CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200701307

A New Aptameric Biosensor for Cocaine Based on Surface-Enhanced Raman Scattering Spectroscopy

Jiwei Chen, Jianhui Jiang,* Xing Gao, Guokun Liu, Guoli Shen, and Ruqin Yu*[a]

Abstract: The present study reports the proof of principle of a reagentless aptameric sensor based on surface-enhanced Raman scattering (SERS) spectroscopy with "signal-on" architecture using a model target of cocaine. This new aptameric sensor is based on the conformational change of the surface-tethered aptamer on a binding target that draws a certain Raman reporter in close proximity to the SERS substrate, thereby increasing the Raman scattering signal due to the local enhancement effect of SERS. To improve the response performance, the sensor is fabricated from a cocaine-templated mixed self-assembly of a 3′-terminal tetramethylrhodamine (TMR)-labeled DNA aptamer on a silver colloid film by means of an alkanethiol moiety at the 5′ end. This immobilization strategy optimizes the orientation of the aptamer on the surface and facilitates the folding on the binding target. Under optimized assay conditions, one can determine cocaine at a concentration of 1 μM, which compares favorably with analogous aptameric sensors based on electrochemical and fluorescence techniques. The sensor can be readily regenerated by being washed with a buffer. These results suggest that the SERS-based transducer might create a new dimension for future development of aptameric sensors for sensitive determination in biochemical and biomedical studies.

Keywords: aptamers · biosensors · cocaine · colloids · Raman spectroscopy

Introduction

Surface-enhanced Raman scattering (SERS) is a phenomenon observed for a range of different molecules. It occurs when molecules are attached to the surface of certain nanostructured materials.[1] The enhancement effect is generally explained by two enhancement mechanisms, one being a long-range electromagnetic (EM) effect such as "hot spots", and the other being a chemical enhancement that results from the charge-transfer excitation of chemisorbed molecules.[2,3] In recent years, it has been reported that even single molecule detection is possible by SERS, suggesting that an enhancement factor of as much as 10^{14}–10^{15} could be reached.[4–6] Besides high sensitivity, Raman spectra have some advantages over their fluorescent counterparts, such as monitoring molecule excitement at any wavelength, alleviated photobleaching, and showing narrow peak widths (ca. 20 cm^{-1}).[7] Therefore, these intrinsic advantages of Raman spectroscopy make SERS very attractive for the development of various biosensing devices for use in immunoassay and nucleic acid detection.[8–16] Initial trials along this route were demonstrated by Dou et al. based on an enzyme-linked immunoassay format in which the enzyme-reaction product was adsorbed on silver colloids to generate an SERS signal, which would allow indirect assay of the antigen.[8] An alternative design for such biosensors is to utilize certain metal nanoparticles labeled with Raman-active dyes as an SERS tag of the analyte.[9–15] SERS-active nanostructures labeled with different dyes and oligonucleotide probes were proposed by Mirkin's group for multiplexed detection of nucleic acids and proteins.[11,12] Vo-Dinh and co-workers recently reported a piloted reagentless SERS-based biosensor,[15] which utilized a molecular beaconlike oligonucleotide probe immobilized on silver colloids. It exhibited a decreased SERS signal upon hairpin-to-duplex change through hybridization with the target DNA sequences, a conformation change typical of fluorescent molecular beacons.[16] This reagentless biosensor efficiently utilized SERS as a local effect that was strongly dependent upon the distance between the Raman label and the signal-enhancing platform. For the fluorescent molecular beacon, the departure of the quencher moiety from the fluorophore causes an increase in

[a] J. Chen, Prof. J. Jiang, X. Gao, G. Liu, Prof. G. Shen, Prof. R. Yu
State Key Laboratory of Chemo/Biosensing and Chemometrics
College of Chemistry and Chemical Engineering
Hunan University, Changsha 410082 (China)
Fax: (+86) 731-8821916
E-mail: jianhuijiang@hnu.cn
rqyu@hnu.cn

the fluorescent signal ("signal-on"), whereas in the case of SERS the departure of the Raman label from the signal-enhancing platform results in a "signal-off" mechanism. It was therefore of interest to us to search for an alternative sensor design strategy to realize a "signal-on"-type SERS-based sensor. Our attention was focused on the use of aptamers.

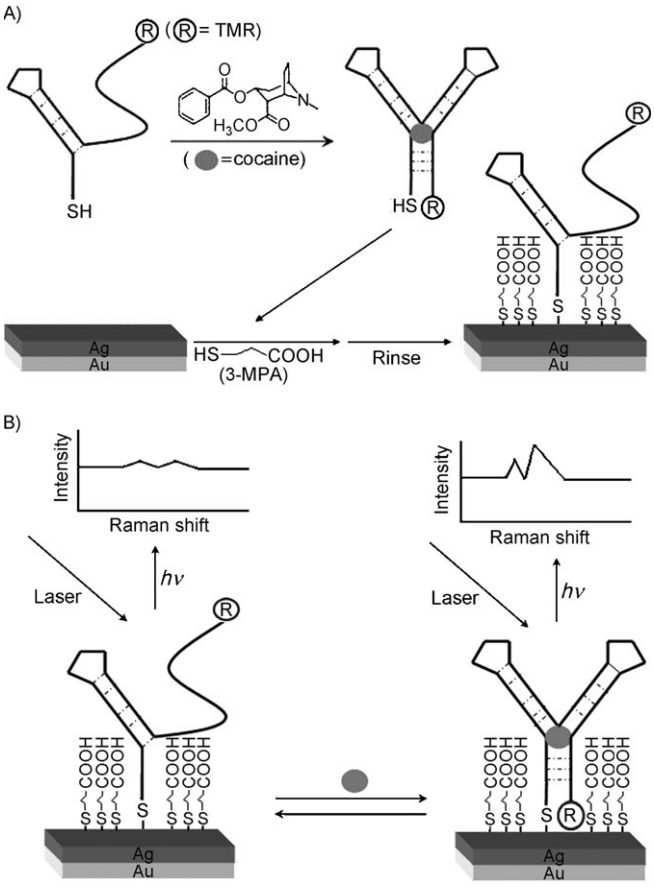
Aptamers constitute a novel affinity recognition element for biosensor development. As short, single-stranded DNA or RNA oligonucleotides screened by using a combinatorial in vitro selection process called SELEX, aptamers can bind specifically to targets with high affinity.[17,18] Targets binding to aptamers range from small organic molecules and biomolecules to entire organisms.[19,20] In contrast to traditional molecular recognition elements in sensory devices, aptamers offer considerable advantages such as adaptability to various targets, convenience in screening, reproducibility for synthesis, versatility in labeling, immobilization, signaling, and regeneration. Aptamers have also been employed in a variety of sensory designs, including homogeneous assays using fluorescence[21–23] and colorimetric[24,25] detection, as well as heterogeneous sensing with quartz crystal microbalance (QCM) measurements[26] and electrochemical transduction.[27–29] Most of these sensors were fabricated based on the interaction between the aptamer and a protein such as IgE,[26] thrombin,[27] and platelet-derived growth factor (PDGF).[29] Biosensors were also developed by using the corresponding aptamers for detecting small molecules such as adenosine and cocaine.[25,28]

