Purpald Reagent to Enhance Surface Plasmon Resonance Imaging Toward Sensing of Formaldehyde

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PURPALD REAGENT TO ENHANCE SURFACE PLASMON RESONANCE IMAGING TOWARD SENSING OF FORMALDEHYDE

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Symbols and Abbreviations

SPRi	Surface Plasmon Resonance Imaging
ppm	Parts per million
ppb	Parts per billion
FA	Formaldehyde
RI	Refractive Index
Au	Gold
SAM	Self-Assembled Monolayer
CCD	Charge Coupled Device

Abstract

Formaldehyde (FA) is the smallest aldehyde with the formula HCHO. An elevated FA concentration (despite at ppm-ppb levels) in the human body reflects abnormal health conditions. Moreover, HCHO is an environmental pollutant. Detecting the ultra-low ppb concentrations of FA is challenging due to the small size of this molecule in comparison to large biomolecule markers (e.g., DNA, protein, antibody). Herein, we demonstrate an ultra-sensitive HCHO detection strategy by utilizing Surface Plasmon Resonance imaging (SPRi) and the Purpald reagent (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) to complex with HCHO. The Purpald reagent is well known to react specifically with formaldehyde to form a purple solution, whereas in the absence of FA, the reagent is colorless. Translation of this fundamental chemistry reaction into clinical relevance using our SPRi microarray, which measures refractive index changes, allowed us to detect HCHO concentrations as low as 30 ppb. To our knowledge, this is the lowest detection level known for HCHO using SPRi. The future goal for this research is to further lower the detection limits by using metal nanoparticles, and to apply this method for the detection of formaldehyde present in biological matrices such as urine and blood serum.

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I. Introduction

Small organic molecules can have an adverse effect on human health. Formaldehyde (FA), the smallest aldehyde with the formula HCHO, is a concern for many health effects. FA is a probable carcinogen, and an elevated level of FA in the human breath can be related to lung cancer.[1] Moreover, formaldehyde is a major pollutant in numerous manufacturing processes, such as in the production of wood resins. Products such as cleaning agents, tobacco, insulators, fertilizers, and paints can contain FA.[2,3]

Detection of FA is essential in many areas because small concentrations of formaldehyde can have adverse effects on the human being and environment. FA is extremely irritating to the eyes, airway, skin, and gastrointestinal tract. Concentrations as low as 0.1 ppm have caused airway irritation. A concentration around 5 ppm can cause airway irritation, a cough, wheezing, and chest tightness. Concentrations of FA over 50 ppm can have a severe pulmonary impact, leading to illnesses such as pulmonary edema, pneumonia, and bronchial irritation. A formaldehyde concentration of 100 ppm is life-threatening.[4]

Because low levels of FA can have such a negative impact, there are limits to the amount of formaldehyde that people can be exposed to, which is called the formaldehyde permissible exposure limit (PEL). The World Health Organization (WHO) set the FA concentration in residential indoor area to not exceed 82 ppb. The maximum daily reference dose of FA was established by the United States Environment Protection Agency and it is 0.2 mg per day. However, these amounts are too low to have a profound impact, and trouble arises when FA from manufacturing is added.**Error! Bookmark not defined.**

Surface Plasmon Resonance Imaging (SPRi) is a spectroscopic detection method that measures pixel intensity changes on a gold surface. This phenomenon involves monochromatic polarized light hitting the gold surface through a prism. When the energy of the incident light matches the resonance frequency of the oscillating electrons on gold ("plasmons"), plasmon waves are generated on the gold surface that is in contact with a dielectric medium (e.g., water, buffer). Free electrons on Au surface absorb the light and convert the light photons to surface plasmon waves.[5] SPRi includes a Charge Coupled Device camera (CCD) that monitors the pixel intensity changes on the gold surface and represents it as differential images that show the intensity in each spot (see figure 1). Through real-time experiment, the changes in the intensity can be monitored over time, which can be used to study binding events on the Au surface. SPRi is extremely sensitive to the refractive index (RI) change on or above the gold surface for up to a 300 nm distance. Any incremental change of the RI will alter the pixel intensity of the gold surface. A change in the color of any analyte solution in contact with the SPRi Au surface can also alter the refractive index property, which is sensitive to SPRi detection.[6]

