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Molecularly Imprinted Au Nanoparticles Composites on Au Surfaces for the Surface Plasmon Resonance Detection of Pentaerythritol Tetranitrate, Nitroglycerin, and Ethylene Glycol Dinitrate

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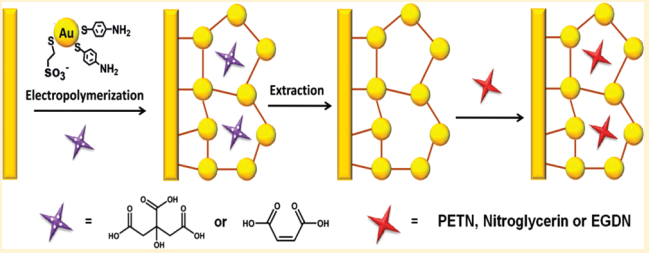
Supporting Information

ABSTRACT: Molecularly imprinted Au nanoparticles (NPs) composites are generated on Au-coated glass surfaces. The imprinting process involves the electropolymerization of thioaniline-functionalized Au NPs (3.5 nm) on a thioaniline monolayer-modified Au surface in the presence of a carboxylic acid, acting as a template analogue for the respective explosive. The exclusion of the imprinting template from the Au NPs matrix yields the respective imprinted composites. The binding of the analyte explosives to the Au NPs matrixes is probed by surface plasmon resonance spectroscopy, SPR, where the electronic coupling between the localized plasmon of the Au NPs and the surface plasmon wave leads to the amplification of the SPR responses originating from the dielectric changes of the matrixes upon binding of the different explosive materials. The resulting imprinted matrixes reveal high affinities and selectivity toward the imprinted explosives. Using citric acid as an imprinting template, Au NPs matrixes for the specific analysis of pentaerythritol tetranitrate (PETN) or of nitroglycerin (NG) were prepared, leading to detection limits of 200 fM and 20 pM, respectively. Similarly, using maleic acid or fumaric acid as imprinting templates, high-affinity sensing composites for ethylene glycol dinitrate (EGDN) were synthesized, leading to a detection limit of 400 fM for both matrixes.



The selective and highly sensitive detection of explosives attracts substantial efforts in homeland security research.[1,2] Different analytical procedures for the detection of explosives were reported, and these include optical,[3] electrochemical,[4] surface acoustic wave,[5] or competitive immunoassay[6] methods.

Recently, we have introduced imprinted Au NPs composites as ultrasensitive and selective matrixes for the electrochemical or surface plasmon resonance (SPR) analyses of the TNT or RDX explosives.[7] The key element of this approach involves the generation of molecularly imprinted sites in Au NPs matrixes. In contrast to the well-established principles of preparation of molecularly imprinted matrixes in organic or inorganic polymers (e.g., TiO_2, SiO_2),[8] the synthesis of molecularly imprinted matrixes of metal nanoparticles (NPs), e.g., Au NPs, demonstrates significant advantages emerging from the nanoscale dimensions of the building blocks of the composite sensing interface. According to this principle, Au NPs modified with electropolymerizable thioaniline units are electropolymerized onto Au-coated surfaces in the presence of a molecular template that acts as a structural analogue for the respective substrate analyte and exhibits affinity interactions with the thioaniline modifying groups, or with the electrogenerated bis(aniline) bridging units. Such affinity interactions could be π-donor−acceptor, H-bonds, acid−base, or electrostatic interactions. The electropolymerization of the functionalized NPs onto a thioaniline-modified surface, and the subsequent removal of the template molecules from the resulting bis(aniline)-cross-linked Au NP matrixes, resulted in the formation of molecularly imprinted contours that bind the analyte by complementary affinity interactions. For example, by the electropolymerization of the functionalized NPs in the presence of picric acid or Kemp's acid, imprinted Au NPs composites for the ultrasensitive detection of TNT or RDX were, respectively, demonstrated. The binding of the substrates to the imprinted sites was monitored by SPR spectroscopy. This spectroscopic method probes the effect of dielectric changes occurring at thin metal surfaces, such as Au or Ag films, on the resonance of the surface plasmon wave.[9] In fact, this method was extensively used to probe the formation of antigen−antibody,[10] substrate−receptor complexes,[11] or DNA hybridization[12] on the metal films, to follow biocatalytic transformations,[13] and to investigate the