In the present study, we report a proof of principle of a reagentless aptameric sensor based on SERS with a "signal-on" architecture using a model target of cocaine. Here the SERS as a local effect dependent upon the distance of the Raman reporter to the substrate surface is utilized in such a way that the SERS signal generated would increase with the increase of analyte concentration.[30,31] With an appropriate aptamer immobilized on an SERS substrate, target binding induces the aptamer molecule to undergo a conformational transition that draws a certain labeled Raman reporter in close proximity to the substrate, thereby increasing the SERS signal. This novel biosensor combines the advantages of aptamers, such as high specificity and affinity, and the benefits of SERS, which include enhanced sensitivity, alleviated photobleaching, noninvasive sampling, and microsized sampling spots, as well as increased multiplexity due to narrow bands, which offers tremendous opportunity for the development of miniaturized transducers with desired performance such as high throughput, specificity, and sensitivity. In this work the SERS substrate was prepared by using an Ag colloid film on a polished gold disc. This SERS substrate demonstrated strong and reproducible scattering signals. Batch-to-batch variations in the substrates could be mitigated substantially by appropriate normalization of the SERS intensities. With an aptamer sequence engineered for the model target cocaine,[21] a biosensor was developed by using a mixed assembled monolayer of 3-mercaptopropionic acid (MPA) and a 5′-terminal thiolated oligonucleotide aptamer with a tetramethylrhodamine (TMR) moiety at the 3′ end in the presence of cocaine. The introduction of a mixed self-assembly of MPA with a cocaine-aided well-folded aptamer was observed to be very beneficial to SERS signaling due to the fact that it offered something similar to "molecular-imprinted" cages for the folded aptamer, thereby facilitating the folding of the aptamer into the appropriate conformation and mitigating steric hindrance on the substrate surface during conformation transition. Moreover, the biosensor can be readily regenerated through a brief wash with buffer solution. These results revealed that the SERS-based transducer creates a new dimension for future development of novel aptameric sensors.

Results and Discussion

Analytical principle: In our preliminary experiments we noticed that straightforward assembly of the aptamer on the surface could not produce a sensor with significant SERS response to the analyte. As a consequence, we utilized a "cocaine template" in the fabrication of the aptameric sensor. Scheme 1 depicts the analytical principle of the aptameric sensor.

In the fabrication of this sensor (Scheme 1A), cocaine is introduced into the aptamer solution as a "template" to



Scheme 1. Schematic diagram for the preparation (A) and analytical principle (B) of the aptameric sensor for cocaine.

form a complex with the aptamer, thus allowing the aptamer molecule to fold into a stable three-way junction. Assembly of the aptamer–cocaine complex on the SERS substrate surface by means of the 5′-terminus alkanethiol group causes the TMR moiety at the 3′ end to be located in close proximity to the substrate. It is important to note that cocaine and MPA are introduced in the self-assembly process. This offered something similar to "molecular-imprinted" cages for the folded aptamer, thereby facilitating the folding of the aptamer into the appropriate conformation and mitigating steric hindrance on the substrate surface during conformation transition. Removal of the cocaine template by rinsing with phosphate buffer solution (PBS) induces the aptamer to partially unfold and draw the TMR moiety away from the substrate, which results in the formation of an aptameric sensor. Therefore, with the templated assembly strategy one could obtain an aptameric sensor with immobilized aptamer molecules in ideal orientation so as to have little steric hindrance to each other when they are refolded on binding to cocaine.

The response mechanism of the resulting aptameric sensor lies in the fact that the SERS enhancement effect, E_R, depends strongly on the distance, d, between the Raman reporter and the roughened substrate ($E_R \propto d^{-12}$).[30,31] As shown in Scheme 1B, in the absence of the target, most of the DNA probes in the solution will be away from the surface due to the thermal motion of DNA strands and the electrostatic repulsion from the negatively charged surface with the mixed assembled monolayer of MPA and DNA probes. So the TMR moiety remains away from the substrate and yields a weak SERS background signal. In response to the cocaine analyte, the surface-immobilized aptamer binds to cocaine and forms a three-way junction, in which the TMR moiety comes in close proximity to the SERS substrate, generating an enhanced SERS signal that indicates the presence of the cocaine target. Therefore, the SERS signal from the reporter increases substantially with the addition of cocaine. Apparently, the sensor, unlike the SERS-based "molecular sentinel",[15] exhibits an enhanced signal with increased target concentration, thereby demonstrating a "signal-on" architecture that is beneficial for signal gain.

Characterization of the silver aggregate substrate: Figure 1 shows the TEM image of silver nanoparticles in the as-prepared state and the SEM image of the prepared SERS substrate film of silver colloids on a gold disc. One can observe that the silver nanoparticles are almost monodispersed and exhibit spherulitic facets. An average size of 40 nm with a standard deviation of 2 nm was estimated by using 100 nanoparticles in the TEM investigation (Figure 1A). The SEM image in Figure 1B shows that the silver particles are closely packed and form a large aggregate film with homogeneous morphology in the SERS substrate film. It was observed that the aptameric sensor gave a very reproducible signal after more than ten washes, thus indicating that the SERS substrate is very stable and no particles are lost in the rinsing steps. The formation of a stable colloid film was also demonstrated previously.[32,33] The stability of the colloid film might be attributed to the vacuum desiccation step that induced a high salt concentration and dehydration on the colloid surface. This reduced the electrostatic repulsion and caused a strong interaction between the silver nanoparticles as well as between the silver colloids and the metal support. Thus a stable colloid film could be obtained as a sensitive SERS substrate. Because aggregates of silver or gold nanoparticles had been demonstrated as very effective SERS substrates,[34] this substrate was expected to show high SERS activity to allow enhanced sensitivity in the aptameric sensor. Moreover, it was observed that this substrate was mechanically very stable and showed reproducible SERS activity when subjected to washing and rinsing treatment with water or salt solution during the experiments.

