride [$Ru(NH_3)_6Cl_3$] (≥99%) were obtained from Acros Organics (Geel, Belgium). Potassium hexacyanoferrate [$K_3Fe(CN)_6$] (≥99.0%) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents were used without further purification. Human anti-IgE antibodies

from mouse and human IgE were obtained from Abcam (Cambridge, MA, USA). The human IgE was generated using human IgE kappa light chains with monoclonal hybridomas. Fetal bovine serum (FBS) was purchased from Biological Industries Ltd. (Kibbutz Beit Haemek, Israel).

2.2 Apparatus

A CH Instruments Electrochemical Workstation 6124E System (Austin, TX, USA) was used for all electrochemical experiments. A three-electrode system was employed, where the working electrode was a modified screen-printed carbon electrode, the reference electrode was a 3 M NaCl, Ag/AgCl electrode, and the counter electrode was a Pt wire. An MP220 Mettler Toledo pH meter was used to measure the pH values of the buffer solutions. The SPCEs (diameter: 5 mm; electrode surface area: 0.196 cm^2) were purchased from Zensor R&D (Taichung, Taiwan).

2.3 Preparation of the polymelamine-modified SPCE (SPCE*-polymelamine)

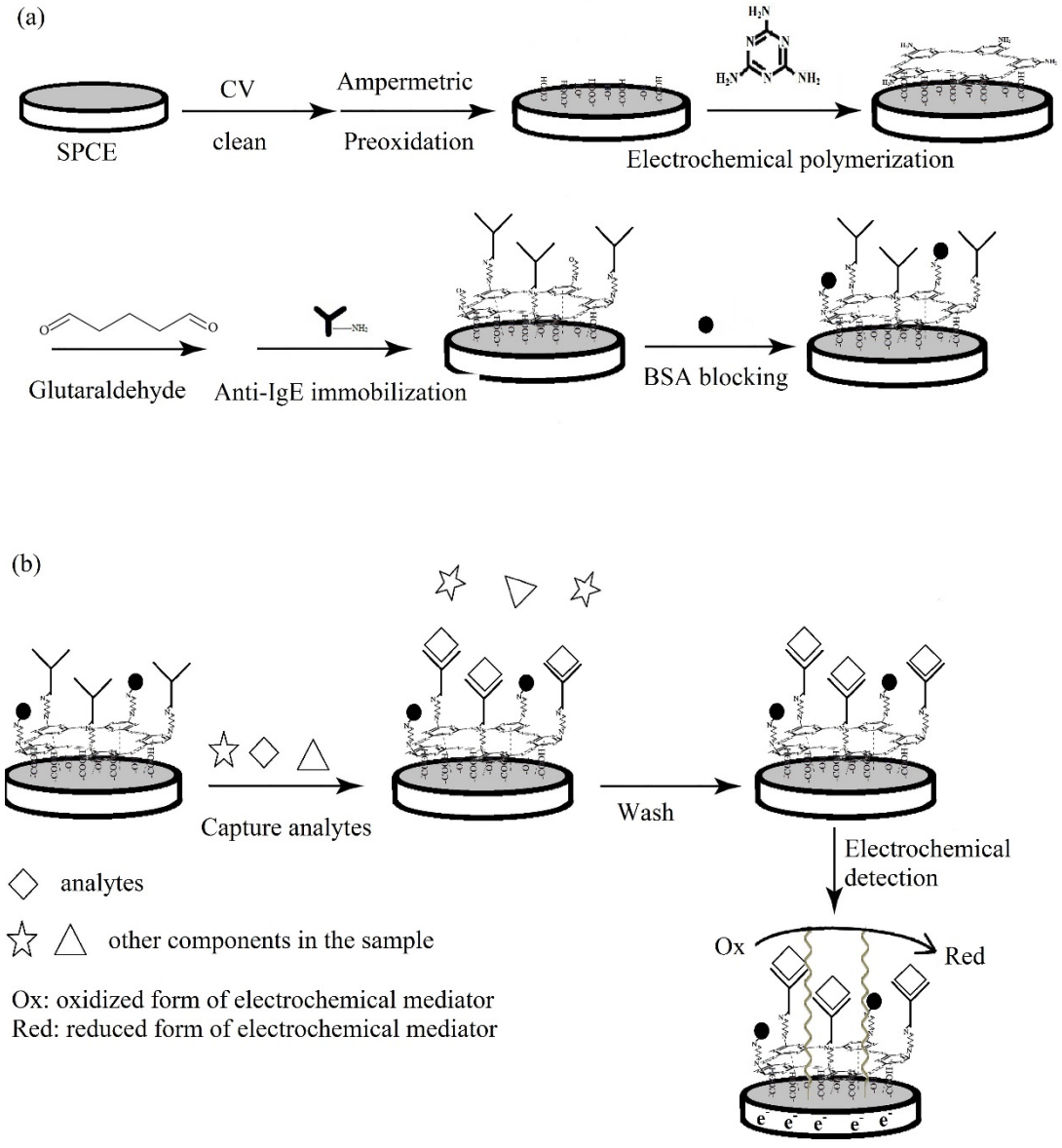
The electrochemical polymerization of melamine was carried out according to the literature [42] with slight modification of the cleaning procedure. Prior to electrochemical deposition of the polymelamine, the SPCE surface was cleaned by cyclic voltammetry between 1.8 and −0.6 V at a scan rate of 0.3 V/s for 20 cycles in 0.1 M phosphate buffer solution (PB, pH 7.4). Subsequently, the cleaned SPCE was anodically pre-oxidized at 2.0 V for 300 s in 0.1 M PB. The electrodeposition of polymelamine was then carried out via cyclic voltammetry between 0.2 to 1.5 V at a scan rate of 0.1 V/s in a 0.1 M HCl solution containing 1.0 mM melamine.

2.4 Preparation of the anti-IgE-modified SPCE*-polymelamine

A drop (50 µL) of the 1 vol% glutaraldehyde (GA) solution was applied to the surface of the SPCE*-polymelamine electrode and allowed to stand for 1 h at room temperature (24–26°C). After washing with PB, a drop of the anti-IgE solution (50 µL, 0.5 µg mL^{-1} in PB) was applied to the electrode surface, and allowed to stand for 1 h at room temperature. After rinsing with PB, the electrode was incubated in a 1% (wt/v) BSA solution, which reacted with the free carbonyl groups to prevent non-specific adsorption on the electrode surface.

2.5 Detection of IgE using the SPCE*-polymelamine-anti-IgE biosensor probes

A drop of the IgE solution (50 μL, various concentrations in PB) was applied to the SPCE*-polymelamine-anti-IgE surface and allowed to stand for 30 min at 25 °C. After this time, the electrode surface was rinsed with a 0.1 M PB solution, then incubated in PB at 4 °C prior to measurement. The electrodes were then immersed in a solution of 5.0 mM $Ru(NH_3)_6Cl_3$ in 0.1 M PB (pH 7.4) and chronoamperometry was carried out at 0.0 V for 60 s, then −0.3 V for 0.5 s. The overall process for preparation of the biosensor probes and subsequent electrochemical detection is illustrated in Scheme 1.



Scheme 1. (a) Pretreatment of electrodes and preparation of the biosensor probes. (b) Detection of IgE using electrochemical mediators.

2.6 Statistical analysis

The chronoamperometric currents of $Ru(NH_3)_6^{3+}$ were reported as an average of 10 data points collected between 0.2 and 0.21 s. All experiments were repeated in triplicate. The data shown in the figures are expressed as the mean ± the standard deviation. The statistical significance of the data was determined using Student's t-test in Microsoft Excel (Redmond, WA, USA). Differences with p values of <0.05 were considered significant.

