Surface Plasmon Resonance Refractive Index Sensing using OpenSPEC™

Overview

OpenSPEC is a powerful visible absorbance spectrophotometer that can be used for a host of applications, such as colorimetric assays, enzyme kinetics, chemical quantification, and more. OpenSPEC's capabilities can be extended to enable solution-phase surface plasmon resonance measurements by the use of colloidal gold nanoparticles in a cuvette. Gold nanoparticles generate localized surface plasmon resonance (LSPR). LSPR allows for the label-free study of binding interactions occurring on the surface on the nanoparticles. In this application note, we demonstrate the SPR capabilities of OpenSPEC by quantifying changes in the bulk refractive index of a solution upon addition of glycerol. Glycerol is added to the nanoparticle solution, causing a change in the refractive index, which shifts the absorbance maximum to longer wavelengths. The OpenSPEC software can quantify in real-time changes to the absorbance spectrum (absorbance peak position, height, FWHM, and more).

Materials & Equipment

- OpenSPEC Instrument or OpenSPR with cuvette holder attachment
- Gold nanoparticle stock mixture (100 nm diameter)
- Deionized Water (DI water)
- Glycerol
- 1 cm path length micro cuvette

Procedure

1. Follow all safety precautions outlined in the material MSDS
2. Following the typical start up procedure found in the OpenSPEC manual, set up the OpenSPEC instrument and software

3. A 1 mL mixture of 100 nm gold nanoparticles stabilized in citrate buffer was pipetted into the micro cuvette.
4. An absorbance spectrum of the gold mixture was saved as the initial spectrum i.e. 0% Glycerol
5. 10 µL of glycerol was pipetted into the gold nanoparticle mixture to create a 1% v/v glycerol solution.
6. Once the absorbance spectrum of the new glycerol mixture is shifted and stable, the spectrum was saved as a .csv file.
7. This procedure was repeated for each of the other concentrations of glycerol: 2%, 4%, 7%, 11%, 14%, 17%, 20% and 23%.

Results & Discussion

The saved spectra were normalized and plotted in Figure 1. The absorbance curve shifts to the right with increasing glycerol concentration as the refractive index of the solution increases. The reduction in the intensity of the peak is caused by the dilution of the initial stock solution of nanoparticles by the addition of glycerol.

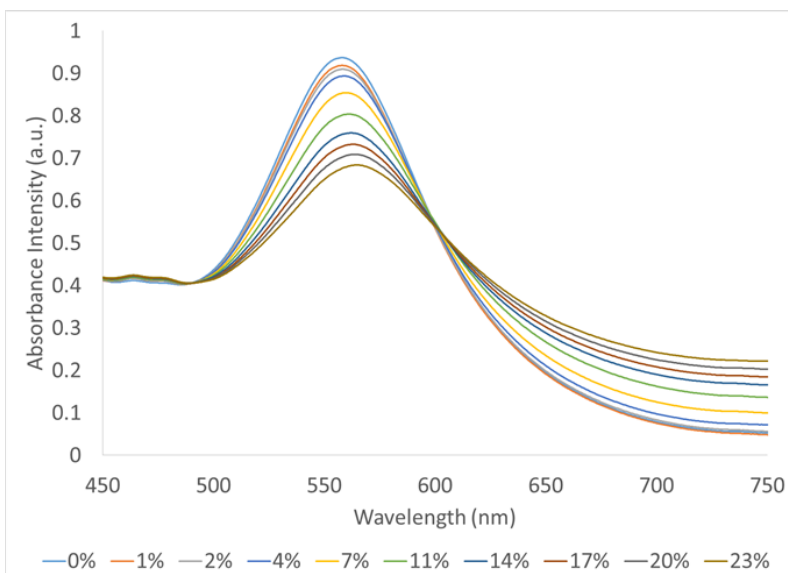

Figure 1. Absorbance spectra at each glycerol concentration

The peak of each spectrum was plotted versus the glycerol concentration of the mixture as seen in Figure 2. The peak shift is linearly proportional to the refractive index change of the mixture.