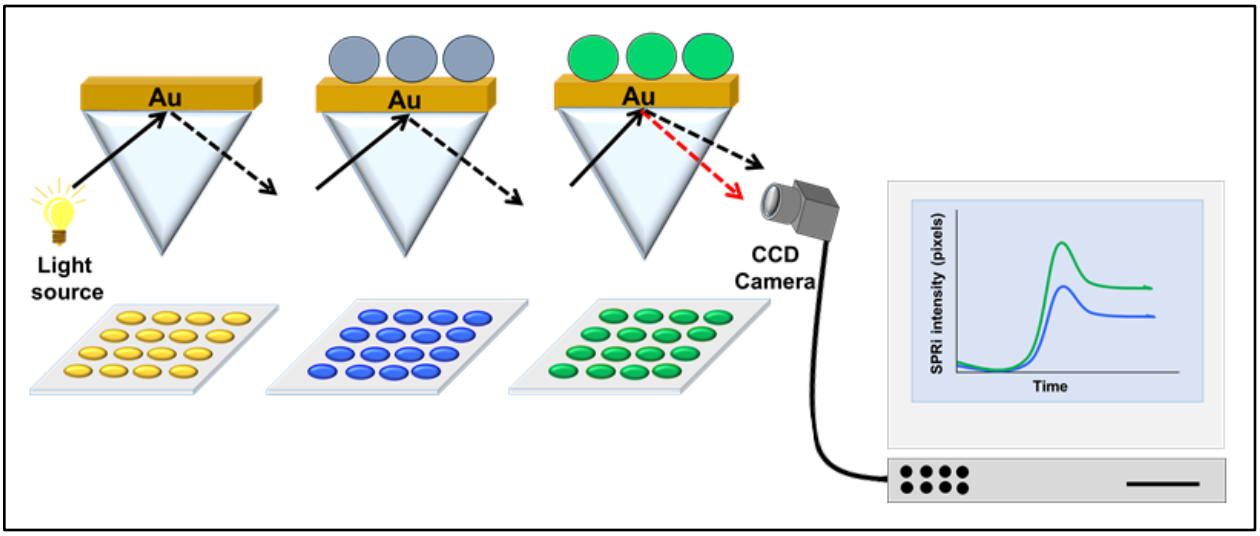
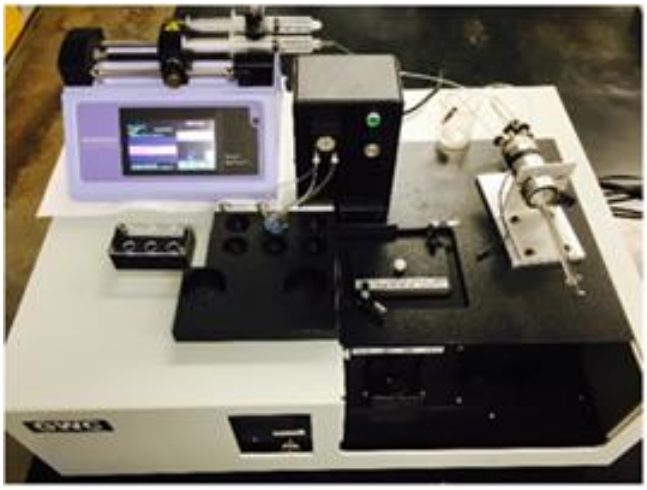


Figure 1. Surface Plasmon Resonance imaging principle.

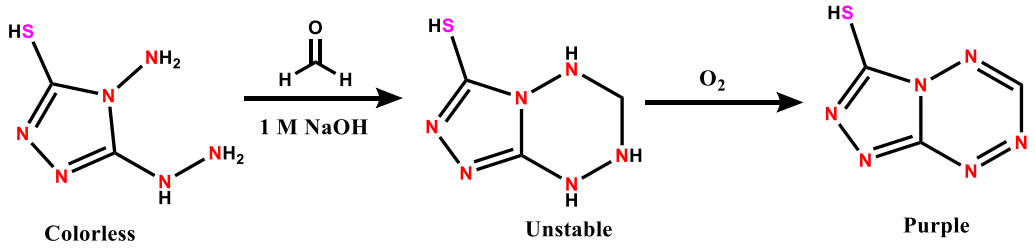
SPRi is an effective diagnostic tool because it is label-free, can be analyzed in real-time, and has high levels of sensitivity and specificity.[7] Additional advantages of SPRi include the identification of specific vs. nonspecific binding, a high sensitivity to changes in the refractive index, and rapid analysis. The applications of SPRi include protein-protein interaction, biological molecule detection, antibody-antigen interaction, organic and inorganic molecule detection, and surface modification of the gold chip. The overall picture of the SPRi instrument used in this study is shown in picture 1.



Picture 1. The SPRi instrument used in this study.

