

# Structure and Properties of *Bombyx mori* Silk Fibers Grafted with Methacrylamide (MAA) and 2-Hydroxyethyl Methacrylate (HEMA)

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## SYNOPSIS

Silk fibers were graft-copolymerized with methacrylamide (MAA) and 2-hydroxyethyl methacrylate (HEMA) in aqueous media, using a chemical redox system as an initiator. High weight gains (300%) were obtained with both monomers, the weight gain being linearly related to the amount of monomer contained in the reaction system. The reaction efficiency attained 95–100%. The extent of homopolymerization was negligible for the MAA grafting system, while large amounts of poly-HEMA covered the surface of silk fibers beyond 70% weight gain. The fiber size increased linearly with the weight gain. The moisture content of MAA-grafted silk fibers was highly enhanced by grafting. The severe grafting conditions caused a partial degradation of the tensile properties of silk fibers, as well as of the degree of fiber whiteness. Following grafting, the breaking load slightly increased, while elongation at break and energy decreased. The decomposition temperature of grafted silk fibers shifted upwards. The Raman spectra of untreated silk fibers showed strong lines at 1667 (amide I), 1451, 1227 (amide III), 1172 and 1083  $\text{cm}^{-1}$ . Overlapping of the lines characteristic of both silk fibroin and grafted polymer was observed in the spectra of grafted silk samples. The vibrational mode of the amide III lines of silk fibroin was significantly modified by grafting. © 1996 John Wiley & Sons, Inc.

## INTRODUCTION

The chemical modification of silk fibers by means of grafting techniques is potentially effective in improving the functional performance and promote a wider utilization of silk.<sup>1,2</sup> Methacrylamide (MAA) and 2-hydroxyethyl methacrylate (HEMA) have been widely applied on industrial scale as grafting agents for silk fibers.<sup>1,3–8</sup> Compared to other vinyl monomers used for silk grafting, such as styrene (St) and methyl methacrylate (MMA), MAA and HEMA are hydrophilic, so that grafted silk fibers usually exhibit an increased equilibrium moisture regain.<sup>1</sup> This fact, along with the appreciable bulkiness and

fullness of handle they confer on silk, has been considered responsible for the improved comfort of grafted silk fabrics.

The physical properties and textile behavior of MAA- and HEMA-grafted silk fibers are closely related to the amount of weight gain attained.<sup>1,3,7</sup> For textile applications, the most common weight gain values range from 20 to 45%. These values, besides ensuring a noticeable increase of silk weight, which is one of the main targets of the grafting techniques, may result in an appreciable improvement of some inferior textile performances<sup>1</sup> (crease recovery, thermal stability, wash and wear characteristics, etc.). However, when the weight gain exceeds the above limits, partial impairment of the intrinsic properties of silk fibers (handle, luster, dyeability) may occur.

Silk fibroin fibers exhibit an unusual combination of strength and toughness that distinguish them

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from other natural and synthetic fibers. These characteristics, as well as their chemical properties and good biocompatibility, have made silk an attractive material not only for textile but also for nontextile uses.<sup>9,10</sup> Applications in the biotechnological (enzyme immobilization) and biomedical (sutures) fields have already been reported.<sup>11</sup> Degummed silk fibers have been used as substrate. However, grafted silk fibers may present characteristics exploitable for nontextile applications. In this respect, the amount of weight gain might be increased over the limits currently adopted for textile purposes.

In a recent study, Tsukada et al.<sup>12</sup> reported a new method for the preparation of MMA-grafted silk fibers with high weight gain (>500%). Weight gain values higher than 100% can be obtained with MAA by means of the conventional reaction system with ammonium persulfate as an initiator.<sup>1</sup> In this work, we studied the reaction conditions for the preparation of MAA- and HEMA-grafted silk fibers with high weight gain values. Because the grafting techniques may induce some degradation on silk fibers (loss of tensile properties, yellowness), the effect of various reaction parameters was systematically studied. Grafted silk fibers were characterized for their physico-mechanical, thermal, and morphological properties. Raman spectroscopy was used to study the structural changes induced by grafting. While x-ray and IR measurements have been extensively applied to the study of the crystalline structure and molecular conformation of silk fibers, very little is known about Raman spectra of *Bombyx mori* silk fibroin.<sup>13-15</sup> Because this technique is a powerful tool for studying polymer conformation, morphology and structure modification at the molecular level, we thought it would be of interest to adopt this approach for the structural characterization of grafted silk fibers.

## EXPERIMENTAL

### Materials

Silk yarn, obtained from reeling of *Bombyx mori* cocoons, was degummed with an aqueous solution of 0.7% Marseille soap, at 98–100°C for 1 h. The yarn was thoroughly rinsed several times with deionized water and then dried at room temperature before being used as grafting substrate.

Reagent grade methacrylamide (MAA) and 2-hydroxyethyl methacrylate (HEMA), purchased from Wako Pure Chemicals, Ltd., were used without further purification.

