Adapting the Turkevich Method to Synthesize Colloidal Rhodium Nanocrystals

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

Department of Chemistry
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2018

Abstract

The Turkevich method has been used widely for the synthesis of gold nanocrystals. Despite its success, it has not yet been applied to the synthesis of rhodium nanocrystals, which have great potential in myriad applications due to their special optical and chemical properties. Herein, it is shown that the Turkevich method, with the appropriate modifications, can be adapted to enable access to rhodium nanocrystals. Products are generated under a wide range of experimental conditions. In most cases, quasi-spherical aggregates of ultrasmall rhodium grains are obtained. Possible means of controlling the aggregate size are proposed. The method can also yield large, anisotropic rhodium nanocrystals, although control over the geometry of this product is not achieved. Further optimizations are suggested toward obtaining monodisperse, single-crystal rhodium nanoparticles for eventual use in applications.
Acknowledgments

I am grateful to Prof. Neil A. Coombs, Ilya Gourevich, and Audrey Darabie for their guidance in collecting and interpreting data using electron microscopy techniques. Over the course of my work, I have developed a passion for electron microscopy that I hope to cultivate in the future, and I am thankful for the opportunities that they have created for me.

I am privileged to have collaborated with Caroline Pao, a fellow graduate student, on a book chapter titled *Photonic Nanoparticles for Cellular and Tissular Labeling*, which could not have been completed on time without her assistance.

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Above all else, I am indebted to my research supervisor, Prof. Gilbert C. Walker, who has been extremely understanding, caring, and supportive. Thank you so much for the extraordinary opportunity to have done research in your lab. You have given me endless encouragement and freedom that I need to grow as a scientist, and have pushed me beyond my limits again and again to complete work that I always thought was beyond my ability. Words cannot describe my immeasurable appreciation for you and for all that you’ve done for me. You are not only my colleague, but also my friend. I am ecstatic to be able to continue working with you in the near future and know that we will achieve great things together.
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<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>percent sign</td>
</tr>
<tr>
<td>([\text{AuCl}_4]^-)</td>
<td>tetrachloroauration(III) coordination complex</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>Ag</td>
<td>silver</td>
</tr>
<tr>
<td>(\text{Ag}_2\text{S})</td>
<td>silver(I) sulfide</td>
</tr>
<tr>
<td>Au</td>
<td>gold</td>
</tr>
<tr>
<td>(\text{Au}^{3+})</td>
<td>gold(III) ion</td>
</tr>
<tr>
<td>(c(\text{Rh}^{3+}))</td>
<td>injection molar concentration of rhodium(III) ion</td>
</tr>
<tr>
<td>Cd</td>
<td>cadmium</td>
</tr>
<tr>
<td>(\text{CdSe})</td>
<td>cadmium(II) selenide</td>
</tr>
<tr>
<td>(\text{CIS})</td>
<td>copper indium sulfide</td>
</tr>
<tr>
<td>(\bar{d})</td>
<td>mean nanoparticle size</td>
</tr>
<tr>
<td>D</td>
<td>polydispersity index</td>
</tr>
<tr>
<td>DMF</td>
<td>(N,N)-dimethylformamide</td>
</tr>
<tr>
<td>e.g.</td>
<td>exempli gratia</td>
</tr>
<tr>
<td>et al.</td>
<td>et alia</td>
</tr>
<tr>
<td>eV</td>
<td>electronvolt</td>
</tr>
<tr>
<td>(F)</td>
<td>Fisher statistic</td>
</tr>
<tr>
<td>(\text{H}_2\text{O})</td>
<td>water</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>HAuCl₄</td>
<td>hydrogen tetrachloroaureate(III)</td>
</tr>
<tr>
<td>HCit²⁻</td>
<td>hydrogen citrate</td>
</tr>
<tr>
<td>i.e.</td>
<td>id est</td>
</tr>
<tr>
<td>InAs</td>
<td>indium(III) arsenide</td>
</tr>
<tr>
<td>K₂CO₃</td>
<td>potassium carbonate</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>kV</td>
<td>kilovolt</td>
</tr>
<tr>
<td>LSPR</td>
<td>localized surface plasmon resonance</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mol</td>
<td>mole</td>
</tr>
<tr>
<td>n(citrate)/n(Rh³⁺)</td>
<td>molar ratio of citrate to rhodium(III) ion</td>
</tr>
<tr>
<td>n(K₂CO₃)/n(Rh³⁺)</td>
<td>molar ratio of potassium carbonate to rhodium(III) ion</td>
</tr>
<tr>
<td>n(PVP)/n(Rh³⁺)</td>
<td>molar ratio of poly(vinylpyrrolidone) to rhodium(III) ion</td>
</tr>
<tr>
<td>n(TA)/n(Rh³⁺)</td>
<td>molar ratio of tannic acid to rhodium(III) ion</td>
</tr>
<tr>
<td>Na₃Cit • 2H₂O</td>
<td>sodium citrate tribasic dihydrate</td>
</tr>
<tr>
<td>NC</td>
<td>nanocrystal</td>
</tr>
<tr>
<td>NIR</td>
<td>near-infrared</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
</tr>
<tr>
<td>NP</td>
<td>nanoparticle</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>$p$</td>
<td>probability value</td>
</tr>
<tr>
<td>Pb</td>
<td>lead</td>
</tr>
<tr>
<td>PbS</td>
<td>lead(II) sulfide</td>
</tr>
<tr>
<td>phNP</td>
<td>photonic nanoparticle</td>
</tr>
<tr>
<td>PVP</td>
<td>poly(vinylpyrrolidone)</td>
</tr>
<tr>
<td>QD</td>
<td>quantum dot</td>
</tr>
<tr>
<td>RCF</td>
<td>relative centrifugal force</td>
</tr>
<tr>
<td>Rh</td>
<td>rhodium</td>
</tr>
<tr>
<td>Rh$^{3+}$</td>
<td>rhodium(III) ion</td>
</tr>
<tr>
<td>RhCl$_3$ • xH$_2$O</td>
<td>rhodium(III) chloride hydrate</td>
</tr>
<tr>
<td>SAXS</td>
<td>small-angle X-ray scattering</td>
</tr>
<tr>
<td>Si</td>
<td>silicon</td>
</tr>
<tr>
<td>sP</td>
<td>semiconducting polymer</td>
</tr>
<tr>
<td>sPNP</td>
<td>semiconducting polymer nanoparticle</td>
</tr>
<tr>
<td>$T$</td>
<td>temperature</td>
</tr>
<tr>
<td>$t$</td>
<td>time</td>
</tr>
<tr>
<td>TA</td>
<td>tannic acid</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscope</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>$V$</td>
<td>total volume of reaction mixture</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>$V(\text{Rh}^{3+})$</td>
<td>injection volume of rhodium(III) ion</td>
</tr>
<tr>
<td>XANES</td>
<td>X-ray absorption near edge structure</td>
</tr>
<tr>
<td>ZnS</td>
<td>zinc(II) sulfide</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>alpha value</td>
</tr>
<tr>
<td>$\mu\text{A}$</td>
<td>microampere</td>
</tr>
<tr>
<td>$\pi$</td>
<td>pi (bonding)</td>
</tr>
<tr>
<td>$\pi^*$</td>
<td>pi star (anti-bonding)</td>
</tr>
</tbody>
</table>
Chapter 1
Rhodium Nanocrystals

