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BIURET STAINING AND X-RAY MICROANALYSIS FOR LOCATING GRAFTED POLY(METHYL ACRYLATE) ON WOOL FIBERS

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Abstract

Biuret reagent was used to stain wool fibers with copper so the location of a small amount of poly(methyl acrylate) grafted onto the fibers could be determined by energy dispersive x-ray microanalysis of copper. The grafted polymer was determined to be located in regions of the fibers where cuticle had been previously damaged. The amount of grafted polymer present was too small for secondary electron imaging to be useful for locating the polymer grafts.

KEY WORDS: scanning electron microscopy, copper staining, x-ray microanalysis, graft polymerization, Biuret reagent, wool fiber, cuticle.

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Introduction

We previously reported that recycled wool fibers were processed into fabric and then a small amount of poly(methyl acrylate) was grafted onto the fibers [1]. The fabric was found to exhibit increased abrasion resistance without increased stiffness. This suggested that grafted polymer was located primarily in damaged cuticle regions rather than in all cuticle regions of the fibers.

The large structural variation among different fibers in non-grafted recycled wool fabric and the small amount of poly(methyl acrylate) present on the fibers made it impossible to ascertain clear morphological differences between grafted and non-grafted fibers using secondary electron imaging. Energy dispersive x-ray microanalysis (EDX) could not be used to locate the grafted polymer directly since poly(methyl acrylate) did not contain elements we could detect. Energy dispersive x-ray microanalysis of osmium tetroxide stained fibers failed even though osmium tetroxide staining has been widely used to differentiate among various morphological components of wool because osmium concentrations detected by EDX in our samples did not vary enough to distinguish among undamaged cuticle, damaged cuticle and grafted poly(methyl acrylate).

We devised a procedure to indirectly determine the location of grafted poly(methyl acrylate) on the wool fibers using EDX. This procedure used the classic Biuret reagent which reacts with proteins to form copper-containing complexes [2,3]. Since wool fibers are proteinaceous, they can be stained by copper in the Biuret reagent. On the other hand, pieces of poly(methyl acrylate) treated with Biuret reagent for extended periods of time followed by EDX analysis for more than 200 s showed no detectable copper. Therefore, if a fiber had been previously grafted with poly(methyl acrylate) and then subsequently placed in the Biuret reagent, we expected less copper to be detected in fiber regions covered with polymer grafts because the polymer would mask the wool protein and reduce copper staining.

Attempts to locate copper by x-ray imaging failed because the concentration of copper on Biuret stained fibers was necessarily small since we desired to restrict

staining to the wool fiber surface rather than stain the fiber bulk. Consequently, we counted copper K_α x-rays in undamaged and visibly damaged regions of the wool fibers and compared counts from grafted and non-grafted fibers.

Materials and Methods

This study is based on a rather detailed process reported elsewhere [1]. In that study, recycled wool fabric was subjected to a pad, rinse and cure/dry treatment sequence. The pad bath contained methyl acrylate monomer, ceric ammonium sulfate for initiation of polymerization, an organic surfactant to aid mixing, sulfuric acid for pH adjustment and distilled water. After immersion of fabric in the treatment bath, it was rinsed in distilled water, cured and dried in air. The treatment process used in this study was similar except individual fibers rather than fabric were treated.

In preliminary experiments in which we compared individual whole fibers, variability among fibers was so great that data interpretation after Biuret staining was impossible. Recycled wool fabric contains fibers from many different sources and fibers vary greatly in chemical composition and physical condition. As a result of this variability, we cut whole fibers in half, grafted one half of each with polymer while the other half was not grafted, and compared the fiber halves to one another. Pairing of fiber halves in this manner minimized chemical and physical differences among fiber specimens and provided statistically meaningful data from only six fibers.

The various steps involved in this procedure are illustrated schematically in Figure 1. Individual fibers were removed from the untreated, recycled wool fabric and each fiber was cut into halves approximately 15 mm long. Each fiber half was attached to a carrier so that the fiber half could be easily handled and identified. Plastic carriers could not be used because they poisoned the treatment bath, so we used tantalum foils approximately 10 mm x 5 mm as carriers. Tantalum foil was readily available, inexpensive, soft enough to crimp the fibers in place and was relatively stable in the acidic treatment bath. A foil was folded in half across its long axis, one end of a fiber was secured in place by crimping the foil with pliers and the foil was notched in an identifiable way.

