

The pH Responsiveness of Fluorescein Loaded in Polysaccharide Composite Films

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–Totals– 10 pages, 11 figures

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Preparation of phosphate buffers (PB) and calculation of these ionic strength.

The preparation of each PB (10 mM, pH 2.0, 3.0, 5.8, 7.4, and 8.0) was conducted by previously reported procedures with several modifications.¹⁾ Initially, 0.20 M of NaH₂PO₄ aqueous solution (aq.) and Na₂HPO₄ aq. were prepared, respectively. The pH 5.8 of PB was prepared as follows; 4.0 mL of the Na₂HPO₄ aq. and 46.0 mL of the NaH₂PO₄ aq. were mixed and diluted in 100 mL of measuring flask. The pH 7.4 and 8.0 of PB were obtained by the same procedure, and 40.5 mL of the Na₂HPO₄ aq. and 9.50 mL of the NaH₂PO₄ aq. were used to obtain pH 7.4 of PB. To produce pH 8.0 of PB, 47.3 mL of the Na₂HPO₄ aq. and 2.7 mL of the NaH₂PO₄ aq. were used. On the other hand, pH 2.0 and 3.0 of PB were prepared by mixing 0.20 M of NaH₂PO₄ aq. (50 mL) and 6 M of HCl aq. (0.90 and 0.40 mL, respectively).

The ionic strength of each PB was calculated by equation (A).

$$I = \frac{1}{2} \sum_{i=1}^n C_i Z_i^2 \quad (\text{A})$$

Here, c_i is the molar concentration of the ion and z_i is the charge number of the ion. Each ionic strength of PB (pH 2.0, 3.0, 5.8, 7.4, 8.0) was 0.007, 0.010, 0.011, 0.022, and 0.027, respectively.

Calculation method for the loading amount of FL in CS/CHI films.

The amount of FL loaded on the CS/CHI films was calculated with Eq. S1 from the molar amount of initial FL amount in the CS solution (n_0), the amount of FL in the supernatant after PIC gel formation (supernatant 1) (n_1), and the amount of FL contained in the washing solution (supernatant 2) (n_2). The amount of each FL (n_1 and n_2) was obtained from measurement of the absorbance of FL-derived peak at 492 nm using a UV-vis spectrophotometer. The specific procedure is as follows. The volumes of the supernatant 1 and supernatant 2 were measured using graduated cylinders. Each supernatant was dispensed with a micropipette and was diluted by PB at pH 8.0 to 100 times (supernatant 1) and 10 times (supernatant 2). Then, measurement

of UV-Vis spectra of these solution was performed. From the results of the UV-vis measurement, the n_1 and n_2 are calculated using the following Eq. S2. The amount of loading FL was calculated from the difference from the number of moles of FL added initially (n_0) (Eq. S1).

$$\text{The amount of loading FL (\%)} = n_0 - n_1 - n_2 \quad (\text{S1})$$

$$\text{The amount of FL (n, mol)} = A \times V \times d / (1000 \times \varepsilon) \quad (\text{S2})$$

where A is the absorbance of FL solution, ε is molar extinction coefficient V is amount of the solution (mL), and d is dilution factor (10 or 100).

Reference

- 1) Z. Tamura, T. Morioka, M. Maeda, and A. Tsuji, Spectrophotometric estimation of the pK_a values, absorption spectra and structural formulas of molecular species in aqueous solutions of fluorescein and sulfonefluorescein. *Bunseki Kagaku*, 1993, **43**, 339–346.

