# Robust Color Fastness of Dyed Silk Fibroin Film By Coupling Modification Dyeing with Aniline Diazonium Salt

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**ABSTRACT**

*Colored silk fibroin (SF) film can change the proportion of transmitted light composition and therefore shows potential application in optoelectronics area. However, few methods are reported for the preparation of colored SF film with good color fastness. Herein, colored SF film was prepared by coupling modification dyeing (CM-dyeing) of SF tyrosine residues in solution. SF film shows excellent color fastness due to the formation of the azo covalent bond formed by electrophilic substitution reaction between SF tyrosine residues and aniline diazonium salt. Furthermore, compared with the undyed film, the dyed SF film is effectively preserved its mechanical property by the mild dyeing conditions (up to 99.02 % on average). Therefore, this facile method presents an alternative approach to construct colored SF film with outstanding performance and benefits its relevant applications.*

KEYWORDS: *Fibroin membrane, CM-dyeing, Tyrosine residue, Diazonium salt, Color fastness.*

## INTRODUCTION

SF film is an excellent natural polymer material, which possesses good biocompatibility, biodegradability and non-toxicity characteristics. Due to the outstanding properties, SF film has been widely used in the field of biomedical materials, cosmetics, drug release materials and biosensor [1,2]. Specially, the colored SF film can also change the proportion of transmitted light composition because of the selective absorption of light, which displays potential application in the field of optics, photonics, electronics and optoelectronics [3-5].

The silk dyeing has been intensively studied and the dyeing method can be tentatively classified: 1) transgenic method. Using the

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transgenic technology constructs silkworms which can produce colored silk [6]. This method is environmentally friendly while with the drawbacks of high cost, complicated process, and limited colors (poor color-depth with rare color of yellow, green and pink) [7]. 2) feeding dyes to domesticated silkworms. However, this method is limited with low efficiency, high cost and poor color fastness. 3) traditional method of preparation materials firstly followed by a after finish process. This is an efficient method to achieve colored silk. In this method, the acid dyeing, acid mordant dyeing, and reactive dyeing are the common technology [8]. Nevertheless, the acid dyeing process favors to poor wet fastness, the acid mordant dyeing process leaves lots of heavy metals which is avoided in environmental protection, and the reactive dyeing process produces highly concentrated-salt colored wastewater that shows bad effect on the water quality and human being healthy [9]. Additionally, in the traditional dyeing process, the mechanical properties of colored SF film will be seriously affected by the subsequent dyeing steps [10]. Meanwhile, more dyeing wastewater is produced and the silk will be easily damaged under the alkaline fixing condition during the reactive dyeing process [11].

It seems like that dyeing the SF solution will be an effective way to obtain better color fastness and mechanical property. However, the structure and properties of the color SF film will also be damaged by dyeing SF solution with acid or reactive dyes, as well as the SF macromolecules due to the harsh dyeing conditions [12]. In addition, the SF molecules favor to reunion because of the pH alteration during dyeing process. Therefore, preparation the colored SF film under mild external dyeing conditions is under urgent demand.

The tyrosine, the main component of SF, can be easy to react with chemical reagents [13-15]. Moreover, the tyrosine residue is an important electrophilic reaction site for the electronic induction effect of phenolic hydroxyl and hydroxyl benzene group [16-19]. The reactivity of tyrosine residues and its correlation with the process factors have also been insensitively studied [20-22]. For example, Murphy reported the protein labeling based on electrophilic substitution using diazonium salt as an electrophilic reagent to attack phenolic group in the SF tyrosine [23]. Joshi also researched protein labeling tracking from tyrosine residue modification based on Mannich type reaction [24]. Prior to this study, we reported a benzotriazole structure in-situ synthesized on the tyrosine residues to improve the light stability [16]. Meanwhile, the silk coloration based on CM-dyeing by a series of arylamine diazonium has been also researched in our previous work [25]. These researches demonstrate the CM-dyeing with diazonium salt is an efficient approach to obtain colored silk-based materials including the SF film.

Herein, the SF solution was dyed based on CM-dyeing of tyrosine residues used the aniline diazonium salt as an electrophilic reagent.

