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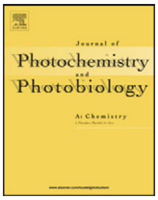

Spectral dependence of gold nanorods on the optical properties of substrates and adsorption of polypeptides

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ABSTRACT

Gold nanorods (NRs) wrapped with poly(styrene sulfonate) were fixed on glass or indium tin oxide (ITO) substrates that had been modified with polycationic polymer or 3-aminopropyltrimethoxysilane layers. The spectral properties of the NRs were affected by the refractive indices of the substrates and their surface conditions. Surface modification with poly(styrene sulfonate) and avidin molecules shifted the longitudinal surface plasmon (SP) bands to longer wavelength when the substrates were placed in air. In water and a buffered solution, red shifts of the SP bands were also observed, but the shift shifts were not as large as that in the air. It was found that refractive indices around the polymer-wrapped NRs were sensitive to the adsorption of organic molecules even in the aqueous media.

1. Introduction

Gold nanorods (NRs) are rod-shaped nanoparticles that exhibit two surface plasmon (SP) bands, one in the visible region and the other in the near infrared (NIR) [1–3]. These bands are assigned as transverse and longitudinal SP oscillations, respectively [4–7]. Because of their unique optical properties, NRs have been studied extensively in the fields of colloid science [8–10], laser chemistry [11–13], and bioscience [14–18].

The SP bands of gold nanoparticles are responsive to the refractive indices of their surroundings. Changes in the refractive index are observable as shifts in the SP bands. These shifts are useful in the fabrication of sensors that detect solvents or adsorbates [19–21]. In the case of NRs, the position of the longitudinal SP band is expected to be more sensitive to changes in the refractive index than the SP bands of spherical particles [4,5]. Thus, NRs are a potential probe for sensing refractive index in the NIR region. Shifts in SP bands caused by changes in the surrounding refractive index have been described previously [22,23]. NRs fixed on a glass substrate show a solvatochromic shift of 36 nm when exposed to water and chloroform (refractive indices of 1.33 and 1.45, respectively) [22]. A shift of 40 nm induced by exposure to ethanol and then toluene (refractive indices of 1.36 and 1.45, respectively) has also been reported [23]. A rough theoretical estimation predicted that changing the refractive indices from 1.3 to 1.5 induces a red shift of 45 nm in the longitudinal SP bands [4,5], which is larger than the changes observed experimentally [23]. This discrepancy mainly arises from the refractive indices of the glass substrates and molecules used for surface modification. Thus, to understand the spectral changes of NRs adsorbed on a substrate, the discontinuous refractive indices at an interface should be examined. Previously, Vernon et al. discussed the contribution of discontinuous refractive indices to spectroscopic properties using a theoretical model [24]. In this work, the spectroscopic responses of NRs that are affected by the surfaces of substrates have been determined. Polyion-wrapped NRs were prepared and then fixed on glass and indium tin oxide (ITO) substrates. Additional surface modification with water-soluble polymers or avidin molecules (MW. ~67,000) affected the spectroscopic properties of the NRs. These spectral changes are discussed in view of the changes in refractive index at the interface between the NR and the substrate.

2. Experimental

NRs were synthesized using a photochemical method [8] in a joint research project conducted between Mitsubishi Materials Co. and Dai-Nihon-Toryo Co. Ltd. The initial zeta potential of the NRs was +20 mV, which originates from a molecular bilayer of hexadecyltrimethylammonium bromide (CTAB) on the surface of the NRs. A typical procedure for the surface modification of NRs is as follows.

A solution of the as-prepared NRs was centrifuged twice at 15,000 × g for 10 min and then decanted to remove excess CTAB molecules from the solution. The positively charged surfaces of the NRs were wrapped with poly(styrene sulfonate) (PSS, Mw.

