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APPLICATION NOTE

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

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Key Words

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Thermo Scientific NanoDrop One/One^{C} Microvolume UV-Vis Spectrophotometer

Introduction

Gold nanoparticles (AuNPs) have played a significant role in advancing a wide array of biomedical applications, including molecular diagnostics, targeted drug delivery, nucleic acid delivery, and photothermal therapies. AuNPs' unique optical properties, biocompatible features (e.g., low toxicity, low immune reactivity), and ease of synthesis have facilitated their rapid implementation in biomedical research.

Perhaps the most important characteristic of AuNPs is their surface plasmon resonance (SPR). SPR can be described as the collective oscillation of surface electrons when AuNPs are irradiated with light. This SPR property depends on the size and shape of the AuNPs and the surrounding colloidal medium. For example, 13 nm diameter AuNPs that are suspended in water have a peak resonance wavelength of ~520 nm. AuNPs' SPR properties can be used for sample characterization, particularly to evaluate the concentration of AuNPs within suspension, since the optical density of the sample at the peak resonance wavelength may be related to concentration via Beer's Law. Traditionally, UV-Vis spectrophotometers with a 1 cm pathlength are used to measure the optical density of AuNPs at their SPR wavelength; however, this method has several disadvantages. One is that the fixed pathlength of standard spectrophotometers limits the linear range of the instrument. If an AuNP sample is too concentrated such that it has an extinction coefficient outside the linear range of the instrument, the sample needs to be diluted so that its extinction falls within the linear range of the spectrophotometer. This increases the likelihood for the measurement to be inaccurate due to pipetting errors. Another disadvantage of standard spectrophotometers is that they require large sample volumes (ranging from 0.5 mL to 3 mL), which may not be available after synthesized AuNPs have been modified with any surface-bound moieties and subsequently purified.

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The Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer is uniquely suited to characterize the SPR band of AuNP preparations because it can overcome these disadvantages. The instrument utilizes short, variable pathlengths (0.03–1.0 mm), thus allowing measurements of highly concentrated AuNP preparations without the need for dilution. In addition, the NanoDrop One Spectrophotometer takes sample measurements by using a minimal sample volume (1 to 2 µL); thus, researchers can retain most of their AuNP preparation for use in downstream applications. In this application note, we describe our research on the accuracy of the NanoDrop One instrument for quantification of the SPR band in solid spherical AuNPs with a diameter of 13 nm.

Experimental procedures

The AuNPs used in this experiment were synthesized according to the Frens method.[1] Briefly, gold chloride (Sigma-Aldrich, St. Louis, MO) was dissolved in deionized (DI) water and brought to a boil under reflux. Sodium citrate was added while stirring vigorously, and the solution reacted for 15 min until the color changed from pale yellow to deep red. The synthesized AuNPs were then filtered to purify the sample. The diameter of the AuNPs was measured via transmission electron microscopy utilizing a Zeiss LIBRA 120 instrument, which revealed the AuNPs were 13 nm in diameter. For spectrophotometry, the AuNPs were placed in a 1 cm cuvette and scanned on a traditional spectrophotometer from 850 nm to 450 nm, after subtracting the baseline with water. The optical density (OD) of the AuNPs was determined at the peak SPR wavelength (521 nm) and the concentration calculated using this value in Beer's Law, where 2.7×10^8 M^{-1} cm^{-1} was used as the extinction coefficient for 13 nm AuNPs. This revealed the initial AuNP sample had a concentration of 12 nM. To produce samples of various concentrations, the AuNPs were pelleted by centrifugation at 12000 *xg* for 5 minutes, and then systematically diluted in water to prepare samples ranging from 150 nM to 10 nM. The SPR characteristics of these AuNP samples were then evaluated using both a traditional spectrophotometer and a NanoDrop One Spectrophotometer. To measure the SPR of AuNPs in highly concentrated suspensions by spectrophotometry, the AuNPs were diluted 100-fold in deionized water and SPR data collected using a traditional spectrophotometer as described above; this dilution step was not required for analysis with the NanoDrop One Spectrophotometer. For measurements on the NanoDrop One Spectrophotometer, 2 µL aliquots were placed directly on the sample pedestal, which was cleaned using a lint-free Kimwipe®. The auto-pathlength option was turned on in the NanoDrop One Software for each measurement.