## EXPERIMENTAL

### Materials and chemicals

The raw silk fiber was supplied by Qingyang Sanfang Silk Co., Ltd. Aniline, hydrochloric acid (HCl, 37%), sodium nitrite ($NaNO_2$), sodium carbonate ($NaCO_3$), sodium hydroxide (NaOH), and N, N-dimethylformamide (DMF) were supplied by Aladdin Chemicals Co., Ltd.

### Preparation of silk fibroin

The raw silk was firstly degummed using $Na_2CO_3$ solution. The raw silk was immersed into the 0.5 wt.% $Na_2CO_3$ solution in a liquor-to-silk ratio of 50:1, and this operation was repeated twice at (the temperature of 100° C for 30min in a water bath. The degummed silk was then rinsed thoroughly with distilled water and dried at 40°C. Afterwards, the degummed silk fiber was dissolved in a $CaCl_2$-$CH_3CH_2OH$-$H_2O$ ($CaCl_2$/$CH_3CH_2OH$/$H_2O$, 1:2:8 in molar ratio) ternary solvent which was prepared in advance, and the ternary solvent was called $CaCl_2$ solvent for short in this paper. The degummed silk fiber was added into the $CaCl_2$ solvent in a liquor-to-silk ratio of 20:1. The solution was kept at 50°C for 6h to swell the silk fiber, and then stirred at 80°C to form a clear solution. The fibroin solution was dialyzed continuously using dialysis bag (Union Carbide Corporation, cutoff 8000-14000) with water for 72 h (changing the water every 4 h to remove $CaCl_2$ and $CH_3CH_2OH$), and the concentrated fibroin solution was prepared through polyethylene glycol concentration.

### Preparation of diazonium salt

Aniline (1.98 g, 0.02 mol) was added into a mixture of HCl (6 ml, 0.06 mol) and super pure water (10mL) to form a homogeneous solution under mechanical stirring. The solution was then cooled down to 5°C. Meanwhile, $NaNO_2$ (1.656 g, 0.024 mol) was dissolved in super pure water (5 mL) to prepare a sodium nitrite solution. Which was then added into the aniline solution and stirred for 40 mins at 5°C. The obtained clear diazonium salt solution was kept on ice under dark.

### CM-dyeing (coupling modification dyeing) of silk fibroin

The aniline diazonium salt (0.01 mol) solution was slowly added into the 10 wt.% concentrated silk fibroin solution (20 g) at 5°C. Meanwhile, the pH of the solution was adjusted to 8 by a dilute solution of $Na_2CO_3$ (2 g/L). The CM-dyeing was kept at 5°C under constant stirring for 60 min. After dyeing, the silk fibroin solution was dialyzed again as described previously to remove the salt and other small molecules. The CM-dyeing mechanism was described in Figure 1.

### Preparation of SF and CM-dyed SF film

The dyed fibroin solution was dropped into a PTFE plate blank with the depth of 0.5 mm. The samples were air-dried at room temperature for about 40 h to obtain the CM-dyed SF film.

### Measurements

UV2600 spectrophotometer (Shanghai Tianmei Co., Ltd, China) was used to analyze the silk fibroin solution