MAA- and HEMA-grafted silk fibers were prepared as follows. Dried silk fibers (0.4 g) were immersed in a reaction system containing formic acid (HCOOH, 0.4 and 0.8 mL for MAA and HEMA grafting system, respectively), different amounts of monomer (0.1, 0.2, 0.3, 0.5, 0.7, and 1.2 g), and initiator (ammonium persulphate, APS), in order to keep the monomer/initiator ratio constant (100:1). The material-to-liquor ratio of 1:10 and 1:20 was maintained for MAA and HEMA, respectively. The reaction system was heated to 80°C in 45 min and maintained at the same temperature for 1 h. At the end of the reaction, the silk samples were washed with tap water, dried at 105°C for 2 h, and placed in a desiccator over silica gel before measurements. As control samples, silk fibers were treated in the same reaction system as mentioned above, without MAA or HEMA. Blank samples were prepared in the same way, varying the concentration of reagents.

Weight gain and grafting efficiency were calculated from the weight increase of silk yarn as follows:

$$\text{Weight gain (\%)} = (W_2 - W_1)/W_1 \times 100$$

$$\text{Efficiency (\%)} = (W_2 - W_1)/W_3 \times 100$$

where  $W_1$  and  $W_2$  and  $W_3$  denote the weight of the original silk sample, that of the same sample after grafting, and that of the monomer used, respectively.

### Measurements

Tensile properties of silk fibers were measured with an automatic tensile testing machine (Tensilon UTM-II, Toyo Baldwin Co., Ltd.) in standard conditions (20°C and 65% R.H.). Each value is the average of 20 measurements.

Color change of silk yarn was evaluated with a Shimadzu spectrophotometer (UV-3100S). The L/b ratio was calculated on the basis of the colorimetric parameters  $L^*$  and  $b^*$  provided by the computer system (UV-2100/3100) connected to the spectrophotometer. Because the parameters  $L^*$  and  $b^*$  denote lightness and yellowness, respectively, the change in the L/b ratio may be considered as an index of the color change of the samples, for instance, the lower the index, the higher the degree of yellowness.

Heat flow DSC measurements were performed on a Rigaku Denki instrument (DSC-10A) at a heating rate of 10°C/min. The DSC range and sample weight were 2.5 mcal/s and 2 mg, respectively. The open aluminium pan was swept with  $N_2$  gas during the course of the analysis.

NIR-FT Raman spectra were obtained by means of a Perkin-Elmer 1700X spectrophotometer (500 scans), equipped with a Spectron Laser System Continuous Wave Raman module. An Nd/YAG Laser (1064 nm) was used as exciting source.

The surface of MAA- and HEMA-grafted silk fibers was examined with a JEOL JAX-333S scanning electron microscope, after gold coating, at 15 keV acceleration voltage.

## RESULTS AND DISCUSSION

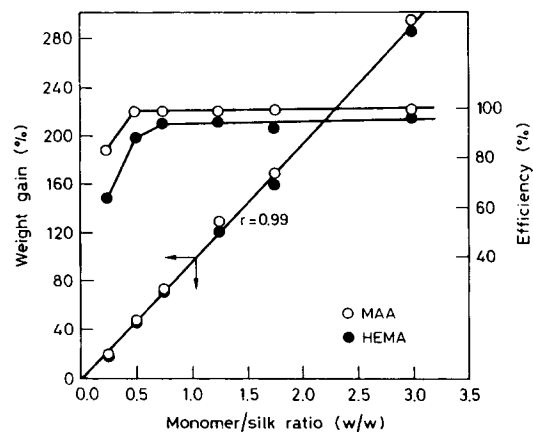
### Reactivity of MAA and HEMA

Grafted silk samples with different weight gains were prepared by varying the monomer concentration in the reaction system, the other parameters (time, temperature, liquor ratio, monomer/initiator ratio) being constant. Extremely high weight gain values, approaching about 300%, were obtained. The weight gain of both MAA- and HEMA-grafted silk fibers increased linearly with the amount of monomer added to the reaction system (Fig. 1). Linearity in the low weight gain range (< 50%) has been reported by other authors.<sup>3</sup>

The values of grafting efficiency were calculated on the basis of the weight gain and plotted as a function of the monomer/silk ratio (Fig. 1). It is interesting to note that both MAA and HEMA grafting systems exhibited a sharp increase of efficiency at low monomer/silk ratios (0.25–0.75 w/w), and then attained a plateau. While MAA reached 100% conversion, the equilibrium value of HEMA remained slightly lower (95%). However, SEM observations (see below) showed the presence of large amounts of homopolymer attached to the surface of HEMA-grafted silk fibers, especially at high monomer/silk ratios. Therefore, the remarkably high efficiency of HEMA is due to the contribution of both graft-copolymer and homopolymer.

A high efficiency, combined with a reduced level of homopolymerization, is a prerequisite for the industrial application of grafting techniques. In fact, the presence of homopolymer attached to the fiber surface may draw negative consequences on the physical properties and functional performances of silk fibers. MAA seems to meet the above requirements. Moreover, the present results show that, if needed, weight gain values much larger than those currently used on industrial scale may be attained, with a noticeably high grafting efficiency.

