

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Gold Nanoparticles for Biosensor Development

A thesis presented in partial fulfillment of the degree of

Doctor of Philosophy in

Chemistry

Institute of Fundamental Science, Massey University

Palmerston North, New Zealand

Xiuqian Jiang

2009

ABSTRACT

Gold nanoparticles, are one of the most widely investigated nanoparticles (NP) and are normally synthesized by the reduction of metal salts in citrate solution. The reason for studying this nanostructured material from a technological standpoint is mainly the anticipated application in different areas based on optical properties explained with plasmon resonance. The main work of this study was to develop different sensing systems using gold nanoparticles. Three techniques have been utilized, being lateral flow immunoassay (LFIA), surface plasmon resonance (SPR), and surface-enhanced Raman scattering (SERS).

A one-step semi-quantitative LFIA strip test was developed using colloidal gold coated by a partially-purified polyclonal antibody (pAb) raised in sheep as a signal generator, and bovine serum albumin-Estriol-16-glucuronide (BSA-E3-16G) conjugates as the capture agent spotted onto a nitrocellulose membrane as the test line. In this system, gold nanoparticles were applied for visualising the response. The application of the strip sensor to urinary samples from pregnant woman proved successful.

A quantitative evaluation of low levels of E3-16G in liquid media was developed based on SPR, which used the same pAb-nanogold conjugates employed for the LFIA analysis. The assay can be carried out directly on any urine samples without sample pretreatment. In this system, gold nanoparticles were utilized as high mass label to improve the sensitivity of the assay.

A SERS probe was developed which comprised of Raman reporter molecules (RRM) and gold NPs. Results showed that the conducting polymer materials of 3'-[(E)-2-(4-R-phenyl)ethenyl]-2',2':5',2''-terthiophene (R-pe3T, where R is NO₂ or NH₂) showed significant enhancement. Moreover, high bio-activity groups included in the compounds make them potential candidates for the development of a SERS based sensing system.

ACKNOWLEDGMENTS

I would like to take this opportunity to offer my heart felt thanks to a number of people without whose help and support this thesis would never have been possible. I must start with my supervisor **Dr. Mark Waterland** who has given me the chance to come to New Zealand and work here. I am sure there are only a few students who ever have the pleasure of working for as wonderful a researcher and educator as I have had. I will always be grateful for all his guidance, understanding and support. I must also express great gratitude to **Assoc. Prof. Len Blackwell**. It has been a great pleasure working with and learning from him. I appreciate his assistance in writing this thesis. A very special thanks for **Assoc. Prof. Ashton Partridge** who has supported me throughout my study with his patience and knowledge whilst allowing me the room to work in my own way. In addition I would like to thank the MacDiarmid Institute for providing me with financial support to conduct my studies, and the Institute of Fundamental Science at Massey University providing the support and equipments I have needed to produce and complete my thesis.

I have had the pleasure of working with a number of very talented and knowledgeable people in the MacDiarmid Institute at Massey University. I would like to deeply thank Dr. Wayne Campbell, giving me so many advices about conducting polymers and providing compounds for SERS work, Dr. Krishanthi Jayasundera who synthesized the ligand (E3-16G-OEG) for SPR study. Dr. Steve Kirk, Adam Stephenson, Gaile Dombroski, Nyree Parker, Helen Hsu, Indu Sharma and Emad Al-Imarah, it is my pleasure to work with them.

A number of people in the collaboration have provided invaluable support and information. I would therefore like to thank Dr. Jenness Guthrie, Dr. Delwyn Cooke, Dr. Yinqiu Wu, and Dr. Jing Yuan.

My parents have always been an important source of encouragement and support. Nothing I can say can do justice to how I feel about their support throughout all my entire life. I must acknowledge my mum and my fiancé in particular. Without their care, their support and their love in the last few months this thesis would never have started much less finished.

