SERS Study of the Controllable Release of Nitric Oxide from Aromatic Nitrosothiols on Bimetallic, Bifunctional Nanoparticles Supported on Carbon Nanotubes

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ABSTRACT A hybrid system comprising bimetallic nanoparticles supported on carbon nanotubes (CNTs) was engineered to maximize the surface-enhanced Raman scattering signal from solution by generating a high density of hot spots with reproducible enhancing activity and long-term colloidal and optical stability. CNT@AgAu was employed as a bifunctional material to catalyze and monitor the controlled release of nitric oxide from aromatic nitrosothiols, as a function of the gold content.

KEYWORDS: SERS • NO • nitrosothiols • kinetics • metal nanoparticles

1. INTRODUCTION

The astonishing pace of discovery on the bioregulatory roles of nitric oxide (NO) (1, 2), including neurotransmission, hormone secretion, vasodilatation, bacterial cell adhesion, and anticarcinogen properties (3), demands new methods for generating NO in a controlled manner (4), to facilitate both an improved understanding of the function of NO in physiology and the development of NO-associated therapies. *S*-Nitrosothiol (RSNO) (5) is one of the better known NO-releasing systems. RSNOs have much the same physiological properties of NO itself, particularly of vasodilation and of inhibition of platelet aggregation (1). They have also been identified in bodily fluids, notably as *S*-nitrosoglutathione and *S*-nitosoalbumins (6). Indeed, the current belief is that NO is transported around the body as RSNO (mostly as *S*-nitosoalbumins) and they can decompose in vivo, generating NO (7). Recently, several platforms for the controlled release of NO have been described that are more appealing than earlier systems, based on substances that undergo spontaneous thermolysis decomposition. NO release confined to small areas has been demonstrated in polymer films, hydrogels, self-assembled monolayers, and free nanoparticles (NPs) (8). The study of controllable NO release in the therapeutic window ($10^{-3}-10^{-9}$ M) has been carried out using highly sensitive NO detection techniques such as electrochemistry or chemiluminescence; unfortunately, acquisition times for these techniques are not suitable for the study of fast kinetics. On the other hand, ultrafast techniques for kinetic studies such as stopped-flow require high RSNO concentrations, above 10^{-3} M. In this context, surface-enhanced Raman scattering (SERS) spectroscopy has become one of the most powerful and ultrasensitive analytical techniques (9). The applicability of this analytical tool may be further expanded to new research fields by simply optimizing the design of the optical enhancer, either free or supported metallic nanostructures that are responsible for enhancing the electromagnetic field (10) to each specific analytical problem.

In this letter, we present the use of an engineered bimetallic nanosystem supported on carbon nanotubes (CNT@AgAu). The CNT@AgAu system maximizes the Raman signal in solution by generating a high density of hot spots within the material, with reproducible intensity and long-term colloidal and optical stability. CNT@AgAu was used in this particular example as a bifunctional material for a sensitive study of the controlled release of NO at physiological pH from aromatic nitrosothiols, catalyzed by metallic gold present in the supported NPs. In this case, gold was used as the main catalytically active element, while silver mainly provided the strong electromagnetic field required to increase the signal. Indeed, the catalytic activity of NPs is improved onto carbon supports (11). Moreover, the deposition of NPs on the external walls of CNTs facilitates contact with the reactant, as compared to those trapped in the pores of carbon black matrixes (11). By using this material in conjunction with SERS detection, we were able to study the catalytic effect of gold on the kinetics of NO release. Our results additionally show that through tuning of the gold content in the NP composition the release speed of NO can be controlled.

2. EXPERIMENTAL SECTION

Tetrachloroauric acid ($HAuCl_4$; ACS reagent), silver nitrate ($AgNO_3$; ACS reagent), benzenethiol, poly(allylamine hydrochloride) (PAH; $M_w \approx 70\,000$), sodium dodecyl sulfate (SDS), triso-