Colloidal nanocrystals (NCs) or nanoparticles (NPs) are entities, on the scale of 1–100 nm in at least one dimension, that exist as a stable dispersion in a fluid. NCs possess shape- and size-dependent electrical, magnetic, and optical properties. In particular, the class of plasmonic, noble-metal NCs is well-studied and offers exciting applications in, e.g., plasmon-enhanced Raman spectroscopy,\(^1\) in vivo diagnostics and multimodal imaging,\(^2\) enhanced detection of chiral molecules,\(^3\) and generation of hot electrons for photocatalysis.\(^4\) This thesis is centered on the synthesis of NCs comprising rhodium (Rh) metal. In this introductory chapter, I begin by providing a brief overview of the catalytic applications of Rh NCs. I also summarize the state of the art of synthesis of Rh NCs. Finally, I delineate my intended contributions to this field.

1.1 Catalytic Applications of Rh NCs

Elemental Rh (atomic number 45) is a chemically inert transition metal. It is classified as both a noble metal and platinum-group metal. Rh possesses an electron configuration of [Kr] 4d\(^8\) 5s\(^1\), and has a common oxidation state of +3. Interest in nanocrystalline Rh has been driven principally by its catalytic properties. In the past, Rh NCs have been used extensively to catalyze the hydrogenation of aromatic systems, as covered in detail by Yuan et al. in their review article.\(^5\) Rh NCs are also excellent electrocatalysts in both oxidation and reduction reactions, such as the ethanol oxidation reaction and evolution of hydrogen from ammonia borane.\(^6\)–\(^13\)

The plasmonic properties and photocatalytic applications of Rh NCs are now becoming better understood. Rh NCs are known to support localized surface plasmon resonances (LSPRs) in the ultraviolet (UV) region, whose energy surpasses that of the LSPRs of more commonly used plasmonic NCs, \(i.e.,\) those comprising gold (Au) and silver (Ag).\(^14\) This property gives Rh NCs an edge over other noble-metal NCs for the applications listed above. Out of many candidates for UV plasmonics, including magnesium, aluminium, and gallium, Rh has been identified as the best choice given its good optical response in the UV, as well as its resistance toward oxidation, which can diminish or destroy the optical response of other metals.\(^15\)
Zhang et al. have demonstrated that alumina-supported Rh nanocubes can plasmonically photocatalyze the hydrogenation of carbon dioxide, leading to the almost exclusive formation of methane. Yet, in the absence of UV illumination, carbon monoxide and methane are produced in equal amounts. More recently, Kuo et al. have shown that concave Rh nanotetrahedra can photocatalyze the evolution of hydrogen from ammonia borane.

1.2 Synthesis of Rh NCs

The state of the art of fabricating Rh NCs is the polyol method, which provides control over the shape and size of the Rh NCs. In this method, a rhodium(III) (Rh^{3+}) precursor, typically a halide hydrate salt, is reduced by a polyol, most often ethylene glycol, at moderate temperatures, i.e., 140–160 °C. Reduction of Rh^{3+} occurs in the presence of an encapsulating agent, almost always poly(vinylpyrrolidone) (PVP) and, optionally, a shape-control agent such as a halide salt. Using this method, researchers have produced nanoscale Rh cubes, icosahedra, tetrahedra, triangular plates, and tripods. For example, Zhang et al. employed the polyol method to produce PVP-encapsulated Rh nanocubes, showing that the LSPR is red-shifted from 4.51 to 3.25 eV as the cube edge length increases from 15 to 59 nm.

Other techniques have been used successfully, but on a smaller scale. Jiang et al. synthesized mesoporous Rh NCs by reducing sodium hexachlororhodate(III) with L-ascorbic acid onto polymeric micelle templates in a solvent system consisting of water (H_{2}O) and N,N-dimethylformamide (DMF). Zhang and coworkers generated Rh tetrahedra, concave tetrahedra, and hexagonal nanosheets by reducing rhodium(III) acetate with glucose in DMF, with shape control provided by altering the ratio of oleylamine to 1-octadecene. Kuo et al. prepared concave Rh tetrahedra with defect-rich surfaces, capped with cetyltrimethylammonium bromide, by reducing rhodium(III) bromide hydrate using formic acid. Zhang et al. employed a solvothermal method wherein rhodium(III) chloride is reduced by an alkylamine in the presence of PVP, obtaining a variety of shapes including novel cyclic penta-twinned Rh nanobranches. Kang et al. used Ag NCs as templates in a galvanic replacement reaction, obtaining access to Rh nanoshells, Rh nanoframes, and porous Rh nanoplates.
1.3 Thesis Structure

In Chapter 2, the adaptation of a well-established method, currently used for the synthesis of Au NCs, to the synthesis of Rh NCs is motivated and described. A portion of the associated synthetic space is mapped out.

In Chapter 3, the author’s contributions to a book chapter are detailed. This work was done in collaboration with Caroline Pao, a doctoral student in the Walker group, and has been submitted and accepted for publication as:

Chapter 2
Adapting the Turkevich Method to Synthesize Rh NCs

2.1 The Turkevich Method

The Turkevich method, first reported in 1951, is the most common and simplest method used to generate Au NCs with sizes in the range 12–16 nm. In the original method, an aqueous solution of sodium citrate is injected into a boiling, aqueous solution of hydrogen tetrachloroaurate(III) (HAuCl₄). Citrate functions as a buffer that fixes the pH through the interconversion of different species (Scheme 2.1). It also serves as a reducing agent (Scheme 2.2) that converts gold(III) (Au³⁺) to Au as well as a ligand that coordinates to the NC surface through negatively charged carboxylate groups, imparting electrostatic colloidal stability.

![Scheme 2.1](image1)

Scheme 2.1. Acid–base equilibria between different citric acid species.

![Scheme 2.2](image2)

Scheme 2.2. Oxidation of citric acid into dicarboxyacetone.