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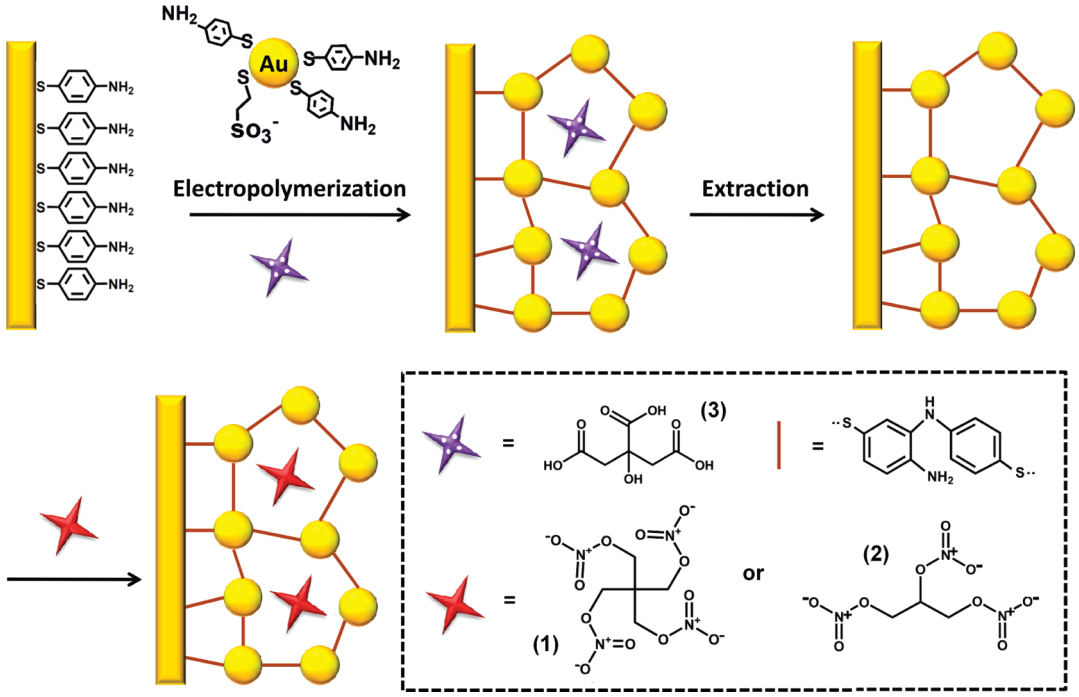
Scheme 1. Schematic Presentation for the Electropolymerization of a Composite of Bis(aniline)-Cross-Linked Au NPs for the Sensing of PETN or NG Using Citric Acid as an Imprinting Template



chemical properties of polymer films linked to surfaces.[14] For systems with low coverage of the analytes, or upon the association of low-molecular-weight substrates, the dielectric changes at the Au surface are too small to allow detectable SPR spectral changes. Different methods to enhance the dielectric changes upon binding the analyte to the surface were reported by implementing latex particles,[15] liposomes,[16] or secondary proteins[17] as amplifying labels for the recognition events. One effective method to amplify the recognition process has involved the conjugation of the recognition events to metallic NPs, e.g., Au NPs. The coupling between the localized plasmon of the NPs and the surface plasmon wave was found to significantly affect the SPR spectrum.[18] Thus, minute changes of the dielectric properties as a result of the binding of the analyte could be amplified by the conjugation of metallic NPs to the sensing complex. For example, the detection of DNA or antigen−antibody complexes was amplified by the labeling of the recognition complexes with Au NPs.[19] This amplification paradigm represents the major advantage of the imprinted bis(aniline)-cross-linked Au NPs composites. Since the association of the analytes to the imprinted sites in the Au NPs composites alters the local dielectric properties at the Au surface, even small dielectric changes as a result of the low coverage of the imprinted sites are amplified by the coupling of the localized Au NPs plasmons with the SPR wave associated with the Au surface.

