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Journal Name

ARTICLE

Surface-enhanced Raman Scattering Behaviours of 4-Mercaptophenyl boronic Acid on Assembled Silver Nanoparticles

Shuangshuang Li[a,b], Qun Zhou*[a] , Wenya Chu[a], Wei Zhao[a], Junwei Zheng*[a,b]

Molecular recognition based on specific intermolecular interactions is essential for the design of sensors with high selectivity. Herein, we report surface-enhance Raman scattering (SERS) behaviours of 4-mercaptophenyl boronic acid (MPBA) on self-assembled silver nanoparticles and its interaction with the D-glucose. It is demonstrated that the orientation and existing form of the MPBA strongly depend on the pH value of the media. The surface immobilized MPBA can be reversibly associated with OH$^-$ in solution, along with a molecular orientation alteration. Self-condensation reaction among the OH$^-$-associated MPBA molecules results in irreversible conversion of OH$^-$-associated MPBA to anhydride, which may hinder the interaction between D-glucose and the B-moiety of MPBA. However, the self-condensation reaction can be diminished under optimized conditions. By taking advantage of the difference in the kinetics of dissociation of the OH$^-$-associated MPBA and D-glucose associated MPBA in acidic media, a proper scheme of the SERS detection of D-glucose is proposed to illuminate the spectral interference of OH$^-$-associated MPBA, which exhibits SERS features similar to those of D-glucose associated MPBA species. Based on those strategies, the SERS detection of D-glucose can be achieved in the physiologically relevant concentration range.

Introduction

During the last couple of decades, interest in arylboronic acids for saccharide sensing applications has increased significantly due to that the boronate group has both a strong affinity and noticeable specificity to saccharides by bonding to 1,2 and 1,3-diols to form cyclic boronate ester.[1-3] Various sensing techniques have been developed based on molecular recognition and fast ester formation of arylboronic acid with sugars in aqueous solution, including fluorescence,[4,5] colorimetry,[6,7] dynamic light scattering,[8] electrochemistry,[9-11] and surface Plasmon resonance (SPR).[12,13] To achieve high affinity and selectivity for particular saccharides, such as D-glucose, a broad range of appropriately designed boronic acid compounds have to be synthesized in order to employ those techniques.[14,15] Compared with those detection techniques, surface-enhanced Raman scattering (SERS) exhibits several significant advantages, such as ultrahigh sensitivity and spectral selectivity.[16-18] Recently, SERS technique has been attempted for the detection of sugars and glycoproteins. In most cases, SERS sensing of saccharides is based on the changes in SERS spectral features upon the binding of saccharides to boronic acid moieties. Sun et al.[19] reported an active SERS substrate composed of nearly aligned silver nanorods adsorbed with a monolayer of 4-mercaptophenyl boronic acid (MPBA) for quantitative detection of glucose. The specific binding of glucose with the boronic acid motif in MPBA can lead to significant increase in the absolute intensity of the SERS signal of MPBA, which was ascribed to orientation change and charge transfer effect. In contract, Torul et al.[20] pointed out that only the boronic acid functional group displayed less signal change in the SERS response upon addition of glucose. By using gold nanorod particles and a gold coated slide modified with the two component self-assembled monolayers consisting of 3-mercaptophenyl boronic acid and 1-decanethiol, they constructed glucose SERS detection platforms, which provide a linear glucose concentration range of 2-16 mM with detection limit of 0.5 mM. On the other hand, in a SERS detection study of fructose on MPBA modified quasi three-dimensional plasmonic gold nanostructure array, Sun took advantage of the bands at 1587 and 1574 cm^{-1}, which were assigned to the 8a and 8b modes of MPBA, respectively, and particularly sensitive to binding of fructose, to correlate the relative intensity of the bands to the concentration of fructose.[21] Besides those, a few different SERS detection approaches have also been developed. Gupta et al.[22] established a SERS glucose biosensor based on mercaptophenyl boronic acid terminated Ag@AuNPs/graphene oxide, the linearity range of glucose was obtained as 2-6 mM with detection limit of 0.33 mM. Moreover, a boronate-affinity sandwich assay approach was employed for the specific and sensitive determination of trace glycoproteins by using boronate-affinity-functionalized silver nanoparticles as SERS probes.[23]

a. College of Chemistry, Chemical Engineering and Materials Science, and Key Lab of Health Chemistry and Molecular Diagnosis of Suzhou, Soochow University, Suzhou 215123, P. R. China.
b. College of Physics, Optoelectronics and Energy, Soochow University, Suzhou 215006, P. R. China.

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Nevertheless, most of those studies mainly focused on the sensing capabilities of the SERS systems, dealing only briefly with structure of the boronic acids and the nature of the molecular recognition process. In fact, the SERS detections involve immobilization of molecules with boronic acid moieties on the metallic surfaces, which may lead to the properties of the functional molecules different from those in a solution. To gain an insight into the behaviours of the immobilized molecules and intermolecular interactions between the functional groups in molecular recognition is therefore highly desirable for proper design of SERS sensors. Herein, we systematically investigated the SERS behaviours of MPBA immobilized on assembled silver nanoparticles (AgNPs) in various media. It was demonstrated that the association of OH^- to MPBA may lead to self-condensation of MPBA to form anhydride, which may in turn, affect the binding of D-glucose through the formation of esters. On the other hand, a properly designed SERS detection strategy was proposed to illuminate a spectral interference from the similarity between the SERS spectra of D-glucose associated MPBA and OH^--associated MPBA species.

