

# Dyeing of Chitin Nanofibers With Reactive Dyes and Preparation of Their Sheets and Fiber Reinforced Materials

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## Research Article

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# Abstract

Blue, red and yellow chitin nanofibers were obtained using reactive dyes. Since the chitin nanofibers and the dye are covalently bonded, they are resistant to hot water and detergents. Since the dyed chitin nanofibers can be mixed at an arbitrary ratio, purple, orange, and green nanofibers corresponding to the mixed colors could be obtained. The dyed chitin nanofibers were evaluated by the reflection spectrum and the color space ( $L^*a^*b^*$ ). From the viewpoint of reaction yield and color properties, the appropriate ratio of the reactive dye to chitin was 5% at the maximum. The morphology of chitin nanofibers was maintained even after the introduction of the reactive dye. A resin reinforced with dyed chitin nanofibers was prepared. By combining with nanofibers, it is possible to give color and improve mechanical strength to resin while maintaining the transparency of the resin to some extent.

# Introduction

Chitin is a polysaccharide in which acetylglucosamine is linearly bound, and has a chemical structure similar to cellulose. It is contained in the exoskeleton of crabs, shrimp, insects, and in the cell walls of mushrooms and fungi. Chitin is known as a biomass with the second largest resources after cellulose. All natural chitin exists as nanofibers. Chitin nanofibers contained in crab and shrimp shells form a complex with a protein, and the complex spontaneously aggregates to form a hierarchical structure (Raabe et al. 2006). The gap is filled with calcium carbonate to give elasticity to the shell. By applying method for producing cellulose nanofibers, technique for producing chitin nanofibers has been reported (Ifuku et al. 2009). That is, chitin nanofibers can be obtained by isolating chitin from crab shells and mechanically disintegrating it with water. The resulting chitin nanofibers are similar in shape to cellulose nanofibers and are 10–20 nm wide. Compared with conventional chitin powder, it can be dispersed homogeneously in water, so it is easy to process, mold (Torres-Rendon et al. 2015), and modify chemically (Ifuku et al. 2014). It has become easier to evaluate biological functions *in vitro* and *in vivo* tests, and various biological functions for animals and plants have been clarified. For example, chitin nanofibers accelerate wound healing (Izumi et al. 2015), suppress inflammation (Izumi et al. 2016), and promote hair growth (Azuma et al. 2019). By taking chitin nanofibers, inflammation of the intestinal tract is suppressed (Azuma et al. 2012). Moreover, chitin nanofibers promote plant growth (Aklog et al. 2016) and induce disease resistance (Egusa et al. 2015). By utilizing these biological functions, chitin nanofibers are commercially available as the functional ingredient. Furthermore, since chitin nanofibers are composed of extended crystalline structure like cellulose nanofibers, they have excellent mechanical properties (Nair and Dufresne 2003). It has been reported that it is used as a reinforcing material to strengthen the material by taking advantage of such characteristics (Ifuku et al. 2011). Since arthropods such as crustaceans and insects biosynthesize chitin nanofibers to acquire a hard shell, their use as a reinforcing material reasonable. For example, chitin nanofibers can significantly improve the elastic modulus and breaking strength of a resin and suppress thermal expansion. Further, the nanofibers thinner than visible light are less likely to cause light scattering at the interface between the resin and the nanofibers due to the size effect, so that the transparency of the resin can be maintained.

Cellulose is one of the three major natural fibers. Cotton, hemp, and regenerated cellulose fibers such as rayon, are used on a large scale for mainly clothing. Many dyes for dyeing cellulosic fibers have been developed in order to improve fashionability as costumes. Among cellulosic dyes, reactive dyes are chemically bonded to cellulose molecules, so they are excellent in stability and fastness. Most of the reactive dyes are introduced through the hydroxyl group of cellulose *via* an ether bond or an ester bond. There are several reports on the dyeing of cellulose nanofibers. Uesaka *et al.* dyed the cellulose nanofibers produced by mechanical treatment, with a reactive dye, and prepare the composite material of the dyed cellulose nanofibers and the thermoplastic resin (Uesaka and Inouchi 2020). Khatri *et al.* dyed cellulose acetate produced by electrospinning, with reactive dyes (Khatri et al. 2014). Since chitin also has a hydroxyl group, cellulosic reactive dyes could be used to dye chitin. If chitin nanofibers can also be dyed with reactive dyes, it will promote the use of chitin nanofibers as reinforcing materials. For example, dyed chitin nanofibers can color resins that are difficult to dye directly, in addition to the reinforcing effect. However, as far as the authors know, there are no reports on the dyeing of chitin nanofibers. Therefore, in this study, we dyed chitin nanofibers with reactive dyes and evaluated their color properties. In addition, composites of the nanofiber and the resin were prepared and their color properties were evaluated.