Furthermore, this method to imprint molecular recognition sites into the cross-linked Au NPs matrixes can be extended to the analytes (or analogues) that lack direct affinity interactions with the thioaniline sites associated with the NPs or the bis(aniline) units that bridge the Au NPs. This is achieved by the coimmobilization of a ligand or a receptor site to the thioaniline-functionalized Au NPs. The association of the analyte to the ligand/receptor sites, the subsequent polymerization of the Au NPs on the Au surface, and the removal of the analyte bound to the ligand associated with the bis(aniline)-cross-linked Au NPs composite result in the specifically imprinted matrix. For example, the electropolymerization of thioaniline-modified Au NPs cofunctionalized with a phenylboronic acid ligand in the presence of saccharides,[20] or vicinal diol-functionalized antibiotics as analytes,[21] resulted (after the removal of the analytes) in molecularly imprinted matrixes for the respective analytes. The formation of complexes between monosaccharides or antibiotics and the phenylboronic acid ligands provided the motif for the generation of the imprinted sites. Sensi tive, selective, and even chiroselective detection paradigms of analytes by the imprinted Au NPs composites were demonstrated.[22] Similarly, the coimmobilization of cysteine, as a zwitterionic ligand on the thioanline-functionalized Au NPs, enabled the electrochemical synthesis of imprinted Au NPs for a series of amino acids.[20,22] The electrostatic interactions between the amino acid analytes and the cysteine ligand resulted in supramolecular structures that formed the imprinted sites. For these systems, both selectivity and chiroselectivity were demonstrated. It should also be noted that, in all of the systems, SPR spectroscopy was used to probe the sensing of the different analytes.

RESULTS AND DISCUSSION

In the present study, we report on the preparation of molecularly imprinted Au matrixes for the detection of pentaerythritol tetranitrate, PETN, nitroglycerin, NG, and ethylene glycol dinitrate, EGDN. As the carboxylic acid residues exhibit similar dimensions and structural features to the nitro groups in the explosives, and since ionic and/or H-bonds between the carboxylate and the anilinium residues could provide affinity

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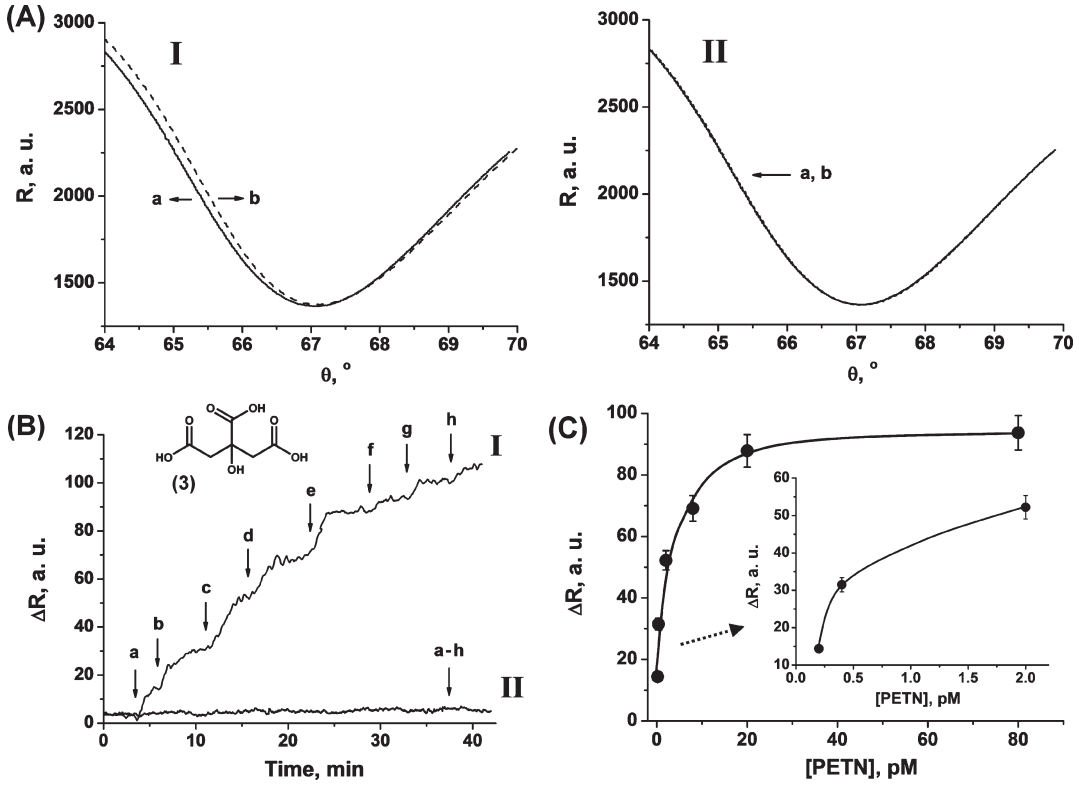