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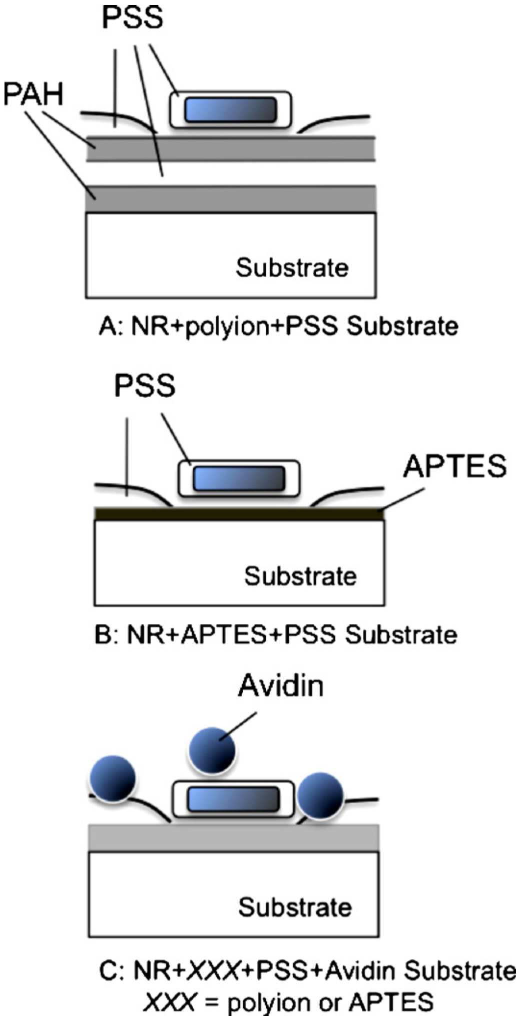

Fig. 1. Schematic illustration of NR-modified substrates.

70,000, Aldrich) through electrostatic interactions to obtain PSS-NR [25,26].

Glass and ITO substrates were used for the experiments. The substrates were modified with water-soluble polymers using layer-by-layer methods [22,27] and the silane-coupling agent 3-aminopropyltriethoxysilane (APTES). For the polymer modification, a hydrophilic substrate was sequentially immersed in a solution of poly(allylamine hydrochloride) (PAH, Mw. 15,000, Aldrich) (1 mg/mL, 20 min), a solution of PSS (3 mg/mL, 20 min), and finally another solution of PAH. A multilayered polyionic-polymer film was obtained using this procedure. The outermost layer on the substrate is positive because of the presence of PAH. For the APTES modification, a hydrophilic substrate was immersed in a solution of APTES (3.4 mM) in 2-propanol for 1 h, and then washed with water and dried in air. The obtained cation-modified substrates were then immersed in a solution of PSS-NRs to deposit NRs on the polyionic and APTES layers. Four substrates containing deposited NRs were prepared: NR + polyion and NR + APTES on both glass and ITO substrates. After immersion in the solution of PSS-NRs for $\sim$24 h, the substrates were washed with water, and then immersed in a solution of PSS (2 mg/mL, 1 h). Additional PSS modification suppressed translocation of the NRs on the surface of the substrate. The resulting substrates (NR + polyion + PSS (Fig. 1a) and NR + APTES + PSS on glass or ITO substrates (Fig. 1a) possess an anionic surface because of the presence of PSS as the outermost layer. The substrates modified with NRs were immersed in a solution of avidin (0.15 μM) to deposit avidin on the anionic surfaces *via* electrostatic interactions. The surfaces containing adsorbed avidin are noted as NR + *XXX* + PSS + Avidin (*XXX* = polyion or APTES, Fig. 1c).

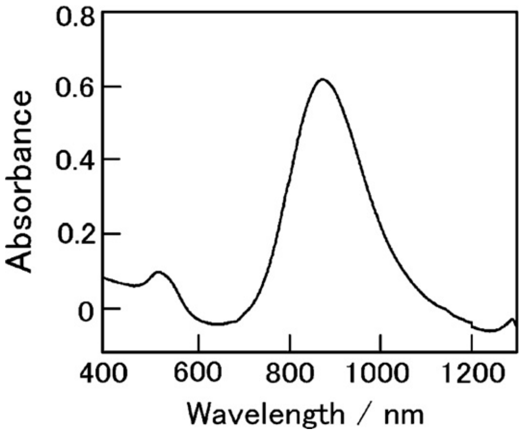

Fig. 2. Absorption spectrum of colloidal NRs wrapped with PSS.

Absorption spectra of the NR solutions were obtained on a V-570 JASCO spectrophotometer. To obtain the spectra of the substrates in solution, each substrate was immersed in a solution filling an optical cell. All measurements were performed at room temperature. The zeta potentials of the NRs were measured on a Malvern Zetasizer Nano ZS instrument. Zeta potentials were calculated using a theoretical model with spherical particles. Thus the value obtained qualitatively indicated the sign and magnitude of the zeta potential of the NRs. This was sufficient for relative evaluation of the surface charge of the NRs in different environments.

