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Synthesis and Grafting Efficiency of Poly(vinyl alcohol)-*graft*-Fibroin Peptides

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ABSTRACT

A new product of poly(vinyl alcohol)-*g*-fibroin peptides (PVA-*g*-FP) is produced by graft-copolymerization of PVA with allyl fibroin peptides (AFP). The AFP was obtained from a nucleophilic substitution reaction of fibroin peptides (FP) with allyl chloride. The optimal parameters of preparing the PVA-*g*-FP, corresponding to the acquired grafting efficiency, 10.1 wt%, were selected on the basis of the orthogonal experiments design of $L_{16}4^5$, and the influence of reaction conditions on the grafting efficiency are discussed in detail, according to the data obtained from the orthogonal experiments. The FP was successfully modified and grafted onto the PVA macromolecules, which was confirmed by the analyses of the FTIR spectra of AFP and PVA-*g*-FP, and the reaction mechanisms for the formation of AFP and PVA-*g*-FP are proposed. The results of the orthogonal experiments have indicated that, relative to other parameters, the reaction temperature and the molar ratio of potassium peroxide sulphate (KPS) to AFP seemed to be more effective on the grafting efficiency. The highest grafting efficiency of FP was obtained under the reaction temperature of 60ºC for 3 h reaction time, with AFP/PVA weight ratio of 3/7, and molar ratio of KPS/AFP of 1/20, having a solution concentration of 10 wt%. The product may be potentially useful in the fields of biomaterials, textile industry, and chemical engineering.

Key Words:

biodegradable;
graft copolymers;
proteins;
radical polymerization;
synthesis.

INTRODUCTION

Poly(vinyl alcohol) (PVA) is a widely used, non-toxic, water-soluble, biocompatible, and biodegradable synthetic polymer with excellent mechanical properties, such as flexibility and good film-forming quality with excellent gas barrier ability [1-3]. It is well suitable for fabricating biomaterials due to the hydrophilicity and ease of modification through its hydroxyl groups, as well as commercial availability in a wide range of molecular weights at low price. However, sometimes PVA has the disadvantage of low chemical reactivity with some bioactive compounds at low temperature. The route to solve this problem may be the creation of new more reactive functional groups in its structure that enable the coupling reactions of bioactive agents more efficiently.

The modification of conventional organic polymers is an important technique in the preparation of new

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materials with improved properties. Covalent bonding of amino acids onto polymers main chain [4-8] or side chain [9-16] is another example of chemical modification of polymers for biomedical application. Because amino acids are naturally occurring compounds, polymers based on amino acids are expected to be non-toxic, biocompatible, and biodegradable. Fibroin peptides (FP), with a molecular weight of 90 Da, obtained from the hydrolysates of the silk fibroin protein are widely used as biomaterials [17-25], possessing good water-solubility characteristics which facilitates processing and utilization, and also has many side groups such as amino, carboxyl and hydroxyl, facilitating the modifications for exploiting functional materials. Thus, FP may be suitable to modify the PVA molecules.

In this work, we reveal a new technology for covalent bonding of FP onto PVA main chain, by means of graft-copolymerization of PVA with alkyl fibroin peptides (AFP), for which no report of the technology and the resultant material are found in relevant literature. An experimental orthogonal analysis has been carried out, and the influence of the reaction conditions on the grafting efficiency of the FP is discussed in detail. The resultant product of poly(vinyl alcohol)-*g*-fibroin peptides (PVA-*g*-FP), may have potential applications in biomaterials and other biomedical fields.

EXPERIMENTAL

Materials

Fibroin peptides powder, average molecular weight 90 Da, food degree, was purchased from Huzhou Xintiansi Bio-tech Co. Ltd, Zhejiang Pro, PR China. Allyl chloride, a pure degree chemical, distilled before use and was purchased from Jiachen Chemical Co., Shanghai, PR China. Poly(vinyl alcohol), with degree of polymerization: 1700±50 and degree of saponification: 99.9 mol% was purchased from Organic Chemical Engineering Plant, Beijing City, PR China and it was washed by distilled water before use. Potassium peroxide sulphate (KPS), analytical grade was purchased from Standard-tech Co. Ltd, Tianjin City.