Figure 1. (A) SPR curves corresponding to (I) the citrate-imprinted bis(aniline)-cross-linked Au NP composite (a) before and (b) after the addition of PETN, 2 pM, and (II) the nonimprinted bis(aniline)-cross-linked Au NP composite (a) before and (b) after the addition of PETN, 2 pM. (B) Sensograms corresponding to the changes in the reflectance intensities, at a constant angle $\theta = 65.0°$, upon addition of variable concentrations of PETN: (a) 200 and (b) 400 fM and (c) 2, (d) 8, (e) 20, (f) 80, (g) 200, and (h) 400 pM to (I) the citrate-imprinted Au NPs matrix and (II) the nonimprinted matrix. (C) Calibration curve relating the reflectance changes to the concentrations of PETN on the citrate-imprinted matrix. The inset shows the lower concentration region of the calibration curve. Error bars correspond to a set of $N = 5$ measurements. All measurements were performed in ethanol.

interactions between the carboxylic acids and the electrogenerated bis(aniline)-cross-linked Au NPs composite, we argued that the structurally related carboxylic acids could act as templates for the generation of imprinted sites for structurally analogous nitro-substituted explosives. Scheme 1 outlines the method to synthesize an imprinted Au NPs composite for the detection of PETN (**1**) or NG (**2**). The thioanline-functionalized Au NPs were electropolymerized on a thioaniline-modified Au-coated glass surface in the presence of citric acid (**3**) as an imprinting template. The resulting bis(aniline)-cross-linked matrix was then rinsed to remove the imprinting template molecule. The electropolymerization of the Au NPs and the elimination of the imprinting template were followed by SPR spectroscopy (see Figure S1, Supporting Information). A nonimprinted Au NPs composite was similarly prepared by the electropolymerization of the modified Au NPs in the absence of the imprinting template. Figure 1A exemplifies the SPR spectra observed upon the treatment of the citrate-imprinted Au NPs matrix: (panel I) and the nonimprinted Au NPs composite (panel II) with 2 pM PETN. Whereas a noticeable shift in the SPR curve is observed for this low PETN concentration on the citrate-imprinted composite, no shift is evident for the nonimprinted matrix. Figure 1B, curve I, shows the reflectance changes of the citric acid-imprinted Au NPs composite upon interaction with different concentrations of PETN. As the concentration of PETN increases, the reflectance changes are intensified, consistent with a higher content of PETN that binds to the composite. For comparison, Figure 1B, curve II, depicts the reflectance values observed upon the treatment of the nonimprinted Au NPs composite within the same PETN concentration range. Evidently, only minute reflectance changes are observed, implying that PETN does not bind to the nonimprinted Au NPs matrix. The calibration curve corresponding to the reflectance changes of the citrate-imprinted composite upon treatment with different concentrations of PETN is shown in Figure 1C. The detection limit for analyzing PETN by the citrate-imprinted matrix is 200 fM. Assuming a Langmuir-type association of the explosive to the imprinted composite, we derived an association constant of $K_a = 9.5 \times 10^{11}\ M^{-1}$ for the binding of PETN to the imprinted sites (for further details, see the Supporting Information). The sensing interface can be, however, regenerated, and upon rinsing-off of the bound PETN, we were able to reactivate the sensing performance of the Au NPs matrix for at least three times without any noticeable degradation of the sensing composite. Furthermore, we find that the structure of the imprinting carboxylic acid template is extremely important to generate an effective sensing matrix. Imprinting of the Au NPs composite using an isocitric acid, as a template, yields a matrix with a poor sensing performance, Figure S2, Supporting Information. It is intriguing that, although citric acid acts as an effective imprinting analogue for analyzing PETN, the stereoisomer isocitric acid leads to imprinted sites of poor affinity for the explosive. Although this phenomenon is not fully understood, presumably the OH group associated with the isocitric acid interacts via H-bonds with the

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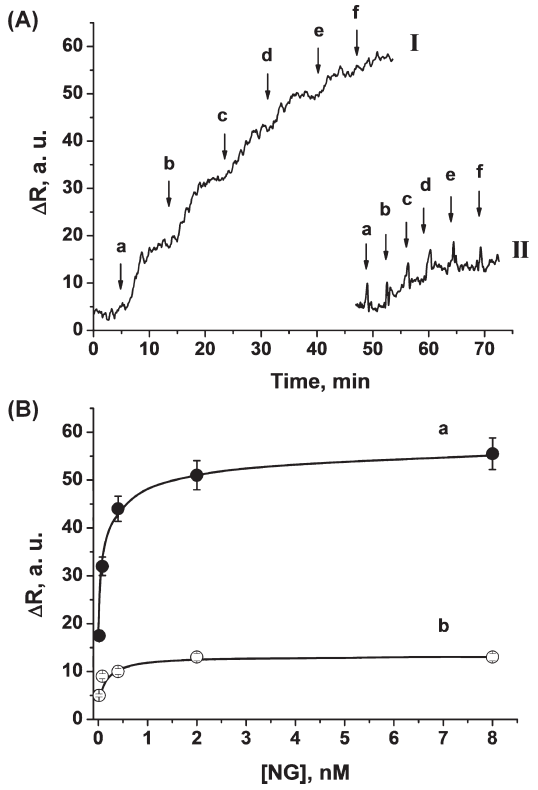

