Synthesis, Photoluminescence, chromatographic and electrophoretic studies of monolayer-protected gold nanoparticles

Man Chin Paau
Hong Kong Baptist University

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THESIS TITLE: Synthesis, Photoluminescence, Chromatographic and Electrophoretic Studies of Monolayer-Protected Gold Nanoparticles

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Issued by Graduate School, HKBU
Synthesis, Photoluminescence, Chromatographic and Electrophoretic Studies of Monolayer-Protected Gold Nanoparticles

PAAU Man Chin

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Principal Supervisor: Prof. CHAN Wing Hong

Hong Kong Baptist University

February 2016
Declaration

I hereby declare that this thesis represents my original work carried out in the Department of Chemistry, Hong Kong Baptist University since my registration for the degree of PhD in September 2009 and has done independently. This thesis has not been previously included in a thesis, dissertation or report submitted to this or other institution for a degree, diploma or other qualification.

Signature: ____________________________

Date: February 2016
Abstract

This thesis mainly consists of three parts. This first part is the synthesis of ultrasmall (< 2.0 nm) thiolated α-cyclodextrin-capped gold nanoparticles (α-CD-S-AuNPs). Per-6-thio-α-cyclodextrins were firstly synthesized and were employed to protect gold nanoparticles (AuNPs) from aggregation. These α-CD-S-AuNPs (core size < 2.0 nm) display remarkably strong blue emissions at 478 nm when excited at 400 nm. The 1.4 nm-sized α-CD-S-AuNP shows photoluminescence enhancement in the presence of tetraalkylammonium ions but is strongly quenched by Hg(II). We found that the α-CD-S-AuNP possesses ultrahigh sensitivity and good selectivity for the determination of Hg (II) with the limit of detection at 49 pM (9.7 ppt).

In the second part of this work, two liquid chromatographic methods have been developed and their efficiencies in separating samples of polydisperse gold nanoparticles protected with N-acetyl-L-cysteine ligand (NAC-AuNPs) and other ultrasmall ligand-protected gold NPs are compared. The total elution time for analysing a NAC-AuNPs sample by ultra high-performance liquid chromatography (UHPLC) is ten times shorter than that of high-performance liquid chromatography. The major attributes of UHPLC are smaller sample volume (1–2 µL) and better
separation efficiency. More importantly, our proposed UHPLC method has been successfully applied to evaluate and compare polydisperse NAC-AuNPs products synthesised with the one-phase and two-phase Brust-Schiffrin methods. The results indicate that the two-phase method would harvest AuNPs product with smaller core size and less dispersity.

The third part of this work is to describe a novel and effective capillary electrophoretic method to study positively charged, sub-nanometer-sized, water-soluble gold nanoclusters protected by N,N'-dimethylformamide (DMF-AuNC). The effects of buffer concentration, pH, and % ethanol (EtOH) on the electrophoretic mobility of the cationic DMF-AuNC are investigated. The optimum CE conditions are found to be 30 mM phosphate run buffer in 20 v/v % EtOH (pH 7.0) and an applied voltage of 15 kV. We find that the addition of SDS to the run buffer can enhance the separation of cationic DMF-AuNC, attributing to the attachment of the charged SDS to the AuNC surface with a concomitant effect on changing the charge-to-size ratio of the cationic DMF-AuNC.
Acknowledgements

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Environmental and Human Health Risk Assessment of Persistent Toxic Substance. I also need to express my wordless and deepest gratitude and love to my parents, husband, son and younger brother for their love, support and encouragement during this period.
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<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>α-CD-I</td>
<td>Per-6-Iodo-α-Cyclodextrin</td>
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<tr>
<td>α-CD-S-AuNP</td>
<td>Thiolated α-cyclodextrin-capped gold nanoparticle</td>
</tr>
<tr>
<td>α-CD-SH</td>
<td>Per-6-Thio-α-Cyclodextrin</td>
</tr>
<tr>
<td>Au</td>
<td>Gold</td>
</tr>
<tr>
<td>AuMPCs</td>
<td>Monolayer-protected Au nanoclusters</td>
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<tr>
<td>AuNC</td>
<td>Gold nanoclusters</td>
</tr>
<tr>
<td>AuNPs</td>
<td>Gold nanoparticles</td>
</tr>
<tr>
<td>B-S</td>
<td>Brust-Schiffrin</td>
</tr>
<tr>
<td>Bu₄N⁺</td>
<td>Tetrabutylammonium</td>
</tr>
<tr>
<td>Bu₄N⁺F⁻</td>
<td>Tetrabutylammonium fluoride</td>
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<tr>
<td>CE</td>
<td>Capillary electrophoresis</td>
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<tr>
<td>CDs</td>
<td>Cyclodextrins</td>
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<tr>
<td>CZE</td>
<td>Capillary zone electrophoresis</td>
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<tr>
<td>DDI</td>
<td>Distilled deionized</td>
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<tr>
<td>DHB</td>
<td>2,5-Dihydroxybenzoic acid</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N'$-dimethylformamide</td>
</tr>
<tr>
<td>DMF-AuNCs</td>
<td>$N,N'$-dimethylformamide-protected gold nanoclusters</td>
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<tr>
<td>DMSO-$d_{6}$</td>
<td>Dimethylsulfoxide-$d_{6}$</td>
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<td>Abbreviation</td>
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<tr>
<td>Et$_4$N$^+$</td>
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<td>ESI</td>
<td>Electrospray ionization</td>
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<td>His-AuNPs</td>
<td>Histidine-protected gold nanoparticles</td>
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<tr>
<td>HLB</td>
<td>Hydrophilic-lipophilic balanced sorbent</td>
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<td>HNO$_3$</td>
<td>Nitric acid</td>
</tr>
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<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<td>Hg (II)</td>
<td>Mercury (II)</td>
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<tr>
<td>IC</td>
<td>Ion chromatography</td>
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<td>MALDI-TOF</td>
<td>Matrix-assisted laser desorption ionization time-of-flight</td>
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<td>Me$_4$N$^+$</td>
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<td>Nanoclusters</td>
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<td>Sodium Hydroxide</td>
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<td>PDA</td>
<td>Photodiode array</td>
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<td>Pr$_4$N$^+$</td>
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<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
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<td>SEC</td>
<td>Size exclusion chromatography</td>
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<td>SPR</td>
<td>Surface plasmon resonance</td>
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<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
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<td>Tetraoctylammonium bromide</td>
</tr>
<tr>
<td>UV-vis</td>
<td>Ultraviolet-visible</td>
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<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
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Chapter 1 Introduction

1.1. Gold Nanoparticles

Nanoparticles (NPs) have, by definition, one or more dimension in the nanometer scale approximately 1-100 nm in size and subsequently show novel properties from their molecules or a comparable bulk materials. In recent years, there has been renewed interest and activity in the field of synthesis, characterization, and applications of metallic NPs as the transition from microparticles to NPs was seen to lead to immense changes in the physical and chemical properties of a material. The idea that gold (Au) sols are composed of small metallic particles was first proposed in 1857 by Faraday. Faraday noticed reversible color changes of thin films (dried colloidal Au) upon mechanical compression. Upon application of pressure, the films were green, like thin continuous Au films, while they appeared bluish-purple when the pressure was released, displaying more the color of wine red solutions of the particles. Recently Kawakami et al. observed color changes of Au films from black to red when the pressure was increased. The gold nanoparticles-(AuNPs) films were produced by gas deposition method. In the literatures, two major methods have been reported for the synthesis of AuNPs: gas-phase Au cluster synthesis followed by solution-phase encapsulation and liquid
phase Au growth using alkyl thiols as surfactants. Size, morphology, and crystalline phase NPs all determine the properties of nanostructured materials. Therefore, the key to controlling properties lies in the control of size, morphology, and phase of NPs. The desired size can be obtained by careful control of preparation parameters, such as temperature, pressure, concentration, pH, presence of complex-formers, and the hydrodynamics of solution. Method for determining the particle size will be discussed later.

1.2. Synthesis of Gold Nanoparticles

The synthesis of NPs is a complex process and hence there is a wide range of techniques available for producing different kinds of NPs. Nanometer-sized Au particles have attracted broad attention in various research fields. In this regime, size is an important factor that may dramatically affect a particle’s physical and chemical properties, such as its ability to be involved in catalysis. Extensive investigations have continued to demonstrate that both the size and shape of AuNPs can affect their properties. During the past few decades, various procedures for the synthesis of NPs have been introduced that allow AuNPs to be obtained with a more monodisperse nature (e.g., size and shape).

The gold hydrosols were prepared by reduction of an aqueous solution of hydrogen
tetrachloroaurate(III) with phosphorus dissolved in carbon disulfide, reported in 1857 by Faraday et al.\textsuperscript{19} Later in 1951, the most popular approaches for the synthesis of AuNPs that using citrate reduction of hydrogen tetrachloroaurate(III) (HAuCl\textsubscript{4}) in water was introduced by Turkevitch et al., in which citrate acts as both reducing and stabilizing agent and provides AuNPs of ca. 20 nm.\textsuperscript{20} Frens et al. proposed a method where the ratio between the gold salt to sodium citrate was varied to obtain AuNPs of prechosen size (between 16 and 147 nm).\textsuperscript{21}

Synthesis of AuNPs within the pores of mesoporous silica has been receiving considerable attention. Chen et al. loaded the pores of the mesoporous silica with AuNPs synthesized in-situ by sonochemical reduction of hydrogen tetrachloroaurate(III) (HAuCl\textsubscript{4}).\textsuperscript{22} More recently, Ghosh et al. synthesized and entrapped AuNPs within the pores of propylamine and propylthiol-functionalized MCM-41 materials by auto-reduction of aqueous chloraurate ions, which occurs via silanol groups present on the inner surface of the mesopores. This method is attractive in that it is simple and environmentally benign due to auto-reduction.\textsuperscript{23} Araki et al. reported the template synthesis of gold nano-wire and NPs onto mesoporous silica FSM-16 (Au/FSM-16).\textsuperscript{24} Lim et al. synthesized Au nanolayer-encapsulated silica particles by a surface seeding and shell growing method.\textsuperscript{25} Liu et al. described the fabrication of self-assembled AuNPs attached to
3-aminopropyltrimethoxysilane (APTMS) modified fused silica, by a spin coating method, where advantages include shorter fabrication time, higher uniformity, and better reproducibility compared to the immersion method. Seitz et al. described the preparation of AuNPs by chemical reduction of AuCl₄⁻ and attached to APTMS-treated glass plates. The coupling agent was proved to be very effective in immobilizing the NPs. Cant et al. conducted similar work which involved the formation of aminosilane self-assembled films and subsequent attachment of functionalized AuNPs, as a precursor to the growth of multilayer films consisting of alternating polyelectrolyte/AuNP layers. Chen et al. very recently showed photochemical production of AuNPs in monolithic porous silica by using a novel excimer ultraviolet (UV) source. Fixed-size AuNPs were synthesized using the newly developed excimer UV lamp at 222 nm. Wescott et al. developed a colloidal assembly for the study of plasmon–plasmon interactions between AuNPs. Colloidal aggregates of controlled size and interparticle spacing were prepared on silica particles. After immobilization of isolated AuNPs onto silica NPs, the surfaces of the adsorbed AuNPs were functionalized with 4-aminobenzenethiol. This molecular linker facilitated the attachment of more AuNPs to the ‘parent’ AuNPs, forming small NP aggregates. Metal clusters in the gas phase are essentially unstable due to dangling bonds
present on their surfaces. This led Mafune and Kondow to develop a method of preparing AuNPs by laser ablation of a Au plate in a surfactant solution (surfactant-controlled formation by laser ablation in solution). Thus NPs are covered with surfactant molecules, that are weakly bound to their surfaces; hence the stabilized NPs are free of passivative distortion. Kawakami et al. prepared AuNPs films by the gas deposition method. These NPs were generated by a nanosecond pulsed Nd:YAG laser ablation of a Au substrate under a low-pressure inert gas atmosphere. Swihart has recently reviewed examples and advances in vapor-phase methods for NP preparation. Microemulsions are colloidal ‘nano-dispersions’ of water in oil (or oil in water) made stable by a surfactant film. These thermodynamically stable dispersions can be considered as true nanoreactors, which can be employed to carry out chemical reactions, in particular to synthesize NPs. Manna et al. described the synthesis of dodecanethiol-capped nanospherical Au metal particles using a biphasic Winsor II type microemulsion of diethyl ether/aerosol-OT/water.

1.3. Monolayer-Protected Gold Nanoparticles

NPs can be categorized based on single or multiple materials into simple and core/shell or composite NPs. As the name implies, simple NPs are made from a
single material while composite and core/shell NPs are composed of two or more materials. The core/shell type NPs can be broadly defined as comprising a core (inner material) and a shell (outer layer material). The choice of shell material of the core/shell NPs is generally strongly dependent on the end application and use.

A very important aspect of modern nanotechnology is the assembly of two- and three-dimensional materials and structures in which particles are closely packed without initiation of uncontrolled aggregation. Monolayer-protected clusters (MPC) are of great interest in catalysis, molecular electronics and chemical sensing. Typical MPC are NPs consisting of Au cores with peripherally bound self-assembled organothiolate monolayers with overall diameters less than 5 nm. Such NPs are soluble in organic solvents due to the organic thiolate capsule which facilities processing and thin-film decomposition.

Earlier synthesis by Brust et al. involving the use of a two-phase (water-toluene) via the reduction of $\text{AuCl}_4^-$ by sodium borohydride ($\text{NaBH}_4$) in the presence of an alkanethiol (RSH) resulted in 1–3 nm NPs bearing a surface thiol coating as the following reactions:
Scheme 1.1. Brust-Schiffrin method for two-phase synthesis of AuNPs by reduction of gold salts in presence of external thiol ligands.