The high grafting efficiency of MAA is worthy of some comment. Besides other factors, the reactivity



**Figure 1** Weight gain and grafting efficiency of MAA- and HEMA-grafted silk fibers as a function of the monomer/silk ratio.

of vinyl monomers towards silk depends on the chemical structure of the molecule. For example, by comparing the efficiency of methacrylate derivatives, such as MAA and HEMA, with that of the corresponding acrylic acid derivatives (acrylamide, 2-hydroxyethyl acrylate), the former attained about 100%, while the latter never exceeded 20–30% (Tsukada et al., in preparation).

### Moisture Content and Size

MAA-grafted silk fibers exhibited a marked increase of moisture content as a function of the weight gain (Table I). The values of “observed” moisture content increased almost linearly in the low weight gain range (100%), and then showed a tendency to attain a saturation value at higher weight gains. The values of moisture content calculated on the basis of the amount of silk in the grafted sample fit a straight line ( $r = 0.998$ ). These results confirm the effectiveness of MAA grafting in enhancing the moisture absorption of silk. The increased hygroscopicity of MAA-grafted silk fibers has been recognized to play a positive role in improving the comfort of silk fabrics.

HEMA was expected to enhance the hygroscopicity of silk as well. However, the moisture content of HEMA-grafted silk fibers remained rather low, even at high weight gains. This behavior should be primarily attributed to the presence of homopolymer adhering to the surface of silk fibers, which might have interfered with the absorption of moisture.

Among the morphological changes induced by grafting, the increase of the transverse dimension is one of the most noticeable effects. These changes may influence to some extent the functional prop-

**Table I** Weight Gain and Moisture Content of MAA- and HEMA-Grafted Silk Fibers

Weight Gain (%)	Moisture Content (%)	
	Observed <sup>a</sup>	Calculated <sup>b</sup>
Control	8.0	—
MAA		
19.9	8.7	10.4
48.5	13.5	20.0
73.2	16.0	27.7
129.3	20.0	43.8
168.7	24.0	64.5
291.9	27.0	105.6
HEMA		
18.1	10.6	12.5
47.7	10.2	15.1
71.8	10.3	17.7
120.5	10.6	23.4
159.2	9.9	25.7
283.4	11.8	45.2

<sup>a</sup> Referred to the total weigh of the sample (silk + grafted polymer).

<sup>b</sup> Referred only to the amount of silk in the sample.

erties of silk fibers, such as tensile behavior, handle, luster, etc. As shown in Figure 2, the size of grafted silk fibers increased linearly with increasing weight gain. The linear relationship between weight gain and size has already been reported for other vinyl monomers,<sup>16</sup> although the range of weight gain explored rarely exceeded 100%. The results obtained in this work show that this relationship is valid also at extremely high weight gains, as those obtained with MAA.

### Tensile Properties

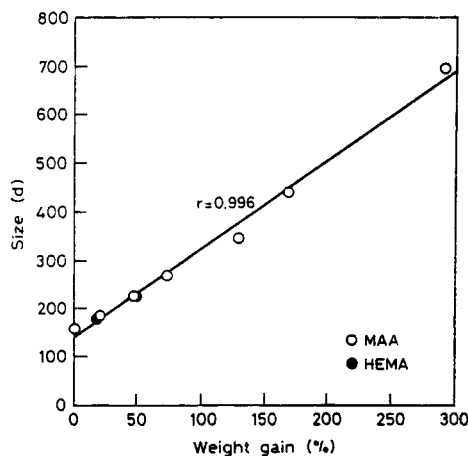
Because the grafting reaction may affect the tensile properties of silk fibers, the influence of various reaction parameters has been systematically studied by preparing a series of blank samples. The results of the tensile measurements are listed in Table II. All blank treated samples (Control, A-F) exhibited a significant loss of breaking load, elongation at break, and energy. Comparing the treated samples among them, only sample F is significantly different from the others, while sample E is at the limit for the rejection of the null hypothesis (*t*-test).

These results lead to some interesting comments. The behavior of the control sample demonstrates that a wet treatment, conducted at high temperature and neutral pH, may cause a partial degradation of the tensile properties of silk fibers, in good agree-

ment with the results reported by Vogt et al.<sup>17</sup> A selective effect of the chemicals used for the grafting reaction appears only when the amount of formic acid is doubled (sample F), while the effect of formic acid in combination with a high amount of initiator (sample E) may be questionable. A specific influence of the initiator alone can be excluded, at least on the basis of the results obtained in this work.

The tensile properties of MAA- and HEMA-grafted silk fibers as a function of the weight gain are shown in Figure 3. The values of breaking load of MAA-grafted silk fibers [Fig. 3(A)] exhibited a tendency to increase with increasing weight gain, as shown by the slight positive slope of the curve. This behavior is in good agreement with previously reported results.<sup>1</sup> HEMA-grafted silk fibers showed slightly lower breaking load values, probably due to the higher amount of formic acid used in the reaction system (hydrolytic degradation). As expected from the relation between size and weight gain (see Fig. 2), the tenacity of grafted silk fibers decreased [Fig. 3(B)]. The drop is steeper at low than high weight gains, probably due to the slight increase of breaking load.