Figure 2. (A) Sensograms corresponding to the changes in the reflectance intensities, at a constant angle $\theta = 65.0°$, upon addition of variable concentrations of NG: (a) 20, (b) 80, and (c) 400 pM and (d) 2, (e) 8, and (f) 40 nM to (I) the citrate-imprinted Au NPs matrix and (II) the nonimprinted matrix. (B) Calibration curves relating the reflectance changes to the concentrations of NG on (a) the citrate-imprinted and (b) the nonimprinted matrixes. Error bars correspond to a set of $N = 5$ measurements. All measurements were performed in ethanol.

two adjacent carboxylic acid groups, leading to their configurational distortion. Thus, the resulting imprinted sites are sterically perturbed to bind effectively PETN. Also, upon the application of dicarboxylic acids, such as succinic acid or fumaric acid, as imprinting templates, Au NPs composites that lack sensing abilities toward PETN were generated. These results emphasize the significance in the selection of an appropriate template analogue for the target explosive (see also further discussion for other explosives).

Nitroglycerin exhibits, however, structural similarity to the citric acid imprinting template, and thus, we anticipated that the citrate-imprinted matrix would also sense the NG explosive. Figure 2A, curve I, shows the reflectance changes of the citrate-imprinted matrix upon interaction with different concentrations of NG. For comparison, Figure 2A, curve II, depicts the reflectance changes of the nonimprinted Au NPs composite upon interaction with different concentrations of NG. Only at high NG concentrations, above 2 nM, minute reflectance changes are observed. Figure 2B, curve a, shows the calibration curve for analyzing NG by the citrate-imprinted Au NPs composite. The reflectance changes level off to a saturation value at a concentration of ca. 2 nM, consistent with the saturation of the imprinted sites. From the calibration curve, we derived the association constant of NG to the citrate-imprinted sites, $K_a = 2.5 \times 10^{10}\ M^{-1}$. The detection limit for analyzing NG by the

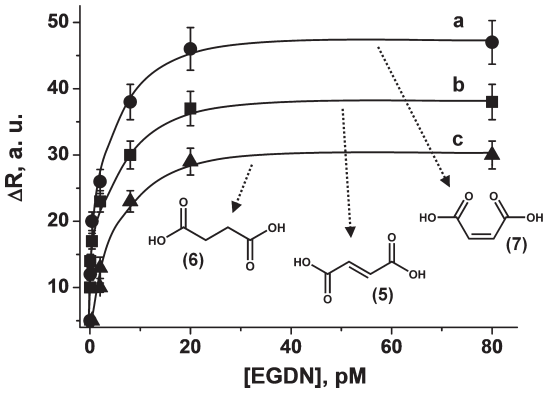

Figure 3. Calibration curves relating the reflectance changes to the concentrations of EGDN on (a) the maleic acid-imprinted, (b) the fumaric acid-imprinted, and (c) the succinic acid-imprinted bis(aniline)-cross-linked Au NPs matrixes. Error bars correspond to a set of $N = 5$ measurements. All measurements were performed in ethanol.

imprinted composite corresponds to 20 pM. Evidently, the detection limit for analyzing PETN by the citrate-imprinted Au NPs matrix is ca. 100-fold lower as compared to the analysis of NG on the same matrix. This enhanced sensitivity is also reflected by the significantly higher association constant (ca. 40-fold higher) of PETN to the citrate-imprinted sites. The enhanced association of PETN to the citric acid-imprinted sites, as compared to the NG binding to these sites, may be attributed to the fact that, in addition to the three carboxylic acid residues that mimic the $-ONO_2$ functionalities of PETN, the additional OH group present in the imprint molecule (citric acid) acts cooperatively in generating a binding domain for the fourth $-ONO_2$ group present in PETN. That is, the imprinted sites are structurally optimized to accommodate the substrate with four $-ONO_2$ groups. Figure 2B, curve b, shows the calibration curve for analyzing NG by the nonimprinted matrix. The reflectance changes are minute, and they do not allow the detection of NG. We also find that the imprint of other carboxylic acids, such as isocitric, succinic, or maleic acids does not lead to Au NPs matrixes that allow effective sensing of NG at the low-concentration range.