2.1.1 Mechanistic Studies of the Turkevich Method

A large body of work exists on the mechanistic study of the Turkevich reaction. Significant contributions to this field have been made by Polte and coworkers within the past eight years. In a landmark study, Polte et al. coupled in situ small-angle X-ray scattering (SAXS) and X-ray absorption near edge structure (XANES) to elucidate the mechanism of Au NC formation and growth in the Turkevich method. They identified four steps of nucleation and growth (Figure 2.1): (1) rapid formation of nuclei; (2) coalescence of nuclei into larger particles; (3) slow growth of...
particles sustained by ongoing precursor reduction and coalescence; and (4) rapid growth of particles to their terminal size, as determined by the complete consumption of precursor. Polte and coworkers also investigated the effect of increasing the temperature or precursor concentration, deducing that the mechanism remains unchanged but that the particles nucleate and grow faster.\footnote{30}

![Figure 2.1. Mechanism of Au NC nucleation and growth in the Turkevich method, as deduced by Polte et al. from in situ XANES and SAXS data. Reprinted with permission from Mechanism of Gold Nanoparticle Formation in the Classical Citrate Synthesis Method Derived from Coupled In Situ XANES and SAXS Evaluation. Jörg Polte, T. Torsten Ahner, Friedmar Delissen, et al., Journal of the American Chemical Society, Copyright 2010 American Chemical Society.}

Later, Polte et al. improved their experimental setup to increase the temporal resolution of in situ SAXS, allowing more information about the initial stages of the reaction to be collected. In particular, the authors were able to estimate the Au NC growth rate, which was equated to the Au$^{3+}$ reduction rate, and relate it to the four steps of nucleation and growth identified in the previous work. Initially, the growth rate is large, corresponding to rapid nucleation and a fast increase in the number of particles. When coalescence and slow growth take place, the reduction rate decreases. Finally, the reduction rate increases sharply as the remaining 70–80% of Au$^{3+}$ precursor is reduced, enabling rapid growth of Au NCs until they attain their terminal size.\footnote{31}

In another work, Polte has summarized the group’s mechanistic studies on the synthesis of Au NCs, rejecting the commonly applied La Mer model of nucleation and growth. On the basis of the group’s data, he concluded that the terminal particle size is determined not by thermodynamic stability, as it is in models based on classical nucleation theory, but by colloidal stability.\footnote{32}

Wuithschick and coworkers next used time-resolved SAXS to examine, among other aspects of the reaction, the growth mechanism, how and when the final NC size is determined, and how
different synthetic parameters influence NC growth. Several key findings were made. First, the four-step seed-mediated growth mechanism was confirmed. Next, it was deduced that the terminal NC size is determined at the end of the seed particle formation step, and that the size is determined by the number of seed particles. The effects of several parameters were also resolved: the concentration of citrate is not important provided that it can buffer a neutral pH after mixing; higher concentrations of HAuCl₄ lead to a larger number of smaller NCs; inverting the order of addition leads to smaller NCs; and higher temperatures cause larger seed particles to form more quickly.³³

Most recently, Kettemann et al. have used SAXS to investigate the role of citrate protonation, finding that seed crystals are generated exclusively from the reduction of tetrachloroaurate(III), [AuCl⁴⁻], by hydrogen citrate (HCit²⁻), and that it is principally the concentration of HCit²⁻ that determines the rate of reduction. The authors applied this conclusion to reproducibly generate Au NCs with a polydispersity of 10% (±0.1 nm) by fixing the pH at 5.6, at which the fraction of citric acid in the form HCit²⁻ is maximized.³⁴

Recently, reports have emerged on the interaction of citrate with the Au NC surface. Monti et al. used molecular dynamics simulations to characterize the adsorption dynamics of trisodium citrate on Au NCs in H₂O. They discovered that citrate, preferentially fully deprotonated, binds strongly to the Au surface through the coordination of one or more carbonyl groups. Citrate prevents the migration of surface Au atoms and limits the extent to which surface reconstruction can take place. Moreover, citrate molecules were found to be interconnected through metal adatoms, forming staple motifs. Finally, sodium ions were located within the citrate monolayer.³⁵

Al-Johani et al. have employed a number of complementary techniques to study how citrate binds to and stabilizes Au NCs. They identified three distinct binding modes: monocarboxylate bridging, monocarboxylate monodentate, and dicarboxylate bridging. The relative amounts of citrate and Au determine the fractions of these binding modes. Furthermore, Au surface atoms were found to exist mainly in the zero oxidation state. The presence of sodium ions near the Au surface was also confirmed.³⁶

2.1.2 Variations of the Turkevich Method

A number of modifications have been made to the Turkevich method by different researchers in an effort to improve the quality of the product and to extend the applications of the method. It should
be mentioned that the mechanistic conclusions of Polte and coworkers may not necessarily apply to these variants, as that group focused on only the original Turkevich method.

One of the earliest refinements of the Turkevich method is the Frens method, reported in 1973. Frens adjusted the ratio of citrate to HAuCl₄ to create Au NCs (16–147 nm) larger than possible with the original Turkevich method. However, the larger NCs obtained through this method were polydisperse and irregularly shaped.³⁷

In recent work, Xia et al. have constructed a new model for the citrate-based reduction of HAuCl₄ using dynamic light scattering and zeta-potential measurements. They found that nucleation and growth proceeds through complexes of gold(I) and sodium acetone dicarboxylate, and that the process is described adequately by the La Mer model. Although this contradicts the conclusions of Polte and coworkers, the authors were nevertheless able to apply their findings to prepare quasi-spherical Au NCs using the Frens method, achieving sizes spanning the range 2–330 nm.³⁸

Building on the literature, Piella et al. optimized a reaction system that incorporates tannic acid (TA) as a competing reducing agent. Moreover, aqueous potassium carbonate (K₂CO₃) was added to adjust the pH. Piella and coworkers determined values of pH, TA concentration, and temperature that yielded the smallest and least polydisperse Au NCs possible, attaining a best size distribution of 3.6 ± 0.4 nm. The authors then demonstrated the use of their Au NCs in controlled seeded-growth reactions, wherein premade NCs are present in the reaction mixture at the time of injection so that no new NCs form and existing NCs grow. By keeping all parameters constant, Au NCs can be grown to the desired size simply by choosing the appropriate number of growth steps. In doing so, Piella et al. generated Au NCs ranging in size from 3.6 to 13.1 nm.³⁹

2.1.3 Synthetic Objectives

Given the widespread success of the Turkevich method in the synthesis of Au NCs, it is surprising that the method has not yet been adapted to the synthesis of Rh NCs. The Turkevich method would present a greener alternative to the polyol method, and may enable access to new NC geometries not otherwise available. Additionally, the Turkevich method would provide a convenient, seeded growth alternative to the use of continuous injection in the polyol method in order to obtain large Rh NCs. To the author’s knowledge, large Rh NCs have not yet been investigated, but may present
multipolar plasmonic modes. Work by Hastings et al. has shown that imaging Au NCs at multipolar modes can greatly enhance their Raman scattering efficiency, making them promising materials in a variety of applications.  

Although the reaction system of Piella et al. may not be applicable as-is to the synthesis of Rh NCs, the author believes that it may be adapted with the appropriate modifications. Accordingly, this chapter describes how the reaction system developed by Piella and coworkers can be modified to permit the synthesis of Rh NCs, and explores the associated synthetic space.