Further work by Brust et al. entailed synthesizing thiolated ligand, \( p \)-mercaptophenol.\(^{38} \) The most important prerequisite for NP synthesis by the borohydride reduction method is the knowledge of and control over the kinetic parameters determining their nucleation and growth.\(^6 \)

Following the initial\(^{37} \) synthetic report and subsequent characterizations, a number of additional important features of alkanethiolate (RS)-MPCs have been elucidated:

(a) The RSH:AuCl\(_4^+\) mole ratio governs the average core size produced in the synthesis, which is appealing for examining the size dependence of important physical and chemical properties.\(^{18,39} \)

(b) The crude synthetic MPC product is modestly polydisperse (in core size) but can be separated into rather monodisperse
samples by fractional precipitation. Electrochemistry of room-temperature solutions of monodisperse clusters exhibits quantized capacitive (Coulomb staircase) charging steps and further displays a transition from metal-like to molecule-like core charging characteristics with decreasing MPC core size. (c) Cluster functionalization can be achieved by simple place-exchange reactions with \( \omega \)-functionalized alkanethiolates, providing a path to explore clusters functionalized with multiple electroactive moieties. (d) The coupling reactivity of \( \omega \)-functionalized alkanethiolate/Au MPCs has been demonstrated, expanding the scope of MPCs available as large, polyfunctional chemical reagents that are in many ways analogous to dendrimers and hyperbranched polymers. Au MPCs have been discussed in the context of nanoscale electronic devices, multifunctional catalysts and chemical sensors. Potential applications also exist in biological chemistry, including biosensors, for example, on binding reactions with clusters functionalized with receptor and/or reporter sites, and as materials useful in the emerging field of biolistics. Access to these and other applications would be facilitated by a wide range of MPC solubility properties including water-solubility. MPCs having partially or fully \( \omega \)-carboxylic acid functionalized alkanethiolate ligand shells (and related examples) are soluble in polar solvents such as ethanol and acetone but exhibit sparing to negligible solubility in water. This behavior
suggests that water-solubility must be sought by minimizing (or eliminating) the methylene spacer content and by including polar elements between the thiol group and ω-terminal sites. Examples that meet these requirements are thiol-containing biomolecules such as tiopronin and coenzyme A.52

After the NPs have been synthesized, it should be well characterized so that we can justify its potential analytical applications which are largely dependent on their particle size and the properties of monolayer molecules on their surfaces.

1.4. Characterization of Gold Nanoparticles

The characterization of AuNPs is critical because of the presence of shell material on the core surface. Hence, a suitable characterization technique is always required for the both the core and shell. The most significant characterization techniques used are the same as those used for single particles, but one technique may not be sufficient. Depending on the characterization techniques and different instruments, analysis can be classified as described in the following sections.

1.4.1. Core Size

The particle sizes of AuNP can be preliminarily studied by UV-visible absorption spectroscopy. Spherical AuNPs show a strong absorption band in the visible region
of the electromagnetic wave at about 520 nm. It originates from coherent collective electron oscillations coupled through the surface to the applied electromagnetic field. This absorption, namely surface plasmon absorption, is absent for very small particles (< 2 nm) as well as for bulk Au.\textsuperscript{53,54} The plasmon absorption maximum also depends on the size of particles but also the methods of preparation and the properties of the monolayer-forming molecules. Link \textit{et al.} reported the plasmon absorption maximum red-shifts with increasing particle diameter ($\lambda_{\text{max}} = 517, 521, 533, \text{ and } 575 \text{ nm for the } 9, 22, 48 \text{ and } 99 \text{ nm particles, respectively}$) where the particles were stabilized with citrate ions.\textsuperscript{55} Moreover, a similar trend was observed for AuNPs modified with thiolated-$\beta$-cyclodextrin ($\lambda_{\text{max}} = 507, 516, 518, \text{ and } 520 \text{ nm for the } 2.7, 3.6, 4.9 \text{ and } 6.4 \text{ nm particles, respectively}$).\textsuperscript{56} On the other hand, the AuNP capped with mercaptopropionate displayed a surface plasmon absorption at about 530 nm. However, the absorption intensity was enhanced with increasing size of AuNPs without large peak shifts.\textsuperscript{57} The absorption characteristics only provide a rough estimate of the size of the NP. Microscopic and other techniques are of crucial importance for accurate measurement of the particle size, dispersity of the NP and the correlation between the change in particle size and absorption characteristics.

Furthermore, the core dimensions of NPs can be easily studied by scanning
tunneling microscopy (STM), atomic force microscopy (AFM), transmission electron microscopy (TEM), small angle X-ray scattering (SAXS), matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS), and X-ray diffraction. TEM reveals concomitant observation of the self-ordering and regular core-core spacing found in thin films. Knowing and controlling the dispersity of core size is crucial. However, it is an expensive and time-consuming technique and no separation processes are involved.

Capillary electrophoresis (CE) has rapidly emerged as one of the most powerful NPs separation techniques. This technique is not limited to the separation of small molecules but characterizing nanometer-sized spherical AuNPs. A linear relationship was obtained between the electrophoretic mobility and the size of AuNPs. The charge of a AuNP arises from the sorption of citrate ions onto the NP’s surface during the preparation process and the subsequent formation of an electric double layer. The electric potential associated with this double layer stabilizes the NPs and prevents their agglomeration. Surfactants have been employed as stabilizers for the size-selective preparation of metal NPs; ionic surfactants surrounding the metal cores prevent the agglomeration of NPs by electrostatic repulsion. These NPs behave in manner similar to charged particles.

If the NPs are well characterized, researchers would like to explore its potential
applications. The studies of NPs in CE have become more popular, not only its large surface can serve as a platform for the separation of analytes but also it can suit the development of miniaturized analytical methods.

1.4.2. Elemental Analysis

Monolayer shell of NP could be examined by other techniques. Elemental analysis is consistent with free alkanethiolate and alkanethiolate bound to the surface of Au core. The average number of ligands per core is derived from a combination of X-ray photoelectron spectroscopy (XPS) or thermogravimetric analysis (TGA) and core size analysis. Besides, the composition of AuNPs can be determined by mass spectrometric measurement. Whetten and coworkers have succeeded in fractionation of AuNPs protected by monolayers of glutathione (GSH) by using gel electrophoresis and identified the most abundant species as \( \text{Au}_{28}(\text{SG})_{16} \) by MALDI-MS and electrospray ionization (ESI)-MS.

1.5. Photoluminescence Properties of Gold Nanoparticles

AuNPs have attracted great research interest with regard to their use in biosensors, solar cells, catalysis, and nanophotonics due to their unusual chemical physical properties. One of the recent topics of unique properties of the NPs concerns
photoluminescence (PL). The overall quantum efficiency of the visible photo-luminescence from copper and Au films is very low (~$10^{-10}$), as observed by Mooradian et al.\textsuperscript{74} several decades ago. Recently, several groups have observed that orders-of-magnitude higher quantum efficiencies are seen when the metal specimen is in the nanometer dimension.\textsuperscript{75-78} Huang et al. reported a highly efficient, visible wavelength fluorescence for water-soluble AuNPs protected by monolayers of tiopronin. For excitation at 451 nm, the emission of tiopronin-AuNPs occurs in a broad peak centered at about 770 nm and its quantum yield of the luminescence of AuNPs with 1.8 nm diameter cores was estimated as 0.003±0.001.\textsuperscript{79} More recently, the unusual fluorescence enhancement behavior of AuNPs has been investigated.\textsuperscript{80-85} Wang et al. prepared two AuNPs protected by alkanethiols with pyrene units. Unusual fluorescence enhancement was observed after their fresh solutions were aged. By prolonging the aging time, more and more excimers were formed in the solutions of these two AuNPs. Formation of more and more excimers will result in the generation of more rigid aggregates, and hence the some non-radiative processes can be reduced. Thus, the lifetimes of excimers will be prolonged after the solutions of these two AuNPs have been aged.\textsuperscript{80}

A very important aspect of fluorescence phenomenon of AuNPs is their application to chemical sensing such as the detection of Hg(II) in aqueous media.\textsuperscript{86-89} Hg(II) is
a well-known chemical pollutant and its toxicity can be dangerous even at low concentration levels. Therefore, the rapid and sensitive detection of trace Hg(II) in the environment is of great importance. A new AuNP-based sensor for detecting Hg(II) in aqueous solution has been developed by Huang et al. Rhodamine B molecules that are highly fluorescent in bulk solution fluoresce weakly when they are absorbed onto AuNP surfaces as a result of fluorescence resonance energy transfer and collision with AuNPs. In the presence of Hg(II), rhodamine B molecules are released from the AuNP surface and thus restore the fluorescence of rhodamine B.

1.6. Separation of Gold Nanoparticles

Conventionally, TEM is used to measure the size and shape of metal particles. TEM, however, is a time-consuming technique that does not include a separation process by which size-dependent properties can be deduced. In addition, it is difficult to infer an ensemble’s average properties, such as average diameter or shape, based on the limited regions typically examined by TEM. This statistical uncertainty arises partly because of human subjectivity when deciding which areas of the grid to image and photograph, as well as size segregation effects that may occur during the drying process, which may provide a non-representative sample of
clusters in a given region. The use of separation techniques for the characterization of NPs has the advantage of allowing particle size distributions to be measured simultaneously with the physical properties (e.g., absorbance, conductivity) of the particles. In addition, such an analysis can be performed using a small sample volume to obtain the true, unbiased, ensemble average of the size and optical properties of the solution.61

1.6.1. Liquid Chromatography

Liquid chromatographic methods, traditionally used to separate molecules and polymers according to their sizes and chemical properties, including size exclusion chromatography (SEC),12,90,92-96 reversed-phase high performance liquid chromatography (HPLC),97-99 and ion exchange chromatography (IC)100 have been extensively studied. HPLC and SEC have been utilized and combined with TEM to successfully characterize nanometer-sized metal clusters.11,90,91

SEC in which porous material is utilized as the stationary phase, is a method for the separation of macromolecules in solution according to their sizes. The smaller analyte more readily penetrate into the pores, thereby hindering its progress through the column, while larger analytes are transported forward more easily with the mobile phase. This technique, using columns containing 25-μm silica particles
was combined with TEM to characterize nanometer-sized spherical AuNPs ranging in size from 3 to 20 nm.\textsuperscript{90} It was also employed for the size analysis of semiconductor particles.\textsuperscript{101,102} Liu \textit{et al.} reported the effect of mobile-phase additives including sodium citrate, sodium chloride, and sodium dodecyl sulfate (SDS) on separation of AuNPs by SEC.\textsuperscript{103} The addition of SDS surfactant to the mobile phase facilitates the size separation of AuNPs while AuNPs will adsorb on a column stationary phase when sodium citrate and NaCl are used as mobile-phase additives during particle size analysis by SEC. The problem of applying SEC for the separation of nanometer particles is the irreversible adsorption of the particles by column packing material due to the high surface area of stationary phase and high surface activity of NPs. This problem limits the types of columns that can be used for the separation of NPs.\textsuperscript{104}

Recently, Murray \textit{et al.} used ion-pair chromatography for separations of samples of charged, polydisperse, water-soluble AuNPs protected by $N$-acetyl-L-cysteine (NAC) and tiopronin ligands.\textsuperscript{105} The mobile phase contained tetrabutylammonium fluoride ($\text{Bu}_4\text{N}^+\text{F}^-$) as ion-pairing reagent, MeOH as organic modifier and phosphate buffer. Our research group\textsuperscript{106} also used ion-pair chromatography for separation of polydisperse AuNPs protected with monolayers of NAC using a
gradient elution program with a mobile phase of methanol (MeOH)/water containing Bu₄N⁺F⁻ and sodium chloride. The separated fractions were collected and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to determine the number of Au atoms in the fractions. In addition, our developed HPLC method was also successfully applied to analysis AuNPs obtained from various synthesis reaction times, demonstrating that the reaction’s behavior follows the nucleation-growth-disintegration process. Although our previous work demonstrated that ion-pair chromatography can be applied to separate polydisperse water-soluble AuNPs, it involves long analysis time and the resolutions are not good enough.

In the recent years, separation science was revolutionized with the introduction of ultra-performance liquid chromatography (UHPLC). Significant advances in instrumentation and column technology were made to achieve dramatic increases in resolution, speed and sensitivity in liquid chromatography. UHPLC have been utilized and combined with quadrupole time-of-flight mass spectrometry to successfully discover the presence of pesticide metabolites in food sample. The development and validation of a multi-residue UHPLC-tandem mass spectrometry (UHPLC-MS/MS) method for quantification and conformation of 11 triazine related compounds in surface and wastewater samples at the nanogram per liter
level has also been described by Benvenuto et al.\textsuperscript{108} In additional, UHPLC-MS/MS has also been developed for the simultaneous quantification and confirmation of the 20 most consumed pharmaceuticals in urban wastewater and surface water samples.\textsuperscript{109}

1.6.2. Capillary Electrophoresis

Water-soluble AuNPs are charged in aqueous state and migrate in the electric field and thus electrophoretic method should be a good candidate for analyzing and separating water-soluble AuNPs. Our research group\textsuperscript{60} described an effective CE technique for separating samples of negatively charged, polydisperse water-soluble AuNPs protected by monolayers of NAC. The larger core sizes AuNPs emerged first from the capillary. The addition of aliphatic alcohols to the run buffer can improve the separation of AuNPs by reducing the electroosmotic flow (EOF) and changing the selectivity between the AuNPs. Our proposed CE method provides a powerful tool to evaluate and separate the water-soluble AuNP products.

Schnabel \textit{et al.}\textsuperscript{110} reported the use of CE for the size-based characterization of colloidal Au particles (mean core diameters between 5.2 and 14.6 nm). This investigation was carried out in free solution with Au particles without any sieving additive. At the highest ionic strength of 6 mM, a good linear dependence of the
mobility on the reciprocal of the core radius allows the size-based characterization of the AuNPs. Several reports on separation of AuNPs by CE have been developed by Liu and his coworkers.\textsuperscript{61-63,111-113} The typical background electrolyte contained sodium dodecyl sulfate (SDS) as an additive. Adding SDS surfactant to the running buffer enhances the capability of CE to separate AuNPs.

1.7. Cyclodextrins in Capillary Electrophoresis

Cyclodextrins (CDs), also known as Schardinger dextrins, cycloamyloses, and cycloglucoamyloses, comprise a family of cyclic oligosaccharides obtained from starch by enzymatic degradation. They were discovered in 1891 by Villiers,\textsuperscript{114} but the first detailed description of the preparation and isolation was made in 1903 by Schardinger.\textsuperscript{115} Investigations of CD chemistry have been on the increase for several decades.\textsuperscript{116-131} The reasons for the enormous effort in the study of CDs are that they are the first and probably the most important example of relatively simple organic compounds which exhibit complex formation with other organic molecules; they are excellent models of enzymes which led to their use as catalysts, both in enzymatic and nonenzymatic reactions; and they are natural products and readily available for most researchers. The most characteristic property of CDs is their remarkable ability to form inclusion complexes with a wide variety of guest
molecules ranging from organic or inorganic compounds of neutral or ionic nature to noble gases. Complexing ability can also be improved by chemically modifying the CD molecules. Recent interest in the use of chemically modified CDs for various purposes has generated a number of reviews dedicated to the syntheses and application of CD derivatives.\textsuperscript{132-134}

In capillary zone electrophoresis (CZE), CDs have been successfully used as additives in the carrier system for the separation of structural isomers and structurally related compounds. The use of CDs as chiral recognition agents in the carrier system has made CE a useful technique for the enantiomeric separation of a wide variety of chiral compounds, such as terbutaline and propranolol,\textsuperscript{135} dansyl-DL-amino acids,\textsuperscript{136} DL-tryptophan and (±)-epinephrine,\textsuperscript{137} ephedrine, norephedrine, norepinephrine, isoproterenol,\textsuperscript{138} quinagolide,\textsuperscript{139} ergot alkaloids,\textsuperscript{140} terbutaline and propranolol.\textsuperscript{141}

More recently, Jira \textit{et al.}\textsuperscript{142} reported that the use of anionic/cationic chiral and achiral ion-pairing reagents with CDs was a promising new method of separating acidic and basic enantiomers in CE. The separation depends on the type of CD and ion-pairing reagents, the buffer pH, and the concentration of CD and ion-pairing reagents. In addition, tetraalkylammonium reagents has been successfully used to reverse the electroosmotic flow (EOF) in β-CD-modified CE for improving the
resolution of the cationic enantiomers.\textsuperscript{143}

1.8. \textbf{Aims of the Project}

The introduction of organic molecules on AuNPs surface not only stabilizes these nanoentities in different solvents but also provides the desired surface functionality. Our research group is particularly interested in studying the surface attachment of AuNPs with cyclodextrins (CDs). CDs are regarded as a representative of the supramolecular host compounds used to functionalize the NPs due to their high water-solubility, low toxicity and specific recognition ability towards many model substrates. Its binding ability has been well recognized. Unfortunately, the PL properties of AuNPs capped by thiolated $\alpha$-CD ($\alpha$-CD-S-AuNPs) have not been investigated in details. In the first part of my work, the PL emissions of $\alpha$-CD-S-AuNPs with different sizes are studied in order to choose a typical size of AuNPs with stronger PL intensity for further investigation. In addition, the unusual enhancement effect of tetraalkylammonium ion on small-sized $\alpha$-CD-S-AuNPs is also described. To the end, our small-sized $\alpha$-CD-S-AuNPs are successfully applied to determine Hg(II) with ultrahigh sensitivity and excellent selectivity.