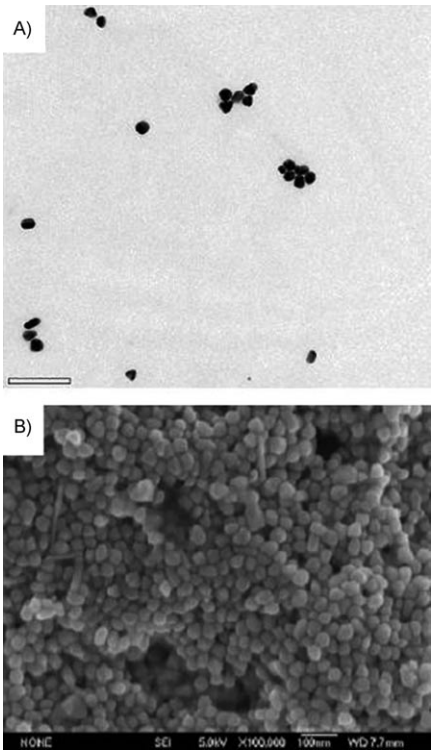


Figure 1. A) TEM image of silver nanoparticles in the as-prepared state (scale bar = 200 nm). B) SEM image of the SERS substrate film of silver colloids (scale bar = 100 nm).

Response performance of the sensor: Figure 2A depicts the SERS spectra obtained by using the aptameric sensor before and after cocaine binding. In the absence of the target, a small SERS signal is observed with five typical Raman bands appearing in the region from 1100 to 1700 cm^{-1} (curve a). The peaks of these Raman bands are located at 1648, 1533, 1510, 1351, and 1215 cm^{-1}. The assignments of major Raman bands for TMR in the 1700–1100 cm^{-1} region are given in Table 1.[35] With the addition of the cocaine target, the SERS signal exhibits an appreciable enhancement by about 1.6 times (curve b) and there is

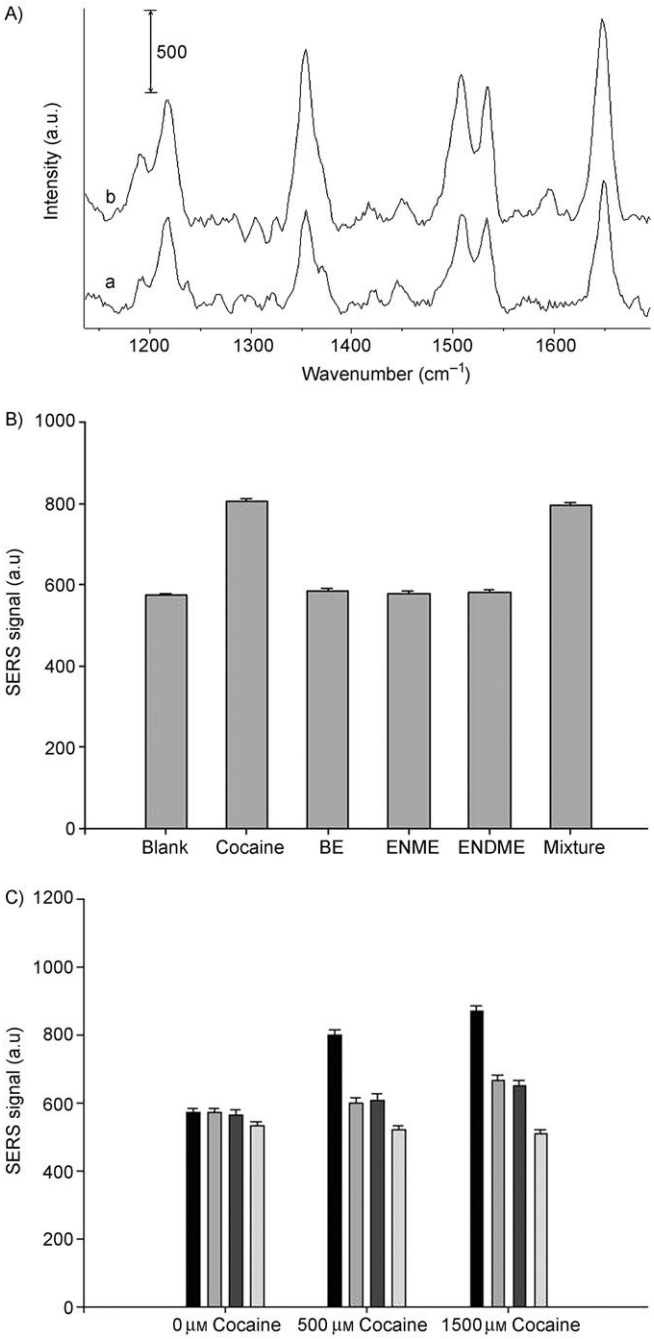


Figure 2. A) SERS spectra of the aptameric sensor in the absence (a) and presence (b) of cocaine (3.126 mM). B) SERS signals of the aptameric sensor at 1648 cm^{-1} for cocaine (500 μM), BE (1 mM), ENME (1 mM), ENDME (1 mM), and a mixture of cocaine (500 μM), BE (1 mM), ENME (1 mM), and ENDME (1 mM). C) SERS signal at 1648 cm^{-1} using the sensor prepared with templated assembly of 10 μM aptamer (the first column in each bar set), with nontemplated assembly of 10 μM aptamer (the second column in each bar set), with nontemplated assembly of 0.2 μM aptamer (the third column in each bar set), and with 10 μM arbitrary DNA sequence (the fourth column in each bar set) in response to 0 (the left bar set), 500 (the middle bar set), and 1500 μM cocaine (the right bar set). Cocaine aptamer: 5′-SH-$(CH_2)_6$-GAC-AAG-GAA-AAT-CCT-TCA-ATG-AAG-TGG-GTC-(TMR)-3′. Arbitrary DNA sequence: 5′-SH-$(CH_2)_6$-GAC-AAG-GAA-AAT-GGA-AGT-TAG-AAG-TGG-GTC-(TMR)-3′. The error bar is the standard deviation.

Table 1. Assignments of major Raman bands for TMR in the 1700–1100 cm^{-1} region.