Preparation of Allyl Fibroin Peptides (AFP)

The FP was dissolved in a water/ethanol solvent system, and the aqueous solution of sodium hydroxide were added in a four-necked flask, equipped with a mechanical stirrer, a thermometer, a burette filled with pre-weighed allyl chloride, and a water cooling condenser. After mechanically stirring for 10 min at 55ºC, the allyl chloride was added dropwise into the flask. The reaction was stopped until there were almost no allyl chloride drops falling from the condenser (about 3 h), and after that, the pH of the solution was adjusted to 7 by adding dilute aqueous solution of hydrogen chloride. Then, the resultant solution was evaporated at 40ºC with a rotary evaporator, until a sticky solid was obtained.

The sticky solid was dissolved in hot ethanol for 8 h, and the ethanol solution was separated by filtration, the target product, temporally named as allyl fibroin peptides (AFP) appeared as a waxy and sticky solid with a light brown colour was obtained after evaporating the ethanol solution to a constant weight under vacuum at 40ºC [26].

Preparation of PVA-g-FP

The aqueous AFP and PVA solutions with different concentrations, mixed in certain weight ratios as shown in Table 1, were added to a four-neck flask equipped with a mechanical stirrer, water condenser, thermometer, and nitrogen inlet. The mixed solutions were stirred for 30 min to eliminate the oxygen through ventilating nitrogen. After the temperature was raised to 70ºC, precalculated amount of KPS, dissolved in water, was added to the mixed solutions, and the reaction of graft-copolymerization was kept for predetermined time under stirring and nitrogen atmosphere at 70°C. After the reaction was stopped, the solution was poured into a large amount of ethanol and deposited out. Then the deposition was filtered under vacuum and washed by deionized water for 24 h in order to obtain pure PVA-*g*-FP. The final product was vacuum-dried for further test.

An orthogonal analysis experiments were carried out according to the design of $L_{16}4^5$ [26]. The reaction conditions such as, temperature, time, AFP/PVA weight ratio, KPS/AFP molar ratio, and weight concentration of the solution were taken as the determining factors and their role on the grafting

efficiency are illustrated in Figures 1-5. Data summarized from the results of the orthogonal analysis are discussed in detail, as well. All the data reported in Figures 1-5 are mean values summarized from the results of $L_{16}4^5$ and standard deviations were calculated from four sets of relevant data.

Measurement of Grafting Efficiency

The grafting efficiencies of FP were determined in terms of the weight percentage of the FP in PVA-*g*-FP, which were obtained from the nitrogen content of the product using the method of Kjeldahl determination. The mathematical relationship is as follows:

$$X(\%) = \frac{(V_1 - V_2) \times N \times 0.014 \times F}{m \times \frac{10}{100}} \times 100 \quad (1)$$

Where X is the content of FP (wt%), V is the volume of standard HCl solution (mL) used, N is the concentration of standard HCl solution (mol/L), 0.014, a constant which is a weight magnitude of the nitrogen corresponding to 1 mL standard HCl solution with a concentration of 1 mol/L, m is the weight of the sample (g), and F is the coefficient of the transition from nitrogen to FP (5.26).

Measurement of FTIR Spectra of AFP and PVA-*g*-FP

A spectrometer TENSOR37 from BRUKER was employed to obtain the FTIR spectra of the AFP and PVA-*g*-FP. The AFP was vacuum-dried at room temperature and used in a form of a KBr tablet. The PVA-*g*-FP was dissolved in hot water and prepared as a film by solution casting and the film was dried before use.

RESULTS AND DISCUSSION

Results of the Orthogonal Experiments

From the obtained data of the orthogonal analysis, the optimal parameters of graft-copolymerization, resulting in a highest grafting efficiency of 10.1 wt%, were found as follows: reaction temperature: 60ºC, reaction time: 3 h, the weight ratio of AFP/PVA: 3/7, and the molar ratio of KPS/AFP: 1/20, as well as the concentration of the solution of 10 wt%. Furthermore, from the data summarized from the results of the orthogonal analysis, as given in Table 1, it is concluded that, the most effective factor is the molar ratio of KPS/AFP, with the highest value of F ratio of 2.069, and the least effective one is the weight ratio of AFP/PVA, with the lowest value of F ratio of 0.203.