## Experimental

### Materials

The chitin nanofiber aqueous dispersion derived from crab shell was purchased from Marine Nanofiber Co., Ltd. The reactive dye was purchased from Sumika Chemtex Co., Ltd. Based on the report by Uesaka *et al.*, Blue, yellow, and red bifunctional reactive dyes (Sumifix Supra Blue BRF 150% gran, Sumifix Supra Yellow 3RF 150% gran, Sumifix Supra Brilliant Red 150% gran) were used (Uesaka and Inouchi 2020). The liquid detergent (soaping liquid N-3) was purchased from Iwase Shoten. Other chemicals were purchased from Wako Chemicals and used as received.

### Dyeing for chitin nanofibers

The chitin nanofibers were dyed with a reactive dye according to Fig. 1. That is, blue, red, and yellow reactive dyes were added into chitin nanofiber aqueous dispersion (230 g) having a concentration of 1.0 wt% under stirring at room temperature. The weight of the dye was 0.1–40% (2.3–92 mg) of the dry weight of chitin. After 5 minutes, 1.84 g of sodium sulfate was added. After another 10 minutes, 7.36 g of additional sodium sulfate was added. Sodium sulfate increases the ionic strength in water, so that the reactive dye aggregates in the vicinity of the chitin nanofibers. After another 20 minutes, 0.96 g of sodium carbonate was added. After 5 minutes, the reaction mixture was heated to 60 °C. After 20 minutes, 0.345 g of sodium carbonate was added again. After another 10 minutes, 2.415 g of last sodium carbonate was added. Sodium carbonate makes the aqueous solution alkaline condition and activates the reactivity of the reactive dye. After 40 minutes, the reaction mixture was cooled to room temperature.

### Washing of dyed chitin nanofibers

The chitin nanofiber aqueous dispersion dyed in blue, red, and yellow was suction filtered with a membrane filter. The chitin nanofibers on the filter were collected, suspended in 130 mL of distilled water, and filtered again. The collected chitin nanofibers were suspended again in 130 mL of distilled water, neutralized with acetic acid, heated to 80 °C, and stirred for 20 minutes. Liquid detergent (0.46 g) was added to the suspension, and the mixture was stirred at 90 °C for 10 minutes. The suspension was cooled to room temperature and then filtered. Finally, the collected chitin nanofibers were suspended in 130 mL of distilled water and filtered. The collected chitin nanofibers were suspended again in 130 mL of distilled water and stored in refrigerator. A series of filtrates were also stored to determine the reaction yield of the reactive dye.

## **Preparation of dyed chitin nanofiber sheet**

An aqueous dispersion containing 0.2 g of dyed chitin nanofibers in dry weight was suction-filtered using a membrane filter. The wet chitin nanofiber sheet was collected and hot-pressed at 100 °C for 30 minutes to obtain a dry non-woven sheet.

## **Preparation of dyed chitin nanofiber sheet/acrylic resin composite sheet**

Bifunctional acrylic monomer (tricyclodecanedimethanol dimethacrylate) was used for complexation with dyed chitin nanofiber sheet. 2-Hydroxy-2-methylpropiophenone was added as a photopolymerization initiator in an amount of 2 %. A dyed chitin nanofiber sheet cut into 20 mm squares was immersed in the acrylic monomer for 2 days. A dyed chitin nanofiber sheet immersed in acrylic monomer was sandwiched between slide glasses, and the monomer was polymerized by irradiating both sides with ultraviolet rays at  $40 \text{ mWcm}^{-1}$  for 60 seconds using an ultraviolet curing device (SPOT CURE SP-7, Ushio Inc.). After polymerization, the composite sheet was peeled off from the slide glasses to obtain the dyed chitin nanofiber/resin composite sheet.

## **Reaction yield of reactive dye**

The reaction yields of the reactive dye with chitin nanofibers were determined from the absorbances of the four filtrates after washing. That is, the reaction yields were calculated indirectly from the amount of unreacted dye. A calibration curves were prepared by measuring the absorbances at 612 nm, 542 nm, and 418 nm, which are the absorption bands of the aqueous solutions of the blue, red, and yellow reactive dyes whose concentrations were adjusted in advance. An ultraviolet-visible spectrophotometer (UV-2600i, Shimadzu Corporation) was used to measure the absorbances.

## **Ultraviolet-visible spectrophotometer**

The reflection spectra of the chitin nanofiber sheet dyed in blue, red, and yellow were measured using an ultraviolet visible spectrophotometer (UV-2600i, Shimadzu Corporation) set with an integrating sphere (ISR-2600, Shimadzu Corporation). For color property test, the  $L^*a^*b^*$  color space defined by the International Commission on Illumination was calculated using color measurement software (COL-UVPC,

Shimadzu Corporation). The light transmittance spectra of the chitin nanofiber/resin composite sheet were also measured.