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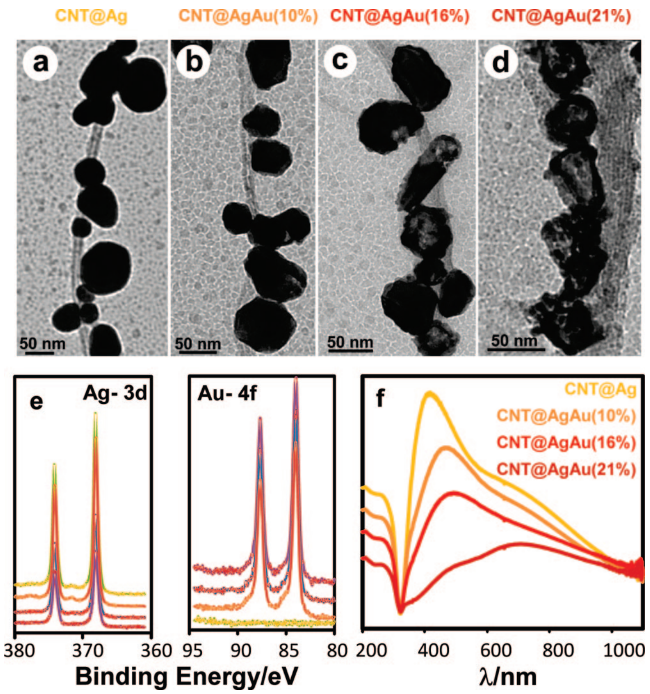


FIGURE 1. (a−d) TEM images of Ag or AuAg NPs supported on CNTs, corresponding to gold percentages of 0% (a), 10% (b), 16% (c) and 21% (d). XPS surface composition analysis (e) and UV−vis spectra (f) for Ag and AgAu NPs on CNTs. See the color code in parts a−d or f.

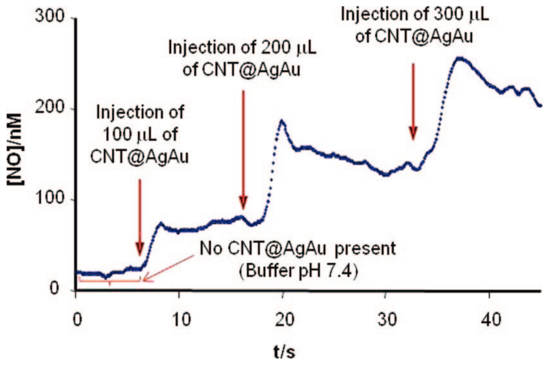


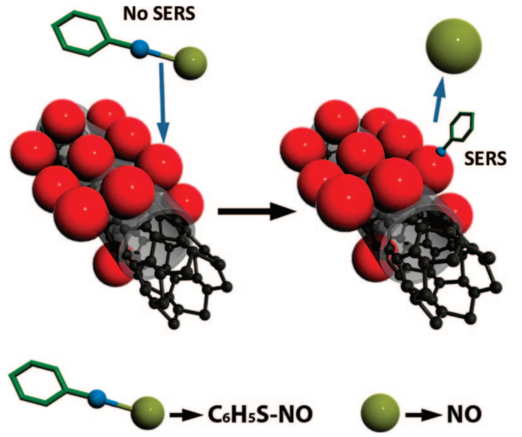
FIGURE 2. Time evolution of the NO concentration in a RSNO solution, with the addition of different amounts of a CNT@AgAu colloid.

dium citrate, and methanol were purchased from Sigma-Aldrich. Sodium nitrite was purchased from Merck and hydrochloric acid from Panreac. Multiwalled carbon nanotubes (CNTs) were supplied by Nanolab, Boston, MA.

Preparation of Silver−Gold Bimetallic Nanostructures. Silver−gold bimetallic NPs were prepared as reported earlier (14a). Briefly, 500 mL of silver colloid was prepared by using the citrate reduction method (13). The colloidal suspension was then diluted to 1 L with Milli-Q water (Millipore) and titrated with a 1 mM $HAuCl_4$ solution under vigorous stirring at room temperature, with 30 min intervals between additions (from 10 up to 30 mL in 10 mL aliquots). Samples of 100 mL were stored after each addition.

CNT@NP SERS Active Substrates. A total of 45 mg of multiwalled CNTs (Nanolab; 15 ± 5 nm diameter, 5−20 μm length, and 95% purity) was sonicated for 4 h in 300 mL of a pH 10 NH_4OH solution containing NaCl (0.5 M) and PAH (3 g). The sample was extensively washed and resuspended in water (0.11 mg mL^{-1}). A total of 4 mL of CNTs@PAH (0.022 mg mL^{-1}) was added dropwise to the NP suspension under sonication. Sonication was maintained for 2 min, and NP assembly was allowed to proceed during 1 h at rest.