The Purpald reagent (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) reacts specifically with formaldehyde to form a purple solution. The color intensity of the solution is directly related to the formaldehyde concentration. Other aldehydes, such as hexanal, show no color change with the Purpald reagent. Due to the specificity of the Purpald reagent, it has been used to detect FA in different samples such as alcoholic beverages, as well as to

perform quantitative measurements. The Purpald reaction mechanism with FA is shown in scheme 1.[8] Purpald reacts with FA and the transparent solution turns purple when the reaction takes place in 1.0 M NaOH (pH= 14). Altering the color of the solution will change the overall refractive index of the Purpald reagent in the solution, which is detectable by SPRi technology. Thus, the Purpald reagent can be used to enhance SPRi imaging of FA. In a previous study, FA-gold interaction in solution, based on the physical adsorption on Au surface by SPRi method, was studied and the LOD was 100 ppm.[9] Therefore, increasing the sensitivity of SPRi toward detection of FA is desirable.



Scheme 1. Reaction mechanism for determination of formaldehyde using the Purpald reagent.

Herein, for the first time, we utilized the sensing of FA with different concentrations in aqueous solutions by integrating SPRi technology advantages with the Purpald reagent. Altering the color of Purpald reflects changes in the refractive index. Thus, the Purpald reagent has been used to enhance SPRi toward detection of FA. The detection limit we achieved in this study was found to be 30 ppb FA.

II. Experimental

- Materials and Apparatus

Formaldehyde 37% was purchased from Sigma-Aldrich. Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) was purchased from Alfa Aesar. Solid sodium hydroxide was purchased from EMD Millipore. Real-time SPRi experiments were performed using a GWC SPRimager-II (Horizon SPR imager model) attached to a Pico Plus Elite Pump11 (Harvard Apparatus, Massachusetts, U.S.A.) and a dual injector valve (Rheodyne model 9725i PEEK injector, IDEX Health & Science LLC, California, U.S.A.). Glass chips (18 mm x 18 mm), each coated with 16 gold spots and one reference spot, were used with the SPRimager instrument. All experiments were run at room temperature with an incident light wavelength of 800 nm. SPRi differential images were collected with the Digital Optics V++ software. [7]

- Procedures

1.0 and 0.1 M NaOH solutions were prepared in Millipore water. 20 mM Purpald solution was then prepared in 1 M NaOH. 5 mL solutions with formaldehyde concentrations of 1.0, 0.5, 0.1, 0.05 and 0.03 ppm were prepared in 1.0 M NaOH and 1000 μL of a 20 mM Purpald solution was added. After 5 minutes, to oxidize the intermediate by O_2, the vials were shaken for approximately 5 minutes until a stable purple color resulted. The control was a 1.0 M NaOH solution containing 1000 μL of 20 mM Purpald solution with no FA in it. Both solutions, the one containing FA and control (with no aldehyde) were injected simultaneously into the SPRi dual channels (one channel of 8 spots for FA sample and one channel of 8 spots for control). Once the SPRi signal reached a maximum response, the flow rate (150 μL/min) of the solutions was slightly decreased to allow 15 minutes incubation time, then the gold chip was washed with 0.1 M NaOH. The SPRi intensity differences were calculated based on the baseline shift in the sample and control sensograms, and then converted into a differential image to find the net change in pixel intensity for various HCHO concentrations. The SPRi intensities were monitored using CCD camera over time (real-time analysis). Using the V++ software, the differential images were taken every second.

