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Metallic Colloid Wavelength-Ratiometric Scattering Sensors

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Abstract

Gold and silver colloids display strong colors as a result of electron oscillations induced by incident light, which are referred to as the plasmon absorption. This absorption is dependent on colloid–colloid proximity, which has been the basis of absorption assays using colloids. We now describe a new approach to optical sensing using the light scattering properties of colloids. Colloid aggregation was induced by avidin–biotin interactions, which shifted the plasmon absorption to longer wavelengths. We found the spectral shift results in changes in the scattering at different incident wavelengths. By measuring the ratio of scattered intensities at two incident wavelengths, this measurement was made independent of the total colloid concentration. The high scattering efficiency of the colloids resulted in intensities equivalent to fluorescence when normalized by the optical density of the fluorophore and colloid. This approach can be used in a wide variety of assay formats, including those commonly used with fluorescence detection.

At present, there is intense interest in the optical properties of noble metal colloids. Such colloid suspensions display brilliant colors as a result of intense light absorption and scattering, a fact first recognized by M. Faraday.¹ These properties are due to electron oscillations in the metallic particles induced by the incident light field giving rise to plasmon absorption.^{2–3} These properties have great potential for the control and manipulation of light in nanophotonic devices.⁴ As examples, the interaction of light with a surface plasmon can increase the output of light-emitting diodes,⁵ multiphoton excitation can be dramatically enhanced,⁶ and light can be efficiently transmitted through subwavelength apertures when the surfaces are coated with a thin metallic film.^{7–8} The optical properties of metallic colloids have also found use in biotechnology.^{9–10} Colloid–colloid proximity induced by surface-bound DNA has been used to measure DNA hybridization¹¹ and to develop optical sensors for metal ions.^{12–13}

To date, all reported biological applications of metal colloids have been based on measurements of the plasmon absorption, as seen by direct absorption measurements or the visual color.^{14–15} However, it has been reported that the light scattered from individual colloids can be equivalent to the intensity of 10^5 fluorescence molecules.^{16–18} Upon first examination, there appeared to be little use of the scattered light for sensing. The scattered light does not have the information content of fluorescence and did not appear to provide an opportunity for measurements that are not sensitive to total intensity, such as anisotropy or wavelength-ratiometric measurements. However, we recognized that shifts in the plasmon absorption should be detectable by the extent of light scattered at various incident wavelengths. We used avidin/biotin binding to induce clustering of colloids, which modified the plasmon resonance. We found that the ratio of light scattered at two incident wavelengths could be used to determine the extent of colloid aggregation. Importantly, the ratio of scattered intensities was independent

of the total colloid concentration over a wide range of colloid concentrations. Additionally, the scattered intensity was somewhat brighter than a solution of rhodamine B at the same optical density. These results indicated that wavelength-ratiometric light scattered by colloids can be a new generic approach to bioaffinity assays, such as DNA hybridization and immunoassays.

MATERIALS AND METHODS

Chemicals

Avidin from egg white was obtained from Sigma (A-9275). Biotinylated bovine serum albumin (B-BSA) was obtained from Sigma (A-6043). Sodium citrate (trisodium salt of citric acid dihydrate) was obtained from Sigma (C-0909). Gold colloid was prepared from hydrogen tetrachloroaurate (III) trihydrate purchased from Aldrich (52,091-8). Millipore purified water was used for all solutions.