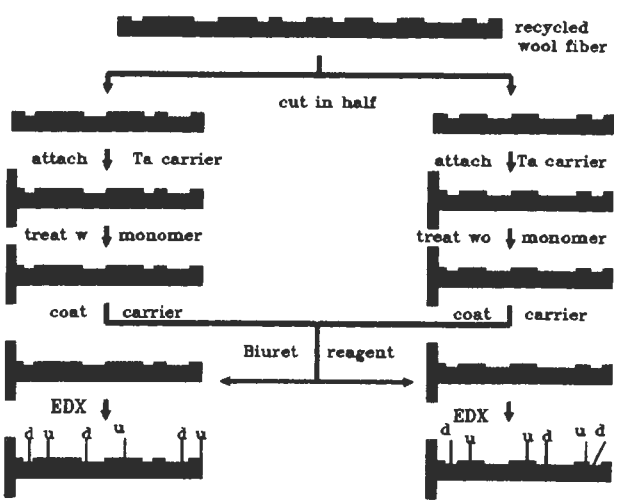

Figure 1. Schematic illustration of experimental procedure with one fiber having damaged surface on one side. Key: d = damaged cuticle region; u = undamaged cuticle region; w = grafting treatment with monomer; wo = grafting treatment without monomer; EDX = energy dispersive x-ray microanalysis for copper.

After attachment of each fiber half to a tantalum carrier, one half of each original fiber was randomly selected to be treated with the graft polymerization process. The other half of each fiber was treated similarly but with methyl acrylate monomer excluded from the treatment bath. After treatment, all samples were dried and conditioned at a temperature of 294 $\pm$ 1 K (70 $\pm$ 2 F) and a relative humidity of 65 $\pm$ 2%.

After conditioning the fibers, the tantalum carriers were coated by immersing them into a solution of poly(methyl methacrylate) in chloroform, removing them and allowing the chloroform to evaporate. Care was taken to completely immerse the tantalum into the solution but minimize the contact of the solution with the fibers. This coating process was repeated until a film approximately 0.25 mm thick coated each carrier.

After coating the carriers with poly(methyl methacrylate), all fiber halves were stained simultaneously with Biuret reagent consisting of 0.15% $CuSO_4 \cdot 5H_2O$, 0.6% sodium potassium tartrate and 3.0% sodium hydroxide in distilled water [2,3]. The staining procedure involved immersing fibers in the Biuret reagent for three minutes, rinsing them in deionized water for three minutes and then drying them in air. The three minute immersion time was chosen to maximize copper deposition on the fiber surfaces and to minimize deleterious effects of the basic (pH = 11.5) Biuret reagent on the wool fiber morphology.

Colloidal graphite paste was used to attach each fiber half and its carrier to carbon planchets mounted on aluminum stubs. Since tantalum L_{α_2} and copper K_α x-rays are close in energy (8.14 and 8.04 kV, respectively), care had to be taken to prevent tantalum from contributing x-rays to the copper peak. Consequently, each carrier was coated with a thick layer of colloidal graphite paste when attaching specimens to the planchets even though each tantalum carrier had been covered with a 0.25 mm thick layer of poly (methyl methacrylate) as previously described. Finally, fibers were evaporatively coated with carbon to decrease charging.

Scanning electron microscopy and EDX were performed with an ETEC Autoscan U-1 scanning electron microscope equipped with an Ortec energy dispersive x-ray detector and a Norland-Inotech multichannel analyzer. The specimen was positioned as described by Roomans [4] and only areas between his positions number 1 and number 2 were analyzed. Energy dispersive x-ray microanalysis was performed using a 20 kV accelerating voltage for a period of