We then attempted to develop an imprinted matrix for the sensing of EGDN (**4**) explosive. On the basis of the imprinting rationale described for the generation of imprinted matrixes for PETN or NG using the structurally related carboxylic acids, we argued that fumaric acid (**5**), succinic acid (**6**), or maleic acid (**7**) may act as imprinting templates for EGDN. Accordingly, we used each of these dicarboxylic acids to generate the respective imprinted Au NPs composites. Figure 3 shows the calibration curve observed upon the treatment of the imprinted Au NPs composites (using **5**, **6**, or **7** as imprinting templates) with variable concentrations of EGDN. We find that the three Au NPs matrixes reveal comparable sensing capabilities for EGDN. Maleic acid as an imprinting template (**7**) yields the superior sensing matrix (highest reflectance changes along the EGDN concentration profile), while the second-best sensing matrix is formed upon the imprinting of **5** into the composite. The Au NPs composite imprinted with **6** reveals the lowest sensing features. Previous spectroscopic (IR) studies have analyzed the most plausible configurations of EGDN and found that the favored existence of the explosive is when the nitro substituents are in a "gauche" conformation with a lower population of the $-ONO_2$

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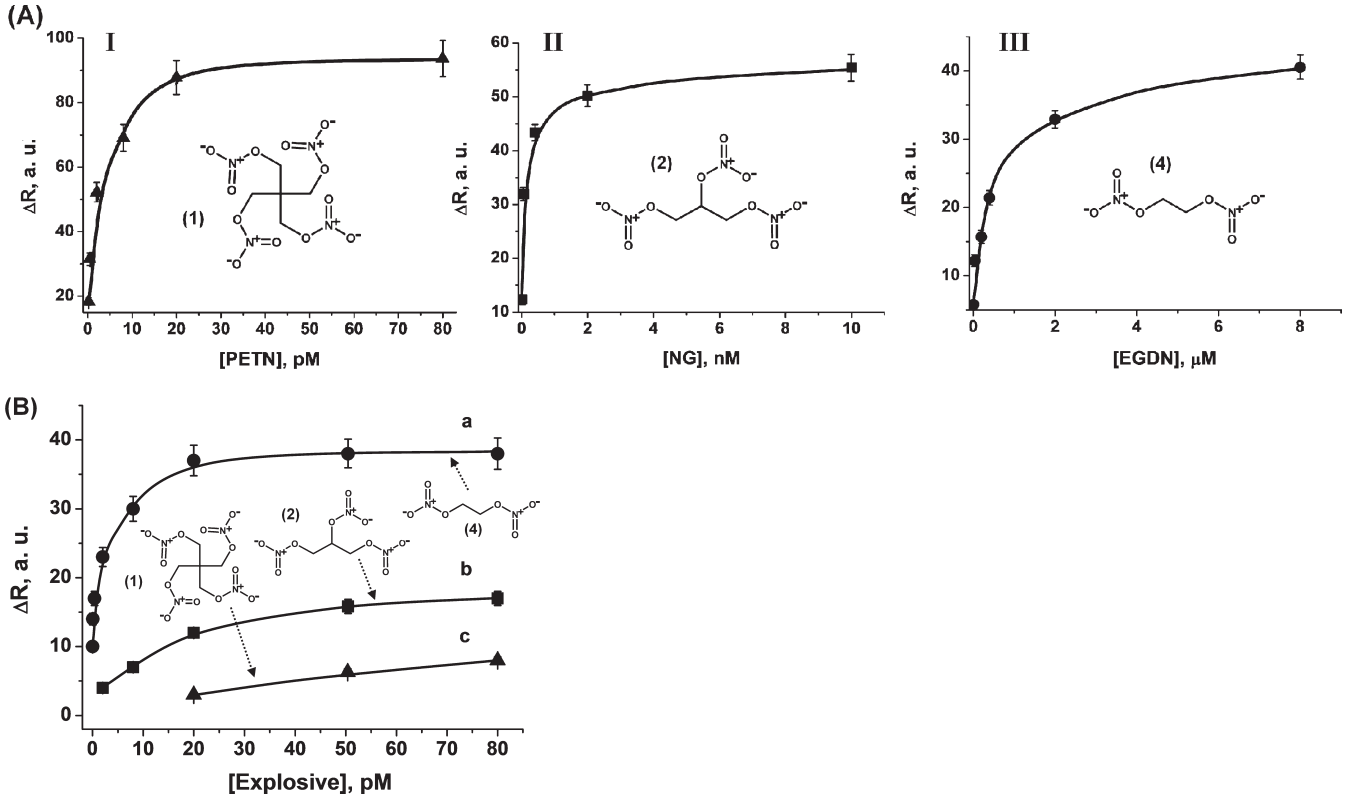