3. Results and discussion

The zeta potential of the PSS-NR solution was −28.3 mV. The absorption spectrum of the PSS-NRs shows a double peak that is typical of colloidal NRs (Fig. 2), indicating the modification of PSS on the NRs without the formation of aggregates. Fig. 3 shows the absorption spectra of the NR + polyion, NR + polyion + PSS, NR + polyion + PSS + Avidin layers on glass substrates. All of the spectra exhibit characteristic SP bands, indicating that the NRs have been deposited on the substrates without remarkable aggregation. An SEM image of the NR + polyion substrate (Fig. 4) also supports this interpretation, and also shows that the deposited NRs are not oriented. The longitudinal SP band appears at 816–887 nm, while the colloidal PSS-NRs (Fig. 2) have a SP band at 900 nm. This large shift originates from the absence of water molecules around the colloidal PSS-NRs. Surface modification with PSS (Fig. 3b) and avidin (Fig. 3c) induced further red shifts of the SP band. This indicates that the presence of organic molecules around the NRs gives higher refractive indices. Thus, the longitudinal SP bands responded the refractive indices of the environment surrounding the NRs.

The positions of the longitudinal SP bands of the NR-modified glass and ITO substrates at each surface modification step are shown in Table 1. In all cases, absorption spectra were obtained in air, and the bandwidths were not affected by the surface modifications. Thus, aggregation of the NRs was not induced by additional surface modification, and changes in the refractive index induced the peak shifts. As shown in the second column of Table 1, surface modification with APTES gave SP bands at longer wavelength for each surface modification step. The position of the peak ranged from 855 to 903 nm. The observed red shifts of the SP bands indicate that the refractive indices around the NRs increased. The thin APTES layer contributed to the higher refractive indices arising from the presence of a glass substrate ($n = 1.54$). The polymer layers, on the

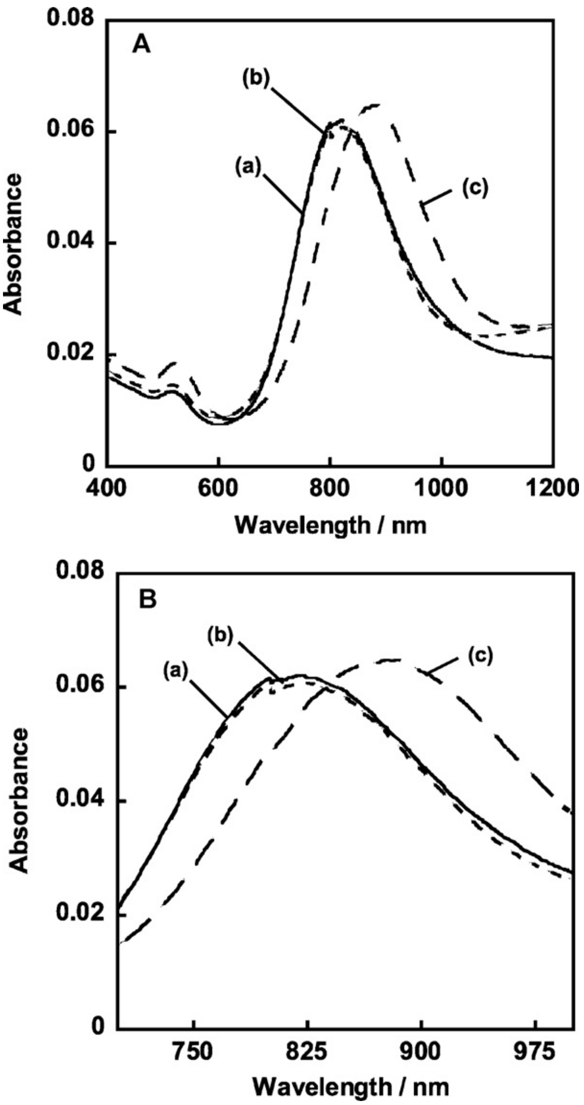

Fig. 3. [A] Absorption spectra of NR + polyion (a), NR + polyion + PSS (b), NR + polyion + PSS + Avidin (c) glass substrates. [B] Enlarged longitudinal SP bands of [A].