In the second part of my work, an efficient UPLC technique has been developed to separate and analyze water-soluble monolayer-protected AuNPs. \textit{N}-acetyl-L-cysteine-protected AuNPs (NAC-AuNPs) have been well separated and
characterized in our previous studies,\textsuperscript{105,106} thus it is chosen for further investigation in the second part of this work. The advantages of using UPLC are described in term of comparing the analysis time, separation performance and sample injection volume with HPLC. Furthermore, the potential use of our proposed UPLC method to analyze various polydisperse NAC-AuNP products synthesized from different conditions including the one- and two-phase Brust-Schiffrin methods is also investigated.

Last but not least, an efficient CE technique has been developed to separate and analyze positively charged, sub-nanometer-sized, water-soluble gold nanoclusters protected by $N,N'$-dimethylformamide (DMF-AuNC). This is the first report on simultaneously resolving various positively charged gold nanocluster species present in an as-synthesized product by CE. Solid-phase extraction method using a hydrophilic-lipophilic balanced sorbent is employed to extract the positively charged DMF-AuNC which are synthesized using a surfactant-free DMF reduction method. Besides the effect of buffer concentration, pH, and ethanol (EtOH) on the electrophoretic mobility of cationic DMF-AuNC is studied, the effect of adding SDS to the run buffer is also investigated in detail.
Chapter 2 Experimental

2.1. Chemicals and Reagents

$N,N$-Dimethylformamide (DMF), glacial acetic acid, hydrogen tetrachloroaurate(III) trihydrate (HAuCl$_4$•3H$_2$O > 99.9%), mercury(II) acetate, potassium hydrogensulfate, sodium borohydride (NaBH$_4$, 99%), sodium methoxide, tetrabutylammonium (Bu$_4$N$^+$) bromide, tetraethylammonium (Et$_4$N$^+$) bromide, tetramethylammonium (Me$_4$N$^+$) bromide, tetrapropylammonium (Pr$_4$N$^+$) bromide, tetrabutylammonium fluoride (Bu$_4$N$^+$F$^-$ > 98%), sodium dodecylsulfate (SDS) and methanesulfonic acid were purchased from Aldrich (Milwaukee, WI, USA).

2,5-Dihydroxbenzoic acid (DHB, 98 %), iodine, thiourea, tetraoctylammonium bromide (TOA$^-$Br$^-$) and triphenylphosphine were from Sigma (St. Louis, MO, USA). $N$-Acetyl-$L$-cysteine (NAC > 99%) and ammonium acetate were obtained from International Laboratory (South San Francisco, CA, USA). $L$-Histidine was obtained from TCI Tokyo Chemical Industry (Tokyo, Japan). Acetone, dimethylsulfoxide (DMSO), ethanol (EtOH) and methanol (MeOH) of HPLC grade were purchased from Labscan (Bangkok, Thailand). Trisodium phosphate, hydrochloric acid (HCl) and sodium hydroxide (NaOH) were obtained from Farco Chemical Supplies (Beijing, China). Sodium dihydrogen phosphate dihydrate and
disodium hydrogen phosphate dihydrate were obtained from Fluka (Buchs, Switzerland). Nitric acid (HNO₃) was purchased from BDH (Poole, England). Hydrofluoric acid (HF) was obtained from Riedel-da Haën (Seelze, Germany). Ammonia solution was purchased from Ajax Chemical (Auburn, Australia). α-Cyclodextrin was obtained from Acros Organics (Geel, Belgium). Dimethylsulfoxide-d₆ (DMSO-d₆) was purchased from Armar Chemicals (Dottingen, Switzerland). Sodium chloride was obtained from Beijing Chemical Works (Beijing, China). Potassium chloride was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). All reagents were of analytical grade or above. Water was purified by a Milli-Q-RO4 water purification system (Millipore, Bedford, MA). Distilled deionized (DDI) water was used unless otherwise stated.

2.2. Synthesis of Gold Nanoparticles

2.2.1. Thiolated α-Cyclodextrin-Capped Gold Nanoparticle (α-CD-S-AuNP)

2.2.1.1. Synthesis of Per-6-Iodo-α-Cyclodextrin (α-CD-I)

α-CD-I was prepared as described in a previous article¹ with slight modifications. Briefly α-CD (6.48 g, 6.66 mmol) was added to a stirred solution of triphenylphosphine (31.5 g, 120 mmol) and iodine (30.5 g, 120 mmol) and the solution was stirred at 80°C for 15 h. It was then concentrated under vacuum to half
of its volume and the pH was adjusted to 9–10 by the addition of sodium methoxide in MeOH (3.0 M, 45 mL) with simultaneous cooling. The solution was kept at room temperature for 30 min to destroy the formate esters formed in the reaction. It was then poured into 800 mL of MeOH to form precipitate, washed with MeOH and acetone successively. The resulting precipitate was dried superficially. After further vacuum drying, α-CD-I was recovered as white powder: 1H NMR (400 MHz, DMSO-d6) δ 3.20–3.52 (18 H, H-2, H-4, H-6a), 2.52–3.72 (12 H, H-3, H-5), 3.80 (6H, H-6b), 4.95 (6 H, H-1), 5.74 (6 H, 3-OH), and 5.88 (6 H, 2-OH).

2.2.1.2. Synthesis of Per-6-Thio-α-Cyclodextrin (α-CD-SH)

6.71 g α-CD-I was dissolved in 40 mL of DMF and 2.118 g thiourea was then added. The reaction mixture was heated to 70°C under a N2 atmosphere. After 19 h, DMF was removed under reduced pressure to give a yellow oil product, which was dissolved in 150 mL DDI water. 1.82 g NaOH was added and the reaction mixture was heated to a gentle reflux under a N2 atmosphere. After 1 h, the resulting suspension was acidified with aqueous KHSO4 and the precipitate was collected by centrifugation, washed thoroughly with DDI water, and dried. To remove the last traces of DMF, the product was suspended in 300 mL DDI water and a minimum amount of KOH was added to give a clear solution; the product was then
re-precipitated by acidifying with aqueous KHSO₄. The resulting α-CD-SH fine precipitate was carefully centrifuged and freeze-dried as off-white powder.¹⁴⁵,¹⁴⁶ ¹H NMR (400 MHz, DMSO-d₆) δ 2.18 (6 H, SH), 2.79 (6 H, H-6a), 2.79 (6 H, H-6a), 3.12 (6 H, H-6), 3.30–3.41 (12 H, H-2, H-4), 3.75–3.77 (12 H, H-3, H-5), 4.91 (6 H, H-1), 5.60 (6 H, 3-OH), and 5.72 (6 H, 2-OH).

2.2.1.3. Synthesis of α-CD-S-AuNP

The synthetic procedures of α-CD-S-AuNP were similar to our previous work.⁵⁹,¹⁰⁵,¹⁰⁶ In a typical synthesis, 9.0 mg HAuCl₄·3H₂O and 24.4 mg α-CD-SH were co-dissolved in 2.0 mL of 6:1 v/v DMF/acetic acid, giving a yellow solution. After stirring for 30 min, NaBH₄ (80 mg) in 0.20 mL of EtOH was added with rapid stirring at room temperature (~20 °C). The dark-brown suspension formed was stirred for an additional 24 h, and acetone was then added to precipitate the crude α-CD-S-AuNP product. The residues were collected by centrifugation. Then, it was purified by dialysis with the crude product dissolved in 30 mM phosphate buffer (pH 12). This solution was loaded into 24-mm flat width segments of Spectr/Por® cellulose ester dialysis tubes (Spectrum Laboratories, Rancho Dominguez, CA) and placed in a 1-L beaker of 30 mL phosphate buffer (pH 12) over the course of 7 days. The dark-brown α-CD-S-AuNP solutions were collected
from the dialysis tubes, and excess amounts of HCl were added to precipitate the product which was then collected by centrifugation. This product was washed by DDI water until the supernatant fluid was neutral and dried in a Virtis freeze-drier (Gardiner, NY). The above preparation procedures for synthesizing \( \alpha \)-CD-S-AuNP were based on 1:1 \( \alpha \)-CD-SH/Au molar ratio. Other \( \alpha \)-CD-S-AuNPs synthesized with different \( \alpha \)-CD-SH/Au molar ratios and constant \([\text{AuCl}_4^-]\) were also prepared.

2.2.2. \textit{N}-Acetyl-L-Cysteine-Protected Gold Nanoparticle (NAC-AuNP)

2.2.2.1. One-phase Brust-Schiffrin synthesis

The synthesis of NAC-AuNPs in one-phase system has been described previously.\textsuperscript{59,60,106} Briefly, 0.0182 g \( \text{HAuCl}_4 \cdot 3\text{H}_2\text{O} \) and 0.0228 g NAC were dissolved in a 2.0 mL MeOH/glacial acetic acid (6:1 v/v) solvent mixture at 0°C to form an orange solution with white suspension. The mixture was then reacted with NaBH\(_4\) (0.0357 g) in 0.45 mL ethanol to form a dark brown solution. The reaction mixture was stirred for another 30 min and acetone was added to precipitate the crude NAC-AuNPs. The residues were collected by centrifugation, re-dissolved in a minimum amount of DDI water and the pH was adjusted to \textit{ca.} 1 by dropwise addition of concentrated HCl. The crude NAC-AuNPs was precipitated again and washed by acetone. It was further purified by dialysis in DDI water. The
NAC-AuNPs solution was loaded into 24-mm flat width segments of Spectr/Por cellulose ester dialysis tubes (Spectrum Laboratories, Rancho Dominguez, CA, USA), which were then placed in a 1-L beaker of DDI water and stirred slowly, recharging with fresh water ca. every 24 h over the course of 7 days. The dark-brown NAC-AuNPs solutions were collected from the dialysis tubes and the solvent was removed by a stream of nitrogen (N₂ ≥ 99.999%) at < 25°C. The purified NAC-AuNPs product is very soluble in water.

2.2.2.2. Two-phase Brust-Schiffrin synthesis

The synthesis of NAC-AuNPs in two-phase system followed the B-S method with slight modifications. An aqueous solution of 0.0380 g HAuCl₄·3H₂O in 2.0 mL H₂O (yellow) was mixed with 0.0528 g TOA⁺Br⁻ in 2.0 mL DCM in a 1:1 mole ratio. The two-phase mixture was vigorously stirred until all AuCl₄⁻ was phase-transferred from aqueous solution to DCM by TOA⁺. The DCM portion was collected and its pH was adjusted to ca. 2–3 by dropwise addition of concentrated HCl. Then, 2.1 mL of MeOH/glacial acetic acid (6:1 v/v) solvent mixture containing 0.0475 g NAC was added and stirred for 30 min. The solution was placed in an ice bath. NaBH₄ (0.0730 g) in 0.95 mL ethanol was added to the solution and stirred for another 3 h. Acetone was then added to precipitate the crude
NAC-AuNPs product. The residues were collected by centrifugation, re-dissolved in a minimum amount of DDI water and the pH was adjusted to ca. 1 by dropwise addition of concentrated HCl. The crude NAC-AuNPs product was precipitated again and washed by acetone. Finally, it was purified by dialysis as described above.

2.2.3. Histidine-protected Gold Nanoparticles (His-AuNPs)

The synthesis of His-AuNPs has been described previously. Briefly, 1.0 mL of 10 mM HAuCl₄ aqueous solution was mixed with 3.0 mL of 100 mM histidine aqueous solution at room temperature. The mixture was left for 2 h to allow reaction to proceed. Afterward, the solvent was removed by a freeze-dryer. The His-AuNPs product was kept at 4 °C prior to further liquid chromatographic analyses.

2.2.4. N,N'-dimethylformamide-protected Gold Nanoclusters (DMF-AuNCs)

The synthesis of DMF-AuNCs has been described previously. In summary, 150 μL of 0.10 M HAuCl₄ aqueous solution was added to 15 mL of DMF at 140 °C and refluxed for 6 h under vigorous stirring. Afterward, the solvent was removed under vacuum and the brown residue was further dried by a stream of nitrogen gas and
stored in a desiccator. For preparing the positively charged DMF-AuNCs, residue was re-dissolved in DDI water for SPE. The 1 mL SPE cartridge packed with hydrophilic-lipophilic balanced (HLB) sorbent (Waters, Milford, MA, USA) was pretreated by rinsing with 1 mL DDI water, 1 mL MeOH, and then 1 mL DDI water consequently before use. The DMF-AuNC in DDI water passed through the HLB sorbent SPE and the aqueous eluent was collected and dried by a stream of nitrogen (N₂) gas for further analysis. In addition, 1 mL MeOH was used to elute the adsorbed DMF-AuNC on the HLB sorbent SPE. The MeOH eluent was collected and dried by a stream of N₂ for further analysis. The aqueous eluent was determined to contain the positively charged DMF-AuNC species while the MeOH eluent contains the neutral DMF-AuNC species (*vide infra*).

### 2.3. Characterization Methods

#### 2.3.1. UV-Visible Absorption and Photoluminescence Spectroscopy

All UV-visible absorption spectra were acquired with a Varian Cary 300 Scan UV-visible absorption spectrophotometer (Palo Alto, CA) over the wavelength range from 220 to 700 nm for characterizing DMF-AuNC. The PL properties of α-CD-S-AuNPs were recorded by a PTI QM4 spectrofluorometer (Lawrenceville, NJ). All the α-CD-S-AuNPs solutions for spectral studies were prepared in DMSO.
The PL spectra of DMF-AuNC solutions were recorded on a PTI QM4 spectrofluorometer (Birmingham, NJ, USA). All the DMF-AuNC solutions for spectral studies were prepared in DDI water.

