Adsorption Behavior of Curcumin onto Cetyltrimethylammonium Chloride Reverse Micelles Immobilized on a Glass Surface As Studied by Polarized Visible Attenuated Total Reflection Spectrometry with a Glass Slab Optical Waveguide

Takuya NISHIWAKI,* Kiichi SATO,* Kin-ichi TSUNODA,† Hiroaki HORIUCHI,* and Terufumi FUJIWARA**

* Department of Chemistry and Chemical Biology, Gunma University, Tenjin-cho, Kiryu 376-8515
** Department of Chemistry, Graduate School of Science, Hiroshima University, Kagamiyama, Higashi-Hiroshima 739-8526

† To whom correspondence should be addressed.

E-mail: tsunoda@gunma-u.ac.jp
Abstract

Polarized Visible attenuated total reflection spectrometry with a glass slab optical waveguide revealed that a hydrophobic dye, curcumin, adsorbed onto the cetyltrimethylammonium chloride (CTAC) reverse micelles immobilized on a glass surface in two forms, i.e., in an un-dissociated form, curcumin that adsorbed onto the reverse micelles was perpendicular to the surface plane, while in a dissociated form, that was parallel to the plane, implying that the former may be located in the CTAC monolayer while the latter may be located in the reverse micellar water phase immobilized on the plane.

Keywords: Polarized Visible attenuated total reflection spectrometry, slab optical waveguide, reverse micelle, cetyltrimethylammonium chloride, curcumin.
Introduction

Reverse micelles are nanometer-sized droplets of water surrounded by a surfactant monolayer which are dispersed in a non-polar organic solvent, where the polar head-groups of surfactant molecules are concentrated in the micellar water core, while the surfactant hydrophobic chains are directed into the bulk organic solvent. They provide unique reaction fields and have been applied to various fields, in particular, to analytical chemistry.\(^1\)\(^-\)\(^5\) Fujiwara’s group has intensively studied reverse micelles of cetyltrimethylammonium chloride (CTAC) and has successfully applied them to the media for various chemiluminescence methods.\(^5\)\(^-\)\(^8\) Moreover, they have tried the immobilization of the reverse micelles onto porous silica gels\(^9\) as well as silica surface, where the stable structure of silica surface/water phase/CTAC/non-polar organic solvent was supposed to be formed; it is assumed that the adsorption of the CTAC molecules may be caused by an electrostatic interaction between the positively charged CTAC head-groups and the silica surface covered with the water phase to form aggregates on the surface, and the CTAC hydrophobic hydrocarbon chains may orient away from the surface in the CTAC monolayer formed by the association of the surfactants. The immobilized reverse micelle onto the silica surface was successfully applied to the reaction medium for the synthesis of nylon-6,6 immobilized on the silica surface;\(^10\) in the earlier work, it was demonstrated that the nylon-6,6 nanoclusters formed lay perpendicular to the glass surface plane, while location and orientation of reactants in the immobilized reverse micelles was unclear. In spite of the importance of the reverse micelles used as the reaction media, however, information on the characterization of the immobilized reverse micelles has been limited because of lack of analytical methods applicable to them.

Visible attenuated total reflection (ATR) spectrometry with a slab optical waveguide (SOWG) is a highly sensitive, especially surface-sensitive method based on the absorption of
evanescent wave at the multiple total internal reflection process; it can be even two to four orders of magnitude more sensitive than conventional absorption spectrometry.\textsuperscript{11, 12} Moreover, it is applicable to molecules that are not fluorophores, and information on the orientation of adsorbed molecules can be obtained with polarization (linear dichroism) measurements.\textsuperscript{13-15}

We have been using glass SOWGs for chemical sensing of traces of various compounds,\textsuperscript{16-18} electrospectrochemistry,\textsuperscript{19, 20} and the characterization of modified and unmodified glass surfaces using dye molecules with polarization measurements.\textsuperscript{14, 21, 22}

In this study, polarized Visible ATR spectrometry with a glass SOWG was applied to the study on the adsorption behavior of a hydrophobic dye, curcumin, onto the CTAC reverse micelle immobilized on a glass surface to characterize the immobilized CTAC reverse micelle.

\textbf{Experimental}

\textit{Reagents and chemicals}

Curcumin \textit{(1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione)}, and cetyltrimethylammonium chloride (hexadecyltrimethylammonium chloride, CTAC) were from Wako Pure Chemical. Cyclohexane, 1-hexanol and other ordinary chemicals were also from Wako Pure Chemical. These reagents were of analytical reagent grade and were used without further purification. Water was purified with a Milli-Q system.