## Scanning electron microscope

The dyed chitin nanofiber sheets were coated with a 2 nm platinum layer with an ion sputter (JFC-1600 JEOL Ltd.). Then, the morphologies of nanofibers were observed with a field emission type scanning electron microscope (JSM-6700F, JEOL Ltd.).

## Results And Discussion

### Reaction of chitin nanofiber aqueous dispersions with reactive dye

Chitin nanofibers were dyed with blue, red and yellow reactive dyes. Reaction dyes were added at 1% by weight to chitin. All nanofibers were colored bright blue, red, and yellow (Fig. 2). These nanofibers were stably dispersed in water as well as unstained nanofibers. The reaction yields of the dyes to the chitin nanofibers were measured from the amount of the unreacted dyes collected from washing filtrate. Since the reaction yields of the blue, red, and yellow reactive dyes were 92.3%, 96.9%, and 94.6% respectively, these dyes reacted with chitin nanofibers almost quantitatively. The reactive dyes used in this study are bifunctional reactive dyes (Fig. 3). Chromophore and a sulfonylethyl sulfate sodium salt are bound to monochlorotriazine. Sulfonylethyl sulfate group ( $-\text{SO}_2\text{CH}_2\text{CH}_2\text{OSO}_3^-\text{Na}^+$ ) is converted to highly reactive vinyl sulfone group ( $-\text{SO}_2\text{CH}=\text{CH}_2$ ) under alkaline condition and reacts with the hydroxyl group of cellulose ( $-\text{SO}_2\text{CH}_2\text{CH}_2\text{-O-Cell}$ ). In addition, the monochlorotriazine group occurs a substitution reaction with the hydroxyl group of cellulose with the elimination of hydrochloric acid. The fact that the dye was hardly desorbed by washing with liquid detergent and hot water suggests that the reactive dye is covalently bonded to the hydroxyl group of chitin, as well as the reaction with cellulose. The dyed chitin nanofibers were mixed in an arbitrary ratio without coagulation. Dyed chitin nanofiber dispersions having 12 different colors were obtained (Fig. 2).

### Preparation of dyed chitin nanofiber sheets and its color properties

Blue, red and yellow chitin nanofiber sheets were obtained by filtration and heat pressing. They had bright colors (Fig. 4). In addition, blue, red, and yellow chitin nanofibers were mixed in the same ratio, and the nanofiber sheets were prepared in the same manner. The sheets exhibited a color corresponding to subtractive color mixture. That is, when blue and red, red and yellow, and yellow and blue dyed nanofibers were mixed, purple, orange, and green colored sheets were obtained, respectively (Fig. 4). Figure 5 shows the reflection spectra of the six-color dyed chitin nanofiber sheets. The blue, red, and yellow dyed chitin nanofiber sheets have absorption bands around 612 nm, 542 nm, and 418 nm, respectively. The reflected light that is not absorbed corresponds to blue, red, and yellow. In other words, the complementary colors

of the absorbed wavelength light correspond to blue, red, and yellow. The sheets exhibited in the mixed colors of purple, orange, and green had an absorption band derived from the original dyed nanofibers, respectively. Table 1 shows the color space  $L^*a^*b^*$  of the six types of dyed nanofiber sheets. Lightness value of the color is represented by  $L^*$ . The  $a^*$  axis is relative to the green–red opponent colors, with negative values toward green and positive values toward red. The  $b^*$  axis represents the blue–yellow opponents, with negative numbers toward blue and positive toward yellow. The lightness ( $L^*$ ) was highest in the yellow sheet ( $L^*=65.0$ ), followed by the orange ( $L^*=52.7$ ) and green ( $L^*=47.7$ ) sheets containing yellow-dyed nanofibers. In the red direction ( $a^*$ ) and green direction ( $-a^*$ ), the red ( $a^*=38.3$ ) and green ( $a^*=-8.8$ ) dyed nanofiber sheets were the highest, respectively. In the yellow direction ( $b^*$ ) and blue direction ( $-b^*$ ), the yellow ( $b^*=41.0$ ) and blue ( $b^*=-28.7$ ) dyed nanofiber sheets were the highest, respectively.

The scanning electron microscopic images of chitin nanofibers dyed blue, red, and yellow are shown in Fig. 6. Fibrous structure with a width of 10–20 nm could be observed in each case. They were similar to the original chitin nanofibers (Ifuku et al 2009). Thus, it was confirmed that the morphology of the chitin nanofibers was maintained even when they reacted with the dye.