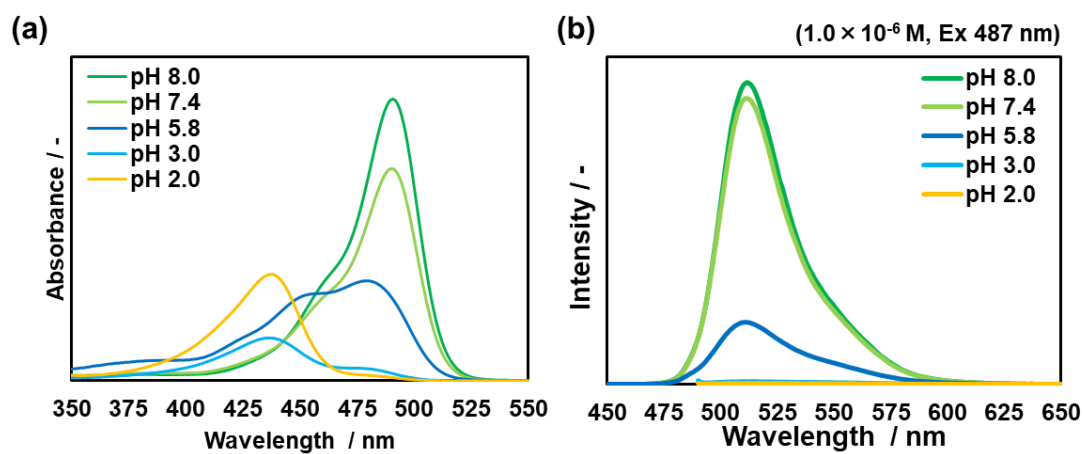


Figure S1. (a) UV-vis spectra and (b) fluorescence spectra of FL in different pH of buffer solutions.

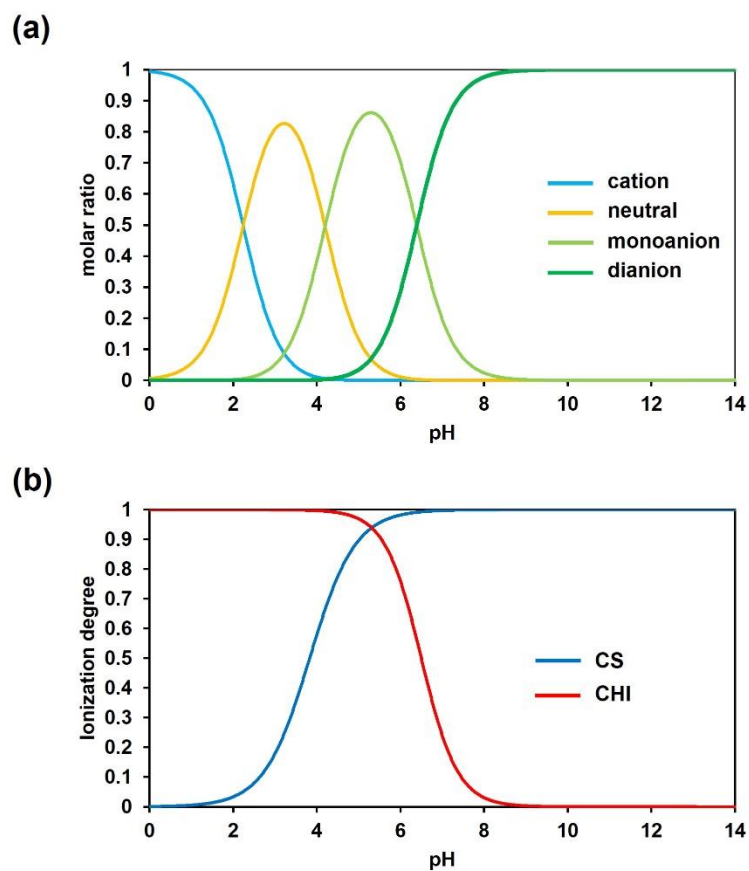


Figure S2. (a) Molar ratio of each ionic structure of FL; (b) ionization degree of CS and CHI.



Figure S3. Photographs of fluorescence of FL in buffer solutions. (From left to right, pH 2.0, pH 3.0, pH 5.8, pH 7.4, pH 8.0)

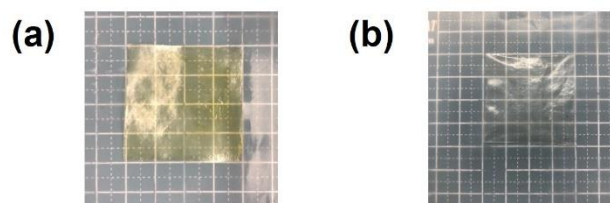


Figure S4. Photographs of CS/CHI films prepared by hot press techniques at (a) 120 °C and (b) 35 °C.