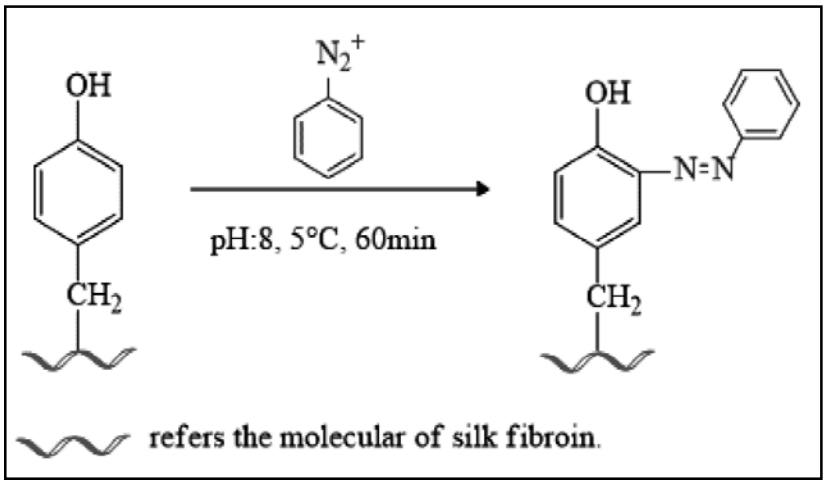


Fig. 1. Reaction mechanism of the CM-dyeing

before and after CM-dyeing, and the measurement wavelength ranged from 250 to 600 nm. X-ray diffraction analysis of the film sample was conducted on a D/max-IIIB X-ray Diffractometer (Rigaku Co., Japan) at 40 kV and 30 mA with a wavelength of 0.154 nm Cuk$\alpha$ radiation. The scanning region of the diffraction angle (2$\theta$) was from 5° to 45° with an angular step of 0.02° and a scanning rate of 2 °/min. The IRPrestige-21 fourier transform infrared (FTIR) spectrometer (Shimadzu Co., Japan) was used to qualitatively analyze the infrared spectra over the range of 500-4000 $cm^{-1}$ with a scanning speed of 32 $s^{-1}$ and a resolution of 4 $cm^{-1}$. Coloration of CM-dyeing SF films were computed using illuminant D65 and 10° observer data, and the rub color fastness was measured based on ISO 105-X12:2016. Meantime, DMF was used as solvent to strip the color of CM-dyeing SF films. Stripping was carried out at a liquor ratio of 30:1 for 15 min under 40 °C each time. K/S value of the CM-dyeing SF films was measured after one time or twice stripped. The mechanical properties of membrane samples were tested by YG021 textile strength machine (Wenzhou Fangyuan Instrument Co., Ltd, China). All samples were cut into 30 mm×5 mm rectangular strip before measuring. All samples were tested 5 times and the average value was recorded.

## RESULTS AND DISCUSSION

### Characterization of fibroin solution

It was evident that the UV-vis spectrums of silk fibroin and CM-dyed silk fibroin solutions were quite different from Figure 3. There was no any characteristic absorption peak between 300 and 600 nm for silk fibroin solution. However, after the CM-dyeing with the aniline diazonium, a characteristic absorption peak at 325 nm together with a weaker absorption peak at 400 nm was observed, which could be ascribed to a large conjugate structure. The conjugated structure containing tyrosine residue and aniline molecules connected by azo bond (-N=N-) was proposed. In the reaction process, electrophilic substitution reaction was occurred between aniline diazonium salt and the ortho position of phenolic hydroxyl in tyrosine residues. Compared with tyrosine residue, the conjugation system was enhanced after CM-dyeing, which resulted that the characteristic absorption moves to long wave direction.

The FTIR spectra of the fibroin and CM-dyed fibroin solutions are presented in Figure 4. The spectra were used to confirm the coupling reaction of aniline diazonium and tyrosine residues in fibroin molecules. Compared with the characteristic absorption peaks of the fibroin solution, two new absorption peaks were observed in the spectrum of the azo-dyed fibroin. The peak frequency 1542 $cm^{-1}$ was ascribed to the characteristic absorption band of "N=N" bond stretching [27,28], the other peak appearing at 1234 $cm^{-1}$ was attributed to the stretching vibration of "C-N" [29]. Both the peaks indicated the aniline diazonium was covalently linked to the silk fibroin molecules through CM-dyeing and then the azo structure was formed [25].

### Characterization of fibroin film

As seen in Figure 5, the diffraction peak shapes of SF film and CM-dyed SF film were quite similar. Peaks at 19.66° and 22.56° were correspond to the structure of silk I and silk II, respectively. Both of them were the characteristic signals of silk fibroin [30,31]. Therefore, it could be conformed that the two SF films had the same structure. In other words, the CM-dyeing did not affect the structure of silk fibroin film. Their crystallinity was calculated using the Herman's method [32] and the values were 46.19% and 42.94%, respectively. The introduction of benzene ring