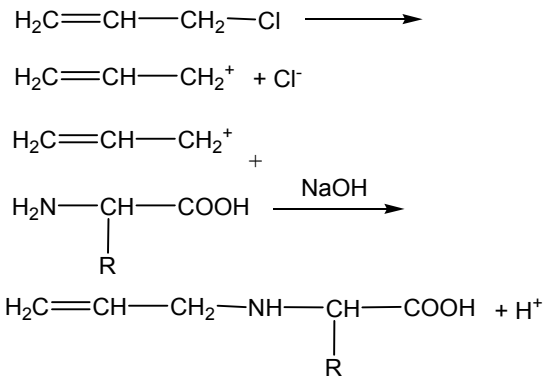
Reaction Mechanisms

It is clear that, the allyl chloride is reactive in alkaline medium, due to the electrophilicity of the allyl cation dissociated from the allyl chloride. Thus, when the allyl cations collide with FP amino groups, which are nucleophilic, a substitution reaction takes place according to the mechanism suggested as Scheme I.

The graft-copolymerization mechanism is shown in Scheme II, with the AFP copolymer as the resultant product of reaction (3), and the expected reaction product (7). Since, the products of reactions (8) and (9) are difficult to separate from the expected product they are usually ignored due to their negligible contents relative to the final products.

Table 1. Variance analysis of the conditions.

Conditions	Quadratic sum of deviation	Degree of freedom	F Ratio	Critical value of F	Significance
Temperature (ºC)	22.627	3	1.860	2.490	null
Time (h)	5.690	3	0.468	2.490	null
AFP/PVA (wt/wt)	2.465	3	0.203	2.490	null
KPS/AFP (N/N)	25.170	3	2.069	2.490	null
Solution concentration (wt%)	4.884	3	0.401	2.490	null
Error	60.840	15	-	-	-



(R represent the side group of amino acids or peptieds)

Scheme I. Reaction mechanism of FP with allyl chloride [26].

Influence of Reaction Temperature on Grafting Efficiency

The data of Figure 1 were obtained from the analysis of the orthogonal experiments. The variation of the grafting efficiency with the reaction temperature increases is shown in Figure 1. This change may be due to the competition between the homo-polymerization reaction rate of the AFP as shown in reaction (3) of Scheme II, and the rate of graft-copolymerization of AFP with PVA as shown in reactions (5), (6), and (7) of Scheme II. If the rate of homo-polymerization accelerates faster than that of graft-copolymerization the final grafting efficiency will be lower. These comparisons are made for reaction temperatures at 70°C and 80°C with the case at 60°C. On the contrary, if the rate of graft-copolymerization accelerates faster than that of homo-polymerization, the final grafting efficiency will be higher, as in the case of 90°C reaction temperature, which is being compared with the case at 80°C.

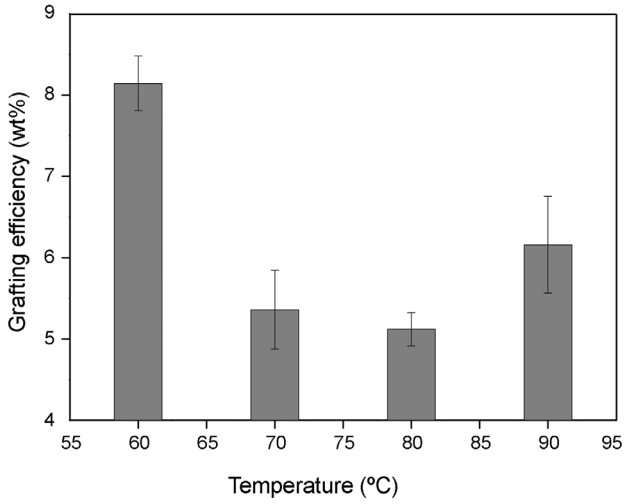
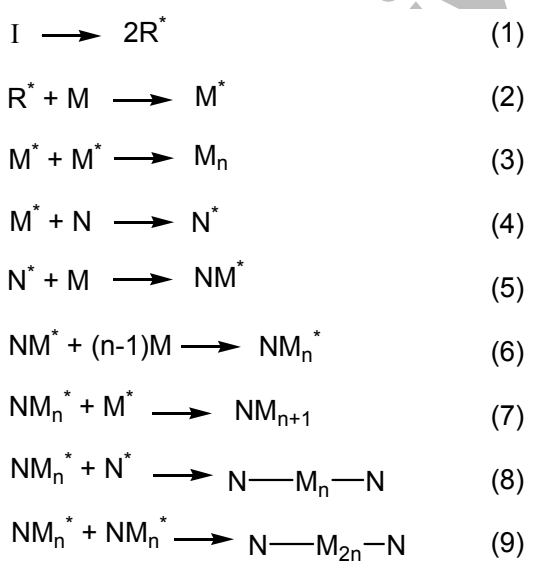


Figure 1. Influence of temperature on the grafting efficiency of the FP.