Vibrational frequency [cm^{-1}]	Band assignment
1648	aromatic C–C stretching
1533	aromatic C–C stretching
1510	aromatic C–C stretching
1351	aromatic C–C stretching
1215	aromatic C–H in-plane bending

no significant change of relative intensity among these major bands in the 1700–1100 cm^{-1} region, thereby indicating that the TMR reporter is located close to the SERS substrate after the target binding. Although almost all the major bands showed a similar change after the addition of cocaine, to maximize the signal-to-noise ratio the band at 1648 cm^{-1} was chosen as the signal for cocaine quantification due to the fact that it gave the strongest signal and ideal peak profile. This observation was in good agreement with the assay principle. In the absence of the target, most of the aptamer molecules remain partially unfolded and induce the TMR reporter to be separated from the substrate, which results in a small SERS signal. Upon addition of target, the conformational change of the aptamer molecule draws the TMR reporter in close proximity to the SERS substrate, thus clearly enhancing the SERS signal. In addition, we investigated the response of the aptameric sensor to a series of cocaine metabolites including benzoylecgonine (BE), ecgonine methyl ester (ENME), and ecgonidine methyl ester (ENDME). The SERS signals are plotted in Figure 2b. It is clear that the sensor shows excellent specificity for cocaine against its analogues.

To demonstrate the benefit of the templated assembly for the aptameric sensor, the response behavior of aptameric sensors prepared according to a "templated" or "nontemplated" assembly was compared. One sees from Figure 2C that without the templated assembly, the aptameric sensor only shows 4.7 and 16.2% increases in SERS signals at 1648 cm^{-1} in response to 500 μM and 1500 μM cocaine, respectively. In contrast, 39.5 and 51.8% increases in SERS signals are obtained by using an aptameric sensor prepared by templated assembly. This result revealed that templating could mitigate the steric hindrance during the folding of the aptamer upon binding to the cocaine target, thereby improving the sensitivity of the aptameric sensor to the analytical target. Note that such improvement of response performance by using the templated assembly was not a result of the lowered surface density of the aptamer. Actually, an inspection of the response using an aptameric sensor prepared with diluted aptamer solution resulted in even poorer performance, as shown in Figure 2C. Because target-induced conformation change for aptamers is a general phenomenon commonly involving folding or unfolding of oligonucleotide stands, templated assembly of aptamers might be a very promising approach for the development of aptameric sensors.

To make the aptameric sensor applicable to quantitative determination, the reproducibility of the sensor is of crucial

significance. Therefore, the reproducibility of the aptameric sensor at different spots on an SERS substrate over an area of several hundred μm^2 and on substrate films prepared in different batches was investigated by using the Raman band at 1648 cm^{-1}. As can be seen from Figure 3, the SERS signal

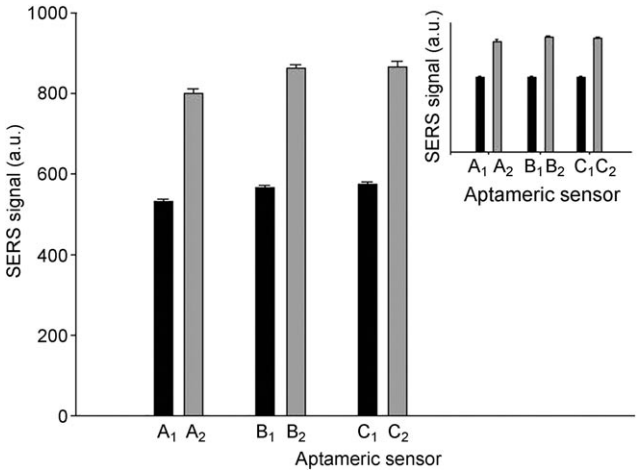


Figure 3. SERS signal of the aptameric sensor at 1648 cm^{-1} measured before (A_1, B_1, C_1) and after (A_2, B_2, C_2) addition of cocaine (1.5 mM) from three different batches of aptameric sensors. Inset: the SERS signal obtained after the intensities for the blank were adjusted to be equal. The error bar is the standard deviation.

of TMR increased after the addition of 1.5 mM cocaine on substrate films prepared in three batches. To clearly demonstrate the reproducibility of the aptameric sensors, SERS signals obtained on substrate films prepared in different batches were all normalized by the blank signal. The spot-to-spot relative standard deviations in each sensor are all below 8.9% either before or after the addition of cocaine. Upon the addition of the target of the same concentration, three sensors prepared in three batches respectively show a 51.8, 51.3, and 51.3% increase in the SERS signals at 1648 cm^{-1}. Here one notices that the standard deviation for the signal ratio over different batches of aptameric sensors is 0.3%, which was significantly improved in comparison with spot-to-spot relative standard deviation of 8.9%. This indicated that the prepared SERS substrate still had some variation over different spots such that the blank signals could show relatively large standard deviations. However, if one used the ratio of the SERS signal for the sample to that of the blank as the measure for quantification, this could largely eliminate the effect of spot-to-spot variation in the SERS substrate, and desirable reproducibility could be achieved for the SERS-based aptameric sensor prepared in different batches. Alternatively, one could adjust the focal plane of the confocal microscope to such a position that the blank signals were equal at different spots, and then the response of the sample could be measured at the same spot. In subsequent studies, this strategy was employed for the determination of all SERS signals.

As one can see from Figure 3, the ratios of the signal to the blank for a certain cocaine concentration from different modified surfaces are very close to each other, although the absolute spectra might show substantial variations. This indicated that using the ratio of the signal to the blank as the measure for quantification of cocaine circumvented the requirement of adjusting the sampling area exactly at the focal plane and mitigated the signal variation resulting from different modified surfaces. These findings suggested that quantitative detection for cocaine could be achieved by using the developed aptameric sensor.

