科技部補助專題研究計畫成果報告
期末報告

發展結合磁場之電化學生物感測器

計 畫 類 別 ：個別型計畫
計 畫 編 號 ：MOST 102-2113-M-040-002-
執 行 期 間 ：102 年 08 月 01 日至 103 年 12 月 31 日
執 行 單 位 ：中山醫學大學醫學應用化學系(含碩士班)

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報 告 附 件 ：出席國際會議研究心得報告及發表論文

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中 華 民 國 104 年 03 月 10 日

中 文 摘 要 ： 本研究分兩部分，首先直接將酵素 label 在磁性顆粒上，然後將磁性顆粒沉積在流動系統中， 注射酵素之受質(substrate)，探討酵素固定化後之活性及其穩定性，將酵素固定在磁性顆粒上，可以快速回收，不須加強酸或強鹼，使其可重複使用，將酵素固定在磁性顆粒上並透過磁場將它結合流動系統(flow system)，反應時間可由流速控制。本研究將 HRP 透過 biotin –strepavidin 之 interaction 固定在磁性顆粒上， 其活性上可保留 90%，在流動系統中重複反應 50 次後， 尚有 70% 的活性。把酵素透過 magnetic deposition 方式固定在 flow system 內, 除了當 sensor probe 之外亦可作為 enzyme inhibitor screening，如應用於篩選酵素活性抑制劑如 ACE 抑制劑。
第二部分則是設計磁性微盤與傳統 ELISA 結合，將 probe 標示在磁性顆粒上，分散於微盤中與分析物進行類均勻溶液的反應，可以縮短 antigen 與 antibody 反應時間，減少清洗次數，提高反應效率，降低偵測極限。酵素反應後的 substrate 可以光學方法或電化學方法偵測。此部我們以兔子血清中 IgG 的偵測為 model，成功證明方法的可行性， 相關成果已發表在 SCI 期刊。 若用光學方法偵測，此操作與傳統 microplate reader 結合，不須改變設備及操作習慣，只要清洗微盤時把微盤置於自製磁盤上即可，自製磁性微盤非常便宜，具備臨床應用的潛能， 值得推廣。

中文關鍵詞： 酵素固定化，生物指標成分偵測

英 文 摘 要 ：

英文關鍵詞： enzyme immobilization，biomarker detection

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（□期中進度報告/■期末報告）

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共同主持人：

計畫參與人員：張哲偉、吳伯宜、呂亦暄、廖奐瑄

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

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中　華　民　國 104 年 3 月 25 日

壹、前言

電化學感測器(Electrochemical sensor) 是具靈敏度、儀器設備較便宜的檢測方法，2009 及2010 發表之文獻較之前倍數成長,每年多超過 一千篇以上。Electrochemical biosensor 之關鍵技術在於把生物選擇性之探針(probes)固定(immobilized)在電極上，為保留 probe 之生物活性，immobilization 方法最常用 self-assembling monolayer 方法，使用之電極以金電極為主，近幾年有結合金奈米顆粒於電極表面以增加偵測之靈敏度之趨勢，而 electrochemical biosensor 結合奈米磁性顆粒(magnetic nanoparticles) 的文獻則從 2007 開始逐漸增加, 從Scopus 的資料庫搜尋結果顯示2007~2013 含review 文獻並不多, 因金與蛋白質(antibody or enzyme) 中 cysteine 的S 易形成共價鍵，因此即使使用 Fe_3O_4 奈米磁性顆粒其外層仍包覆一層金，真正只用 Fe_3O_4 奈米磁性顆粒沒包覆金外層的，其做法仍是把 Fe_3O_4 奈米磁性顆粒加入電極製作之paste 中, 然後填入 4 mm 內徑之玻璃管中做成電極，其目的在利用 Fe_3O_4 奈米磁性顆粒的導電性增加碳電極 (carbon paste)的信號靈敏度。無論是結合奈米金還是奈米磁性顆粒之電化學感測器，過去文獻顯示電極均須一支一支的加工(probe immobilization)，本研究欲開發結合 magnetic deposition 的方式使電極表面具有選擇性偵測之 probe，且 functional magnetic particles 可批次生產，一次的量可使用在數百甚至數千次的沉積與偵測，增加 sensor run-to-run 之再現性，開發方便、快速、靈敏之生物指標(biomarkers)偵測器。

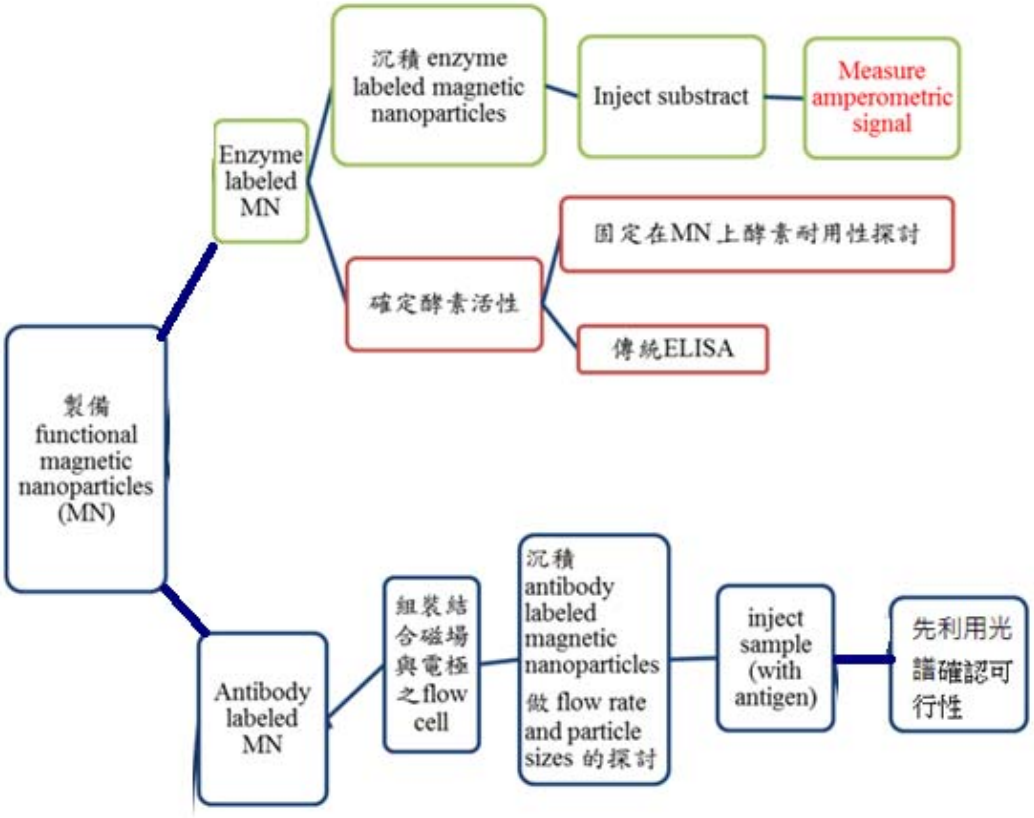
貳、研究目的

本研究將透過永久磁鐵將 antibody/enzyme labeled magnetic nanoparticle 沉積於電極上，作為選擇性偵測之 probe, antibody 可選擇性地與注入流動系統的樣品中的 antigen 結合，電極表面因增加蛋白質層而改變電子傳遞速率及阻抗，因此不需用 2^{nd} antibody 的反應做定量偵測，透過電流信號或阻抗變化與分析物標準品濃度做校正曲線，即可定量未知物；流動系統同時將樣品中其他未鍵結的物質流洗掉，此方法既可省去二抗(2^{nd} antibody)的費用，與

傳統 immunoassay 比較又可省去反覆清洗的步驟。必要時 antibody/enzyme labeled magnetic nanoparticle 可回收再利用。

一般酵素用來催化反應， 酵素可重複使用，但在均勻溶液中，通常會加入強酸或強鹼終止反應，酵素回收不易且酵素非常昂貴，如本研究室之前用的 angiotensin-converting enzyme (ACE)， 1U 就要兩萬八千元。 因此本研究分兩部分，操作流程如下圖，首先直接將酵素 label 在磁性顆粒上，然後將磁性顆粒沉積在流動系統中， 注射酵素之受質(substrate)，探討酵素固定化後之活性及其穩定性，本研究希望將酵素固定在磁性顆粒上，可以快速回收，不須加強酸或強鹼，使其可重複使用，但產物與反應時間有關，時間控制將嚴重影響實驗再現性，我們將它結合流動系統(flow system)，反應時間可由流速控制。另一方面若把酵素透過 magnetic deposition 方式固定在 flow system 內, 除了當 sensor probe 之外亦可作為 enzyme inhibitor screening，如應用於篩選酵素活性抑制劑如 ACE 抑制劑。第二部分則將抗體固定在磁性顆粒上，將標示抗體之磁性顆粒固定在流動系統，注入樣品測試電化學信號之改變，但此部分因本實驗採用網版印刷碳電極，血液樣品含豐富的蛋白質很快將探電極毒化，因此我們改良設計，利用自製磁性微盤與傳統 ELISA 結合，可快速偵測血液中生化指標成分，且因操作易與傳統 ELISA 結合，較容易被臨床應用者接受。

以下結報我們分兩部分撰寫， 第一部分酵素固定化與流動系統應用之可行性探討，第二部分為標示磁性顆粒與磁盤結合在 ELISA 上之應用， 此部分我們已整理發表在 chemistry central journal 上， 所以直接附上發表之論文。


製備 functional magnetic nanoparticles (MN)
Enzyme labeled MN
沉積 enzyme labeled magnetic nanoparticles
Inject substract
Measure amperometric signal
確定酵素活性
固定在MN上酵素耐用性探討
傳統ELISA
Antibody labeled MN
組裝結合磁場與電極之flow cell
沉積 antibody labeled magnetic nanoparticles
做 flow rate and particle sizes 的探討
inject sample (with antigen)
先利用光譜確認可行性

第一部分　酵素固定化與流動系統應用之可行性探討

I.實驗原理與相關文獻回顧

1.1 HRP 結構與特性

HRP是從horseradish roots (Amoracia rusticana)，分離出來的酵素。 分子量44kDa，由鐵血紅素基團與兩個鈣離子所構成的兩個金屬中心，其中血紅素基團為由鐵原子位於四個porphyrin ring中心構成平面結構，而鐵有兩個可鍵結的位置，分別接上proximal histidine residue (His170)，位於血紅素基團平面的上下方。proximal histidine residue (His170)上有一個Histidine，於氧化還原反應中，可與H_2O_2之氧原子鍵結[1] (如圖1.1)，圖1.2 為HRP的 3D結構[2]。

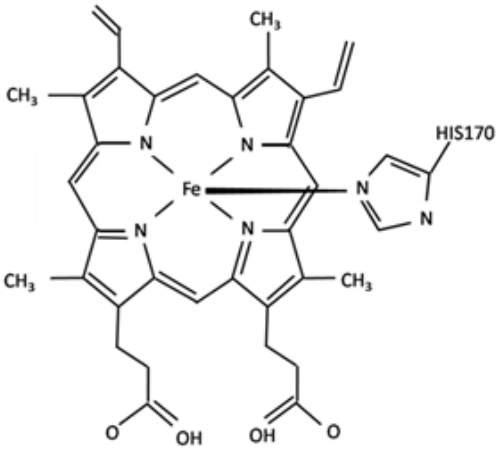


圖1.1 HRP分子結構[10]