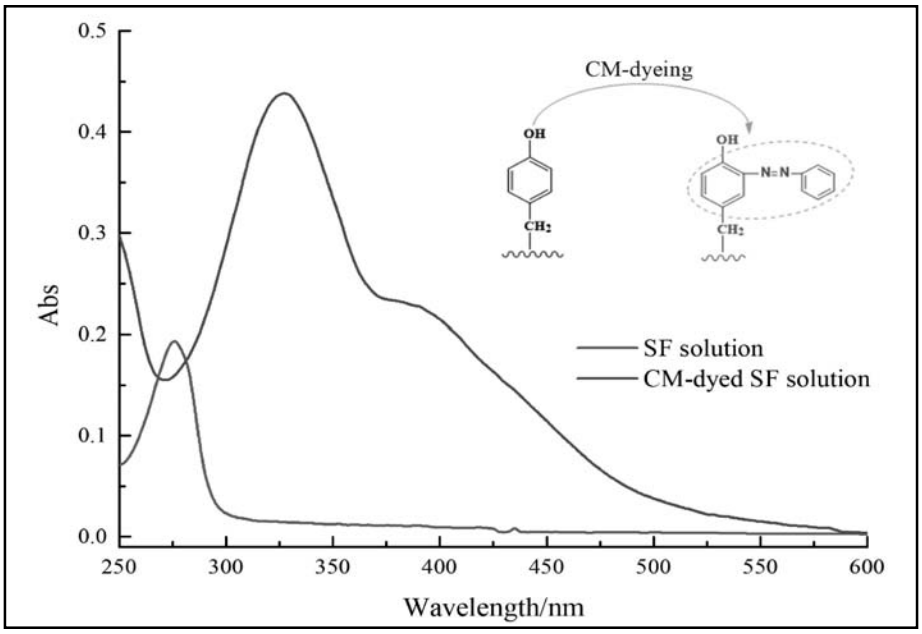


Fig. 2. UV-vis spectrum of fibroin before and after CM-dyeing.

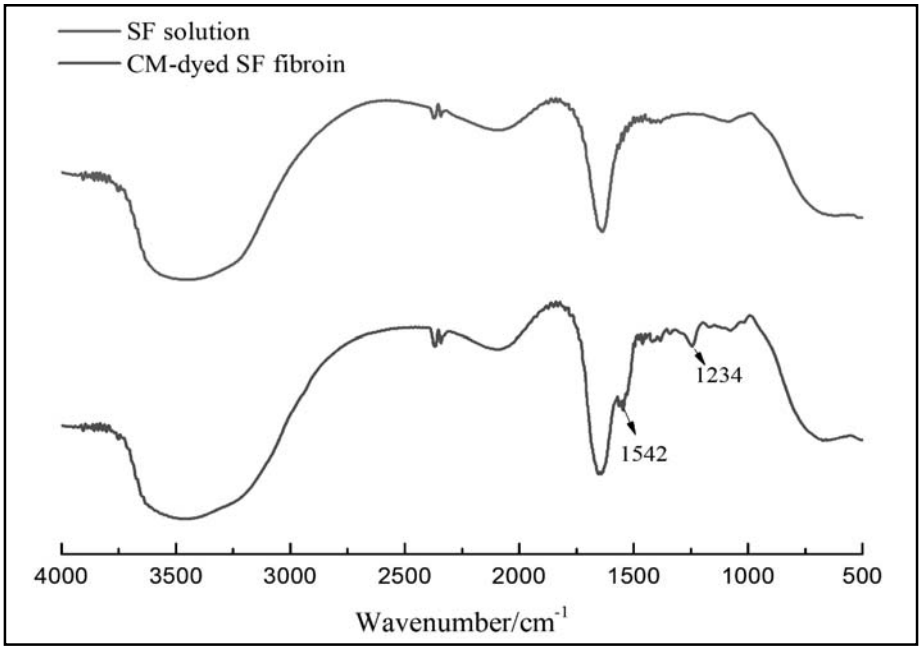


Fig. 3. FTIR spectrum of fibroin before and after CM-dyeing.

into fibroin molecules affects the regular arrangement of its molecular chains, which is inferred to be the reason for the decrease of crystallinity caused by CM-dyeing.