3. Results and Discussion

3.1 Preparation and characterization of the polymelamine-modified SPCE

The reversible redox reaction of $Fe(CN_6)^{3-}$ was examined by cyclic voltammetry using both SPCE and the anodically-treated SPCE (SPCE*), as outlined in Figure 1a. As indicated, when SPCE* was employed, the reversible redox reaction was enhanced compared to that of the untreated SPCE (i.e., ΔE = 141 mV vs. 677 mV for SPCE* and SPCE, respectively), which implies an increased active surface area and an increase in surface hydrophilicity on the SPCE* [43]. In addition, Figure 1b shows the reversible redox voltammogram of the SPCE*-polymelamine electrode carried out in a 0.1 M HCl solution, which reflects the intrinsic properties of polymelamine.

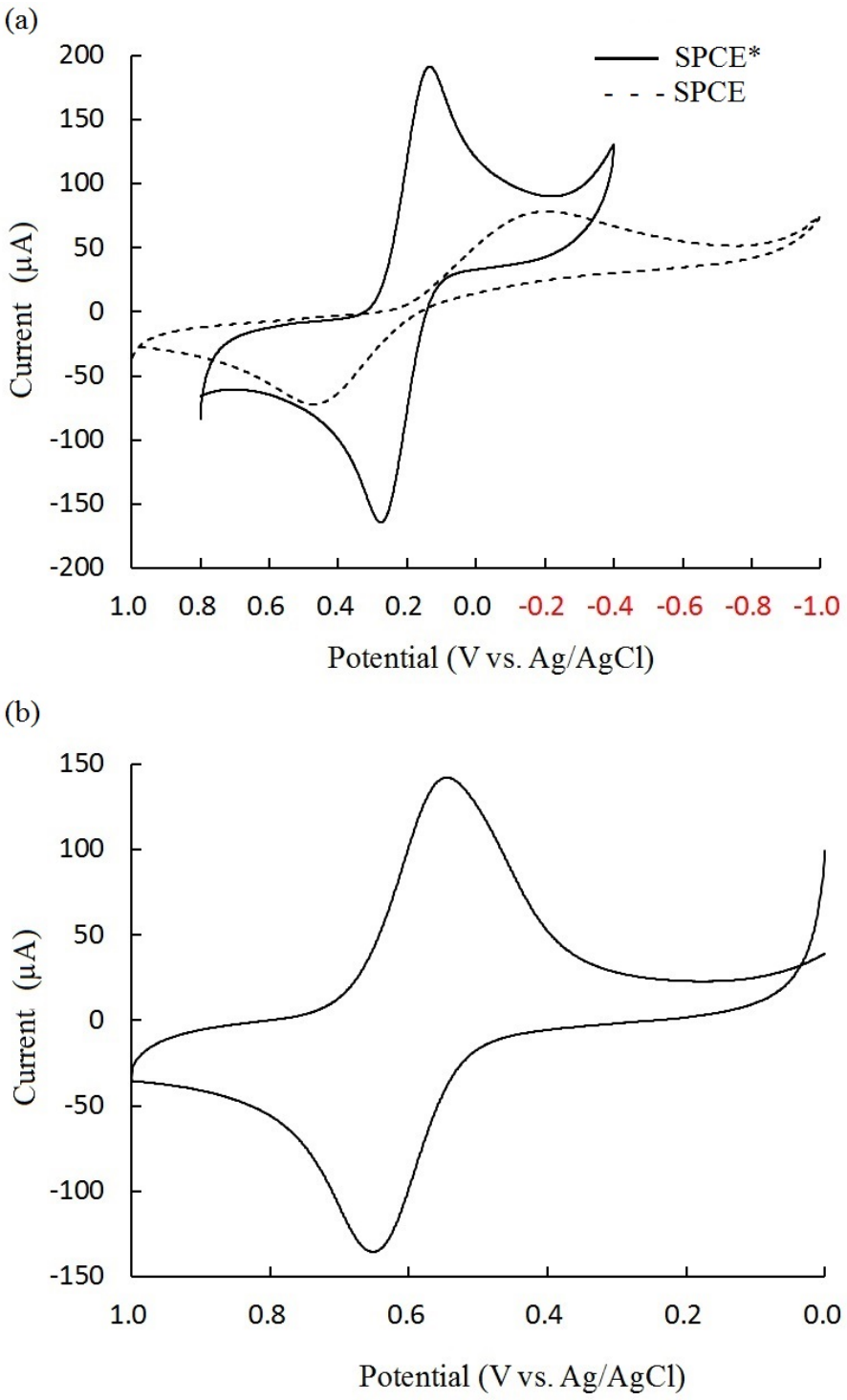


Figure 1. (a) The effect of using the anodically-treated SPCE (SPCE*) on the reversible redox reaction of $Fe(CN)_6^{3-}$ (5.0 mM) in pH 7.0 PB. The solid line and the dashed line represent the cyclic voltammograms (CVs) obtained from the SPCE* and the bare SPCE, respectively. (b) The intrinsic cyclic voltammogram of polymelamine obtained using SPCE*-polymelamine in a 0.1 M HCl solution.

As reported by Baskar et al[42], the intrinsic reduction reaction can be described according to equation (1), while the dependence of the reduction potential on the solution pH and on the Nernst expression is shown in equation (2). In addition, Figure 2a shows the linear relationship between the peak potential of polymelamine and the solution pH, where the slope of −0.0594 indicates that the m/n ratio was equal to 1 for reaction (1):

$$\mathrm{Ox} + \mathrm{n}e^- + \mathrm{m}H^+ \leftrightarrow \mathrm{Red} \quad (1)$$

$$\mathrm{E} = E^0 - \frac{0.05916}{n} log \frac{[Red]}{[OX]} - \frac{0.05916\, m}{n}\, pH \quad \text{at } 25\ ^\circ\mathrm{C} \quad (2)$$

Where Ox is the oxidized form of polymelamine and Red is the reduced form of polymelamine. As shown in Figure 2b, the redox peak currents exhibited a linear relationship to the square root of the scan rate, which indicates that the electrochemical redox was diffusion controlled, despite the polymelamine being adsorbed on the electrode surface. In addition, the broadened reduction peak at higher solution pH values further confirms that the redox reaction of polymelamine involved the transfer of two electrons and two hydronium ions (data not shown). These results were consistent with those reported by Baskar et al.[42], who concluded that the polymerization of melamine took place due to the structure of melamine allowing radical cations to undergo head-to-head coupling, which resulted in the formation of a dimer through NH-NH bonding, followed by the generation of trimers and tetramers through head-to-head coupling between the radical cations of the non-dimerized amines. In addition, we found that the electrochemical polymerization of melamine reached a steady state after 20 cycles. We therefore aimed to achieve the stable absorption of a polymelamine film on the SPCE surface, where–NH_2 functional groups remained available for further cross-linking to allow the polymelamine-modified SPCE to be employed in the preparation of a biomolecular probe. As such, the reproducibility and stability of the SPCE*-polymelamine material was then investigated. Figure 3a shows the variation in melamine electropolymerization reproducibility on the SPCEs with increasing cycle number, where 10 cyclic voltammetry cycles resulted in better reproducibility of polymelamine deposition on the SPCEs. In addition, Figure 3b shows the stability of the resulting polymelamine-

modified electrodes, indicating that the polymelamine film was firmly adhered to the electrode surface after soaking in PBS for 3 h.