2.3.2. \(^1\)H NMR Spectroscopy

\(^1\)H NMR spectra were recorded on a Varian 400 MHz FT-NMR Spectrometer Inova 400 (Palo Alto, CA). 2.0 mg samples were dissolved in 0.5 mL DMSO-\(d_6\) for measurements.

2.3.3. Mass Spectrometry

All mass spectra were acquired on an Autoflex matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer (Bruker-Daltonics, Bremen, Germany).

2.3.3.1 NAC-AuNP Samples

The selected Fractions 1–8 in HPLC separation was collected manually based upon appropriate UV signal threshold and the pH was adjusted to \(\leq 4\) by glacial acetic acid. The MeOH content was removed by purging with a stream of \(N_2\) at room temperature. The concentrated fraction passed through a C18 solid-phase extraction
(SPE) column (Alltech). An appropriate amount of 50 mM ammonium acetate buffer (pH 4) was used to sweep away Bu₄N⁺F⁻ from the AuNPs sample in the SPE column. After that, MeOH was applied to the SPE column to elute all AuNPs. The eluate was collected and preconcentrated by a stream of N₂ prior to mass spectrometric analysis. Sample solution of AuNPs in MeOH was mixed (1:1 v: v) with a 0.10 M solution of 2,5-dihydroxybenzoic acid in 1:1 water: MeOH. Then 4 μL of this solution was deposited on a stainless steel MALDI target plate and air-dried. The sample was immediately inserted into the instrument and irradiated by the third harmonic (355 nm) of a continuum Nd:YAG pulsed laser.

2.3.3.2 Positively Charged DMF-AuNC Sample

The positively charged DMF-AuNC sample was dissolved in DDI water and then mixed in 1:1 v/v with a 1.0 M solution of DHB in MeOH/H₂O (1:1 v/v). 5.0 μL of this solution was deposited on a MALDI target plate and air-dried. The sample was irradiated by a pulsed N₂ laser at 337 nm and the laser intensity was minimized to avoid excessive AuNC fragmentation.
2.4. Analytical Separation

2.4.1 High-Performance Liquid Chromatography

All high-performance liquid chromatographic separation was conducted on a Waters (Milford, MA, USA) instrument consisting of a 2695 separations module capable of gradient elution and a 2998 PDA detector. Absorption spectra (1.2 nm resolution) were taken over 250–800 nm. The chromatographic column (150 × 4.6 mm i.d. stainless steel) was packed with 5 μm C18 bonded silica with 100 Å pore size (Grace Davison Discovery, Deerfield, IL, USA). The 3.0 mg/mL AuNPs sample solution in mobile phase was pre-filtered through a 0.45-μm, 13 mm i.d. cellulose acetate membrane syringe filter (Alltech, Deerfield, IL, USA) before injection. The mobile phase consisted of Bu₄N⁺F⁻, MeOH and DDI water. An aqueous solution of 50 mM Bu₄N⁺F⁻ as eluent I was prepared as the mobile phase. The mobile phase was filtered through a 0.45-μm cellulose acetate membrane filter (Alltech). The injection volume was 20 μL and the column temperature was maintained at 25°C. The column was pre-equilibrated with at least 40 mL of mobile phase. A gradient elution program was used at 0.40 mL/min, in which eluent I was 50 mM Bu₄N⁺F⁻ aqueous solution and eluent II was pure MeOH. The elution program was applied as follows: 43% I (57% II) for 8.0 min, linearly decreased to 38% I from 8.0 to 48 min, maintained at 38 % I from 48 to 68 min, linearly
decreased to 35% I from 68 to 80 min, and kept at 35% I from 80 to 120 min. The mobile phase components and the gradient elution program were optimised in regard to various AuNPs samples (*vide infra*).

### 2.4.2 Ultra High-Performance Liquid Chromatography

All ultra high-performance liquid chromatographic separation was conducted on a Waters (Milford, MA, USA) Acquity UPLC H-Class System. Absorption spectra (1.2 nm resolution) were taken over 250–800 nm. The chromatographic column (100×2.1 mm i.d. stainless steel) was packed with 1.7 μm C18 bonded silica with 100 Å pore size (Waters). The 3.0 mg/mL AuNPs sample solution in mobile phase was pre-filtered through a 0.45-μm, 13 mm i.d. cellulose acetate membrane syringe filter (Alltech) before injection. The mobile phase consisted of Bu₄N⁺F⁻, MeOH and DDI water. An aqueous solution of 50 mM Bu₄N⁺F⁻ (eluent I) was prepared as the mobile phase. The mobile phase was filtered through a 0.45 μm cellulose acetate membrane filter (Alltech). The injection volume was 2.0 μL, and the column temperature was maintained at 40°C. The column was pre-equilibrated with at least 4 mL of mobile phase. A gradient elution program was used at 0.40 mL/min, in which eluent I was 50 mM Bu₄N⁺F⁻ aqueous solution and eluent II was pure MeOH. The elution program was applied as follows: linearly decreased from 43 % I (57 % II) to
38 % I from 0.0 to 4.2 min, maintained at 38 % I from 4.2 to 6.4 min, linearly decreased to 35% I from 6.4 to 12.2 min, and kept at 35% I from 12.2 to 12.4 min. The mobile phase components and the gradient elution program were optimised with reference to different AuNPs samples (vide infra).

2.4.3 Capillary Electrophoresis

All electrophoretic experiments were conducted on a Beckman P/ACE MDQ CE system (Fullerton, CA, USA) in conjunction with a diode-array detector monitoring at 254 nm. Uncoated fused-silica capillaries with 50 μm i.d. and 375 μm o.d. from Polymicro Technologies (Phoenix, AZ, USA) were used. The total and effective lengths of the capillary were 40.0 and 30.0 cm, respectively. A detection window was fabricated ca. 10.0 cm from the capillary outlet. DMF-AuNC samples in the run buffer at a concentration of ca. 3.0 mg/mL were injected hydrodynamically at 0.5 psi for 5 s. Positive polarity (15 kV) was applied at the capillary inlet, i.e., electroosmotic flow (EOF) toward the cathode. The capillaries were pretreated by flushing with DDI water (5 min), 1.0 M NaOH (15 min), 0.10 M NaOH (15 min), DDI water (30 min), and run buffer (30 min) before use. Between each analysis, the capillary was rinsed sequentially with DDI water (2 min), 0.10 M NaOH (2 min), DDI water (2 min), and run buffer (5 min). All CE experiments were performed at
25 °C. The run buffer was prepared by dissolving an appropriate amount of sodium phosphate in DDI water and its pH was adjusted by the amount of sodium dihydrogen phosphate and disodium hydrogen phosphate solutions. The pH of the buffer was measured by an Orion model 410A pH meter (Allometrics Inc., Baton Rouge, LA, USA). The DMF-AuNC sample was dissolved in 250 μL 30 mM phosphate buffer (pH 7.0). The composition of the buffer solution varied according to the experiments being studied and will be discussed in the subsequent sections (*vide infra*). All run buffers and DMF-AuNC samples for CE analysis were filtered through 0.45-μm, 13 mm i.d. cellulose acetate syringe filters (Alltech, Deerfield, IL, USA) before use.

### 2.4.4 Ion Chromatography

The ion chromatograph (IC) used in this study was a Dionex ICS-1100 system (Sunnyvale, CA, USA) equipped with a DS6 heated conductivity cell and a 100 μL injection loop. Cation Self-Regenerating Suppressor (CSRS® ULTRA II, 4 mm) was employed with 59 mA applied current. A cationic exchange column (4.0 mm i.d., 250 mm) with carboxylic/phosphonic groups on 8.5 μm silica gel (model IonPac® CS12A) was used. In this work, the eluent solution was 20 mM methanesulfonic acid at a flow rate of 1.0 mL/min. The eluent was degassed with
ultrasonication for 15 min before use.
3.1. Introduction

Recently in modern nanochemistry research, the modification of AuNPs with multidentate functional molecules is fruitful.\textsuperscript{150-152} Cyclodextrins (CDs) are cyclic oligomers of six, seven, or eight glucose molecules (Scheme 3.1).

\begin{center}
\textbf{Scheme 3.1.} Chemical structures of \(\alpha\)-, \(\beta\)-, and \(\gamma\)-cyclodextrins.\textsuperscript{157}
\end{center}

The outside of the CDs toroid is hydrophilic due to the hydroxyl groups, imparting the molecules with good water-solubility; whereas the interior is relatively hydrophobic because of the glycosidic oxygen bridges.\textsuperscript{153} As such, CDs are regarded as a representative of the supramolecular host compounds used to functionalize the NPs due to their high water-solubility, low toxicity and specific
recognition ability towards many model substrates. Thiolate-modified CDs and other CD derivatives have been successfully chemisorbed onto Au surfaces. Most of these works were performed with β-CD whereas some used α-CD derivatives.\textsuperscript{145,154-156}

In addition, the effect of initial molar ratio of α-CD-SH to AuCl\textsubscript{4}\textsuperscript{−} precursors (α-CD-SH/Au ≥ 1) on AuNP particle size and composition of α-CD-S-AuNPs has been investigated in detail in our pervious work.\textsuperscript{158} The synthesis of AuNPs (core size less than 2.0 nm) capped by α-CD-SH has been studied and characterized by infrared spectroscopy, UV-visible absorption spectroscopy, and high-resolution TEM. HAuCl\textsubscript{4}•3H\textsubscript{2}O is reduced by NaBH\textsubscript{4} in the presence of α-CD-SH to produce α-CD-S-AuNPs. The particle size of the as-synthesized α-CD-S-AuNPs is highly dependent on the α-CD-SH/Au. The average AuNP core size increases progressively from 1.4 ± 0.5 nm to 1.7 ± 0.6 nm, and then to 1.9 ± 0.6 nm according to the α-CD-SH/Au 1:1, 2:1 and 3:1, respectively (Figure 3.1). On the other hand, the average AuNP core size decreases progressively from 4.1±0.9 nm to 3.5±0.7 nm, and then to 2.7±0.7 nm according to the α-CD-SH/Au 1:10, 1:5 and 1:3, respectively (Figure 3.2).
Figure 3.1. HRTEM images and core size histograms of α-CD-S-AuNP synthesized with different molar ratios of α-CD-SH/Au: (A) 1:1, (B) 2:1 and (C) 3:1.
Figure 3.2. TEM images and core size histograms of α-CD-S-AuNP synthesized with different molar ratios of α-CD-SH/Au: (A) 1:10, (B) 1:5 and (C) 1:3.
When the $\alpha$-CD-SH/Au is kept $\geq 1$, $\alpha$-CD-S-AuNPs (core size $< 2.0$ nm) are acquired and their size increases with increasing $\alpha$-CD-SH/Au. It is postulated that the increase in particle size is attributed to the inter-hydrogen bond between the $\alpha$-CD-SH molecules at higher concentrations with a concomitant decrease in the availability of free $\alpha$-CD-SH to stabilize the AuNP surface. By contrast, when the $\alpha$-CD-SH/Au is controlled at $< 1$, larger $\alpha$-CD-S-AuNPs (core size $> 2.5$ nm) with typical surface plasmon bands are obtained and the particle size increases with the decrease in $\alpha$-CD-SH/Au. The average chemical compositions of such AuNPs in the empirical formula $\text{Au}_x(\alpha$-CD-S)$_y$ have been further determined by thermogravimetric analysis, mass spectrometry and atomic absorption spectroscopy.

Furthermore, the PL properties of AuNPs have been recognized and applied in biosensors because of their dimensional similarities with biomacromolecules and significant size-dependent optical and electronic properties.$^{89}$ To our knowledge, the PL properties of CD-S-AuNPs and its application in Hg(II) sensing have not been studied. In this work, we firstly observe that these small-sized $\alpha$-CD-S-AuNPs also display strong blue emissions which have potential applications in optical chemo/biosensors. These small-sized $\alpha$-CD-S-AuNPs also exhibit unusual enhancement in PL intensity via the interaction with tetraalkylammonium ion based
on the aggregation-enhanced emission phenomenon. By contrast, it displays PL quenching in the presence of Hg(II). The small-sized α-CD-S-AuNPs has been successfully applied to determine Hg(II) with ultrahigh sensitivity and excellent selectivity.

3.2. Results and Discussion

3.2.1. Photoluminescence Properties of α-CD-S-AuNP

It has been reported that AuNPs possess size-dependent PL behavior and its PL is attributed to transitions between the filled 5d^{10} band and 6sp^{1} conduction band of the Au atom.\textsuperscript{159-162} As such, the PL properties of our small-sized α-CD-S-AuNPs were investigated. All the α-CD-S-AuNPs synthesized with α-CD-SH/Au 1:1, 2:1 and 3:1 in DMSO display strong blue emissions when excited at 400 nm (Figure 3.3).

The possibility of emission arising from adventitious impurity of the reagents or synthesis was carefully scrutinized. None of the solutions including α-CD-SH used in the whole synthetic process exhibited fluorescence emission. Overall, there is firm evidence that the emission originates from α-CD-S-AuNPs itself. Our small-sized α-CD-S-AuNPs display strong blue light emission at 478 nm which is similar to other AuNPs reported in the literature.\textsuperscript{79} In contrast, no observable PL
properties was identified for α-CD-S-AuNPs of larger core size (> 2.0 nm). Among the small-sized α-CD-S-AuNPs studied, the 1.4 nm-sized α-CD-S-AuNP produces stronger PL intensity as a result of the lower density of energy states and electronic states present in the smaller AuNPs, which probably minimize the number of internal nonradiative relaxation pathways. Therefore, the 1.4 nm-sized α-CD-S-AuNP was chosen for further investigation.