There is no significant difference in the characteristic absorption peaks between the two different SF films as observed from Figure 5. The structure of silk fibroin could be divided into two structural models, one was silk I known to be rich in helical structures, and the other one was silk II known to be rich in β-sheets. The infrared absorption peaks of each structure in amides I, II and III were also different. The peak at 3286 $cm^{-1}$ was the N-H stretching vibration of silk fibroin. The peaks at 1622 and 1510 $cm^{-1}$ were attributed to the amides I, II of β-sheets, respectively. And the peak at 1236 $cm^{-1}$ belonged to the amides III of random coils. In summary, there was no obvious structural differences between the two different SF films from the FTIR analysis.

As can be seen from Table 1, the average breaking strength of SF film and CM-dyed SF film is $105.44\times10^{-2}$ N/$mm^2$ with the SD of $1.83\times10^{-2}$ and $104.41\times10^{-2}$ N/$mm^2$ with the SD of $1.92\times10^{-2}$, respectively, meanwhile, the average breaking elongation is 2.87 % with the SD of 0.37 and 2.76 % with the SD of 0.42, respectively. It can be found that there is no significant difference in tensile properties between two SF films. Thus, it can be concluded that CM-dyeing has little damage to the mechanical properties because of its mild conditions.

TABLE 1. Tensile properties of two different SF films

| Samples | Breaking strength / N×$mm^{-2}$ | | | | Breaking elongation / % | | | |
|---|---|---|---|---|---|---|---|---|
| | Max. $\times10^2$ | Min. $\times10^2$ | Average $\times10^2$ | *SD** $\times10^2$ | Max. | Min. | Average | *SD** |
| Blank SF film | 107.37 | 101.96 | 105.44 | 1.83 | 2.93 | 2.82 | 2.87 | 0.37 |
| CM-dyed SF film | 107.77 | 101.53 | 104.41 | 1.92 | 2.84 | 2.66 | 2.76 | 0.42 |

Ten SF and CM-dyed SF films were tested in this experiment, respectively, and the values in this table were based on the above test.

*SD refers to the standard deviation.*

The coloration of SF film and CM-dyed SF film is shown in Figure 6 and 7. As presented in Figure 6, it can be observed the CM-dyed SF film has towering spectral reflectance only in the range of 550~700 nm, while the reflectance is zero in the range of 400~550 nm. Whereas, the SF film is achromatic, which has an almost stable spectral reflectance across the entire band of 400~700 nm. However, the K/S value of the SF film is zero over the entire 400~700 nm interval, while the CM-dyed SF film has a K/S value of 10 or higher in the 400~550 nm interval as observed from Figure 8. This indicates that a high color depth can be obtained through the CM-dyeing method.

As can be seen from Figure 8, the K/S value of the CM-dyed SF film increases as the dosage of aniline diazonium salt increasing. During the CM-dyeing, the tyrosine residue