Figure 4. (A) Calibration curves relating the reflectance changes to the concentrations of (I) PETN, (II) NG, and (III) EGDN on the citric acid-imprinted bis(aniline)-cross-linked Au NPs matrix. (B) Calibration curves relating the reflectance changes to the concentrations of (a) EGDN, (b) NG, and (c) PETN on the fumaric acid-imprinted bis(aniline)-cross-linked Au NPs matrix. Error bars correspond to a set of $N = 5$ measurements. All measurements were performed in ethanol.

groups in a trans conformation.[23] The cis configuration of the carboxylic acid substituents in maleic acid or the trans configuration of the carboxylic acid functionalities in fumaric acid represent rigid structures that provide good analogues for the respective gauche/trans conformations of EGDN, suggesting that this leads to the more effective sensing matrixes. We also observed that the nonimprinted Au NPs composite lacked any affinity for the association of EGDN, and no reflectance changes were observed in this concentration range. Thus, we conclude that the imprinting of the dicarboxylic maleic acid in the Au NPs composite is essential to yield an effective sensing matrix for EGDN. From the calibration curve, we estimate the association constant of EGDN to the imprinted sites to be $K_a = 8.1 \times 10^{11}$ M^{-1}.

Finally, we examined the selectivity of the carboxylic acids-imprinted matrixes toward the analysis of the three explosives: PETN, NG, and EGDN. Figure 4A shows the calibration curves corresponding to the analysis of PETN (curve I), NG (curve II), and EGDN (curve III) by the citric acid-imprinted Au NPs composite. While PETN is detected with a sensitivity that corresponds to 200 fM, the NG and EGDN explosives yield detectable reflectance responses starting at concentrations above 100 pM and 400 nM, respectively. These results clearly indicate that the citric acid template that includes three carboxylic acid substituents and one OH functionality yields an imprinted contour that allows an effective binding for the four nitrate ester substituents associated with PETN. The EGDN that is sterically smaller and includes only two nitro substituents does not fit to the resulting imprinted sites, leading to low binding affinities and to poor performance of the matrix toward sensing EGDN. We also found that, compared to the other carboxylic acid imprint molecules, the fumaric acid exhibited the highest selectivity toward EGDN among the different explosives. Figure 4B shows the calibration curves corresponding to the analysis of EGDN (curve a), NG (curve b), and PETN (curve c) by the fumaric acid-imprinted Au NPs composite. Evidently, EGDN shows the best sensing performance on the fumaric acid-imprinted composite, whereas both NG and PETN are inefficiently sensed by this matrix, as reflected by the lower reflectance changes measured for these explosives at a similar concentration range. These results clearly indicate that the imprinting procedure is highly efficient and facilitates the analysis of picomolar, or subpicomolar, concentration range of the explosives and that, by the appropriate selection of the imprinting template, highly selective matrixes for each of the three explosives can be prepared.

CONCLUSIONS

The present study has demonstrated the fabrication of imprinted Au NPs composites for the detection of PETN, NG, and EGDN. The imprinting process was successfully achieved by applying di- or tricarboxylic acids as structural analogues for the nitrate ester substituents associated with the target explosives. The study has demonstrated that the structure of the carboxylic acids and their steric rigidification are of utmost importance to yield high-affinity sites for the respective explosives. The study revealed the preparation of highly sensitive and selective imprinted composites for the different explosives. The electropolymerization

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method used to synthesize the imprinted Au NPs provides an effective approach to prepare arrays of Au NPs that can be addressed with predesigned specific recognition matrixes for the respective explosives. Also, by employing the oligocarboxylic acid templates, sensing platforms for other explosives may be produced and, particularly, the multiplexed analysis of explosives may be performed. Furthermore, the present study has implemented the SPR readout method. The intimate interaction of the imprinted sites with the analytes, and the resulting recognition complexes formed within the Au NPs matrixes suggest that other amplified readout signals, such as surface-enhanced Raman spectroscopy, SERS, may also improve the sensitivity of the systems.