Both elongation at break and energy at break [Fig. 3(C-D)] exhibited a rather similar trend, decreasing linearly as the amount of weight gain increased. Taking into account that the values of breaking load remained essentially unchanged, the change in elongation behavior should be mainly attributed to a selective effect of the grafted polymer on the extensibility of silk fibers. The polymer grown in the course of the grafting process filled the space available within the fiber matrix, therefore perturbing



**Figure 2** Relationship between weight gain and size of grafted silk fibers. High weight gain HEMA-grafted samples were not used due to the extensive homopolymerization that occurred on the surface of silk fibers.

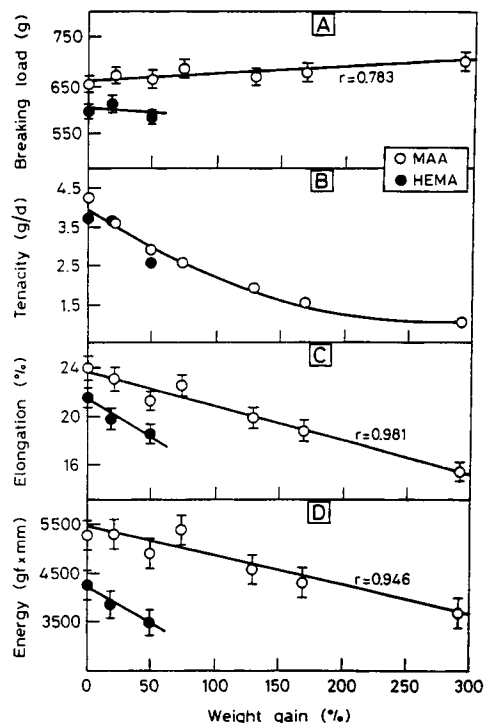
the arrangement of the fibroin chains in the amorphous regions and partially hindering their mobility when subjected to tension. Energy decreased as well, because the drop of elongation occurred at constant breaking load values.

### Fiber Yellowness

Yellowing of silk fibers may occur during grafting.<sup>18</sup> To overcome this drawback, suitable aftertreatments are conducted, such as bleaching with sodium hydrosulfite. To study the influence of the grafting process on silk yellowing, silk samples were treated with different amounts of reagents (HCOOH, APS), without grafting agent. The degree of yellowness of silk fibers was evaluated spectrophotometrically, by means of the L/b ratio.<sup>19</sup>

Silk fibers untreated, blank treated with water, and formic acid showed L/b values of 31.3, 21.1, and 20.2, respectively. These results suggest that a wet treatment conducted at high temperature caused a decrease of the degree of whiteness of silk fibers. Moreover, the samples treated with water and formic acid did not differ significantly, suggesting that the acid had only a negligible effect on silk yellowing. The addition of initiator to the reaction system caused a further decrease of the L/b ratio, either with water (14.2) or formic acid (11.5). The combination HCOOH-APS appeared more aggressive towards silk.

To verify the influence of the initiator on silk yellowing, samples were treated with increasing amounts of APS, simulating the conditions of the grafting reaction. The L/b values of the blank samples, as well as those of MAA-grafted silk fibers, were plotted as a function of the amount of initiator



**Figure 3** Breaking load (A), tenacity (B), elongation at break (C), and energy (D) as a function of the weight gain. High weight gain HEMA-grafted samples were not used due to the extensive homopolymerization that occurred on the surface of silk fibers.

in the reaction system (Fig. 4). The data fit a linear regression curve, whose correlation coefficient ( $r = 0.834$ ) passed the test of significance. The null hypothesis can, therefore, be rejected, and it is possible to conclude that there is association between the two variables.

**Table II** Effect of the Grafting Conditions on the Tensile Properties of Silk Fibers

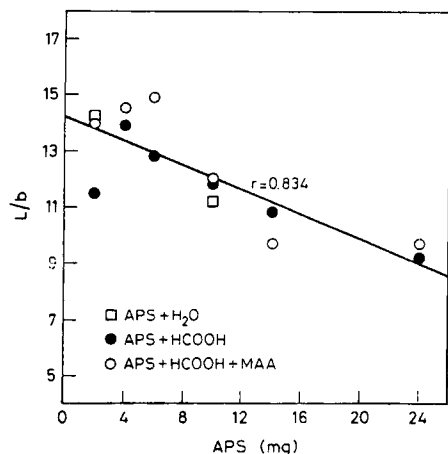
Sample	H <sub>2</sub> O (mL)	HCOOH (mL)	APS (mg)	Breaking Load (g)	Elongation (%)	Energy (gf × mm)
Untreated	—	—	—	749 ± 19	26.9 ± 1.0	6961 ± 319
Control	4	—	—	638 ± 17	24.4 ± 0.9	5184 ± 304
A	—	4	—	634 ± 16	24.4 ± 1.0	5236 ± 314
B	4	—	2	655 ± 18	24.6 ± 1.0	5433 ± 315
C	—	4	2	654 ± 18	23.9 ± 0.9	5275 ± 306
D	4	—	10	657 ± 18	25.0 ± 1.0	5515 ± 313
E*	—	4	10	617 ± 15	23.0 ± 0.9	4713 ± 296
F†	—	8	2	597 ± 16	21.5 ± 0.8	4253 ± 313

No. of measurements per sample = 20.