Table of Contents

Abstract.....	i
Acknowledgements	ii
Abbreviations	viii
List of Figures.....	xi
List of Tables.....	xvii
List of publications.....	xix

Chapter 1: Introduction

1.1 Background theory of gold nanoparticles	1
1.1.1 The formation of spherical nanoparticles.....	2
1.1.2 The formation of rod-shaped nanoparticles.....	4
1.1.3 Plasmon resonance.....	5
1.2 Application of gold nanoparticles to biological sensing	7
1.2.1 Lateral flow assay	7
1.2.2 Surface plasmon resonance sensing.....	10
1.2.3 Surface-enhanced Raman scattering	13
1.3 Thesis overview	17

Chapter 2: Gold nanoparticles and conjugates

2.1 Gold nanostructure	18
2.1.1 Introduction.....	18
2.1.2 Spherical nanoparticles	21
2.1.2.1 Amount of reductant and particle size.....	21
2.1.2.2 The stability of NPs.....	24
2.1.2.3 Dynamic Light Scattering	25
2.1.3 Rod-shaped nanoparticles	27
2.1.3.1 Seed mediated synthesis.....	27
2.1.3.2 Synthesis in the presence of AgNO ₃	28
2.2 Gold conjugates.....	32
2.2.1 Finding optimal conditions	33

2.2.1.1 Titration for synthesis of pAb-Au conjugates	34
2.2.1.2 Titration for synthesis of 2° antiserum-Au conjugates.....	35
2.2.2 Antibody/antiserum-Au conjugates	37
2.2.2.1 pAb-Au conjugates	37
2.2.2.2 2°antiserum-Au conjugates	39
2.3 Conclusion	39

Chapter 3: Lateral flow immunoassay

3.1 Introduction.....	41
3.2 Lateral flow immunoassay	44
3.2.1 Assays developed with pAb-Au conjugates.....	46
3.2.2 Assays developed with 2°antiserum-Au conjugates	50
3.3 Application of LFIA	54
3.4 Conclusion	57

Chapter 4: SPR Biosensor-based immunoassay

4.1 Introduction.....	59
4.2 Development of SPR assay	60
4.2.1 Synthesis of E3-16G-OEG-OVA conjugates.....	61
4.2.2 Immobilization of ligands onto the CM5 chip surface.....	62
4.2.3 Binding performance of pAb/pAb-Au conjugates on the chip surface	64
4.2.4 Inhibition assay developed with pAb.....	66
4.2.5 Enhanced assay developed with pAb-Au conjugates.....	67
4.2.6 Enhanced assays developed with 2°Ab-Au and 2° antiserum.....	68
4.3 Determination of E3-16G in urinary samples.....	72
4.3.1 Cross-reactivity of the polyclonal anti-E3-16G antibody	72
4.3.2 The matrix effect of the urine in the SPR immunoassay.....	73
4.3.3 Precision and accuracy of the immunoassay.....	75
4.3.4 Determination of E3-16G.....	75
4.4 Conclusion	79

Chapter 5: Surface-enhanced Raman scattering

5.1 Introduction.....	81
5.2 Structures of Raman reporter molecules	83

5.3 Study of substituted porphyrins	86
5.3.1 Compound 5.1	86
5.3.1.1 SERS signals from Au and Ag sol.....	86
5.3.1.2 Concentration and size dependence studies of Au sol.....	89
5.3.1.3 pH dependence study of SERS	90
5.3.2 Compound 5.2 and 5.3	94
5.3.3 Conclusion	98
5.4 Study of thiophene class	99
5.4.1 Compound 5.4	99
5.4.1.1 Concentration dependence study of Raman signals	100
5.4.1.2 Signal enhancement in the presence of KCl.....	101
5.4.1.3 Oxidation caused by FeCl ₃	101
5.4.2 Compound 5.5	102
5.4.2.1 Concentration dependence study of Raman signals	104
5.4.2.2 Oxidation caused by FeCl ₃	105
5.4.3 Conclusion	106
5.5 Study of substituted terthiophene.....	107
5.5.1 Compound 5.6	107
5.5.2 Compound 5.7	109
5.5.2.1 Oxidation caused by Au ³⁺	110
5.5.2.2 Effects of the reaction conditions on the Raman signals.....	112
5.5.2.3 Oxidation caused by FeCl ₃	114
5.5.2.4 Characterization of dimer-Au nanocomposites	116
5.5.3 Compound 5.8	119
5.5.4 Conclusion	121
5.6 Conclusion	123
Conclusions and future work.....	125
Conclusion	125
Future work.....	128
Appendix.....	131
I: Experimental	132
1. Chemicals.....	133

2. Instruments	134
3. Software	135
4. Recipes	136
4.1 Nanoparticles	136
4.2 Gold-Antibody conjugates	138
4.3 Lateral flow immunoassay	141
4.4 SPR-based immunoassay	145
4.5 Surface-enhanced Raman Scattering	151
II: Reference	157

