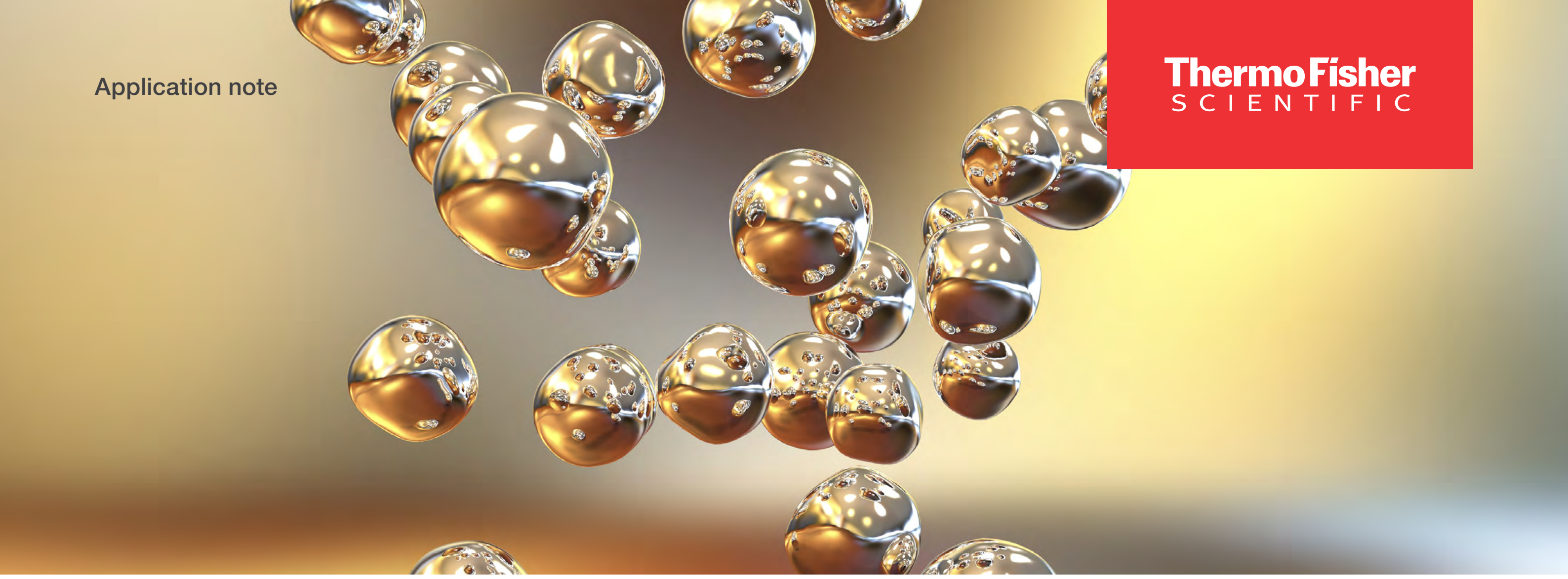
Application note



Quantification of gold nanoshells using the NanoDrop One Microvolume UV-Vis Spectrophotometer

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Introduction

Nanoshells (NS) are spherical nanoparticles consisting of a dielectric (e.g., silica) core surrounded by an ultrathin metallic shell, most commonly gold. One of most important characteristics of gold NS is their strong surface plasmon resonance (SPR). By changing the relative core size and shell thickness, the plasmon resonance of the NS can be tuned to specific wavelengths across the visible and near-infrared (NIR) regions of the electromagnetic spectrum.[1] More precisely, NS with thinner shells have peak extinctions that are red-shifted to longer wavelengths relative to NS with thicker shells but a similar core diameter. Because of their highly favorable optical, physical and chemical characteristics, as well as their benign toxicity profile, NS have been used in numerous biomedical applications, including as imaging contrasts agents and as photothermal therapeutics, with proven preclinical and clinical success.[2–7]

To properly utilize NS in different applications, it is crucial to be able to accurately determine their concentration. Traditionally, UV-Vis spectrophotometers are used to measure the optical density (OD) of the NS in suspension. The OD of the sample at the peak resonance wavelength can then be utilized to calculate the concentration via Beer's Law. Conventional cuvette-based UV-Vis spectrophotometers have been utilized for characterizing NS, but they have presented several drawbacks. First, the use of a standard fixed pathlength (10 mm) for cuvette samples limits the linear range of the instrument. NS are often produced in high concentrations with an OD that falls outside the linear range of the spectrophotometer, requiring samples to be diluted before measurement. Sample dilution is time-consuming and increases the likelihood of inaccurate measurements due to pipetting errors. In addition to this, traditional spectrophotometers often require large sample volumes (ranging from 0.5 mL to 3 mL) which is not ideal for preserving samples that may be especially precious following lengthy purification and modification.

The Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer presents a low volume alternative to the use of a cuvette-based spectrophotometer. The NanoDrop One instrument uses surface tension to hold a small volume (1 to 2 µL) of sample between two pedestals, which allows researchers to retain most of their valuable samples for downstream applications. Additionally, the variable, auto-ranging pathlengths (0.03 to 1.0 mm) of the NanoDrop One Spectrophotometer allow users to measure highly concentrated samples without dilution by expanding the instrument's dynamic range. In this application note, we describe our research on quantification of gold NS with a diameter of 150 nm using the NanoDrop One instrument.

