Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 978–980

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

# Total internal reflection resonance light scattering at solid/liquid interfaces

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ARTICLE INFO

*Article history:*
Received 18 January 2008
Received in revised form 26 March 2008
Accepted 26 March 2008
Available online 8 April 2008

*Keywords:*
Resonance light scattering
Total internal reflection
Solid/liquid interface
Macromolecule

ABSTRACT

Total internal reflection (TIR) technique is an interface-specific tool and resonance light scattering (RLS) is of high sensitivity. The combination of both approaches is introduced into the solid/liquid interface for the first time. The behaviors of mixture of TPPS and BSA at the interface have been studied with total internal reflection resonance light scattering (TIR-RLS). The preliminary experimental results indicate that TIR-RLS is a good approach to study the interaction and distinguish the states of macromolecules at the solid/liquid interface.



## 1. Introduction

Bauer et al. [1] first studied the resonance light scattering (RLS) phenomena in molecular absorption system from basic theory to experiment. RLS is a special elastic scattering produced when the wavelength of Rayleigh scattering is located at or close to its molecular absorption band. The scattering intensity is enhanced several orders of magnitude in comparison with single Rayleigh scattering. RLS is a novel technique for the study of aggregates. Scattered light originates from the fluctuations of the solution refractive index. Enhanced light scattering intensity is observed at wavelengths of light absorption by aggregated species when strong coupling exists among their chromophores. Since Pasternack et al. [2,3] established the RLS technique to study the bio-macromolecules on an ordinary fluorescence spectrometer, the RLS technique has received a great deal of extensive and intensive studies. In recent years, RLS technique has been widely applied in biomedical analysis [4–6]. Many detergents and other amphipathic molecules like porphyrins can form aggregates [7] which scatter light strongly and resonantly. Borissevitch et al. [8] has studied the aggregation formation due to the interaction of TPPS and BSA in solution using RLS technique.

However, RLS method still suffers from limited selectivity and fails to distinguish light signals of analytes with those of their coexisting foreign substances. To solve these problems, liquid/liquid system has been introduced [9]. At interface, analytes can be well separated from its coexisting substances and be enriched to the interface, and thus better selectivity and sensitivity can be achieved. Total internal reflection (TIR) is the excitation of an evanescent field coupled with total internal reflection of light at interface [10,11]. Huang et al. has proposed the combination of RLS and TIR at liquid/liquid interface [9,12,13] for the determination of micro-amount of bio-macromolecules and medicines. However, the technique has not been used at the solid/liquid interface, which is also a highly selective interface for some substances. In this paper, TIR-RLS was first introduced into the solid/liquid interface. The interfacial behavior of protein at the glass/water interface was investigated.

## 2. Experimental

### 2.1. Reagents and chemicals

TPPS (99%, Porphyrin Products Inc.) exhibits a large molar absorption coefficient of ca. $5 \times 10^5\ M^{-1}\ cm^{-1}$. Bovine serum albumin (BSA) (Shanghai Huashun Biology Inc.) was dissolved in 0.1 M phosphate buffer solution (PBS) (pH 7.4, including 0.1 M NaCl) and stored at 4 °C. Buffer solutions were prepared with 0.01 M potassium hydrogen phthalate ($KHC_8H_4O_4$) (99.8%, Shanghai Reagent Inc.) and pH adjusted using 0.01 M NaOH or HCl. All solutions were prepared with deionized and double-distilled water. The pH values of the solutions were measured by a Delta320 pH meter (Mettler Toledo).

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doi:10.1016/j.jpba.2008.03.029

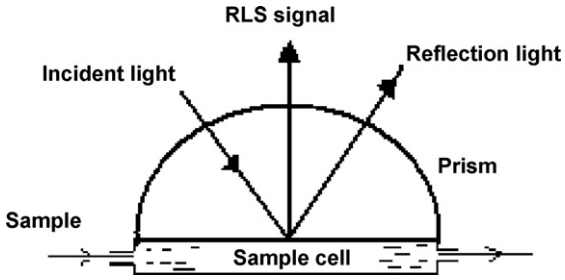


**Fig. 1.** Schematic drawing of the flow TIR-RLS cell.

### 2.2. Apparatus and procedure

All TIR-RLS spectra were obtained on a laboratory-constructed versatile spectrofluorimeter similar to the one previously described [14,15]. A flow glass cuvette with a cylindrical prism (BK7 glass) was used as the TIR-RLS sample cell (Fig. 1). When a beam of light irradiates onto the interface of the cell wall and the solution in the sample cell at an incidence angle greater than the critical angle, it undergoes total internal reflection. The evanescent wave induces the RLS signal of molecules at the interface region. Thus, RLS spectra could be obtained at the solid/liquid interface by TIR technique. In order to get the TIR-RLS spectra, we set the excitation wavelength equal to the emission wavelength and scan both wavelengths simultaneously (constant-wavelength difference between both wavelengths $\Delta\lambda = 0$).