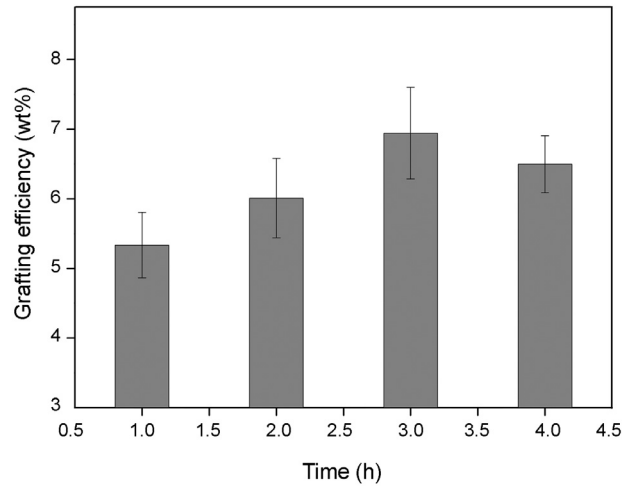


(I: initiator; R*: active centre; M: AFP molecule; N: PVA molecule)

Scheme II. Graft-copolymerization reaction mechanism of AFP with PVA.

Influence of Reaction Time on Grafting Efficiency

Figure 2 shows the variation of the grafting efficiency with the increases in reaction time.



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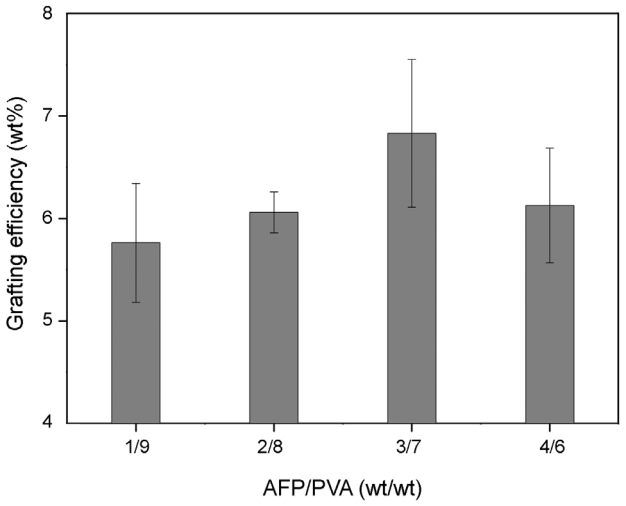

Figure 3. Influence of the ratio of AFP/PVA on the grafting efficiency of the FP.

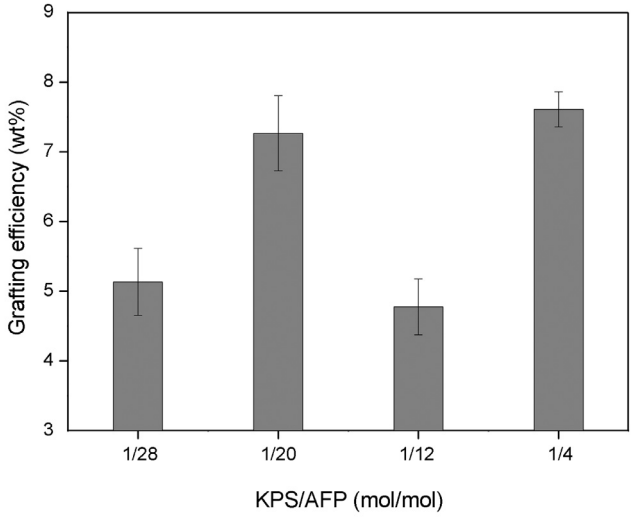

Figure 4. Variation of the grafting efficiency of the FP with the increasing of initiator.

Generally speaking, with the increases in reaction time, the molecules of AFP would be gradually exhausted, resulting in a gradually increased grafting efficiency. However, unnecessary extended time does little contribution to the grafting efficiency, and even it might lead to a lower grafting efficiency due to the dissociation of the expected product under agitation at higher temperature.