\textit{Measurement system}

Figure 1a shows a schematic diagram of the slab optical waveguide (SOWG) used in this study; this is the same as that used in our previous papers.\textsuperscript{18-22} Briefly, cover glass [22 mm x 50 mm x 170 \( \mu \text{m} \) thickness, borosilicate glass \( (n_0 = 1.5255) \), Matsunami Glass Ind., Ltd.] was used
for the core, which was fixed to slide glass with a double-sided adhesive tape, where the light pass remained uncovered with the tape for the air to be the clad. A flow cell was constructed on the SOWG using a PTFE block and a silicone rubber spacer. Figure 1b shows a schematic diagram of the polarized Visible ATR spectrometric measurement system with the glass SOWG, which is basically the same as that in our previous paper; one multichannel charge-coupled device (CCD) detector with a modulation system of the source light using a light chopper was used in the previous system, while two detectors were used in this study to monitor the TE and TM mode signals separately at the same time. A xenon arc lamp (150 W, Hamamatsu Photonics) was used as the light source. The source light was collimated with an optical fiber collimator and was then coupled with the SOWG through a coupler prism (LaSF08, \( n_D = 1.8785 \)). The incident angle of the source light was set at 35 degrees. The guided light was out-coupled through another coupler prism. The out-coupled light was divided with a polarized beam splitter (S312-20-550, Surugaseiki, Co., Ltd., Japan) into the TE and TM mode beams respectively, which were detected by two multichannel CCD detectors (PMA-11, Hamamatsu Photonics). The signals were processed by personal computers to obtain visible ATR spectra. When the detectors were changed to each other, the identical absorption signals were obtained, i.e., there was no substantial difference between the two detectors.

**Immobilization of reverse micelle onto the SOWG surface**

CTAC reverse micelles immobilized onto the SOWG surface were prepared based on the method of Fujiwara’s group. A well made of a PTFE block [10 mm (w) x 10 mm (l) x 10 mm (h)] was built on the SOWG surface (the cover glass), which was treated with Piranha solution prior to the modification. One mL of a mixed solution of 1-hexanol and cyclohexane [the molar fraction \( X_h \) of 1-hexanol was 0.1] containing 44 mmol dm\(^{-3}\) CTAC was placed in the well, then a small amount of 1 mol dm\(^{-3}\) NaOH aqueous solution was added to the mixed
solution in the well. After gently mixed with pipetting, it was left to stand for 10 min to form reverse micelles in the area of the SOWG surface, onto which immobilization of the reverse micelles was completed. Then, the solution was carefully removed from the well with a micro-pipet, and the SOWG was set for the measurements. The value of $R (= [\text{H}_2\text{O}]/[\text{surfactant}])$ was important to decide the stability of the reverse micelle: the conditions of $R = 2, 4, 8$ (1.6, 3.2, 6.4 mm$^3$ of 1 mol dm$^{-3}$ NaOH solution, respectively) were tested in the experiments: $R = 4$ was found to be the optimum. Thus, this condition was used throughout this experiment.

Polarization Measurements of curcumin with SOWG

One mL of a mixed solution of 1-hexanol and cyclohexane ($X_h$ of 1-hexanol = 0.1) was first sent to the flow cell at the flow rate of 10 cm$^3$ h$^{-1}$ using a syringe pump and the baseline spectra were obtained with the two detectors for both TE and TM modes, then, one mL of the mixed solution containing 50 $\mu$mol dm$^{-3}$ curcumin was also sent at the same flow rate to obtain the respective polarized spectra.

Quantum chemical calculation

To obtain the optimized molecular structure of curcumin, DFT calculations were carried out at the B3LYP/6-31+G(d,p). The transition dipole moments were estimated for the optimized structures by the TD-DFT method at the B3LYP/6-31+G(d,p). All calculations were performed with the Gaussian 09W.

Results and Discussion
The adsorption of curcumin onto the SOWG with and without the reverse micelle immobilization was tested using a usual Visible ATR measurement system with the SOWG, where the polarized beam splitter was removed and one detector was used. The strong light absorption of curcumin was observed using the SOWG with the reverse micelles, while almost no absorption was observed using that without the reverse micelles. Moreover, the SOWGs with and without the reverse micelle immobilization were observed using a fluorescence microscope, when curcumin was applied. Uniform fluorescent image due to curcumin was observed with the SOWG with the reverse micelles, while almost no fluorescent signal was observed with the SOWG without the reverse micelles. These results may show that the reverse micelles were almost uniformly adsorbed onto the SOWG surface by our preparation method and uptake of curcumin by the adsorbed reverse micelles was caused.