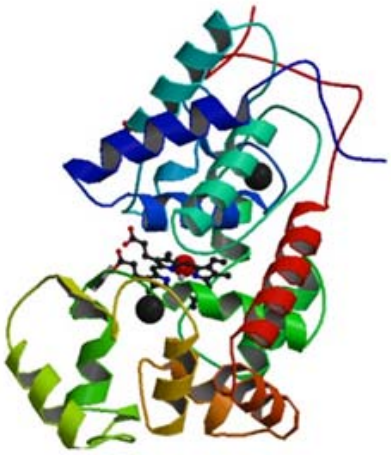


圖1.2 HRP 3D結構[10]

HRP的受質種類不少，主要可以分為冷光和呈色兩大類。對於受質的選擇，主要考慮的是靈敏度、背景和使用的方便性和穩定性。過去常使用的的受質有o-phenylenediamine (OPD) ，2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) 和3,3',5,5'-tetramethybezidine(TMB)[3]

其中OPD化性不穩定，易潮解，再現性沒有TMB好。ABTS靈敏度低，呈色反應慢、時間長。也有文獻指出，OPD與ABTS於Ames test中[4]，易產生突變。

而TMB靈敏度比OPD和ABTS好，且呈色時間短[5]，不會產生突變也無致癌

基因[6]。且TMB 具有電化學氧化還原性質，可利用光學方法或電化學方法偵測[7]。

2. HRP 催化 TMB 與雙氧水反應原理

HRP催化TMB與雙氧水反應，產生兩種不同顏色的產物。當TMB與H_2O_2的mole數比為2:1時，產生的第一個產物為為TMB失去一個電子的氧化產物，呈現藍色，是diamine和diimine 之charge-transfer中間體，其與自由基產生快速的平衡，在波長370及650nm有吸收峰。當加酸中止反應， 生成第二的產物是TMB失去兩個電的子氧化(diimine)產物，呈現黃色，可由最大吸收波長450nm偵測(如圖1.3)[8]。

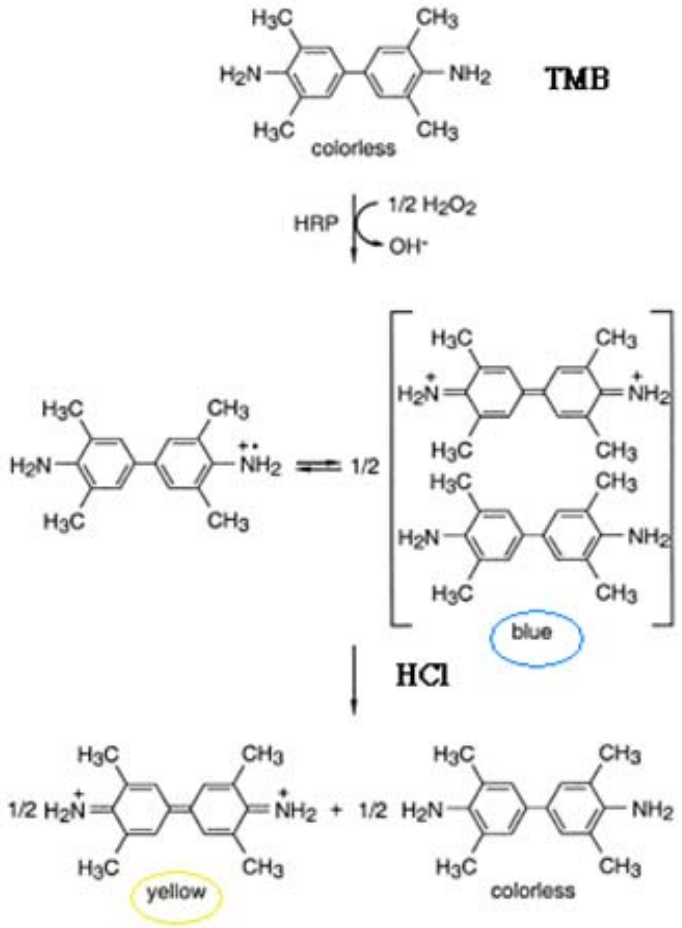


圖1.3 HRP催化H_2O_2與TMB反應機構圖[8]

1.3 磁性奈米粒子特性

磁性奈米粒子，具備磁性的性質是因為擁有不成對電子的元素存在化合物當中，一般有鐵、鈷、鎳這三種元素為成分的化合物時，通常都會有磁性性質的表現。若將這三個元素與其他元素結合或取代，則可以形成各種不同特性的磁性材料，這些材料隨著磁性的差異而有不同的應用價值。而磁性奈米粒子又可稱為「磁流體(ferrofluid)」，這是因為從外觀看起來像是一種擁有磁性的流體。微觀之下，這是磁性奈米粒子穩定的分散在溶劑中且沒有產生聚集的現象，在磁鐵靠近時因為產生吸引力所以可以看到被吸引的現象[9]，所以可以利用磁場快速將其與其他物質分離。在無外加磁場下，該流體無自發性磁偶極。但當有磁場施加在該流體時，液體中的磁性粒子之磁矩會傾向沿著外加磁場方向，因而產生了磁偶極。而當外加場移除時，由於磁性奈米粒子受水分子熱擾動的作用，

再度呈現出零磁偶極。這現象即所謂超順磁(superparamagnetism)[10]。

目前最常使用的磁性顆粒以四氧化三鐵為主，由於裸露的磁性顆粒具有高反應性且易氧化，在加上其不溶於水。故通常會於外層包覆一層物質，如高分子聚合物、二氧化矽、碳等，如此一來，可保護氧化鐵避免直接裸露於空氣中造成氧化，也可避免其於長時間下造成聚集而能穩定的分散在水中，易可在表面上衍生其他的官能基和蛋白質或抗體進行鍵結，故被廣泛運用於酵素、生化、核磁共振影像等[11]。

1.4 生物素(Biotin) 與鏈黴抗生物素蛋白(Streptavidin) 鍵結原理

生物素(Biotin)為維生素B群之一，又被稱為維生素H(vitamin H)，其分子量為244.3Da，結構如圖1.4(a)所示。1916年Bateman於雞蛋蛋白中發現存在於細胞中的微量物質，稱為avidin，分子量為72kD，其結構是由四個相同的單體(monomers)所構成，其每一個單體能與一個Biotin分子進行專一性的結合。

而鏈黴抗生物素蛋白(Streptavidin)為從Streptomyces avidin細菌中純化出的蛋白質，其分子量為60kDa。結構同樣也具有四個相同的單體，結構如圖1.4(b)所示，能與Biotin分子形成具有專一性結合的Biotin – Streptavidin複合物[12](如圖1.5)。Biotin與Avidin、Streptavidin等物質之間有很強的親和力，能形成很強的非共價性鍵結($K_f=1.0\times10^{15}M^{-1}$)[13]。

在生化感測的領域上，例如酵素連結免疫分析(Enzyme-linked immunosorbent assay ；ELISA)、蛋白質活性分析(Electrophoretic mobility shift assay ； EMSA) 、親和層析法(Affinity chromatography)等，經常藉由Streptavidin(Avidin)與biotin間快速且強大的非共價鍵結能力，將不易鍵結的生物分子，分別與Streptavidin和biotin共價鍵結，形成化合物，再進行結合。通常Streptavidin會和固相結合，如 magnetic bead、microtiter plate、biosensor chip；biotin會與核酸、蛋白質、抗體結合。而利用biotin作為連接分子的處理的過程，被稱為生物素化(biotinylation)。本研究利用此特性將HRP固定(immobilize)於dynabead®上。

(a) (b)

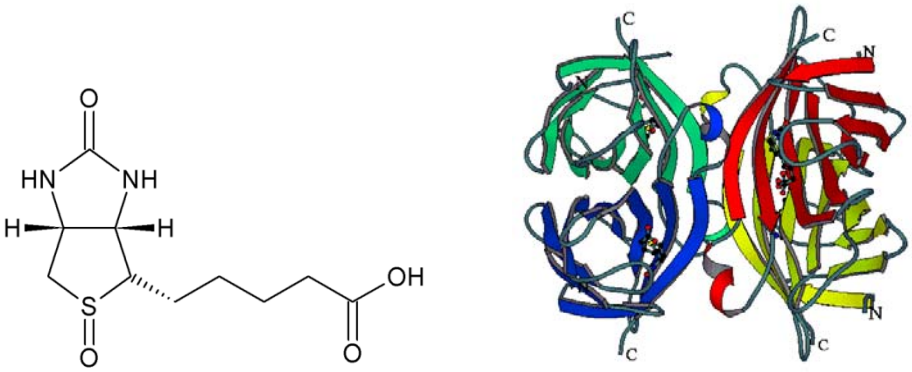


圖1.4 (a)生物素(Biotin)[14]與(b)鏈黴抗生物素蛋白(Streptavidin)[15]結構圖

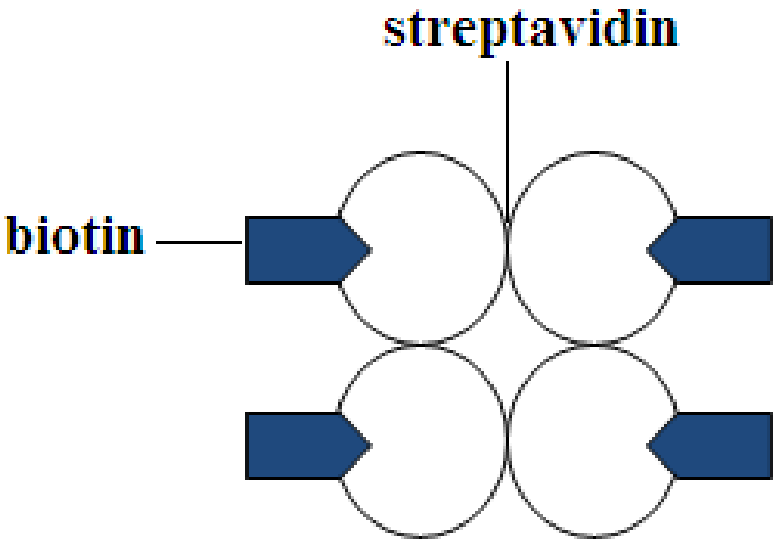


圖1.5 生物素(Biotin)與鏈黴抗生物素蛋白(Streptavidin)鍵結

1.5 流動注入分析(Flow injection analysis,FIA)原理

由Ruzicka 和Hansen於1975 年所提出，目的是要解決實驗室大量的定量分析工作。其原理為利用幫浦(pump)帶動連續流動的載體溶液(carrier solution)，將樣品注入注射閥(injection valve)，進樣分為裝載(Load)與注射(inject)兩個階段。Load時，以注射針將樣品注入Loop，而多餘的的樣品會流出(如圖1.6a)，以確保固定體積的樣品進入流體通路；扳至inject時，Loop與pump通道連接，藉由載體

溶液將樣傳送到偵測器進行偵測(如圖1.6b)。

FIA可以配合光學偵測器或電化學偵測器，將分析樣品導入系統中偵測，也可進一步運用於免疫分析與生化分析。FIA特性為分析快速、再現性佳、試劑用量少、可於短時間內分析大量樣品、易於自動化及系統多變化性等優點，使其被廣泛使用。近幾年來，FIA更與高解析度與精密儀器連結，例如光學系統、電化學、FT-IR、MS、NMR等，做樣品的偵測。