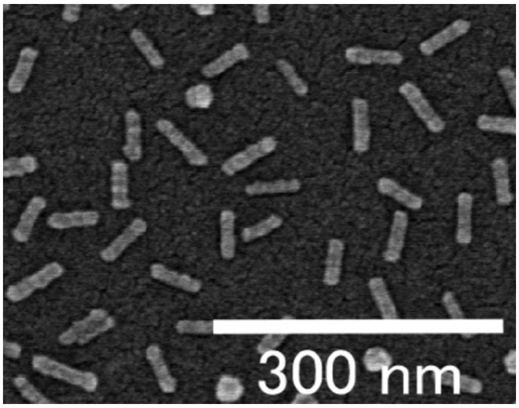

Fig. 4. SEM image of the NR + polyion + PSS glass substrate.

Table 1
Positions of longitudinal SP bands of NRs on glass and ITO substrates modified with organic molecules, in air.

XXX:	On glass		On ITO	
	Polyion	APTES	Polyion	APTES
NR + *XXX*	816	855	846	855
NR + *XXX* + PSS	824	828	851	871
NR + *XXX* + PSS + Avidin	887	903	898	926

Peak wavelength/nm in the air.

other hand, were relatively thick (about 6 nm for the three layers PAH/PSS/PAH [27]), which was enough to decrease the contribution from the substrate.

In the case of the ITO substrates (third and fourth column in Table 1), the higher refractive index ($n = 1.72$) of ITO shifted the peaks to longer wavelength (846–926 nm). The polyion and APTES modifications of the ITO substrates showed the same contribution as they did for the glass substrate; that is, the APTES-modified substrates showed SP bands that were red-shifted compared with those of the polyion-modified substrates. The NR + APTES + PSS + Avidin substrate (bottom of Table 1) exhibited a SP band at 926 nm. This peak appeared at wavelength longer than 900 nm, which is where the peak of colloidal NRs in water is observed (Fig. 2). It was found that the refractive index around the NRs on the NR + APTES + PSS + Avidin ITO substrate, which were wrapped with PSS and avidin and deposited on the APTES-modified ITO substrate, was larger than that of colloidal NRs wrapped with PSS and dispersed in water ($n = 1.33$).

The NR + polyion + PSS glass substrate was immersed in phosphate buffer (PB, 100 mM, pH 7.4) and then the substrate was washed with a small amount of water and dried under a stream of air. Before the PB treatment, the longitudinal SP band appeared at 820 nm. After PB treatment, the band shifted to 889 nm, corresponding to a red shift of 69 nm. When the substrate was immersed in water again, the peak shifted back to 820 nm, which means that the large change is reversible (closed triangles in Fig. 5). The use of a diluted PB solution (5 and 10 mM) resulted in smaller peak shifts. Thus, the large red shifts in Fig. 5 are probably caused by the ionic strength of the buffer solutions. PB (0.1 M) shows a slightly higher refractive index than that of water (PB: 1.335, water: 1.333). However, it is unlikely that this small difference in refractive index could cause such a large shift. Additionally, the spectra of colloidal PSS-NRs in a PB solution did not show any significant shifts (data not shown). Thus, the reversible spectral changes induced by subsequent immersion in PB and water probably originate from structural changes in the polyion layers on the substrate and surface of the NRs. In water, the charged groups on the polymer chains tend to make the polyions more rigid [28,29]. A straightforward effect of

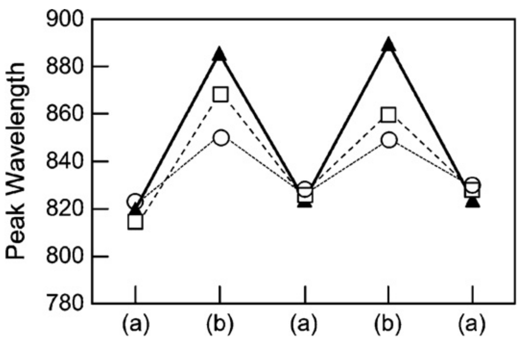

Fig. 5. Positions of the longitudinal SP bands of a NR + polyion + PSS glass substrate immersed alternatively in water (a) and PB (b). The concentrations of the PB solutions were 5 (open circle), 10 (open square), 100 mM (filled triangle).

Table 2
Positions of longitudinal SP bands of NRs on glass and ITO substrates modified with organic molecules, in water.

XXX:	On glass		On ITO	
	Polyion	APTES	Polyion	APTES
NR + *XXX*	913	919	915	935
NR + *XXX* + PSS	909	919	908	935
NR + *XXX* + PSS + Avidin	914	933	929	944

Peak wavelength/nm in water.