Scheme 1. Schematic Representation of the Catalytic Decomposition of Aromatic Nitrosothiols onto CNT@AuAg, Yielding NO and the SERS Active Benzenethiol



Kinetic Study of NO Release on CNT@NP. *S*-Nitrosothiol (RSNO; 2×10^{-5} M) was generated in situ by mixing benzenethiol (fresh solution in methanol, 0.01 M) with an acidic solution of nitrous acid in excess (3×10^{-5} M), in the presence of SDS (40 mM). The final methanol content was 3 wt %. RSNO decomposition was carried out in a buffer medium (phosphate buffer, pH 7.4) catalyzed by CNT@NP (2 mL), to reach a final volume of 3 mL. SERS spectra were acquired in real time with 200 ms intervals from 0 to 70 s, in a Jobin Yvon HR Raman system. The samples were excited with a 633 nm laser line. Kinetic data were always satisfactorily fitted by the first-order integrated rate equations, and therefore k_{obs} denotes the pseudo-first-order rate constant:

$$\frac{d[RS-CNT@AuAg]}{dt} = k_{obs}[RSNO]$$

The influence of CNT@NP on NO release was confirmed by means of a World Precision Instrument, a ISO-NOP meter, based on direct amperometric detection, with a short response time (<5 s) and a sensitivity range between 1 nM and 1 mM. The sensor was accurately calibrated by mixing standard solutions of $NaNO_2$ with 0.1 M H_2SO_4 and 0.1 M KI, producing NO according to the reaction

$$4H^+ + 2I^- + 2NO_2^- \rightarrow 2H_2O + 2NO + I_2$$

3. RESULTS AND DISCUSSION

Bimetallic NPs were prepared by using a standard galvanic replacement reaction (12), starting from citrate-reduced silver colloids (13) as sacrificial templates (14). The reason for choosing these NPs is that they produce large enhancements from a virtually clean surface, which is required for efficient catalysis and SERS (15). Either silver or bimetallic nanoalloys were physisorbed on polyelectrolyte-coated CNTs (CNT@NP) (16). Shown in Figure 1a−d are representative transmission electron microscopy (TEM) images of the hybrid materials, where the gradual replacement of silver by gold can be observed, through the generation of hollow (silver−gold) particles (Figure 1c,d). The bimetallic nature of the nanoalloys was established by X-ray photoelectron spectroscopy (XPS; Figure 1e). Sacrificial Ag NPs

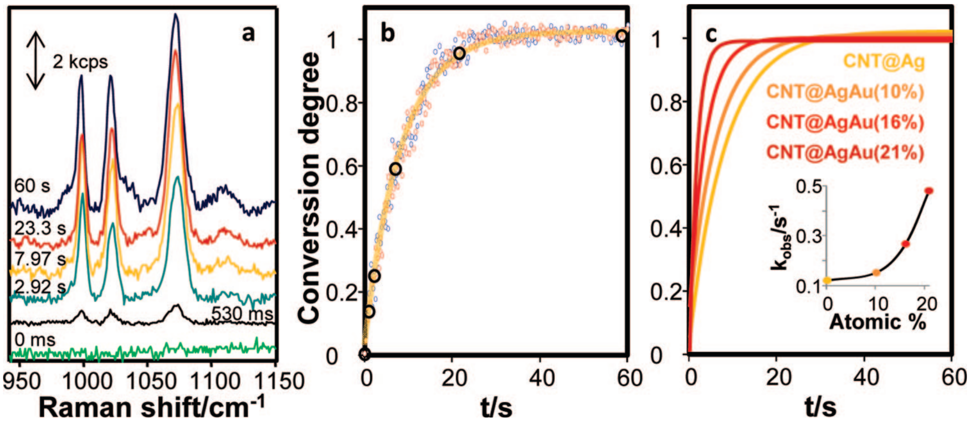


FIGURE 3. (a) Benzenethiol SERS spectra acquired at different reaction times (defined by the open circles in part b) on CNT@Ag. (b) Two different experimental sets of data (blue and red dots) measured by SERS on CNT@Ag. The yellow, solid line is the fit to a first-order kinetic model. The acquisition time per scan was 200 ms using a 633 nm laser line. (c) Kinetic fit for reactions on CNT@Ag and CNT@AgAu with varying composition. The inset shows the exponential increase of k_{obs} with the gold content at the particle surface.