Experimental

Materials

4-Mercaptophenyl boronic acid and polyvinylpyridine (PVP) were purchased from J&K Technology and Acros Organics, respectively. The other chemicals were analytical reagent grade and used as received without further purification.

Experimental Procedures

The preparation and assembly of AgNPs were according to the protocol reported previously.[24] The average diameter of the particles was about 60 nm, estimated from the SEM image (Fig. S1). The PVP-derivaterized glass slides were used to immobilize the AgNPs. Self-assembled monolayer of MPBA was obtained by immersing the glass slides modified with AgNPs into 1 mM

MPBA ethanol solution for 2 h. Then the substrates were rinsed with ethanol three times and dried in air. All the SERS spectra obtained in solutions were acquired with the substrates directly immersed in the corresponding solutions.

Characterization

The surface morphologies of the samples were measured on a Hitachi S4700 FE-SEM microscope. The SERS spectra were recorded on a RENISHAW Invia Raman microscope equipped CCD detector and a 50×(NA 0.5) microscope objective to focus the laser beam onto a spot of ~2 μm^2. A 633 nm laser was used as excitation source. The Raman band of the silicon wafer at 520 cm^{-1} was used to calibrate the spectrometer.

Results and discussion

SERS spectra of MPBA on AgNPs

The SERS spectrum of MPBA adsorbed on the assembled AgNPs and the ordinary Raman spectrum (ORS) of MPBA neat solid state is compared in Figure 1. The assignments of Raman bands of MPBA are summarized in Table 1.[25] The SERS spectrum was obtained directly after the self-assembled AgNPs substrate adsorbed with MPBA was removed from the MPBA ethanol solution, completely washed with pure ethanol, and dried in air. The bands at 2563 and 907 cm^{-1} arising from the S-H stretching mode (ν_{S-H}) and C-S-H bending band mode
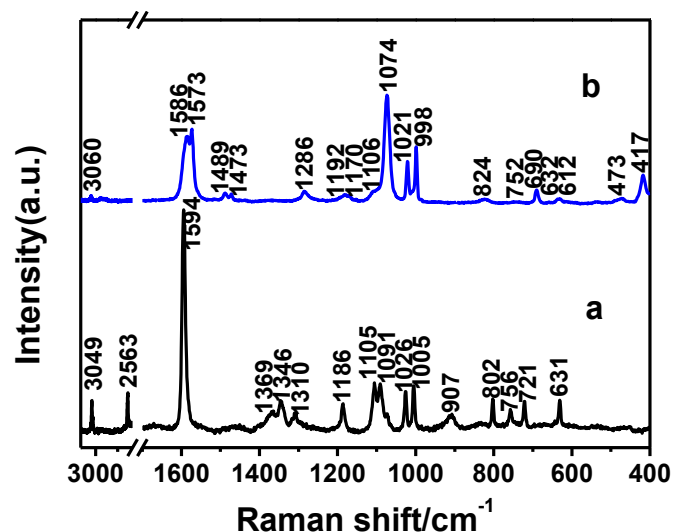
Table 1 Raman frequencies and assignments of MPBA

Assignment	Solid	SERS
$2(a_1),\nu_{CH}$	3049	3060
ν_{SH}	2563	-
$8a(a_1),\nu_{CC}$	1594	1586
	-	1573
$19a(a_1),\nu_{CC}$	-	1489
	-	1473
ν_{BO}	1369	-
ν_{BO}	1346	-
ν_{BO}	1310	-
$3(b_2),\beta_{CH}+\beta_{BOH}$	-	1286
$9a(a_1),\beta_{CH}+\beta_{BOH}$	1186	1192
	-	1170
$15(b_2),\beta_{CH}$	1105	1106
$1(a_1),\beta_{CCC}+\nu_{CS}$	1091	1074
$18a(a_1),\beta_{CH}$	1026	1021
$12(a_1),\beta_{CCC}$	1005	998
β_{CSH}	907	-
$10a(a_2)\gamma_{CH}$	802	824
$11(b_1),\gamma_{CH}$	756	752
$4b(b_1),\gamma_{CCC}$	-	-
$6a(a_1),\beta_{CCC}+\nu_{CS}$	721	690
ν_{CS}	631	632
$6b(b_1),\beta_{CCC}$	-	612
$16b(b_1),\gamma_{CCC}$	-	473
$7a(a_1),\beta_{CCC}+\nu_{CS}$	-	417

ν, stretching; β, in plane bending; γ, out of plane bending.

Figure 1 (a) Ordinary Raman of neat solid MPBA and (b) SERS spectrum of MPBA adsorbed on AgNPs.

