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Adsorption Behavior of a Water-soluble Porphyrin at the Glass-water Interface as Studied by Synchronous Total Internal Reflection Fluorescence Spectroscopy

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Abstract: Total internal reflection fluorescence spectroscopy (TIRF) and synchronous scanning technique were combined to study the adsorption behavior of the meso-tetrakis (4-sulfonatophenyl) porphyrin (TPPS) at the glass-water interface without any surfactant. The pH dependence of synchronous fluorescence signal at the interface was analyzed. Both unprotonated ($TPPS^{4-}$) and diprotonated (H_2TPPS^{2-}) forms of TPPS were observed at the interface. But the interface favored the adsorption of. The apparent estimated pK_{a2} value shifted from 5.00 in the bulk solution to 2.7 at the interface. STIRF provides a good technique to study multi-component systems at the interface.

Keywords: Total internal reflection fluorescence, synchronous fluorescence, water- soluble porphyrin, pH dependence, glass-water interface.

Interfacial analysis has attracted more and more attention due to its fundamental and biological importance. TIRF[1-3] spectroscopy has been proven to be a highly selective way to study the interfacial region. TIRF can be easily applied to *in situ* observation of an interface, such as liquid/liquid and solid/liquid interface. Synchronous fluorescence spectroscopy[4,5] is very useful for multi-component analysis. The combination of TIRF and synchronous scanning technique has been suggested in a previous paper[6]. In this paper, we use synchronous TIRF to investigate the adsorption behavior of meso-tetrakis (4-sulfonatophenyl) porphyrin (TPPS) at the glass-water interface. The adsorption of the two forms of TPPS could be obtained synchronously. Watarai *et al.*[7] has studied the adsorption of TPPS at the glass-solution interface by using total internal reflection absorption spectroscopy in the presence of surfactant. We could observe the adsorption of TPPS at the glass-water interface without any surfactant. TPPS is a kind of water-soluble porphyrin with special importance in biological science. The porphyrin ring system contains two pyrrole nitrogen atoms that are able to accept two protons. The porphyrin used to exist in anionic forms in aqueous solution such as diprotonated TPPS (H_2TPPS^{2-}), monoprotonated TPPS ($HTPPS^{3-}$) and unprotonated TPPS ($TPPS^{4-}$) depending on pH. However monoprotonated TPPS existed in very narrow pH range

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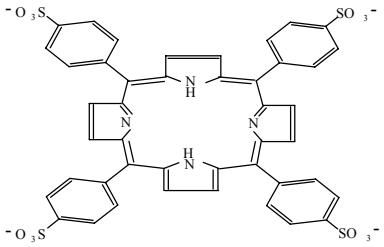
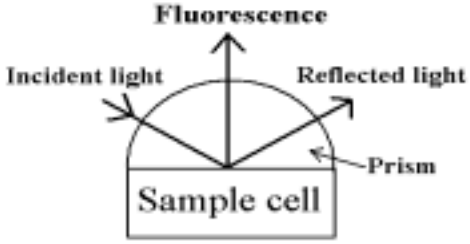
Molecular structure of TPPS



TIRF cell



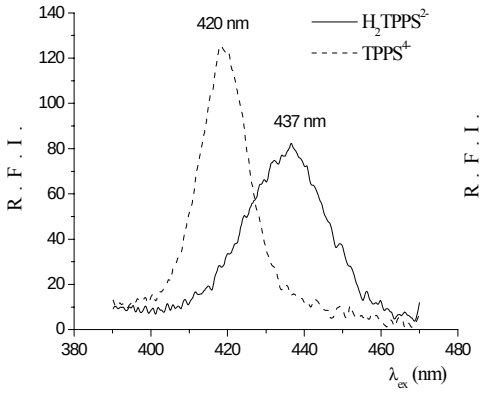
and could not be observed by fluorimetry.

All spectra were obtained on a laboratory-constructed computer (IBM)-controlled spectrofluorimeter similar to that in the reference[4], except for the TIRF cell. The cylindrical prism (BK7 glass) was attached to the sample cell, replacing the triangular prism[6] for better result. The buffer solution series were prepared by using 0.01mol/L potassium hydrogen phthalate ($KHC_8H_4O_4$) and NaOH or HCl. The pH values were measured by Delta320pH meter (Mettler Toledo). 1×10^{-6} mol/L TPPS was used throughout.