2.2 Reaction Template

In previous experiments, the author has observed that citrate is unable to colloidally stabilize Rh NCs. Therefore, the inclusion of a stabilizing agent was necessary. PVP was incorporated for this purpose, given its reliability in both aqueous and polar organic media alike. Additionally, the volume of the reaction mixture was scaled down by a factor of three relative to the method of Piella et al. This smaller volume is more economical and appropriate for reaction prototyping.

Each reaction is constructed by choosing values for the following parameters: the concentration $c$(Rh$^{3+}$) and volume $V$(Rh$^{3+}$) of precursor to be injected, the molar ratio $n$(citrate)/$n$(Rh$^{3+}$) of citrate to Rh$^{3+}$, the molar ratio $n$(K$_2$CO$_3$)/$n$(Rh$^{3+}$) of K$_2$CO$_3$ to Rh$^{3+}$, the molar ratio $n$(TA)/$n$(Rh$^{3+}$) of TA to Rh$^{3+}$, the molar ratio $n$(PVP)/$n$(Rh$^{3+}$) of PVP to Rh$^{3+}$, the total volume $V$, the temperature $T$, and the time $t$. It must be mentioned that, although the pH is a critical parameter, it is not listed since it is controlled only indirectly through other parameters, namely $n$(K$_2$CO$_3$)/$n$(Rh$^{3+}$).

A visual representation of the reaction is provided in Scheme 2.3.

![Scheme 2.3. Proposed citrate reduction method for the synthesis of Rh NPs. The dashed line represents a polymeric shell that provides each NP with steric colloidal stability.](image-url)

In the following series of experiments, the following parameters were locked: $V$(Rh$^{3+}$) = 1 mL, $n$(PVP)/$n$(Rh$^{3+}$) = 2, $V$ = 51 mL, and $t$ = 15 min. All other parameters were varied independently. The prototype reaction was then constructed using the parameters in Table 2.1. Hereafter, these
parameters will be referred to as the *standard parameters* and the product accessed through them, the *standard product*.

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c(\text{Rh}^{3+}) / \text{mM}$</td>
<td>8</td>
</tr>
<tr>
<td>$n(\text{citrate})/n(\text{Rh}^{3+})$</td>
<td>16</td>
</tr>
<tr>
<td>$n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+})$</td>
<td>32</td>
</tr>
<tr>
<td>$n(\text{TA})/n(\text{Rh}^{3+})$</td>
<td>1/16</td>
</tr>
<tr>
<td>$T / ^\circ\text{C}$</td>
<td>80</td>
</tr>
</tbody>
</table>

*Table 2.1.* The standard parameters used to construct the prototypical reaction.

## 2.3 Experimental

### 2.3.1 Materials

All materials were used as received. Type 1 water (H\textsubscript{2}O, 18.20 M\(\Omega\) cm) was used exclusively. Rhodium(III) chloride hydrate (RhCl\(_3\) \(\cdot\) \text{xH}_2\text{O}, 206261, 39\% Rh), sodium citrate tribasic dihydrate (Na\(_3\)Cit \(\cdot\) 2H\(_2\)O, for molecular biology, C8532, \(\geq\)99\%), tannic acid (TA, 403040, ACS reagent), and poly(vinylpyrrolidone) (PVP, PVP40, average molecular weight 40 kg/mol) were obtained from Sigma–Aldrich (St. Louis, MO). Potassium carbonate (K\(_2\)CO\(_3\), anhydrous, certified ACS, \(\geq\)99\%) was obtained from Fisher Scientific (Fair Lawn, NJ).

### 2.3.2 Methods

#### 2.3.2.1 Synthesis of Rh NCs

At the start of each day, fresh stock solutions of Na\(_3\)Cit \(\cdot\) 2H\(_2\)O, K\(_2\)CO\(_3\), TA, and PVP were prepared. Into a three-neck, round-bottom flask charged with an octagonal, Teflon\textsuperscript{®}-coated magnetic stir bar, the appropriate solutions were introduced and H\(_2\)O was added to a total nominal volume of $V - V(\text{Rh}^{3+})$ while stirring at 500 rpm. Next, the flask was equipped with a thermometer adapter, stopper, and water-cooled condenser. A thermometer was inserted into the solution, which was heated to the temperature $T$ in a temperature-controlled silicone-oil bath. A solution of RhCl\(_3\) \(\cdot\)
xH₂O was then prepared and a volume \( V(\text{Rh}^{3+}) \) of this solution was injected into the hot reducing solution. After a time \( t \), the flask was removed from heat and the system was permitted to cool to room temperature. Finally, the solution was stored in a BD Falcon™ tube.

### 2.3.2.2 Purification

Neat reaction mixtures were purified by ultrafiltration using an Amicon™ Ultra-0.5 mL Centrifugal Filter Unit with a molecular weight cut-off of 100 kg/mol. Each sample was centrifuged at a relative centrifugal force (RCF) of 14 000 for 10 min at least three times. The concentrate was diluted to 0.5 mL between each cycle with H₂O. The concentrate was then recovered by inverse centrifugation at a RCF of 2000 for 1 min and diluted with 0.1–0.5 mL of H₂O.

### 2.3.2.3 Transmission Electron Microscopy

Each specimen was prepared as follows: a purified sample of colloidal Rh NCs was drop-casted onto the metallic (hydrophilic) face of a clean 200-mesh Cu grid coated with a Formvar/carbon film (Ted Pella, No. 01801) and allowed to dry inside a fume hood under ambient conditions.

Specimens were characterized using a Hitachi HT-7700 transmission electron microscope (TEM) operating in bright-field mode. The instrument was operated at an accelerating voltage of 80 kV, with the emission current falling in the range 18–22 μA. Images were analyzed using ImageJ 1.51j8 software.

### 2.3.2.4 Calculation and Comparison of Size Distributions

Each size distribution was generated from 400 manually sized particles. Because of the morphological diversity of the samples, the size of each particle was taken as the longest distance spanning the particle. The mean and sample standard deviation for each set of sizes were calculated using the “AVERAGE” and “STDEV.S” functions, respectively, in Microsoft® Excel® 2016. The polydispersity index \( \bar{D} \) was calculated using the equation

\[
\bar{D} = \frac{n}{\Sigma_i d_i} \left[ \frac{\Sigma_i (d_i)^4}{\Sigma_i (d_i)^2} \right],
\]

where \( n \) is the number of particle sizes and \( d_i \) is the size of the \( i \)th particle. Size distributions were compared using a two-tailed \( t \)-test with \( \alpha = 0.05 \). Calculations were performed automatically using
the “t-test: Two-Sample Assuming Equal Variances” and “t-test: Two-Sample Assuming Unequal Variances” modules in the Excel® Analysis ToolPak add-in. The (in)equality of variances was determined using a two-tailed F-test with $\alpha = 0.025$, with calculations performed automatically using the “F-Test Two-Sample for Variances” module in the Excel® Analysis ToolPak add-in.