(β_{CSH}),respectively, in ORS completely disappeared in the SERS spectrum, indicating that MPBA was adsorbed on the silver surface as thiolate after losing its thiol proton, as for other thiols. According to the SERS surface selection rule based on the electromagnetic enhancement mechanism, it can be expected that the in-plane modes would be weak for flat-oriented aromatic rings, whereas for a standing-up orientation they should be fairly strong.[26, 27] From the SERS spectrum in Figure 1, it can be seen that the SERS spectrum is dominated by the bands at 1074, 1021 and 998 cm^{-1}, which are assigned to the $\beta_{CCC}+\nu_{CS}$ (1), β_{CH} (18a), and β_{CCC} (12) modes, respectively, and belong to in-plane modes. A pair of intense bands at 1586/1573 cm^{-1} is ascribed to the original and OH$^-$-associated forms of in-plane ν_{CC} (8a), which will be discussed later. Moreover, in the high frequency region, the aromatic C-H stretch mode at 3060 cm^{-1}, which is often employed for determining the surface geometry of a planar molecule adsorbed on a silver surface,[28] can be observed in the SERS spectrum. Additional clue about the orientation of the adsorbed MPBA molecules comes from the fact that the out of plane modes, such as γ_{CCC} (16b) and γ_{CH} (10a) were observed at 824 and 473 cm^{-1}, although their intensities were relatively weak. All those observations indicate that most likely, the adsorbed MPBA molecules originally obtained from the ethanol solution would adopt a tilted orientation in their state with respect to the surface of AgNPs.

The tilted orientation of the adsorbed MPBA molecules makes it possible that the boronic acid group in the molecules would be free and sensitive to pH value of the environment. The SERS spectra of the MPBA molecules on AgNPs were examined in the solutions with different pH values, the typical results obtained in pH 1.0, 7.0, and 13 solutions are presented in Figure 2. The results obtained at other pH values are also shown in Fig. S2 in Supporting Information. Under acidic condition (pH=1.0), the ν_{CC} mode was located at 1586 cm^{-1}. As the pH value was increased, a new band at 1573 cm^{-1} appeared and intensity increased with increasing of pH value.
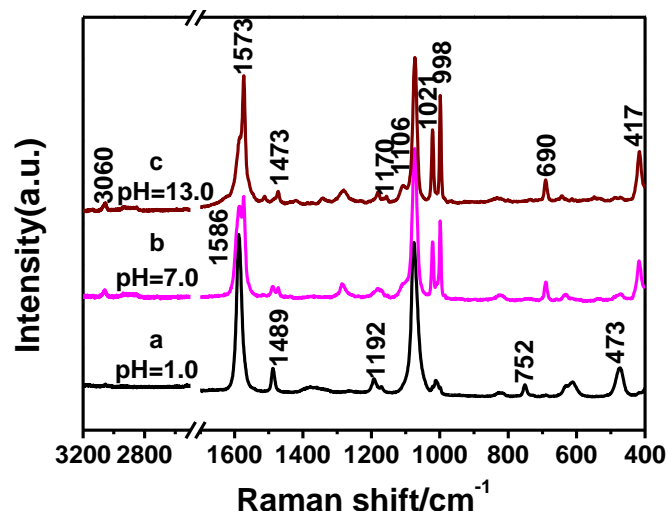

Figure 2 SERS spectra of MPBA adsorbed on AgNPs in pH (a) 1.0, (b) 7.0 and (c) 13 buffer solutions.

The intensity of this band became comparable to that of the band at 1586 cm^{-1} at pH 7.0, and maximized at pH 13. Similar pH dependent behaviour of the ν_{CC} mode has been reported for 4-mercaptobenzoic acid and 4-mercaptobyridine, which has been ascribed to protonation/deprotonation of the carboxylic group or pyridine group have carboxyl group or pyridine group.[29-31] In this case, since both MPBA molecules and assembled AgNPs are stable in this pH range (Fig. S3, Supporting Information), we believe that a similar acid-base conversion process could also occur for the adsorbed MPBA molecules. However, the special molecular structure of MPBA would lead to a mechanism of acidity different from that for carboxylic acid or pyridine, where proton abstraction occurs from the -COOH or pyridine groups. Boronic acid in MPBA is trigonal planar moiety containing a sp^2-hybridized boron. The vacant p-orbital on boron is responsible for the Lewis acidity of boronic acid and is capable of accepting a lone pair, i.e. from a base.[32] In this case, the presence of OH$^-$ can result in the conversion of MPBA to OH$^-$-associated form, which contains B-moiety with a tetrahedral configuration because of the sp^3-hybridization of the B atom. The bands at 1586 and 1573 cm^{-1} in the SERS spectra are highly sensitive to such an acid-base conversion. Those two bands was previously assigned to the 8a and 8b modes of MPBA by Sun et al.[21] Based on above observation, in contrast, we prefer to ascribe those two bands to the ν_{CC} modes of MPBA and its corresponding OH$^-$-associated form, respectively. They can be referred to as the marker bands of acidity of the adsorbed MPBA molecules.