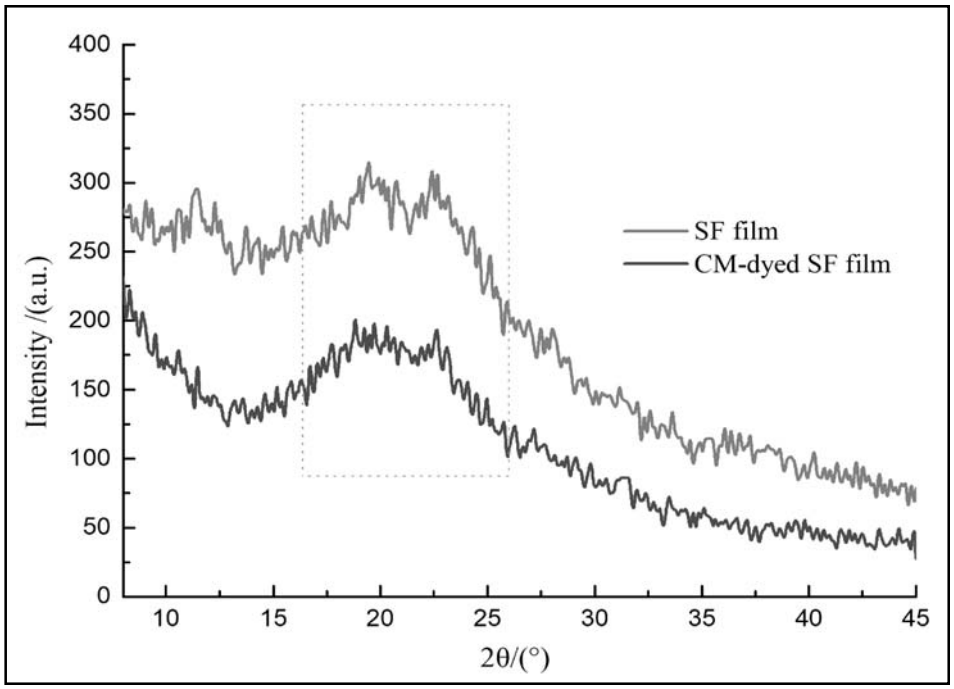


Fig. 4. XRD spectrum of two different SF films

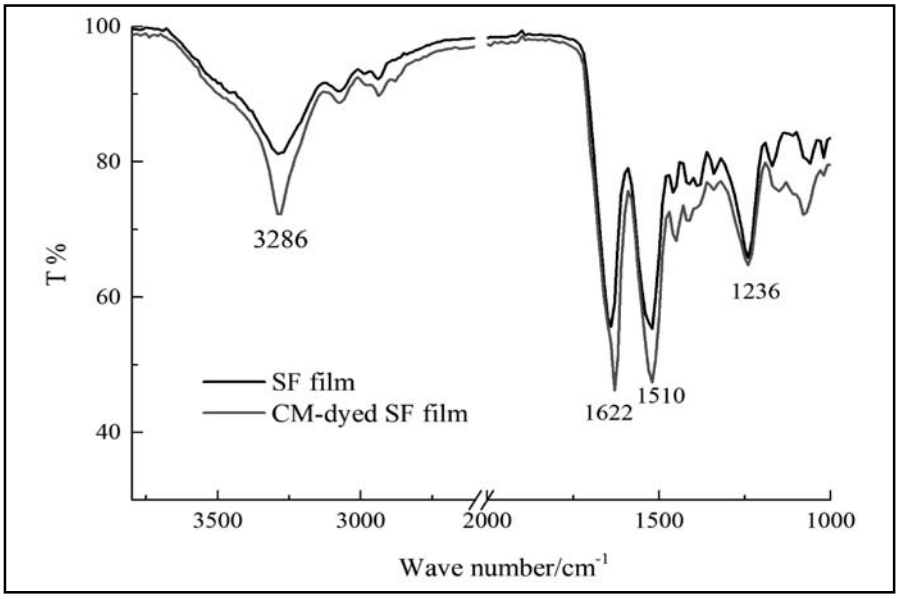


Fig. 5. FTIR spectrum of two different SF films

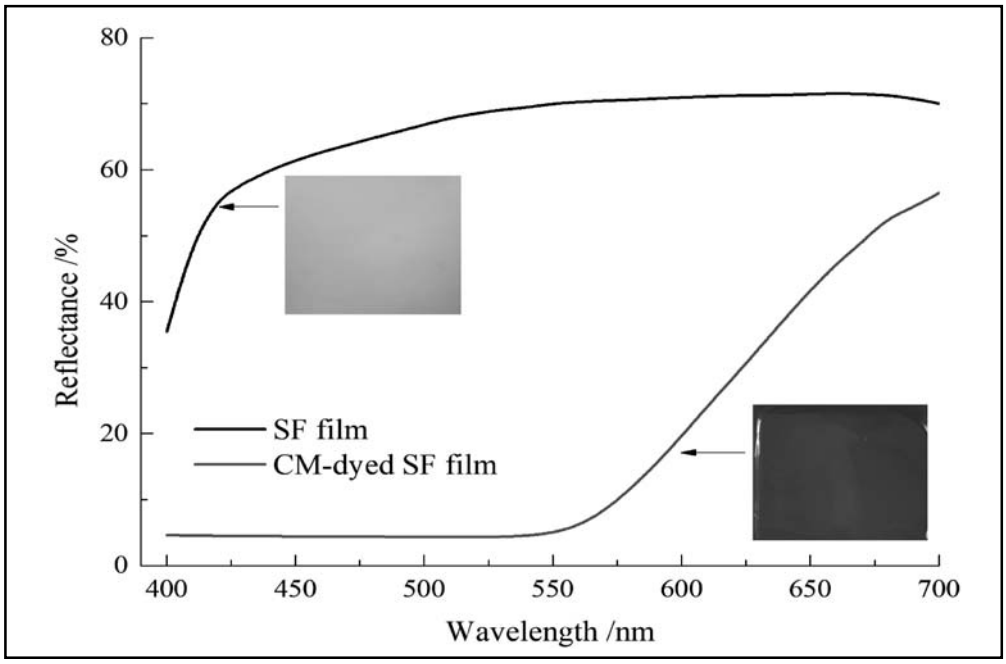


Fig. 6. Reflectance curves of two different SF films

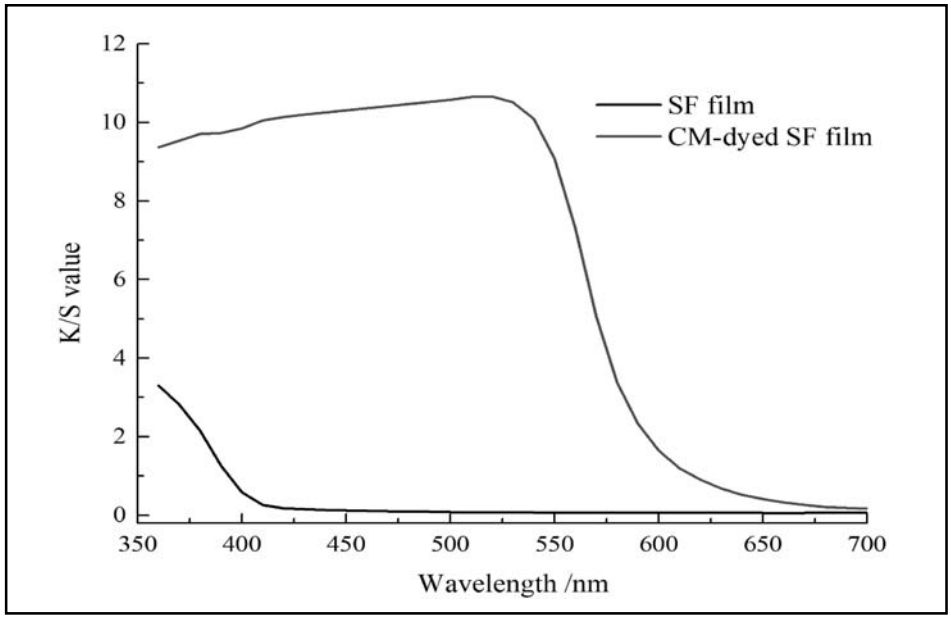


Fig. 7. K/S curves of two different SF films

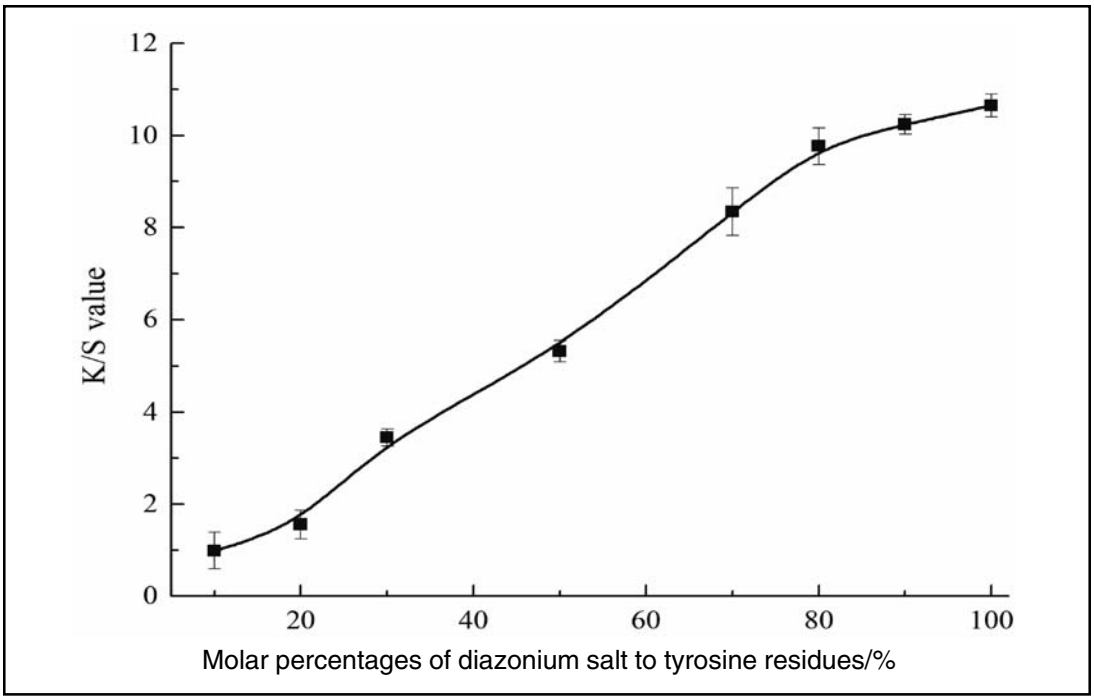


Fig. 8. K/S value of the CM-dyed SF film with the increasing of diazonium salt dosage

in silk fibroin acts as the dyeing site. The amount of diazonium salt can continuously increase when the tyrosine residue completely reacts. However, the coupling modification reaction cannot be carried out, which results that the color depth of the CM-dyed SF film cannot continuously increase. Consequently, the SF film with different K/S values can be prepared through altering the dosage of diazonium salt. Furthermore, SF films with different colors also can be obtained through CM-dyeing of fibroin with diazonium salts of different chromophores.