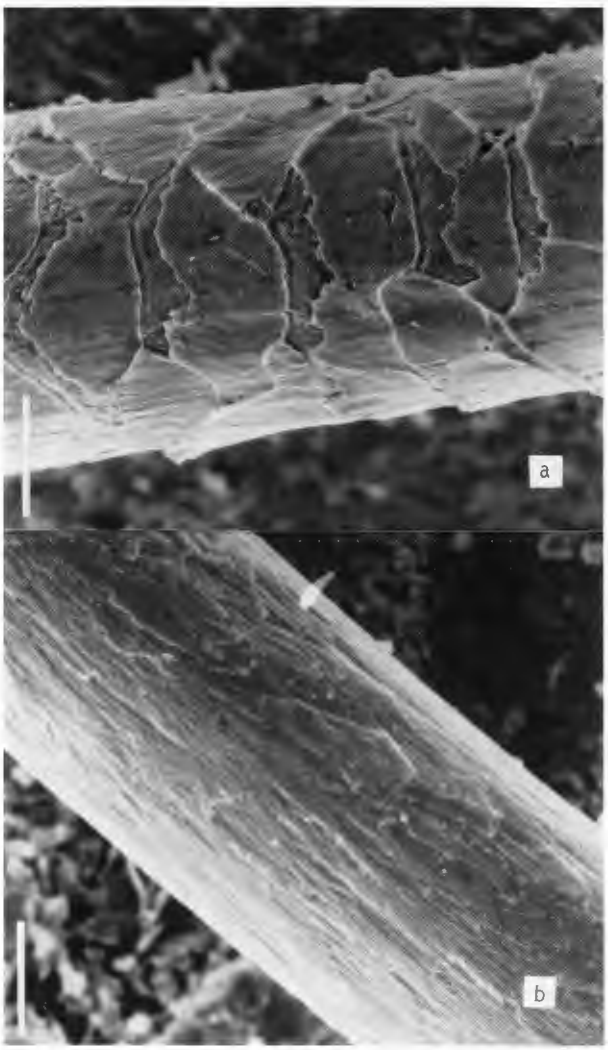

Figure 2. Recycled wool fibers showing: a) displaced cuticle, and b) underlying cortex exposed by severely damaged cuticle.

100 s. Longer analysis times caused visible changes in regions of the fibers where cuticle damage existed. The size of the electron probe used during EDX was unknown, but a reduced area was analyzed.

Three regions on each fiber half where cuticle damage exposed underlying cortex were randomly selected and analyzed by EDX in the vicinity of the copper K_α x-ray peak. In addition, undamaged cuticle immediately adjacent to each damaged region also was analyzed. To illustrate this procedure in Figure 1, damaged areas are labeled "d" whereas adjacent undamaged areas are labeled "u". Background counts under the copper K_α peaks were estimated using a linear regression equation determined from counts in five multichannel analyzer channels immediately before and five immediately after the peak boundaries. Background counts calculated by the regression equation were summed over all the channels in the peak to estimate the total background. The number of copper

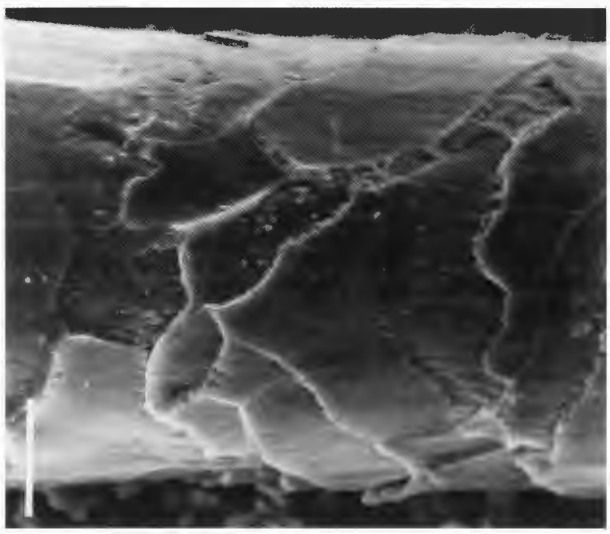

Figure 3. Recycled wool fiber after grafting with poly(methyl acrylate).

counts associated with each peak were estimated by subtracting the total background from total peak counts.

Results

Figure 2 shows secondary electron images of the recycled wool fibers extracted from the fabric prior to any treatment. These images show the great variability in fiber size and morphology that exists in recycled wool fabric. Mechanical damage can be seen to include chipped and displaced cuticular material as shown in Figure 2a, as well as severely damaged cuticle with disruption of the underlying cortex as shown in Figure 2b. After treatment by graft polymerization, no morphological changes in the fibers could be clearly identified in secondary electron images. A typical fiber grafted with poly(methyl acrylate) is shown in Figure 3. When compared with the ungrafted fibers in Figure 2, the grafted fiber in Figure 3 shows a lack of distinctive morphological features that could be used to directly identify poly(methyl acrylate) grafts on the wool fibers.

Coating the tantalum carriers with graphite paste and poly(methyl methacrylate) was necessary because we found that the amount of copper detected in the damaged areas on each of the fiber halves increased with increasing proximity to the tantalum carriers if they were not coated. We concluded that this effect was due to tantalum exerting a synergistic effect on the absorption of copper during staining rather than copper contributing tantalum x-rays to the copper K_α peak. The reasons for this conclusion were (1) increases in copper were observed only in damaged areas of fibers, (2) direct excitation of tantalum carriers that had been coated with poly(methyl methacrylate) and colloidal graphite did not produce detectable tantalum x-rays, and (3) coating the carriers with poly(methyl methacrylate) prior to Biuret staining eliminated the copper concentration gradient.

