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Surface enhanced infrared absorption spectroscopy based on gold nanostars and spherical nanoparticles

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HIGHLIGHTS

- Signal enhancement from two analytes at plasmonic nanostars and nanospheres were compared for SEIRAS in IR-ATR.
- The signal obtained from nanostars was at least 2-times higher in comparison with nanospheres.
- Up to 10-times signal enhancement at plasmonic nanostars for SEIRAS was observed.
- Dependence of enhancement on the number of nanostars at the internal reflection element surface was demonstrated.
- SEIRA signal was correlated to the concentration of analyte molecules present within the evanescent field.

GRAPHICAL ABSTRACT

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ABSTRACT

Plasmonic anisotropic nanoparticles possess a number of hot spots on their surface due to the presence of sharp edges, tips or vertices, leading to a high electric field strength surrounding the nanostructures. In this paper, we explore different plasmonic nanostructures, including anisotropic gold nanostars (AuNSTs) and spherical gold nanoparticles, in surface-enhanced infrared absorption spectroscopy (SEIRAS) in an attenuated total reflection (ATR) configuration. In our experiments, we observed up to 10-times enhancement of the infrared (IR) absorption of thioglycolic acid (TGA) and up to 2-times

Abbreviations: ATR, attenuated total reflection; AuNSTs, gold nanostars; BSA, bovine serum albumin; c-NPs, citrate-capped spherical nanoparticles; CTAB, cetyltrimethylammonium bromide; EF, enhancement factor; RSD, relative standard deviation; IR, infrared; IRE, internal reflection element; MCT, mercury–cadmium–telluride; La-NPs, laser-ablative gold nanoparticles; LOD, limit of detection; LOQ, limit of quantification; PH, plasmon hybridization; SAMs, self-assembled monolayers; SEIRAS, surface-enhanced infrared absorption spectroscopy; SEM, scanning electron microscopy; SERS, surface enhanced Raman scattering; Si, silicon; TEM, transmission electron microscopy; TGA, thioglycolic acid.

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enhancement of signals for bovine serum albumin (BSA) protein on plasmonic nanostructure-based films deposited on a silicon (Si) internal reflection element (IRE) compared to bare Si IRE. The dependence of the observed enhancement on the amount of AuNSts present at the surface of the IRE has been demonstrated. Quantitative studies with both, TGA and BSA were performed, observing that the SEIRA signal can be correlated to the concentration of analyte molecules present within the evanescent field. The calibration curves in the presence of the AuNSts showed enhanced sensitivity as compared with the bare Si IRE. We finally compare efficiencies of anisotropic AuNSts and spherical citrate-capped and “bare” laser-synthesized gold nanoparticles as SEIRAS substrates for the detection of TGA and BSA. The signal obtained from AuNSts was at least 2 times higher for TGA molecules in comparison with spherical gold nanoparticles, which was explained by a more efficient generation of hot spots on anisotropic surface due to the presence of sharp edges, tips or vertices, leading to a high electric field strength surrounding the AuNSts.

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1. Introduction

The enhanced electromagnetic field strength of gold nanoparticles (AuNPs) due to plasmon excitation [1] may be exploited for surface-enhanced vibrational spectroscopies such as surface-enhanced infrared absorption spectroscopy (SEIRAS) [2]. SEIRAS is capable of providing molecular information on analyte molecules located in close vicinity to metallic islands or nanostructures. Furthermore, material-specific vibrational absorptions in the IR-fingerprint region enable unambiguous molecular identification [3]. SEIRA in an attenuated total reflection (ATR) configuration was first reported by Hartstein et al. [2]. ATR spectroscopy takes advantage of total internal reflections within an internal reflection element (IRE) or waveguide [4,5]. SEIRAS in an ATR configuration was previously used to investigate chemical interactions between SEIRA-active layers and various chemicals [6], for the analysis of biological species [7], to monitor the adsorption kinetics of bovine hemoglobin on AuNP films with an improved detection sensitivity (i.e., up to 3 orders of magnitude) [8] and to study the adsorption behavior of cytochrome C at carboxylic acid and hydroxyl terminated self-assembled monolayers immobilized at gold surfaces [9]. In addition, López-Lorente et al. in situ monitored the synthesis of bare AuNPs mediated by stainless steel as reducing agent via SEIRA-ATR spectroscopy [10,11].