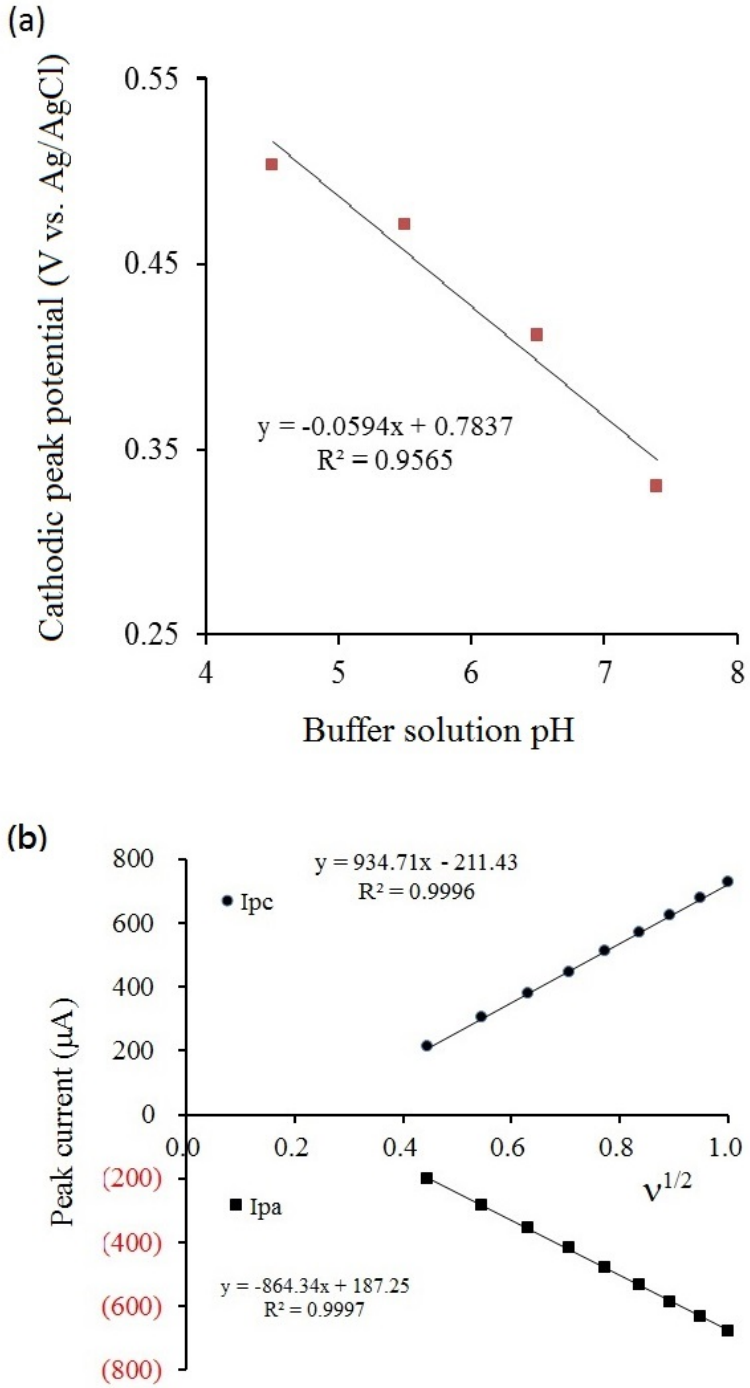


Figure 2. (a) Relationship between the polymelamine reduction peak potential and the pH of the buffer solution. (b) Relationship between the redox peak currents and the

square root of the scan rate. Ipc = cathodic peak current; Ipa = anodic peak current; ν = scan rate.

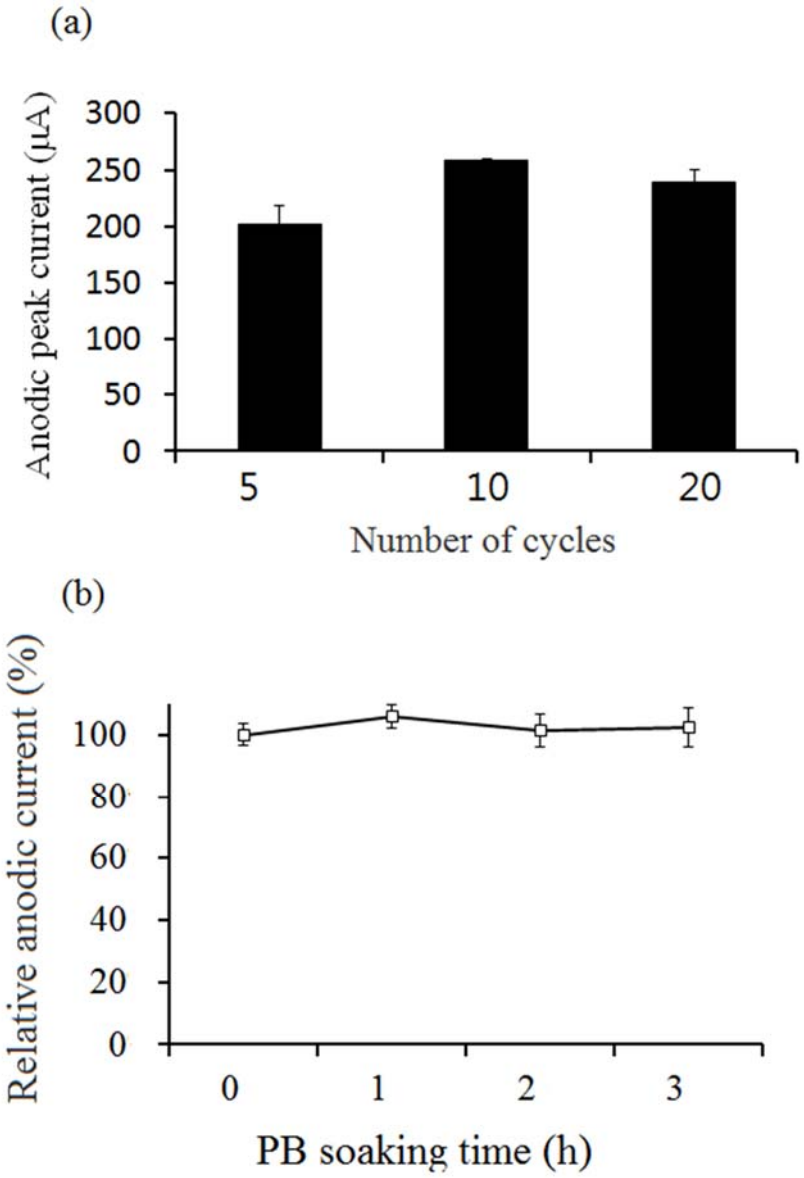


Figure 3. Optimization of the melamine electropolymerization conditions. The intrinsic polymelamine redox signals were measured by cyclic voltammetry between 1.0 and 0.0 V at a scan rate of 0.1 V/s in a 0.1 M HCl solution. The polymelamine-modified electrode was employed as the working electrode. (a) The electrodeposition of polymelamine on the SPCE* surface was carried out via cyclic voltammetry between 0.2 and 1.5 V at a scan rate of 0.1 V/s in a 0.1 M HCl solution containing 1.0 mM melamine over a number of cycles. (b) The stabilities of the polymelamine-

modified electrodes measured according to the relative anodic current after soaking in PB solution for 0–3 h.

