Journal Name

ARTICLE TYPE

Cite this: DOI: 10.1039/xxxxxxxxxx

Probing Specific Gravity in Real-time With Graphene Oxide Plasmonics†

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Received Date
Accepted Date

DOI: 10.1039/xxxxxxxxxx

www.rsc.org/journalname

Specific gravity (SG), the ratio of density of a substance to the density of a reference material, is a standard indicator of concentration of an analyte in a given solution. SG is routinely used for product quality assessment in food industries. However, currently available commercial SG meters, such as hand-held refractometers and density meters, are highly sensitive to humidity, temperature, and do not allow real-time measurements. For these reasons, SG detection is often time-consuming which leads to unwanted interruptions in food manufacturing process. Therefore, highly sensitive, label-free, and real-time sensors for the detection of SG are urgently needed for food quality control. In this context, we develop a graphene oxide (GO)-coated gold (Au) surface plasmon resonance (SPR) sensor, for the first time, used to measure SG of food samples in real-time. SG values of sample solutions are correlated with refractive indices (RI) of these solutions, which are captured by the SPR measurements, with a sensitivity of 10^5 SPR response units. Moreover, the use of GO coating provides a strong enhancement of plasmonic resonances due to their optoelectronic properties, sometimes doubling the sensitivity of SPR response units per RI unit ($2x10^5$) when compared to conventional Au SPR chips ($1x10^5$). We also validate our sensor performance by measuring the SG in real food samples. Our results demonstrate a highly sensitive, efficient, high throughput, and reproducible approach for SG measurements in food industry settings, and open new opportunities to utilize improved SPR sensor technology for many other label-free analytical sensing applications.

1 Introduction

Over the years, specific gravity (SG) has been widely used as an efficient and convenient indicator to estimate concentrations of various compounds in aqueous solutions[1]. Specifically, SG has been used to evaluate quality of products in food industry; while in healthcare settings, SG has been used to analyze body fluids such as blood, saliva and urine for early and accurate disease diagnostics. For instance, Moore *et al.* discovered that changes in SG of the blood plasma and brain fluids corresponded to formation of edema in nephritis[2,3]. In addition, SG values of plasma and urine can give an insight into hydration levels of body associated with diseases such as diabetes, cancer, and ischemic strokes[4].

In food industry, measurements of SG assist in estimation of food quality during manufacturing processes. For instance, SG is used in the brewing industry: the Plato chart (beer strength measurement table) lists sucrose concentration by weight against its corresponding SG values[5]. Similarly, in soft drinks, fruit juices, and honey, sucrose concentration by weight is deduced from a *A. Brix* table that uses SG to determine sugar content[6]. In addition, in food industry, SG sensors have been used to detect deleterious or pathogenic microbes, and production of certain food byproducts, such as glutamate, that can have immediate or disastrous effects on a living organism[7]. Therefore, to optimize food manufacturing processes for the best return on assets and improved food quality, reusable, real-time sensors for assessing concentration of food analytes are urgently needed.

SG is a dimensionless quantity defined as the ratio of density of the solution of interest to the density of a reference solution. For the SG measurement of an unknown aqueous solutions, the density of water at 4°C is commonly used as a reference solution parameter. Traditionally, hydrometer and weighing balance are routinely used for the evaluation of SG values[8]. Both of these methods rely on physical measurement of the weight and volume of a given sample. Recently, optical prism based refractometer scales, sensitive to changes in the refractive index (RI) of the media, have also been used for the SG quantification. This ap-

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proach is convenient, but relies heavily on user-handling skills where measurements are prone to semantic errors resulting from differences in the judgment from user to user[9]. Alternative approaches such as hydraulic pressure based instruments, vibrating element transducers and ultrasonic sensors have been proposed to measure SG. These methods involve use of an electronic readout displays which eliminate source of semantic errors in measurements[10]. However, most of these methods have so far found limited applications to measure SG of food samples, primarily because these methods require human interventions (eg. stopping the mixing process at specific time intervals) during the manufacturing process[11]. In addition, these density based measurements tend to yield low sensitivity, poor signal reproducibility, and higher instrumentation cost.