2.3.2.5 pH Measurement

The pH of selected reaction mixtures was checked prior to the injection of Rh$^{3+}$ precursor using a pH electrode (89231-590, VWR International) connected to a Water Quality Meter (850081, Sper Scientific). Before each set of measurements, the electrode was calibrated using a pH-7 buffer. Measurements were taken at 80 °C with the Water Quality Meter configured appropriately.

2.4 Results and Discussion

No obvious transformations were observed upon the injection of aqueous Rh$^{3+}$ precursor into the hot reducing solution. Although the solution immediately acquired a pale gold colour, this can be attributed to the dilution of the red/copper-coloured aqueous Rh$^{3+}$ precursor and is not necessarily indicative of chemical reduction. The pH of the hot solution was measured to be ~8.5 prior to injection. At this pH level, citrate should exist predominantly in the tribasic form ($pK_{a,3} \approx 6.4$).

A low-magnification TEM image of the product generated using the parameters in Table 2.1 is shown on the following page (Figure 2.2). Several observations can be made: (1) the specimen comprises nanosized particles; (2) under the conditions in Table 2.1, spheroidal/quasi-spherical particles with mean size 21.7 ± 5.2 nm (24%) and polydispersity index 1.15 are generated; (3) the particles form disordered superstructures on the TEM grid; and (4) the image contains predominantly mass-thickness contrast and there is no obvious diffraction contrast.

This proves that the Turkevich method may be adapted to the synthesis of Rh NPs. That there is no obvious diffraction contrast in the image suggests that the NPs are not crystalline. To determine whether the NPs comprise amorphous Rh or may be aggregates of small Rh NCs, additional images were taken at larger magnifications. A representative image is presented as Figure 2.3.
Figure 2.2. TEM image of the standard product at a magnification of 15k. The scale bar corresponds to a distance of 500 nm. The brightness and contrast have been enhanced for improved clarity.

At larger magnifications, the NPs are seen to consist of small grains. Most grains are not sufficiently well-defined for a size distribution to be calculated, but a size range of 1–3 nm can be estimated. Rh NCs of this size may be too small to have plasmonic properties, or may possess broad LSPRs in the vacuum UV that cannot be accessed with the instrumentation available.

This product most closely resembles that of Ewers et al., who obtained spherical aggregates of Rh NPs (1–3 nm) with diameters of 12–70 nm by reducing aqueous RhCl₃ using sodium borohydride in the presence of PVP. These aggregates were found to also form superstructures upon evaporation of solvent. Ewers and coworkers used SAXS to show that the aggregates do exist in dispersion and are not an artifact of solvent evaporation during specimen preparation. The sizes of the grains and aggregates obtained by Ewers et al., and their tendency to form superstructures when the solvent is evaporated, gives credibility to the notion that the Rh NPs described above may also be aggregates of ultrasmall Rh NCs.
Figure 2.3. TEM image of the standard product at a magnification of 100k. The scale bar corresponds to a distance of 100 nm. The brightness and contrast have been enhanced for improved clarity.

Using this reaction as a reference point, the next objective was to investigate the influence of pH under otherwise identical conditions. In three separate reactions, K$_2$CO$_3$ was added with the ratio $n$(K$_2$CO$_3$)/$n$(Rh$^{3+}$) equal to 64, 16, and 0, resulting in pre-injection pH levels of approximately 9, 8, and 7, respectively.

When the pH increases from ~8.5 to ~9, the mean NP size decreases to 17.3 ± 6.3 nm (37%) and the polydispersity index increases to 1.38. This distribution is statistically distinct from the standard size distribution. Furthermore, images taken at higher magnifications suggest that the aggregates consist of grains ~1 nm in diameter. It is likely that the aggregates obtained at the larger pH level are smaller because the grain size is smaller, although it may also be that the aggregates generated at the larger pH level contain fewer grains.

At pH ~8 and ~7, the characteristics of the products change dramatically (Figure 2.4): (1) large, aggregated, and anisotropic NCs are obtained; (2) most NCs are on the order of 100–200 nm in
size, although a size distribution was not calculated given the morphological diversity of the sample; (3) diffraction contrast due to particle thickness variation is observed on some NCs; and (4) the boundaries of some NCs have a textured, grain-like appearance. Additionally, despite having been processed in the same manner as other samples, these samples present a noticeable degree of contamination, which may have facilitated the aggregation of the NCs on the grid.

Figure 2.4. TEM image of the product obtained with the standard parameters but with \( n(K_2CO_3)/n(Rh^{3+}) = 16 \) at a magnification of 10k. The scale bar corresponds to a distance of 1000 nm. The brightness and contrast have been enhanced for improved clarity.

Moving forward, it will be useful to label the two types of products that have been generated so far. Aggregates of Rh grains will be referred to as Class 1 NPs. Large, anisotropic Rh NCs will be referred to as Class 2 NPs.

Next, two reactions were executed with the standard parameters but with \( T \) set to 60 and 70 °C. At 60 °C, Class 1 NPs with average size 21.6 ± 8.0 nm (37%) and polydispersity index 1.36 were obtained, whereas a temperature of 70 °C led to Class 1 NPs with average size 19.2 ± 6.0 nm (31%) and polydispersity index 1.24. The two products have statistically distinct size distributions. However, whereas the size distribution of the standard product is not significantly different from
the product obtained at 60 °C, it is statistically distinct from the product obtained at 70 °C. This suggests that there may not be a clear relationship between the geometry of the product and the temperature, and that these differences in NP size could be attributed to conditional variations between different reactions. Interestingly, small, isolated NPs were seen in both samples. These NPs were not sized as a result of inadequate resolution and poor image contrast. The fraction of these small NPs appeared to be much larger in the 60 °C-sample than in the 70 °C-sample, suggesting that the temperature may play a role in the assembly of Rh NPs into aggregates, with higher temperatures leading to more efficient and complete assembly. Indeed, no such isolated NPs are observed when the standard parameters are employed.

The importance of temperature to the assembly could be rationalized as follows. At higher temperatures, polymers encapsulating aggregates of Rh NCs have a less rigid structure and can accommodate additional grains. In contrast, the polymers do not have the conformational flexibility at lower temperatures necessary to take in more grains once they have become part of an existing aggregate, leading to the isolation or limited clustering of those grains.

In another series of reactions, the ratio \( n(TA)/n(Rh^{3+}) \) was varied with all other parameters held constant. In addition to the standard reaction, with \( n(TA)/n(Rh^{3+}) = 1/16 \), values of 1/8, 1/32, and 0 were used. In all cases, Class 1 NPs were obtained. The size distributions and polydispersity indices are listed in Table 2.2.