The rub fastness of the CM-dyed SF film with the highest color depth was also tested. Meanwhile, the color stripping was carried out using DMF as solvent. Every stripping process was conducted for 15 min at 40 ! using a liquor ratio of 30:1. The K/S value changes after one time and twice stripping is also shown in Table 2.

TABLE 2. Rub fastness and K/S value of CM-dyed SF film after DMF stripping

| Rub fastness | | K/S value (λ = 520nm) | | |
|---|---|---|---|---|
| Dry | Wet | Before stripping | One-time stripping | Twice stripping |
| 5 | 4-5 | 10.653 | 10.423 | 10.219 |

As shown in Table 2, the dry and wet rub fastness of the CM-dyed SF film are 5 and 4-5 grade, respectively, indicating the CM-dyed SF film exhibits good rub fastness. It is impossible to obtain such high color fastness through ionic or other weak bonds. Meanwhile, the CM-dyed SF film also shows good stripping fastness. Before stripping, the K/S value is 10.653, after one-time stripping, the K/S value is 10.423 and only decrease by 2.16 %. Even after twice stripping, K/S value can be 10.219 and decrease by 4.07 %. As we all know, DMF is a strong polar solvent, the weak chemical bonds such as van der Waals gravitation, ion bonds, hydrogen bonds formed on fibroin molecules during dyeing can be broken and the coloration can fade obviously or disappear. In the present work, the good fastness of the CM-dyed SF film is attributed to the coupling modified reaction of aniline diazonium salt and tyrosine residue in fibroin. The results are in accordance with the previous study that Color Base Red G diazonium salt reacts with silk and achieves excellent color fastness.

## CONCLUSIONS

In the present work, the CM-dyeing of SF film was carried out based on an electrophilic substitutive reaction between the aniline diazonium salt and tyrosine residue in the silk fibroin. The resulting CM-dyeing SF film displayed high color depth and excellent color fastness to physical rubbing and chemical DMF stripping. Moreover, due to the mild reaction conditions, the structure and mechanical performance of the CM-dyed SF film were properly protected compared with the SF film. Therefore, CM-dyeing can provide an alternative approach to get colored SF film with excellent color fastness and good mechanical properties.

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