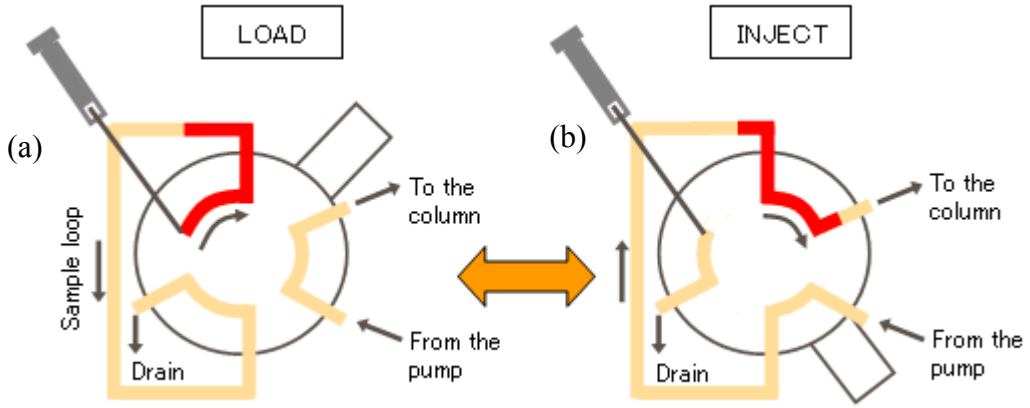


圖1.6 injection valve進樣注射通路示意圖[16]

1.6.網版印刷電極(screen printed carbon electrodes；SPCE)

電極在電化學分析法中扮演重要的角色，由早期的汞滴電極、光滑碳電極(glass carbon electrode)逐漸被具有便宜、可拋、多變等性質的網版印刷電極所取代。本實驗所使用禪譜公司生產之網版印刷電極，利用印刷技術將碳膠、銀膠等各類不同膠材，印刷於不易溶解於水的聚丙烯基板。其結構概可分為底層、反應區與絕緣區三個部分，印刷於底層的膠材一般選擇有高導電性、易烘乾特性的材料，而在反應區域則大多以碳粉和有機溶劑混合作為基礎膠體，最後刷上絕緣膠固定反應面積[17](如圖1.7)。

為達到快速篩檢、現場監控目的，利用電腦科技製版技術將一般實驗室所使用的三電極系統(工作電極、參考電極、輔助電極)整合於一紙板上(如圖1.8)，改以試片型式呈現。使用上通常只需將試片插入待測樣品，或是將少量的待測物滴上試片，即進行測試。在所有電極系統體積減小後，不僅提高檢測設備之攜帶性，同時大幅縮減了所需成本與簡化測定的步驟，更提昇其應用價值。

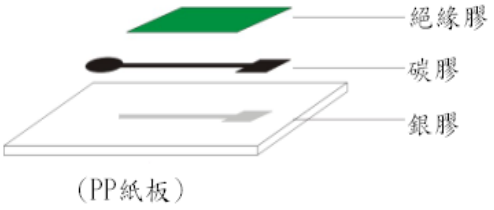


圖 1.7 網版印刷電極剖面圖 [17]

圖 1.8 三碳網版印刷電極示意圖
(a)輔助電極(b)工作電極(c)參考電極

II. 實驗方法

2.1 HRP 固定化(immobilizing)於磁性顆粒(dynabead®)方法

圖2.1為HRP 固定於dynabead之操作示意圖，其步驟說明如下:

1.使用pH=7.4 PBS 清洗Dynabeads- Streptavidin後，加入pH=7.4 PBS 使其分散

2.加入biotin-HRP，於室溫下，360度rotation反應30分鐘

3.靜置於磁鐵上，吸出上清液

4.使用pH=7.4 PBS 清洗Dynabeads-HPR三次

5.加入 pH=7.4 PBS 分散

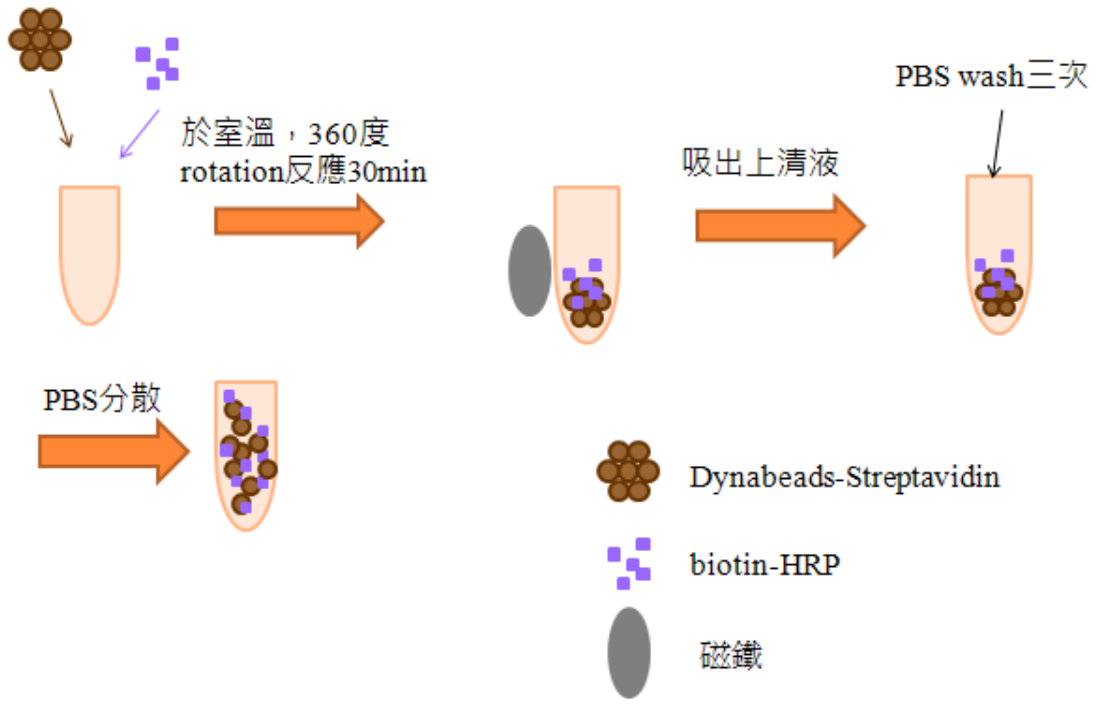


圖 2.1 HRP 固定化於磁性顆粒示意圖

2.2 Flow injection analysis (FIA)連接光學系統偵測

圖2. 2為FIA連接光學系統偵測由HPR催化生成的產物示意圖，其步驟說明如下:

1.將Dynabeads-HPR固定於外圍包附磁鐵的自製槽(Dynabeads-HPR trap)

2.注射樣品 (不同濃度之H_2O_2 與固定濃度的TMB混合)

3.藉由stop flow的方式(如圖2.3)，使其反應2分鐘後，恢復流速 = 0.5ml/min

4.利用370nm偵測其產物吸收值

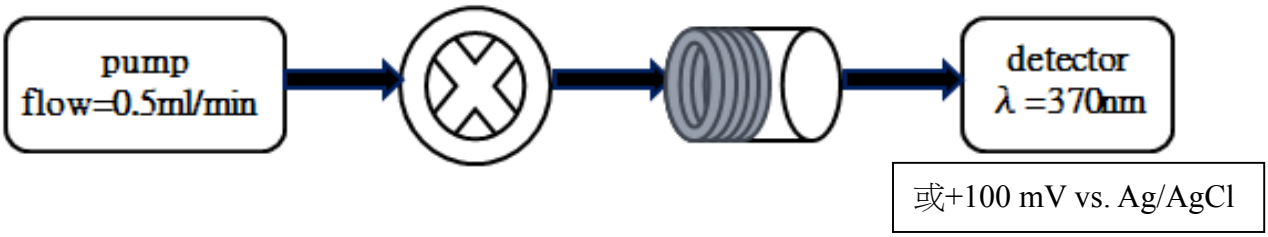


圖2.2 FIA連接光學/電化學系統偵測示意圖

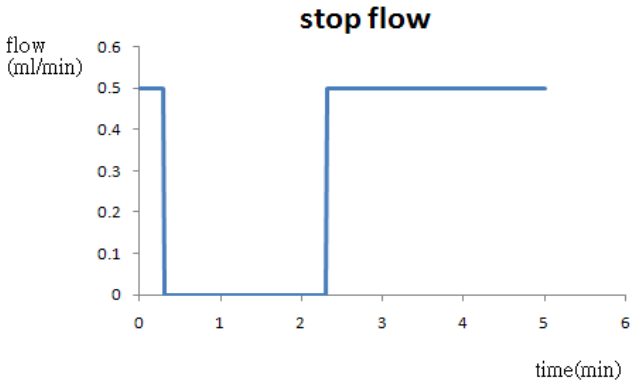


圖2.3 stop flow 方法圖

2.3 In-situ 安培法(Amperometric)偵測

圖2.4為使用電化學系統偵測H_2O_2組裝示意圖，其步驟說明如下:

1. 加入Dynabeads-HPR於含有PBS buffer容槽中，使其均勻分散於溶液中
2. 將網版印刷三碳電極與Ag/AgCl參考電極固定於反應槽中
3. 使用安培法(Amperometry)，施加一個固定的電位於工作電極表面，再逐次累加H_2O_2，使HRP催化H_2O_2反應，隨著時間記錄電極產生的電流訊號。

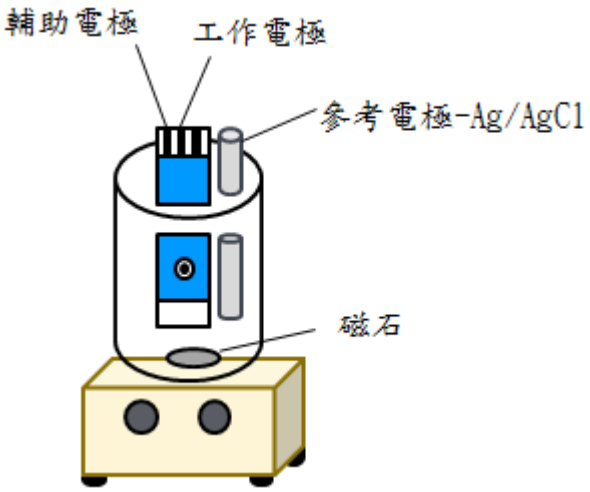


圖2.4 電化學系統組裝示意圖

III. 實驗結果與討論

3.1.HRP 活性定量最佳化條件探討

3.1.1反應時間最適化

圖3.1為不同反應時間所得之HRP校正曲線，反應時間為2min及3min時，R^2分別為0.9995與0.9988，均有良好的線性關係， 為了減省時間，往後反應時間用2min即可。

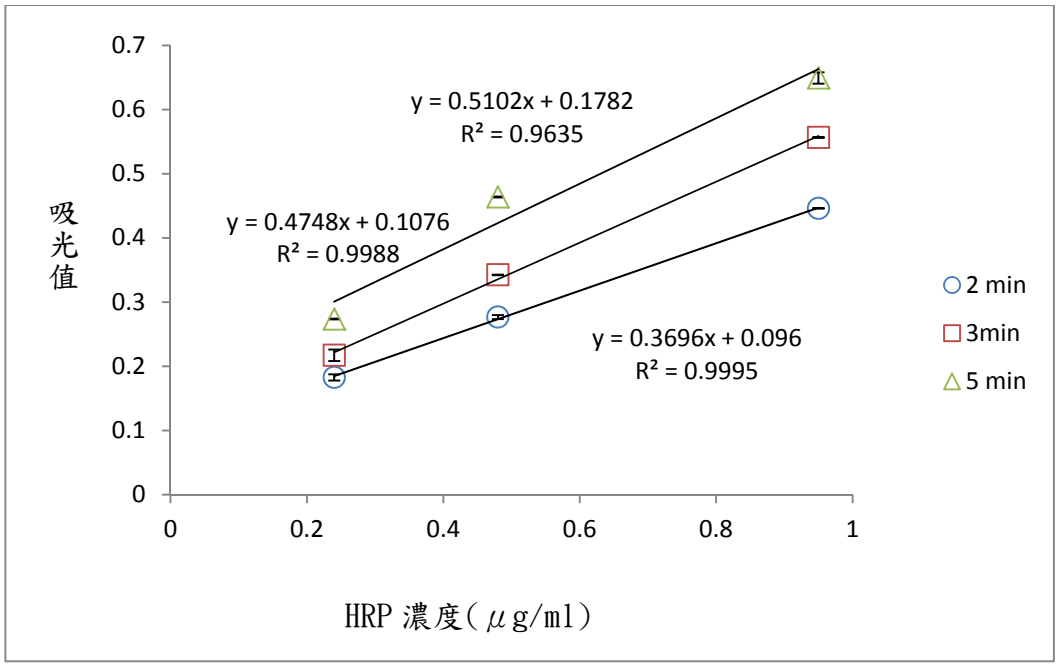


圖 3.1 HRP 於不同反應時間下所得之校正曲線(n=3)