Several other spectral features in Figure 2 are also worth noting, as compared to those in the SERS spectrum in Figure 1. In pH 1.0 solution, the in-plane modes, such as the bands at 1021 and 988 cm^{-1} for β_{CH} (18a), and β_{CCC} (12) modes, became very weak, in contrast, the out-of-plane modes at 752 cm^{-1} for γ_{CCC} (16b) and 473 cm^{-1} for γ_{CH} (10a) gained moderate intensity. As the pH value of the media was increased, the 1021 and 988 cm^{-1} bands became distinct. The relative intensities of these two bands increased with the pH value of the media. Unlike the observation for the 1573 cm^{-1} band, however, only slight increase in the relative intensity of those two bands was observed, as the pH value of the media became higher than pH 7.0. At the same time, the out of plane modes at 752 cm^{-1} for γ_{CCC} (16b) and 473 cm^{-1} for γ_{CH} (10a) correspondingly became weaken with increasing of pH value and eventually disappeared in the basic media. Considering the SERS surface selection rule, we believe that the adsorbed MPBA molecules may adopt a flat orientation under acidic condition, as indicated by the relative weak intensity of the in plane modes and intensification of the out-of plane modes, whereas they takes a perpendicular orientation in basic media, leading to increase in relative intensity of the in-plane modes and disappearance of the out of plane modes. This behaviour may be interpreted in terms of conversion of MPBA and its OH$^-$-associated form in different pH media. The pK$_a$ value for the immobilized MPBA molecules on metal substrate is about 9.2 estimated by the electrochemical method,[9] which means that under acidic condition the MPBA molecules mainly exist in its original form, while they would be in OH$^-$-associated form in
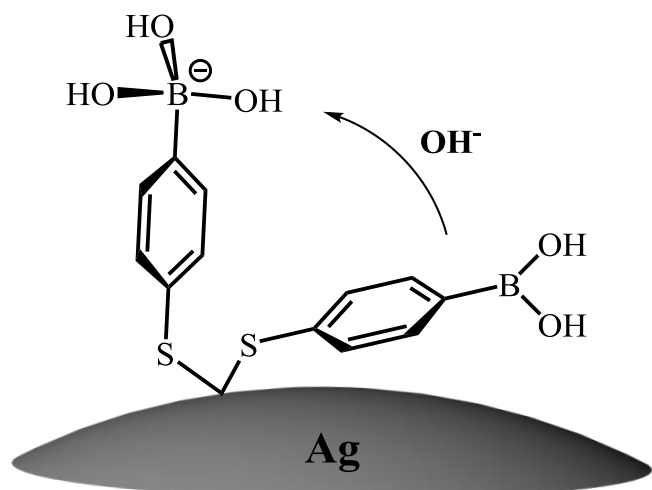
Figure 3 Schematic illustration of orientation alteration immobilized MPBA in acidic and basic solutions.

Figure 4 SERS spectra of MPBA on AgNPs obtained step-by-step: (A) (a) pH 1.0, 10 s, (b) pH 13, 10 s and (c) pH 1.0, 10 s; (B) (d) pH 1.0, 10 s, (e) pH 13, 5 min, and (f) pH 1.0, 10 s.

basic media. The electrostatistic repulsion between the negative charged OH$^-$-associated MPBA molecules in basic media would lead to the adsorbed molecules to be favourable to take a perpendicular orientation. On the other hand, due to lack of the electrostatistic interaction, the MPBA molecules in acidic media would adopt a flat orientation. Thus, it is reasonable that the orientation of the adsorbed MPBA molecules on the AgNPs can be altered with the pH value of the media, as schematically illustrated in Figure 3. It should be emphasized that the intensity of the 1586 cm^{-1} band relative to the 1573 cm^{-1} band, which correspond to the ν_{CC} modes of MPBA and OH$^-$-associated MPBA, may mainly be determined by the pH value of the media. The orientation change of the adsorbed MPBA could not have influence on the relative intensity of those two bands, although the absolute intensities of the bands, as the in plane modes, vary with the molecular orientation. Based on the above observations, it is concluded that the bands at 1021 and 988 cm^{-1} for β_{CH} (18a), and β_{CCC} (12) modes are strongly related to the orientation of the adsorbed MPBA molecules and, thereby, can be referred to the orientation sensitive marker bands.