Surface plasmon resonance (SPR) is the collective resonance of free electrons, which can be initiated by incident light via prism, grating, or waveguide coupling. SPR occurs at the interface of a metal and a dielectric, and it is highly sensitive to RI variations occurring in the vicinity of the sensor surface[12]. Since, at a certain angle, photons hitting the surface of the lower RI material can excite plasmons at the surface of the metal film, incident photons are transformed into surface plasmons[13]. As a result, there is a decrease in the intensity of the reflected light, which can be correlated to the angle at which photons are transformed into plasmons. By detecting magnitude of this change and angle at which dip in the energy occurs, we can measure the value of the RI for any system[14]. Based on this principle, SPR machine records angles at which dips in the SPR signal intensity take place, and later gives out the readout as SPR response units plotted against time. Since a change in the SG of a solution essentially shifts the RI of this solution, SPR can be readily used for quantification of SG in aqueous solutions. Recently, Chen *et al.* used an SPR-based fiber-optic sensor for urine SG detection, where one side of the optic fiber was coated with a 48 nm thick gold (Au) film. This Au coating substantially enhanced sensitivity towards urine SG detection when compared with bare optic fibers[15]. Similarly, Chiu *et al.* utilized SPR principle in order to perform immunoaffinity biosensing by coating surface of the SPR chip with aqueous solution of graphene oxide (GO)[16,17]. GO is a 2D plasmonic material with optoelectronic properties which can enhance plasmonic resonances[18]. Thereafter, in this work, they functionalized carboxylic groups of the GO with biocompatible ligands in order to initiate molecular bindings to the surface of the modified SPR chip. Fabricated GO-modified SPR sensing pushed the limit of protein detection from ng/mL to pg/mL scale.

Recognizing the need for rapid, simple, and reliable method to assess food quality, and motivated by the utility of SPR for potential SG measurements at high resolution, we develop GO-enhanced SPR sensors to determine the SG of aqueous food samples, for the first time. First, the RI and SG values are correlated to achieve SG detection with a sensitivity in the range of 1×10^5 SPR response units. By coating the conventional Au SPR chip surface with a thin layer of GO, the sensitivity of the SPR sensor is enhanced up to two times (2×10^5 SPR response units per each SG unit) when compared to that of the bare SPR sensor substrate[19]. By using GO-modified SPR sensors, we conducted systematic SG measurements for standard solutions containing sodium chloride (NaCl), sugar ($C_{12}H_{22}O_{11}$), and ethanol (C_2H_5OH) at different concentrations, followed by real-time SG measurements in real food samples (green tea, apple juice, and soy sauce). GO-based Au SPR sensors developed in this work are highly sensitive and reusable (regeneration coefficient $>$ 90%) with respect to the real-time detection of SG in aqueous solutions. This provides alternative and improved sensing strategies to measure SG for applications in food industry.

2 Experimental Methods

2.1 Materials

Sodium chloride (assay 99%, CAS# 7647-14-5), ethanol (assay 95%, CAS# 64-17-5) were purchased from Sigma-Aldrich (Tokyo, Japan). White sugar (Mitsui sugar, Japan), green tea (Ayataka, Coca-Cola Company, Japan), soy sauce (Kikkoman, Japan), and apple juice (Tominaga & Co., Ltd., Japan) were purchased from a local grocery store (Tabata, Ishikawa, Okinawa, Japan). Deionized (DI) water from an 18.2MΩ cm^{-1} Milli-Q Integral 3 water purification system (Millipore, Germany) was used as a reference solution, washing buffer, and a rinsing solution. Graphene oxide dispersion at $\phi = 0.4$ wt% was obtained by adding GO sheets (GrapheneA, Spain) to distilled water and diluting the sample on a precision balance.

2.2 Standard Test Sample Preparation

For each of the measurements, three sets of aqueous solutions containing salt (NaCl), sugar ($C_{12}H_{22}O_{11}$), and ethanol (C_2H_5OH) were prepared. Each set of samples contained six different concentrations of analyte dissolved in 3 mL of Milli-Q water. This resulted in 18 samples of Milli-Q water with dissolved salt, sugar, and ethanol in amounts varying from 100 to 600 mg. Experimentally measured values of RI and SG of these samples are obtained for calibration purposes and listed in Table S1† in ESI.

2.3 Real Food Sample Preparation

To produce real food samples, we used same approach as for preparation of the standard test samples. We made three sets of samples, each containing six different concentrations of the analyte. First set of samples was prepared by dissolving 100-600 mg of NaCl in soy sauce. Whereas, second and third sets contained 100-600 mg of $C_{12}H_{22}O_{11}$ dissolved in green tea and apple juice. Apart from that no other parameters in real food samples were changed.

2.4 RI and SG Correlation

Prepared standard test and real food samples contained different amounts of analyte. This affects the RI and SG values of the bulk media[20]. Therefore, to calibrate the SPR sensor, it is essential to correlate the RI with the SG value for each set of samples individually. To quantify the RI values, we used MASTER-SUR/Nα clinical refractometer (ATAGO CO., LTD, Japan). This refractometer measures RIs with an accuracy of ±0.0005 units by determin-