Effect of the ratio of aptamer to 3-MPA in mixed self-assembly: Efficient immobilization of the aptamer plays a crucial role in the detection sensitivity. The present study introduced the mixed self-assembled monolayer of a thiolated aptamer and thiol compounds for the immobilization of the aptamer so as to control the surface density and mitigate the steric hindrance in conformation changes.[36,37] MPA is a commonly available reagent that is negatively charged as an oligonucleotide, therefore it might provide a hydrophilic environment beneficial to mitigating the nonspecific adsorption on the sensor surface. One could also use other hydrophilic thiol compounds with hydroxyl or carboxyl terminal groups to make the mixed monolayer. As shown in Figure 4, the SERS signal enhancement is strongly dependent upon the ratio of aptamer to 3-MPA in the mixed self-assembly process, and the optimum ratio is achieved at a ratio of 1:4. One could assume that too much 3-MPA would induce an insufficient loading of aptamer on the substrate, thus deteriorating the signal enhancement. In contrast, a deficient amount of 3-MPA presumably led to large steric hindrance and unfavorable conformation of the aptamer; this would prevent a sensitive response to the target. Therefore, the ratio of 1:4 was selected for the immobilization of aptamer on the substrate by using a mixed self-assembled monolayer.

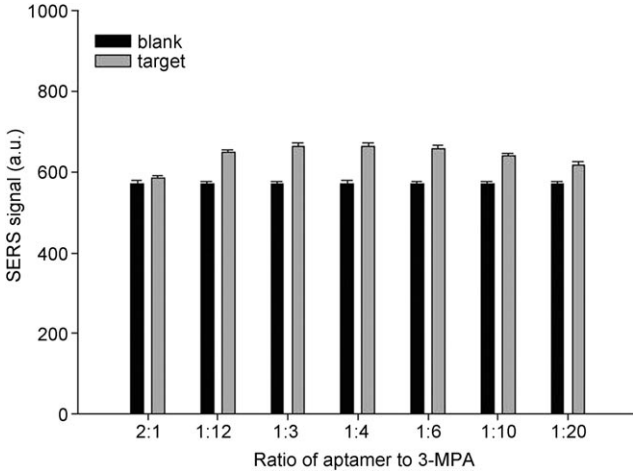


Figure 4. SERS signal at 1648 cm^{-1} as a function of the ratio of aptamer to 3-MPA. The MPA was stored in sodium chloride (1 M) and PBS buffer (10 mM, pH 7.0), and was diluted using PBS to the concentration desired by the corresponding ratios. The error bar is the standard deviation.

Effect of ionic strength: The cation concentration has a significant effect on the conformation and recognition ability of the aptamer.[21,23] We investigated the effect of two cations, Mg^{2+} and Na^{+}, on the performance of the aptameric sensor. As shown in Figure 5, these two metal ions have a distinct effect on the SERS signal enhancement on cocaine binding. It can be observed in Figure 5A that with an increase of Na^{+} concentration, the SERS signal increment increases and plateaus over 600 mM. In contrast, the SERS signal shows the maximum increase in an Mg^{2+}-free buffer and decreases with increasing Mg^{2+} concentration, as shown in Figure 5B. Because metal ions are expected to reduce the electrostatic force between the negatively charged phosphate backbones in DNA structures, the increase in the concentration of Na^{+} is beneficial for the stabilization of the folding of the aptamer structure, which results in better intercalation between aptamer and cocaine with a stronger SERS signal. On the other hand, the increase of Mg^{2+} would improve the stability of the stem moiety of the aptamer, thereby leading to a high background signal for the performance of the SERS sensor with a smaller signal increment. By considering the overall effect of the metal ions, the optimal assay buffer of the aptameric sensor was obtained at 1 M Na^{+} concentration without any Mg^{2+}, and such a condition was used throughout subsequent experiments.

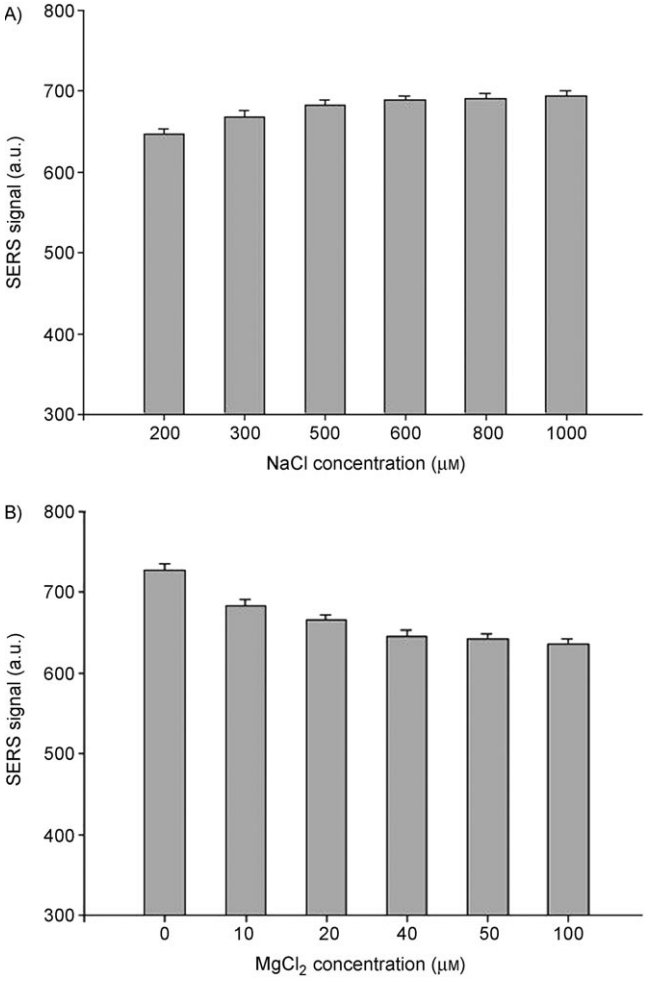


Figure 5. SERS signal of the aptameric sensor as a function of NaCl (A) and $MgCl_2$ (B) concentration. In the experiment the sample solution contained 100 μM cocaine. The error bars are the relative standard deviation.

Regeneration of the sensors: In practical applications, reusability has always been a desired feature for biosensors.[38] The regeneration of a DNA sensor is mostly achieved by either a thermal or a chemical method. The interaction of cocaine with the aptamer was relatively weak with a dissociation constant (k_d) of 100 μM. Therefore, in a cocaine-free buffer with sufficient volume, the cocaine–aptamer complex would dissociate, and the system could be regenerated. In this process, to ensure that most of the cocaine–aptamer complex dissociated at the surface, the SERS aptameric sensor was regenerated through dropping an aliquot (50 μL) of 10 mM phosphate buffer (pH 7.0) containing 1 M NaCl on the substrate, removing the buffer, and then rinsing the substrate again with a second aliquot (50 μL) of buffer followed by removal of the buffer. In this case, the concentration of cocaine in the first rinsing buffer was at most 2.9×10^{-3} μM, as estimated by the density of a common thiolated DNA self-assembled monolayer (ca. 2.8×10^{12} molecules cm^{-2}).[39] Therefore, it could be estimated that the ratio of free aptamer to aptamer–cocaine complex was at least 3.4×10^{4}, which would indicate that most of the cocaine–aptamer complex dissociated at the surface and the sensor interface was regenerated after the first rinsing. From the above discussion, one might conclude that the templating process as well as the regeneration procedure used in our experiments was compatible with the dissociation constant of the cocaine–aptamer complex.