## 3. Results and discussion

BSA could induce the aggregate of TPPS and appear the resonance light scattering of TPPS aggregates in the solution [8,16]. Fig. 2 shows the RLS spectra of the mixture of TPPS and BSA in the bulk and at the hydrophilic glass/water interface. We could observe RLS spectra both in the bulk and at the interface. On the other hand, our experimental results confirm no RLS spectra appearing for either pure *meso*-tetra(4-sulfonatophenyl)porphyrin (TPPS) or pure BSA both in the solution and at the glass/water interface in the experimental condition. This indicates that there are no aggregates for TPPS or BSA in separate solutions, where TPPS and BSA exist as monomers.

From Fig. 2, we can see that RLS spectra are different in bulk between pH 3.2 and pH 5.5. It is well known that the p$K_a$ values of TPPS are: p$K_{a1}$ = 4.8 and p$K_{a2}$ = 5.0. So TPPS exists as diprotonated TPPS ($H_2TPPS^{2-}$) at pH 3.2 and deprotonated TPPS ($TPPS^{4-}$) at pH 5.5. $H_2TPPS^{2-}$ exists as zwitterionic species carrying negative charges at the peripheral $SO_3^-$ group and positive charges at the center of the porphyrin ring. The main RLS spectral peaks are at 490 and 470 nm at pH 3.2 and pH 5.5, which are due to the J-aggregates of diprotonated and deprotonated TPPS, respectively. The RLS intensity is weak in the bulk solution, indicating only a few aggregates in it. The behaviors of H- and J-aggregates of TPPS in the aqueous solution have been largely reported [7,8,17–19]. BSA can induce TPPS to aggregate as J- or H-aggregates of TPPS. The schematic representation is shown in Fig. 3(A). Aggregates of TPPS are mainly controlled by electrostatic force and hydrophobic forces. The characteristic RLS spectra peaks of TPPS H-aggregates are at 420 nm and J-aggregates are at 490 or 470 nm (for diprotonated and deprotonated TPPS, respectively). No H-aggregates were observed in our experimental condition.

Interestingly, the main peaks of RLS spectra at the glass/water interface are at 470 nm both at pH 3.2 and pH 5.5 (Fig. 2, solid line), the same as those of the RLS spectra of J-aggregate of deprotonated TPPS in the bulk solution. The results indicates that TPPS exists mainly as J-aggregates of deprotonated TPPS at glass/water interface. It can also be observed that the intensity of RLS at the interface is very strong, which indicates that there are a large amount of aggregates at the glass/water interface. The same spectral peak position of TIR-RLS at pH 3.2 and pH 5.5 at 470 nm can be ascribed to a shift of apparent p$K_a$ at the solid/liquid interface. Yao and Li [20] also found that the apparent p$K_a$ of TPPS at glass/water surface shifts to lower pH with total internal reflection fluorescence spectroscopy. Thus, at the interface, TPPS exists as deprotonated form even at pH 3.2. The shift of apparent p$K_a$ should be due to the polarity change of microenvironment at the interface in comparison with in the bulk solution. De Luca et al. [4] found that the RLS spectral peak of TPPS J-aggregates was at 470 nm in dichloromethane. At the glass/water interface, BSA is adsorbed onto the interface and forms a hydrophobic microenvironment. The polarity of the glass/water interface is close to that in the organic solvents. Fig. 3(B) shows the schematic representation of J-aggregates of TPPS at the glass/water interface in the presence of BSA. Thus, TIR-RLS may provide a good way to study the interaction and distinguish the states of macromolecules at the solid/liquid interface.