Although parameter sets with \( n(TA)/n(Rh^{3+}) \) equal to 1/8 and 1/32 led to smaller NPs than the set of standard parameters, there does not appear to be a direct correlation between the concentration of TA and the particle size. Interestingly, the largest ratio of 1/8 compromised the ability of the aggregates to form superstructures on the grid, as the aggregates were present as mostly isolates or small clusters. Moreover, the same ratio led to a small population of Class 2 NPs. That both Class 1 and Class 2 NPs can be generated under the same conditions suggests that there are multiple, competing mechanisms for Rh NP nucleation and growth.
Table 2.2. Mean NP size $\bar{d}$ and polydispersity index $\mathcal{D}$ of Class 1 NPs generated at different values of $n(TA)/n(Rh^{3+})$. An asterisk represents a size distribution that is statistically distinct from the standard size distribution at $p < 0.05$.

<table>
<thead>
<tr>
<th>$n(TA)/n(Rh^{3+})$</th>
<th>$\bar{d}/nm$</th>
<th>$\mathcal{D}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/8</td>
<td>17.0 ± 4.7 (28%)*</td>
<td>1.22</td>
</tr>
<tr>
<td>1/16</td>
<td>21.7 ± 5.2 (24%)</td>
<td>1.15</td>
</tr>
<tr>
<td>1/32</td>
<td>19.3 ± 5.2 (27%)*</td>
<td>1.25</td>
</tr>
<tr>
<td>0</td>
<td>21.7 ± 6.1 (28%)</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Another reaction was constructed with the standard parameters, but with the citrate concentration halved, *i.e.*, $n(\text{citrate})/n(Rh^{3+}) = 8$. Again, this generated Class 1 NPs, this time with a size distribution of $31.5 \pm 20.2$ nm (64%) and polydispersity index of 1.98. This distribution is statistically distinct from that of the standard product. Of note is the large polydispersity index, which implies that a large excess of citrate is needed for a narrow size distribution.

Additional reactions were carried out starting from parameter sets that had previously generated Class 2 products. For the first reaction, the standard parameters were used except with $n(K_2CO_3)/n(Rh^{3+}) = 0$ (pH ~ 7) and $n(\text{citrate})/n(Rh^{3+}) = 32$, *i.e.*, the citrate concentration was doubled at neutral pH. For the second reaction, the standard parameters were used with $n(K_2CO_3)/n(Rh^{3+}) = 0$ (pH ~ 7) and $n(TA)/n(Rh^{3+}) = 1/8$, *i.e.*, the TA concentration was doubled at neutral pH. Each reaction yielded aggregates of Rh NPs with mean NP sizes of $13.7 \pm 3.1$ nm (22%) and $10.9 \pm 2.5$ nm (23%), respectively, and polydispersity indices of 1.14 and 1.16, respectively (Figure 2.5). These size distributions are statistically distinct from the standard size distribution and from one another. They should not, however, be treated as accurate representations of the true particle sizes since the boundaries of the aggregates were poorly defined. This is a consequence of two factors: (1) the small size of the aggregates leads to little mass-thickness contrast, and (2) the aggregates are packed together and in close contact with one another.

Nevertheless, the size distributions do reveal that the aggregate size can be decreased by increasing either the citrate or TA concentration at neutral pH. Although these products are more uniform
than earlier products, the aggregates have highly irregular morphologies and can no longer be
described as spheroidal or quasi-spherical. Furthermore, the particles assemble differently than
spheroidal aggregates, forming networks instead of disordered arrays.

**Figure 2.5.** TEM images of the Class 1 NPs obtained with the standard parameters but with (a) $n(\text{citrate})/n(\text{Rh}^{3+}) = 32$ and (b) $n(\text{TA})/n(\text{Rh}^{3+}) = 1/8$ at a magnification of 30k. The scale bar corresponds to a distance of 200 nm. The brightness and contrast have been enhanced for improved clarity.

In yet another such reaction, the standard parameters were used except with $n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+})$ = 0 (pH ~ 7) and $T= 90 \ ^\circ\text{C}$. This yielded Class 1 NPs with a size distribution of 12.4 ± 2.9 nm (24%) and polydispersity index of 1.16 (Figure 2.6). Again, the differences between this mean size, the standard mean size, and the mean sizes obtained with $n(\text{citrate})/n(\text{Rh}^{3+}) = 32$ and $n(\text{TA})/n(\text{Rh}^{3+})$ = 1/8, both with $n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+}) = 0$, are statistically significant. Although these NPs were irregularly shaped, they had more quasi-spherical character than other NPs synthesized at pH ~ 7.

Overall, we see that the standard set of parameters with $n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+}) = 0$ yields smaller and more irregularly shaped Class 1 NPs when the reduction kinetics are increased by increasing the temperature or concentration of either reducing agent.

Finally, reactions were run with the standard parameters but with $c(\text{Rh}^{3+})$ equal to 4 and 16 mM. In both cases, Class 2 NPs were obtained, mostly in the size range 100–200 nm (Figure 2.7). Each
sample also contained a small population of Class 1 NPs (not shown), again suggesting that there may be multiple, competing mechanisms for NP nucleation and growth.

![TEM image of Class 1 NPs](image)

**Figure 2.6.** TEM image of the Class 1 NPs obtained with the standard parameters but with $n(K_2CO_3)/n(Rh^{3+}) = 0$ and $T = 90 \, ^{\circ}C$ at a magnification of 60k. Small grains are clearly visible. The scale bar corresponds to a distance of 100 nm. The brightness and contrast have been enhanced for improved clarity.

### 2.5 Conclusions

The Turkevich method, as optimized by Piella and coworkers and adapted by the author, can be used to synthesize colloidal Rh NCs. An important modification is the integration of PVP as a stabilizer, since citrate is unable to stabilize Rh NCs colloidally in H$_2$O. Under most parameter sets, the method generated aggregates comprising ultrasmall Rh grains 1–3 nm in size. These aggregates, termed Class 1 NPs, presented a spheroidal/quasi-spherical shape, and had sizes of 11–32 nm with polydispersity indices of 1.1–2.0. At smaller sizes (11–14 nm), Class 1 NPs were irregularly shaped and could no longer be described as spheroidal/quasi-spherical. Furthermore, larger Class 1 NPs had a tendency to form disordered superstructures on the specimen grid, with smaller NPs forming networks.
Figure 2.7. TEM images of the Class 2 NPs obtained with the standard parameters but with \( c(\text{Rh}^{3+}) = 4 \text{ mM} \) (a) and 16 mM (b) at magnifications of 5k and 10k, respectively. The scale bars correspond to distances of 2000 nm and 1000 nm, respectively. The brightness and contrast have been enhanced for improved clarity.

Under other parameter sets, large, aggregated, and anisotropic NCs mostly on the order of 100–200 nm were obtained. These were termed Class 2 NPs. All attempts to control the geometry of Class 2 NPs were unsuccessful and resulted instead in Class 1 NPs. Therefore, most of the following discussion is centered on Class 1 NPs.