3.2 Preparation of the SPCE*-polymelamine-anti-IgE biosensor

As indicated in Figure 2a, it was necessary to carry out all electrochemical measurements at a pH value of 7.4 to maintain an optimal antibody activity and binding affinity to the target species. However, as the reduction peak of ferricyanide overlapped with that of polymelamine in solution at pH 7.4, we employed $Ru(NH_3)_6Cl_3$ as an electrochemical mediator. Thus, to confirm the feasibility of this system, we investigated the immune pairing of IgG with anti-IgG antibodies, due to the costs involved with using the IgE immuno-pair. However, the preparation of the SPCE*-polymelamine-IgG biosensor was the same as that employed for the preparation of the SPCE*-polymelamine-anti-IgE biosensor. Figure 4a shows the cyclic voltammograms of SPCE*-polymelamine, SPCE*-polymelamine-IgG, and SPCE*-polymelamine-IgG-anti-IgG in a buffer solution at pH 7.4 containing 1 mM $Ru(NH_3)_6Cl_3$. As shown, the two reduction peaks were well separated, thus indicating that the intrinsic reduction of polymelamine was not affected by the presence of either IgG or anti-IgG on the outer polymelamine layers. However, the reduction currents of $Ru(NH_3)_6^{3+}$ decreased slightly upon the adsorption of proteins on the electrode surface, as the protein obstructed electron transfer from the electrode to $Ru(NH_3)_6^{3+}$. As electrochemical impedance spectroscopy (EIS) can quantitatively measure both resistances and capacitances in an electrochemical cell, it can provide a snapshot of the coating status. As such, Figure 4b shows the variation in impedance of the electrochemical cells prepared using the different working electrodes. All measurements were performed in 0.1 M PB containing 1 mM $Ru(NH_3)_6^{3+}$ between 0.1 Hz and 100 kHz at −0.3 V (taking an average of the anodic and cathodic peak potentials of $Ru(NH_3)_6^{3+}$) with a 5 mV amplitude sine wave. Protein labeling was employed to introduce a dielectric layer on the electrode; however, due to the steric hindrance of the proteins, it was not possible to completely cover the electrode surface with the labeling layer. In this context, Figure 5a shows the equivalent circuit employed in the biosensor system, while Table 1 shows the simulation results obtained from Figure 4b, which indicate that the high frequency impedance was significantly affected by protein coverage. As shown in Figure 5b, the majority of the

current flow in the solution channels occurs under and between adjacent protein molecules at relatively low frequencies (<10 Hz); however, at higher frequencies (>100 Hz) the current begins to flow directly through the insulating proteins (Fig. 5c) [44]. Thus, although the system impedance increased when IgG was bound to polymelamine, the resistance of the electrolyte solution (Rs) and the resistance of charge transfer from the polymelamine (Rct_1) remained relatively constant, while the resistance of charge transfer from the electrode to the $Ru(NH_3)_6^{3+}$ (Rct_2) in solution increased, due to an increase in the dielectric protein layer on the electrode surface. The successful immobilization of the antibody onto the electrode surface via cross-linking between the polymelamine $–NH_2$ moieties and the antibody using glutaraldehyde and successful binding to the target analyte were therefore confirmed from agreement of the cyclic voltammetry and EIS results.

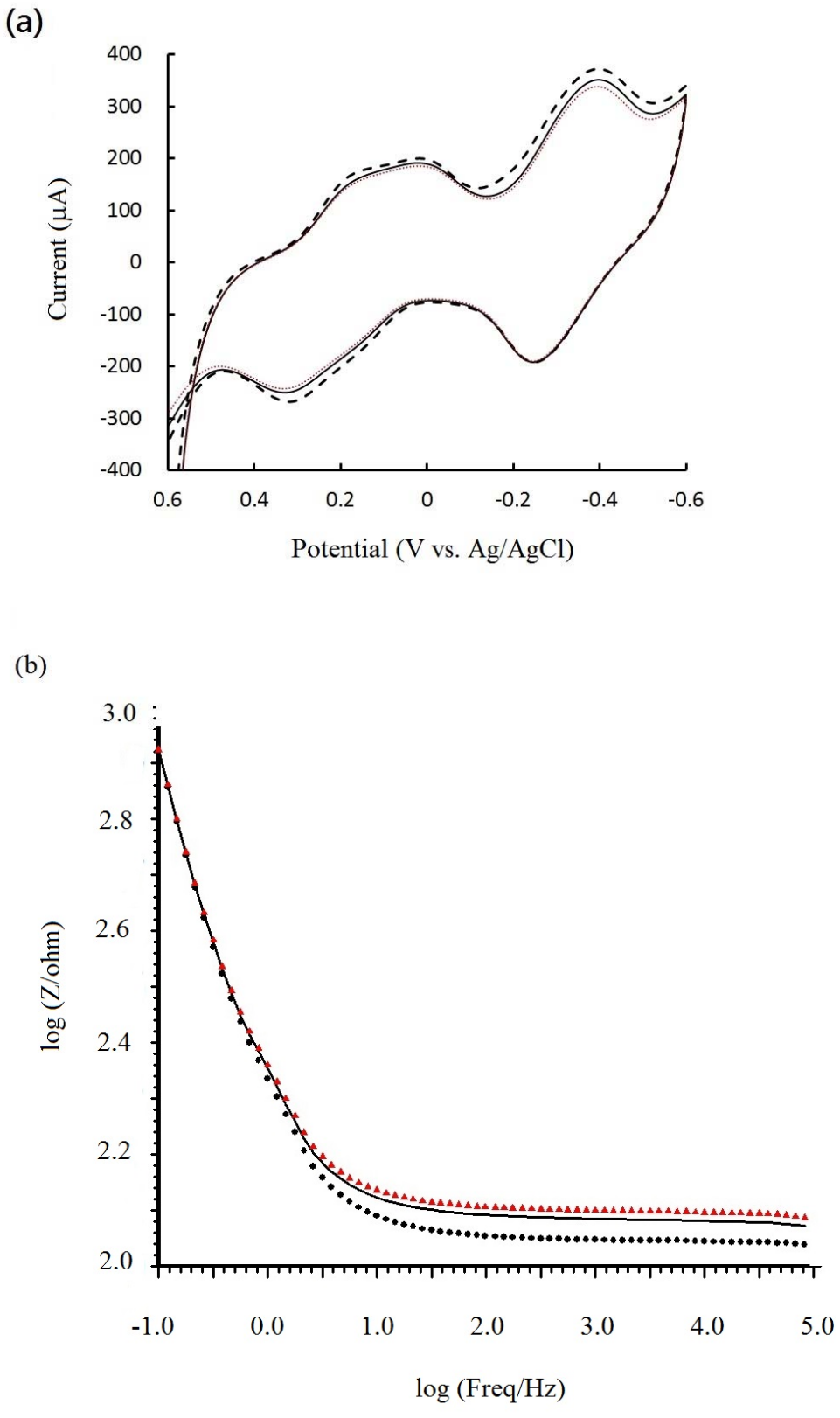


Figure 4. (a) Cyclic voltammograms of SPCE*-polymelamine (blacked dashed line),

SPCE*-polymelamine-IgG (solid line), and SPCE*-polymelamine-IgG-anti-IgG (red dotted line) in a PB solution containing 1 mM $Ru(NH_3)_6Cl_3$. (b) EIS Bode plots of the three electrodes in a PB solution containing 1 mM $Ru(NH_3)_6Cl_3$.