In polarization (linear dichroism) measurements, the electric field vector of TE mode light is parallel to the surface plane of the SOWG, while that of TM mode light is almost perpendicular to the plane. Thus, the light absorptions in TE and TM modes are due to the respective components of the transition moment vector of the adsorbed dye molecules. By comparing the ATR spectra in TE and TM modes, obtained is information on the orientation of the transition moment vector of the adsorbed dye molecules. For example, when ATR spectra in TE and TM modes are almost identical in shape to each other, the average orientation angle of the vector can be calculated.\textsuperscript{13, 14} Figure 2 shows the polarized ATR spectra of curcumin with the SOWG with the reverse micelle immobilization in TE and TM modes; the absorption spectra of TE and TM modes are considerably different from each other; $\lambda_{\text{max}}$ in TM mode was ca. 420 nm and that in TE mode was ca. 470 nm. To clarify the chemical species of curcumin, absorption spectra of curcumin in cyclohexane with various pH conditions were measured with a conventional spectrophotometer, where a small amount of aqueous solution of pH indicated in Fig. 3 was added to cyclohexane containing 10 $\mu$mol dm$^{-3}$ curcumin, vigorously shaken, and
then the organic phase was applied for the measurements. As shown in Fig. 3, curcumin in a
dissociated form in the organic phase (probably an ion-pair of dissociated curcumin and Na\(^+\))
causeda red shift of the absorption spectrum, i.e., from ca. 420 nm to ca. 470 nm in \(\lambda_{\text{max}}\). Fig.
3 was compared with the literatures.\(^{24,25}\) In the literatures, curcumin (H\(_3\)cur) is shown to have
three pK\(_a\)s (8.39, 9.88, 10.51, respectively),\(^{24}\) thus, i.e., four dissociation forms (H\(_2\)cur, H\(_3\)cur\(^-\),
H\(_{\text{cur}^2}\)\(^-\) and cur\(^3\)\(^-\)) as shown in Fig. 4. Considering the pK\(_a\) values and the spectra in the literatures,
\(^{24,25}\) the spectra of pH 4.4 and 5.8 in Fig. 3 were due to H\(_3\)cur; that of pH 9.7 might be due to the
mixture of H\(_2\)cur and H\(_{\text{cur}^2}\)\(^-\); that of pH 14 was due to cur\(^3\)\(^-\). Thus, comparing the spectrum of
TM mode in Fig. 2 with those of Fig. 3 and the literatures,\(^{24,25}\) curcumin in an un-dissociated
form (H\(_3\)cur) could mainly be observed in TM mode. In addition to the comparison of the
spectra, considering the experimental condition where 1 mol dm\(^{-3}\) NaOH aqueous solution was
used (see Experimental), that in the dissociated form (cur\(^3\)\(^-\)) could mainly be observed in TE
mode.

Moreover, the optimized molecular structures and the transition dipole moments of H\(_3\)cur and
cur\(^3\)\(^-\) obtained by quantum chemical calculation were also shown in Fig. 4 (although only the
result of the keto form for H\(_3\)cur was shown, the similar result was also obtained for the enol
form). The direction of transition dipole moment of those forms (shown with the arrows in Fig.
4) was almost the same as that of the long axis of the species as shown in Fig. 4. Thus, it could
be concluded that curcumin in an un-dissociated form was perpendicular to the surface plane,
while that in a dissociated form (cur\(^3\)\(^-\)) was parallel to the plane. The quantum chemical
calculation also predicted the red-shift in the spectrum of the dissociation from (cur\(^3\)\(^-\)) compared
with that of the un-dissociated form (H\(_3\)cur), which was also in accordance with the
experiments.