Typically, as a first step in SEIRAS studies AuNPs [8,12,13] are periodically structured at the crystal surface providing for two-dimensional arrays. Deposition techniques include sputtering [14], lithography [15] and vapor phase or wet chemical deposition [16]. In a second step, analyte molecules are administered at the arrayed gold surface. In the present study, gold nanostructures were interacted and assembled with analyte molecules in colloidal solution prior to the formation of AuNP arrays at Si ATR waveguide surfaces by adapting a procedure from Seelenbinder et al. [17].

Notably, the signal enhancement strongly depends on the sharpness of AuNP edges, tips or vertices, which are directly related to the intensity of the electromagnetic field surrounding AuNPs [18]. From that point of view, anisotropic AuNPs such as nanorods [19], nanoshells [20], or nanocages [21] are potential amplifiers. However, these types of AuNPs have several limitations including elaborate and time-consuming synthesis. In contrast, AuNSts synthesized via seed-mediated methods [22] require a minimum of neat compounds in a straightforward procedure, and appear to be excellent electromagnetic field enhancers owing to the large number of hot spots at the surface associated with the sharp tips. Moreover, according to the plasmon hybridization (PH) model [23] the core-tip structure serves as an antenna increasing both, the excitation cross section and the electric field enhancement of nanostar plasmon modes [24]. To the best of our knowledge, only a

few research groups have applied IR-ATR spectroscopy for monitoring the growth kinetics of such particles [25] and have analyzed the surface chemistry of the AuNSts in details [26]. Last but not least, AuNSts have not been applied in ATR-SEIRAS to date.

In this work, different gold nanostructures (nanostars, ligand-protected or bare spherical nanoparticles) have been deposited together with analytes on a Si IRE and their responses in ATR SEIRAS compared. In our tests, so prepared SEIRAS substrates were used to study the signal enhancement of bovine serum albumin protein (BSA) and thioglycolic acid (TGA). TGA, which was previously investigated in various analytical studies [27,28], was selected as an exemplar of small molecule. BSA, was selected as an extensively studied example of a large molecule within the family of serum albumins, and is among the most important blood carrier proteins [29]. The present study demonstrates that for both TGA and BSA examples an enhancement of the IR-signal due to the presence of the AuNPs was achieved, as compared to IR-ATR results obtained at the bare Si surfaces. This enhancement directly related to the amount of Au nanostructures present within the evanescent field. SEIRA signals obtained with AuNSts have also been compared to results obtained at alternative gold nanostructures with a spherical shape using both, citrate-capped and bare Au nanospheres. The presented results demonstrate that the signal enhancement produced by AuNSts is elevated in comparison to spherical AuNPs, which is explained by a much larger electromagnetic field strength at sharp tips on their surface. These data demonstrate a high potential of gold nanostars in surface-enhanced infrared absorption spectroscopy.

2. Materials and methods

2.1. Chemicals

Gold (III) chloride trihydrate ($\text{HAuCl}_4 \times 3\text{H}_2\text{O}$), trisodium citrate dihydrate ($\text{C}_6\text{H}_5\text{O}_7\text{Na}_3 \times 2\text{H}_2\text{O}$), hydrochloric acid (HCl), L(+)-ascorbic acid (AA), silver nitrate (AgNO_3), thioglycolic acid (TGA), bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, USA) at the highest purity grade available. Milli-Q water (resistivity $18.2 \text{ M}\Omega \text{ cm}$ at 25°C ; Millipore, USA) was used in all preparations. Prior to synthesis, the glassware was washed with a mixture of nitric acid and hydrochloric acid (HNO_3/HCl 1:3 mixture, aqua regia).

2.2. Synthesis of gold nanostars

AuNSts were fabricated by a modified seed-mediated growth protocol by Yuan et al. [29] with minor modifications [30]. Seeds were prepared using the citrate reduction method introduced by

Grabar [31]. For the AuNSt synthesis, 150 μL of 1 M HCl and 1.5 mL of the seed solution were added to 50 mL of 0.25 mM HAuCl₄ water solution at room temperature under vigorous stirring. Then, 1.5 mL of 2 mM AgNO₃ and 0.75 mL of 0.1 M AA were simultaneously added. The solution was stirred for 30 s while its color turned from light red to dark grey. The AuNSts were concentrated by centrifugation to an Au amount equal to 500 $\mu\text{g mL}^{-1}$, as determined via Inductively coupled plasma atomic emission spectroscopy (ICP-AES).