Influence of AFP/PVA Weight Ratio on Grafting Efficiency

As shown in Figure 3, at first the FP grafting efficiency is increased and then it is decreased with the increase in AFP/PVA weight ratio. A higher weight ratio of AFP/PVA means a higher concentration of AFP in the reaction system which gives the polymer more opportunity to graft-copolymerize. However, when higher concentration of AFP is introduced in the reaction system, the rate of homo-polymerization might also be greatly increased at the expense of the rate of graft-copolymerization. This is accounted for the decreased grafting efficiency when the weight ratio of AFP/PVA was changed from 3:7 to 4:6.

Influence of KPS/AFP Molar Ratio on Grafting Efficiency

Figure 4 demonstrates the variation in grafting efficiency with the increased KPS/AFP molar ratio. In general, a greater amount of initiator may produce more active centres, facilitating both graft-copolymerization and homo-polymerization.

A lower content of initiator may lead to a higher rate of graft-copolymerization than homo-polymerization, resulting in a higher grafting efficiency as the case of 1:20 in Figure 4. However, a higher content of initiator may simultaneously lead to a higher rate of homo-polymerization relative to graft-copolymerization, resulting in lower grafting efficiency as the case of 1/12.

Furthermore, an excessive amount of initiator not only creates too many active centres accelerating the homo-polymerization, it may also lead to cross-links as shown by reactions (8) and (9) in Scheme II, resulting in anomalous increase of grafting efficiency as the case of 1:4 in Figure 4. Although the change in grafting efficiency seems to be anomalous with the increases in initiator content, the influence of KPS/AFP molar ratio on the grafting efficiency is the most effective parameter, according to the data given in Table 1 with the highest value of 2.069 for F ratio.

Influence of Solution Concentration on Grafting Efficiency

It is clear that a higher solution concentration of AFP means a higher monomer concentration, providing more opportunities for monomers to collide with each other. But at the same time, a higher PVA

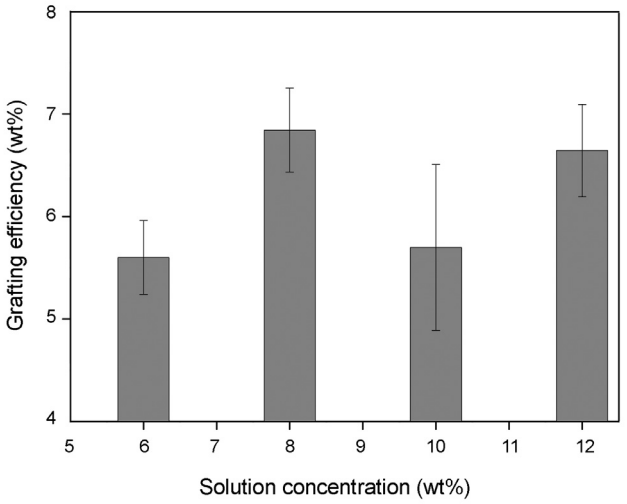

Figure 5. Variation of grafting efficiency of the FP with the increasing of the solution concentration.

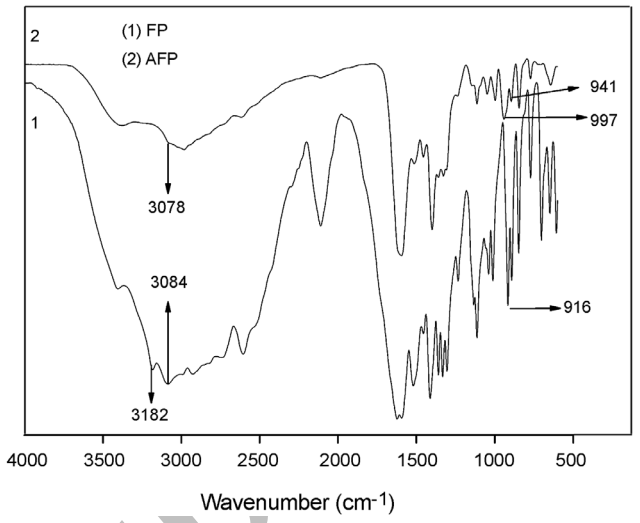

Figure 6. FTIR spectra of FP and AFP [26].

concentration inhibits the movement of small molecules due to its higher molecular weight and viscosity. As shown in Figure 5, the differences in grafting efficiencies of different solution concentrations are indefinite, because the highest magnitude of the grafting efficiency is only 1.242 wt% greater than the lowest magnitude. In addition, the data given in Table 1 must be taken into consideration as well.