This conclusion may be explainable if it is assumed that upon curcumin uptake, i.e.,
adsorption of curcumin onto the immobilized CTAC reverse micelle, the non-ionic curcumin
may be located in the CTAC monolayer perpendicular to the surface plane, while the ionic curcumin may be located in the water phase, as the image picture is shown in Fig.5. The neutral curcumin molecule can be likely distributed from the organic solvent phase to the surfactant hydrophobic layer due to its hydrophobicity. On the other hand, penetration of the curcumin into the water phase may presumably bring about its dissociation and an enhancement in the distribution of curcumin in the dissociated form to the water phase is likely due to the presence of NaOH in the phase. It should be noted that the absorption spectrum of curcumin in 1 mol L\(^{-1}\) NaOH aqueous solution showed the larger red shift: its \(\lambda_{\text{max}}\) was ca. 550 nm. Thus, the curcumin in the dissociated form still existed near the CTAC monolayer as shown in Fig.5. Because the positively charged CTAC head-groups carrying three methyl groups possess a relatively hydrophobic behavior, it may be reasonable to consider that the negatively charged curcumin may be in contact directly with the charged hydrocarbon surface of the CTAC monolayer, near which hydrophobic as well as electrostatic interactions are significant.

The surface coverage of curcumin on the SOWG was roughly estimated using the relative sensitivities (S/cm) of the SOWG\(^{11,14,16}\) (95 for TM mode and 85 for TE mode, respectively: relative errors of these values were estimated as about 20 \%) and the estimated molar absorption co-efficients of \(\text{H}_3\text{cur}\) and \(\text{cur}^3\) [\(\varepsilon_{\text{max}} = 1.1 \times 10^5 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}\) (calculated from Fig. 3) and 4.2 x 10\(^4\) \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}\) (calculated based on the literature\(^{25}\)), respectively]. Obtained were ca. 0.3 molecules nm\(^{-2}\) for \(\text{H}_3\text{cur}\), and ca. 1.5 molecules nm\(^{-2}\) for \(\text{cur}^3\). Considering the molecular sizes of the species [1.9 nm x 0.7 nm x 0.2 nm for both \(\text{H}_3\text{cur}\) (keto form) and \(\text{cur}^3\)], \(\text{cur}^3\) might exist as almost monolayer, while \(\text{H}_3\text{cur}\) less than one tenth of monolayer. On the other hand, in the previous study, estimated was the surface coverage of CTAC on the silica surface, i.e., ca. 1.8 molecules nm\(^{-2}\). Thus, one molecule of curcumin might exist in about six molecules of CTAC in the CTAC monolayer.

In summary, polarized Visible ATR spectrometry with a glass SOWG revealed that
curcumin adsorbed onto the CTAC reverse micelle-immobilized glass surface in two forms as shown in Fig.4. This study shows that the CTAC reverse micelles immobilized on the glass surface may well-preserve the nature of the reverse micelles in a non-polar solvent and may provide useful systems for their characterization as well as for unique reaction media. Moreover, the present method was found to be useful for their characterization. Further studies using various dyes including fluorophores are now in progress.

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Figure Captions

Fig. 1 (a) Schematic diagram of a slab optical waveguide. (b) Schematic diagram of the measurement system. See text for detail.

Fig. 2 Polarized Visible ATR spectra of curcumin with the SOWG. See text for the experimental conditions.

Fig. 3 Visible absorption spectrum in cyclohexane with conventional spectrophotometer. A small amount of aqueous solution of pH indicated in this figure was added to cyclohexane containing 10 μmol dm⁻³ curcumin, vigorously shaken, and then the organic phase was applied for the measurements.

Fig. 4 Dissociation of curcumin (H₃cur) and the optimized molecular structures and the transition dipole moments of H₃cur and cur⁻ obtained by quantum chemical calculation. The values of pKₐs are from literature 24. See text in detail.

Fig. 5 Illustrative summary of the adsorption behavior of curcumin onto the reverse micelle immobilized on a SOWG.

*1 As the sensitivity of the SOWG method to curcumin in bulk solution is very low (its effective path length is about one hundredth of the conventional method), it is substantially insensitive to the SOWG method in this experimental condition.

*2 The sickness of the water phase was unknown, because it was difficult to control the amount of water in the experiment. Thus, the sickness of the water phase in this figure is just schematically drawn.
"3 The penetration depth of the evanescent wave should be estimated as ca. 50 nm. Thus, ray of light in this figure does not reflect the real situation, but is also just schematically drawn.
Fig. 1 (a) Schematic diagram of a slab optical waveguide. (b) Schematic diagram of the measurement system.
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Graphical Index

Non-polar organic solvent phase

CTAC monolayer

Water phase

Core (Cover glass)

Curcumin not detected with SOWG measurements
Curcumin insensitive to TM mode measurements
Curcumin insensitive to TE mode measurements

Ray of light