Figure 3.3. Photoluminescence spectra of α-CD-S-AuNPs (13.5 µM) synthesized with different molar ratios of α-CD-SH/Au: (a) 1:1, (b) 2:1 and (c) 3:1. The spectra were acquired at an excitation wavelength of 400 nm.
To ensure that the emission band is indeed PL signals from the α-CD-S-AuNP, the emission spectra at various concentrations (7.29–13.5 μM) of the 1.4 nm-sized α-CD-S-AuNP were acquired and displays in Figure 3.4. No observable shift in the emission band is found. In addition, the PL intensity is linearly related to the concentration of α-CD-S-AuNP (inset of Figure 3.4), inferring that α-CD-S-AuNPs can disperse well in solvent. These results further demonstrate the potential of the PL properties of small core size metal NP, especially those smaller than 2.0 nm, for luminescence sensing.
Figure 3.4. PL spectra of various concentrations of 1.4 nm-sized α-CD-S-AuNP: (a) 7.29, (b) 7.49, (c) 7.70, (d) 7.93, (e) 8.17, (f) 8.42, (g) 8.70, (h) 8.99, (i) 9.30, (j) 9.63, (k) 9.98, (l) 10.4, (m) 10.8, (n) 11.2, (o) 11.7, (p) 12.3, (q) 12.9, and (r) 13.5 µM. The inset displays the linear relationship between the PL intensity at excitation/emission wavelengths of 400/478 nm and the concentration of α-CD-S-AuNP.
3.2.2. **Enhancement Effect of Tetraalkylammonium Ion**

Most attention has been focused on the investigation of chromophore-AuNPs composites where AuNPs always play as photoluminescent quencher to quench the molecular excitation energy.\(^{86,89,163}\) However, the aggregation-enhanced emission phenomenon attracts more and more attention in chromophore-AuNPs and other organic NPs in recent years.\(^{81-85,164}\) This phenomenon can be explained by the fact that the energy relaxation processes through non-radiant channels are blocked by the restriction of intramolecular motions in the aggregate state. As a result, the energy relaxes through the radiant pathway and the emission of this kind of compounds is enhanced. In our previous work, we successfully induced the linking of our \(\alpha\)-CD-S-AuNPs by \(\text{Bu}_4\text{N}^+\) ion.\(^{165}\) Figure 3.5 depicts the PL spectra of \(\alpha\)-CD-S-AuNPs as a function of the concentration of \(\text{Bu}_4\text{N}^+\). It is observed that the emission intensity increases successively with the increasing concentration of \(\text{Bu}_4\text{N}^+\). It has been reported that the formation of \(\beta\)-CD-modified AuNPs assemblies is directed by the host-guest interaction of the surface-attached CDs on AuNPs and a triangular shape osmium complex.\(^{166}\)
Figure 3.5. PL enhancement effect of tetrabutylammonium ion on 1.4 nm-sized α-CD-S-AuNP (13.5 µM): (a) 0.00, (b) 2.38, (c) 4.55, (d) 6.52, (e) 8.33, (f) 10.0, (g) 11.5, (h) 12.9, (i) 14.3, (j) 15.5, and (k) 16.7 mM Bu₄N⁺. The inset displays the relationship between the PL enhancement effect at excitation/emission wavelengths of 400/478 nm and the concentration of various tetraalkylammonium ions (Me₄N⁺, Et₄N⁺, Pr₄N⁺, and Bu₄N⁺).
Since each Bu₄N⁺ carries four butyl chains, the butyl chains can enter the hydrophobic cavities of different α-CD-S-AuNPs with a concomitant effect of linking the α-CD-S-AuNPs together. This linkage restricts the intramolecular vibration of α-CD-S-AuNPs, thus minimizing the number of internal non-radiative relaxation pathways. In addition, the PL enhancement of other tetraalkylammonium ions with different carbon chain lengths on the PL of α-CD-S-AuNPs was investigated and depicted in the inset of Figure 3.5. The PL enhancement effect (%) is defined as \( \frac{(I - I_o)}{I_o} \) where \( I_o \) and \( I \) are the PL intensity in the absence and presence of tetraalkylammonium ion, respectively. The enhancement effect follows the trend: Bu₄N⁺ > Pr₄N⁺ > Et₄N⁺ ≈ Me₄N⁺, indicating the possible formation of inclusion complexes between the α-CD-S-AuNPs and the larger enhancement effect for the longer carbon chain length tetraalkylammonium ions. This aggregation-enhanced emission phenomenon is consistent to our previous report and is useful for this type of AuNPs which have potential applications in optical sensing tetraalkylammonium ions.

### 3.2.3. Quenching Effect of Mercury(II) Ion

Mercury is one of the toxic heavy metals and it exists in metallic, inorganic and organic forms. Hg(II) ion, the most stable inorganic form of Hg, is a caustic and
carcinogenic species and possesses high celler toxicity even at low concentrations.167,168 These problems have prompted researchers to develop efficient and sensitive methods for trace analysis of Hg(II) in the environment. Besides conventional methods, nanosensor based on AuNPs86,87,89,163 and semiconductor quantum dots (QDs)169-173 have attracted considerable interest in recent years. Herein, we demonstrate a highly sensitive and selective method for ultra-trace determination of Hg(II) based on PL quenching on small-sized α-CD-S-AuNPs. Figure 3.6 displays the PL emission spectra of α-CD-S-AuNPs as a function of the concentration of Hg(II) from 0.00 to 26.4 nM. The observed PL intensity centered at 478 nm (excitation 400 nm) gradually decreases with increasing concentration of Hg(II) and there is no optical shift of PL emission band. The PL intensity is almost completely quenched at 33.7 nM (Figure 3.7A). The PL quenching of α-CD-S-AuNPs is achieved through facilitating non-radiative electron relaxation process from α-CD-S-AuNPs to Hg(II). This effect can be employed to develop a PL quenching method for the determination of Hg(II).
Figure 3.6. PL of 1.4 nm-sized α-CD-S-AuNP (13.5 µM) in the presence of various concentrations of Hg(II): (a) 0.00, (b) 0.0962, (c) 0.337, (d) 0.524, (e) 0.637, (f) 0.962, (g) 1.84, (h) 2.64, (i) 3.37, (j) 4.04, (k) 4.66, (l) 5.24, (m) 5.77, (n) 6.27, (o) 6.73, (p) 9.62, (q) 13.2, (r) 18.4, (s) 23.3, and (t) 26.4 nM. The PL intensity was monitored at excitation/emission wavelengths of 400/478 nm.

Figure 3.8 displays the Stern-Volmer plot of \( I_o/I \) versus [Hg(II)], where \( I_o \) and \( I \) are the PL intensity in the absence and presence of Hg(II), respectively. The curve displays good linear relationship at a low concentration range (0.0962–10.0 nM) of Hg(II) as shown in inset of Figure 3.8. However, it turns to a step upward curvature at higher Hg(II) concentration (> 10.0 nM), indicating that the quenching of α-CD-S-AuNPs is more effective at higher Hg(II) concentration. Both dynamic and static quenching seem to act together, suggesting a more complex quenching model.
(vide infra). Electron transfer and ion-binding can possibly lead to decrease of PL emission with the former playing an important role.\textsuperscript{170,171}

The limit of detection (LOD) based on the 3$\sigma$ at the blank is 49 pM (9.7 ppt), which is much lower than the maximum level (2.0 ppb) of Hg in drinking water permitted by the US EPA. The relative standard derivation for six replicate measurements of a 3.37 nM Hg(II) solution is 1.29%. Our approach can develop an ultrasensitive $\alpha$-CD-S-AuNP-based nanosensor for the determination of Hg(II).

Moreover, it was observed that the $\alpha$-CD-S-AuNPs solution would change from colorless to light red in the presence of micromolar concentration of Hg(II). The PL emission spectrum of $\alpha$-CD-S-AuNPs is hypsochromic shifted under higher concentration of Hg(II) as depicted in Figure 3.7A. It is possible that dynamic quenching of $\alpha$-CD-S-AuNPs is dominant under low concentration of Hg(II) (< 10 nM). When the concentration of Hg(II) increases, both dynamic and static quenching come to play a dominant role, leading to the upward curvature of the Stern-Volmer plot (Figure 3.8). This conclusion is supported by the UV-vis absorption spectra of $\alpha$-CD-S-AuNPs with Hg(II) as depicted in the inset of Figure 3.7A.
Figure 3.7. (A) Photoluminescence spectra of 1.4 nm-sized α-CD-S-AuNP (13.5 µM) in the presence of various concentrations of Hg(II): (a) 0.00, (b) 9.62 nM (c) 33.7 nM, (d) 23.1 µM, and (e) 44.8 µM. The inset displays the UV-vis absorption spectra of α-CD-S-AuNP in the presence of various concentrations of Hg(II): (a) 0.00, (b) 96.2 pM, (c) 9.62 nM, and (d) 23.1 µM. (B) Photoluminescence spectra of (a) 1.4 nm-sized α-CD-S-AuNP (13.5 µM) in the presence of 44.8 µM Hg(II), (b) α-CD (13.5 µM), and (c) α-CD (13.5 µM) in the presence of 44.8 µM Hg(II). The excitation wavelength is 400 nm.
Figure 3.8. Stern-Volmer plot of \( I_0/I \) against [Hg(II)]. The inset displays the linear relationship of the Stern-Volmer plot at the low concentration range (0.0962–10.0 nM) of Hg(II). \( I_0 \) and \( I \) are the PL intensities of \( \alpha \)-CD-S-AuNP at excitation/emission wavelengths of 400/478 nm in the absence and presence of Hg(II), respectively.

The UV-vis absorption spectrum does not change in the presence of 0.00–10.0 nM Hg(II). By contrast, the absorption increases drastically when there is 23.1 \( \mu \)M Hg(II), possibly attributing to the formation of larger Au-HgNPs, i.e., deposition of Hg(II) onto AuNPs, resulting in an increase of the absorption intensity in UV-vis absorption spectrum of AuNPs. By contrast, \( \alpha \)-CD does not show any photoluminescence regardless of Hg(II) as shown in Figure 3.7B, inferring that the AuNPs indeed play an importance role in determining the photoluminescence properties of \( \alpha \)-CD-S-AuNPs.
Finally, to test the selectivity of our proposed PL α-CD-S-AuNP-based quenching method, the PL emission responses in the presence of various metal ions including Hg$^{2+}$, Ag$^+$, Cu$^{2+}$, Co$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, Mg$^{2+}$, Fe$^{2+}$, and Cd$^{2+}$ at a concentration of 3.37 µM (except Hg$^{2+}$ and Ag$^+$ at 3.37 nM), 1000 times greater than that of Hg$^{2+}$, were studied and plotted in Figure 3.9.

![Graph showing quenching effect of various metal ions on PL intensity](image)

**Figure 3.9.** The quenching effect of various metal ions on 1.4 nm-sized α-CD-S-AuNP (13.5 µM). The concentration of all metal ions except Hg$^{2+}$ and Ag$^+$ (3.37 nM) are 3.37 µM. The PL intensity was monitored at excitation/emission wavelengths of 400/478 nm.

The quenching effect (%) is defined as $\frac{(I_o-I)}{I_o}$ where $I_o$ and $I$ are the PL intensity in the absence and presence of metal ion, respectively. It is evident that high
concentrations of other ions do not produce any noticeable effect on the emission signals of $\alpha$-CD-S-AuNPs as compared to [Hg$^{2+}$]. Hence, our as-prepared $\alpha$-CD-S-AuNPs should show excellent selectivity for ultra-trace determination of Hg(II) in nanomolar range.

3.3. Conclusion

All $\alpha$-CD-S-AuNPs of core diameters smaller than 2.0 nm possess strong PL properties which are in contrast to the larger ones. It is observed for the first time that tetraalkylammonium ions can enhance PL of $\alpha$-CD-S-AuNPs via the formation of a rigid $\alpha$-CD-S-AuNPs network. Since traalkylammonium ion is able to form inclusion complexes with $\alpha$-CD-S-AuNPs in the hydrophobic cavities of the surface-attached thiolated $\alpha$-CD, it can induce the inter-linkage of $\alpha$-CD-S-AuNPs. Finally, it was determined that the 1.4 nm-sized $\alpha$-CD-S-AuNPs can be applied for PL sensing Hg(II). The sensitivity of $\alpha$-CD-S-AuNPs to Hg(II) is very high and the LOD can be down to 49 pM. It also possesses remarkable selectivity over other metal ions. Our non-toxic $\alpha$-CD-S-AuNPs is especially beneficial as compared to the commonly used semiconductor QDs that contain toxic metals. This important feature will pave the way for ultrasensitive detection of Hg(II) using $\alpha$-CD-S-AuNP.
Chapter 4 Role of UHPLC in evaluating as-synthesised monolayer-protected gold nanoparticles products

1. Introduction

An attractive aspect of recent development is that AuNPs of 1–2 nm are somewhat approaching molecule-like and they possess interesting and size-dependent properties including optical absorption,\textsuperscript{174,175} quantized electrical charging\textsuperscript{176} and catalytic activity\textsuperscript{177-179}. In the previous chapter, we observe that the photoluminescence property of the as-prepared AuNPs is highly dependent on the core size of $\alpha$-CD-S-AuNPs. Thus, recent techniques on synthesizing high-quality monodisperse NPs have attracted considerable attention.\textsuperscript{7} When developing a new synthetic protocol, it would be ideal if the final NP products can be characterized quickly in terms of particle sizes so that the synthetic conditions could be optimized.

While transmission electron microscopy (TEM) is generally employed to characterise the size of NPs,\textsuperscript{60,63,105,106,180-183} it is an expensive and time-consuming technique and no separation processes are involved. In addition, dynamic light scattering is commonly applied to characterise the size of NPs. Although it is a simple method, it cannot determine accurately particle size smaller than 10 nm. Other disadvantages are that this technique only assesses the hydrated size of NPs
and again it does not involve any size separation NPs mixtures. Thus, there is also a high demand for fast and efficient methodology for separation and purification of polydisperse NPs products.

Up till now, many researchers have developed a variety of approaches to achieve advanced particles separation and/or to narrow down the size distribution of polydisperse AuNPs samples. Among them, high-performance liquid chromatography (HPLC) has been one of the most important techniques for separation of polydisperse AuNPs. Although polydisperse AuNPs can be successfully studied by HPLC with C18 bonded silica columns, the total elution time is still relatively long (ca. 60–120 min) and the separation efficiency is moderate. Since the development of ultra high-performance liquid chromatography (UHPLC), it is nowadays feasible to perform faster and more efficient separation of complex mixtures. UHPLC has rapidly emerged as an efficient and powerful separation tool in a wide range of fields, from peptide analyses to drug impurity profiling. This technology provides better resolution, higher separation efficiency, higher sensitivity and faster analysis. To our knowledge, UHPLC has not been applied to separate and analyse AuNPs.

In this work, we report for the first time the UHPLC separation of as-synthesised polydisperse N-acetyl-L-cysteine-protected-AuNPs (NAC-AuNPs) products and
other ultrasmall ligand-protected AuNPs. The results demonstrate that UHPLC indeed provides very fast and efficient separation of AuNPs as compared to HPLC. In conjunction with a photodiode array (PDA) detector, the optical spectra of the separated NPs can be visualised and are found to be size-dependent. Moreover, our proposed UHPLC methodology can be applied to study the effect of synthetic conditions on the quality of as-synthesised AuNPs products in terms of size and dispersity. Particular focus is emphasised on comparing the one-phase with the two-phase Brust-Schiffrin (B-S) syntheses. Remarkably, the two-phase synthetic procedure produces more monodisperse AuNPs products with a higher proportion of small AuNPs in comparison to the one-phase B-S method. Our proposed UHPLC methodology provides a fast route for better understanding of an as-synthesised AuNPs products.