Figure 6 depicts the regeneration results of the aptameric sensor. In this experiment the sensor was used for the detection of cocaine in different matrices including phosphate buffer as well as 50-, 10-, and 5-fold dilutions of human serum, and then was regenerated by using a target-free phosphate buffer solution. The performance of ten cycles of detection and regeneration is shown in Figure 6. It can be observed that in phosphate buffer and 50- and 10-fold dilutions of human serum the SERS signal increased by about 40% in response to 0.5 mM cocaine, and recovered to the initial blank value after regeneration in ten detection and regeneration cycles. The standard deviation for the SERS signal increase is 1.8, 2.0, and 2.9%, respectively, in phosphate buffer and 50- and 10-fold dilutions of human serum for ten detection and regeneration cycles. This indicated that the aptameric sensor could be regenerated in such matrices as phosphate buffer and 50- and 10-fold dilutions of human serum. In contrast, the detection and regeneration of the sensor in more complicated matrices such as 5-fold diluted serum suffered from some difficulty. As one sees in Figure 6, the SERS signal only increases by about 25% in 5-fold diluted serum in response to 0.5 mM cocaine, and the signal increases become slightly smaller after each regeneration, indicating that the sensing interface might be contami-

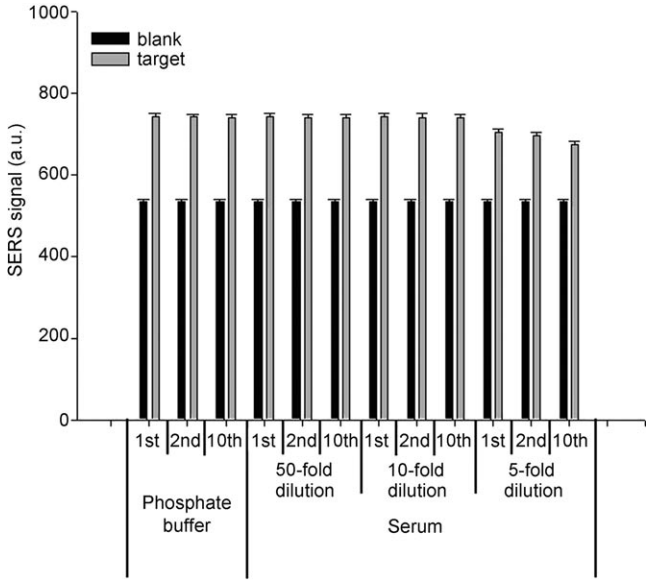


Figure 6. SERS signal at 1648 cm^{-1} from three rounds of detection and regeneration with 500 μM cocaine in phosphate buffer and 50-, 10-, and 5-fold dilution of serum, respectively. The error bar is the standard deviation.

nated by a relatively high loading of nonspecific adsorption of protein in such a complicated matrix. The results revealed that the surface could also be regenerated in pure buffer as well as in 50- and 10-fold diluted sera, but would significantly lose sensitivity in a more concentrated serum matrix such as 5-fold diluted serum. Therefore, one could expect that the aptameric sensor held its potential for determining cocaine in some pure matrices such as buffer or 10-fold diluted serum, but was not applicable in a 5-fold diluted serum specimen or more complicated matrices. In these cases, a preliminary dilution of these matrices or dialysis of the specimens to obtain protein-free samples was required. Though this cost some time and labor, aptameric sensors might still be a realistic approach to detect small molecules in complex matrices.

SERS-based detection of cocaine: Under optimized assay conditions, the SERS aptameric sensor was employed to detect cocaine in a PBS buffer. As shown in Figure 7, the SERS signal increases with increasing cocaine target concentration ranging from 1 to 3200 μM. According to the three-times standard-deviation rule, a detection limit of 1 μM could be obtained. The performance is comparable to immunoassay techniques for cocaine.[40] In comparison to those reported by aptameric sensors for cocaine that use fluorescent[21] or electrochemical signals,[28] the SERS-based senor improved the detection limit approximately 10-fold. Furthermore, because each Raman reporter has a fingerprint spectrum and many dyes can be excited at the same wavelength, it is expected that the SERS-based aptameric sensor furnishes a tremendous capacity for multiplexed assay by labeling multiple reporters on different aptamers that recognize different targets.

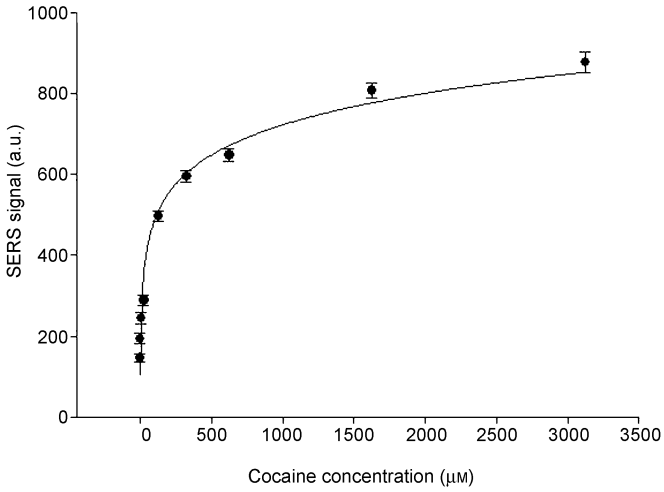


Figure 7. The response curve for the SERS-based aptameric sensor to cocaine in PBS buffer. The error bar is the standard deviation.