2.3. Synthesis of spherical nanoparticles

2.3.1. Citrate-capped spherical nanoparticles (c-NPs)

40-nm citrate-capped spherical nanoparticles (c-NPs) were prepared by the reduction of HAuCl₄ with sodium citrate by Grabar's method [31]. Briefly, 2.1 mL of 1% sodium citrate were added quickly to a boiling 0.03% HAuCl₄ solution (50 mL), resulting in a change in the color of the solution from pale yellow to deep purple-red. The c-NPs were concentrated by centrifugation to an Au concentration equal to 500 $\mu\text{g mL}^{-1}$, as determined by ICP-AES.

2.3.2. Laser-ablative synthesis of spherical gold nanoparticles (la-NPs)

Laser-ablative gold nanoparticles (la-NPs) were prepared by pulsed laser ablation using a modified protocol by Kabashin et al. [32] via a Ti/sapphire laser system (Hurricane Spectra Physics Lasers, wavelength 800 nm, repetition rate 1000 Hz, pulse duration 110 fs). A two-step approach was applied: (i) direct laser ablation of a gold target (Goodfellow, 99.95%), and (ii) laser fragmentation of the obtained colloidal solution.

During the first step, the gold target was immersed in 18 M Ω deionized water and irradiated with laser radiation. The liquid volume and its level over the target were 4 mL and 4 mm, respectively. Laser fluence was controlled by a half-wave plate and was fixed at 150 $\mu\text{J/pulse}$. Radiation was focused on the surface of the target by a 50 mm lens. In order to avoid ablation from the same area, the gold target was moved constantly. Total ablation time was 20 min. As a result, a colloidal solution of dispersed la-NPs was obtained. In order to narrow their size distribution, a second step of laser fragmentation was used. In this case, the collected solution was irradiated with the same laser radiation focused 10 mm below the surface level using the same laser fluence. The laser fragmentation step was performed for 1 h under magnetic stirring. Thus obtained la-NPs were concentrated by centrifugation to an Au amount equal to 500 $\mu\text{g mL}^{-1}$.

2.4. Instrumentation

Infrared spectroscopic studies were performed using a Vertex 70 FTIR spectrometer (Bruker Optics, Ettlingen, Germany) equipped with a BioATR II unit (Bruker Optics, Ettlingen, Germany). The ATR crystal surface interfacing with the sample was made from a circular section of a silicon wafer providing 8–10 internal reflections. IR radiation is coupled into this silicon ATR plate via a secondary zinc selenide (ZnSe) ATR crystal providing a hemispherical shape for efficient coupling of radiation from the FTIR spectrometer into the Si ATR waveguide. A liquid nitrogen cooled mercury–cadmium–telluride (MCT) detector (Bruker Optics, Ettlingen, Germany) was used for signal recording. Data acquisition and processing was performed using the OPUS 6.5 software package (Bruker Optics, Germany), and the Essential FTIR spectroscopy toolbox (Operant LLC, USA).

Scanning electron microscopy (SEM) images were acquired using a Helios Nanolab 600 scanning electron microscope (FEI Corp., Eindhoven, the Netherlands). The Au concentration was obtained

via inductively coupled – atomic emission spectrometry (ICP-AES) using an Ultima 2 system (HORIBA Jobin Yvon, Tokyo, Japan).

2.5. IR-ATR studies

For IR-ATR measurements, 20 μL of mixtures of aqueous solutions of analyte and AuNPs in equal volumes (1:1) were deposited onto the Si ATR waveguide. Then, spectra were acquired for 1 min each until total evaporation of the liquid (several hours). IR-ATR spectra were recorded by averaging 100 scans in the spectral range of 4000–500 cm^{-1} at a spectral resolution of 4 cm^{-1} . For ATR measurements of pure analyte samples, the AuNPs colloid solution was replaced by an equal amount of water, thus keeping the analyte concentration and total sample volume the same. Prior to analyzing each set of samples, a background spectrum was acquired. For better comparability, a baseline correction was performed.

3. Results and discussion

3.1. Characterization of Au nanostructures

The morphology of the synthesized Au nanostructures was investigated via SEM and TEM. As shown in Fig. 1, AuNSts with an outer diameter of approximately 60 nm, which provide approximately 12–15 tips at a length of 15 nm were obtained.