In the cases of 4-mercaptobenzoic acid and 4-mercaptopyridine, it has been demonstrated that the protonation/deprotonation process is reversible, as indicated by the reversible variation of the relative intensity of the SERS characteristic bands for the protonated and deprotonated forms of the molecules. In this case, we also examined the reversibility of the association/dissociation process of OH$^-$ for MPBA molecules adsorbed on the AgNPs by probing the behaviours of the characteristic bands at 1574 and 1586 cm^{-1} in the media with different pH values, as shown in a series of SERS spectra in Figure 4. The SERS measurements were carried out in a step-by-step fashion in highly acidic and basic solutions. That is, the substrate was firstly immersed in pH 1.0 buffer solution for 10s. Then the substrate was removed and immersed in a pH 13 buffer solution for 10s. After that, the substrate was transferred back to pH 1.0 buffer solution and immersed for 10s. The SERS spectra were recorded in each
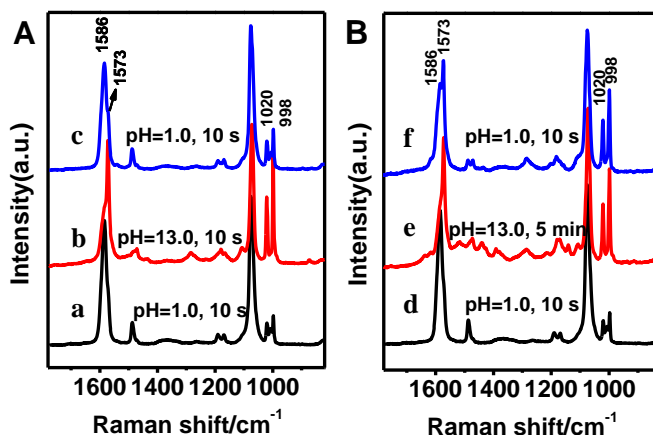
step. Comparing the SERS spectra obtained in acidic solution and basic solution in Figure 4A (Spectrum a and b), it can be seen that MPBA can be quickly converted to OH$^-$-associated form in a pH 13 solution, as indicated by the rapid intensity changes of the bands at 1586 and 1573 cm^{-1}. As the substrate was immersed in a pH 1.0 solution again, the most of the intensity of the 1587 cm^{-1} band can be recovered in 10 s (Figure 4A, Spectrum c), indicating that the dissociation process is also a fast process. However, to certain extent, residual intensity of the 1573 cm^{-1} band, along with the intensity loss of the 1587 cm^{-1} band, means that a fraction of the MPBA molecules in OH$^-$-associated form were not converted to MPBA at pH 1.0. Moreover, prolonging immersing time of the substrate in a pH 13 solution seems to result in more extent of MPBA molecules remaining in the OH$^-$-associated form (Figure 4B). For example, after the substrate was immersed in a pH 13 solution for 5 min, the intensity of the band at 1573 cm^{-1} became even higher than that of the band at 1586 cm^{-1} in the SERS spectrum measured in a pH 1.0 solution again (Figure 4B, Spectrum c). Because the dissociation process of the OH$^-$-associated MPBA is a fast process, the above results imply that the OH$^-$-associated MPBA molecules could be irreversibly converted to other species, which exhibit SERS spectral features similar to those of the OH$^-$-associated MPBA.

Self-condensation of MPBA on AgNPs

To verify this argument, the controlled experiments were performed for the SERS measurements. The substrate was immersed in a pH 13 solution for 2 h, ensuring that the possible irreversible conversion of the OH$^-$-associated MPBA reached the equilibrium state. Then, the substrate was transferred to a pH 1.0 solution and the SERS spectra were measured every 30 min. As shown in Figure 5, the SERS spectrum is initially dominated with the features similar to that of OH$^-$-associated MPBA, namely, the 1573 cm^{-1} band is more intense than the 1586 cm^{-1} band. The intensity of the 1573 cm^{-1} band, relative to that of the 1586 cm^{-1} band, slowly decreases

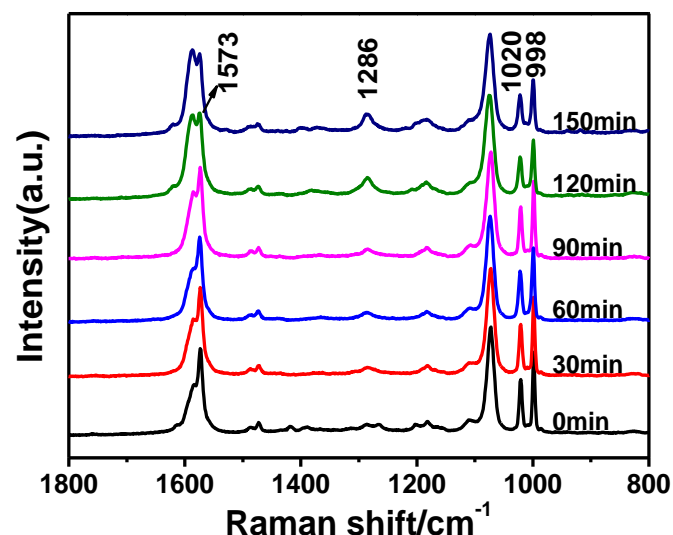Physical Chemistry Chemical Physics Accepted Manuscript

Figure 5 Time dependent variation of SERS spectra of MPBA in pH 1.0 buffer solution after the substrate was immersed in pH 13 solution for 2 h.

Figure 6 Schematic illustration of OH^--association and self-condensation of MPBA on AgNPs.

with time. After 120 min, the intensities of the two bands become comparable. The results suggest that the product of the possible irreversible conversion process of OH^--associated MPBA can also be converted to the original form of MPBA, although such a conversion is quite slow. Considering the similarity between the SERS spectra of the OH^--associated form of MPBA and the product of the irreversible conversion, we propose that the adsorbed MPBA molecules in OH^--associated form under the basic conditions may be irreversibly converted to a more stable new structure. In the present case, except for the OH^- anions, there is no other species that could react with OH^--associated MPBA. Therefore, the possible mechanism for the irreversible conversion could be stemmed from the self-condensation among the OH^--associated MPBA molecules. In fact, the formation of boronic acid anhydride (boroxine trimer) has been reported by Barriet et al.[33] in an X-ray photoelectron spectroscopy (XPS) study of the self-assembled monolayer of MPBA on gold under vacuum. They observed that a ratio of O_{1s}/B_{1s} was equal to 1.3, which was consistent with the substantial dehydration of boronic acid with the formation of anhydride. Similarly, in this case, the OH^--associated MPBA molecules adsorbed on AgNPs could also condense with each other to form anhydride, as schematically illustrated in Figure 6. The strong intensity of the orientation sensitive bands at 1021 and 988 cm^{-1} indicate that the benzene rings in the anhydride are nearly perpendicular with respect to the surface of AgNPs. Moreover, the slow SERS spectral change observed in Figure 4, indicates that hydrolysis of anhydride to original form of MPBA under acidic conditions is a quite slow process.