**Table 1** Chromatic parameters for the chitin nanofiber sheets dyed blue, red, and yellow and sheets in which they are mixed at a ratio of 1: 1

	Yellow	Yellow + Red	Red	Red + Blue	Blue	Blue + Yellow
$L^*$	65.0	52.7	47.7	43.9	45.8	51.6
$a^*$	5.1	31.3	38.3	10.8	-8.7	-8.8
$b^*$	41.0	15.5	-3.1	-21.1	-28.7	3.3

## Color properties of chitin nanofiber sheets with different amounts of blue dye

The dyeing was performed by changing the weight ratio of the blue reactive dye to the chitin nanofibers (0.1, 1, 5, 10, 20, 40%). Table 2 shows the reaction yields of the reactive dyes when the ratio of the reactive dyes was 1, 5, 10, and 20%. When the ratio of the reactive dye was 10% or less, the reaction yield was more than 90%, but when the ratio was 20%, the reaction yield decreased to 80%. The dye reacts with the hydroxyl groups present on the surface of the chitin nanofibers. When it was 20% or more, the dye was in excess and could not react quantitatively. Chitin nanofiber sheets with different amounts of reactive dyes were prepared by dyeing, washing, suction filtration and heat pressing. The appearance and reflection spectrum of the sheet are shown in Fig. 7. When the ratio of the reactive dye was 0.1–5%, the

dyed chitin nanofiber sheet became darker blue depending on the ratio of the reactive dye. On the other hand, when the ratio of the reactive dye was 5–40%, the chitin nanofiber sheet became darker black as the ratio of the reactive dye increased. The blue reactive dye has an absorption band around 612 nm and exhibits blue color as its complementary color. The absorption increases depending on the ratio of the reactive dye, but when it is more than 5%, it almost reaches a plateau. On the other hand, the blue reactive dye absorbs visible light of other wavelengths depending on its ratio. And when the ratio is 40%, it absorbs most of the visible light. This is the reason why the chitin nanofiber sheet became dark black. Table 3 shows the chromatic parameters  $L^*a^*b^*$  of the dyed chitin nanofiber sheet. The brightness ( $L^*$ ) was highest when the dye ratio was 0.1% ( $L^*=62.3$ ), and decreased as the ratio of the reactive dye increased. The redness ( $a^*$ ) and greenness ( $-a^*$ ) values were small and fluctuated slightly in the range of -0.3 to -6.0 depending on the ratio of reactive dyes. The blueness value ( $-b^*$ ) was -10.9 when the ratio of reactive dye was 0.1%, and increased to -28.7 when the ratio was 1%. Also, at 5%, there was almost same (-28.1). On the other hand, when it was 5% or more, the absolute value of blueness ( $-b^*$ ) decreased depending on the ratio of the reactive dye. This is in good agreement with the results of the reflectance spectrum (Fig. 7). That is, when the reactive dye is 5% or more, the absorption around 612 nm derived from blue is almost leveled off. On the other hand, the dyed chitin nanofibers also absorb visible light of other wavelengths. From the above, it was clarified that the appropriate ratio of the blue reactive dye was 5% at the maximum.

**Table 2.** Ratio of blue reactive dye added to chitin nanofibers and its reaction yield

Ratio of blue reactive dye (%)	1	5	10	20
Reaction yield (%)	92	96	95	80

Table 3

Chromatic parameters for the chitin nanofiber sheets dyed blue with different ratio of dye.

Ratio of blue reactive dye (%)	0.1	1	5	10	20	40
$L^*$	62.3	45.8	32.1	27.8	25.0	23.8
$a^*$	-6.0	-8.7	-4.9	-3.1	-1.8	-0.3
$b^*$	-10.9	-28.7	-28.1	-24.5	-20.2	-12.5

## Preparation of dyed chitin nanofiber/resin composite

A chitin nanofiber sheet dyed with a 1.0% reactive dye was composited with a bifunctional acrylic resin. The refractive index of this resin is close to that of chitin nanofibers (Ifuku et al. 2011). The appearance is shown in Fig. 8. Blue, red and yellow chitin nanofiber/resin composite sheets were obtained. It has been reported that the elastic modulus and strength at break of the resin can be improved and the coefficient of thermal expansion can be reduced by chitin nanofibers. The dyed chitin nanofibers not only improved the mechanical properties of the resin, but also improved the design due to the color elements. However, the resulting composite sheet was opaque. This is due to the large absorption of dyed chitin nanofibers. Therefore, in order to reduce the absorption to make the composite transparent, the ratio of the reactive dye was reduced to 0.1%. The appearance and transmission spectra of dyed chitin nanofiber sheets and these resin composites are shown in Fig. 9. The dyed chitin nanofiber sheets were light blue, red, and yellow, and hardly transmitted visible light, but when combined with the resin, it transmitted visible light. For example, the linear light transmittances of the blue, red, and yellow composite sheets at the wavelength of 600 nm were 4%, 22%, and 6%, respectively. In conclusion, by using dyed chitin nanofibers as reinforcing fillers, it was possible to improve the mechanical properties of the acrylic resin and to provide color property and a certain degree of transparency at the same time.