Bare spherical nanoparticles synthesized by laser ablation (la-NPs) had a size distribution ranging from 20 nm to 40 nm (Fig. 2a). Despite the absence of any protective ligands, la-NPs colloids remain stable due to electric repulsion effect associated with a strong negative charging of the formed nanoparticles [33]. Citrate-capped spherical nanoparticles (c-NPs) with a diameter of approximately 40 nm (Fig. 2b) were prepared by the reduction of HAuCl₄ with sodium citrate, acting both as a reducing agent and as a capping agent following Grabar's method [31].

3.2. ATR-SEIRA studies at TGA

FTIR spectra for (a) TGA in water at acidic pH and (b) the deprotonated form of TGA in a basic solution (pH $\sim$ 11.5). In the acidic trace (red line), the broad band between 3600 and 2500 cm^{-1} corresponded to the O–H stretch of a carboxylic acid, including the convolution of C–H stretching bands with the broad O–H feature. The signal at 2550 cm^{-1} indicates the intact thiol group and the stretch at 1700 cm^{-1} represents the carbonyl group characteristic of carboxylic acids.

Thioglycolic acid (TGA, HS–CH₂–COOH), a.k.a., mercaptoacetic acid has been selected as model analyte for this study. An IR-ATR spectrum of pure TGA is shown in Fig. 3c. The strongest signal around 1709 cm^{-1} represents the carbonyl band associated with carboxylic acids (ν (C=O)) [34]. The C–S stretch and the C–H bend are located between 685 and 1000 cm^{-1} , respectively. The stretching feature at 2560 cm^{-1} indicates the intact thiol group, whereas the band at 1145 cm^{-1} (ν (C–O)) and a broad band between 3600 and 2500 cm^{-1} correspond to the O–H stretch of a carboxylic acid, respectively [34,35].

The evaporation kinetics of TGA and mixtures of TGA and Au nanostructures deposited at the surface of the Si waveguide were monitored by in situ ATR-SEIRA. For this purpose, two types of solutions were used: (i) 10 μL of AuNSts, c-NPs or la-NPs (initial Au concentration 500 $\mu\text{g mL}^{-1}$) and 10 μL of TGA (initial TGA concentration 4 $\mu\text{g mL}^{-1}$), and (ii) replacing the AuNSt solution with the equivalent amount of water for maintaining a final TGA concentration of 2 $\mu\text{g mL}^{-1}$ and a total volume of 20 μL , similar to the experiment in the presence of Au nanostructures. In our experiments, we recorded the enhancement of SEIRAS signals for all three

Fig. 1. SEM (a) and TEM (b) images, and size distribution (c) of gold nanostars (AuNSts).

types of gold nanostructures compared to control samples. Since we monitored the strongest enhancement for gold nanostars, we will mostly present data for these nanostructures, but all conclusions are valid for spherical nanoparticles as well. In the final subsection we summarize and compare data for the investigated nanostructures. Fig. 3 shows exemplary spectra of the TGA solution (a), and of the mixture of TGA and AuNSts (b) vs. time until complete solvent evaporation (red curves) occurs (without baseline correction). A comparison of conventional ATR and ATR-SEIRA spectra of fully evaporated TGA (in black) and TGA + AuNSts (in red) is shown in (d).

Evidently, since air was used as the background spectrum the spectra during the first few minutes are dominated by the water absorption (i.e., O-H bending and stretching modes). As water evaporates, a decrease in the water absorption features is observed together with the appearance of the absorption features of TGA, which emerged with enhanced sensitivity if TGA was adsorbed at the AuNSts sedimenting at the Si waveguide surface. The comparison of the enhanced intensity achieved in the IR-ATR spectrum of TGA in the presence of the AuNSts is shown in Fig. 3d.

Minute variances in the TGA spectrum in the wavelength range 1200–1300 cm^{-1} are explained by the changes in pH value of the solution [36]. According to the SEM images (Fig. S-1, Supporting

Fig. 2. (a) SEM image of spherical nanoparticles obtained laser ablation and their size distribution (inset). (b) SEM image of citrate-capped spherical nanoparticles and their size distribution.