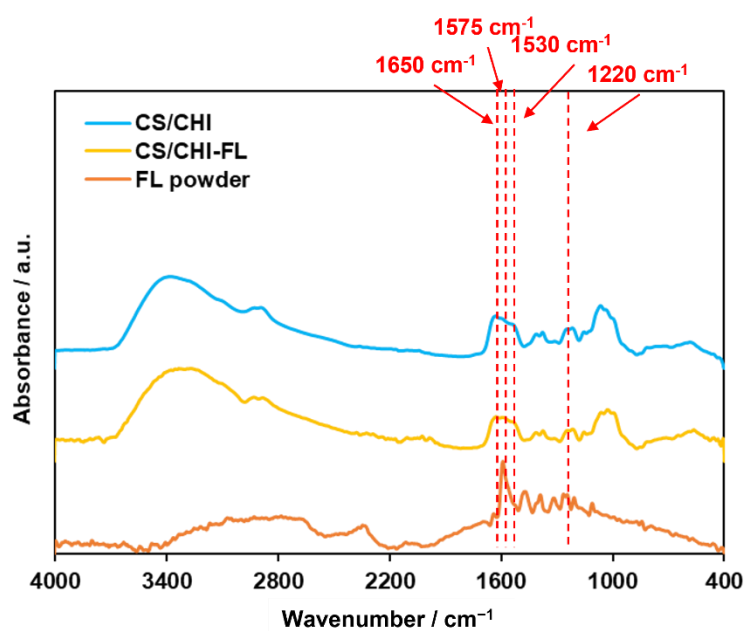


Figure S5. FT-IR spectra of FL powder, CS/CHI film, and CS/CHI-FL film.

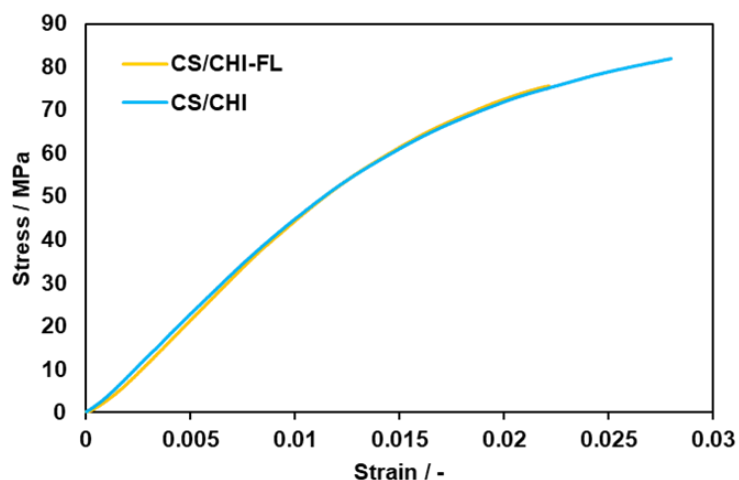


Figure S6. Stress-strain curves of CS/CHI film and CS/CHI-FL film.



Figure S7. Photographs of solubility tests for confirmation of FL solubility in PB with different pH. More than 100 mL of PB was necessary for the dissolution of 5 mg of FL at pH 2.0, 3.0, and 5.8. At pH 7.4 and 8.0, 9 mL and 5 mL of PB were needed for the complete dissolution of 5 mg of FL.