The pH dependence of TPPS in the bulk solution could be divided into three pH regions: below 4 (the main form was H_2TPPS^{2-}), between 4 and 6 (both H_2TPPS^{2-} and $TPPS^{4-}$ existed), and above 6 (the main form was $TPPS^{4-}$). The equilibrium was as follows: H_2TPPS^{2-}═$HTPPS^{3-}$+H^+(pK_{a1}), $HTPPS^{3-}$═$TPPS^{4-}$+H^+(pK_{a2}). And the pK_a values in the bulk solution obtained in this work was: pK_{a1}=4.76 and pK_{a2}=5.00 by synchronous fluorometric titration. Synchronous scanning technique allowed the simultaneous analysis of different components of TPPS. Combining TIRF spectroscopy, the technique was extended to characterize both H_2TPPS^{2-} and $TPPS^{4-}$ simultaneously at the interface. Only H_2TPPS^{2-} or $TPPS^{4-}$ could be observed in a single spectrum by normal TIRF spectroscopy with selective excitation wavelength. Based on the bulk measurement in the solution, the synchronous fluorescence intensity of $TPPS^{4-}$ was about three times weaker than that of H_2TPPS^{2-}. But the synchronous spectra of TPPS with pH 1.23 and pH 6.30 at the interface (**Figure 1**) showed that the interfacial synchronous fluorescence intensity of $TPPS^{4-}$ was much higher. The fluorescence quantum efficiency of either H_2TPPS^{2-} or $TPPS^{4-}$ adsorbed at the interface could be assumed to be the same as that in the bulk, and the adsorption is correlative positively with the synchronous fluorescence intensity. So the stronger interfacial signal of $TPPS^{4-}$ indicated that $TPPS^{4-}$ adsorption was much more than H_2TPPS^{2-} adsorption at the interface.

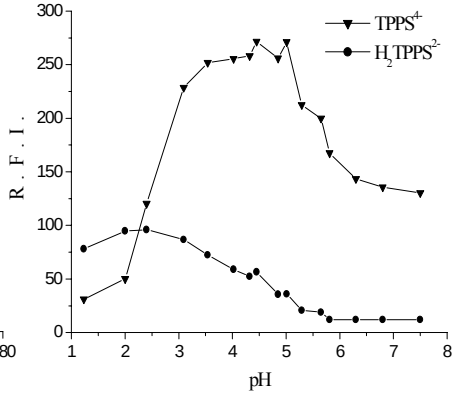
The pH dependence of TPPS at the interface was very different from that in the bulk solution (**Figure 2**). The intensity of $TPPS^{4-}$ increased from pH 1.23 to pH 3.5, and reached a constant value (pH 3.5-5.0), while H_2TPPS^{2-} had its leading role in the bulk solution. Consequently the pK_{a2} value apparently shifted to a much lower pH region at the interface (about 2.7), compared with that in the bulk solution. The continuous decrease of the adsorption of $TPPS^{4-}$ above pH 5 at the interface was observed, which might be caused by more negative environment induced on the glass

Figure 1 Synchronous TIRF spectra of TPPS at the interface (Δλ=230 nm).



pH=1.23 (solid line), pH=6.30 (dotted line).

Figure 2 pH dependence of TPPS at the interface from synchronous TIRF spectra



The intensities were measured at 420 nm for $TPPS^{4-}$, 437 nm for H_2TPPS^{2-}, respectively.

surface. With increasing pH an increase in concentration of the hydroxide ion induced more negative charges on the glass surface. As a result, $TPPS^{4-}$ tended to have an affinity with the bulk solution rather than with the electrically charged negative interface. H_2TPPS^{2-} showed slightly absorption at the glass-solution interface. The adsorption of H_2TPPS^{2-} decreased from pH 2 to pH 6. The pK_{a1} value did not change so much as the pK_{a2} value. It could be concluded that the interface favored the adsorption of $TPPS^{4-}$. Using synchronous TIRF technique, the apparent pK_a values of TPPS at the glass-water interface can be estimated.

Acknowledgments

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