Abbreviations

E3-16G	Estriol-16-glucuronide
SPR	Surface plasmon resonance
SERS	surface-enhanced Raman scattering
LFIA	Lateral flow immunoassay
BSA	bovine serum albumin
RRM	Raman reporter molecule
R-pe3T	3'-[(E)-2-(4-R-phenyl)ethenyl]-2'2':5',2''-terthiophene
NP	nanoparticle
NR	NanoRod
MTP	multiple twinned particles
CTAB	cetyltrimethylammonium bromide
R	aspect ratio
GS	growth solution
LFA	Lateral flow assay
RU	Reaction Unit or Resonance Unit
DDC	Direct Digital Control
TEM	transmission electron microscopy
Ab	antibody

pAb	polyclonal primary antibody
Au-Ab	nanogold-antibody
2°AS	anti-sheep serum raised in rabbit
E1-3G	Estrone-3-glucuronide
E3-3G	Estriol-3-glucuronide
E3-17G	Estriol-17-glucuronide
LOD	low limit of detection
TM	Tris-maleate
CV	coefficient of variation
RIA	radio immunoassay
HPLC	high performance liquid chromatography
LC	liquid chromatography
MS	mass spectroscopy
OVA	ovalbumin
OEG	oligoethylene glycol
FC	flow cell
FR	flow rate
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
NHS	N-hydroxysuccinimide

EAH	ethanolamine-HCl
RelResp	relative response
OD	optical density
PEG	poly ethylene glycol
PdG	pregnanediol glucoronide
PVP	polyvinylpyrrolidone
EDOT	2,3-dihydrothieno[3,4-b]-1,4-dioxin
PEDOT	poly(2,3-dihydrothieno-1,4-dioxin)
DCM	dichloromethane
ZnTXP	5,10,15,20-Tetrakis(3',5'-dimethylphenyl)porphyrinato zinc(II)
ZnTPP	5,10,15,20-Tetraphenylporphyrinato zinc(II)

List of Figures

<u>FIGURE</u>	<u>PAGE</u>
1.1	Portraiture of Michael Faraday1
1.2	Change of total free energy, volume free energy and surface free energy as a function of nucleus size3
1.3	The critical radius increase with increasing the reacting T.....4
1.4	The growth of NRs5
1.5	A typical absorption spectrum of spherical NPs6
1.6	A typical absorption spectrum of rod-shaped NPs6
1.7	Schematic diagram of the analytical device for a lateral flow assay8
1.8	Half strip and Mircotiter plate for assay development8
1.9	Schematic explanation of the total internal reflection and the evanescent wave under this condition 11
1.10	Schematic illustration of a Surface Plasmon Resonance sensor12
1.11	Schematic illustration of surface-enhanced Raman scattering (SERS) experiment16
2.2	One-step seed mediated synthesis20
2.3	Three-step seed-mediated synthesis20
2.4	Absorption spectra of the six spherical NPs samples produced with different amount of reductant22

2.5	Correlation between the plasmon resonance wavelength of the Au nanospheres and the mole ratio of Au ³⁺ to reductant	23
2.6	TEM images of six nano-spheres' samples	23-24
2.7	Absorption spectra of two-week old samples	24
2.8	Size distributions by number of the spherical NPs reduced by sodium citrate	26
2.9	Size distribution by intensity of 20 nm sized commercial Au NPs determined by the Malvern Zetasizer	26
2.10	TEM images of NRs using seed mediated synthesis	27
2.11	Absorption spectra of 8 NRs – 3 samples synthesized in the presence of AgNO ₃	29
2.12	TEM images of NRs-3	29-30
2.13	Correlation between the aspect ratios (TEM) determined experimentally and longitudinal plasmon resonance wavelength of NRs (UV-Vis)	31
2.14	Correlation between the experimentally determined aspect ratio (TEM) and volume of the applied AgNO ₃	32
2.15	Correlation between the plasmon resonance wavelengths of pAb coated Au NPs and the concentrations of the coating pAb at different pH values	34
2.16	Absorption spectra of the 2°AS-Au conjugates synthesized using Au NPs of different pH	36
2.17	Correlation between the plasmon resonance wavelengths of conjugates and the concentrations of coating 2°AS at different pH values	36
2.18	Absorption spectra of the unconjugated Au NPs and two pAb-Au conjugates synthesized using pAb of different concentrations	38
2.19	Absorption spectra of the unconjugated Au NPs and two 2°AS-Au conjugates synthesized using different amounts of antiserum	38