3.1.2 TMB and H_2O_2 濃度最適化

TMB濃度大於0.16mM，會產生沉澱，故TMB 濃度固定為0.16mM。從圖3.2中可看出當H_2O_2 濃度為0.11mM時，R^2為0.9977，HRP活性偵測線性範圍為0.015~0.5μg/ml。但其斜率只有0.8424，較不靈敏。而當H_2O_2 濃度為0.46mM時，R^2為0.9976，HRP活性偵測線性範圍為0.015~0.25μg/ml，從圖中可看到HRP於高濃度下其再現性較差，其線性範圍也較小。當H_2O_2的濃度為0.23mM，R^2為0.9967，HRP活性偵測線性範圍為0.015~0.5μg/ml。其HRP活性偵測線性範圍大，再現性也較佳，故以0.23mM H_2O_2 做HRP之定量反應較適當。

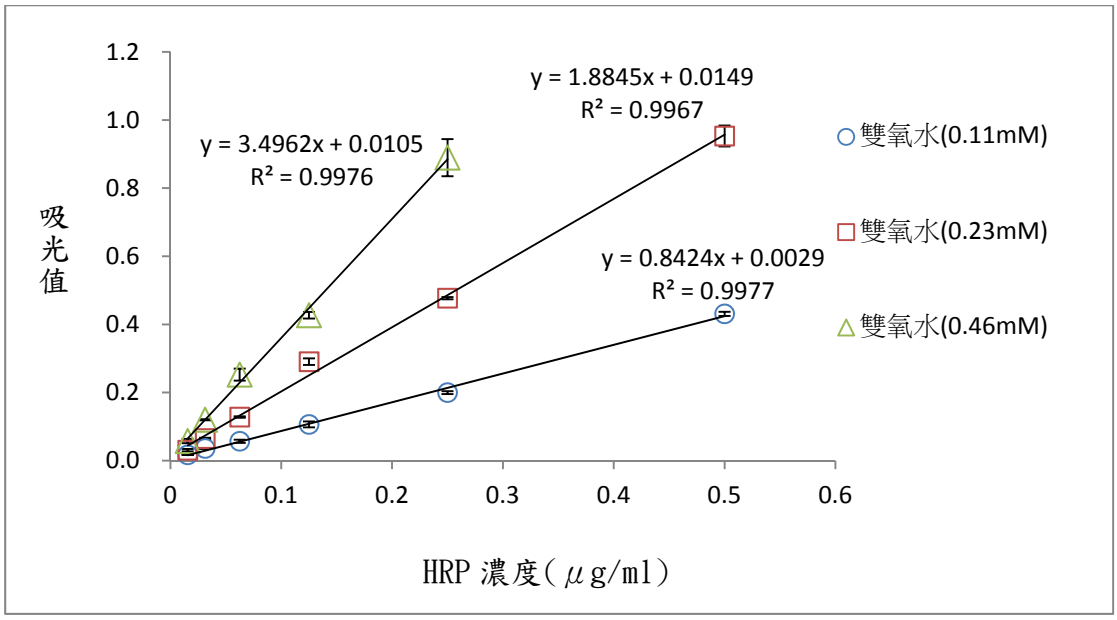


圖 3.2 不同濃度 H_2O_2 之 HRP 活性校正曲線圖 (n=3)

所以後續HRP活性定量所使用條件為取150μl 0.16mM TMB和20μl HRP於微盤三重複，再加入50μl H_2O_2(0.23mM)三重複，反應2分鐘後，加入50μl 1M HCl中止反應，於最大吸收波長450nm測定。

3.2 HRP 活性校正曲線

圖3.3 為HRP為在微盤中，HRP催化H_2O_2與TMB反應2分鐘後，加酸中止

反應，透過波長450nm偵測產物所得之HRP校正曲線吸光值校正曲線圖，其線性範圍在0.015~0.5μg/ml，R^2為0.9967，偵測極限為0.001μg/ml(3*S_b/m)。

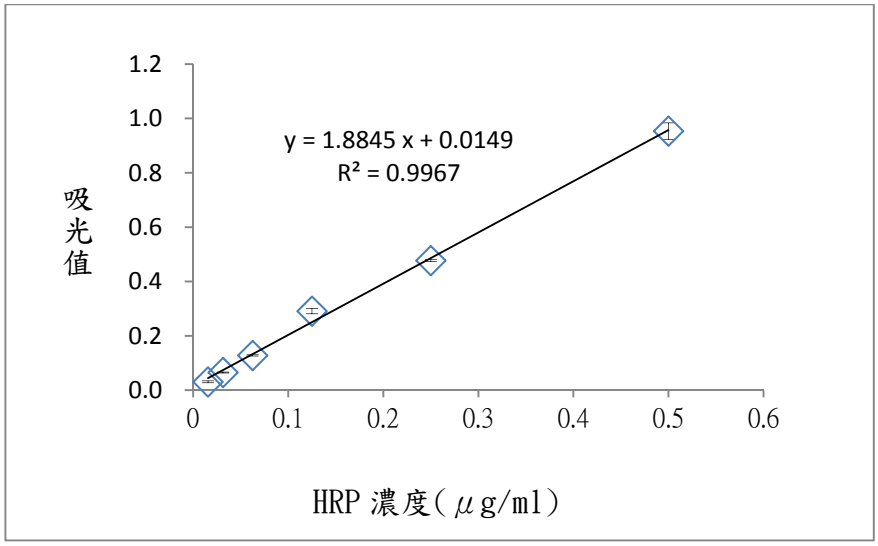


圖 3.3 HRP 吸光值校正曲線圖(n=3)

3.3 Biotin-HRP 活性校正曲線

圖3.4為Biotin-HRP為在微盤中，HRP催化H2O2與TMB反應2分鐘後，加酸中止反應，透過波長450nm偵測產物所得之Biotin-HRP吸光值校正曲線圖，其線性範圍在0.015~1μg/ml，R2為0.9987，偵測極限為0.0009μg/ml(3*Sb/m)。

其斜率相較於HRP略小，因在一定的時間內，產物與反應速率有關，催化反應與催化物莫耳數成正比，Biotin-HRP分子量較HRP大，所以相同體積濃度下，其莫耳濃度較小，與所得結果一致。

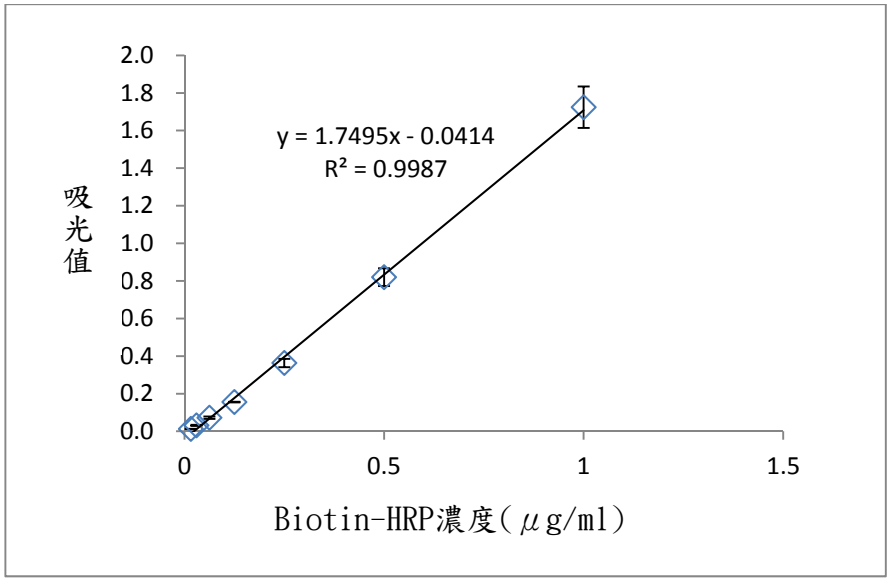


圖 3.4 Biotin-HRP 吸光值校正曲線圖(n=3)

3.4 標定 Dynabeads-HRP 之活性

首先測試Dynabeads-Streptavidin與Biotin-HRP反應後的上清液、wash1、wash2、 wash3的吸光值，其中wash1、wash2、wash3分別為使用pH為7.4的PBS緩衝溶液清洗反應後的Dynabeads之第一次、第二次、第三次溶液，把上述溶液混合，加入TMB與H_2O_2反應2分鐘後，加酸中止反應，透過波長450nm偵測，所得的吸光值帶入Biotin-HRP校正曲線。而測試Dynabeads-HRP之HRP活性，其步驟同上述，加入TMB與H_2O_2反應2分鐘後，加酸中止反應，再利用磁鐵吸住磁性顆粒，吸出上清液，透過波長450nm偵測，所得的吸光值帶入Biotin-HRP校正曲線。

起初加入與Dynabeads-Streptavidin反應的Biotin-HRP為100μg， 反應結果為48.8μgHRP標示在Dynabeads上，未標示上的HRP為41.53μg，因此可知有48.8%的HRP標示在Dynabeads，41.5%的HRP未標示上，9.7%的HRP損失掉，回收率為90.3%。

3.5 酵素固定化的再利用性-- FIA-photodetection 探討

本實驗使用Flow injection analysis (FIA)分析方法，控制流速，利用stop flow的方式控制反應，並由連續流動的溶液清洗磁性顆粒，達到再利用的目的。

先前探討HRP的活性，為加酸中止反應，故使用波長450nm做偵測。但由

於加酸會使酵素失去活性，故後續探討HRP的重複使用性，未加酸終止反應，使用波長370nm做偵測。

3.5.1 最佳流速靜止時間(stop time)

為了使注射的反應溶液(TMB與H_2O_2)能精確的進入反應槽(Dynabeads-HPR trap)，並於線上模擬於微盤反應的2分鐘，故須知道反應溶液從注射閥進入到反應槽的時間。若流速停止時間太早，則反應溶液未進入到反應槽與HRP反應；流速停止時間若太晚，則反應溶液會流出反應槽而無法與HRP反應。

圖3.5顯示，流速靜止(stop flow)時間分別為0.2、0.25、0.3、0.35、0.4 分鐘之訊號，當靜止時間從0.25至0.4 分鐘，訊號會隨著靜止時間的增加而減小，是因有越來越多反應溶液流出反應槽。而當流速靜止時間為0.25分鐘時，所產生的訊號最大。故選擇0.25分鐘，使流速停止，反應2分鐘後，再恢復流速為0.5ml/min，使溶液進入偵測器由波長370nm偵測。