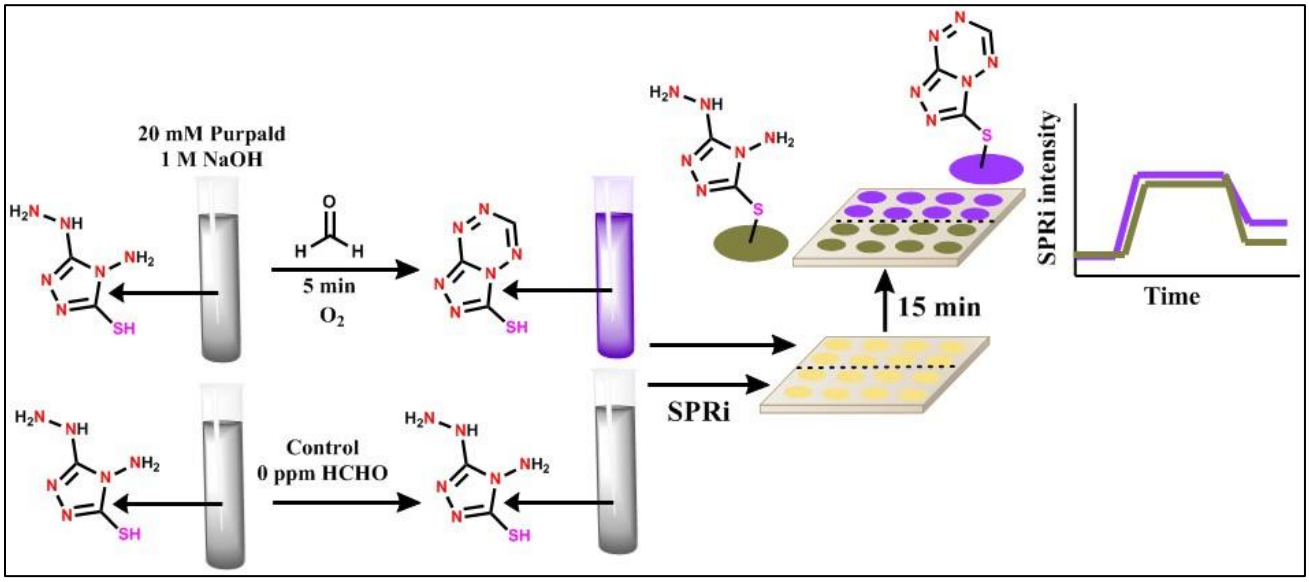


Figure 2. Graphical representation of the detection method used for FA using SPRi and the Purpald reagent.

III. Results and Discussion

Figure 2 represents the method followed for FA detection by SPRi. Two solutions in 1.0 M NaOH were prepared, one containing FA and the Purpald reagent and the second one with Purpald only and no FA. The reaction was performed off-line and then both solutions were injected simultaneously into the SPRi dual channel, followed by

incubation of both solutions for 15 minutes. FA-Purpald stable precursor, as well as the Purpald in the control solution, will form self-assemble monolayer (SAM) on the gold surface via a thiol-gold bond. After washing the surface of both channels with 0.1 M NaOH, the SPRi pixel intensity was found to be higher for FA-Purpald than Purpald alone on the Au surface. Increasing the FA concentration led to an increase in the SPRi signal.

Figure 3 (A) demonstrates the SPRi intensity in each spot. The height of each spot represents the intensity increment due to the formation of SAM. Concentrations of 1000, 100, and 50 ppb FA were used and shown in the SPRi differential images. Higher intensity was given by a larger concentration of FA. SPRi is extremely sensitive to RI changes on the gold surface, and it is clear that FA-Purpald RI is higher than that of Purpald alone. Figure 3 (B) shows the SPRi sensogram, where SPRi pixel intensity was monitored over time. The intensity of SPRi increased with FA concentration in the solution, which is due to the presence of a higher number of FA-Purpald precursors on the surface.