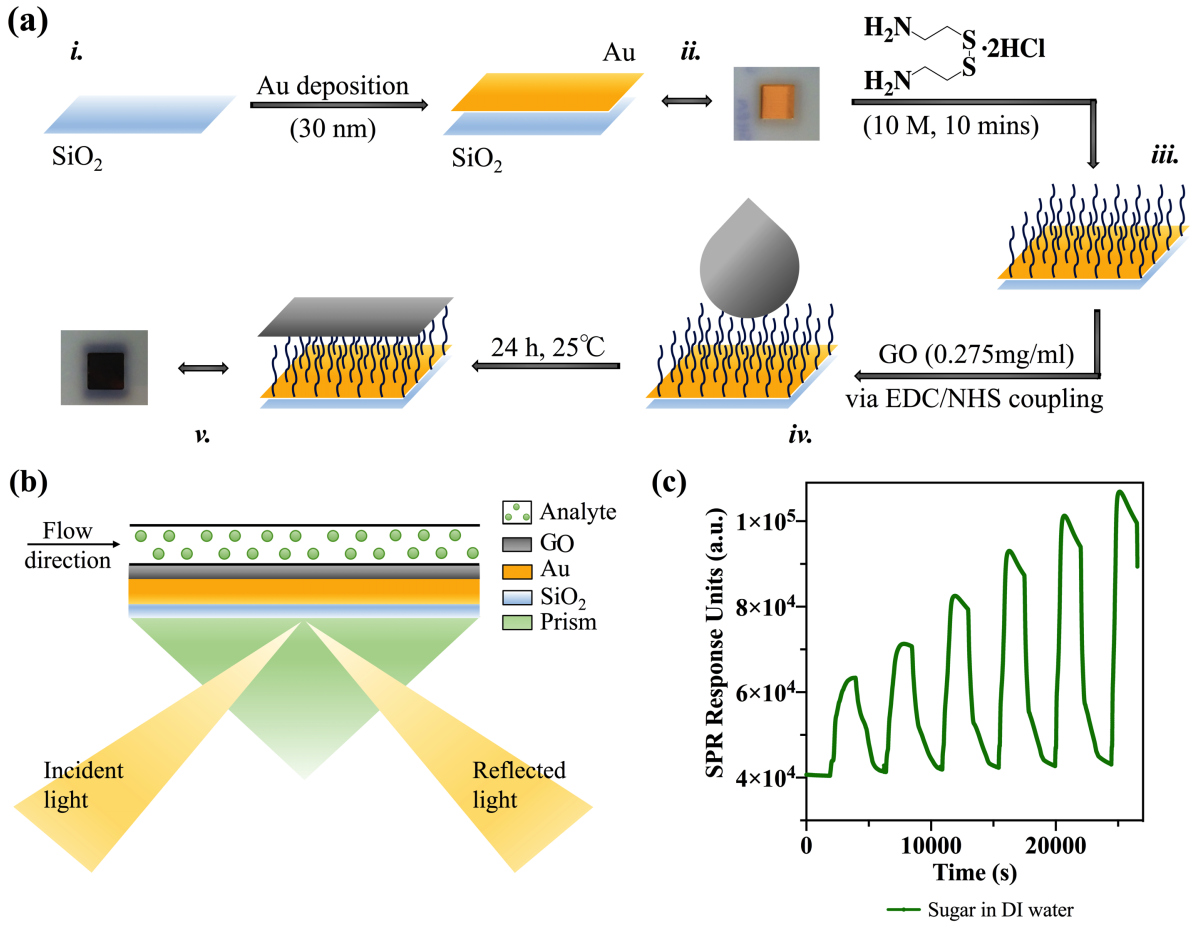

Fig. 1 Sensor fabrication and working principle: a) Schematic illustration of preparation of GO-coated Au based SPR sensor chips; i) Bare glass (SiO_2) substrate; ii) Deposition of 30 nm of Au on SiO_2 (with 8-10 nm Cr layer underneath for Au adhesion, not shown in figure); iii) Functionalization of Au surface with cystamine dihydrochloride (10 M); iv) Drop-casting the GO solution on to the EDC/NHS (0.4 M/0.1 M) wetted sensor surface; vi) Drying overnight for 24 hours to achieve Au-GO SPR chips; b) SPR measurement principle showing Kretschmann configuration where prism is used as an optical coupler to excite and measure SPR. The SPR signal is sensitive to changes in RI of the analyte flowing on the top surface of the sensor; c) Real-time standard SPR response from Au-GO SPR chips. This particular plot was achieved by exposing the sensor surface to different concentrations (from 100 mg to 600 mg) of aqueous $C_{12}H_{22}O_{11}$ solutions.

ing the critical angle of total reflection via prism coupling. Meanwhile, SG values of the samples were recorded by using a portable density/specific gravity/concentration meter with an accuracy of ± 0.001 g/cm^3 (Anton Paar DMA 35 Ex, Japan). This device incorporates oscillating U-tube technique to determine relative and absolute densities of the tested solutions[21]. Collected RI and SG values were then plotted against each other resulting in a linear correlation (R^2 = 0.9997) as it can be seen from Fig. 3a. See more details in Section 3.3 and refer to Figure S1† in ESI.