EXPERIMENTAL SECTION

Chemicals. Succinic acid, fumaric acid, and isocitric trisodium salt were obtained from Aldrich. Citric acid monohydrate was obtained from Merck. Maleic acid was obtained from BDH Chemicals. Dehydrated ethanol was obtained from Biolab. PETN was obtained by the laboratory of Identification and Forensic Science, EGDN was purchased from Restek U.S., and NG was extracted from Nitroderm TTS10 nitroglycerin patches (50 mg per patch) obtained from Novartis. The NG extraction involved the immersion of the patches in diethyl ether, followed by centrifugation and removal of the silica precipitate by decantation. Prior to the experiments, the explosives were diluted in ethanol to the required concentration for analysis.

Nanoparticles Synthesis. Au nanoparticles functionalized with 2-mercaptoethane sulfonic acid and *p*-aminothiophenol (Au NPs) were prepared by mixing a 10 mL solution containing 197 mg of $HAuCl_4$ in ethanol and a 5 mL solution containing 42 mg of mercaptoethane sulfonate and 8 mg of *p*-aminothiophenol in methanol. The two solutions were stirred in the presence of 2.5 mL of glacial acetic acid on an ice bath for 1 h. Subsequently, 7.5 mL of aqueous solution of 1 M sodium borohydride, $NaBH_4$, was added dropwise, resulting in a dark-colored solution associated with the presence of the Au NPs. The solution was stirred for one additional hour in an ice bath and then for 14 h at room temperature. The particles were successively washed and centrifuged (twice in each solvent) with methanol, ethanol, and diethyl ether. A mean particle size of 3.5 nm was estimated using transmission electron microscopy (TEM).

Chemical Modification of the Electrodes. *p*-Aminothiophenol-functionalized electrodes were prepared by immersing the Au slides for 24 h into a *p*-aminothiophenol ethanolic solution, 10 mM. In order to prepare the bis(aniline)-cross-linked Au NPs composite on the electrode, the surface-tethered *p*-aminothiophenol groups were electropolymerized in a 0.1 M HEPES buffer solution (pH = 7.2) containing 2 $mg \cdot mL^{-1}$ of *p*-aminothiophenol-functionalized Au NPs. The polymerization was performed by the application of 10 potential cycles between −0.35 and 0.8 V versus Ag wire quasi-reference electrode, at a potential scan rate of 100 $mV\ s^{-1}$, followed by applying a fixed potential of 0.8 V for 1 h. The resulting films were then washed with the background buffer solution to exclude any residual monomer from the electrode. Similarly, imprinted bis(aniline)-cross-linked films were prepared by adding 0.1 M of the template substance to the Au NPs mixture prior to the electropolymerization process. The extraction of the template molecules from the film was carried out by immersing the electrodes in a 0.1 M HEPES solution, pH = 7.2 for 2 h, and then in ethanol for additional 2 h at room temperature. The full removal of the template molecules from the electropolymerized film was followed by SPR.

Instrumentation. A surface plasmon resonance Kretschmann-type spectrometer NanoSPR 321 (NanoSPR devices, U. S.A.), with a LED light source, λ = 650 nm, and with a prism refraction index of n = 1.61, was used in this work. The SPR sensograms (time-dependent reflectance changes at a constant angle, θ = 65°) represent real-time changes, and these were measured in situ using a home-built fluid cell. Au-coated semitransparent glass slides (Mivitec GmbH, Analytical μ-Systems, Germany) were used for the SPR measurements. Prior to modification, the Au surface was cleaned in hot ethanol, at 60 °C, for 30 min. Electropolymerization was performed using a PC-controlled (Autolab GPES software) potentiostat/galvanostat (μAutolab, type III). A Pt wire (d = 0.5 mm) and a Ag wire (d = 0.5 mm) were used as the counter and reference electrode electrodes, respectively.

Analysis of the Explosives. Carboxylic acid-imprinted Au NPs matrixes were subjected to different concentrations of the respective explosive substances. Sensograms were recorded at a fixed reflection angle corresponding to 65.0°. The detection limit confidence intervals were estimated according to the IUPAC recommendations, where the detection limit (C_L) is "the lowest detectable concentration that can be detected with reasonable certainty" and is given by $C_L = \overline{C}_n + tS_n/(n)^{1/2}$, where $\overline{C}_n$ is the average value of the lowest detectable concentration, t is the Student's factor chosen according to a 95% confidence level, and using n = 5 measurements. S_n is the measured standard deviation for five separate measurements.

ASSOCIATED CONTENT

Supporting Information. SPR curves monitoring the electropolymerization and template extraction processes, sensograms demonstrating PETN detection on the citric acid and isocitric acid-imprinted Au NPs matrixes, and the derivation of the association constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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