To further reveal the self-condensation process of OH^--associated MPBA on the surface of AgNPs, effect of the pH value of the solution on the condensation reaction was investigated by measuring the time dependent SERS behaviours of adsorbed MPBA in the solutions with different pH values. According to the mechanism illustrated in Figure 6,
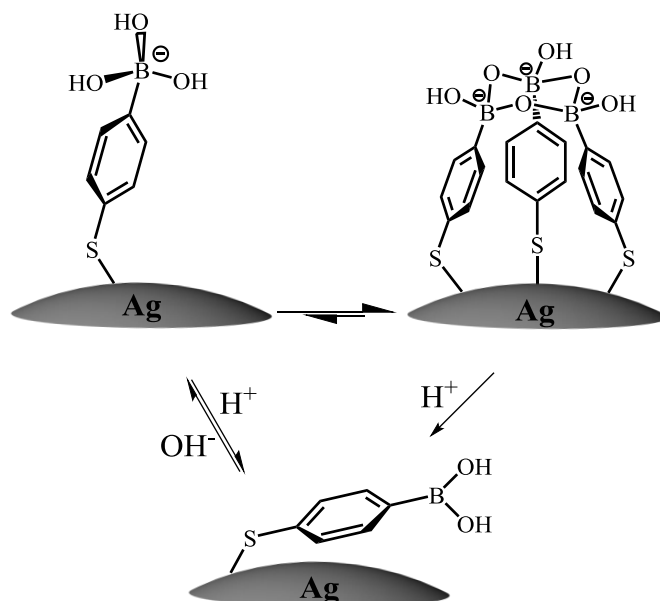
the condensation reaction involves combination of three adjacent OH^--associated MPBA through sharing oxygen between neighbouring B atoms. The kinetic of the reaction should be determined by the concentration of the surface adsorbed OH^--associated MPBA, which would depend on pH value of the media. Due to similarity between the SERS spectra of the OH^--associated form of MPBA and anhydride, it is difficult to assess the degree of anhydride formation from the SERS spectra that are directly measured in the solution with different pH values. Therefore, we conducted the experiments by measuring the SERS spectra in the pH 1.0 solution immediately after the substrate was immersed in the solutions with different pH values for different time. Because the pair of 1574/1586 cm^{-1} bands in the SERS spectra is pH sensitive bands, the relative intensities of these two bands could not be used to predict the extend of anhydride formed. We selected the band at 998 cm^{-1}, which is mainly sensitive to the orientation of the adsorbed molecules, as an indication of anhydride formed on the AgNPs. As mentioned above, anhydride adopts perpendicular orientation to the surface of AgNPs, under this circumstance, the relative intensity of the bands at 998 cm^{-1} should directly reflect the extent of anhydride formation during the immersing process because the conversion of the uncondensed OH^--associated MPBA to its original form is a fast process, and the hydrolysis of anhydride is quite slow. Figure 7 depicts variation of the relative intensity of the 998 cm^{-1} band with time. The detailed SERS spectra are shown in Fig. S4 in Supporting Information. In Figure 7, the intensity of the 998 cm^{-1} band is normalized to that of the 1073 cm^{-1} band, which is not affected by the condensation of the adsorbed MPBA. It can be seen that the existence of OH^- can facilitate the formation of anhydride, probably due to that the condensation between neighbouring OH^--associated MPBA molecules with tetrahedral B-moiety is more sterically favourable than that occurs between MPBA
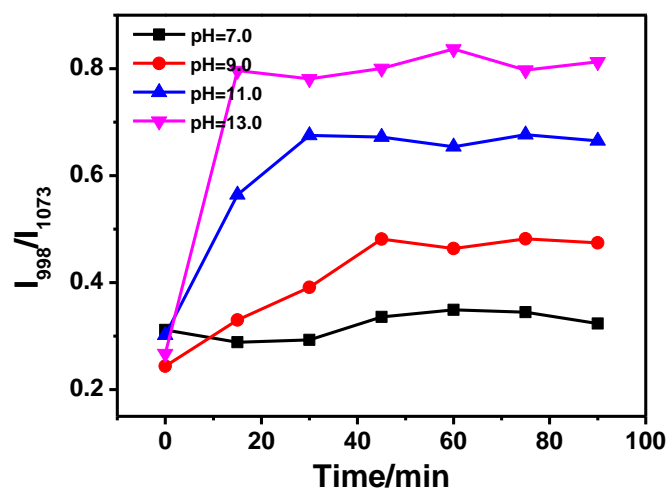
Figure 7 Variation of I_{998}/I_{1073} in the SERS spectra with time