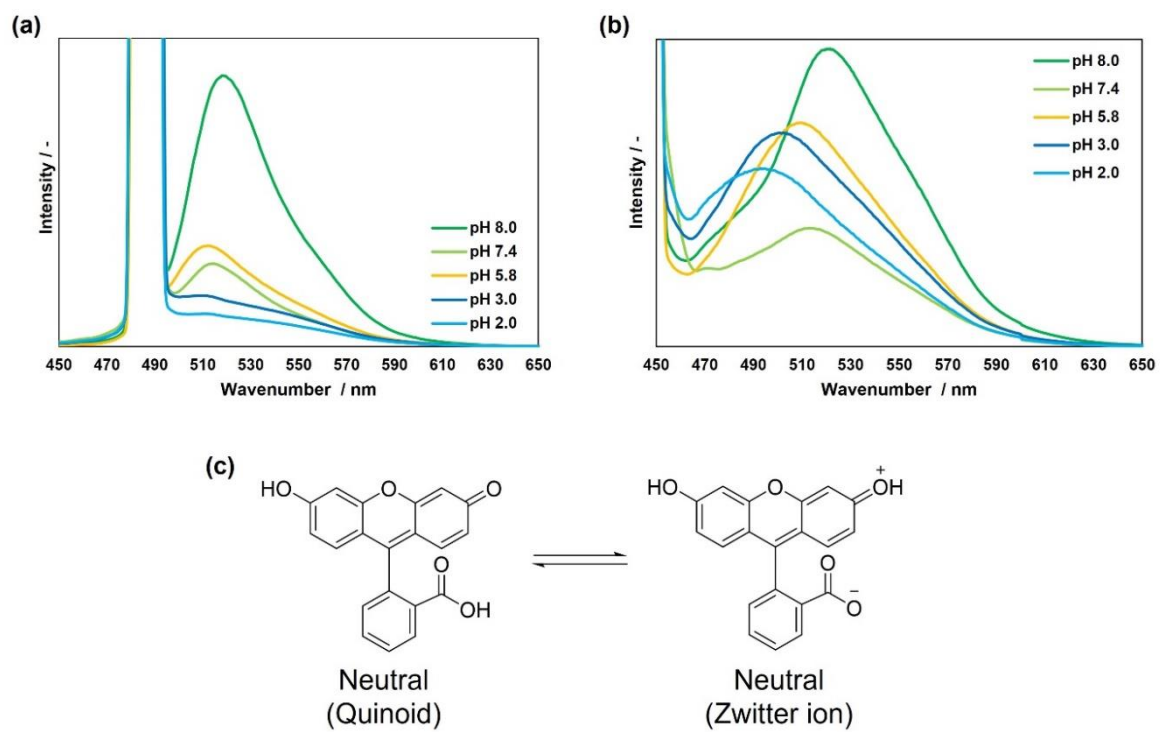


Figure S8. Fluorescence spectra of CS/CHI-FL films after FL release in each pH of PB with excitation wavelength of (a) 487 nm and (b) 445 nm; (c) isomerization of neutral FL.