NanoDrop One Microvolume UV-Vis Spectrophotometer

Experimental procedures

NS were synthesized by published protocols via the Oldenburg method.[1] First 3–5 nm diameter gold colloid was made by the Duff method[8] from hydrogen tetrachloroaurate (III) hydrate ($HauCl_4$) (VWR), tetrakis(hydroxymethyl)phosphonium chloride (VWR), and 1 N sodium hydroxide (Fisher Scientific). The gold colloid was then combined with 120 nm diameter silica spheres functionalized with 3-aminopropyltriethoxysilane (Nanocomposix) and 1 M sodium chloride (NaCl) and rocked for 3–4 days at room temperature to create "seed" nanoparticles. The seed was purified twice via centrifugation at 3000 rpm for 30 minutes each and resuspended in milliQ water to an OD of 0.1 at 530 nm. The diluted seed was mixed with additional $HAuCl_4$ diluted in potassium chloride followed by addition of a small volume of 37% formaldehyde (VWR). The mixed solution was rapidly agitated to form complete gold shells and purified twice via centrifugation at 500 *g* for 15 minutes each. The hydrodynamic diameter of the NS was measured via Dynamic Light Scattering utilizing an Anton Paar® Litesizer® instrument, which revealed the NS were 150 nm in diameter. For conventional spectrophotometry, the NS were placed in 1 cm pathlength disposable cuvettes and read on an Agilent® Cary® 60 UV-Vis spectrophotometer from 1100 nm to 400 nm. The NS concentrations were calculated from Beer's Law using the peak extinction (OD at ~800 nm) as determined by the spectrophotometer and the theoretical extinction coefficient of NS with 120 nm diameter silica cores and 15 nm thick gold shells. This revealed the initial NS had a concentration of 9 pM. The NS were then pelleted by centrifugation at 500 *g* for 15 minutes and diluted in water to prepare samples ranging from 100 pM to 0.5 pM. The OD of these NS were then measured using a NanoDrop One Spectrophotometer by pipetting 2 µL aliquots directly on the sample pedestal. Between measurements, the Nanodrop One sample pedestal was simply cleaned using a lint-free Kimwipe®. The auto pathlength option was turned on in the NanoDrop One software for each measurement.

Results

The absorption spectra of NS with concentrations over a range of 0.5 pM to 100 pM as measured by the NanoDrop One Spectrophotometer are shown in Figure 1A. These spectra reveal the NS have a peak plasmon resonance at ~800 nm, which is consistent with the spectra obtained using a reference spectrophotometer. The corresponding OD of NS at the peak absorbance (~800 nm) was plotted against the concentration (Figure 1B). The plot showed a strong linear relationship over a wide concentration range (0.5 pM to 100 pM) with an R^2 value of 0.9983. Importantly, the spectra produced by the NanoDrop One Spectrophotometer were highly reproducible and precise, with high signal-to-noise ratios (low standard deviation for triplicate measurements at each concentration) and could be utilized to accurately measure the concentration of 150 nm diameter NS up to 100 pM without any dilution required. This concentration is approximately 10–20X higher than what can be accurately measured using a standard cuvette-based spectrophotometer.

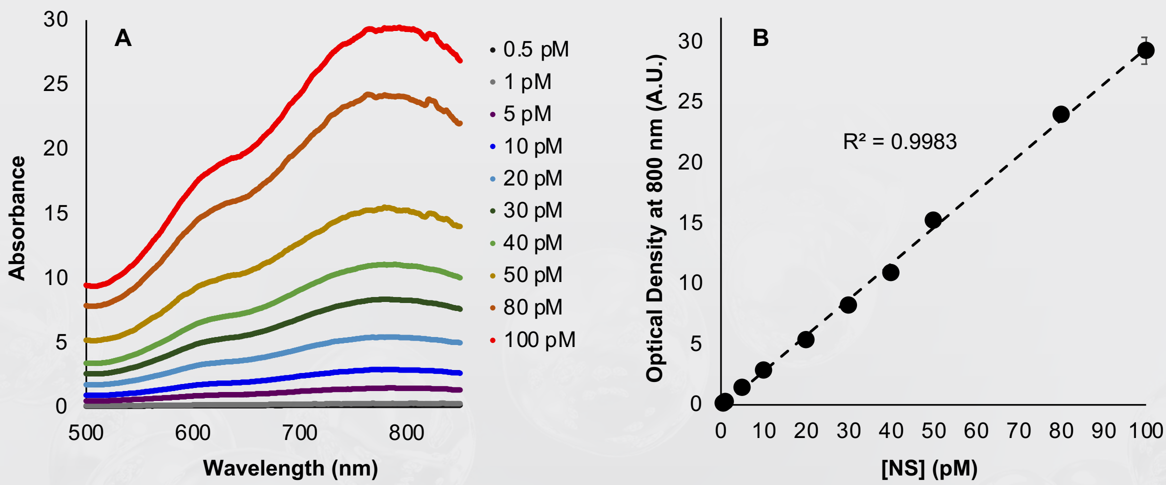

Figure 1: (A) UV-Vis spectra for 150 nm NS at concentrations ranging from 0.5 pM to 100 pM, as measured on the NanoDrop One Spectrophotometer. (B) The OD of NS at 800 nm as measured by the NanoDrop One Spectrophotometer plotted versus known concentration, showing a linear relationship over a large range of 0.5 pM to 100 pM with an R^2 value or 0.9983. n=3.

Conclusions

This study demonstrates that the NanoDrop One Microvolume UV-Vis Spectrophotometer can be easily integrated into the workflow of NS synthesis and characterization to determine an accurate concentration. The NanoDrop One Spectrophotometer was found to be very versatile in the analysis of 150 nm diameter NS concentration. The major asset of the NanoDrop One Spectrophotometer compared to a traditional cuvette-based spectrophotometer is the ability to measure highly concentrated samples in a 1–2 µL sample volume. This advantage is useful for conserving precious samples and preparing doses for in vitro or in vivo studies. The NanoDrop One Spectrophotometer is an ideal, easy-to-use instrument that can be a valuable addition to the workflow of a nanotechnology laboratory.

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