3.1	Chemical structures of Estriols, E3-16G, E3-17G and E3-3G	42
3.2	One-step assay developed with pAb-Au conjugates.....	44
3.3	Two-step assay developed with 2°AS-Au conjugates	45
3.4	Strip tests performed using a series of standards (12 different concentrations).....	46
3.5	Buffer standard curves performed using pAb-Au conjugates	47
3.6	Urine blank and buffer standard curves performed using pAb-Au conjugates	48
3.7	a) Strip tests for the determination of the correct dilution factor of the pAb introduced in the first step. b) Two standard curves performed using 1:5 pAb and 1:10 pAb in the first step	51
3.8	Standard curves performed using the conjugates synthesized with different sized Au NPs	52
3.9	Buffer and urine blank standard curves performed using 2°AS-Au conjugates	53
3.10	E3-16G excretion pattern through a normal menstrual cycle obtained using the LFIA strips	56
3.11	Excretion rates of E3-16G during the first and second trimester determined using the test strips	56
4.1	Development of biosensor-based inhibition assay	60
4.2	Buffer conditions for successful immobilization of ligands onto a CM5 chip surface	62
4.3	Sensorgram for the process of immobilizing ligands onto a CM5 chip surface	63
4.4	Correlation between the concentration of pAb in the samples and the sensor response	65
4.5	Standard curves developed with samples containing 1 µg/mL and 5 µg/mL pAb.....	66

4.6	Standard curves performed with SPR and strip sensor	68
4.7	Correlation between the OD of the 2°Ab-Au conjugates and the related binding response	70
4.8	SPR reference calibration curve performed in the presence of the time-diluted blank urine	74
4.9	A) E3-16G concentration obtained using the SPR biosensor and LFIA strips. B) Excretion pattern of PdG compared with E3-16G concentration values obtained from the SPR biosensor	76
4.10	Excretion of E3-16G during the first two trimesters determined using the SPR biosensor and LFIA	78
5.1	Model of SERS probe.....	82
	Scheme I: Chemical structures of compounds 5.1 to 5.8	84-85
5.2	Chemical structure of porphyrin.....	86
5.3	Normalized UV-Vis absorption spectra of 5.1 and Ag/Au NPs	87
5.4	Raman spectra of solvent (methanol) and 5.1 taken under different conditions	88
5.5	Raman spectra of 5.1 taken using different substrates.....	89
5.6	UV-Vis absorption spectra of the Au NP 2 at different pH value	90
5.7	UV-Vis absorption spectra of samples prepared with 5.1 and the Au-sol of pH 2 to 10.....	91-92
5.8	Raman spectra of samples prepared with 5.1 and the Au-sol of pH 2 to 10	93
5.9	UV-Vis absorption spectra of 5.2 and 5.3	94
5.10	Raman spectra of 5.2 and 5.3 obtained using Au-sol as substrate	95

5.11	Schematic explanation of the orientation of 5.2 and 5.3 on the Au surface.....	96
5.12	pH dependence study of 5.2 and 5.3	97
5.13	Raman spectra of pure 5.4 and 5.4 of different concentrations using the Au-sol as substrate	99
5.14	Correlation between the intensity of the band at 1445 cm^{-1} and the concentration of 5.4	100
5.15	Raman spectra of 5.4 ($25\text{ }\mu\text{M}$) in the Au sol with KCl of different concentrations	101
5.16	Raman spectra of 5.4 recorded with FeCl_3 at different volumes	101
5.17	UV-Vis absorption spectra of a) sample comprising 5.5 and the Au-sol, b) the sample after adding the oxidant of FeCl_3 and c) 0.25 % commercial PEDOT (w/v).....	103
5.18	Raman spectra of 5.5 (10 mM) prepared with ethanol and the mixture of 5.5 ($50\text{ }\mu\text{M}$) and the substrate of the Au sol.....	103
5.19	Correlation between the intensity of bands, 1174 cm^{-1} and 1476 cm^{-1} , and the concentration of 5.5	104
5.20	Raman spectra of 5.5 recorded with FeCl_3 at different volumes	105
5.21	TEM images of the Au sol and aggregated Au NPs	107
5.22	Absorption spectra of a) 5.6 in CHCl_3 , b) the mixture of 5.6 and the Au-sol and c) the Au-sol	108
5.23	Raman spectra of 5.6 of different concentration recorded using the Au sol as substrate.....	108
5.24	UV-Visible absorption spectra of 5.7 in CHCl_3 (a), 5.7 ($2.5\text{ }\mu\text{M}$) with Au-sol before (b) and after (c) adding FeCl_3	109
5.25	Raman spectra of 5.7 recorded under different conditions	111