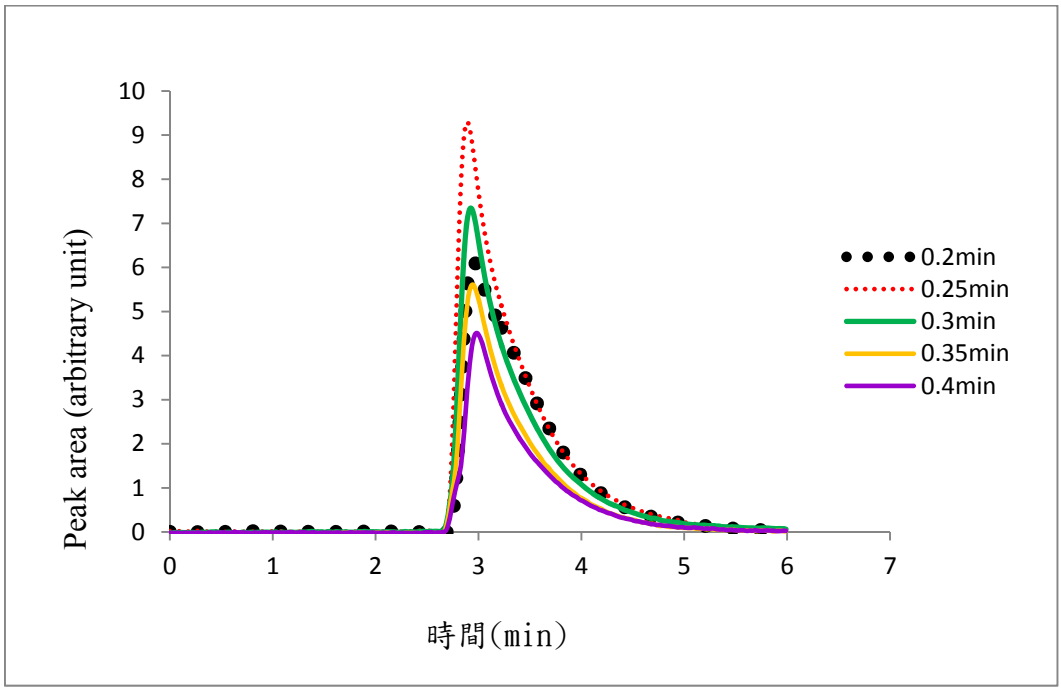


圖 3.5 FIA 結合光學系統偵測之不同 stop time 產生的訊號圖

3.5.2 H_2O_2校正曲線

圖3.6為在FIA系統，HRP催化H_2O_2與TMB反應，透過波長370nm偵測產物

所得之H_2O_2校正曲線，其線性範圍在0.11~0.46mM， R^2為0.9985，偵測極限為0.036mM(3*S_b/m) 。

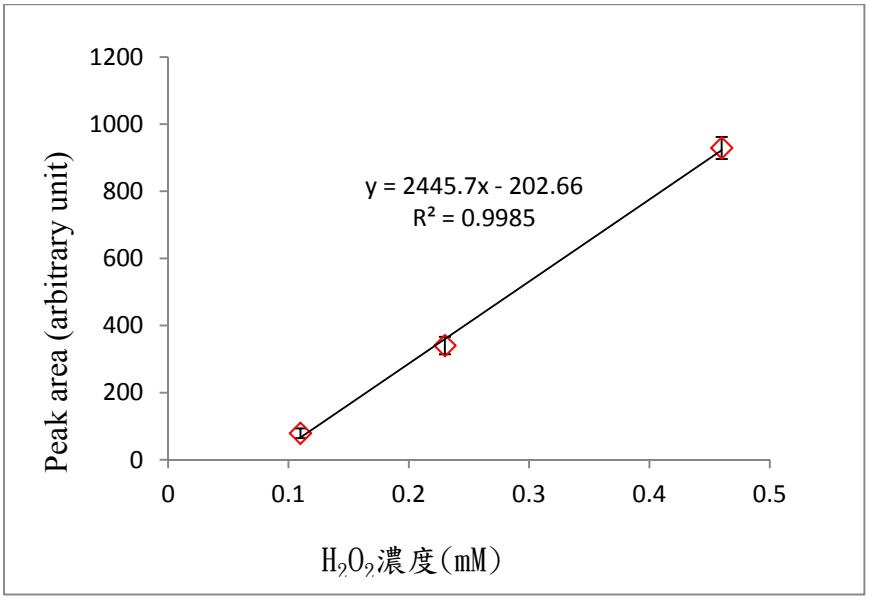


圖 3.6 H_2O_2 校正曲線(n=3)

3.6 Dynabeads-HRP重複使用性

圖3.7為在FIA系統，將Dynabeads-HPR固定於外圍包附磁鐵的反應槽(trap)，注射反應溶液(TMB與H_2O_2)進入反應槽，並藉由stop flow的方式模擬HRP於微盤中催化H_2O_2與TMB反應2分鐘，透過波長370nm偵測產物所得HRP活性重複使用性。其於室溫流動系統中，連續流動11小時，重複使用52次，活性保留為70%。

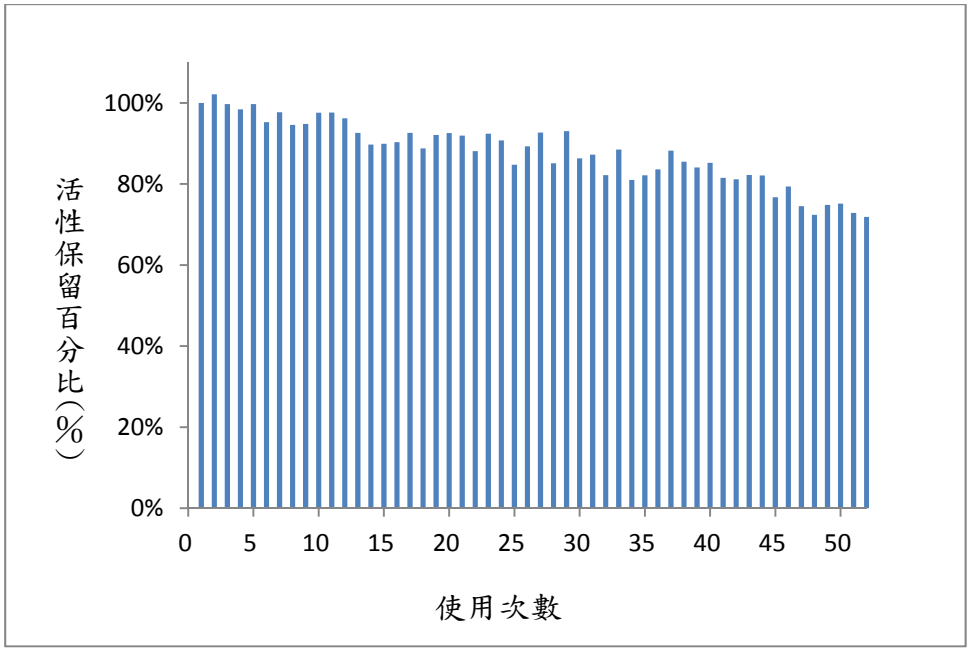


圖3.7 HRP重複使用性

3.7 酵素固定化的再利用性--使用安培法(Amperometric)連續偵測

圖3.8為在靜相反應槽中，透過磁石攪拌溶液(r.p.m為600)，每次逐步累加入雙氧水，使用電位為-0.4V偵測雙氧水之校正曲線。在濃度1.5~20.5mM 的範圍，未經HRP催化的雙氧水，其斜率為 0.055 μA/mM (R^2=0.9991)，而添加dynabead-HRP催化的雙氧水，其斜率為 0.0727 μA/mM (R^2= 0.9984)。由成對母體平均數差異檢定(paired t-test)，計算出P值為$3.34*10^{-6}$，表示經HRP催化之雙氧水訊號與未經HRP催化之雙氧水訊號在95% 可信度下有顯著差異。在攪拌過程中，磁性顆粒會附著在磁石上，可以快速回收利用，dynabead-HRP 重複使用，初步所得之雙氧水校正曲線其斜率再現性 RSD= 5.7%，而故此方法應用於HRP催化雙氧水之電化學偵測及再利用均有可行性。

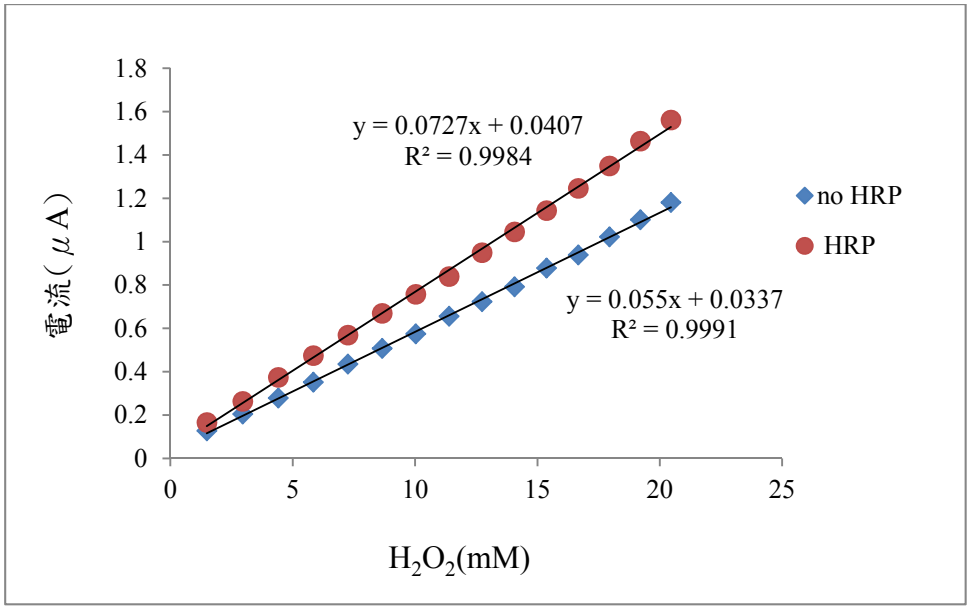


圖3.8　使用電位為-0.4V偵測雙氧水之校正曲線

IV. 結論

在本次的研究中利用固定酵素於磁性顆粒上，其活性定量回收率為90.3%。另外藉由FIA結合光學系統來偵測酵素的再利用性，於室溫下流動偵測11小時(重複使用52次)，其活性依然還保留70%。本研究之設計可以使酵素於均相溶液中快速回收，且較不容易受影響失去活性，並且裝置較為簡單、操作方便；也從本方法中可以看到酵素在使用後回收的可能性，可以改善在工業上只能一次使用的浪費進而降低測定成本並更符合綠色化學的原則。

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第二部分為標示磁性顆粒與磁盤結合在 ELISA 上之應用， 此部分我們已整理發表於 chemistry central journal 上。

Detection of rabbit IgG by using functional magnetic particles and an enzyme-conjugated antibody with a homemade magnetic microplate

Background

In recent years, functionalized magnetic particles have attracted considerable research interest for many biological applications such as biomedicine [1-3], isolation of specific DNA [4], and manipulation of cells [5,6]. In addition, MPs have been used for detecting biomarkers by using superconducting quantum interference device (SQUID) [7-9] and magnetic resonance [10]. We used MPs for detecting biomarkers by using various techniques [11-17], such as (1) predeposited the functionalized MPs in the thin channels coupling with a magnet, then captured the biomarker antibodies and analyzed by counting particles or off-line measurement of fluorescent intensity. (2) detected biomarker antibodies using functional nanomagnetic and fluorescent nanoparticles in magnetic microplate.