Information), AuNSts aggregation during the measurement period was not observed. Detailed information on the detection sensitivity for TGA was obtained by analyzing the strongest band at 1709 cm^{-1} , and the band at 1155 cm^{-1} to exclude potential overlapping with the water band at 1635 cm^{-1} caused by the O–H–O scissoring band [37]. The enhancement factor of the SEIRA substrates prepared by simultaneous deposition of TGA and AuNSts, was calculated following [38]:

$$EF = (I_{\text{SEIRAS}}/I_{\text{ATR}}) \cdot (N_{\text{ATR}}/N_{\text{SEIRAS}}),$$

where I is the intensity (i.e., band height) of the considered TGA band, and N represents the total number of analyte molecules deposited on the Si crystal. Taking into account that the TGA concentration was equal in both solutions, an enhancement factor of approximately 10-fold was calculated considering both TGA bands at 1709 cm^{-1} and at 1155 cm^{-1} for a concentration of AuNSts equal to 250 $\mu\text{g mL}^{-1}$ (higher Au concentrations interfere with the quality of the stars). To optimize the experimental parameters and to demonstrate that AuNSts produce the observed SEIRA effect, the relation between the obtained enhancement vs. AuNSts concentration was investigated for two absorption bands, i.e., at 1709 cm^{-1} (Fig. 4), and at 1155 cm^{-1} (Fig. S-2, Supporting Information). Each concentration level was analyzed in triplicate with the error bars representing the standard deviation of the mean value.

The analytical performance of the developed SEIRA substrate

Fig. 3. Series of ATR and SEIRA spectra without baseline corrections showing the evaporation process of (a) 2 $\mu\text{g/mL}$ TGA solution, and (b) mixture of TGA and Nsts at a final concentration of 2 $\mu\text{g/mL}$ and 250 $\mu\text{g/mL}$, respectively for TGA and AuNsts. (c). Spectrum of pure TGA (solution $\geq 99\%$) recorded on a silicon ATR crystal. (d) Baseline corrected spectrum of TGA (black line) and AuNsts + TGA (red line) at a concentration of 2 $\mu\text{g/mL}$ of TGA in both cases after full evaporation of the solvent. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was further evaluated by studying TGA and AuNsts + TGA solutions at different concentrations of TGA again depositing 20 μL of each solution at the Si waveguide surface after solvent evaporation. A calibration function was established by plotting the height of band at 1709 cm^{-1} vs. the TGA concentration (Fig. 5).

Each concentration level was analyzed in triplicate with the error bars again representing the standard deviation of the mean value. For comparison, a calibration based on the height of the band at 1155 cm^{-1} vs. the TGA concentration is represented in Fig. S-3 (Supporting Information).

3.3. ATR-SEIRA studies at bovine serum albumin

IR-spectroscopy is an excellent tool for the structural characterization of proteins in various environments. However, the detection of relevant absorptions resulting from protein monolayers is challenging due to the low signal strength [39]. To illustrate the potential of AuNPs in IR biospectroscopy, bovine serum albumin (BSA) was selected as an exemplary analyte. Due to its well-characterized structure and properties, BSA is frequently used as a model protein for fundamental studies of protein-nanoparticle conjugates [40–42].

In situ monitoring of the evaporation kinetics of BSA and BSA mixed with Au nanostructures at the Si crystal surface were performed similar to the TGA studies. The final concentration of BSA was equal to 500 $\mu\text{g mL}^{-1}$ in both BSA and BSA + AuNPs solutions. The AuNsts, c-NPs and Ia-NPs concentrations were equal to 250 $\mu\text{g mL}^{-1}$. Series of ATR and ATR-SEIRA spectra for AuNsts vs. time during the evaporation process (without baseline correction) are presented in Figs. S-4a and S-4b. A comparison of conventional ATR and ATR-SEIRA spectra of BSA and BSA + AuNsts after solvent evaporation is presented in Fig. 6.

Fig. 6 (black line) shows a native BSA spectrum with the most pronounced bands around 1650 cm^{-1} , which are associated with the amide I band (i.e., the C=O stretching vibrations of the peptide bond), and the band at 1539 cm^{-1} (i.e., N-H bending vibration/C-N stretching vibration) called the amide II band [43]. The red line in Fig. 6 illustrates spectra of BSA (500 $\mu\text{g mL}^{-1}$) mixed with AuNsts (at a Au mass concentration 250 $\mu\text{g mL}^{-1}$). A distinct signal enhancement was observed for both, the amide I and amide II band. To avoid any influence of potential water vapor bands located near 1600 cm^{-1} , detailed information on the correlation between the signal enhancement and the AuNsts concentration was obtained by analyzing the amide II band at 1539 cm^{-1} (Fig. 7).