Keeping the concentration of TPPS constant, there is a linear relationship between the TIR-RLS intensity at 470 nm and the BSA concentration in solution in the range of $1.5 \times 10^{-8}$ to $2.2 \times 10^{-7}$ M. The linear regression equation is $\Delta I = 60.9 + 35.5C$ with a correlation coefficient of 0.995 ($n = 5$), where $C$ is the concentration of protein

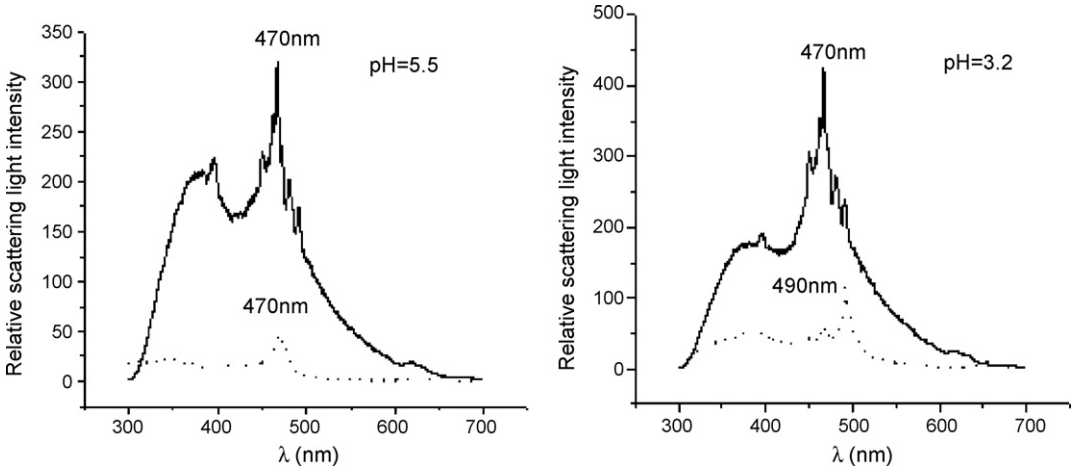


**Fig. 2.** RLS spectra of the mixture of BSA and TPPS in the solution (dotted line) and at the glass/water interface (solid line) at pH 5.5 and pH 3.2, [TPPS] = $5.28 \times 10^{-7}$ M, [BSA] = $8.82 \times 10^{-8}$ M.

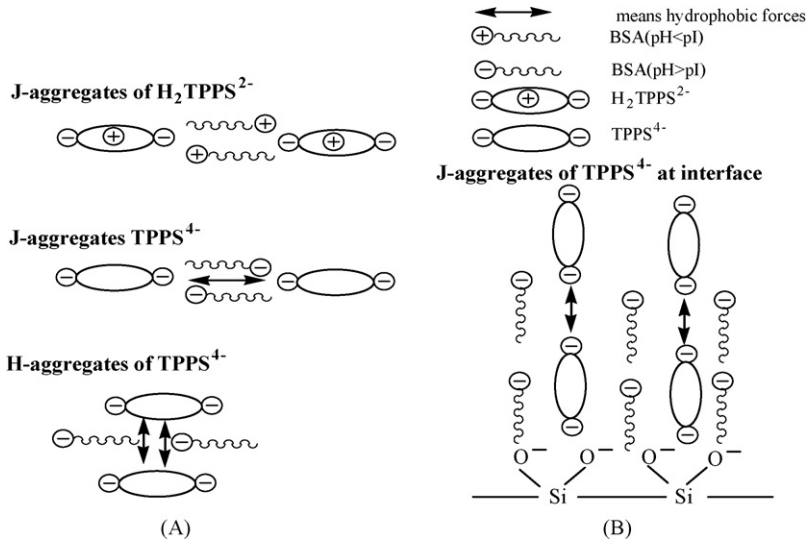


**Fig. 3.** Schematic representation of J-aggregates, H-aggregates of TPPS induced by BSA in the bulk (A) and J-aggregates of TPPS induced by BSA at the glass/water interface (B).

in $10^{-8}$ M. So TIR-RLS provides a new approach to determine BSA. Because it is an in situ technique, it may be used to study the adsorption kinetic of protein at the solid/liquid interface. Furthermore, TIR-RLS would be a useful tool to investigate the characteristics of macromoleculars at different solid/liquid interfaces.

## Acknowledgements

The financial supports from the National Natural Science Foundation of China (no. 20575055), National Basic Research Program of China (973 Program, 2007CB935600) and the Natural Science Foundation of Fujian Province (no. B0410002) are gratefully acknowledged.

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