## **Conclusion**

Chitin nanofibers were dyed with reactive dyes. Blue, red and yellow chitin nanofibers could be obtained. By mixing the nanofibers, the nanofibers having desired color were prepared. Dyeing with reactive dyes can prevent decolorization with hot water or detergent. The morphology of the nanofibers was maintained even after the reaction. The appropriate amount of dye used was clarified. Chitin nanofibers have excellent physical properties. By combining the dyed chitin nanofibers with the resin, it was possible to color the resin while maintaining the transparency of the resin to some extent. Chitin nanofibers are manufactured from crab shells and are biodegradable. In the future, it is expected that such environmentally friendly materials will be effectively utilized as reinforcing materials that can improve the design and mechanical properties of materials that are difficult to dye.

## **Declarations**

### **Ethical approval**

Not applicable

### **Ethical standards**

Not applicable

### **Conflicts of interest**

The authors declare that they have no conflict of interest.

## Human and Animal rights

This is not research involving Human participants and Animals.

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## Figures

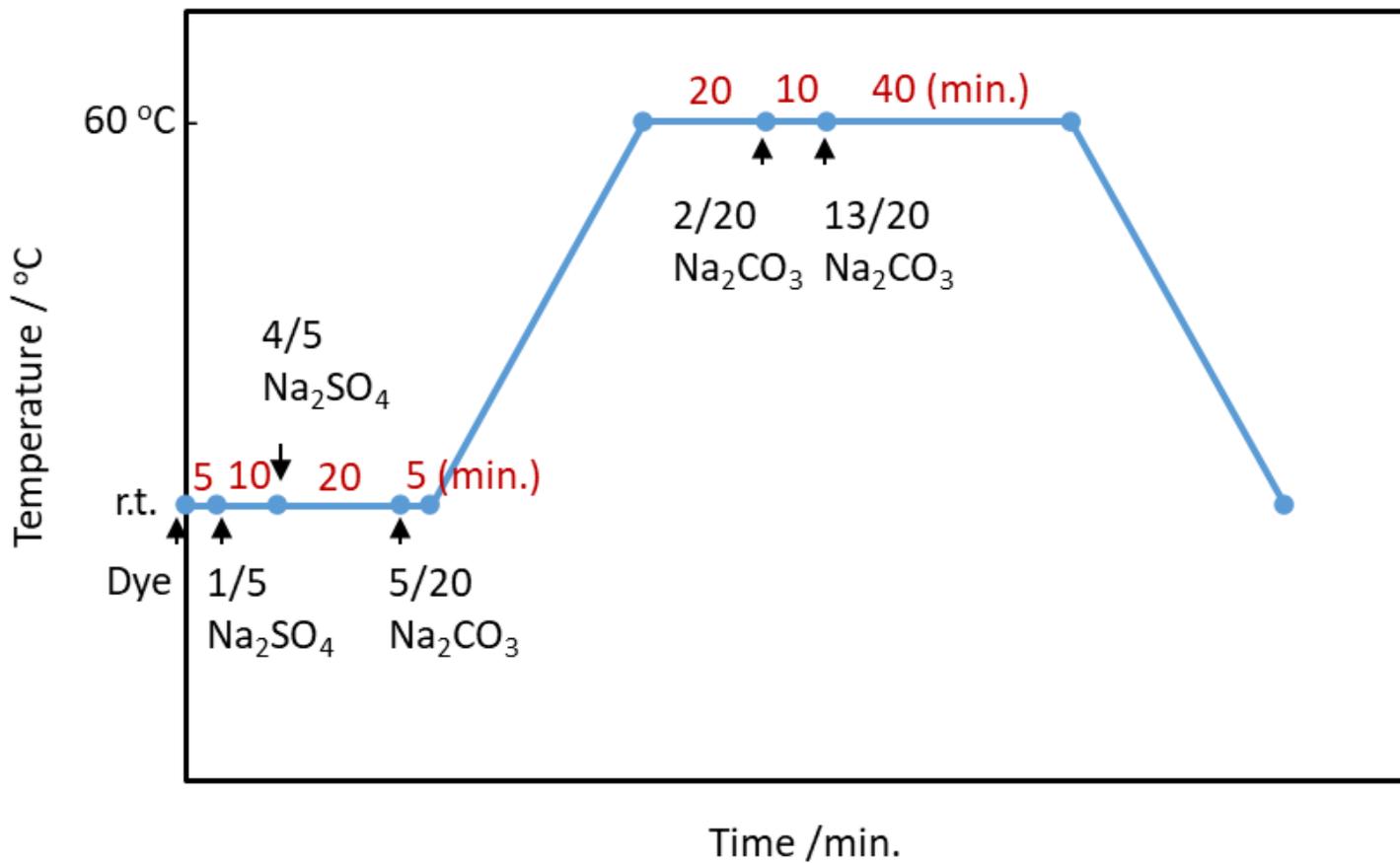
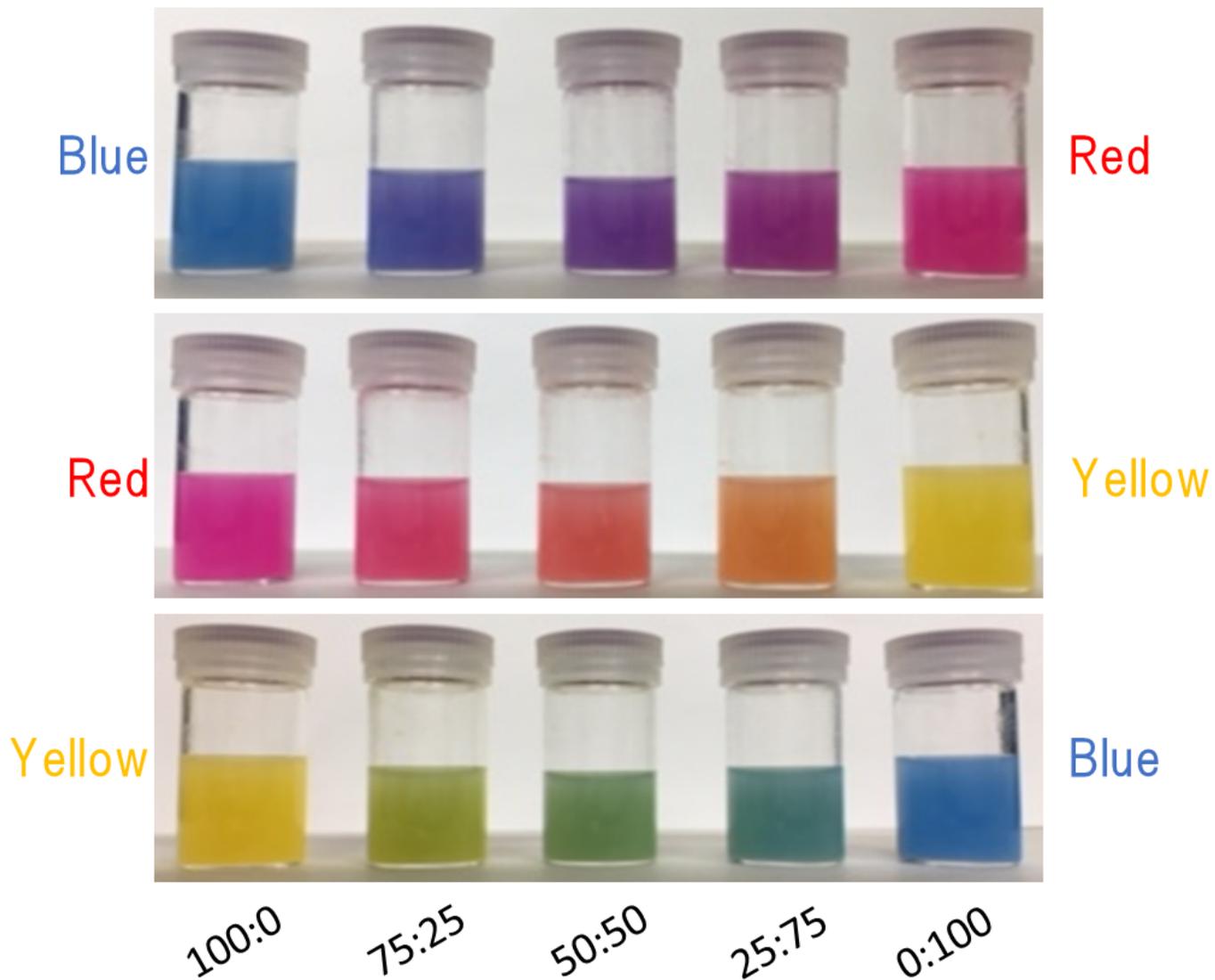