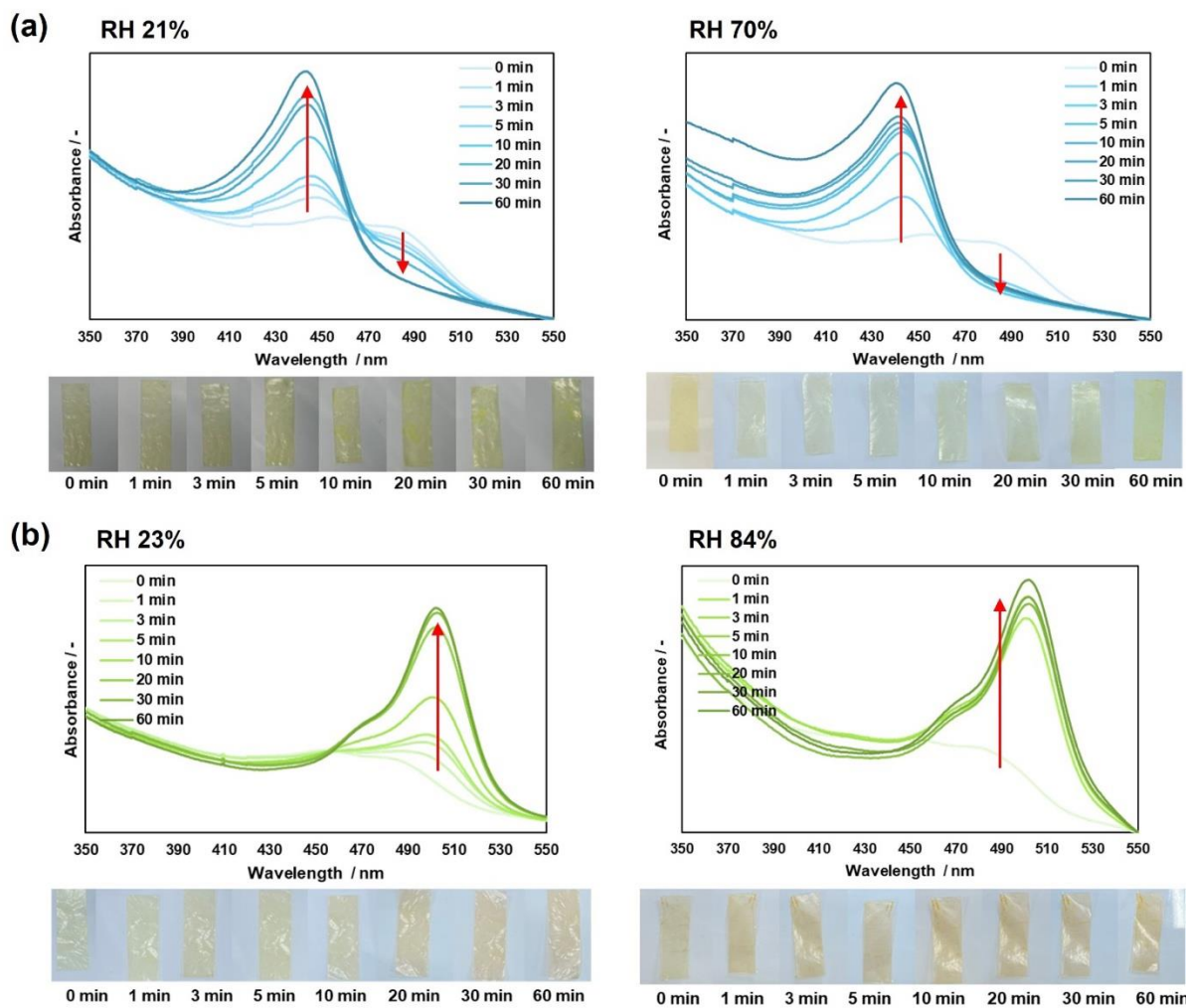


Figure S9. UV-Vis spectra and color change of the CS/CHI-FL films under natural light at low (left) and high (right) relative humidity (RH). (a) Exposed to HCl gas; (b) exposed to NH₃ gas.

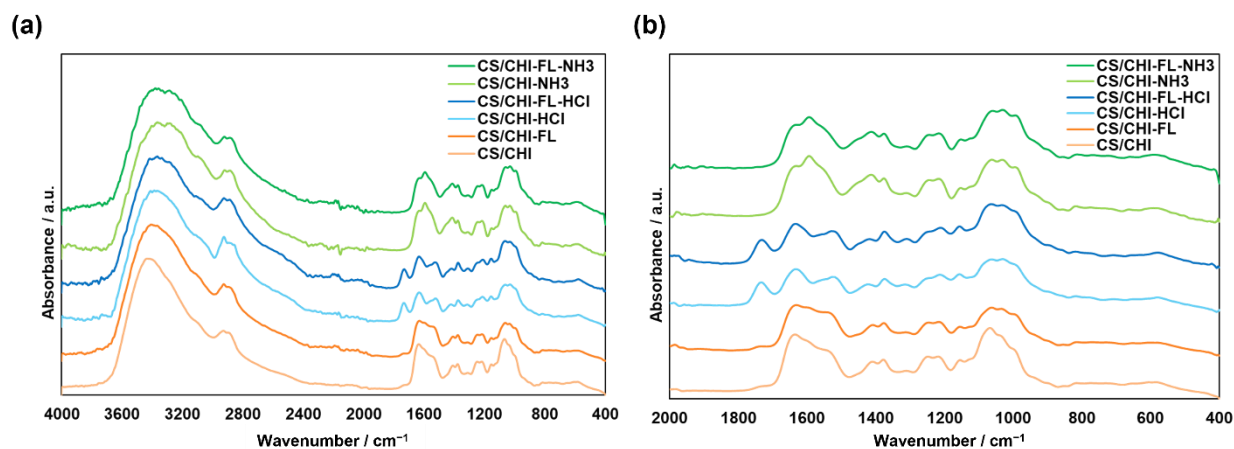


Figure S10. FT-IR spectra of CS/CHI films and CS/CHI-FL films after HCl and NH₃ exposure.

(a) 400–4000 cm⁻¹; (b) 400–2000 cm⁻¹.

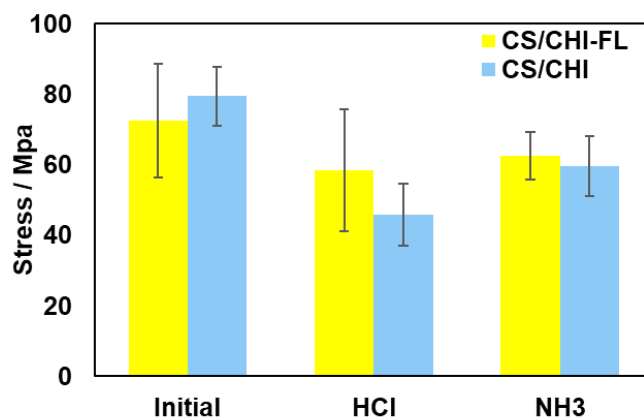


Figure S11. Mechanical strength of CS/CHI films and CS/CHI-FL films after HCl and NH₃ exposure.