Figure 8 SERS spectra of MPBA on AgNPs with and without D-glucose binding in (A) pH 7.0 and (B) pH 1.0 buffer solutions.

molecules with B-trigonal planar moiety. It is worth noting that in pH 7.0 solution, very small amount of the adsorbed MPBA could be converted to anhydride, although near half of the MPBA molecules are converted to the OH$^-$-associated MPBA molecules, as revealed by the comparable intensities of the characteristic bands at 1586 and 1573 cm^{-1} in Figure 2. As the pH value of the media became higher than 7, noticeable anhydrides are formed on the AgNPs. In fact, the amount of formed anhydride strongly depends on the pH value of the media. As can be seen, an equilibrium state of condensation reaction of the adsorbed MPBA can be achieved in 45, 30 and 15 min in the pH 9.0, 11.0 and 13.0 solutions, respectively, indicated by appearance of stabilized I_{998}/I_{1073} ratio. The I_{998}/I_{1073} ratio at equilibrium state also increases correspondingly from about 0.3 to 0.8. Therefore, it seems that the media with high pH value, ensuring enough surface density of the OH$^-$-associated MPBA molecules, is essential for the occurrence of the condensation reaction of the adsorbed OH$^-$-associated MPBA molecules. On the other hand, the formation of strong B-O-B bonds would lead to the hydrolysis of the anhydride energetic unfavorable, because this process involves breaking of series B-O-B bonds in the anhydride structure. This could be the main factor responsible for the very slow SERS spectral change in Figure 5 as the substrate obtained in the media with high pH value was transferred to a pH 1.0 buffer solution.

Binding of D-glucose to MPBA on AgNPs

To gain an insight into the interaction of sugars with the immobilized boronic acid, the SERS spectra of MPBA adsorbed on AgNPs were measured in the presence or absence of D-glucose, the results are shown in Figure 8; As aforementioned, self-condensation of the adsorbed boronic acids could occur to produce surface anhydride in the media with high pH values, which may hinder the binding of D-glucose to the surface through the esterification between D-glucose and B-moiety of MPBA. To illuminate the formation of anhydride, the SERS detection were conducted in a pH 7.0 buffer solution, in which MPBA could scarcely be conversed to anhydride. At the first
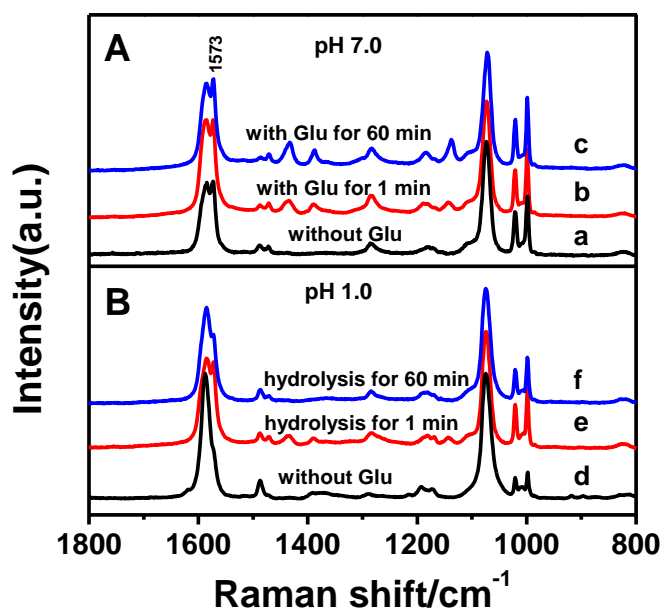
glance, the SERS spectrum (Figure 8b) obtained in the presence of 2 mM D-glucose is quite similar to that without glucose (Figure 8a). Extending the reaction time to 60 min only resulted in negligible spectral change (Figure 8c). Nonetheless, the binding of D-glucose can be confirmed by comparing the SERS spectra obtained as the substrates were transferred into a pH 1.0 solution (Figure 8d-f). In the absence of D-glucose, the OH$^-$-associated MPBA quickly converts to MPBA, as indicated by the intensity changes for the characteristic bands at 1586 and 1573 cm^{-1} (Figure 8d). In contrast, in the presence of D-glucose, negligible spectral change was observed (Figure 8e). The noticeable intensity changes for the bands at 1586 and 1573 cm-1 was obtained only after hydrolysis in pH 1.0 solution for 60 min (Figure 8f). Such spectral behaviours could be interpreted by the mechanism of the esterification of D-glucose with boronate. It has been well documented that D-glucose with *cis*-diol in furanose anomers preferentially interacts with OH$^-$-associated form of B-moieties with tetrahedral configuration.[34] In fact, Springsteen et al. [35] demonstrated that the ratio of equilibrium constants for the esterification of D-glucose with trigonal and tetrahedral B-moieties of phenylboronic acid, which possesses a B-moiety structure similar to that of MPBA, is about 100. Therefore, in the present case, the dominant esterification reaction should occur between the D-glucose and OH$^-$-associated MPBA molecules with tetrahedral B-moieties to form D-glucose-MPBA species. The binding of D-glucose to the immobilized OH$^-$-associated MPBA molecules would not largely disturb the configuration of the B-moiety, as a result, the SERS spectral features relevant to the benzene ring for D-glucose-MPBA would be quite similar to those for OH$^-$-associated MPBA. Furthermore, the fact that only a slight spectral change was observed upon extending the immersion time (Figure 8c) may imply that the immobilized OH$^-$-associated MPBA has high affinity for D-glucose, and equilibrium state of esterification
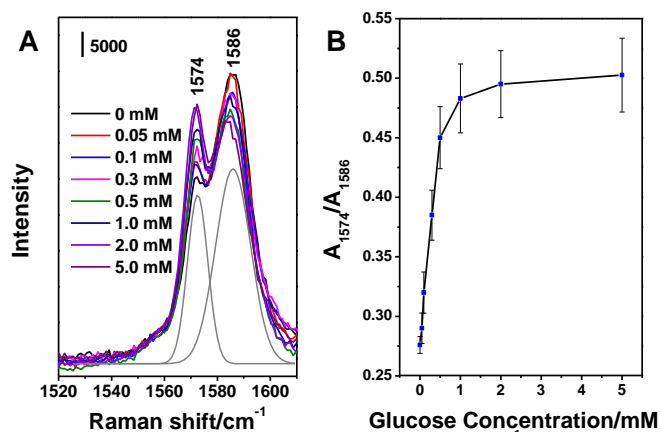
Figure 9 (A) Variation of the 1574 and 1586 cm^{-1} bands with D-glucose concentration. (B) Plot of A_{1574}/A_{1586} against D-glucose concentration.