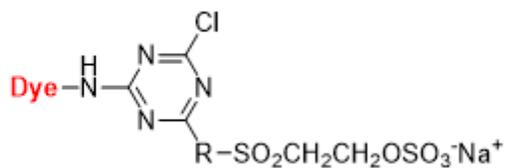
Figure 1

Time schedule for dyeing chitin nanofibers



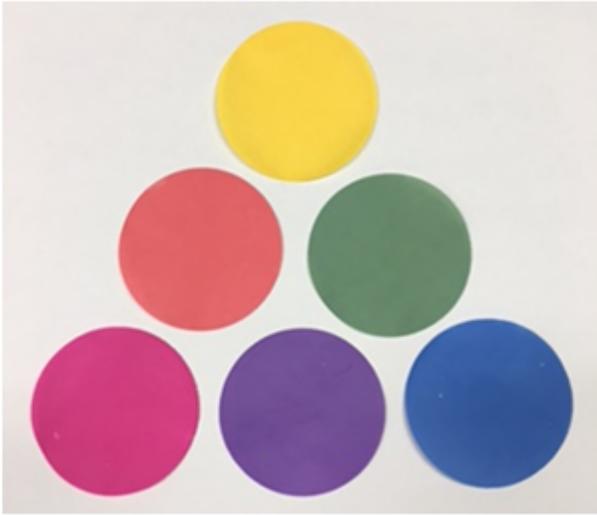
**Figure 2**

Appearance of chitin nanofiber aqueous dispersions dyed in blue, red, and yellow and their mixtures



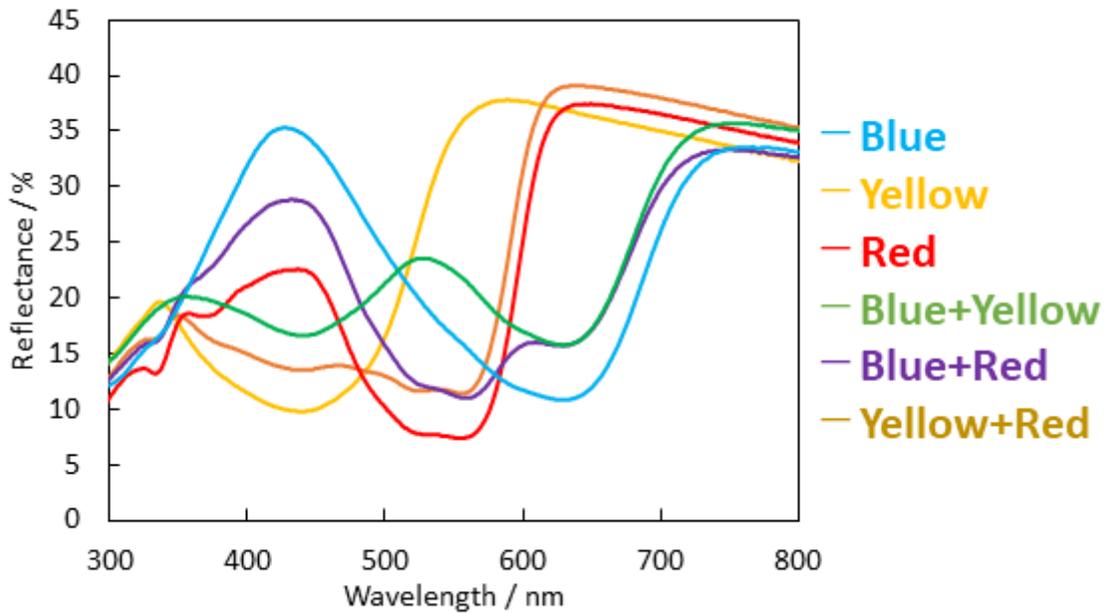
**Figure 3**

Basic chemical structure of reactive dye used in this study



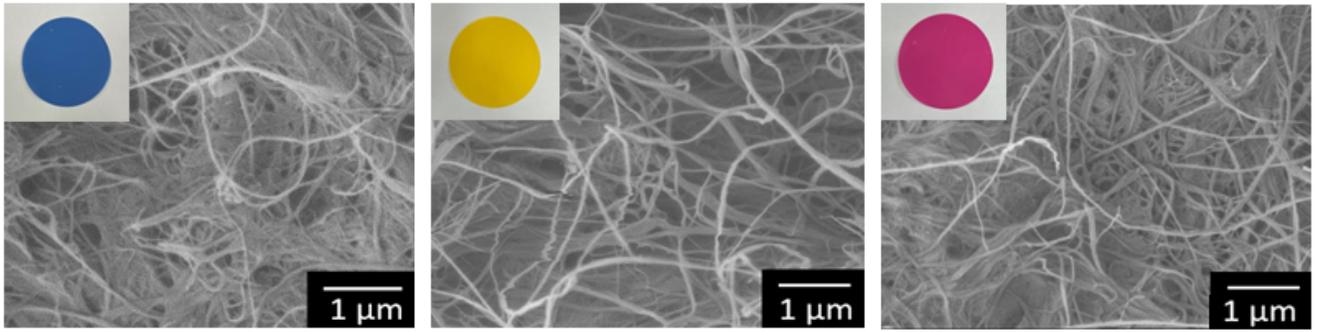
**Figure 4**

Appearance of chitin nanofiber sheets dyed blue, red, and yellow and sheets in which they are mixed at a ratio of 1:1