show fundamental bands at 368.2 ($3d_{5/2}$) and 374.2 ($3d_{3/2}$) eV, with no evidence of the presence of gold. Upon the addition of Au^{III}, Ag 3d bands are progressively damped while Au^0 4f bands at 84.4 eV ($4f_{7/2}$) and 88.1 eV ($4f_{5/2}$) increase. The gold molar ratio at the surface ranges from 0%, for pure Ag NP, up to 21%, for the last bimetallic nanoalloy. The formation of these core−shell structures strongly affects their plasmonic properties as the localized surface plasmon resonance progressively red-shifts as more gold is added (Figure 1e,f). This red shift is mainly attributed to the hybridization of the dielectric constants of silver, gold, and solution (core void) (12, 17).

The influence of gold on NO formation at physiological pH was studied by mixing the nitrosothiol solution with the CNT@AuAg hybrid colloid. The RSNO bond was immediately cleaved because of the high affinity of gold for thiols (18), liberating NO (see Scheme 1). Evidence of NO release by CNT@NP in this process was provided using a sensitive NO electrode, which directly detects the NO concentration by an amperometric technique. As displayed in Figure 2, injection of different amounts of CNT@AuAg hybrid colloid into a vessel containing a solution of RSNO and buffer (pH 7.4) promotes an instantaneous electrode response, proportional to the amount of CNT@NP added. Interestingly, the electrode does not detect any NO before the addition of the CNT@NP suspension, which rules out the possibility that NO release could be due to the well-known copper-ion-catalyzed process (5, 19).

Kinetic analysis of the reaction was carried out for the first time by means of SERS detection. In this respect, the aromatic thiol was used as a spectroscopic label to monitor NO release. The well-known SERS spectra of benzenethiol (Figure 3a) (20) recorded at different times after mixing (open circles in Figure 3b) show a good signal-to-noise ratio, which is sufficient to accurately plot the NO release kinetic curves, even with short acquisition times of 200 ms. Note that the detection limit for this molecule using SERS has been established around the nanomolar range (21). Figure 3b shows the experimental kinetic curves obtained by plotting the intensity of the ring breathing mode (1072 cm^{-1}) versus time for two different experiments, together with the fitting of both data sets to a first-order kinetic model. Both series fit point to point, yielding perfectly reproducible first-order kinetic constants ($k_{\mathrm{obs}} = 0.122$ and $0.123\ s^{-1}$). It is important to note that similar experiments carried out with free (nonsupported) NPs resulted in much lower SERS intensities because no aggregation (and concomitant hot-spot formation) was observed at the first stages of the reaction, resulting in a much poorer signal-to-noise ratio. As the reaction progressed, a decrease in the intensity was recorded because of aggregation and sedimentation, due to the high ionic strength.

Finally, the effect of the gold content on the NO release rate can be clearly seen in Figure 3c. The reaction rate dramatically increased while using gold content in the NPs, while with Ag NPs, the reaction was completed within ca. 30 s, using the alloyed system with the highest gold concentration, this time was reduced to less than 7 s. An exponential effect of the gold content on the reaction time was found (inset of Figure 3c). This nonlinear behavior can be attributed to the intrinsic nature of the bimetallic colloids. The galvanic replacement reaction between silver particles and $HAuCl_4$ involves a number of processes, including diffusion of $HAuCl_4$ to the surface of the template, diffusion of Ag atoms from the core to the surface, formation of Ag^+ and Au^0, deposition of Au atoms on the surface of the template, and alloying or dealloying between Au and Ag atoms, depending on the volume of $HAuCl_4$ solution added, yielding a homogeneous alloy rather than a segregated mixture (22).

4. CONCLUSIONS

We have demonstrated that metal NPs adsorbed onto CNTs can be used as a trigger for the generation of NO from an external RSNO. Besides, the preparation of CNT@AuAg with varying gold contents can be envisaged as an important step toward developing controllable NO-release scaffolds for biological applications. Additionally, we demonstrated that SERS spectroscopy is a suitable technique to follow reaction kinetics, in particular for fast reactions or very low reactant concentrations.

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