Methods

Gold colloids were prepared by the standard citrate reduction of HAuCl_4 , as described previously.^{19–20} A 68-mg portion of HAuCl_4 was dissolved in 200 mL of Millipore purified water (1 mM) and brought to vigorous boiling with stirring. A 20-mL portion of 38.8 mM sodium citrate was added, and the stirring continued for another 10 min. The solution changed from colorless to deep red. After 10 min, the solution was rapidly cooled in an ice water bath with stirring. This method produces a gold nanoparticle colloid with an average diameter of 15 nm and 10% polydispersity.²⁰

The colloids were then coated with biotinylated bovine albumin. A 50-mL portion of the gold colloid was mixed with a 0.5-mL aqueous solution of biotinamidocaproyl-labeled bovine serum albumin (1.44 mg/mL). The mixture was incubated at room temperature for 2 h. The colloid was spun for 1 h in a JA-25.50 rotor at 18 000 rpm (39191g) at 18 °C (Avanti J-25I centrifuge) to pellet the biotinylated bovine albumin-coated gold colloid. The supernatant was carefully removed, and the colloid was resuspended in 1 mM sodium citrate.

To set up the aggregation assay, a series of 3-mL samples of biotinylated bovine albumin-coated gold colloid was prepared in plastic cuvettes and mixed with increasing concentrations of avidin. An aqueous stock avidin solution of 1.6×10^{-6} M was prepared on the basis of an extinction coefficient of ϵ (280 nm) = 90 000 $\text{M}^{-1} \text{cm}^{-1}$. Serial dilutions were prepared and added to each gold colloid sample to achieve the desired final avidin concentrations. Each colloid sample was mixed upon addition of avidin and incubated at room temperature for 1 h. The degree of aggregation was measured by recording the absorption spectrum of each sample using an 8453 Hewlett-Packard diode array spectrophotometer. Light scattering was measured using an SLM 8000 spectrofluorometer with white light LED illumination. The white LED was obtained from Radio Shack and was powered with 3 V from two 1.5-V batteries.

RESULTS

Colloidal suspensions are brightly colored. Figure 1 shows the absorption spectra of gold colloids coated with B-BSA. For preciseness, we note that we use the term “absorption” for convenience. More properly we should use the term “extinction”, because the absorption spectra and visible colors are due to both absorption and scattering. Upon addition of avidin, the absorption spectra shift to longer wavelength with a characteristic increase in the long wavelength absorption. The binding of avidin to the gold colloids coated with B-BSA is relatively rapid and is essentially complete in 15 min at room temperature. The shift in absorption can be seen visually (Figure 2), which has been the basis of some biological assays.
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Since the color of the colloids is due in part to light scattering, we reasoned that the absorption spectral shifts would result in an increase in the scattered intensity for longer wavelength illumination. To choose appropriate wavelengths for the wavelength-ratiometric measurements, the OD values for the monomer gold colloid UV–visible spectrum can be divided by the OD values for the aggregate gold colloid UV–visible spectrum, and the resultant ratio can be plotted versus wavelength, as shown in Figure 2 (bottom). To obtain the best dynamic range for the ratiometric determination, we can choose a wavelength at the peak of the spectrum ratio (530 nm) and a wavelength approaching the minimum of the spectrum ratio (680 nm). The light scattering intensity at 530 nm is also the region of maximum absorption of the gold colloid, as identified by the upper arrow in Figure 1. If one chooses a wavelength at the very minimum of the spectrum ratio, the light scattering intensity may be too low to accurately detect for the ratiometric measurements. In addition, at the 680 nm wavelength, the aggregate gold colloid shows a higher absorption than the monomeric gold colloid, as identified by the other arrow in Figure 1.

Figure 3 (lower left) shows the scattered light intensity for the monomeric colloid as a function of OD at 530 and 680 nm. The amount of light scattering was found to be linear with colloid optical density at least up to an OD of 0.04. The light scattering by the monomer is ~100-fold greater at 530 nm than at 680 nm. Figure 3 also shows the scattered light intensity for the aggregate colloid as a function of OD at 530 and 680 nm (lower right). The light scattering by the aggregate is ~10-fold greater at 530 nm vs 680 nm.