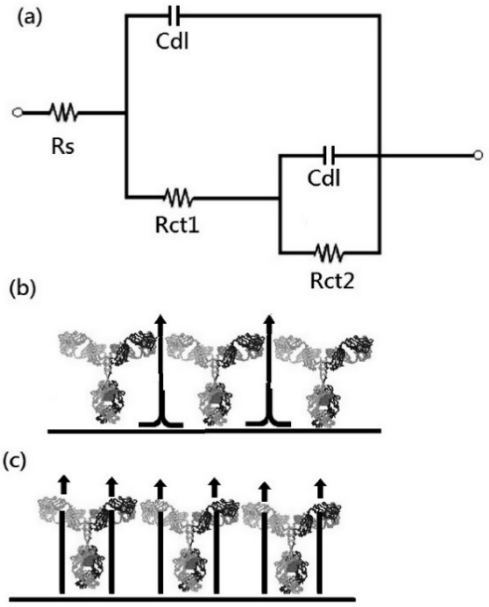


Figure 5. (a) Representation of the equivalent circuits employed in the biosensor system. Also shown are schematic illustrations of the main current flow pathways of the impedimetric measurements at (b) low frequencies and (c) high frequencies.

Table 1. Simulated results from Figure 4b (n = 3)

Electrode	Rs (ohm)	Rct_1 (ohm)	Rct_2 (ohm)
SPCE*-polymelamine	115 (±4)	212 (±4)	3891 (±66)
SPCE*-polymelamine-IgG	123 (±1)	215 (±13)	4150 (±50)
SPCE*-polymelamine-IgG-anti-IgG	128 (±1)	236 (±9)	4254 (±11)

As indicated, little variation in the cyclic voltammogram signals of $Ru(NH_3)_6Cl_3$ were observed, as only a small amount of protein was immobilized on the electrode surface. However, the biosensor system was somewhat complicated due to the intrinsic reduction of polymelamine. As such, various electrochemical methods were investigated for optimization of these sensitive measurements. As the amperometric currents produced at −0.4 V were the sum of the reduction of polymelamine and

$Ru(NH_3)_6^{3+}$ plus interference from the reduction of H^+, we employed chronoamperometric measurements to reduce the contribution from polymelamine in this IgE biosensor. Thus, the initial step was set at 0.0 V to reduce the polymelamine moieties, prior to decreasing the potential to −0.3 V to reduce the $Ru(NH_3)_6^{3+}$. All currents were taken at 0.2 s during the second step to minimize the background current. As shown in Figure 6, no significant differences in the contribution of polymelamine to the reduction current of $Ru(NH_3)_6^{3+}$ were observed after 60 s at 0.0 V. As such, for subsequent experiments, we employed a chronoamperometric method in which the initial step was set at 0.0 V for 60 s, while the second step was set at −0.3 V for 0.5 s. Table 2 shows the reproducibility of the SPCE*-polymelamine-anti-IgE biosensor, where the obtained chronoamperometric currents indicate that the intra-assay precision was 1.2–2.4%, while the interday repeatability was 4.3%.

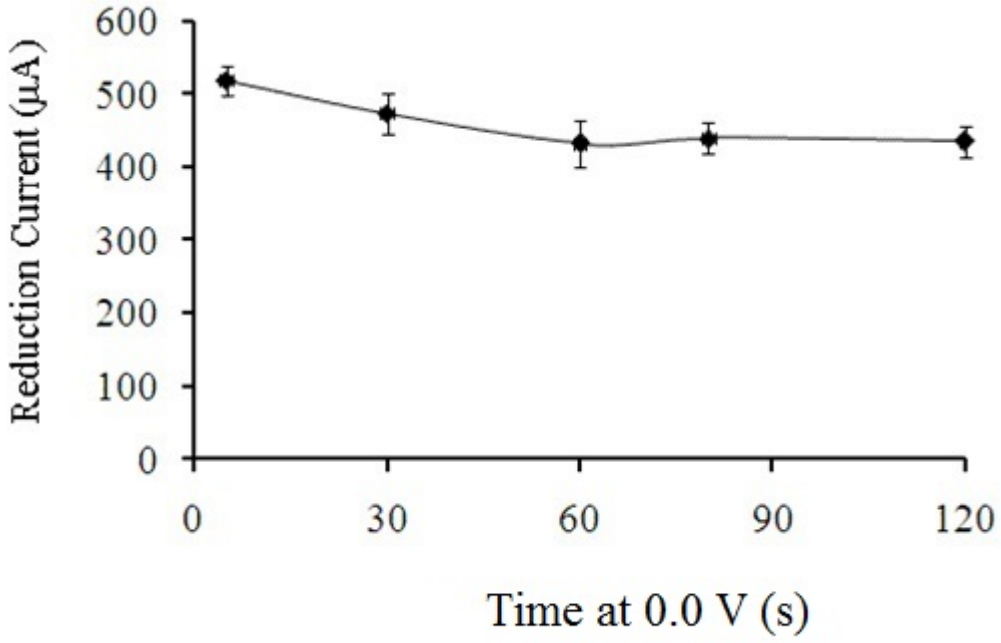


Figure 6. The influence of polymelamine reduction time at 0.0 V on the reduction current of $Ru(NH_3)Cl_3$. The initial step was set at a potential of 0.0 V for 0–120 s, prior to changing to −0.3 V for 0.5 s. The reduction currents were recorded at 0.2 s in the second step.

Table 2. Reproducibility of the SPCE*-polymelamine-anti-IgE biosensor

	Intraday (N = 3)	Interday (N = 9)

Day	Average current (*n*A)	RSD%	Average current (*n*A)	RSD%
Day 1	370	2.1	390	4.3
Day 2	405	2.4		
Day 3	394	1.2		

3.3 Measurement of IgE

Figure 7 shows the calibration curves of IgE, which were obtained by incubation of the SPCE*-polymelamine-anti-IgE electrodes with various concentrations of IgE, and subsequent rinsing with 0.1 M PB to remove any unbound species. The electrodes were then immersed in $Ru(NH_3)_6^{3+}$ solution and analyzed by chronoamperometry, which revealed the logarithmic relationship between the chronoamperometric current and the IgE concentration between 5.3 and 530 fM (i.e., over 2 orders of magnitude). In addition, the $Ru(NH_3)_6^{3+}$ reduction current reached a maximum at low IgE concentrations, but decreased upon increasing the IgE concentration. Furthermore, as the isoelectric point (pI) of IgE is 5.1–5.2 [45], we speculated that the negatively charged IgE at pH 7.4 took part in electrostatic interactions with the positively charged $Ru(NH_3)_6^{3+}$ ions during the first step (0 V), thus resulting in an increase in the reduction current of $Ru(NH_3)_6^{3+}$ at low IgE concentrations. However, upon increasing the IgE concentration, the current decreased due to the dielectric properties of the protein obstructing electron transfer. To confirm this speculation, application of the SPCE-polymelamine-based biosensor for other proteins with a range of different pI values is underway. Moreover, as shown in Table 3, the detection limit for this system 0.64 fM, which is significantly lower than previously reported literature values. This detection limit was calculated from least squares linear regression based on $3s_b/m$, where m is the slope of the least squares linear regression and s_b is the standard deviation of the *y*-intercept [46]. The maximum IgE binding affinity towards the electrode surface depended on the quantity of anti-IgE that was linked to the polymelamine. As the use of human samples requires approval from the clinical trials institutional review board, we instead employed fetal bovine serum to mimic the human serum matrix. Upon spiking the fetal bovine serum with 16 fM human IgE, a recovery of 114 ± 14% was achieved. In addition, there were no statistically significant differences between the measured reduction currents of the SPCE*-

polymelamine-anti-IgE biosensor incubated in fetal bovine serum and those incubated in PBS (Fig. 7c). This indicated that the human anti-IgE probe could selectively bind to human IgE without interference from the complex matrix.