Significance level of  $t(19, 95\%) = 2.09$ .

\*  $t(19, 95\%) \cong 2.09$ .

†  $t(19, 95\%) \gg 2.09$ .



**Figure 4** Relationship between the degree of yellowness of silk fibers and the amount of ammonium persulfate (APS) in the reaction system.

The above results emphasize that two factors may influence the degree of yellowness of grafted silk fibers. The wet treatment itself is responsible for the decrease of fiber whiteness. Additionally, higher amounts of initiator may induce a further loss of fiber whiteness. This effect might be related to the free radical initiation mechanism of the redox system used for silk grafting.

### Thermal Behavior

The thermal behavior was studied by means of differential scanning calorimetry (DSC). DSC thermograms of MAA- and HEMA-grafted silk fibers have already been reported in previous articles.<sup>6,7</sup> Following grafting, the decomposition temperature of silk fibroin slightly shifted upwards. The availability of samples with extremely high weight gain values permitted us to study the thermal behavior in detail.

The DSC thermogram of silk fibroin is characterized by a broad and large endotherm, with a peak temperature at around 315°C, attributed to the thermal decomposition of fibroin chains with oriented  $\beta$  sheet structure.<sup>20</sup> The peak values of untreated and grafted silk fibers were plotted as a function of the weight gain (Fig. 5). The data fit a straight line, whose positive slope indicates that the decomposition temperature of silk fibroin increased with increasing weight gain.

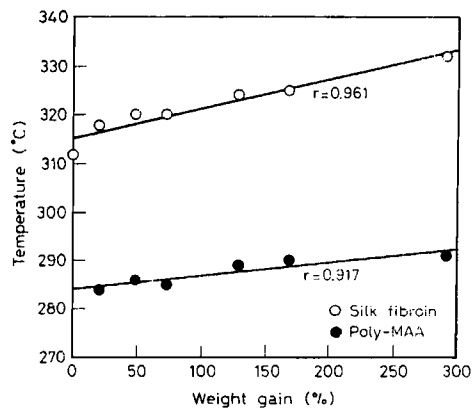
The DSC thermograms of MAA-grafted silk fibers exhibited an additional endothermic transition at about 280°C, attributed to the poly-MAA chains immersed in the silk fiber matrix.<sup>6</sup> It has been observed that the peak temperature of this transition

is linearly related to the weight gain (Fig. 5). As the latter increased, the peak shifted upwards.

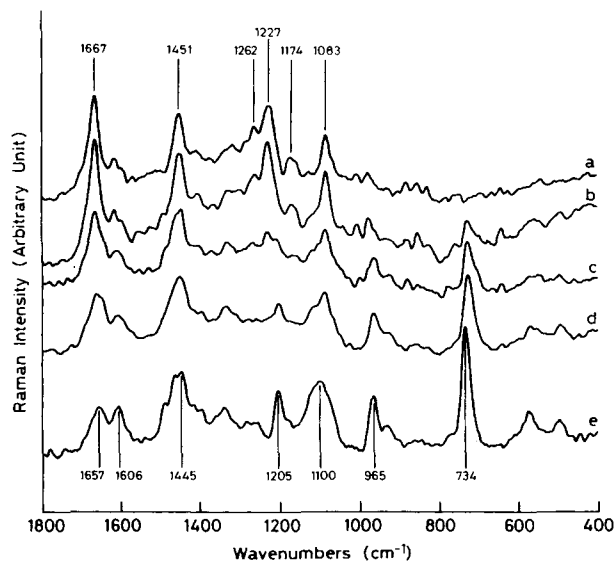
The presence of separate thermal transitions in the DSC curves of MAA-grafted silk fibers may be considered indicative of a poor compatibility between the grafted polymer and the fibrous substrate. However, the behavior of the major endothermic transition attributed to silk fibroin suggests that grafting gave a higher thermal stability to silk fibers. This conclusion well agrees with thermomechanical (TMA), thermogravimetric (TGA), and dynamic mechanical (DMA) data reported in a previous study.<sup>6</sup> The upward shift of the thermal transition attributed to the grafted poly-MAA chains might be related to their molecular weight. In a study on the molecular weight of poly-methyl methacrylate separated from grafted silk fibers,<sup>21</sup> we demonstrated that the increase of weight gain above 50–60% mainly occurred through elongation of the grafted polymer. Therefore, the higher the weight gain, the higher the average molecular weight of grafted polymer chains. The thermal behavior seems to support this hypothesis.

### Raman Spectra

Raman spectra of MAA- and HEMA-grafted silk fibers with different weight gains were recorded in the range 1800–400  $\text{cm}^{-1}$  and displayed as intensity of scatter against frequency shift ( $\text{cm}^{-1}$ ). Because proteins usually exhibit very complicated Raman spectra, due to the contribution of both chemical and conformational features, the spectra of untreated silk fibers, poly-MAA, and poly-HEMA homopolymers were used as reference in order to interpret the spectral changes induced by grafting.