A sandwich enzyme-linked immunosorbent assay (ELISA) is a frequently employed bioanalytical assay that involves using an antibody-labeled solid phase to detect the presence of a substance, generally an antigen, in a liquid sample. An enzyme-conjugated secondary antibody is then added to form a sandwich structure. Thus, the enzyme-catalyzed substrate reaction increases the sensitivity of the immunoassay. The ELISA has been used for diagnosing medical and plant pathologies. In addition, it has been 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 have improved the efficiency of the antigen–antibody reaction by integrating the sandwich immunoassay using functional magnetic and fluorescent nanoparticles in magnetic microplate. The magnetic microparticles were not suitable for direct measurement by microplate reader due the light scatting of the microparticles. In this study, we attempted to overcome this limitation using functional magnetic

microparticles and an enzyme-conjugated antibody in a magnetic microplate. This method has many potential advantages which were reported in previous studies [17], such as (1) the amount of proteins immobilized on the particles is consistent in the same batch, which can be used for performing several reactions. (2) The magnetic microparticles (MPs) with avidin, carboxyl, or amino functional groups are commercial available which made antibody labeling easily. (3) MPs can be dispersed in a solution to yield a pseudohomogeneous reaction with antigens and can be easily separated from the unreactive substances by applying a magnetic force. (4) They can be redispersed in the solution after removing the magnetic force. (5) The enzyme-conjugated antibody can react with substrates pseudohomogeneously, and the products can be easily transferred from one microplate to another. The absorbance of the products can be measured without interference by light scattering caused by magnetic microbeads. The homogenous immunoreactions are more efficient than that of reaction on the surface of microplates [18]. Thus, in using antibody-labeled MPs, the time required for analysis is expected to be less than that required by a conventional ELISA. Most literatures on magnetic particle-based ELISA were processed in tubes [19, 20]. The washing steps were done with one tube by one tube or with commercial magnetic separators, such as fully automated multisampling separators. The automated multisampling separators are expensive. Thus, we fabricated a practical and inexpensive magnetic microplate. The important contribution of our current work is the integration of microplate ELISA with homemade magnetic microplate. The process of the microplate ELISA will be more easily adopted in the clinical laboratory than tube-ELISA and the home made magnetic microplate is inexpensive.

Rabbits are among the most commonly used experimental animals in the areas of biochemical research and medical products. The serum immunoglobulin levels of an animal reflect its immune status. One of our coworkers used rabbits for performing immune experiments. Therefore, we used rabbit IgG as a model analyte to demonstrate our detection method. In this study, MPs were labeled with anti-IgG, then IgG from the sample was bound to anti-IgG-MPs. A secondary antibody conjugated with horseradish peroxidase (HRP) was then used to bind to IgG. In the final step, enzyme substrates were added. The subsequent reaction produced a color change, and the absorbance of the product was measured.

Materials and methods

Chemicals and materials

An affinity isolated antibody, a buffered aqueous solution of biotinlyated antirabbit IgG antibody (whole-molecule), was produced in goat; rabbit IgG purified from a normal rabbit serum by using fractionation and ion-exchange chromatography; phosphate-buffered saline (PBS); dimethyl sulfoxide (DMSO); and 3,3′,5,5′-tetramethybezidine (TMB) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Dynabeads® MyOne™ Streptavidin T1 (streptavidin-coupled superparamagnetic beads 1 μm in diameter) and Novex® HRP-conjugated goat antirabbit IgG (H&L) antibody were purchased from Life Technologies (Grand Island, NY, USA). Triton X-100 was purchased from Tedia (Fairfield, OH, USA). Hydrogen peroxide (H_2O_2, 35%) was purchased from Shimakyu's Pure Chemicals (Osaka, Japan).

Magnetic separator–magnetic microplate

Permanent magnets 6 mm in diameter and 13 mm in length were fixed in the wells of a microplate, and the assembled magnetic microplate was then placed under another microplate to form a magnetic separator. The magnetic field strength of these magnets was 4.1 (±0.2) kG at the top of separator.

Instrumentation

A spectrometer (Flexstation 3 multimode plate reader, Molecular Devices, Sunnyvale, CA, USA) was used to measure the optical intensity.

Functional magnetic particle preparation (anti-IgG-labeled MPs)

Streptavidin-coupled dynabeads were conjugated with biotinylated anti-IgG, based on the extremely high binding affinity of the streptavidin–biotin interaction ($K_d = 10^{-15}$), and further used for developing the pseudohomogeneous immunoassay. An aliquot of 100 μL of biotinylated anti-IgG (3.3 mg/mL) was added to a centrifuge tube containing 10 mL of PBS and 10 mg dynabeads, and the tube was then gently rotated using a MACSmix™ tube rotator for 2 h at 4°C. Anti-IgG-labeled MPs were attracted

by the magnets, and the MPs were washed three times with PBS to remove unreacted anti-IgG. Finally, anti-IgG-labeled MPs were reconstituted with 10 mL of PBS and divided into aliquots of 1 mL each. The aliquots were maintained at 4°C until use. The suspensions of the unreacted and washed PBS were mixed, and the protein concentration was evaluated using a Bradford reagent. The amount of labeled anti-IgG was approximately 18 μg/mg of dynabeads, which was semiquantitative based on the added amount subtracted from the amount left in the suspension. This result was consistent with that claimed by the supplier (biotinylated IgG up to 20 μg/mg of dynabeads).

Procedures for the magnetic sandwich immunoassay

Figure 1 shows the schematic of the reaction steps involved in IgG detection. This is a sandwich-type detection conducted by applying magnetic force and colorimetric detection. The procedures are briefly described as follows: In Step 1, an aliquot of 220 μL of IgG standards or serum samples, and 10 μL of 1 mg/mL anti-IgG-labeled MPs were added to the wells of the microplate. The mixture was then pipetted several times for mixing and incubated for 20 min at room temperature. In Step 2, the microplate was placed on top of a homemade magnetic microplate that attracted MPs to the bottom of the plate, and the remaining solution was gently removed. The microplates were then washed twice with PBS containing 0.1% BSA. In Step 3, 20 μL of anti-IgG-HRP (dilution ratio of 1:1000) and 200 μL of PBS were added to the microplates. The mixture was then pipetted several times for mixing and incubated for 30 min at room temperature. In Step 4, the unreacted anti-IgG-HRP was removed by washing the microplates twice with PBS containing 0.1% triton. The sandwich particles were resuspended in 200 μL of buffer solutions containing the enzyme substrate (TMB/H_2O_2). The enzyme substrates were 0.5 mL of TMB (2.0 mg/mL in DMSO) and 32 μL of 0.75% H_2O_2 freshly mixed with 10 mL of PBS. This is the reported optimized concentration of a TMB substrate [21]. In Step 5, finally, the microplate was placed on top of the homemade magnetic microplate, and 150 μL of the solution was transferred to another microplate. An aliquot of 50 μL of 1 M HCl was added to each well, and the absorbance for each sample was measured at 450 nm. All experiments were performed in triplicate. Notably, the solution of dynabeads mixed with 1 M HCl gradually turned yellow, and the beads caused light scattering.

Therefore, we transferred the products of the enzyme–substrate reaction to another microplate for subsequent measurements.

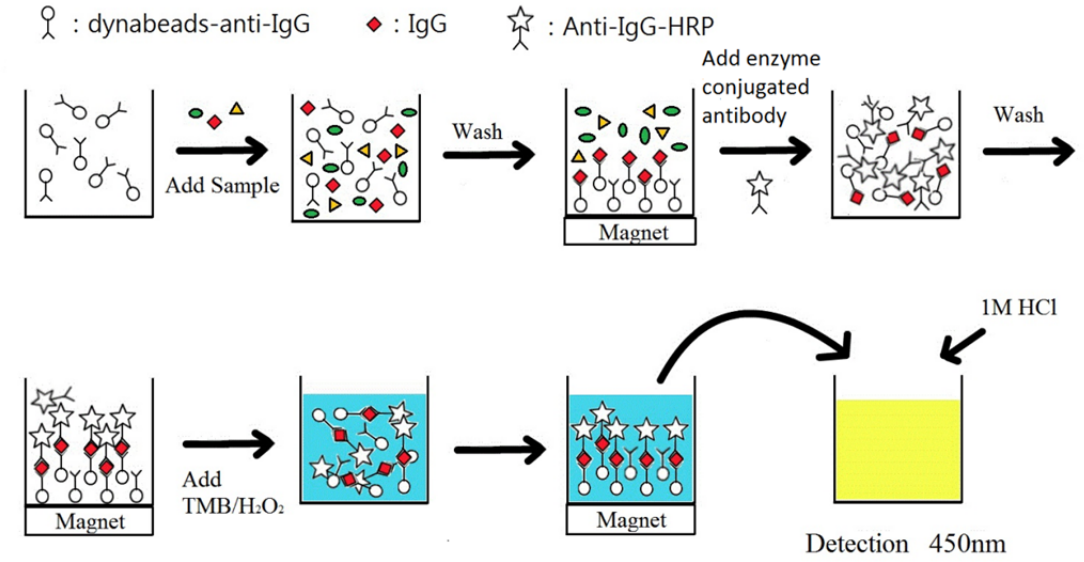


Figure 1 Schematic of immunoassay procedures for a magnetic ELISA.

A calibration curve of the absorbance was established by plotting the measured absorbance versus the various known concentrations of IgG.

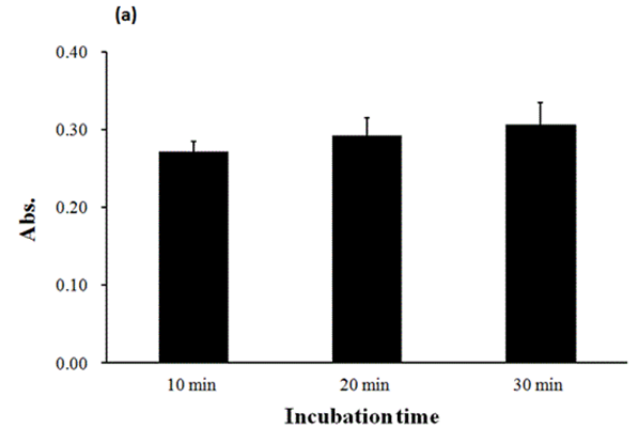
Preparation of serum and spiked samples

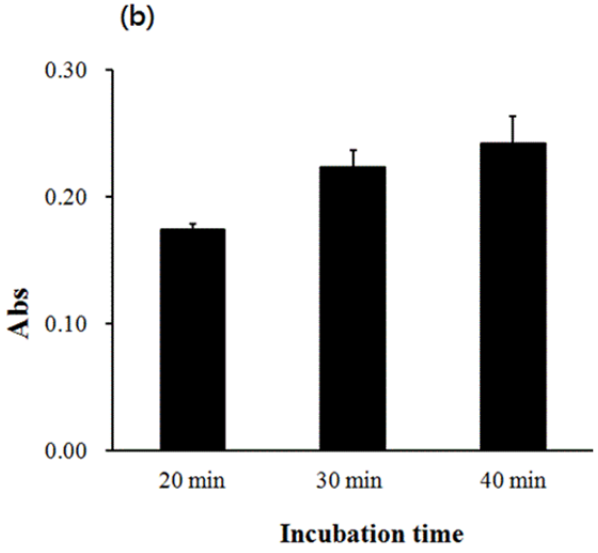
We drew blood from rabbits' ears. The rabbit antisera was precipitated using $(NH_4)_2SO_4$ to a final concentration of 50% and 35% in sequence by 100% saturated $(NH_4)_2SO_4$ solution. The precipitate was redissolved in distilled water equal to half of the original volume and then dialyzed against 2 L PBS for 72 h at 4°C with two changes of buffer. Finally, 0.01 M PBS was added to the original volume. The rabbits were New Zealand white rabbits (body weight, 3.5 kg), which were obtained from Deer-Ho farm (Taichung, Taiwan) for immune experiments conducted by our coworkers. The animal experiments in our study were approved by the Institutional Animal Care and Use Committee at the Chung Shan Medical University (approval no. 1269). Prior to using the ELISA, the serum was diluted 10,000 times using PBS. To demonstrate the practicality of the proposed magnetic ELISA, IgG concentration in spiked serum samples was measured.