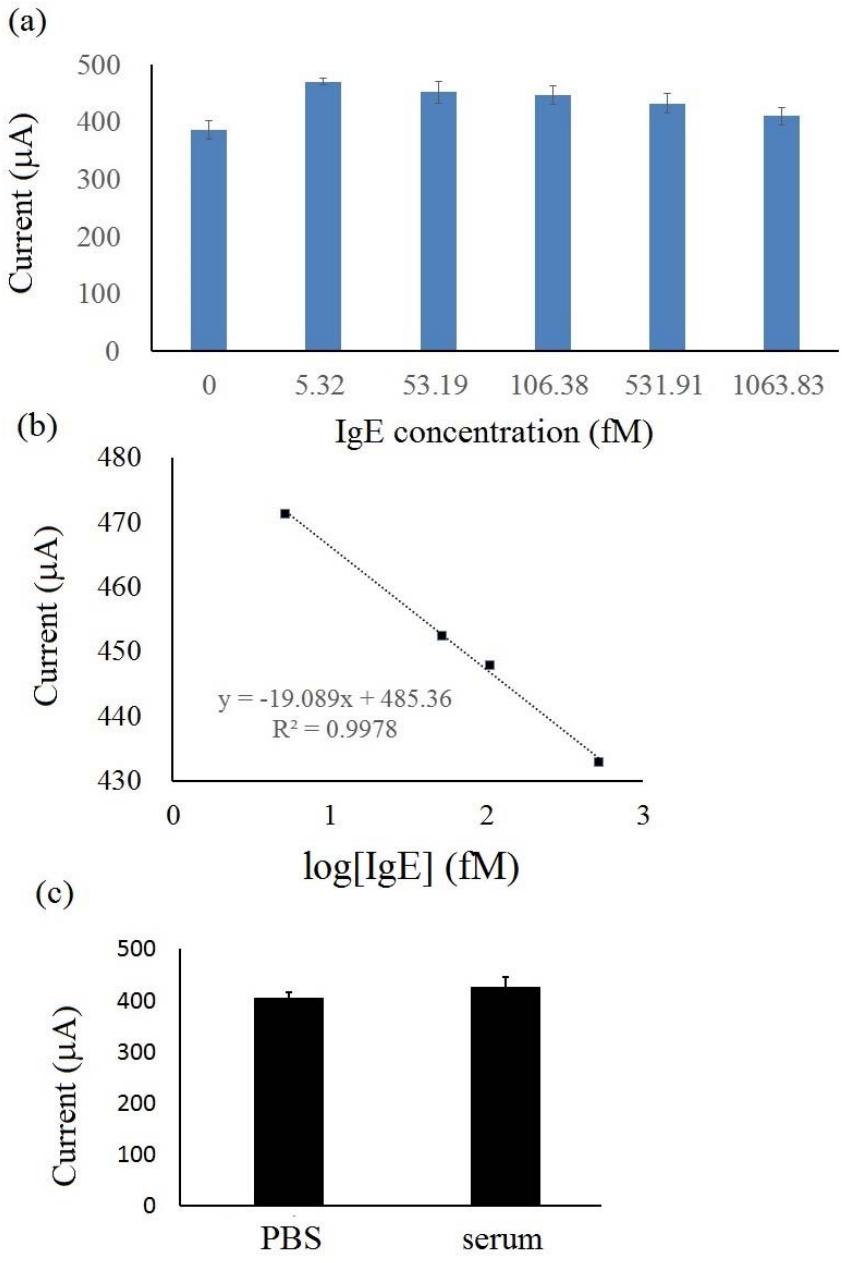


Figure 7. (a) Reduction currents measured after incubating with various IgE concentrations. (b) IgE calibration curves. (c) Reduction currents measured using the SPCE*-polymelamine-anti-IgE electrode following incubation in PBS and fetal bovine serum.

Table 3. Comparison of studies investigating the electrochemical detection of IgE

Electrode	Probe	Method[a]	Linear range (pM)	Detection limit (pM)	Ref.
SPCE	Polymelamine-anti-IgE	CA	0.005–0.5[b]	0.00064	This work
Au	Aptamer	AC	54–36,000[b]	36	[33]
Gold array chip	Aptamer	EIS	2,500–100,000[b]	100	[47]
Glassy carbon	Chit/IL/MWCNT/Aptamer[d]	DPV	500–30,000	37	[48]
Nanocrystalline diamond film	Aptamer	EIS	158–225,263	158[c]	[49]
SPE	AuNPs/Aptamer	SWV	62.5–6250	22.5[c]	[28]
SPE	AuNPs/MB/Aptamer	SWV	1–100,000[b]	0.16	[27]
Au thin film	AuNPs/AgNPs/MUA/Der p2	EIS	0.0625–6,250[b]	0.00625	[32]

[a] CA: c*hronoamperometry*, AC: alternating current voltammetry, EIS: electrochemical impedance spectroscopy, DPV: differential pulse voltammetry, SWV: square wave voltammetry.

[b]Logarithm linearity.

[c]Different concentrations were converted based on the full molecular weight of human IgE (190 kDa).

[d]Chit/IL/MWCNT/aptamer represent the chitosan/*N*-butyl-*N*-methyl pyrrolydinium bis(trifluoremethylsulfonyl) imide/multiwalled carbon nanotube/aptamer.

4. Conclusion

We herein described the preparation of an electrochemical biosensor for immunoglobulin E (IgE) detection based on a screen-printed carbon electrode (SPCE) and polymelamine as the base matrices. The deposition of polymelamine on the SPCE surface produced layers bearing amino functional groups, which interacted with anti-IgE antibodies. This system is advantageous as the costs of the SPCE and melamine

matrices were significantly lower than previously employed gold and silver nanoparticles or electrodes. In addition, our developed biosensor was sensitive, with only small quantities of sample being required for analysis. Furthermore, this assay exhibited a wide operating range (linear between 5.3 and 530 fM), and the detection limit (0.64 fM) was lower than those of previously reported systems. As such, we successfully demonstrated that the IgE assay is suitable for use in a complex serum matrix without any significant interference being observed. We expect that in practice, blood samples can be collected using a lancing device similar to that employed with a blood glucose meter, which will avoid the unnecessary withdrawal of blood from patients. The obtained samples can then be diluted using phosphate buffer prior to analysis. We therefore expect that our IgE biosensor will be useful for monitoring variations in blood IgE levels during the clinical treatment of allergic asthma and rhinitis. Finally, to confirm the proposed mechanism of action, we are currently investigating the application of our SPCE-polymelamine-based biosensor for a range of other proteins. Preliminary results obtained for the SPCE-polymelamine-anti-BSA system towards BSA (pI = 5.3) have exhibited similar tendencies in the context of current variations.