Results

The UV-Vis spectra and standard curve correlating peak SPR with concentration for AuNPs as measured by the NanoDrop One Spectrophotometer are shown in Figure 1A and 1B, respectively. As shown in Figure 1B, the optical density of the AuNPs at the peak SPR is linearly correlated with AuNP concentration over a range of 10 nM to 150 nM with an R^2 value of 0.9996. The instrument measurements were very precise, as the standard deviations for triplicate measurements of all samples were within 1% of the respective absorbance values at each concentration. The spectral measurements produced by the NanoDrop One Spectrophotometer were highly reproducible using small volumes of 2 µL and the instrument could be utilized to reliably evaluate the concentration of 13 nm diameter AuNPs up to 150 nM without any dilutions required.

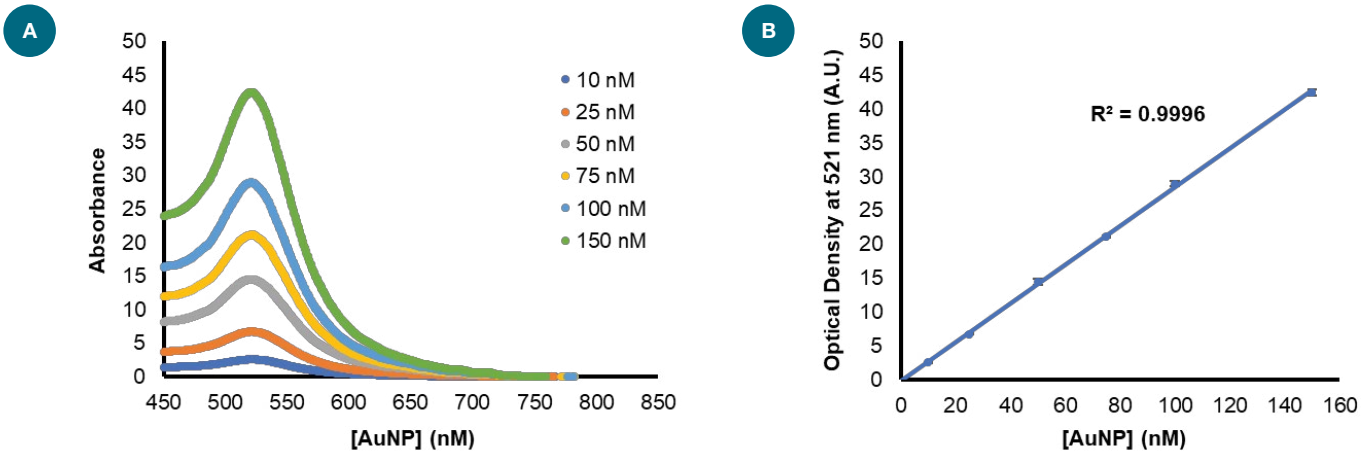

Figure 1: (A) UV-Vis spectra for 13 nm AuNPs at concentrations ranging from 10 nM to 150 nM, as measured on the NanoDrop One Spectrophotometer. (B) The optical density of AuNPs at 521 nm as measured by the NanoDrop One Spectrophotometer plotted versus known concentration, showing a linear relationship over a large range of 10 nM to 150 nM with an R^2 value or 0.9996. n=3.

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Conclusion

The Thermo Scientific NanoDrop One Microvolume UV-Vis Spectrophotometer was found to be a very fast and accurate instrument to evaluate the concentration of 13 nm diameter AuNPs, as it can reliably measure SPR spectra, which can be related to concentration through Beer's Law. The major advantage of the NanoDrop One Spectrophotometer compared to traditional spectrophotometers is that it requires minimum sample preparation and comes with user-friendly software that allows for custom calculations to be performed. The high absorbance capability and low sample volume requirement of the NanoDrop One Spectrophotometer make it ideally suited for metal nanoparticle concentration measurements. While only 13 nm AuNPs were evaluated here, it is likely that the NanoDrop One Spectrophotometer could be used to rapidly and accurately analyze the SPR and concentration of AuNPs of various diameters.

Reference

1. Frens, G., Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. *Nature Physical Science* **1973**, *241*, 20.

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