**Figure 5** Thermal behavior of silk fibroin and grafted poly-MAA as a function of the weight gain.



**Figure 6** Raman spectra of MAA-grafted silk fibers with different weight gain values. (a) Untreated sample; (b) 19.9%; (c) 129.3%; (d) 291.9%; (e) Poly-MAA.

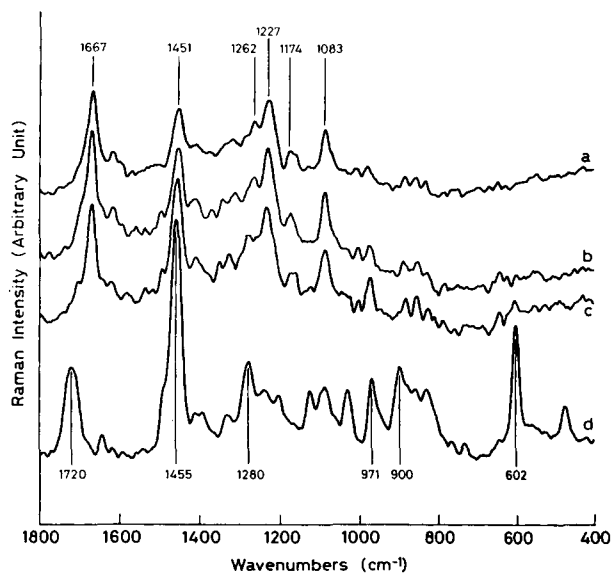
The Raman spectrum of untreated silk fibers [Fig. 6(a)] showed characteristic vibrations in the spectral region 1700–1000  $\text{cm}^{-1}$ . The amide I (C=O stretching vibration) and III (C–N stretching, C–N–H in plane bending) modes, with sharp and strong lines at 1667 and 1227  $\text{cm}^{-1}$ , respectively, are sensitive to the molecular conformation of the polypeptide chains.<sup>14</sup> The position and intensity of these lines is typical of silk fibroin with antiparallel  $\beta$ -sheet structure.<sup>14</sup> In addition, a strong line appeared at 1451  $\text{cm}^{-1}$ , attributed to the  $(\text{CH}_2)(\text{CH}_3)$  bending deformation mode. The region of skeletal stretching vibrations displayed an intense line at 1083  $\text{cm}^{-1}$  (C–N stretching). Among the less intense lines, that at 1172  $\text{cm}^{-1}$ , assigned to the vibration of tyrosine side chains,<sup>14</sup> is characteristic of the  $\beta$ -sheet structure. Similarly to most  $\beta$ -sheet polypeptides, the Raman spectrum of silk fibroin exhibited only weak lines at a frequency  $< 1000 \text{ cm}^{-1}$ .

The Raman spectrum of poly-MAA [Fig. 6(e)] is characterized by strong lines at 1445 and 734  $\text{cm}^{-1}$ , assigned to  $(\text{CH}_2)(\text{CH}_3)$  bending vibration and (C–C) stretching of the aliphatic chain, respectively. The Raman resonances at 1606 and 1100  $\text{cm}^{-1}$  are attributable to the (C=N) and (C–N) stretching, respectively. The spectrum of MAA-grafted silk fibers with 20% weight gain [Fig. 6(b)] showed a Raman vibrational pattern similar to the control sample. The characteristic lines of silk fibroin maintained their position and intensity. A new line appeared at 734  $\text{cm}^{-1}$ , together with another in shoulder form at 1340  $\text{cm}^{-1}$ , both attributable to the

grafted poly-MAA chains. The line at 734  $\text{cm}^{-1}$  could be related to the amount of grafted polymer, because its intensity increased with increasing weight gain above 100%. Overlapping of both silk fibroin and poly-MAA lines was observed in the Raman spectra of the samples with high weight gain [Fig. 6(c,d)]. It is interesting to note that in the sample with 292% weight gain, silk fibroin still exhibited strong and sharp lines at 1667 and 1083  $\text{cm}^{-1}$ . On the other hand, the intensity of the amide III mode decreased significantly. The higher intensity of the line at 1451  $\text{cm}^{-1}$  can be attributed to the contribution of the methylene deformation mode of both grafted poly-MAA and silk fibroin chains.

The Raman spectra of HEMA-grafted silk fibers [Fig. 7(b,c)] showed strong lines at 1667, 1451, 1227, and 1083  $\text{cm}^{-1}$  characteristic of untreated silk fibroin [Fig. 7(a)]. With increasing weight gain, the amide I mode remained unchanged, while amide III slightly broadened. The line at 1451  $\text{cm}^{-1}$  exhibited a sharp increase in the sample with 48% weight gain [Fig. 7(c)], due to the contribution of the grafted poly-HEMA chains, which are characterized by a strong line in this spectral region [Fig. 7(d)]. New lines appeared at 602, 900, and 1280  $\text{cm}^{-1}$ . These can be related to the presence of the grafted poly-HEMA chains.