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科技部補助專題研究計畫出席國際學術會議心得報告

日期：105 年 5 月 19 日

計畫編號	MOST 104－　2113　－M－040－002－		
計畫名稱	電化學方法及物理吸附修飾網版印刷探電極在生物感測器的探討與應用		
出國人員姓名	蔡惠燕	服務機構及職稱	中山醫學大學醫學應用化學系 教授
會議時間	105 年 5 月 12 日 至 105 年 5 月 14 日	會議地點	韓國首爾大學
會議名稱	(中文) 2016 材料與奈米科技國際研討會 (英文)2016 international conference on material science and nanotechnology (ICMSNT 2016)		
發表題目	(中文)利用奈米磁性顆粒與磁盤快速靈敏的檢測生物指標成份 (英文) Rapid and sensitive detection of biomarker by using functional magnetic nanoparticles and microplates		

一、 參加會議經過

1. 參訪延世大學(Yonsei University) Prof. Myeong Hee Moon 研究室，Prof Moon 目前是韓國質譜學會的理事長(President, Korean Society of Mass Spectrometry)，及 Analytical Chemistry (ACS) Editorial Advisory Board，他的研究重點是使用場流分離法（Field-Flow Fractionation ，FFF），納流液相層析(nanoflow liquid chromatography)和電噴霧電離串聯質譜開發各種分離方法離方法以探討/發現

人類疾病生物標誌物(biomarkers)。 很榮幸有這個機會去拜訪他， 初步洽談可能合作的機會。

2. 參加 2016 international conference on material science and nanotechnology (ICMSNT 2016)

此研討會由香港機械工程學會主辦， 地點在韓口首爾大學新校區。學校校門口是一個鋼鐵大三角， 做公車直接進校園內後到工學院大樓還要繞十幾分鐘， 校園內就像一個山區別墅。

首爾大學校門口

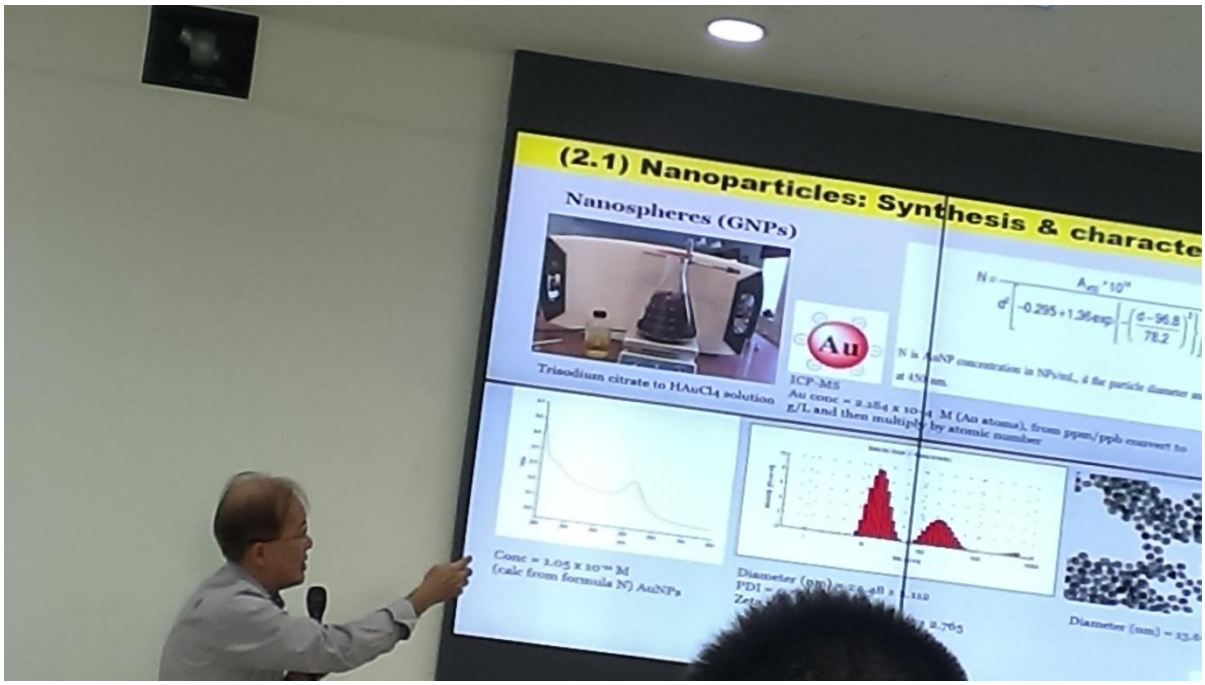
報到


HKSME 2016 CONFERENCES
2016 International Conference on Materials Science and Nan ology
(ICMSNT 2016)
ISMST 2016 &IC3CM 2016
Seoul, South Korea
May 12-14,2016
Supported by
KSME

與會人員合照

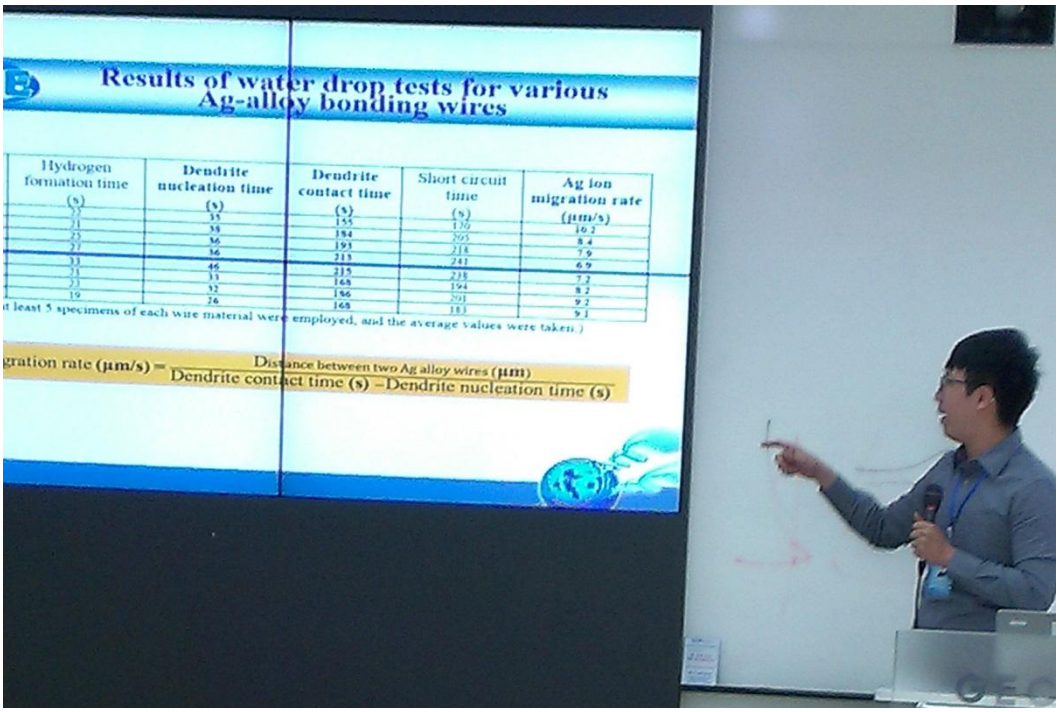
此研討會主要是材料科學， 從材料開發的角度看材料的性質， 會議有兩個 session 同步進行， 我參加的 session 比較偏應用，其中新加坡大學的 Professor Ha Gong 介紹他們合成的奈米物質， 利用 AuNP-linker-Zn 增強螢光強度，應用於生物影像，探討 cell uptake。

Professor Ha Gong



Prof. Chun-IL Kim 來自加拿大 ualberta， 利用數學計算電腦模擬 biomembrane.