4.2. Results and Discussion

4.2.1. Comparison of HPLC with UHPLC Analysis

Initially, a NAC-AuNPs sample was prepared by the one-phase B-S method and then analysed by HPLC-PDA. Figure 4.1A depicts the HPLC chromatogram of this NAC-AuNPs sample separated on a C18 bonded silica column using a mobile phase of 50 mM Bu₄N⁺F⁻ in water and MeOH and the signal was monitored by the PDA at
250 nm. The chromatogram shows at least 12 separated main peaks and no observable peaks were eluted after 120 min of elution (not shown). Bu₄N⁺F⁻ is added into the mobile phase as an ion-paring reagent. The NAC on the Au cluster surface presents as the carboxylate (−COO⁻) functionality which can form an ion-pair with Bu₄N⁺, and thus there is a partition-like intermolecular interaction between the NAC-AuNPs and the C18 bonded silica stationary phase. As a result, NAC-AuNPs can be retained on a C18 bonded silica column.¹⁰⁶

To date, UV-vis absorption spectroscopy is a useful technique for preliminary assessing the core size of AuNPs. Small core Au clusters exhibit a strong UV absorption feature which decays exponentially into the visible region with a superimposed broad band (surface plasmon resonance, SPR) at ~500 nm.¹⁸⁵ The SPR band decreases and is hypsochromic-shifted with the decrease in particle size.¹⁸⁶ As such, the optical properties of Au clusters have been the hot topic of investigation.¹⁸⁷,¹⁸⁸ In addition, since PDA does not alter the sample and can quickly register the spectrum of the solute, it has been widely used as an HPLC detector. Thus, the combination of a spectroscopic technique with chromatographic separation system can be a rapid method to obtain online structural information and is very useful for peak identification or confirmation.
Figure 4.1. (A) Chromatograms (a) and (b) are HPLC and UHPLC separations of a 3.00 mg/mL polydisperse AuNPs product synthesised by the one-phase Brust-Schiffrin method. The inset displays the enlarged view of the UHPLC chromatogram. The peak numbers represent various NAC-AuNPs species. Absorbance chromatograms are detected at 250 nm and offset for clarity. (B) and (C) are the absorption spectra (bottom to top) of Peaks 1–12 shown in chromatograms (a) and (b), respectively. Absorbances are normalised at 250 nm and spectra are offset for clarity.
Herein, UV-vis absorption spectra are utilised to give an evaluation of the core size of the separated AuNPs. The UV-vis absorption spectra of the peaks identified in the chromatogram (Figure 4.1A(a)) are displayed in Figure 4.1B. The absorption spectral feature of the separated NAC-AuNPs is in good agreement with the literature. As a result, we can roughly assign the NAC-AuNPs Peaks 1–8 in chromatogram a (Figure 4.1A). The elution order of AuNPs is from small to large core size. Peaks 1 and 2 are very similar and are the very small AuNPs (Au_{12}). Peak 3 is Au_{13} whereas Peaks 4 and 5 correspond to Au_{15}. For Peak 6, it is Au_{18}. Peaks 7 and 8 are Au_{22}. These peaks were collected as Fractions 1–8 and subjected to TEM analysis without success. It is possible that the amounts of AuNPs in the fractions are too small. Fortunately, these peaks can be further identified and confirmed by MALDI-TOF MS analysis (Figure 4.2). Although we have successfully applied HPLC to separate the polydisperse NAC-AuNPs product, the analysis time is far too long (~2 h) and the resolution of some peaks is still not satisfactory. Hence, UHPLC is subsequently applied to improve the resolution and speed up the analysis.
Figure 4.2. MALDI-TOF mass spectra of Fractions 1–8 (A-H) in Figure 4.1A(a).
UHPLC empowers the use of small particle (1.7 µm) and narrower column to improve chromatographic separation efficiency. Columns with smaller diameters operated at higher linear flow rates can speed up the elution and reduce solvent consumption. Band broadening can also be reduced as the volumes of mixer, tubing, injector and flow cell are smaller. This is especially valuable when sample supply is limited because less sample injection is required. As such, we for the first time attempt to employ UHPLC for separation of polydisperse AuNPs. Chromatogram in Figure 4.1A shows the UHPLC separation of the NAC-AuNPs sample (prepared by the one-phase B-S method) monitored by the PDA detector at 250 nm. The inset displays the enlarged view of the UHPLC chromatogram. Obviously, the chromatographic separation is shortened by about 10 times (~12 min) as compared to the HPLC method (~120 min) at the same flow rate of 0.40 mL/min. The UV-vis absorption spectra of the separated AuNPs peaks (Figure 4.1C) possess more or less the same spectral characteristics to that of the peaks in HPLC chromatogram, inferring that the elution order of these AuNPs is the same. In addition, the resolution is remarkably improved. For instance, Peaks 1 and 2 are unresolved by HPLC but are completely separated by UHPLC. For larger size NAC-AuNPs (Peaks 8–12), they are better resolved by UHPLC. Similarly, faster and better chromatographic separations of other ligand-protected gold nanoparticles
as-synthesised products such as histidine-protected gold nanoparticles (Figure 4.3) and $N,N'$-dimethylformamide-protected gold nanoparticles (Figure 4.4) are obtained by UHPLC as compared to HPLC. Solvent consumption is also greatly reduced as a result of faster elution. In short, UHPLC is a powerful method to improve the separation performance of AuNPs in liquid chromatography with enhancing resolution, sensitivity and speed.
Figure 4.3. Chromatograms (a) and (b) are HPLC and UHPLC separations of a 15.00 mg/mL His-AuNPs sample. The inset displays the enlarged view of the UHPLC chromatogram. The mobile phase consisted of 10 mM pH 5.0 ammonium acetate in water (eluent I, pH adjusted by acetic acid) and MeOH (eluent II). For HPLC separation, the injection volume was 20.0 μL. A gradient elution program was used at 0.70 mL/min and as follows: 100% I for 5.5 min, linearly decreased to 90 % I from 5.5 to 6.5 min, maintained at 90 % I from 6.5 to 9 min, linearly decreased to 80% I from 9.0 to 10 min, and kept at 80% I from 10 to 12 min, linearly decreased to 60% I from 12 to 13 min, and kept at 60% I from 13 to 16 min. For UHPLC separation, the injection volume was 2.0 μL. A gradient elution program was used at 0.70 mL/min and as follows: 100% I for 0.33 min, linearly decreased to 90 % I from 0.33 to 0.45 min, maintained at 90 % I from 0.45 to 0.73 min, linearly decreased to 80% I from 0.73 to 0.84 min, and kept at 80% I from 0.84 to 1.07 min, linearly decreased to 60% I from 1.07 to 1.18 min, and kept at 60% I from 1.18 to 3.00 min. The absorbance chromatograms were monitored at 250 nm and offset for clarity.
Figure 4.4. Chromatograms (a) and (b) are HPLC and UHPLC separations of a 1.00 mg/mL DMF-AuNPs sample. The inset displays the enlarged view of the UHPLC chromatogram. The mobile phase consisted of water (eluent I) and MeOH (eluent II). For HPLC separations, the injection volume was 10.0 μL. A gradient elution program was used at 0.40 mL/min and as follows: 97% I for 11 min, linearly decreased to 90 % I from 11 to 15 min, maintained at 90 % I from 15 to 20 min, linearly decreased to 80% I from 20 to 25 min, and kept at 80% I from 25 to 60 min. For UHPLC separation, the injection volume was 1.0 μL. A gradient elution program was used at 0.40 mL/min and as follows: 97% I for 0.74 min, linearly decreased to 90 % I from 0.74 to 1.19 min, maintained at 90 % I from 1.19 to 1.76 min, linearly decreased to 80% I from 1.76 to 2.32 min, and kept at 80% I from 2.32 to 7.00 min. The absorbance chromatograms were monitored at 250 nm and offset for clarity.
4.2.2. Evaluating the Size Distribution of NAC-AuNPs Synthesised from Different B-S Methods

Understanding the effect of synthetic conditions on the growth of AuNPs is extremely important since different syntheses will affect the qualities of AuNPs in terms of core size, dispersity and number of protecting ligands. So far much effort has been directed to improve NPs core uniformity and monodispersity. Different synthesis methods have their own unique virtues and detriments. Chromatographic techniques offer powerful and useful possibilities. In order to verify the potential application of our proposed UHPLC method, it was applied to study the NAC-AuNPs products prepared by the two synthetic procedures, i.e., one-phase and two-phase B-S methods. The two-phase B-S method is the earliest phase transfer approach for preparing thiol-stabilised metal NPs. After its first publication, a large number of related approaches have been reported that large quantities of relatively monodisperse small-sized NPs were produced. Herein, we employ this two-phase method to synthesise NAC-AuNPs and analyse the as-synthesised NAC-AuNPs product by HPLC and UHPLC.
Figure 4.5. (A) Chromatograms (a) and (b) are HPLC and UHPLC separations of a 3.00 mg/mL polydisperse AuNPs product synthesised by the two-phase Brust-Schiffrin method. The inset displays the enlarged view of the UHPLC chromatogram. The peak numbers represent various NAC-AuNPs species. The absorbance chromatograms are monitored at 250 nm and offset for clarity. (B) and (C) are the absorption spectra (bottom to top) of Peaks 1–9 shown in chromatograms (a) and (b), respectively. Absorbances are normalised at 250 nm and spectra are offset for clarity.
Figure 4.5A displays a co-plot of the HPLC (curve \(a\)) and UHPLC (curve \(b\)) chromatograms from NAC-AuNPs samples synthesised with the B-S two-phase method and monitored by the PDA at 250 nm. Surprisingly, fewer peaks are identified in the chromatogram of the NAC-AuNPs synthesised with the B-S two-phase method than that of the B-S one-phase method (Figure 4.1A), indicating that the AuNPs product synthesised with the B-S two-phase method is relatively more monodisperse. Again, UHPLC provides better separation efficiency and faster elution than that of HPLC. All the major solutes in UHPLC are eluted within 8.0 min and the elution order is from small to large core size of AuNPs. In addition, the majority of these AuNPs are of smaller size. The difference in the dispersity and size of AuNPs produced by the one-phase and two-phase B-S methods can be explained by their different gold precursor species.\textsuperscript{147,191-194} The precursor species of these reactions have been identified. Tetraalkylammonium metal complexes are the precursors of the two-phase reaction, while metal(I) thiolate polymers are the precursors of the one-phase condition. Polar solvent systems favour the formation of metal(I) thiolate polymer.\textsuperscript{147,192} In here, the one-phase B-S reaction conducted in MeOH produces Au(I) thiolate polymer as the main precursor which is insoluble in common solvents, attributing to the intermolecular interactions between the adjacent polymeric units which generates the states of aggregation. The heterogeneous
reaction of the insoluble Au(I) thiolate polymer with the reductant can be very slow. It is reasonable to expect that this factor will produce poor synthetic outcomes.\textsuperscript{52,195-197} This is why the one-phase B-S method results in AuNPs products with higher dispersity and larger core sizes.

In order to verify that the as-synthesised NAC-AuNPs are of smaller sizes, UV-vis absorption spectroscopy was employed. The UV-vis absorption spectra of the peaks in the chromatogram (curve \textit{a} in Figure 4.5A) are in-situ acquired by the PDA and shown in Figure 4.5B. The absorption spectra for Peaks 2–9 show typical spectral characteristics of AuNPs except for the earliest eluting Peak 1. Compared with the absorption spectra of NAC-AuNPs in Figure 4.1B, Peaks 2–4 are very similar and are Au\textsubscript{12}. Peaks 6 and 7 correspond to Au\textsubscript{13} and Au\textsubscript{15}, respectively. Peak 7 is Au\textsubscript{22}. The UV spectrum of Peak 1 is very different from the other peaks. Peak 1 was collected as Fraction 1 from HPLC for further MS analysis. It was purified by SPE to remove the mobile phase matrix and the clean sample was subjected to MALDI-TOF MS analysis.
Figure 4.6 displays the mass spectrum of Fraction 1. The highest mass indicates the presence of $\text{Au}_{10}\text{(NAC)}_{10}$ in Fraction 1. This is in good agreement with another cyclic $\text{Au}_{10}$-ligand$_{10}$ polymer that has the shortest absorption band at the region of $< 300$ nm and our previous work.\textsuperscript{106}

Our UHPLC separation of NAC-AuNPs sample synthesised with the B-S two-phase method is displayed in curve $b$ of Figure 4.5A and the inset displays the enlarged view of the chromatogram. Strikingly, the elution trend and pattern of the chromatogram show good similarity to that of HPLC, except that Peaks 5 and 8 are observed in UHPLC but not in HPLC. It is reasonable to conclude that Peak 4 in
HPLC is better separated and evolved into Peaks 4 and 5 in UHPLC. In addition, it is easier to observe Peak 8 in UHPLC since it provides higher detection sensitivity. These results indicate that UHPLC has much better resolving power, faster elution and higher sensitivity than HPLC. The UV-vis absorption spectra of the peaks for UHPLC separation are depicted in Figure 4.5C. Their absorption spectra show good agreement with that of HPLC separation.