From the experiments and observations above, the following conclusions can be drawn:

- The pH is critical in determining the properties of the product. With \( n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+}) \) equal to 64 (pH ~ 9) and 32 (pH ~ 8.5), Class 1 NPs are obtained. Increasing \( n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+}) \) from 32 to 64 causes the aggregate and grain size to decrease, but polydispersity index to increase. Decreasing \( n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+}) \) from 32 to 16 (pH ~ 8) and 0 (pH ~ 7) instead results in Class 2 NPs.

- With \( n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+}) = 0 \), increasing either \( n(\text{citrate})/n(\text{Rh}^{3+}) \), \( n(\text{TA})/n(\text{Rh}^{3+}) \), or \( T \) yields small, irregularly shaped Class 1 NPs that have a tendency to form networks on the grid.

- There does not appear to be a direct relationship between the Class 1 NP size distribution and the temperature in the range 60–80 °C. The temperature may play a role in determining the
efficiency of assembly of Rh grains into aggregates with $n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+}) = 32$, with $80 ^\circ \text{C}$ leading to complete assembly. However, assembly is incomplete at even $90 ^\circ \text{C}$ with $n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+}) = 0$, implying that both the temperature and pH determine the extent of assembly.

- There does not appear to be a direct relationship between the aggregate size distribution and $n(\text{T}A)/n(\text{Rh}^{3+})$. Different ratios produce different size distributions which, in some cases, are statistically distinct. However, this could be attributed to conditional variations. In contrast, with $n(\text{K}_2\text{CO}_3)/n(\text{Rh}^{3+}) = 0$, increasing $n(\text{T}A)/n(\text{Rh}^{3+})$ from 1/16 to 1/8 results in a significant decrease in the aggregate size, as well as a loss of uniformity in the aggregate shape. Additionally, the ratio of 1/8 was observed to compromise the ability of the aggregates to form superstructures on the grid.

- A large excess of citrate may encourage a narrower size distribution of Class 1 NPs, as demonstrated by the observation that decreasing $n(\text{citrate})/n(\text{Rh}^{3+})$ from 16 to 8 increases the polydispersity index from 1.15 to 1.98.

- Setting $c(\text{Rh}^{3+})$ to 4 and 16 mM, and setting $n(\text{T}A)/n(\text{Rh}^{3+})$ to 1/8, led to both Class 1 and Class 2 NPs, implicating multiple, competing mechanisms for nucleation and growth.

The author proposes three opportunities for future work:

1. continue with systematic parametric variation to develop a more complete map of the synthetic space and identify one or more parameter sets that give rise to UV-plasmonic, monodisperse, single-crystal Rh NCs;

2. following the work of Polte and coworkers, develop a mechanistic understanding of the reaction using in situ SAXS and XANES data, which would guide the work in (1);

3. retain the standard parameters and make alternative changes, including: introducing an agent that forms a more stable complex with Rh$^{3+}$, making the metal ion more difficult to reduce; introducing surfactants per Ewers et al. to control the grain size; changing the solvent composition; and changing the identity or molecular weight of the polymer.
Ewers et al. expressed interest in synthesizing subnanometric Rh clusters, which they hypothesized would result in ferromagnetic aggregate NPs. However, they were unable to synthesize grains smaller than 1 nm. It may be possible to tweak the parameters in the method described herein to generate subnanometric, magnetic Rh clusters, e.g., by increasing the pH.

Overall, the adapted Turkevich method is a promising alternative to the method reported by Ewers et al., offering finer control over the kinetics of reduction. With further optimization, it has the potential to become a reliable, greener alternative to the polyol method, which stands as the current state of the art for the synthesis of Rh NCs.
Chapter 3
Photonic Nanoparticles for Cellular and Tissular Labeling

As part of their studies, the author was tasked with updating the following work in collaboration with Caroline Pao, a doctoral student in the Walker group:


In this third and final chapter, the author’s contributions to this book chapter are described briefly. The second edition, entirely revised, has been submitted and accepted for publication as:


3.1 Photonic Nanoparticles

A photonic NP (phNP) is a NP that emits light in visible or near-infrared (NIR) optical window. Because of their variable electrical, optical, and magnetic properties, phNPs show great promise as a diagnostic tool in the clinician’s arsenal, and are a prime example of how nanotechnology is driving biomedical science.

The advantages of using phNPs over conventional molecular fluorophores were enumerated. Key advantages include: (1) superior geometry-dependent optical properties; (2) diverse surface functionalization chemistries; (3) ability to deliver therapeutic payloads; (4) ability to quantitate small physiologically relevant molecules and physiological parameters, *e.g.*, pH; (5) ability to perform multiplexed analysis and multicolour imaging; and (6) support for multiple, complementary imaging modalities, *e.g.*, optical/magnetic resonance imaging.

Next, considerations that apply to all classes of phNP were covered. The importance of gaining an understanding of how phNPs, biomolecules, and cells interact with one another, and applying that knowledge to design effective phNPs, was stressed. The analysis focused on the biomolecular corona, and how it dictates the interactions between phNPs and the biological milieu. The notion
of engineering the biomolecular corona to overcome the major, unsolved problem of phNP clearance through the mononuclear phagocytic and renal systems was recapitulated. The assessment of phNP toxicity using in vitro and in vivo assays was mentioned. In addition, different surface functionalization techniques for imparting phNPs with colloidal stability and/or a targeting capability were mentioned. The use of amphiphilic polymers, including poly(ethylene glycol)-functionalized phospholipids and co-block polymers, was highlighted.

The importance of optical imaging in the NIR optical window (700–1700 nm) was discussed. In this spectral range, there is little tissue autofluorescence, and less absorption and scattering by blood and tissue. This permits images of higher quality to be acquired more rapidly.

The author next covered two classes of phNP: semiconducting inorganic nanocrystals (quantum dots, QDs), and semiconducting polymer nanoparticles (sPNPs). Both classes of phNP are fluorescent, and their emission can be described using similar excitonic models. These classes have been compared often, as sPNPs are perceived as an emerging, more biocompatible alternative to QDs that offer competitive photophysical properties. For both classes of phNP, the photophysics, synthesis, and toxicity thereof were discussed. Key advances within the past two years were highlighted, and trends and opportunities for additional work were identified.

3.2 QDs

QDs are a type of phNP with an extensive history. A typical QD consists of a binary compound of elements in Groups II and VI (e.g., cadmium(II) selenide, CdSe), III and V (e.g., indium(III) arsenide, InAs), or IV and VI (e.g., lead(II) sulfide, PbS). Today, most QDs have a core/shell structure, with CdSe/ZnS (ZnS = zinc sulfide) core/shell QDs being a common example. Such QDs have been commercialized as dispersions (e.g., Qdot®) and cellular labeling kits (e.g., Qtracker™). Concerns surrounding the toxicity of heavy metals in conventional QDs have motivated the exploration of QDs comprising other materials, such as silicon (Si), silver(I) sulfide (Ag2S), and copper indium sulfide (CIS).