According to the value of F ratio, 0.401, obtained from the statistical analyses of the orthogonal experiment results, the influence of the solution concentration is insignificant. Thus, it can be concluded that the change in the values of grafting efficiency might not be dependent on the solution concentration, but on the scattered data. Nevertheless, the grafting efficiency obtained under the solution concentration of 6 wt% is at lowest level compared to the other three grafting efficiencies obtained with higher solution concentrations.

FTIR Analysis of the AFP and PVA-*g*-FP

Figure 6 shows the FTIR spectra of FP and AFP. By comparison of the two spectra, two distinct differences can be found. First, the characteristic absorption bands in spectrum 1 at 3182 cm^{-1} and 3084 cm^{-1}, corresponding to the stretching vibrations of $-NH_2$ of the primary amine, are disappeared in spectrum 2. The latter only possesses single characteristic absorption at 3078 cm^{-1}, corresponding to the stretching vibration of N-H of the secondary amine. Secondly, spectrum 2 shows the characteristic absorptions at 941cm^{-1} and 997cm^{-1}, corresponding to the out-of-plane bending vibrations of $=CH_2$ of terminal olefin, which are absent in spectrum 1. The results indicate that the allyl chloride molecules are grafted onto FP molecules and the grafting sites might be at the primary amines of the FP molecules [26].

As it is shown in Figure 7, both spectra 1 and 2 have absorption at 1650 cm^{-1}, which correspond to the stretching vibration of C=O. However, absorption appeared in spectrum 1 is due to the existence of

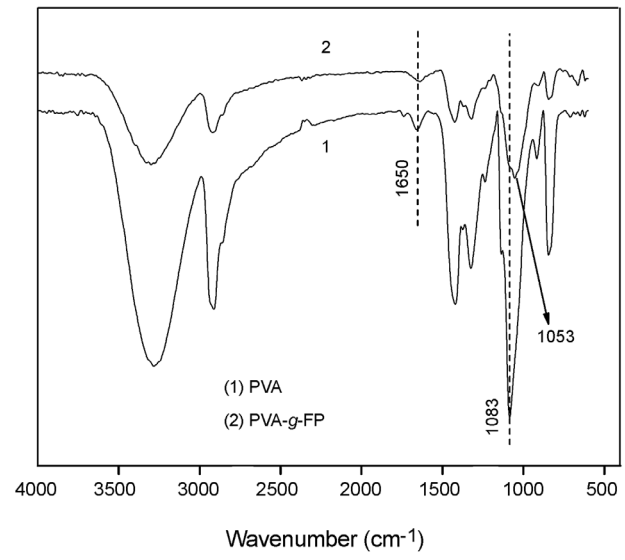

Figure 7. FTIR spectra of PVA and PVA-g-FP.

acetate as impurity in the PVA feed and the one appeared in spectrum 2 might be attributed to the existence of FP grafted onto the PVA which was purified through washing by deionized water.

Furthermore, spectrum 2 possesses shoulder absorptions at 1053 cm^{-1} and 1083 cm^{-1}, probably due to the stretching vibrations of the primary hydroxyl groups, presented in the serine residues of the FP and the secondary hydroxyl groups presented both in FP and in PVA, respectively. Comparing the two spectra, the spectrum 1 only possesses absorption at 1083 cm^{-1}, corresponding to the stretching vibrations of the secondary hydroxyl groups presented in PVA. The results of FTIR confirm that the FP molecules have been successfully grafted onto PVA macromolecules.

CONCLUSION

The fibroin peptides (FP) were chemically modified by nucleophilic substitution reaction with allyl chloride, resulting in an unsaturated mixture of FP derivatives bearing double bonds. FP molecules were successfully grafted onto PVA macromolecules through a reaction of graft-copolymerization by chain transfer. The grafted products were confirmed by FTIR analyses. The orthogonal experiments of the graft-copolymerization were carried out by taking the grafting efficiency of FP as the function of the synthetic parameters. The tests indicated that, the reaction temperature and the content of the initiator greatly influenced the grafting efficiency of the FP. The highest grafting efficiency of FP onto PVA reached 10.1 wt%, at 60°C reaction temperature within 3 h reaction time, and AFP/PVA weight ratio of 3:7, KPS/AFP molar ratio of 1:20, as well as solution concentration of 10 wt%.

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