Results and discussion

Optimization of immunoreaction time

The incubation time of an antibody and antigen is one of the crucial parameters for achieving a satisfactory sensitivity of an immunoassay [17]. Therefore, we varied the immunoreaction time from 10 to 30 min. Figure 2a shows the performance results as a function of the incubation time of anti-IgG-labeled MPs and IgG. The duration of the sandwich immunoreaction (IgG and anti-IgG-HRP) was 30 min. The amount of the HRP–TMB reaction products increased as the reaction time increased. We preliminarily applied 30 min for the enzyme–substrate reaction based on the balance of time consumption and detection sensitivity. The results showed that the intensity increased with longer incubation time; however, no significant difference was observed between the results of reactions performed for 20 min and 30 min (t-test, $p = 0.90$). Therefore, we applied an incubation time of 20 min for the first antibody–antigen reaction, and then further studied the secondary antibody reaction time of the sandwich immunoreaction (IgG and anti-IgG-HRP). Figure 2b shows the performance results as a function of the incubation time of anti-IgG-HRP and MP-anti-IgG-IgG. No significant differences were observed between the results of reactions performed for 30 min and 40 min(t-test, $p = 0.33$). Therefore, we set 20 min for the primary immunoreaction and 30 min for the secondary immunoreaction as the optimized immunoreaction time.


(a)
0.40
0.30
0.20
0.10
0.00
Abs.
10 min
20 min
30 min
Incubation time


(b)
0.30
0.20
0.10
0.00
Abs
20 min
30 min
40 min
Incubation time

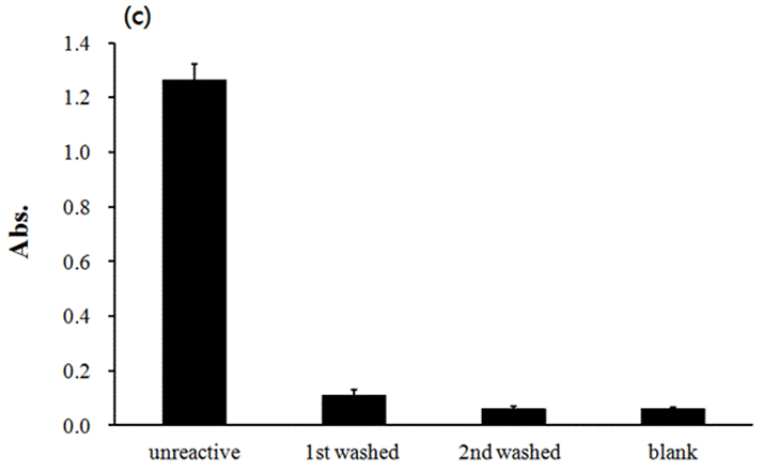

(c)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Abs.
unreactive
1st washed
2nd washed
blank

Figure 2 Optimization of immunoreaction time. (a) Effect of reaction time of anti-IgG-labeled MPs and IgG. **(b)** Effect of reaction time of anti-IgG-HRP with the microparticles to form the sandwich. **(c)** Relationship between washing times and the cleanliness of unreacted anti-IgG-HRP in the solution. "Unreactive" represents aliquots from the suspension of the mixture of anti-IgG-HRP and MP-anti-IgG-IgG after incubation. "1st washed" represents aliquots from the solution of the first washing. "2nd washed" represents aliquots from the solution after the second washing. "Blank " represents aliquots that contained only TMB/H_2O_2 in PBS.

Figure 2c shows the effect of wash times on the cleanliness of the unreactive anti-IgG-HRP. We transferred the suspension of the secondary immunoreaction and washed buffers to another microplate, and then removed 20 μL of each solution to react with 200 μL of PBS containing TMB/H_2O_2. The results from the twice-washed buffer were not different from those of the blank that contained only the TMB/H_2O_2 in buffer. Therefore, we washed the microplate only twice after each immunoreaction step.

Effect of amount of magnetic beads

The amount of dynabeads was relative to the amount of available anti-IgG; more antibodies reacted with more antigens, thereby leading to enhanced sensitivity and a wider dynamic range. By contrast, a large amount of the dynabeads caused particle aggregation. Therefore, we studied the effect of the amount of magnetic beads on sensitivity and linearity. The analysis of variance (ANOVA) statistical results ($p = 0.487$) showed no significant difference in the colorimetric intensities from the immunoreaction containing varied amounts of magnetic beads, as shown in Figure 3. This could have been due to the amount of labeled anti-IgG being considerably greater than the amount of IgG in the solution.

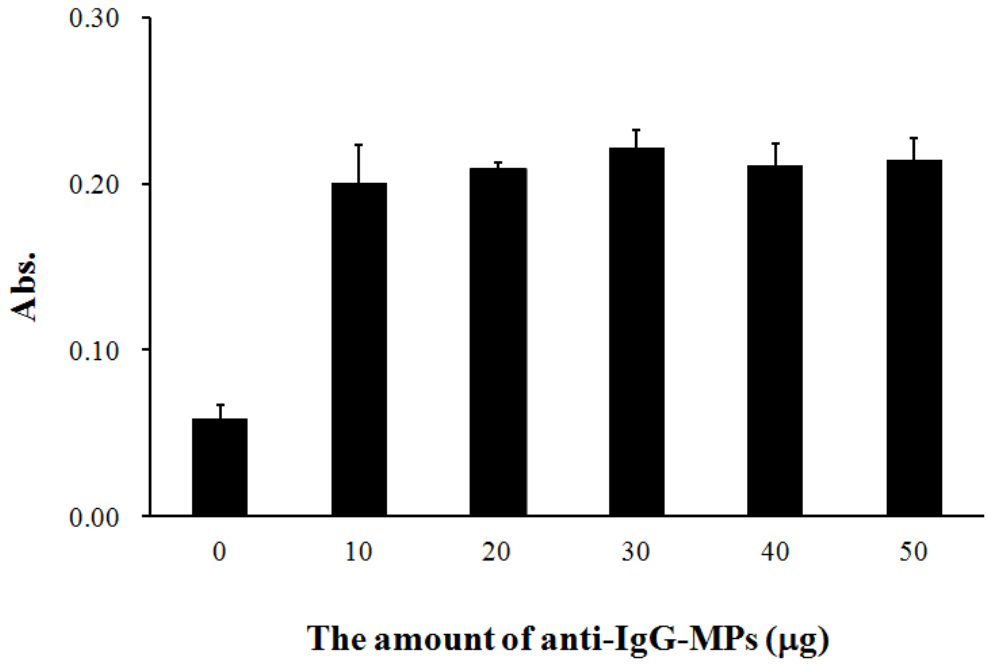


Figure 3 Effect of the amount of magnetic beads on the sensitivity. Here, 22.7 ng/mL (ie. 0.005 μg/well) of IgG with varied amounts of anti-IgG-labeled MPs were incubated for 20 min and washed twice with PBS containing 0.1% BSA. IgG was then reacted with anti-IgG-HRP for 30 min and washed twice with PBS containing 0.1% triton. Furthermore, 200 μL of PBS containing TMB/H2O2 was added, and 150-μL aliquots were transferred to another microplate after 30 min. Finally, 50 μL of 1 M HCl was added to this microplate, and its absorbance was measured at 450 nm.

Regarding the cost of the dynabeads and particle aggregation, we performed dose-dependent measurements of IgG with 10 μg and 20 μg of anti-IgG-labeled MPs.

Calibration curve and reproducibility

Calibration curves were established using commercial rabbit IgG, which was purified from the normal rabbit serum by using fractionation and ion-exchange chromatography. The plots of colorimetric absorbance of varied concentrations of IgG for the optimal reaction time are displayed in Figure 4. The linearity, slope, LOD, and precision are as shown in Table 1. As expected, the upper concentration of the linear range from 20 μg of anti-IgG-labeled MPs was twice that from 10 μg of anti-IgG-labeled MPs. The LOD based on $3S_b/m$ was0.59 ng/mL and 3.4 ng/mL for

10 μg of anti-IgG-labeled MPs and 20 μg of anti-IgG-labeled MPs, respectively. However, no significant difference was observed between their sensitivities.

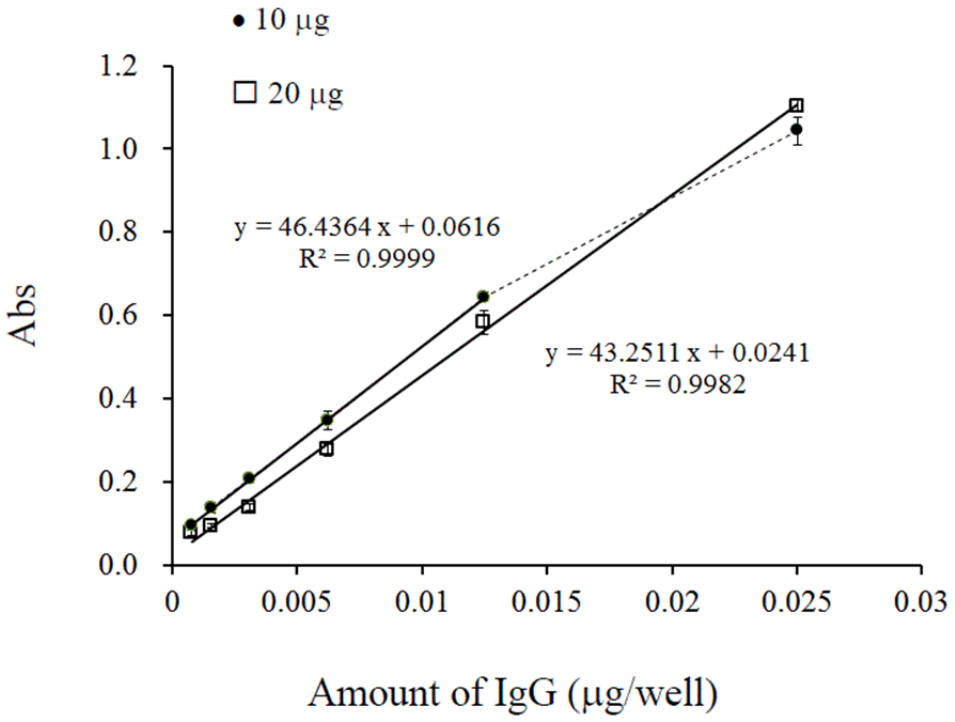


Figure 4 Plots of the calibration curves of IgG. The conditions of immunoreactions were the same as those shown in Figure 3.