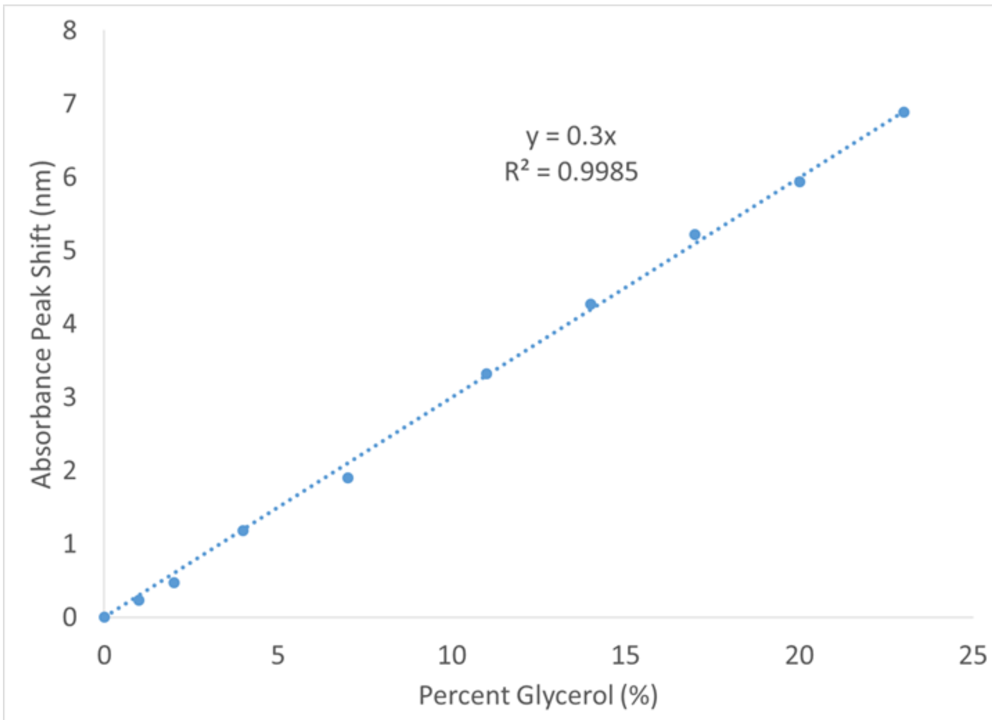

Figure 2. The magnitude of the absorbance peak shift at each glycerol concentration

The absorbance peak position varies linearly with the refractive index of the medium with a slope of 0.3nm per % glycerol, as seen in Figure 2 and as predicted by theory [1]. This phenomenon is what allows LSPR to be used as an effective sensing tool. When molecules bind at or near a sensing array of nanoparticles, the local refractive index changes at the particle surface. This effect is monitored optically through the use of OpenSPEC to quantify the kinetics and specificity of binding.

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We are also able to determine the refractive index sensitivity of the particles, which is calculated as the shift in the peak position per refractive index unit (RIU) change. The change is governed by the equation, [2]

$$R = m\Delta\eta[1 - exp(-d/l)]$$

where R is the transducer response, m is the refractive index sensitivity described above, Δη is the change in the refractive index of the medium, d is the dielectric layer thickness of the analyte binding to the surface and l is the plasmon decay length of the particles. In this case there is no analyte binding so the bracketed term goes to 0. Using known values for the refractive index of glycerol solutions in water, the sensitivity of the particles was calculated to be 183 nm/RIU. With typical noise levels of 5pm, this gives a resolution of 10^{-5} RIU.

This application note demonstrates the ability of OpenSPEC to provide highly sensitive refractive index sensing capabilities in a simple cuvette format. With appropriate surface functionalization of the nanoparticles, OpenSPEC can be used to investigate the binding interactions between a target and a ligand, similar to conventional SPR instruments.

References

[1] K. Mayer and J. Hafner, "Localized Surface Plasmon Resonance Sensors," *Chemical Reviews,* vol. 111, pp. 3823-3857, 2011.

[2] Kedem O. et. al., "Sensitivity and Optimization of Localized Surface Plasmon Resonance Transducers," *ACS Nano*, vol. 5, pp. 748-760, 2011.