Conclusion

This report demonstrated the feasibility and usefulness of SERS as a potential alternative mechanism for the development of aptameric sensors. Cocaine was detected through Raman signal enhancement resulting from a target-induced conformation change. The determination of cocaine could be achieved by covering the range from 1 to 3200 μM under optimized detection conditions. This strategy offers many advantages including alleviated photobleaching, noninvasive sampling, microsized sampling spots, as well as increased multiplexity over the fluorescent sensor. Compared with an electrochemical sensor, the SERS-based sensor has a further benefit in that the DNA probe does not require a specialized procedure in labeling redox groups. Also, the templated assembly technique in the construction of the sensing interface enhances the sensitivity of the aptameric sensor, and its detection limit is better than fluorescent and electrochemical sensors. Furthermore, the aptameric sensor could be regenerated quickly through a simple wash. Therefore, it is expected that SERS-based aptamer sensors might hold promising potential for multiplex and sensitive determination in biochemical and biomedical studies.

Experimental Section

Reagents: Silver nitrate, sodium citrate trihydrate, magnesium chloride, sodium chloride, and 3-mercaptopropionic acid were purchased from Aldrich. Cocaine hydrochloride was obtained from the National Institute for the Control of Pharmaceutical and Biological Products. All chemicals and materials were of analytical grade and used as received. All solutions were prepared with deionized water (=18.32 MΩ) purified by a Nanopure Infinity Ultrapure water system (Barnstead/Thermolyne Corp, Dubuque, IA).

The oligonucleotide aptamer was obtained as HPLC purified grade from Takara Biotechnology Co. Ltd (Dalian, China), and was based on the oligonucleotide sequence previously used by Stojanovic et al.[21] The se-

quence was covalently modified with tetramethylrhodamine (TMR) at the 3′ terminus and a thiol substituent attached to a six-carbon linker at the 5′ end as follows: 5′-SH-$(CH_2)_6$-GAC-AAG-GAA-AAT-CCT-TCA-ATG-AAG-TGG-GTC-(TMR)-3′. TMR was linked to the oligonucleotide by the conjugation of tetramethylrhodamine *N*-hydroxysuccinimide ester with an amino-modified 3′ terminus in sodium carbonate/bicarbonate buffer at pH 9.

Preparation of Ag colloids: Silver colloids were prepared according to the citrate-reduction procedure of Lee and Meisel.[41] An aqueous solution (200 mL) of 10^{-3} M silver nitrate was boiled under vigorous stirring, then 35 mM sodium citrate (5 mL) was added and the boiling was continued for 1 h. The concentration of silver colloids was around 10^{11} particles per milliliter, which was consistent with that reported in previous references.[16] The colloidal solution was stored at 4 °C and protected from light. Because silver colloids might be subjected to unexpected aggregation during storage, sonication of the colloids was performed every time before use. This minimized the aggregation of silver colloids and enabled a reproducible preparation of the SERS substrate, which was naturally of great significance for the development of SERS-based sensors.

Preparation of the substrate: SERS substrates were prepared according to a previously reported procedure[32,33] and slightly modified as follows. Gold discs of 2 mm diameter were polished subsequently with 0.3 and 0.05 μm alumina powder to a mirror finish, followed by ultrasonic cleaning with purified water for about 3 min. Ag colloids were concentrated by 10-fold by centrifuging the solution (10 mL) at 16000 rpm and resuspending it in water (1 mL), and then a 3 μL aliquot was dropped on the gold disc's surface. By putting the colloid-coated gold disc in a vacuum desiccator for 7 min, the nanoparticles on the gold surface were allowed to pack closely and aggregate together. The procedure was repeated another six times. Then, a thin film of closely packed nanoparticles was obtained. This film was attached tightly on the Au surface and was observed to be very stable when it was rinsed with water and salt solutions. SERS substrates prepared in different batches were obtained by using the aforementioned procedure exactly with silver colloids prepared in the same batch on different gold discs. The substrates were stored in a desiccator before use.

Transmission electron micrographs were obtained by using a JEM-3010 electron microscope (JEOL, Japan) with Digitalgraph software at an accelerating voltage of 100 kV. The prepared substrate was analyzed by scanning electron microscopy (SEM) by using a JSM-5600 LV microscope (JEOL, Ltd., Japan).

Fabrication of the aptameric sensor: An aliquot (5 μL) of 10^{-5} M aptamer solution was added to 1.0 mM cocaine solution (100 μL) in a 10 mM phosphate buffer (pH 7.0) containing 10 mM $MgCl_2$ and 1 M NaCl. The mixture was stored at 4 °C in the dark for 7 h. Before the solution was dropped on the substrate, 3-MPA (20 μL) was added and the mixture was strongly shaken for about 1 min. An aliquot (30 μL) of the mixture was dropped on the prepared SERS substrate, followed by incubation for 12 h at 4 °C in the dark. It is of note that maintaining a certain amount of solution on the substrate surface throughout the incubation might keep the DNA aptamer in its natural conformation on the surface. To wash away the cocaine template, the SERS substrate was immersed in a phosphate buffer solution under vigorous stirring for 1 h, with the buffer solution refreshed every 20 min. The aptamer sensor was thus prepared.

Protocol of detection: After the aptamer sensor was mounted on the *XY* stage below the sampling objective of the Raman microspectroscope, 10 mM PBS buffer (20 μL, pH 7.0) was dropped on the surface of the substrate. Raman spectra were collected randomly on a spot in a $50 \times 50\ \mu m^2$ area as the blank. Then cocaine solution (20 μL) was added after the blank solution was removed, followed by the collection of Raman spectra in the same area after 10 min. Because vertical movement of the substrate caused the sampling area to deviate from the focal plane, which would significantly change the SERS signal, the substrate was moved in a series of measurements to keep an equal blank response at different spots. The signal intensity of the band at 1648 cm^{-1} was recorded versus different concentrations of cocaine.

Raman spectra were collected by using a Jobin Yvon Micro-Raman spectrometer (RamLab-010). It comprises an integral Olympus BX40 microscope with a 10× objective that focuses the laser on the sample and collects the backscattered radiation; a notch filter to cut the exciting line; a holographic grating (1800 g mm^{-1}) offering a spectral resolution of 2 cm^{-1}; and a semiconductor-cooled, 1024×256-pixel, charge-coupled device detector. Radiation of 632.8 nm from a He–Ne laser with power of around 5 mW was used as the excitation line. The slit and pinhole were set at 100 and 300 μm. Under this setting, the sampling area was about 10 μm in diameter on the substrate surface. All SERS spectra were acquired with 5 s integration and processed with the software from Jobin Yvon (Labspec4.0). Calibration of the wavenumber was carried out with reference to the 520 cm^{-1} line of silicon.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (nos. 20435010, 20575020, 20675028, 20775023) and the Ministry of Education (NCET-04–0768).