Fig. 4. Height of 1709 cm^{-1} band of TGA + AuNSts solution versus AuNSts concentration. The maximal concentration of AuNSts ($250\text{ }\mu\text{g/mL}$) caused an absorption enhancement factor of 10 for a TGA concentration of $2\text{ }\mu\text{g/mL}$. Measurements were done after total evaporation of solutions.

Fig. 5. Calibration graphs at different concentrations of TGA by measuring the height of the band in the ATR and SEIRAS spectra of TGA and TGA + AuNSts at 1709 cm^{-1} . The calibration curves have linear behavior in the chosen concentration range. AuNSts concentration was similar or all samples and equal $250\text{ }\mu\text{g/mL}$.

Analysis of amide I band is presented in Supporting information (Fig. S-5b). As anticipated, the band height increased, as the BSA mass increased from 0 to $250\text{ }\mu\text{g mL}^{-1}$, and gradually saturated for a BSA mass higher than $250\text{ }\mu\text{g mL}^{-1}$. Both curves fit a linear calibration with a goodness of the fit of $r^2 = 0.993$ for AuNSts + BSA and $r^2 = 0.996$ for native BSA. However, the saturation process can be fitted with a Langmuir isotherm (Fig. S-6) which represent an adsorption process of the protein within the first monolayer, as also shown in literature [44].

According to SEM images (Fig. S-5a, Supporting Information), AuNSts aggregation during the measurements was not evident. Moreover, absence of a shift of the amide I band position in the spectrum of the AuNSts-BSA mixture in comparison to the native BSA spectrum (Fig. S-5b) suggested that the secondary structure of

Fig. 6. Spectra of fully evaporated BSA (black line) and AuNSt + BSA (red line) after baseline correction (c). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 7. Calibration at different concentrations of BSA after evaluating conventional ATR and ATR-SEIRA spectra of BSA and BSA + AuNSt at 1539 cm^{-1} . The AuNSt concentration was similar in all samples and equal to $250\text{ }\mu\text{g/mL}$. All measurements were executed after solvent evaporation.

BSA remained unaffected by the presence of AuNSts [45,46] (Table 1).

SEIRA enhancement factors in the same range were demonstrated for vapor-deposited silver (EF between 5 and 150) [47] and platinum (EF around 10) [48] nanofilms, as well as for wet-chemical deposited spherical gold nanoparticles (EF around 5) [49]. The applied EF calculation method was similar to the approach used in

Table 1

Summarizes the analytical figures of merit obtained for the AuNSts enhanced calibration curves of both analytes TGA and BSA, where LOD is the limit of detection; LOQ is the limit of quantification, EF is the enhancement factor and RSD is the relative standard deviation for the highest concentration.

	TGA (1709 cm^{-1})	BSA (1539 cm^{-1})
Linear range	$0.063\text{--}2\text{ }\mu\text{g}\cdot\text{mL}^{-1}$	$31.25\text{--}250\text{ }\mu\text{g}\cdot\text{mL}^{-1}$
LOD	$0.3\text{ }\mu\text{g}\cdot\text{mL}^{-1}$	$35\text{ }\mu\text{g}\cdot\text{mL}^{-1}$
LOQ	$1\text{ }\mu\text{g}\cdot\text{mL}^{-1}$	$105\text{ }\mu\text{g}\cdot\text{mL}^{-1}$
EF	10	2.5
RSD (%)	4.42	8.4

the present study. Theoretical investigations of nanoshell arrays by finite difference time domain method resulted in electromagnetic SEIRA EF equal to 10^3 [50] or 10^6 [51], while one of the largest SEIRA enhancement factor was experimentally reported as 10^4 [52]. The significant increase in EF factor for AuNSTs may be achieved in future by exploiting two contributing effects, i.e., an increase in particle size enhancing the metallic character [53], and an increase in area filling factor; typical EFs for individualized metal nanoparticles are around 25 [54]. Notably, the EF is also strongly depending on the investigated analyte (i.e., TGA and BSA in the present study).