The findings emerging from the Raman spectroscopy analysis of grafted silk fibers lead to the following comments. Overlapping of the Raman lines typical of both silk fibroin and grafted polymer



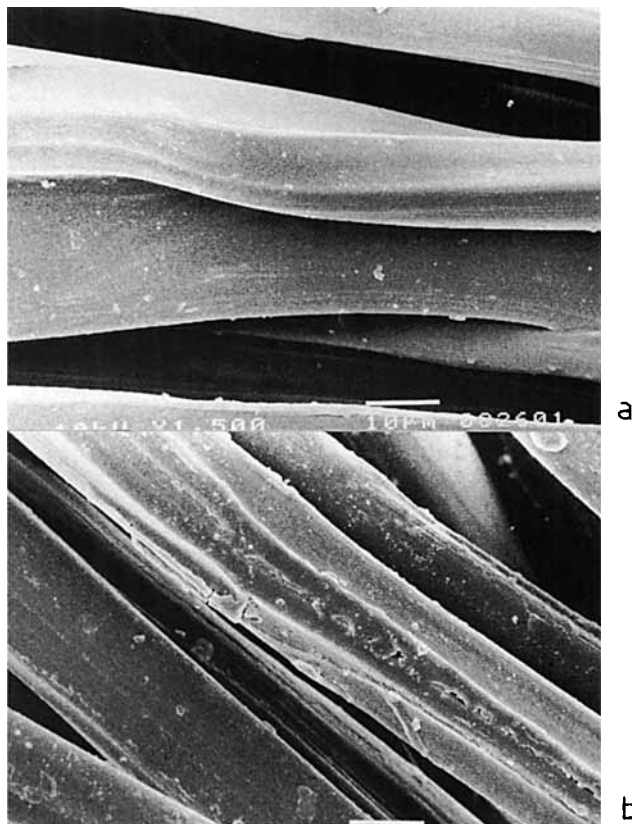
**Figure 7** Raman spectra of HEMA-grafted silk fibers with different weight gain values. (a) Untreated sample; (b) 18.1%; (c) 47.7%; (d) Poly-HEMA.

chains is the most typical feature observed. The  $\beta$  sheet conformation of silk fibroin fibers remained unchanged regardless of grafting. This is confirmed by the absence of any shift of the conformational sensitive amide I and III lines. This result is consistent with x-ray and IR measurements previously reported.<sup>3,6-8</sup> The behavior of the amide III mode is worthy of some comment. This region is characterized by the presence of a strong line at  $1227\text{ cm}^{-1}$ , accompanied by a weak line at  $1262\text{ cm}^{-1}$ , which were attributed to the  $\beta$ -sheet conformation and to the disordered structure of silk fibers,<sup>14</sup> respectively. Raman investigation of poly-(Gly-Ala) with anti-parallel  $\beta$ -sheet structure, a model polypeptide for silk fibroin, exhibited a similar shape for the amide III mode.<sup>14</sup> As the weight gain of MAA- and HEMA-grafted silk fibers increased, the intensity of the line at  $1227\text{ cm}^{-1}$  progressively decreased, while that at  $1262\text{ cm}^{-1}$  remained unchanged or slightly increased. The presence of the grafted polymer in the amorphous regions of the fibers might have influenced the vibrational modes of the fibroin chains. This behavior, as well as the tendency to band broadening, might be interpreted as an increase of the degree of disorder of silk fibers induced by grafting. These data are consistent with previous results on the optical properties of MAA- and HEMA-grafted silk fibers, showing that birefringence decreased with increasing weight gain.<sup>6,7</sup>

### Surface Characteristics

The morphological characteristics of MAA- and HEMA-grafted silk fibers were examined by scanning electron microscopy. The surface of MAA-grafted silk fibers with 48.5% weigh gain [Fig. 8(A)] was as smooth as that of untreated silk fibers, while the presence of foreign deposits attributable to homopolymerization was observed on the surface of the sample with a weight gain of 291.9% [Fig. 8(B)]. However, taking into account the high weight gain value attained, the amount of homopolymer is negligible. Therefore, it is possible to conclude that the graft-copolymerization of MAA onto silk fibers proceeded with high efficiency, the formation of graft-copolymer being favored under the reaction conditions adopted.

HEMA-grafted silk fibers with 47.7% weight gain exhibited a smooth and clean surface [Fig. 9(A)]. At 71.8% weight gain, a thin polymeric film seems to cover part of the fiber surface, forming bridges between adjacent silk fibers [Fig. 9(B)]. As the weight gain increased further, a thick layer of poly-HEMA almost completely covered the surface of the fibers