2.5 SPR Measurements

Samples were analyzed using a Biacore T200 system, an advanced SPR instrument for measuring RI values. As depicted in Fig. 1b, Biacore T200 incorporates the most common optical setup for SPR also known as Kretschmann configuration. This setup involves the use of a prism coupler to excite surface plasmons. Moreover, Biacore T200 quantifies RIs of the sample directly on the surface of the chip. For this reason, we can evaluate concentration of the analyte in the sample as well as identify if there are any interactions between the analyte and the SPR chip surface.

The Biacore T200 was equipped with an automated system of sample injection. In this experiment, we used Milli-Q water as a washing buffer and a reference solution for all of the experiments. All of the measurements were conducted at a flow rate of 30 μLmin^{-1}. For each measurement, we started with introducing reference solution for 300 s. Next, we injected the first sample containing 100 mg of the analyte and analyzed it for 300 s. After the first measurement, we washed the sensor surface with Milli-Q water for 100 s, then injected the next sample containing 200 mg of the same analyte. Fig. 1c illustrates a real-time standard SPR response acquired from six aqueous sugar solutions, where baseline corresponds to a washing buffer (Milli-Q water) and the six peaks are due to the six concentrations of the analyte

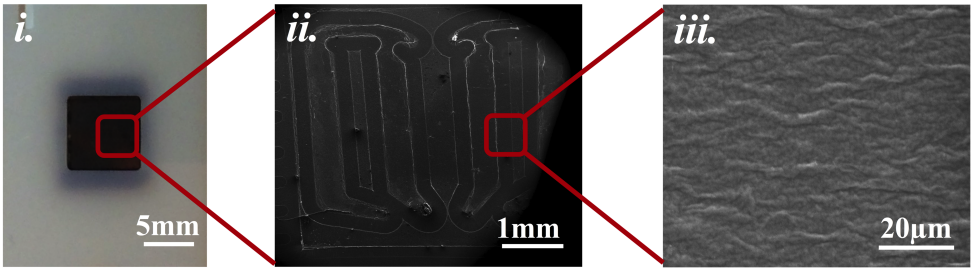

Fig. 2 Surface characterization of Au-GO SPR sensor chip: Image capture of the sensor chip shown in panel *(i)*, *(ii)* and *(iii)* show the SEM images (30 x, 1400 x magnification using FEI Quanta 250 FEG) of the top surface of the fabricated sensor. The imprints shown in *(ii)* are from the microfluidic channels of flow cell clamped on the top of the sensor surface. *(iii)* show the zoomed in surface of GO at 1400 x magnification showing topography of deposited GO layer surface

(from 100 mg to 600 mg of sugar). By taking the average from four different sensors, we acquired single SPR response value for each of the samples. These average SPR responses were plotted against their corresponding SG data points. Standard deviation values for calculations of the average SPR response were found to be within ±1%. Thus, the error bars are smaller than the symbol sizes and cannot be visualized when plotted on the graphs, as discussed later in Results and Discussion section.

3 Results and Discussion

3.1 Sensor Fabrication

A conventional SPR chip consists of a chromium (Cr) layer covered with a thin gold (Au) film on the top of the glass (SiO_2). To enhance magnitude of the detected SPR signal, we coated surface of the Au SPR chip with a thin layer of GO. GO is known to possess unique optical and electronic properties, which leads to improved plasmonic sensing characteristics upon coupling with materials such as Au[22]. In our work, sensors were fabricated by coating surface of the conventional SPR chips (GE healthcare Life Sciences, Japan) with aqueous GO (0.275mg/ml) solution by following the procedure outlined in Fig. 1a. GO was attached to the top Au layer of the chip by using cystamine ($C_4H_{14}Cl_2N_2S_2$) linker molecules. First, in step *iii* in Fig. 1a, we covered surface of the Au SPR chip with 10 M cystamine dihydrochloride ($C_4H_{12}N_2S_2$) solution, and then allowed it to dry for 10 mins at room temperature (25°C). These linker molecules attach to the Au film via covalent bonds formed between Au atoms from the surface of the SPR chip and sulfur (S) atoms of cystamine. After removing excess cystamine dihydrochloride solution from the chip surface, we introduced mixture of EDC (1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide·HCl) and NHS (*N*-hydroxysuccinimide) in 4:1 (EDC:NHS) ratio as shown in Fig. 1a(*iv*). Next, we allowed the EDC/NHS mixture to set for 5 mins in order to ensure its even distribution throughout the entire surface of the chip[23]. A thin layer of aqueous solution of GO (0.275 mg/ml) was drop-casted on top of the cystamine dihydrochloride coated sensor (Fig. 1a(*iv*)). EDC/NHS mixture reacted with carboxylic (-COOH) groups of GO to turn these functional groups into ester moieties. Since newly formed ester groups of GO were more reactive towards amino groups (-NH_2) of cystamine, GO molecules covalently attached to primary amino acid groups present at the sensor surface. This resulted in the formation of strong amide bonds between amino groups of cystamine and ester moieties of the activated GO molecules. Thereafter, in step *v* in Fig. 1a, the modified SPR chips were incubated for 24 hours at ambient temperature (25°C) until complete solvent evaporation was achieved. After GO was attached to the Au film, surface of the chip was washed with DI water to remove excess GO molecules from the sensor[16]. As a result, conventional Au SPR chip was coated with a thin layer of GO.