5.26	Peak areas of bands at 1180 cm ⁻¹ and 1332 cm ⁻¹ are plotted versus the concentration of 5.7	112
5.27	The peak areas of the band around 1380 cm ⁻¹ , before and after adding the Fe ³⁺ , are plotted versus time. The insert shows absorption spectra recorded at different time before adding the Fe ³⁺	113
5.28	Raman spectra of 5.7 recorded with FeCl ₃ at different volumes	115
5.29	TEM images taken before and after adding Fe ³⁺ to the mixture (5 μM 5.7 and Au-sol).....	116
5.30	SEM images of dimer-Au nanocomposites and EDX spectrum	117-118
5.31	Correlation of the average hydrodynamic diameter and the reaction time	119
5.32	UV-Visible absorption spectra of 5.8 in CHCl ₃ (a), 5.8 (5μM) with Au-sol before (b) and after (c) adding FeCl ₃	120
5.33	Raman spectra of 5.8 recorded under different conditions	121
Scheme 1 : Schematic illustration of multianalytes immunoassay based on a parallel application of SPR and SERS		129
Scheme 2 : how to synthesize the conjugates of E3-16G-OEG-OVA		145

List of Tables

<u>TABLE</u>	<u>PAGE</u>
2.1 Six Au nanospheres prepared according to different mole ratios of Au ³⁺ to reductant	22
2.2 Plasmon resonance wavelength of fresh and two weeks old samples	25
2.3 NRs-3 samples prepared by adding AgNO ₃ of different volumes to the growth solution	29
2.4 Aspect ratios of the 8 samples determined by TEM (R _{TEM}) and calculated with the Equation (2-2) (R _{Equation})	31
3.1 pH dependence study of the urine matrix effect in the first format	49
3.2 Conductivity (C) dependence study of the urine matrix effect in the first format	49
3.3 Reproducibility of test strips and synthesis of the conjugates	50
3.4 Recovery and precision of the LFIA	55
4.1 Binding response (FC2-1) according to the concentration of pAb in the samples.....	64
4.2 Relative responses on FC2-1 caused by the 2°Ab-Au conjugates in the presence of pAb of different concentrations.....	69
4.3 Non-specific binding in FC1 and FC2 caused by the 2°AS of 25 µg/mL in different running buffers (A to F)	71
4.4 Specific binding of 2°AS of different concentrations prepared with running buffer E obtained in the presence of pAb	71
4.5 Cross-reactivity of anti-E3-16G pAb to similar compounds	73
4.6 The effect of diluting urine samples on the response to E3-16G binding in an SPR assay	74

4.7	Recovery and precision of SPR assay	75
4.8	Further pre-dilution of time-diluted urinary samples of pregnant volunteer C.....	78
A.	Conjugates synthesized using Au NPs and pAb or antiserum	139
B.	Further pre-dilution of time diluted urinary samples of pregnant volunteer	143
C.	Matrix designed for the determination of the optimum concentration of KCl and the LOD of the compound 5.1	151

List of Publications

1. The first paper based on the SPR work discussed in Chapter Four has been published in *Steroids*.

Jiang, X. Q., Waterland, M., Blackwell, L., Wu, Y. Q., Jayasundera, K. P., & Partridge, A. (2009). Sensitive determination of estriol-16-glucuronide using surface plasmon resonance sensing. *Steroids*, 74(10-11), 819-824.

2. The second paper based on the applications of Lateral flow immunoassay and SPR assay in human urinary samples (discussed in Chapter Three and Four) has been published in *Analytical methods*.

Jiang, X. Q., Waterland, M., Blackwell, L., & Partridge, A. (2009). Determination of Estriol 16-glucuronide in human urine with surface plasmon resonance and lateral flow immunoassays. *Analytical Methods*, 2010, 2, 368-374.

3. The third paper based on the surface enhanced Raman scattering (Chapter Five) has been finished and is going to be submitted to *Small* soon.

Jiang, X. Q., Waterland, M., & Partridge, A. (2010).