4.3. Conclusion

In summary, we have, for the first time, adopted a reversed-phase UHPLC methodology to separate and study polydisperse NAC-AuNPs synthesised via the one-phase and two-phase B-S methods, demonstrating that these two synthesis methods indeed produce different AuNPs products in terms of quality, quantity and size distribution. More importantly, it is anticipated that the proposed UHPLC methodology can be applied to investigate AuNPs protected by many other water-soluble ligands with an extremely fast, sensitive and environmental friendly approach. It can also be a useful analytical tool to study the synthesis conditions in relation to the quality of the NPs product so that metal NPs of the desired core size or dispersity can be harvested at specific experimental conditions.
Chapter 5 Capillary Electrophoretic Study of Cationic Gold

Sub-nanoclusters Protected by $N,N'$-dimethylformamide

5.1 Introduction

Sub-nanometer-sized metal clusters (NC) (< 2 nm) consisting of only several tens of atoms have recently attracted considerable attention in many areas of research including bioscience, chemistry, material science, and physics owning to their unique electronic structures and the subsequent unusual physical and chemical properties. The electronic properties of such NC transit from a bulk-like continuum electronic states to molecule-like discrete electronic orbital levels in the absorption and fluorescence features. As a result, they are distinct and different from the bulk materials or much larger nanoparticles (NP). However, the minute size of the NC brings difficulty in their synthesis. To date, various methods for the preparation of NC in the presence of stabilizing agents have been reported. Among various NC, gold NC (AuNC), especially the thiolated monolayer protected clusters, have been extensively investigated in the past few decades because of the relative ease of manipulating the monolayer chemistry. Previous works have demonstrated that alkanethiols, tiopronin, $N$-acetyl-L-cysteine, homocysteine, and many other organic molecules can be used to passivate AuNC.
Although all of these methods for preparing fluorescent AuNC are good control over cluster sizes and stabilities, the impurities are introduced by the use of surfactants. Therefore, the design and synthesis of surfactant-free but still stable NC with well-defined surface properties is highly worthwhile and ongoing challenge. Recently a surfactant-free solution synthesis of silver, gold, platinum, palladium NC by \(N,N'\)-dimethylformamide (DMF) reduction has been reported. In this reaction, no further stabilizing surfactant is needed. DMF is expected to be a weak reducing agent as well as stabilizing ligand. Using the surfactant-free reduction method, highly fluorescent AuNC protected by DMF (DMF-AuNC) was successfully synthesized. Moreover, such AuNC provides a good platform for further functionalization with various capping ligands including thiocystic acid, 1-dodecanethiol, dodecylamine, thiocholine bromide, 11-mercaptoundecanoic acid, polyvinylpyrrolidone, triphenylphosphine to show tunable optical behavior, which have great potential for use in sensing platforms and novel biomarkers. However, disadvantages remain that the final product of DMF-NC has to be purified by removing the larger gold nanoparticles (AuNP) as a byproduct via centrifugation and passing through a silica gel column. Later on, this synthesis was improved by Kawasaki et al. In their method, DMF-AuNC was obtained without the formation of AuNP and bulk metals using a hot injection process for the
homogeneous reduction. In addition, the as-prepared DMF-AuNC in solution was found to have high thermal stability, dispersion stability in various solvents and photochemical stability in spite of the absence of ligands such as thiolate compounds. Regrettably, the resulting products were confirmed to be a mixture of various-sized AuNC with a cluster number less than 20 including at least Au$_8$ and Au$_{13}$ by their photoluminescence (PL) spectra of as-synthesized DMF-AuNC. But the PL studies of as-synthesized DMF-AuNC only represent the summation or average properties of all DMF-AuNC species in the mixture sample. In fact, size is a crucial factor that determines their chemical and physical properties. As such, there is high demand for fast and efficient technology for separation and purification of polydisperse NC products.

So far, a variety of separation techniques have been developed to narrow down the size distribution of polydisperse AuNP/AuNC products by our research group including reverse-phase high-performance liquid chromatography (RP-HPLC), capillary electrophoresis (CE), and size-selective precipitation. Among them, RP-HPLC has been successfully demonstrated to fractionate the as-synthesized DMF-AuNC. The fractions were collected and further characterized precisely by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Unfortunately, RP-HPLC is unable to
separate and analyze charged NC; thus, other efficient analytical separation methodology is necessitated. CE is considered as one of the most powerful and efficient separation approaches for separating ions in solution according to their charge-to-size ratio. In CE system, a single sample can be rapidly analyzed using a volume as low as several nanoliters with high efficiency and resolution. Therefore, it has been used to separate a variety of different sized AuNP.$^{59,60-63,111,165}$

Herein, we employ CE to separate cationic DMF-AuNC. In theory, the cationic species are eluted before the neutral species, and then lastly is the anionic species under the cathodic flow of CE. The decrease in the migration time of analyzed species will result in decreasing the separation performance. In order to enhance the separation of cationic species, an additive would be introduced into the run buffer. Over the past two decades, the use of sodium dodecyl sulfate (SDS) as an additive in separation buffers is an established practice that allows significant advances in the separation of AuNP.$^{61-63,111-113}$ Liu et al.$^{61}$ illustrated that using a run electrolyte containing SDS can enhance the separation of AuNP by CE. Adding SDS to the run buffer allows the charged surfactant to associate onto the surface of the AuNP and cause a change in the charge-to-size ratio of AuNP, which is a function of the surface area of AuNP and the concentration of surfactant in run electrolyte. At high concentration of SDS in the run electrolyte in which the surface of AuNP is fully
occupied with SDS, a linear relationship exists between the electrophoretic mobility ($\mu_e$) and AuNP ranging from 5.3 to 59.0 nm. $^{61-63}$ Although adding SDS surfactant is useful to the separating AuNP in CE, it has never been used for enhancing the separation of AuNC ($< 2$ nm).

The purpose of this work is to investigate the water-soluble cationic portion of DMF-AuNC which is most commonly neglected. The as-synthesized AuNC product was cleansed up by a solid-phase extraction (SPE) cartridge based on a hydrophilic-lipophilic balanced sorbent and the charged DMF-AuNC was subsequently analyzed by CE. In order to optimize the CE conditions, parameters including run buffer concentration, pH, and ethanol (EtOH) were studied in detail. In addition, the effect of SDS in the run electrolyte on the electrophoretic separation of AuNC was assessed. To our knowledge, this is the first report to successfully employ SDS to enhance the separation capacity of CE for cationic DMF-AuNC.
5.2 Results and Discussion

5.2.1. SPE Clean-up of As-synthesized DMF-AuNC Sample

Our previous work has successfully applied RP-HPLC to separate the neutral DMF-AuNC species in an as-synthesized AuNC sample. Unfortunately, the first eluted peak, which was speculated to contain the positively charged AuNC species, was not retained or separated on the C4 bonded silica column. As such, CE will be an ideal analytical separation technique to study these positively charged AuNC species. SPE is a potent sample preparation technique to remove the undesired impurities as well as preconcentrate the positively charged AuNC species. The method is that the sample passing through the stationary phase of SPE is collected if it contains the desired analytes. Otherwise, they can be removed from the stationary phase for collection by rinsing with an appropriate eluent if the sample retained on the stationary phase includes the desired analytes. Herein, we utilize the SPE cartridge containing a hydrophilic-lipophilic balanced sorbent to remove the neutral DMF-AuNC species from the as-synthesized AuNC sample while the positively charged DMF-AuNC species in the water eluent was collected for CE analysis. Moreover, the neutral DMF-AuNC species adsorbed on the SPE cartridge were subsequently eluted by MeOH and subjected to CE analysis.
Figure 5.1. Electropherograms of DMF-AuNC: (a) eluted with DDI water and (b) MeOH in SPE HLB cartridge. The run buffer is 30 mM phosphate (pH 7.0). Electropherograms are offset for clarity and ease of comparison.
Figure 5.1 displays the electropherograms of DMF-AuNC eluted with DDI water and MeOH in the SPE cartridge, respectively. Electropherograms are offset for clarity and ease of comparison. The electropherogram in Figure 5.1a confirms that positively charged DMF-AuNC species are migrated out first and follow by the neutral marker (DMF) at the later migration time. More detailed discussion on $\mu_e$ of AuNC will be in the following sections (*vide infra*). By contrast, only one single peak is found in the electropherogram of MeOH eluent in Figure 5.1b and its migration time is the same as the neutral marker DMF. In essence, the electropherograms suggest that an as-synthesized DMF-AuNC product eluted with water and MeOH in the SPE HBL cartridge comprise positively charged and neutral DMF-AuNC species, respectively.

The UV-visible absorption and PL spectra of the positively charged and neutral DMF-AuNC are co-plotted in Figure 5.2. The absorption spectra are normalized at 220 nm to remove the effects of concentration differences to allow focus on spectral shape and band position. In general, the absorption spectra of both types of DMF-AuNC decay in approximately an exponential fashion into the visible region with the absence of the typical surface plasmon resonance band at 520 nm, indicating that they have core dimensions $< 2$ nm. Most literatures suggest that smaller core AuNP possesses a sharper
decrease in absorbance compared to that of the larger core ones from shorter to longer wavelengths. Moreover, larger core AuNP has relatively higher absorbance than that of the small core AuNP in the visible light region. In brief, their absorption spectra show that the positively charged DMF-AuNC has smaller core size than that of the neutral ones. In addition, the positively charged DMF-AuNC possesses a shoulder absorption peak at ca. 275 nm which is consistent with our earlier work.\textsuperscript{149} The PL spectra of the positively charged (Figure 5.2c) and neutral (Figure 5.2d) DMF-AuNC also demonstrate that the positively charged DMF-AuNC has smaller core size than that of the neutral DMF-AuNC, owning to the blue shift in the PL spectrum. The emission peaks of the positively charged and neutral DMF-AuNC are at 420 and 475 nm respectively under an excitation wavelength of 329 nm, which are consistent to the emission and shoulder peaks of another as-synthesized DMF-AuNC sample.\textsuperscript{214}
Figure 5.2. UV-visible absorption spectra of DMF-AuNC: (a) eluted with DDI water and (b) MeOH in SPE cartridge. The spectra are normalized at 220 nm for ease of comparison. Photoluminescence spectra of DMF-AuNC: (c) eluted with DDI water and (d) MeOH in SPE cartridge. The spectra were acquired at an excitation wavelength of 329 nm.
Figure 5.3 shows the MALDI-TOF MS of the cationic DMF-AuNC and the inset displays the higher \( m/z \) range 2250–2720 Da. The mass peaks are assigned to \( \text{Au}_x(\text{DMF})_y \), where \( x \) and \( y \) denote the numbers of Au atom and intact DMF ligand, respectively. The number next to the mass peaks are the \( x \) and \( y \) values. The possible largest clusters are computed as \([\text{Au}_{10}\text{DMF}_7]^+\) and \([\text{Au}_{10}\text{DMF}_9]^+\), representing the largest cationic NC species in the fraction of DMF-AuNC eluted with DDI water.

On the other hand, these cationic DMF-AuNC species were analyzed by IC using a strong cation-exchange column and the chromatogram is depicted in Figure 5.4a. For comparison, a standard mixture of Na\(^+\) (5.0 ppm) and K\(^+\) (5.0 ppm) was also analyzed by the same column (Figure 5.4b). Two peaks are found and they have retention times close to Na\(^+\), deducing that they are \([\text{Au}_{10}\text{DMF}_7]^+\) and \([\text{Au}_{10}\text{DMF}_9]^+\).

In summary, our SPE HLB cartridge is very effective in purifying the as-synthesized AuNC sample to allow the collection of the cationic AuNC species.
Figure 5.3. MALDI-TOF mass spectrum of cationic DMF-AuNC. The mass peaks are assigned to $\text{Au}_x(\text{DMF})_y$, where $x$ and $y$ denote the numbers of Au atom and intact DMF ligand, respectively. The number next to the mass peaks are the $x$ and $y$ values. The inset displays the higher $m/z$ range 2250–2720 Da and the red dash line indicates the average signal levels.
Figure 5.4. Ion exchange chromatograms of (a) cationic DMF-AuNC and (b) standard mixture of 5.0 ppm sodium and potassium ions at a flow rate of 1.0 mL/min.
5.2.2. Effect of Buffer Concentration on Electrophoretic Mobility of Cationic DMF-AuNC

As mentioned above, since the cationic DMF-AuNC species are positively charged, the electrophoretic method should be a good technique for analyzing them. Figure 5.5A displays the electropherograms of separation of the positively charged DMF-AuNC under different buffer concentrations at pH 7.0. Electropherograms are offset for clarity and ease of comparison. Before addressing the experimental conditions, it is vital to understand the migration order of AuNC in CE so that any change in the $\mu_e$ of cationic DMF-AuNC under various experimental conditions can be interpreted. Three major migration peaks in all electropherograms are designated into Peak 1, 2 and 3, respectively. The $\mu_e$ of cationic DMF-AuNC can be calculated from:

$$\mu_e = \left( \frac{IL}{V} \right) \times \left[ \frac{1}{t} - \frac{1}{t_o} \right]$$

where $l$ is the distance between the inlet and the detector, $L$ is the total length of the capillary, $V$ is the applied voltage, and $t_o$ & $t$ are the migration times of neutral marker and cationic DMF-AuNC, respectively.\textsuperscript{218,219} DMF which is uncharged species was employed to be the neutral marker. Neutral species are not influenced by electrophoretic mobilities, and therefore move through the capillary at the same rate as the EOF. It is observed that the migration time of Peak 3 is exactly the same with that of pure DMF (not shown) under the same experimental conditions. Peak 3 is a neutral species and found to be the residual DMF from the synthesis. As such, Peak 3 can be viewed as an internal neutral
marker in this work. The migration peaks before the EOF marker are the cationic DMF-AuNC species as the CE is under the cathodic flow. Cationic DMF-AuNC solutes reaching the detector before the neutral marker DMF indicates the apparent mobilities (= EOF + $\mu_e$) of cationic DMF-AuNC in the capillary are faster than that of DMF (EOF). The EOF (Peak 3) and $\mu_e$ of Peak 1 and 2 are calculated and shown in Figure 5.5B. Since the EOF is larger than $\mu_e$ of Peak 1 and 2, all solutes are swept along with the buffer solution to the cathode. The positive value of $\mu_e$ indicates that there is an electrostatic attraction force between the cationic DMF-AuNC and the cathode. It is reasonable since cationic DMF-AuNC is positively charged.
Figure 5.5. (A) Effect of buffer concentration on the electrophoretic separation of cationic DMF-AuNC. The run buffer is at pH 7.0 under various concentrations. Electropherograms are offset for clarity and ease of comparison. (B) Plot of EOF and electrophoretic mobility ($\mu_e$) of cationic DMF-AuNC against concentration of run buffer.
It has been reported that the concentration of the run buffer plays an important role in the separation performance of AuNP.\textsuperscript{60} When the capillary temperature is kept constant, increasing the buffer concentration (ionic strength) decreases the EOF as shown in Figure 5.5B because it lowers the zeta potential. By contrast, increasing the buffer concentration does not affect too much the $\mu_e$ of cationic DMF-AuNC. There are only slight variations in $\mu_e$. In addition, the difference in $\mu_e$ between the cationic DMF-AuNC species (Peak 1 and 2) does not change significantly with the change of buffer concentration. In brief, the resolution of cationic DMF-AuNC is slightly enhanced with the longer separation time even though the buffer concentration increases to 50 mM. As a result, 30 mM phosphate run buffer is chosen as the optimal buffer concentration for separation of the cationic DMF-AuNC species in order to reduce the separation time and cost.

5.2.3. Effect of Buffer pH on Electrophoretic Mobility of Cationic DMF-AuNC

Figure 5.6 depicts the electropherograms of the separation of cationic DMF-AuNC using 30 mM phosphate run buffer at various pHs. In all electropherograms, Peak 3 is DMF (as the neutral marker) and the migration peaks before DMF are again cationic DMF-AuNC. It is well established that the buffer pH has a noteworthy effect on the separation of AuNP.\textsuperscript{60,113} EOF increases with the increase in pH
primarily because at higher pH, there is more dissociation of silanol group (Si–OH) to Si–O\(^-\) on the inner capillary wall. The zeta potential is proportional to the surface charge on the capillary wall. The pH of the buffer will also influence the degree of ionization of the solutes and, hence, their \(\mu_e\). Nevertheless, the charge of DMF-AuNC is contributed by the Au core rather than the ionization of the protective ligands (DMF). The effect of buffer pH on the \(\mu_e\) of DMF-AuNC is negligible (not shown).