Table 1 Data of analytical specifications

Amount of anti-IgG-MPs	Slope (Abs/ μg)	R^2	Range (ng/mL)	LOD(ng/mL)	Precision (reproducibility)
10 μg	46.3 (±0.3)	0.9999	56.8 ~ 3.54	0.59	2 ~ 10%
20 μg	43.3 (±0.9)	0.9982	113.6 ~ 3.54	3.4	1 ~ 9%

The importance of precision has often been emphasized for using a bead-based immunoassay in quantitative analysis. The intraassay precision of the analytical method was calculated by analyzing each concentration in triplicate per run. The relative standard deviation was 1%–5% at varied concentration levels, except for 9%–

10% at 3.5 ng/mL. These results implied that the proposed method exhibited satisfactory reproducibility. The bioactivity of highly diluted HRP decayed rapidly. Aliquots of anti-IgG-HRP diluted 100 times were maintained at 4°C. The working solution of anti-IgG-HRP was adjusted to 500–1000 dilutions based on a positive control test of the anti-IgG-HRP–TMB/H_2O_2 reaction, which maintained an absorbance at 1.5. Thus, reproducibilities of the interassay were less than 7%.

Determination of IgG in rabbit serum sample

An aliquot of 220 μL of dilute rabbit serum was incubated with 20 μg of anti-IgG-labeled MPs. The recovery was measured for the spiked IgG in serum at final concentrations of 56.8 ng/mL and 14.2 ng/mL. The IgG concentration in the obtained serum and spiked solutions was measured and interpreted according to the calibration curve. The IgG concentration of the rabbit serum was 7.66 mg/mL (±3%), which is consistent with that reported (5–10 mg/mL) by a previous study [22]. The recovery and coefficient of variation were 100% (±7%) and 116% (±4%) for the spiked concentrations of 56.8 ng/mL and 14.2 ng/mL, respectively.

Conclusion

We developed an ELISA that combines the sandwich immunoassay with MPs and an enzyme-conjugated secondary antibody on a magnetic microplate for determining the IgG concentration in a buffer solution and serum. The high sensitivity of the assay was achieved using the colorimetric method for measuring the activity of the conjugated HRP. The dynamic working range was 114-3.5 ng/mL. The recovery ranged from 100% to 116%, and reproducibility ranged from 1% to 10%. In using antibody-labeled MPs, the time required for analysis was reduced to one-third of that required in using a conventional ELISA. The detection limit was 3.4 ng/mL (i.e. 2.3×10^{-11} M) which was lower than 10^{-9}–10^{-10} M suggested by the vendors of conventional ELISA kits and time-resolved fluorescence [23].The homemade magnetic microplate was practical and inexpensive. This method has satisfactory potential for detecting other biomarkers and in biochemical applications.

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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：

103 年 11 月 12 日

計畫編號	NSC　102-2113-M-040-002-		
計畫名稱	發展結合磁場之電化學生物感測器		
出國人員姓名	蔡惠燕	服務機構及職稱	中山醫學大學 (教授)
會議時間	103 年 10 月 26 日至 103 年 10 月 30 日	會議地點	中國　成都
會議名稱	(中文) 2014 第十三屆中國國際奈米科技討會 (英文) The 13th international conference on nanoscience and technology		
發表題目	(中文) 結合 QCM 與奈米磁性顆粒做生物指標成分分析 (英文) **Biomarker detections using QCM method and magnetic nanoparticles**		

一、參加會議經過

1. 十月二十五日早上 7:35 由台中清泉崗機場出發，在香港轉機飛成都雙流機場，再轉客運至會議飯店，由於會議飯店在成都外郊的都江堰， 從雙流機場到飯店花了近兩個小時! 轉機、轉車到達成都都江堰玉瑞酒店以晚上六點了!。隔天早上 9:00 am 開始進行報到程序。十月二十七日正式由徐友龍博士開幕 開始三天緊鑼密鼓的學術報告。

2. 10 月 27 日 8:30 開幕典禮後，開始兩天半的大會專題演講，最後半天為分組邀請演講。

大會特邀主題報告包括 2010 年諾貝爾化學獎獲得者根岸英一(Negishi Ei-ichi)教授，澳大利亞兩院院士、澳科學院院長 Prof.Chennupati Jagadish，法國國家技術科學院院士，香港城市大學機械工程系呂堅教授,美國國家工程學院院士、香港中文大學工程學院汪正平教授等院士級學者。詳細議程如下

日期	時段	內容
26 日（周日）	全天	報到
26 日（周日）	19：30 — 21：00H	評審組評選優秀論文
27 日（週一）	08：30 — 12：00H	大會開幕式、大會特邀主題報告
27 日（週一）	14：00 — 17：50H	大會特邀主題報告
27 日（週一）	18：30 — 19：45H	大會招待晚宴及頒發優秀論文證書
28 日（週二）	08：30 — 12：00H	大會報告
28 日（週二）	14：00 — 18：00H	大會報告
29 日（週三）	08：30 — 12：00H	分會特邀報告和口頭交流
29 日（週三）	14：00 — 18：00H	分會特邀報告和口頭交流
30 日（週四）	08：30 — 12：00H	考察觀光、自由活動、返程

由西安納米科技學會常務理事長徐友龍教授主持開幕典禮

2010 Nobel Prize laureate Ei-ichi Negishi 分享他成功的經驗及專題演講"Magic power of d-block transition metal catalysis as examplified in Pd-catalyzed cross-coupling and Zr-catalyzed asymmetric carboalumination of alkenes.

大陸國家千人計畫特聘王志明教授主講 Droplet induced epitaxial nanostructures--physical, material, devices and beyond.

澳大利亞科學院院長既澳大利亞技術科學與工程院院士 Prof. Chennupati Jagadish 主講 semiconductor nanowire for optoelectronics and energy applictions.

长江学者,南京航空航天大学 Wanlin Guo 教授主講 Top-down Fabrication into Sub-nanometre Scale in Low-dimensional Systems

美国国家工程院院士，中国工程院外籍院士，美国乔治亚理工学院“董事教授”，香港中文大學工程學院院長 Prof. Ching-Ping Wong，主講 Nano Etching Via Metal-assisted Chemical Etching(MaCE)for Through Silicon Via (TSV) Stacked 3D Integrated Devices (ICs) and 3D

Functional Filling Photonic Crystal Applications

高義華教授（華中科技大學博士生導師、楚天學者）主講新型能源轉換與記憶體件的研究

高興宇博士（中國科學院上海應用物理研究所研究員，中國科學院“百人”）
主講基於同步輻射的納米材料表徵

潘力佳教授（教育部新世纪优秀人才，南京大学电子科学与工程学院博导）
Title：导电高分子微纳结构及其在功能电子器件上的应用

Prof. Irene M.C. Lo (劳敏慈，欧洲自然科学与社会科学院院士)

Title：Water Purification Using Magnetic Nano- and Microparticles: Removal Effectiveness, Particles Reuse and Pollutant Recovery

呂堅教授（法國國家技術科學院院士，香港城市大學副校長）本來是第天早上主講， 因在美國飛機延誤到第二天下午才趕到，主講 Recent Development of the Surface Nanocrystallization: hierarchical nanostructured materials-mechanical properties and applications

呂教授的研究獲得世界 600 多家公司的贊助， 大陸及日本政府的資助。 希望透過 改扁不鏽鋼料結構，減少車體重量 (可以省油)，還能增加阻力、防撞能力。

台灣逢甲大學蔡宜壽教授介紹主題 The application of graphene encapsulated plastic composites¸但演講內容只講構想， 希望找大陸廠商合作。 感覺有失大會邀請演講的主題。

東華大學材料學院副院長王宏志教授主講智能服裝用奈米功能材料，利用電沉積方式組裝 PMMA¸利用磁場組織小顆粒成線狀， 利用電場作用改變化合物而改變顏色。

第二天下午大會邀請演講的大人物一個個不見了，但仍有不少努力學習的學生繼續學習。賴文勇博士(國家青年 973 首席科學家、教育部“新世紀優秀人才”、南京郵電大學教授)主講 Flexible Electronics Based on Stretchable Transparent Electrodes， 利用玻璃基材， 在上面倒上一層 Ag nanowire，然後加上一層 PDMS，待 PDMS 乾了， 撕開 PDMS 即形成一層透明膜， 表面具導電性質， 此方法簡單便宜， 不須昂貴的蝕刻製成， 值得電化學電極製備者學習。

第三天早上 譚平恒博士（中國科學院半導體研究所研究員、國家“傑青”） 主講 Raman scattering of graphene materials ， 如何利用 Raman 定性奈米石墨烯。

第三天下午分組交流， 甚至有廠商代表發表他們新開發的儀器設備在奈米顆粒的應用。羅俊傑(鉑金埃爾默儀器公司市場專員)介紹 single particle ICP-MS as a metrology tool for nanoparticles: theory and application.

二、與會心得

本次會議共聽了 32 場演講，大會邀請數位歐美國家院士級學者演講， 其中多數是中

國人在海外發光發熱。演講內容包括材料開發、物理性質測試、工業及醫療用途應用。議程安排緊湊，中場休息僅有簡單得茶水， 會議地點在一個重新開幕的酒店，週圍並無其他吸引人的地方， 交通也不是很便利，所以讓人可以在會場專心的學習；反觀台灣辦的研討會，中場休息點心水果應有盡有，很像在開 party，常常下一場開始仍有很多人留在外面吃東西聊天。每次會議分不同領域同步進行， 結果每個人都只在自己的領域打轉。到不同國家參加會議，從會議的安排也可看出一個國家對學術與產業的關聯。

三、發表論文全文或摘要

Title: Biomarker detections using QCM method and magnetic nanoparticles

The general methods of biomarker detections are based on enzyme-linked immunosorbent assay (ELISA). Performing an ELISA involves at least one antibody with specificity for a particular antigen and an enzyme-labeled secondary antibody. Between each bioconjugation step, the plate is typically washed with a mild detergent solution to remove any non-specifically bound species; therefore, it is expensive and time-consuming. In this presentation, we integrated a flow system with a permanent magnet and a quartz crystal microbalance (QCM). The magnetic nanoparticles labeled with antibody will be deposited on the electrode surface of QCM due to the attraction of magnetic field; the antibody will catch the antigen in the flow and other components will be washed out by the flow.

The resonant frequency shift（Δf）of QCM is proportional to the amount of nanoparticle deposition and the amounts of analyte captured on the electrode of QCM. This technique can provide a simple and economic method for on line detection of biomarkers.

四、建議

國內學術研討會， 是否可以改掉中場休息大吃大喝的陋習， 簡單的茶水即可， 讓

與會者充分交流。或者到一幽靜的環境開會， 會後有一天或半天知識之旅(參觀學習)，除了科學交流也可增進人文素養。

五、攜回資料名稱及內容

會議論文摘要光碟一片

六、其他: 無

科技部補助計畫衍生研發成果推廣資料表

日期:2015/03/09

科技部補助計畫	計畫名稱: 發展結合磁場之電化學生物感測器
	計畫主持人: 蔡惠燕
	計畫編號: 102-2113-M-040-002- 學門領域: 分析化學

無研發成果推廣資料

102 年度專題研究計畫研究成果彙整表

計畫主持人：蔡惠燕	計畫編號：102-2113-M-040-002-
計畫名稱：發展結合磁場之電化學生物感測器	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	1	1	100%		
		研討會論文	2	1	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	2	100%	人次	碩士人力不足，改以大學部專題生
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

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。)	1. 本計畫設計酵素固定化在磁性顆粒上，在流動系統中利用磁場吸住磁性顆粒，使酵素可以在流動系統中重複再利用， 若可放大設計將其應用於工業製程成， 將可節省製造及分離純化的成本。 2. 本計畫自行設計磁性微盤， 將其與傳統 ELISA 結合， 可提高偵測靈敏度及縮短操作時間。 3. 培訓碩士班及大學部研究人才。

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

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：

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 100 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）

本研究其中一成果乃自行設計磁性微盤，與傳統 ELISA 結合，可以縮短分析時間及提高分析靈敏度。