In comparing the monomeric colloid in the left panel to the aggregate colloid in the right panel, the amount of light scattered at 530 nm for nearly the same optical density is ~3-fold larger for the colloid aggregates, as compared to the monomer. This result seems consistent with previous reports that showed that the scattering cross section increased with the colloid size.^{16–17} However, these reports referred to the size of colloid monomers and not aggregates. This result suggests that the increase in scattered light can be used to measure association reactions, even if the plasmon absorption is not shifted. In comparison, the light scattering at 680 nm is 30-fold larger for the aggregate gold colloid, as compared to the monomeric gold colloid. Hence, long-wavelength scattering could be an even more sensitive indicator of aggregation than scattering at the absorption maximum.

Wavelength-ratiometric measurements are widely used in fluorescence as a means to avoid the dependence of the measurement on signal intensity. We considered whether such ratiometric measurements would be useful with light scattering by colloids. The upper panels in Figure 3 shows the ratio of scattered intensities ($I(530\text{ nm})/I(680\text{ nm})$) for the colloid monomers and aggregates. This ratio is smaller for the aggregates because of their increased scattering intensity at longer wavelengths. We note that a useful change in the scattering ratio with incident wavelength was not obvious, because the increased intensity at 530 nm upon aggregation could have canceled the relative increase at 680 nm.

To be useful for ratiometric sensing, the scattering intensity ratio must be independent of colloid concentrations. Figure 3 (upper panels) shows the intensity ratio as the samples were diluted. The intensities remain constant down to an optical density of 0.004, where background from the sample contributes to the signal. A preliminary study showed that scattering from the samples was detectable close to an optical density of 4×10^{-5} , which resulted in a signal-to-background ratio of 2 (Figure 4). In the future, it seems likely that the detection limit could be increased if the measurements were performed with larger gold colloids or silver colloids, both of which display larger cross sections for scattering.^{16–17} We used the scattering ratio to measure the extent of colloid aggregates (Figure 5). The $I(530\text{ nm})/I(680\text{ nm})$ ratio decreases upon addition of avidin. The large 3-fold range in the scattering ratio is a good dynamic range

for biological affinity arrays. The scattering ratio may vary in different experiments and would be different if the output of the white light source was different for a different light source.

It is of interest to compare the scattered intensity from the colloids with the fluorescence intensity at comparable optical densities. This comparison is important because previous reports have shown that a single colloid can display the intensity equivalent to 10^5 fluorescein molecules.^{16–17} Describing the scattering properties in this way gives a misleading impression that scattered light from colloids can be 10^5 -fold brighter than fluorescence. Figure 6 shows a comparison of the intensity of light scattered from gold colloids at 550 nm with the intensity of rhodamine B excited at 550 nm. Rhodamine B was observed through a monochromator with a band-pass smaller than the width of its emission spectrum. The detection efficiency of our instrument is not very different at 550 and 580 nm. These data show that the scattered light from the colloids at 550 nm is ~2-fold higher than rhodamine B fluorescence at 580 nm at the same optical density (Figure 6), which shows the high sensitivity of colloid light scattering. However, it is important to note that this comparison favors the colloids. The light scattered by the colloids has the same bandwidth as the incident light, whereas the fluorescence is detected with a bandwidth that rejects part of the emission. Additionally, the signal was detected through a vertically oriented polarizer. The light scattered from the colloids was found to be highly polarized. Hence, detection with a different polarizer orientation could change the comparison in favor of rhodamine B.

To be useful for medical sensing, the method must be possible with a simple device. Hence, we examined the gold colloids with illumination by a white light emitting diode (LED). Figure 7 shows the intensity of light scattered over a range of observation wavelengths. These scattering spectra resemble the colloid absorption spectra, but it should be remembered that the intensities are weighted by both the wavelength-dependent output of the LED and the detection efficiency. We found that the intensities of the scattered light and the wavelength of maximum scattering were not reliable indicators of the extent of colloid aggregates (Figure 8). In contrast, the ratio of scattered intensities with white LED illumination was found to be a more reliable indicator of colloid aggregation (Figure 9).