Fig. 1b shows how the GO-modified Au sensor was subsequently used in the Biacore T200 SPR instrument. In SPR instruments, incident light is directed towards the bottom (SiO_2) side of the sensor, so that plasmons are excited on the metal surface (Au)[24]. Since the Au side of the SPR chip was covered with GO, in our measurements plasmons are excited on the GO-coated Au surface of the sensors. As shown in Fig. 1c, measurements from the sensor are plotted as the SPR response units against time. Here, six 3 mL solutions of Milli-Q water containing different amounts of sugar (from 100 mg to 600 mg) were used to demonstrate the standard response from the SPR instrument (Fig. 1c).

3.2 Sensor Characterization

To characterize surface of the GO-modified chip, scanning electron microscopy (SEM) images of the sensor surface were acquired. Image capture shown in Fig. 2(*i*) demonstrates that GO forms a thin black-colored layer on the surface of the SPR chip. At a higher resolution as in Fig. 2(*ii*), we can see imprints of microchannels of flow cells (from the Biacore T200 SPR instrument) developed on the sensor surface. Fig. 2(*iii*) reveals homogeneous coating of the GO layer throughout the sensor. From the Surface Profiler (Bruker Dektak XT, Japan) measurements, thickness of the deposited GO layer was found to be 600 ± 30 nm. This implies that the landscape of the fabricated sensor is homogeneous and that measurements of the RI data taken from different areas of the sensor are expected to be largely consistent across the sensor surface. In addition, we also performed ellipsometry on both Au and GO based SPR chips. We identify that upon addition of GO to the Au, the surface of the sensor becomes less lossy (as indicated by a decrease in the imaginary part of the dielectric constant), essentially highlighting the importance of GO addition on to the Au surfaces. Further details on ellipsometry are described on Fig-

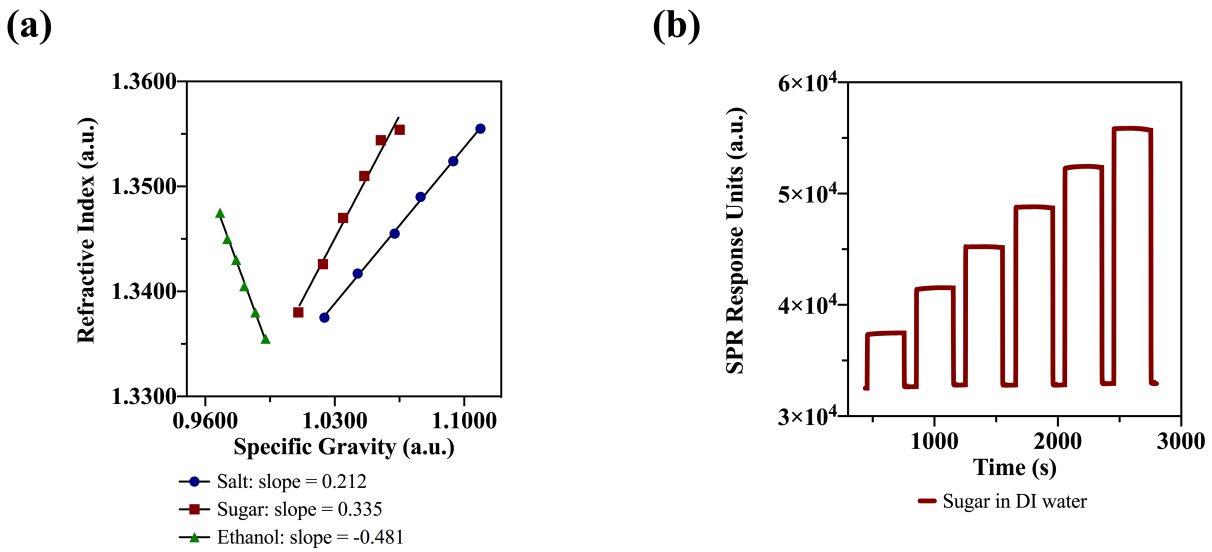

Fig. 3 RI versus SG: a) Shows the relationship between RI and SG for the three sets of aqueous solutions from 100 mg to 600 mg of salt, sugar, and ethanol dissolved in 3 mL of DI water (18.2 MΩ); b) Shows a standard SPR response to varied concentrations of six aqueous solutions when conventional Au SPR chips is used. In this measurement, six solutions with different concentrations of sugar (from 100 mg to 600 mg), values for SG vary from 1.012 to 1.066.

ure S2† in ESI.