That QDs are semiconductors on the nanoscale was mentioned, and the effect of the bulk-to-nanoscale transition on the electronic structure of semiconductors was discussed in terms of band
theory. The concept of quantum confinement was introduced and the dependence of the semiconductor band gap, which determines the emission wavelength, on the QD radius was explained. Trap states, which compromise the optical properties of QDs by decreasing the photoluminescence quantum yield, were introduced. A host of current research was reviewed, which has applied hydrogen-1 nuclear magnetic resonance spectroscopy and density functional theory calculations to show that the displacement of cadmium(II) carboxylate complexes from the QD surface creates surface cadmium (Cd) vacancies and exposes dicoordinated selenium atoms, ultimately generating trap states.\textsuperscript{45–48} The importance of growing a shell of at least one semiconducting material around a QD to create a core/shell QD and eliminate trap states was stated. Moreover, the state of the art of the synthesis of QDs (the hot injection method) was outlined.

The controversial toxicity of QDs containing heavy metals, such as Cd and lead (Pb), was discussed at length. Emphasis was placed on a study by Tsoi \textit{et al.}, in which inconsistencies between \textit{in vitro} and \textit{in vivo} toxicity studies were noted, and a number of recommendations were made for attaining more rigorous conclusions about the toxicity of QDs. These include the standardization of dose metrics, measurement of both the extracellular and intracellular QD concentrations, identification of appropriate \textit{in vitro} models that reflect the \textit{in vivo} QD biodistribution, and use of multiple assays to evaluate toxicity through a number of parameters.\textsuperscript{49} Furthermore, the author highlighted several toxicity studies, including a meta-analysis of the cytotoxicity of Cd-based QDs by Oh and coworkers.\textsuperscript{50} Other studies were noted for their multidimensional approaches. For example, Manshan \textit{et al.} investigated the toxicity of core/shell CdSe/ZnS QDs, encapsulated by electrostatically charged amphiphilic polymers, on two different cell types. In this study, the toxicity profile of each type of QD was expressed in terms of both the administered concentration as well as the intracellular concentration. A crucial finding was that the toxicity profile depends strongly on the concentration by which it is expressed, with the intracellular concentration accounting for the cellular uptake efficiency. Toxicity was assessed in terms of a number of parameters, such as cytotoxicity, oxidative stress, cytoskeletal health, cellular morphology, and gene expression. Surprisingly, both QDs had some positive effects on gene regulation.\textsuperscript{51}

It was noted that the controversy surrounding QDs containing one or more heavy metals has driven interest in QDs free of heavy metals, comprising, \textit{e.g.}, CIS. However, QDs containing Cd and Pb have maintained their relevance. For example, a key trend identified was the synthesis of PbS QDs for bioimaging in the NIR optical window. In addition, other advances were accentuated, such as
the development of QDs bearing InAs cores for high-speed z-sectioned angiography of the mouse brain, among other applications. A growing interest in Si nanostructures was noted. Finally, it was mentioned that a number of recent reports have described QDs supporting both fluorescence and magnetic resonance imaging modalities.

3.3 SPNPs

SPNPs are an emerging class of phNP. A sPNP consists of one or more semiconducting polymers (sPs), e.g., poly(9,9-dioctylfluorenyl-2,7-diyl), that possess extended π-conjugation. Like QDs, sPNPs are attractive probes for bioimaging and are often compared to QDs. SPNPs may be a more biocompatible alternative to QDs that offers competitive photophysical properties.

The photophysics and synthesis of sPNPs were covered briefly. The emission mechanism was described in terms of excitonic recombination across the π and π* molecular orbitals, and strategies for adjusting the band gap were prescribed. The importance of the sP conformation to sPNP photoluminescence was mentioned. Two synthetic approaches, direct synthesis and post-polymerization, were identified and compared.

As with QDs, much discussion was dedicated to toxicity studies centered on sPNPs. This is particularly important, since sPNPs are a novel class of phNP whose biocompatibility should be established early on. A number of comprehensive toxicity investigations were summarized. Of note was a mouse model used by Wu et al. to examine the reproductive toxicity and teratogenicity of sPNPs comprising two sPs encapsulated by an amphiphilic polymer. Wu et al. tracked body weight changes and organ coefficients, ran histological analyses, assessed markers of hepatic and renal function, checked hormone and gene expression levels, and looked at the morphologies of the fetus, placenta, and uterus following injection. Overall, it was deduced that the sPNPs did not pose a reproductive threat to the mouse model.

The author of this thesis concluded that, up to this point, investigations suggest that sPNPs have limited cytotoxicity, but asserted that a larger body of work needs to be established. It was proposed that the recommendations made by Tsoi et al. for QDs are just as relevant to sPNPs, and should be followed by researchers to facilitate the approval of sPNPs for clinical trials.
It was noted that a large number of sPNPs in the past two years have been developed for NIR photoacoustic imaging, a non-invasive and sensitive technique with good penetration depth. The results of a number of these reports were summarized. For example, Qin and coworkers reported sPNPs, equipped with cell-penetrating peptides, for tracking human embryonic stem cell-derived cardiomyocytes transplanted into living mouse hearts. The authors collected three-dimensional photoacoustic information about the relative positions of the transplanted cardiomyocytes and host myocardium.\textsuperscript{54}

### 3.4 Perspectives

Overall, the following trends were identified:

- There is a continued interest in developing nanolabels that operate in the NIR optical window, as exemplified by PbS QDs and sPNPs for photoacoustic imaging.

- Concerns centered on the toxicity of heavy metals have driven the development of QDs comprising Si, Ag\textsubscript{2}S, and C\textsubscript{11}S.

- Intensive efforts have been directed toward the synthesis of multimodal phNPs, which combine complementary imaging modalities to yield more accurate diagnostic information. Examples include manganese(II)- and gadolinium(III)-doped QDs as well as hybrid Au/iron oxide NPs. However, this trend is not observed with sPNPs. The preparation of sPNPs for optical/magnetic resonance imaging may be an area worth exploring.

A number of opportunities were pointed out for future work, with emphasis placed on interdisciplinary work that will help overcome major challenges facing the field:

- A more complete understanding of the biomolecular corona must be attained and applied so that the efficacy of phNPs can be improved by, for example, helping to overcome phNP self-association and non-specific binding.

- More exhaustive toxicity studies need to be carried out for novel phNPs, as most reports contain only a single cytotoxicity assay that evaluates cell viability in terms of metabolic activity. A good starting point would be to assess cell viability in terms of other parameters. The sPNP class would benefit from this especially, as the existing body of work is small.
• Designers of pHNPs should report metrics used by clinicians as well as those employed in chemical analytics. Clinical metrics of efficacy have been tabulated by Chan et al.55
References


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