At high pH (i.e., 9.3), the silanol groups of the capillary are fully ionized to generate a strong zeta potential and dense electrical double layer; consequently, increasing the EOF. Regrettably, the increase in EOF results in less time for the cationic DMF-AuNC solutes to perform the separation in the capillary. Peak 1 and 2 are overlapped at high pH. As the pH decreases to 7.0, there is less surface ionization and a lower zeta potential. The lower EOF allows more time for resolving cationic DMF-AuNC species. The pH drops from 7.0 to 4.3 leading to a further decrease in EOF. A dramatic decrease in EOF results in serious peak broadening; thus, reducing the resolution. In essence, 7.0 is chosen as the optimal pH for separation of the cationic DMF-AuNC.
Figure 5.6. Effect of pH on the electrophoretic separation of cationic DMF-AuNC. The run buffer is 30 mM phosphate at various pHs. Electropherograms are offset for clarity and ease of comparison.
5.2.4. Effect of EtOH on Electrophoretic Mobility of Cationic DMF-AuNC

Organic solvents are favorably applied to enhance the separation selectivity of CE by influencing several variables including viscosity, dielectric constant and zeta potential, and the $\mu_e$ of the solute and EOF.$^{60,220,221}$ In addition, the effect of adding organic solvent to the buffer depends on which and how much solvent is added. In our previous work$^{60}$, MeOH, EtOH and 1-propanol were added to the run buffer to enhance the AuNP separation in CE. However, the extent of their effect on improving the separation is quite different. Since EtOH has a more profound effect on separating AuNP, the effect of EtOH in the run buffer on the separation of cationic DMF-AuNC was investigated in this work.

Figure 5.7A presents the separation of cationic DMF-AuNC at different volume percentages of EtOH in 30 mM sodium phosphate (pH 7.0). The addition of EtOH in the run buffer influences not only the EOF but also the $\mu_e$ of the cationic DMF-AuNC. Figure 5.7B displays the relationship between the EOF and $\mu_e$ of the cationic DMF-AuNC and $v/v$ % EtOH. It is found that the increase in $v/v$ % EtOH is associated with the decrease in EOF. The decrease in EOF is not only due to the increase in run buffer viscosity accordingly but also altering the zeta potential on the capillary walls.$^{220,221}$ The separation window increases with the increase in $v/v$ % EtOH in the run buffer. The $\mu_e$ of the cationic DMF-AuNC (Peak 1 and 2) decrease
with increase in v/v % EtOH shown in Figure 5.7B. Peak 1 and 2 are better resolved when 20% EtOH is used. The enhancement of separation could be explained by the solvation. Solvation is a common way of controlling selectivity through changes in the hydration volume which in turn alter the charge-to-mass ratio of the solute.\textsuperscript{221}. The $\mu_e$ of the cationic DMF-AuNC is related to its charge-to-mass ratio in the run buffer. Hence, the addition of EtOH affects the hydration of cationic DMF-AuNC. In other words, the EtOH alters the double layer thickness of a cationic DMF-AuNC and thus, the charge-to-mass ratio of the cationic DMF-AuNC.
Figure 5.7. (A) Effect of EtOH on the electrophoretic separation of cationic DMF-AuNC. The run buffer is 30 mM phosphate (pH 7.0) under different v/v % EtOH. Electropherograms are offset for clarity and ease of comparison. (B) Plot of EOF and electrophoretic mobility ($\mu_e$) of cationic DMF-AuNC against v/v % EtOH.
5.2.5. Effect of SDS on Electrophoretic Mobility of Cationic DMF-AuNC

Surfactants are molecules that have a hydrophilic, water soluble moiety on one end of the molecule and a hydrophobic, water insoluble moiety on the other. SDS \([\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}^+]\) is an example of a widely used anionic surfactant. On one end of \(\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3^-\) is the hydrophilic \(\text{SO}_3^-\) group, and on the other end is the hydrophobic \(\text{CH}_3(\text{CH}_2)_{11}\) group. Recently, SDS has been employed as an additive to the run buffer for the separation of AuNP.\(^{61-63, 111-113}\) In this study, the effect of SDS on the separation of cationic DMF-AuNC in CE is explored. During the CE separation, samples prepared in a solution are immediately enveloped by the run buffer. This blending offers the possibility of bringing particles into a defined environment, and thus any changes in the charge-to-mass ratios of the analytes can be monitored by changing the composition of the run buffer.

Figure 5.8A depicts the electropherograms of cationic DMF-AuNC at different concentrations of SDS (0.0–30 mM) in 30 mM phosphate run buffers (pH 7.0). In all electropherograms, Peak 3 is DMF (as the neutral marker) and the migration peaks before or after DMF are cationic and anionic DMF-AuNC, respectively. The migration time of Peak 3 does not change much with the increase in SDS concentration. However, the migration times of Peak 1 and 2 change significantly with the variation of SDS concentration. Their migration times increase with the
increase in SDS concentration. At low SDS concentration, Peak 1 and 2 migrate before the EOF (Peak 3). By contrast, they migrate after the EOF when the SDS concentration is higher. Figure 5.8B displays the EOF and $\mu_e$ of DMF-AuNC at different concentrations of SDS. The EOF does not change under different concentrations of SDS while the $\mu_e$ of cationic DMF-AuNC decrease significantly with the increase in SDS concentration until it reaches 10 mM at which the DMF-AuNC species has $\mu_e = 0$. When the concentration of SDS is higher than 10 mM, they resume their $\mu_e$ but move in the opposite direction since they now possess effective negative charges. Their apparent mobilities still migrate toward the cathode. When the concentration of SDS further increases to 30 mM, the $\mu_e$ increases. At 30–70 mM SDS, the $\mu_e$ of DMF-AuNC are the largest and remain constant. The electropherograms at 40–70 mM SDS are similar to that of 30 mM SDS (not shown here).

Figure 5.9 displays schematic representations of the effect of SDS on the cationic DMF-AuNC in the capillary. When SDS is present in the run buffer, the negatively charged SDS molecules interact with the positively charged DMF-AuNC by electrostatic attraction, leading to the decrease in the overall effective positive charge with a concomitant decrease in the overall charge-to-mass ratio of DMF-AuNC (Figure 5.9A). When the SDS concentration is higher than its critical
micelle concentration (8.7) mM, micelles of SDS are formed which allow the partition of DMF-AuNC from the run buffer into the micelles as depicted in Figure 5.9B. As a result, the DMF-AuNC species switches from an overall positive to negative charges and it then migrates after the EOF. Figure 5.10 depicts the UV-vis absorption spectra of positively charged DMF-AuNC with and without SDS. They show a slight difference. The positively charged DMF-AuNC with SDS displays a gradual decrease from shorter to longer wavelength and has relatively higher absorbance in the UV-vis region as compared to that without SDS, indicating that there is the interaction between the SDS and the positively charged DMF-AuNC and leads to an increase in the overall size of DMF-AuNC.\textsuperscript{60,106,162,185} In addition, it is observed that several small peaks are evolved in the electropherograms when the SDS concentration is 10 mM and higher (Figure 5.8A). Obviously, the addition of SDS in the run buffer could increase the separation capability of cationic DMF-AuNC species with the assistance of SDS pseudo-stationary phase. More DMF-AuNC species are resolved by differential partitioning between the micelles (pseudo-stationary phase) and the aqueous run buffer.
Figure 5.8. (A) Effect of SDS on the electrophoretic separation of cationic DMF-AuNC. The run buffer is 30 mM phosphate (pH 7.0) under different concentrations of SDS. Electropherograms are offset for clarity and ease of comparison. (B) Plot of EOF and electrophoretic mobility ($\mu_e$) of cationic DMF-AuNC against concentrations of SDS. The positive and negative $\mu_e$ infer that the DMF-AuNC acquires the effective positive and negative charges under low and high concentration of SDS, respectively.
Figure 5.9. Schematic representation of the effect of SDS on the electrophoretic mobility of DMF-AuNC in CE when the concentration of SDS is (A) lower and (B) higher than its critical micelle concentration.
Figure 5.10. UV-visible absorption spectra of cationic DMF-AuNC: (a) without and (b) with SDS. The spectra are normalized at 220 nm for ease of comparison.
5.3. Conclusion

Our work is the first attempt to separate the cationic DMF-AuNC by CE. 30 mM phosphate buffer (pH 7.0) is employed to lower the EOF to provide wider separation window for the cationic DMF-AuNC. Moreover, 20% v/v EtOH is recommended in the run buffer so that the differences in charge-to-mass ratio between the cationic DMF-AuNC are further enhanced. However, even under the optimal separation conditions, the cationic DMF-AuNC is not well separated well without SDS. To our knowledge, this is the first report on studying the effect of SDS on the separation of cationic Au sub-nanoclusters protected by DMF. This work illustrates that using SDS in run buffer can enhance the separation capability of CE for cationic DMF-AuNC. Adding SDS to the run buffer allows the surfactant to interact with the cationic DMF-AuNC with a concomitant change in the charge-to-mass ratio of DMF-AuNC. The SDS concentration plays an important role in altering the $\mu_e$ of DMF-AuNC. In addition, the SPE cartridge containing HLB sorbent is also the first time to be applied to clean-up the as-synthesized DMF-AuNC sample and allows the water-soluble portion of DMF-AuNC to be collected for CE analysis. It is anticipated that the wealth of information obtained from the CE analysis of cationic DMF-AuNC and the use of SDS in CE (i.e., micellar electrokinetic chromatography) will continue to grow and be used widely.
Chapter 6 Conclusion and Future Work

6.1. Conclusion

In this thesis, we report the synthesis, characterization and analytical separation of various nanoparticles. Such nanoparticles are characterized by different analytical techniques to verify their specific properties. Some of the key points and improvements of the study are summarized at the following:

The ultrasmall (<2.0 nm) α-CD-S-AuNPs are synthesized. It is difficult to synthesize AuNPs with the exactly same core size distribution under the same experimental conditions. This reproducibility also depends on the speed of reactant added, and the quality of α-CD-SH (i.e. the number of the six primary hydroxyl groups converted to thiol groups). The study of these effects on the core size distribution of the as-prepared α-CD-S-AuNPs will be our focus in the near future.

Unfortunately, the manner of α-CD ligands bound to our AuNPs is still not clearly understood. Further work will focus on the study of the crystal structure of α-CD-S-AuNP by X-ray diffraction in order to elucidate the proposed structure in this research. In addition, PL properties of AuNPs have been recognized. To our knowledge, we firstly observe that these small-sized α-CD-S-AuNPs also display strong blue emissions which have potential applications in optical chemo/biosensors.
These small-sized \( \alpha \)-CD-S-AuNPs also exhibit unusual enhancement in PL intensity via the interaction with tetraalkylammonium ion based on the aggregation-enhanced emission phenomenon. By contrast, it displays PL quenching in the presence of Hg(II). The small-sized \( \alpha \)-CD-S-AuNPs has been successfully applied to determine Hg(II) with ultrahigh sensitivity and excellent selectivity.

Most of synthesis methods for AuNPs can only achieve to prepare a complex mixture of components made up of various Au cores and different numbers of capped ligands. A polydisperse AuNPs product only represents the summation or average properties of all individual AuNPs. Thus, there is a high demand for a fast and efficient methodology for separation and purification of polydisperse AuNPs. The UHPLC method can provide a convenient way to separate polydisperse water-soluble AuNPs. In this work, our proposed UHPLC method has been successfully applied to evaluate and compare polydisperse NAC-AuNPs products synthesised with the one-phase and two-phase Brust-Schiffrin methods. The results indicate that the two-phase method would harvest AuNPs product with smaller core size and less dispersity. In addition, the total elution time for analysing a NAC-AuNPs sample by UHPLC is ten times shorter than that of HPLC with smaller sample volume (1–2 µL) and better separation efficiency.

Last but not least, we first attempt to develop CE method for separating the cationic
DMF-AuNC. However, even under the optimal separation conditions, the cationic DMF-AuNC is not well separated without SDS. Adding SDS to the run buffer can enhance the separation of cationic DMF-AuNC, attributing to the attachment of the charged SDS to the AuNC surface with a concomitant effect on changing the charge-to-size ratio of the cationic DMF-AuNC.

6.2. Future Work

6.2.1. Application of α-CD-S-AuNPs

Polycyclic aromatic hydrocarbons (PAHs) are potent atmospheric pollutants that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. Naphthalene is the simplest example of a PAH. As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic. Sirimanne et al.\textsuperscript{211} reported a method for the determination of 16 PAHs and 8 PCDDs in spiked human serum based on cloud point extraction (CPE) and capillary electrochromatography (CEC). Human serum samples spiked with PAHs or PCDDs are extracted using nonionic surfactant Genapol X-080. Subsequently, the surfactant-rich phase is treated with acetonitrile to remove unwanted interfering co-extractants such as proteins to prevent capillary clogging. 16 PAHs or 8 PCDDs are well separated but both migration times are shifted slightly. This phenomenon
may be attributed to the dynamic coating of the C18 stationary phase by the residual surfactant or co-extractants in the sample. But their results still prove to be feasible for analyses of PAHs and PCDDs using CPE-CEC.

New developments in the use of NPs in separation science are taking place, accompanying the trends towards capillary coatings and monolithic stationary phases. In particular, their use in CE and CEC show enhancing performance. While many NP types are being used for separation purposes, the small particle with the biggest role to date in separation science appears to be the alkylthiolated AuNP, a monolayer protected NP.

In addition, molecular recognition phenomena have been extensively studied in the past few decades, probably because of the realization of the widespread importance of noncovalent interactions in biochemistry. A prototypical example of well-studied hosts is CDs which is a class of naturally occurring receptors with cyclic glucopyranose oligomers. Their toroidal shape and strong binding affinity for hydrophobic molecules in aqueous media are very well known. Moreover, various CDs such as native α-, β- and γ-CD, several neutral and selectively substituted charged CD derivatives have been used as chiral selectors for enantiomeric separations in CE. Herein, the investigation of the opportunity to develop α-CD-S-AuNP-based CE separation in the analysis of PAH compounds is
definitely a promising future direction on our research.

6.2.2. Further Investigation of UHPLC for the Separation of AuNPs

In the previous chapter, an UHPLC method has been developed for the separation and analysis of water-soluble AuNP products. This technology provides greater resolution, increased sensitivity and high speed of analysis with the advantages of small volume of sample injection (1–2 µL) and less consumption of solvent. However, this method involves the use of ion-pairing reagent which can shorten the lifetime of a chromatographic column. Moreover, this solvent component cannot be applied directly to the UHPLC/MS system. In addition, it is difficult to collect the sample. Even through the separated fractions can be collected, they are insufficient for most NP characterization methods. Thus, the newly resolved peaks by UHPLC method cannot be identified immediately. For charged AuNPs, another UHPLC method with a more polar column should be tried so as to prevent the use of ion-pairing reagent.
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