PB is the screening of electrostatic repulsions due to the free ions. It is plausible that the refractive indices around the NRs were sensitive to the structural changes of the polyions. The NR + APTES + PSS substrate did not exhibit such remarkable spectral changes. This indicates that the NR + polyion + PSS substrate has the potential to act a novel sensor plate detecting the adsorption of organic molecules on the substrate.

Table 2 shows the positions of the peaks of the NR-modified substrates immersed in water. The spectra were obtained *in situ*, and the spectroscopic properties indicated the absence of aggregation. Because of the contribution from water ($n = 1.33$), all of the peaks shifted to longer wavelength than those measured in air (Table 1). All peaks appeared at wavelengths longer than 900 nm, which is where the peak from colloidal NRs in water is observed (Fig. 2). It was shown that the substrates also contributed to the local refractive indices around the NRs in water. In addition, in water, modifications of the glass and ITO substrates with APTES (second and fourth columns, respectively) resulted in SP bands at longer wavelengths than those arising from modification with polyion (first and third columns). Furthermore, surface modification with PSS and avidin also shifted the peaks to longer wavelengths; for example, compare the wavelengths of the peaks in the second and third rows of Table 2. Even in water, the NR-modified substrates were responsive to the refractive indices of the substrate and organic molecules on the substrates.

Table 3 shows the positions of the peaks from the NR-modified substrates immersed in PB. The positions of the peaks were almost the same as those in water, and they responded to surface modification. The phosphate and its counter cations did not affect the refractive indices around the NRs in an aqueous medium. In contrast, as shown in Fig. 5, the spectra of the dried substrates after immersion in PB showed large shifts. The results in Table 3 indicate that the polymer layers swell in PB and water. When the NR + polyion + PSS substrates are dried, the ionic strength of the solution as it dries probably affects the swelling of the polyion layers.

Table 1 indicates that the shifts in the peaks induced by the adsorption of avidin were 65 and 75 nm for NR + polyion + PSS + avidin (822–887 nm) and NR + APTES + PSS + avidin (828–903 nm) glass substrates in air, respectively. The avidin is a polypeptide whose molecular weight is about 67,000. The bulky polypeptide, in which a certain amount of water should be taken, gave higher refractive index around the gold nanorods. In contrast, for the ITO substrates, the adsorption of avidin induced 47 and 55 nm shifts. Thus, on a glass substrate, the avidin molecules induced larger shifts of the SP bands than those on an ITO substrate. The degree of the spectral change of the NRs induced by the adsorption of organic molecules was tuned by the optical properties of the substrates. In water and PB, the induced spectral changes were independent of the substrate. The average shift before and after the avidin modification was 15 ± 4 nm; this value was obtained by excluding one sample that showed very small peak shift (Table 3, from NR + Polyion + PSS to NR + Polyion + PSS + Avidin). The average shift was much smaller than that in air (Table 1), because of the presence of water, which has a much higher refractive index than air. In other words, the ~15 nm peak shift was originated from the refractive index changes at the solid–liquid interface. This shift is the contribution of the polypeptide chain in water to the refractive index at the interface.

Table 3
Positions of longitudinal SP bands of NRs on glass and ITO substrates modified with organic molecules, in phosphate buffer.

XXX:	On glass		On ITO	
	Polyion	APTES	Polyion	APTES
NR + *XXX*	913	922	915	934
NR + *XXX* + PSS	904	920	922	933
NR + *XXX* + PSS + Avidin	920	936	923	946

Peak wavelength/nm in phosphate buffer.

4. Conclusion

The optical properties of NR-modified substrates are sensitive to the refractive indices of the substrate, surface modification with polyions and APTES, and adsorption of PSS and polypeptides. The optical properties of the NR-modified substrates in buffer solution were similar to those in water, but after drying in air, the SP bands of the NRs showed significant red shifts. These spectral changes are most likely caused by structural changes in the polyion layers. To design an optical sensor using NRs on a plate, the optical properties of the substrate and polyion layers on the substrate and around the NRs should be optimized. In aqueous media, large changes in refractive index are difficult to produce; however, even in PB, the NR-modified glass substrates showed a red shift of 16 nm when avidin molecules were adsorbed on the glass surface. Further improvements of the optical and structural responses of the polyion layers in water will contribute to the application of NRs as practical biosensors.

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