**Figure 8** SEM photographs of MAA-grafted silk fibers with different weight gain values. (a) 48.5%; (b) 291.9%.

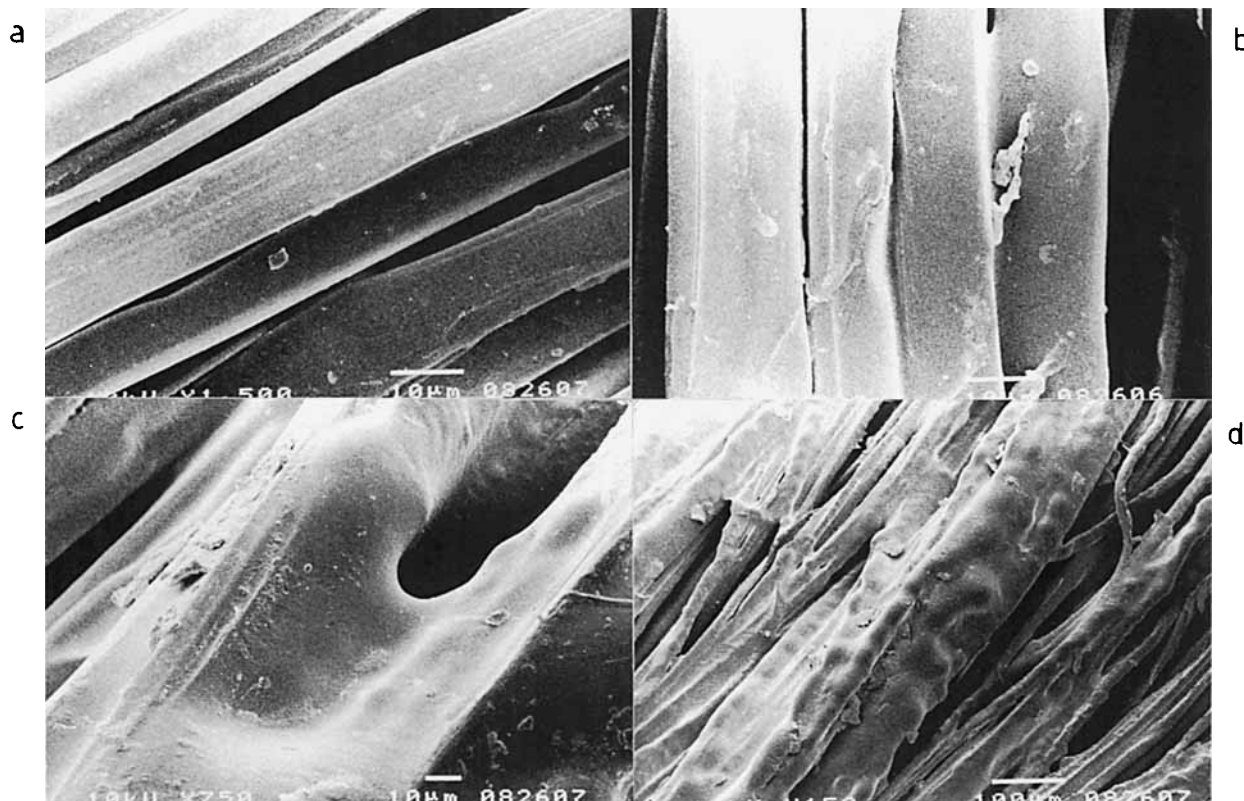
[Fig. 9(C-D)]. The homopolymer is supposed to establish strong interactions with silk, by means of physical forces or chemical bonds, because it is resistant to hot wet treatments in the presence of surface active agents.

### CONCLUSIONS

MAA-grafted silk fibers with high weight gain (up to 300%) can be obtained by means of the conventional reaction system (chemical initiation with APS in aqueous medium). The reaction efficiency approached 100%, while the extent of homopolymerization was negligible. On the other hand, the grafting of HEMA resulted in a high extent of homopolymer formation, especially at high monomer/silk ratios. This behavior can be explained taking into account the different reactivity of the two vinyl monomers.

The conditions of grafting (temperature, time) were found to play the major role in lowering the tensile properties and whiteness of silk fibers. Additional damage, though negligible, may be produced





**Figure 9** SEM photographs of HEMA-grafted silk fibers with different weight gain values. (a) 47.7%; (b) 71.8%; (c) 120.5%; (d) 159.2%.

by the chemicals (HCOOH, APS, monomers). A specific effect of the initiator on silk yellowing has been demonstrated.

The breaking load of grafted silk fibers remained unchanged, while the elongation at break decreased. Moreover, the thermal behavior was characterized by a higher thermal stability. Both effects might be related to the interaction between the grafted polymer and the fibroin molecules in the amorphous regions. In fact, the results of Raman spectroscopy showed that some vibrational modes of silk fibroin (amide III) are strongly modified by the presence of the grafted polymer.

This study represents the first attempt to apply Raman spectroscopy to the characterization of grafted silk fibers. The results obtained seem highly promising for the development of this analytical approach in the conformational study of natural protein fibers. Further studies are in progress concerning the interpretation of the Raman spectra with the view of establishing structure–frequency correlations.

Some of the results reported in this study are particularly attractive in view of developing new textile-based composite materials for biomedical

applications. For example, by optimizing the grafting conditions with HEMA, poly-HEMA chains may grow within the fiber matrix, as well as on the fiber surface, therefore creating a sort of continuous polymeric phase entrapping the textile material. This new type of composite is expected to perform better than the traditional one, due to the close physico-chemical interactions between the two components. While silk fibers may confer the desired mechanical properties on the composite, the polymer in which they are embedded may represent an optimum substrate for the interaction with the biological environment, with the function of preserving the fibers from the degradation phenomena that might be activated as a response of cell interaction. This approach is under study and the preliminary results confirmed the potentiality of the grafting techniques for the preparation of new types of composite materials.

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