DISCUSSION

It is of interest to consider the potential applications of wavelength-ratiometric colloid scattering. This approach uses the same optical geometries in fluorescence and has equivalent sensitivity in terms of signal per optical density unit. It is important to notice that Stokes' fluorescence from the samples generally occurs at longer wavelengths and, thus, does not interfere with the scattering measurements, but it may interfere in the case of small Stokes' shifts. Additionally, scattering can be measured with narrow bandwidth detection, which will further discriminate against more broadly distributed fluorescence. Hence, wavelength-ratiometric colloid scattering is a generic technology that can be used in parallel with fluorescence detection. Each analyte study with this approach may require its own standard curve, which will depend on the extent of aggregation and the distances between the colloids.

Examples of potential applications can illustrate the usefulness of this approach. Consider the efforts to develop glucose sensing in contact lenses for diabetics.²³ In the case of glucose, colloid aggregation could be induced by the well-known system based on dextran and concanavalin A.²⁴ Methods are known for coupling proteins and dextrans²⁵ to metallic colloids. Endogenous glucose in the sample would disrupt the binding and alter the wavelength-ratiometric scattering. Such a glucose sensor could be based on a number of proteins that bind glucose^{26–28} and colloid derivatives to contain bound sugars.²⁹ Colloid glucose sensors could also be based on the well-known interaction of glucose with boronic acids.

The potential simplicity of devices for wavelength-ratiometric scattering is shown in Figure 10. Since light-emitting diodes have recently become available, such an LED could be used for direct illumination of the sample. Scattering at two wavelengths could be isolated with filters. The high intensity of the scattered light should allow detection with solid state detectors, resulting in a simple and robust device. In developing a sensor based on wavelength-ratiometric scattering, it will be necessary to consider the nature of the analyte. Since the signal is obtained from the colloid, the analyte need not be fluorescent. If the analyte is fluorescent, it may be necessary to select wavelengths where the analyte does not absorb or emit. However, scattering from colloids occurs over a wide range of wavelengths, so in many cases, analyte emission may be avoided.

In summary, wavelength-ratiometric scattering by mobile metal colloids provides a new generic approach to sensing that can be performed in parallel with fluorescence measurements. The sensitivity appears comparable to that of fluorescence, and the method may be optimized by the size, shape, and aggregation of the colloids. The method can be applied to any bioaffinity reaction, including protein and nucleic acid assays.

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Figure 1.

Absorption spectra of B-BSA-coated gold colloids upon addition of avidin. In subsequent experiments, light scattering is monitored at 530 and 680 nm, as indicated by the arrows in the lower panel.

Figure 2. Absorption spectra (top) and photographs of monomeric and aggregated colloids with white light illumination. The lower panel shows the ratio of the absorption spectra at 530 and 680 nm.

Figure 3. Colloid concentration-dependent light scattering of gold colloids for illumination at 530 and 680 nm. The upper panels show the ratios of the scattered intensities.

Figure 4. Lower detection limit for 550-nm scattering by aggregated gold colloids as observed with an SLM 8000 spectrofluorometer and white light LED illumination.

Figure 5. Measurements of gold colloid aggregation by wavelength-ratiometric scattering. The optical density of the colloids was near 0.04 at 530 nm.

Figure 6.

Comparison of the scattered light intensity of aggregated colloids at 550 nm with the intensity of rhodamine B excited at 550 nm and observed at maximum emission at 580 nm. The aggregated gold colloids display an absorption maximum of 530–560 nm, and rhodamine B displays an absorption maximum of 550–570 nm.

Figure 7. Wavelength-dependent scattering from colloids with various amounts of avidin and white LED illumination.

Figure 8. Intensity of scattered light by gold colloids (top) and maximum scattering wavelength (bottom) for various avidin concentrations with white LED illumination.

Figure 9. Wavelength-ratiometric scattering by gold colloids for various avidin concentrations and selected observation wavelengths, and white LED illumination.

Figure 10.

Proposed sensing scheme for wavelength-ratiometric scattering with illumination by a white light-emitting diode (LED).