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can be established in a short time. Unlike to the hydrolysis of OH^{-}-associated MPBA, which is a fast process, the dissociation of D-glucose-MPBA seems to be quite slow. After hydrolysis for 60 min, only small fraction of D-glucose-MPBA molecules is dissociated to MPBA, as revealed by the small change in the relative intensity of the bands at 1586 and 1573 cm^{-1} in Figure 8c and f. This spectral behaviour is similar to that of anhydride, indicating that a strong bond is formed between D-glucose and OH^{-}-associated MPBA, which was also confirmed by XPS data (Fig. S5, Supporting Information). On the other hand, the similarity of the SERS spectra of D-glucose-MPBA and OH^{-}-associated MPBA, implies that to employ the SERS technique for direct quantitative detection of D-glucose may be problematic. In particular, the band at 1574 cm^{-1} contains contribution from both OH^{-}-associated MPBA and D-glucose-MPBA species. However, the difference in the kinetics of the hydrolysis reactions of OH^{-}-associated MPBA and D-glucose-MPBA in acidic media (Figure 8) makes the SERS detection still possible. That is, the binding of D-glucose to MPBA is distinguishable in the SERS spectra obtained in pH 1.0 solution. In fact, the D-glucose-MPBA formed is quite stable, as revealed by the XPS characterization (Fig. S5), further ensuring the reliability of the SERS measurements. Based on above consideration, quantitative detection of D-glucose was attempted by monitoring the variation of the intensity ratio of the 1573 and 1586 cm^{-1} bands in the SERS spectra obtained in pH 1.0 media upon D-glucose binding, the results are shown in Figure 9A. In the physiologically relevant concentration range, the intensity of the band at 1573 cm^{-1} increases with increasing of the D-glucose concentration, whereas the band at 1586 cm^{-1} correspondingly decreases. The detection curve of D-glucose is shown in Figure 9B, where the intensity ratio of the bands at 1574 and 1587 cm^{-1} (A_{1574}/A_{1586}) is plotted as a function of the D-glucose concentration. Nevertheless, it should be pointed out that the dynamic linear range of the SERS detection for D-glucose in the present study would be relatively narrow for practical applications, which might be associated with the high affinity of MPBA to D-glucose. The saturated state for D-glucose binding is reached at relatively low concentration, as can be seen from the curve in Figure 9B.

This situation could be improved by adjusting the acidity of the bronoic acid moiety through a proper structural design of the recognizing molecules.[14,15]

Conclusions

The SERS behaviours of MPBA have been investigated on the surface of self-assembled AgNPs. It is revealed that the orientation of MPBA on AgNPs strongly depends on the pH value of the media, changing from flat orientation in an acidic solution to perpendicular orientation in a basic solution, with respect to the surface of AgNPs. The association/dissociation process of OH^{-} to the B-moiety in MPBA is reversible. However, self-condensation could occur among the OH^{-}-associated MPBA to form surface-bonded anhydride, which may hinder the esterification between D-glucose and B-moiety of MPBA. On the other hand, the OH^{-}-associated MPBA, which is essential for binding of D-glucose, exhibits spectral interference to the direct SERS detection of D-glucose, due to the similarity of the SERS spectra between the two species. Nevertheless, under optimized conditions, SERS detection of D-glucose can still be achieved in the physiologically relevant concentration range by taking advantage of the difference in the kinetics of dissociation of the OH^{-}-associated MPBA and D-glucose associated MPBA species. To optimize the structure of the recognizing boronic acid is necessary to improve the dynamic linear range in the detection.

Acknowledgements

Notes and references

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