3.3 Correlation between RI and SG

RI describes how fast light propagates through the medium of a solution, and it is therefore dependent on the character of the species being analyzed[25]. In this work, we modified values of SG for different solution sets by changing concentrations of the analyte in the tested samples. By changing concentration of the given analyte in the solution, we can alter the density and, consequently, the SG value of this solution. As a result, the RI of this solution is modified as well. Therefore, our SG measurement principle relies on first detecting the RI changes in the sample by using SPR-based instrument, and then correlating these RI values to absolute SG values. Table S1† in ESI illustrates RI and SG values reported for solutions containing from 100 mg to 600 mg of salt (NaCl), sugar ($C_{12}H_{22}O_{11}$), and ethanol(C_2H_5OH), which were measured by using refractometer and portable density/specific gravity/concentration meter, respectively. In addition, Fig. 3a demonstrates a linear correlation between experimentally measured RI and SG values for aqueous solutions of salt, sugar, and ethanol dissolved in 3 mL of DI water. See detailed procedure in the Supporting Information, Figure S1†. Note that RI and SG values of samples containing different chemical composition need to be calibrated separately. For example, Basker correlated RI and SG of aqueous glycerol solutions over the range of 0-100% of glycerol at 20°C[26]. However, the formulated relationship only holds true for aqueous solutions containing 0 to 100% of glycerol.

3.4 SPR for SG Measurements

In our studies, we use Biacore T200 system, which can accommodate up to 384 samples in a single measurement. This makes SPR machines the most efficient and high throughput instrument for RI detection. In this system, we use a conventional SPR chip with four inbuilt sensor regions, and expose surface of the sensor chip to the analyte solution. The response from the system is sent to the detector, and an example of the standard readout from six samples with different concentration of sugar is demonstrated in Fig. 3b. Herein, we can see that there is one baseline throughout the measurement at around 3.25×10^4 SPR response units. Whereas, intensity of the response increases proportionally to the increase in the sugar concentration which is dissolved in the tested samples. By exciting plasmons on the surface of the SPR chip, we allow the system to measure the critical angle of total reflection and calculate the RI value by using Equation (1)

$$sin(\theta) = \frac{1}{n_{\text{analyte}}} \times \sqrt{\frac{\varepsilon_{\text{metal}}\varepsilon_{\text{dielectric}}}{\varepsilon_{\text{metal}} + \varepsilon_{\text{dielectric}}}}, \qquad (1)$$

where θ, n_{analyte}, $\varepsilon_{\text{metal}}$, and $\varepsilon_{\text{dielectric}}$ are incident angle of the light, refractive index of the analyte, and permittivity coefficients of the plasmonic metal and dielectric material, respectively[27].

However, depending on the concentration of the analyte in the medium, critical angle of total reflection and consequently measured RI value will change. As a result, by measuring RI of the solution we can identify concentration of the species and later relate this concentration to both absolute and relative density of the analyte in the solution, and quantify SG based on the acquired results. Herein, we use dependence of RI and SG on the analyte concentration to further correlate the first two parameters with each other. According to the results from the GO-modified sensors, SG and RI values for each of the prepared solutions sets have excellent linear dependence ($R^2 = 0.9997$). Linear plots for standard test samples obtained using Au and Au-GO SPR sensors are depicted in Fig. 4a and Fig. 4b, respectively. Responses from GO-coated sensor can be characterized by higher values for slopes,