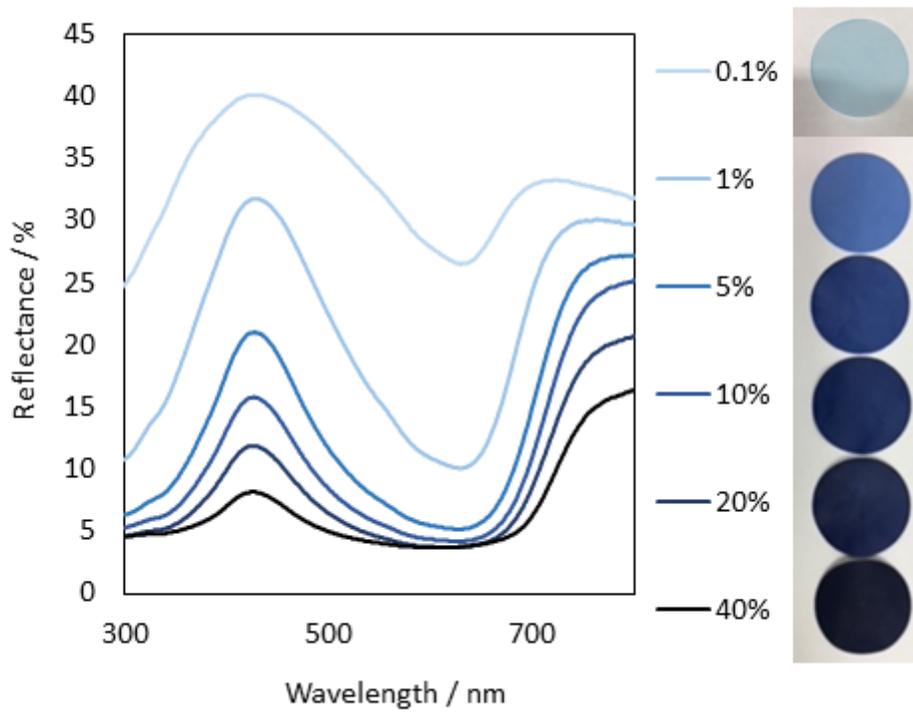
**Figure 5**

Reflection spectra of chitin nanofiber sheets dyed in blue, red, and yellow and sheets in which they are mixed at a ratio of 1:1



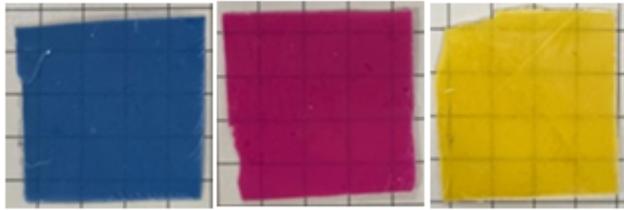
**Figure 6**

Scanning electron microscopic images of three dyed chitin nanofiber sheets



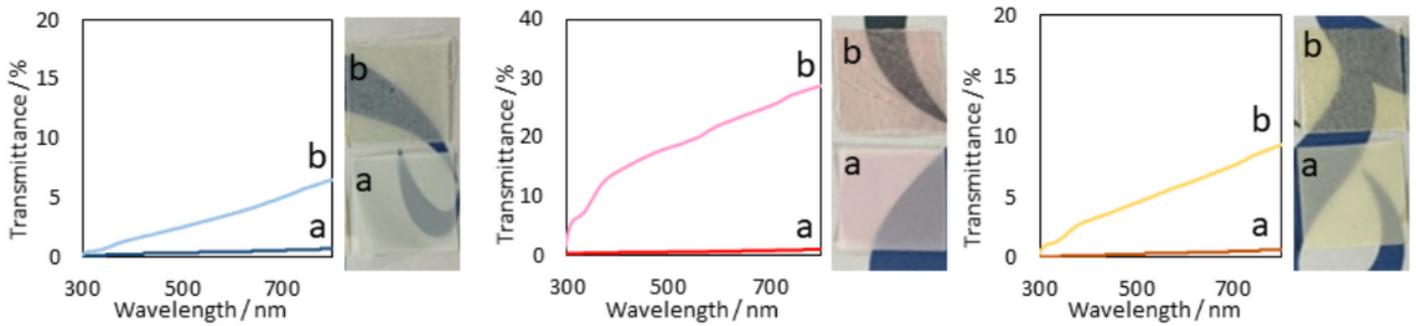
**Figure 7**

Appearance of blue chitin nanofiber sheets with different ratio of dye and these reflection spectra



**Figure 8**

Appearance of chitin nanofiber/ acrylic resin composite sheets dyed with 1% ratio of blue, red and yellow dyes



**Figure 9**

Appearance and transmittance spectra of chitin nanofiber sheets (a) and these resin composite sheets (b) dyed with blue, red and yellow dyes (0.1%)