3.4. Comparison of ATR-SEIRA performance using different types of gold nanostructures

As we pointed out above, we observed the strongest enhancement of SEIRA signals for gold nanostars, but spherical nanoparticles also provided substantial enhancement. In this section, we summarize enhancement factors for all used nanostructures, namely: anisotropic NSTs, bare laser-synthesized gold nanoparticles (la-NPs), and citrate-capped gold nanoparticles (c-NPs). In order to achieve similar experimental conditions, the final Au mass for each AuNPs type were brought to the same value. Fig. 8 represents the comparison of the selected band height both for TGA (1709 cm^{-1}) and BSA (1539 cm^{-1}) mixed with three types of GNPs.

One can see that citrate-capped nanoparticles (c-NPs) provided slightly stronger enhancement compared to laser-synthesized nanoparticles (la-NPs), but anisotropic gold nanostars (NSTs) outperformed both spherical counterparts. In accordance to SERS experiments, the increased enhancement of the citrate capped nanoparticles in comparison to the laser ablated particles can be assigned to the slightly bigger diameter of 40 nm of the citrate capped ones versus the 30 nm of the laser ablated ones [55]. Furthermore, citrate capped nanoparticles innately might hamper analyte adsorption due to the citrate shell, an exchange can be anticipated within the early stages after solution mixing and therefore can be neglected at the experiment time scales [56]. Indeed, SEIRA signals from TGA were about two times stronger for NSTs compared to spherical nanoparticles (Fig. 8a). Although the difference in SEIRA signals for BSA was not so significant, it still reached 20–25% (Fig. 8b). Specific sulfur-gold interaction between TGA and gold nanoparticles is expected. Although the same amount of Au was applied for TGA and BSA, the enhanced band height may partially ascribe to the increased total surface area of nanoparticles. This may account for the less pronounced enhancement factor observed for BSA which has a large molecular volume and no specific interaction with Au. We believe that such a difference in enhancement factors is due to a much larger electromagnetic field strength at sharp tips on the surface of nanostars. Thus, our experiments demonstrated that NSTs the most applicable enhancer agents for Surface enhanced vibrational spectroscopy (SEVS).

4. Conclusions

Various plasmonic nanostructures, including anisotropic nanostars (NSTs) and spherical nanoparticles, were applied for the detection of exemplary analytes in ATR-SEIRA spectroscopy providing enhanced spectral signatures in the infrared regime. This approach enables the detection of molecules close to or at the surface of nanoparticles deposited at Si ATR waveguide surfaces improving the sensitivity of detection by up to 10-fold. In comparison to spherical gold nanoparticles, AuNSTs confirmed superior enhancement due to their anisotropic surface structure. The obtained results demonstrate the utility of Au nanostructures in ATR-

Fig. 8. (a) Band height at 1709 cm^{-1} of evaporated TGA solution and TGA mixed with laser-synthesized nanoparticles (la-NPs), citrate-capped nanoparticles (c-NPs) and AuNSTs. (b) Band height at 1539 cm^{-1} of evaporated BSA solution and BSA mixed with ultrapure la-NPs, c-NPs and AuNSTs. Concentration of each type of GNPs was kept as $250\text{ }\mu\text{g/mL}$. All measurements were executed after solvent evaporation.

SEIRA applications, which is considered of relevance in IR chem/bio sensing schemes as well as IR biospectroscopy, e.g., of proteins and biomarkers. It is anticipated that these findings will aid in enhancing the sensitivity of label-free IR assay technologies via deliberately controlled SEIRA-based signal amplification. This is of particular importance, if extremely low biomarker concentrations have to be detected, as encountered, e.g., in neurodegenerative diseases such as Parkinson or Alzheimer disease. Given the obtained improvements in signal intensity, IR spectroscopy and chem/bio sensing concepts may emerge as a viable alternative vs. more time- and resource-consuming screening technologies currently implemented in conventional medical diagnostic scenarios. Moreover, the number of potential interferants in the MIR requires substantial studies on the competitive behavior of overlapping spectral features by matrix molecules in close vicinity of the SEIRA substrate. Hence, subsequent studies will follow up in detail on these aspects, in order to prove the applicability of the presented SEIRA substrate in real-world scenarios.

Conflict of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.aca.2017.07.045>.

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