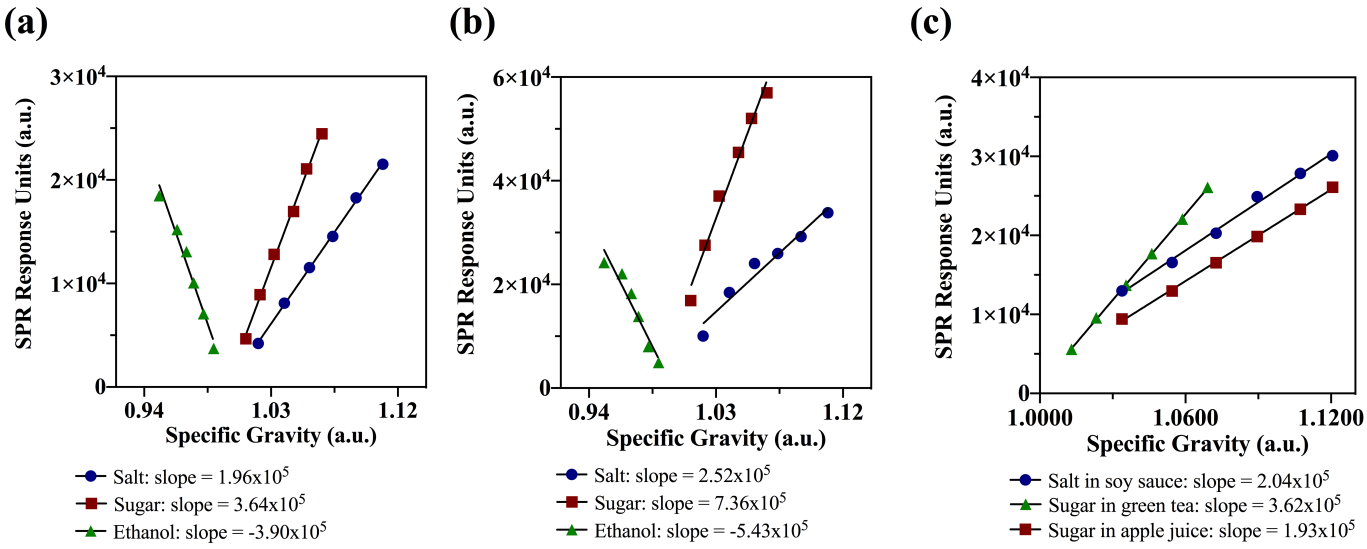

Fig. 4 Measurements of SG using SPR sensors: a) Response of Au and b) Au-GO SPR sensors to 3 sets of standard solutions (100-600 mg of salt, sugar and ethanol dissolved in 3 mL of DI water) of various SG. The response of Au-GO sensor chip has a larger slope than that of the Au chip, suggesting that the Au-GO sensor has better sensitivity towards SG measurements in aqueous solutions. c) SG measurements of real food samples (green tea, soy sauce, and apple juice) using Au-GO SPR sensors.

verifying higher sensitivity upon coating of the Au surface with GO.

To emphasize the importance of our sensor for industrial applications, we tested its applicability in food industry using real food samples. For that, we prepared sets of green tea, soy sauce, and apple juice solutions with different SG values. Real food samples were prepared by varying concentrations of salt, sugar, and consequently SG of the tested samples. From Fig. 4c, we observe that the correlation between SPR response units and SG values of the real food samples follow identical linear trend as that of the standard test samples. Thus, GO-coated Au SPR sensors can be used for detection of SG in aqueous food samples with a sensitivity higher than sensitivity of the standard Au SPR chips.

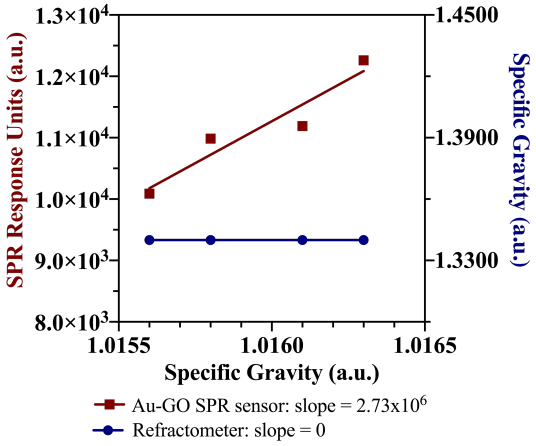

Fig. 5 Comparison test between MASTER-SUR/Nα clinical refractometer and Au-GO SPR sensor: measurements from the hand-held refractometer show zero slope, when Au-GO SPR sensor shows 10^6 SPR response units per SG unit sensitivity with the slope being equal to $(2.73 \pm 0.63) \times 10^6$.

3.5 Sensor Performance

In addition to being highly sensitive to minor changes in SG, fabricated GO-coated sensors have higher resolution capability when compared to SG detectors currently available in the market. This is primarily due to the fact that current SG measuring instruments are mostly based on scales drawn out on glass substrates using RI-sensitive prisms. To demonstrate that, we conducted a comparison test between MASTER-SUR/Nα clinical refractometer and our Au-GO SPR sensor. We prepared four DI water solutions containing $C_{12}H_{22}O_{11}$ in amounts varying from 151.5 mg to 153.3 mg using a precision balance, and measured their RIs and SPR responses using both refractometer and GO-modified SPR sensor. Fig. 5 exhibits results acquired by these two methods. Due to a lower sensitivity of the refractometer, the device could not differentiate among the four tested solutions and resulted in a zero slope. However, when the same samples were analyzed using fabricated Au-GO SPR sensor, we obtained 2.73×10^6 SPR response unit change per each SG unit (Fig. 5). This suggests six orders of magnitude higher sensitivity for the developed sensor when compared to existing SG sensors in the market. In addition, it is to be noted that the developed SG sensor is highly non-specific, which makes it versatile for a wide range of food analytes.