[1] R. K. Chang, T. E. Furtak, *Surface Enhanced Raman Scattering*, Plenum Press, New York, **1982**.
[2] M. Moskovits, *ReV. Mod. Phys.* **1985**, *57*, 783–826.
[3] A. Otto, I. Mrozek, H. Grabhorn, W. J. Akemon, *J. Phys. Condens. Matter* **1992**, *4*, 1143–1212.
[4] S. Nie, S. R. Emory, *Science* **1997**, *275*, 1102–1106.
[5] W. E. Doering, S. Nie, *J. Phys. Chem. B* **2002**, *106*, 311–317.
[6] K. Kneipp, H. Kneipp, I. Itzkan, R. R. Dasari, M. S. Feld, *Chem. Rev.* **1999**, *99*, 2957–2976.
[7] S. P Mulvaney, M. D. Musick, C. D. Keating, M. J. Natan, *Langmuir* **2003**, *19*, 4784–4790.
[8] X. Dou, T. Takama, Y. Yamaguchi, H. Yamamoto, Y. Ozaki, *Anal. Chem.* **1997**, *69*, 1492–1495.
[9] J. Ni, R. J. Lipert, G. B. Dawson, M. D. Porter, *Anal. Chem.* **1999**, *71*, 4903–4908.
[10] J. D. Driskell, K. M. Kwarta, R. J. Lipert, M. D. Porter, *Anal. Chem.* **2005**, *77*, 6147–6154.
[11] Y. C. Cao, R. C. Jin, C. A. Mirkin, *Science* **2002**, *297*, 1536–1540.
[12] Y. C. Cao, R. Jin, J. M. Nam, C. S. Thaxton, C. A. Mirkin, *J. Am. Chem. Soc.* **2003**, *125*, 14676–14677.
[13] W. E. Doering, S. Nie, *Anal. Chem.* **2003**, *75*, 6171–6176.
[14] J. L. Gong, J. H. Jiang, Y. Liang, G. L. Shen, R. Q. Yu, *J. Colloid Interface Sci.* **2006**, *298*, 752–756.
[15] M. B. Wabuyele, T. Vo-Dinh, *Anal. Chem.* **2005**, *77*, 7810–7815.
[16] P. Zhang, T. Beck, W. Tan, *Angew. Chem.* **2001**, *113*, 416–419; *Angew. Chem. Int. Ed.* **2001**, *40*, 402–405.
[17] A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818–822.
[18] C. Tuerk, L. Gold, *Science* **1990**, *249*, 505–510.
[19] L. Gold, B. Polisky, O. Uhlenbeck, M. Yarus, *Annu. Rev. Biochem.* **1995**, *64*, 763–797.
[20] M. Famulok, G. Mayer, *Curr. Top. Microbiol. Immunol.* **1999**, *243*, 123–136.
[21] M. N. Stojanovic, P. de Prada, D. W. Landry, *J. Am. Chem. Soc.* **2001**, *123*, 4928–4931.
[22] R. Nutiu, Y. Li, *Chem. Eur. J.* **2004**, *10*, 1868–1876.
[23] J. Wang, Y. X. Jiang, C. S. Zhou, X. H. Fang, *Anal. Chem.* **2005**, *77*, 3542–3546.
[24] C. C. Huang, Y. F. Huang, Z. H. Cao, W. H. Tan, H. T. Chang, *Anal. Chem.* **2005**, *77*, 5735–5741.
[25] J. W. Liu, Y. Lu, *Angew. Chem.* **2006**, *118*, 96–100; *Angew. Chem. Int. Ed.* **2006**, *45*, 90–94.
[26] M. Liss, B. Petersen, H. Wolf, E. Prohaska, *Anal. Chem.* **2002**, *74*, 4488–4495.
[27] Y. Xiao, A. A. Lubin, A. J. Heeger, K. W. Plaxco, *Angew. Chem.* **2005**, *117*, 5592–5595; *Angew. Chem. Int. Ed.* **2005**, *44*, 5456–5459.
[28] B. R. Baker, R. Y. Lai, M. S. Wood, E. H. Doctor, A. J. Heeger, K. W. Plaxco, *J. Am. Chem. Soc.* **2006**, *128*, 3138–3139.

[29] Y. Zhang, Y. Huang, J. H. Jiang, G. L. Shen, R. Q. Yu, *J. Am. Chem. Soc.* **2007**, *129*, 15448–15449.
[30] R. A. Wolkow, M. J. Moskovits, *J. Chem. Phys.* **1987**, *87*, 5858–5869.
[31] A. Campion, P. Kambhampati, *Chem. Soc. Rev.* **1998**, *27*, 241–250.
[32] J. W. Hu, Y. Zhang, J. F. Li, Z. Liu, B. Ren, S. G. Sun, Z. Q. Tian, T. Lian, *Chem. Phys. Lett.* **2005**, *408*, 354–359.
[33] J. F. Li, Z. L. Yang, B. Ren, G. K. Liu, P. P. Fang, Y. X. Jiang, D. Y. Wu, Z. Q. Tian, *Langmuir* **2006**, *22*, 10372–10379.
[34] A. M. Michaels, J. Jiang, L. Brus, *J. Phys. Chem. B* **2000**, *104*, 11965–11971.
[35] P. Hildebrandt, M. Stockburger, *J. Phys. Chem.* **1984**, *88*, 5935.
[36] L. A. Gearheart, H. J. Ploehn, C. J. Murphy, *J. Phys. Chem. B* **2001**, *105*, 12609–12615.
[37] H. Du, C. M. Strohsahl, J. Camera, B. L. Miller, T. D. Krauss, *J. Am. Chem. Soc.* **2005**, *127* ,7932–7940.
[38] A. P. Abel, M. G. Weller, G. L. Duveneck, M. Ehrat, H. M. Widmer, *Anal. Chem.* **1996**, *68*, 2905–2912.
[39] A. W. Perterson, J. R. Heaton, R. M. Geogiadis, *Nucleic Acids Res.* **2001**, *29*, 5163–5168.
[40] K. Watanabe, K. Okada, H. Oda, K. Furuno, Y. Gomita, T. Katsu, *Anal. Chim. Acta* **1995**, *316*, 371–375.
[41] P. Lee, D. J. Meisel, *J. Phys. Chem.* **1982**, *86*, 3391–3395.

Received: August 23, 2007
Revised: March 8, 2008
Published online: July 30, 2008