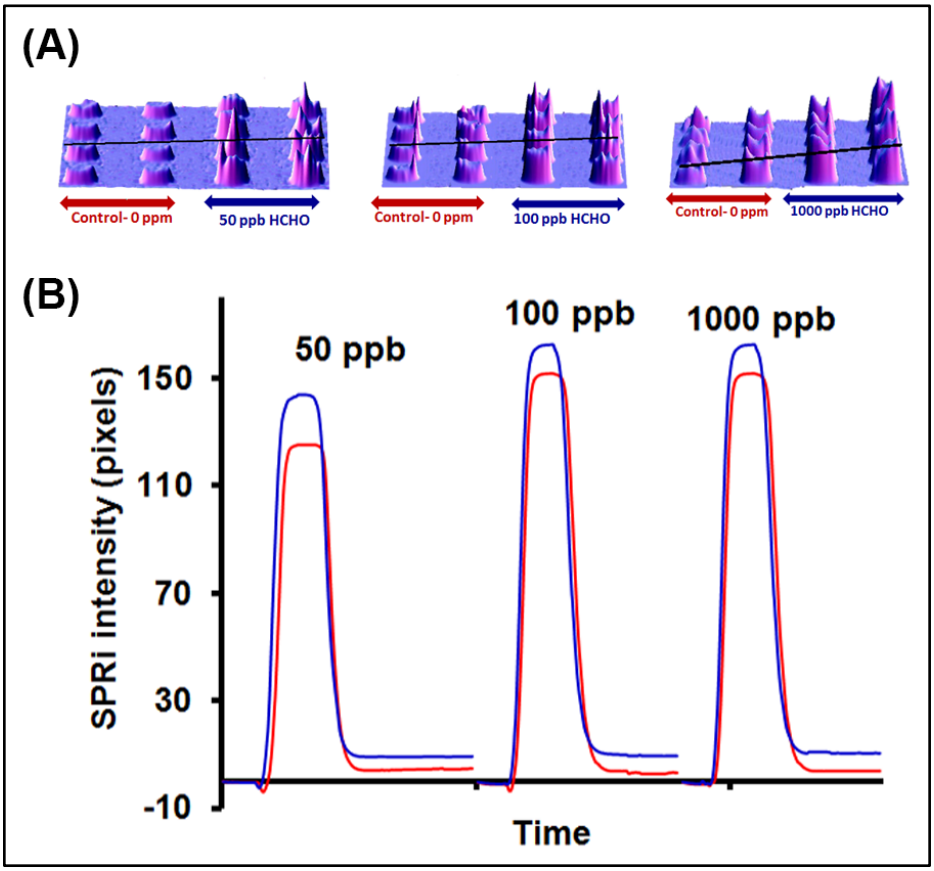


Figure 3. SPRi differential images (A) and sensograms (B) showing the SPRi intensity increase due to the binding of HCHO-Purpald complex on the Au surface, where the blue lines in the sensograms represent solution containing Purpald reagent with FA and the red line shows the control response (Purpald only).

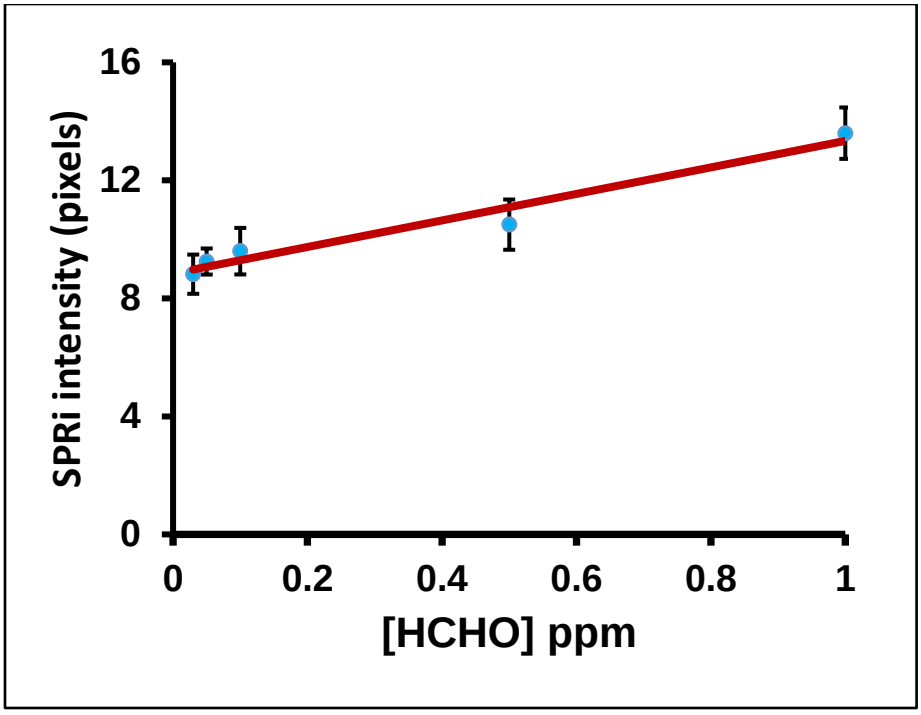


Figure 4. SPRi pixel intensity with increase in concentration of HCHO.

Figure 4 shows the linear relationship between SPRi intensity and the concentration of formaldehyde in ppm. The sensitivity was calculated to be 4.5 pixel.ppm^{-1} from the slope of the line. The limit of detection was found to be 0.03 ppm (i.e., 30 ppb). The ultra-low detection limit achieved in this study is suggested to result from the designed Purpald reagent strategy combined with SPRi detection of the greatly altered refractive index changes that occur upon HCHO complexation with the reagent. The limit of detection using the SPRi method has never been achieved before. Thus, Purpald assisted the SPRi sensitivity toward detection of the smallest aldehyde biomarker.

IV. Conclusion

In this study, we demonstrated that the Purpald reagent enhanced the SPRi sensitivity toward detection of FA. The Purpald reagent specifically reacts with formaldehyde in a 1.0 M NaOH solution and changes the color of the solution from colorless to purple, which can be differentiated from the control solution (no FA) using SPRi technology. The sensitivity and the detection limit were found to be 4.5 pixel.ppm^{-1} and 30 ppb, respectively. The future goal for this research is to lower the detection limits even more, using gold nanoparticles, and to apply this method for the detection of formaldehyde present in biological matrices such as urine and blood serum.

V. References

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