來自台灣的 wire technology co. Josh Chung 介紹電化學方式製造 silver dendrimer 是蠻有趣的， 不過會後聊天時， 他說那不是他們的重點， 那是要證明在他們生產的 wire 不會發生此現象。 但也給我一個增加生化分析電極表面積或製備 flow channel 的一個啟發。



馬來西亞 Prof. Ing Hon Ooi 介紹利用 Sol-gel trasition at 32 ^{0}C,當藥物載體。

Keynote speaker 中央大學林景崎教授， 介紹利用 EIS 探討 corrosion， 會後聊天也讓我學習不少操作 EIS 的技巧。

二、 與會心得

不知是參加者太少， 還是承辦單位要省錢， 在出發前才寄更新議程， 新議程竟然少了一天。 大會把演講者緊奏的排在第二天， 每個人只能講十分鐘， 午餐時間僅 1 小時，下午時段完全沒休息一路講到 6:20 加上時間控制不良, 到 6:45 才結束， 真是疲勞轟炸! 在幾次國際會議後發現亞洲區的國際會議雖然報名費比較便宜， 但 CP 值不高。雖是國際研討會， 但在日本、韓國、大陸舉辦的場次， 發現歐美人士參加的不多， 尤其這次只要來自馬來西亞、大陸、新加玻、台灣，連韓國本地的人都很少。唯一從加拿大來的卻是韓國人,而亞洲區雖來自不通區域，大多是華人。而邀請首爾大學的教授 當 Prof. Dong Il Kwon 當 plenary speaker，竟然沒來， 改由博班學生代打， 讓人有著被騙的感覺。

三、發表論文全文或摘要

Rapid and sensitive detection of biomarker by using functional magnetic nanoparticles and microplates

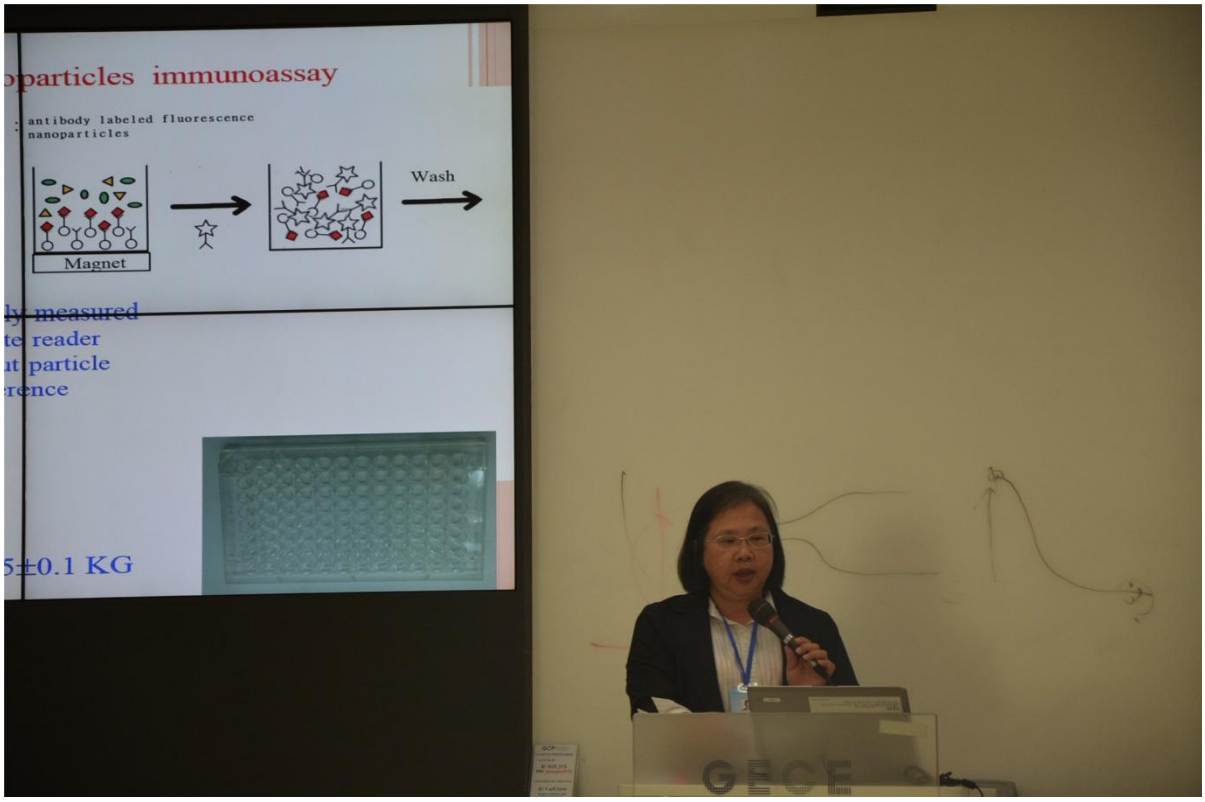
Keywords: Immunoassay, Functional nanoparticles, Magnetic separator.

Abstract.

The enzyme-linked immunosorbent assay (ELISA) has been used for diagnosing medical and plant pathologies. In addition, it is used for quality-control evaluations in various industries. The ELISA is the simplest method for obtaining excellent results; however, it is time consuming because the immunoreagents interact only on the contact surfaces. We will present pseudo-homogeneous immunoassay by using functional magnetic nanoparticles and homemade magnetic microplates for detection of biomarkers. Antibody-labeled magnetic nanoparticles can be dispersed in a solution to yield a pseudohomogeneous reaction with antigens which improved the efficiency of immunoreaction.

We report the preparation and application of functional nanoparticles to detect C-reactive protein (CRP) in magnetic microplates. Anti-CRP labeled magnetic nanoparticles, CRP, and secondary antibody conjugated fluorescent nanoparticles were used in a sandwich immunoassay. The nanoparticles can be homogenously dispersed in the 96-well plates and the fluorescence intensity of the sandwich nanoparticle immunoassay can be directly measured by microplate reader without interfering by nanoparticles. The detection limit of CRP was 1.0 ng/ ml and the linear range was 1.18 ng /ml - 11.8 μg/ ml.

The results revealed that the method involving biofunctional nanoparticles exhibited a lower detection limit and a wider linear range than those of ELISA and most other methods. Using antibody-labeled magnetic particles, the analysis time can be reduced to one-third of that required in using a conventional ELISA. This method demonstrates the potential to replace ELISA for rapidly detecting biomarkers with a low detection limit and a wide dynamic range. The process of the sandwich immunoassay integrated with magnetic microplate will be easily adopted in the clinical laboratory and the home made magnetic microplate is inexpensive.



四、建議

韓國人似乎比較冷漠， 但民族自信心很強， 買東西時問他哪裡生產的， 老闆會說 made in Korea，然後豎起大拇指。 我們國人什麼時候才會以 made in

Taiwan 為傲。

五、攜回資料名稱及內容

會議僅發簡單的議程與報告者的摘要。

六、其他

無

科技部補助計畫衍生研發成果推廣資料表

日期:2017/03/17

科技部補助計畫	計畫名稱: 電化學方法及物理吸附修飾網版印刷碳電極在生物感測器的探討與應用
	計畫主持人: 蔡惠燕
	計畫編號: 104-2113-M-040-002- 學門領域: 分析化學

無研發成果推廣資料

104年度專題研究計畫成果彙整表

計畫主持人：蔡惠燕	**計畫編號：**104-2113-M-040-002-
計畫名稱：電化學方法及物理吸附修飾網版印刷碳電極在生物感測器的探討與應用	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			3		化學年會
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			2	篇	1 篇已投稿，尚在審查中。 1 篇尚在撰寫中
		研討會論文			2		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		

		其他	0		
	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	2	人次	
		碩士生	1		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）					

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：□已發表　■未發表之文稿　□撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   使用網版印刷碳電極開發簡易電化學偵測之文獻很多， 對電極的前處理每一篇文獻都不同， 尚未有人將電極育處理後的穩定性做一有系統的探討，本研究比較物理吸附及電化學沉積的處理方式， 及處理後電極保存的穩定性。對未來biosensor 商業化的開發提供一個選擇。
   利用SPCE*-polymelamine-anti-IgE 生物感測方法，僅需要少量的樣品用於分析，且複雜血清基質並未造成干擾。因此，我們期望這種新穎的系統將有用於監測在過敏性哮喘和鼻炎的臨床治療期間血液IgE水平的變化

4. 主要發現
   本研究具有政策應用參考價值：■否　□是，建議提供機關
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：■否　□是
   說明：（以150字為限）