To evaluate regeneration potential of the fabricated Au-GO SPR sensors, $SPR_{max}\%$ of four different sensors was calculated. $SPR_{max}\%$ is defined in Equation (2).

$$SPR_{max}\% = \frac{(SPR_{max})_{pre}}{(SPR_{max})_{post}} \times 100\%, \quad (2)$$

where $(SPR_{max})_{post}$ is the post-regeneration detection result and $(SPR_{max})_{pre}$ is the pre-regeneration detection result. If SPR_{max}% remains above 90%, the sensor performance should be considered as adequate because the binding efficiency after regeneration remains substantial when compared to the binding efficiency before the regeneration process[28]. Table 1 shows calculations of the regeneration coefficients for 4 fabricated GO-enhanced Au SPR sensors.

Table 1 Calculations of the average regeneration coefficient, according to Equation (2), from SG measurements by using four different GO-enhanced Au SPR sensors

	Sensor 1	**Sensor 2**	**Sensor 3**	**Sensor 4**
$(SPR_{max})_{pre}$	44470	45015	45608	46302
$(SPR_{max})_{post}$	45015	45608	46302	47338
SPR_{max}%	98.789	98.703	98.499	97.811
$\mathbf{SPR_{max}\%}_{\mathbf{average}}$	98.450±0.443%			

Here, the average regeneration coefficient $(SPR_{max}\%)_{average}$ equals to 98.450±0.443% (>90%), which implies high reusability of the GO-enhanced Au SPR sensors.

To estimate the quality of the analytical results, based on Equations (3) & (4), we calculated 95% confidence interval which reflects a significance level of 0.05. The confidence interval for a series of replicate measurements with the average $\bar{c}$ and standard deviation of the mean $s_{\bar{c}}$ is defined as follows:

$$95\%\ \text{Confidence Interval} = \bar{c} \pm \Delta\bar{c}. \tag{3}$$

Here, $\Delta\bar{c}$ is known as confidence limit and is equal to:

$$\Delta\bar{c} = s_{\bar{c}} t_{0.95,\nu}, \tag{4}$$

where, $t_{0.95,\nu}$ is the t-distribution value at the level of significance of 0.05 and for ν degrees of freedom[29]. In our measurements, 95% confidence interval was found to be within ±1% of the range of the acquired data. Thus, error bars of the experimental data are too small to be visualized when plotted on the graph of SPR response units versus SG (Fig. 4). There is a probability of 0.95 to find 95% of the true values within 1% of the experimental results, and hence, there is a low dispersion of data observed.

Thus, in addition to significantly higher magnitudes of sensitivity, our Au-GO SPR sensor for SG detection is also reusable as the average regeneration coefficient of the sensors was calculated to be above 90% (SPR_{max}% = 98.450±0.443%). Confidence intervals calculated using t-test were found to be within ±1% of the obtained SPR response units. Additionally, the GO-enhanced sensors were found to have a significant shelf life as repetitive SG measurements using single sensor for two months resulted in 98% regeneration, suggesting that the developed sensor is highly stable for long term usage. Henceforth, the overall performance of the Au-GO SPR sensor featured with better sensitivity than commercial SG meters, real time and high reproducibility of the measurement along with long term reusability suggests that the developed sensor is highly robust for SG measurements. As a result, we obtained a non-specific SG sensor that can easily be applied for analysis of various food analytes.

4 Conclusions

SPR is a highly surface-sensitive optical technique, which is widely used for various biological applications. Herein, we present a simple but efficient method to apply high throughput nature of SPR sensing for SG measurements, related to food quality and safety assurance. The analytical study of the RI and SG parameters, and of their interdependence has been presented. The SPR measures changes in RI, which are proportional to the changes in SG of a given food sample. Overall, conventional Au SPR sensors can be used for the detection of SG for aqueous solutions with excellent sensitivity (10^5), as the RI measured by SPR instrument exhibits linear dependence on SG values of the tested samples. In addition, it was demonstrated that surface coating of Au SPR chips with a thin layer of GO enhances sensitivity of the sensor towards detection of SG of various aqueous solutions. GO-modified Au SPR sensors were tested using real food samples including green tea, soy sauce, and apple juice. It is found that the fabricated sensors are highly sensitive to minor changes in SG in real food samples as well. Furthermore, statistical analysis of the acquired measurements showed that the new SG sensors are highly reproducible. According to 95% confidence interval calculations, there is a 95% chance that the true values for SG are within ±1% range from the acquired data. Finally, the regeneration coefficient was calculated to be above the threshold of 90%, and thus the modified Au-GO SPR sensors can be characterized as highly reusable. The ability to detect SG values of various samples with the reported sensitivity, in a label-free, real-time, high throughput manner will contribute to a highly accurate product monitoring in food industry.

Conflict of interest

There are no conflicts to declare.

Acknowledgement

We acknowledge financial support from the OIST Graduate University with subsidy funding from the Cabinet Office, Government of Japan. In addition, authors would like to thank Laszlo Szikszai, Alexander Badrutdinov and Alejandro Villar-Briones for their technical support during use of various instruments.

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