Copper K_α counts for six fibers (12 fiber halves) are shown in Table 1. Copper counts were collected from three different regions on each fiber half where the cuticle appeared visibly damaged and three different undamaged regions adjacent to each damaged region. Thus, 72 fiber regions were analyzed. Means for each set of three copper K_α counts also are included in this table. The great variability among fibers in recycled wool fabric can be appreciated from the data in Table 1. For example, the mean copper K_α counts for undamaged areas on each of the six fiber halves treated without monomer ranged from 102 to 786.

Table 2 summarizes the count in Table 1 as grand mean values for each fiber half for each treatment. The great fiber variability inherent in recycled wool fabric made it necessary to evaluate the data in a way that cancelled out some variability. This was accomplished by utilizing paired samples rather than independent samples. The overall means in Table 2 were compared two ways using a Paired "t" Test with each fiber half as one member of a pair and data variability was reduced by comparing only similar fiber regions to one another. That is, damaged fiber regions treated with monomer were compared to damaged fiber regions treated without monomer. Similarly, the undamaged regions treated with monomer were compared to undamaged regions treated without monomer. Copper K_α counts from damaged fiber regions were not compared to counts from undamaged regions because the damaged regions were visibly susceptible to deterioration by the electron beam whereas the adjacent undamaged regions were not visibly affected. Consequently, copper counts would be expected to depend on both the nature of the damage sustained by the fiber and the interaction of the fiber with the electron beam so data variability would be expected to increase.

The probability that mean copper K_α counts for damaged cuticle treated with and without monomer are equal was found to be low ($p = 0.0246$). This indicates that graft polymerization (treatment including monomer) significantly decreased copper K_α counts in damaged regions of the wool fibers. On the other hand, the probability that mean copper K_α counts for undamaged cuticle regions treated with and without monomer are equal was found to be high ($p = 0.7979$). This indicates that graft polymerization did not significantly change the copper K_α counts in undamaged cuticle regions. These two conclusions taken together provide evidence that poly(methyl acrylate) was located primarily in the damaged cuticle regions of the wool fibers.

Use of the Paired "t" Test depends on the assumption that the population of differences between each sample in a pair is normally distributed. The data was evaluated and was found to be normally distributed. However, a signed-rank test which does not assume normality was used to evaluate the data and the statistical results were essentially the same as those of the Paired "t" Test.

Conclusions

Scanning electron microscopy and EDX of wool fibers stained by copper from Biuret reagent allowed the location of small amounts of poly(methyl acrylate) grafted on the fibers to be determined. The grafted polymer was found to be preferentially located in regions where cuticle had been previously damaged rather than in undamaged cuticle regions of the fibers. Secondary electron images were not useful for locating the grafted polymer.

Acknowledgements

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Discussion with Reviewers

G.M. Roomans: Are you sure that biuret staining is restricted to the surface of the fibers? At an accelerating voltage of 20 kV you will penetrate several micrometers into the fibers and a large part of the signal will be generated at some depth in the fibers. Does this affect your results?

Authors: Although our aim was to stain the fiber surfaces, we do not know if staining was actually limited to the surfaces. We do not think that the absence or presence of bulk staining affects our conclusions, however, because all fiber halves were stained simultaneously and we compared copper in similar fiber regions rather than from different fiber regions. If monomer reacted equally with damaged and undamaged regions, we expected a decrease in copper staining in both regions whereas if monomer reacted only with the damaged regions, we expected a decrease in staining in the damaged regions but not the undamaged regions. Although copper detected in the fiber bulk would be expected to obscure these observations, it would not change the basic effects.

TABLE 1. Copper Counts of Fibers After Staining With Biuret Reagent.

Fiber Number		Copper K_α Counts: Half Treated Without Monomer — Damaged Regions	Half Treated Without Monomer — Adjacent Undamaged Regions	Half Treated With Monomer — Damaged Regions	Half Treated With Monomer — Adjacent Undamaged Regions
1		1085	318	1100	683
		1076	350	523	291
		997	520	420	180
	mean	1053	396	681	385
2		2904	408	1144	935
		1307	418	1826	905
		951	485	1068	97
	mean	1721	437	1346	646
3		1618	373	565	156
		2063	713	329	294
		895	524	1012	118
	mean	1525	537	635	189
4		651	360	1883	765
		1747	92	474	290
		2823	192	1251	764
	mean	1740	215	1203	606
5		1496	619	922	233
		1770	954	2134	334
		-	-	1697	361
	mean	1633	786	1584	309
6		526	41	581	61
		1010	154	1292	178
		1736	112	1029	125
	mean	1091	102	967	121
Grand Mean		1461	412	1069	376

TABLE 2. Statistical Summary of Data Using the Paired "t" Test.

Fiber Region Analyzed	Mean Copper K_α Counts: Treated Without Monomer	Treated With Monomer	Mean Difference	Probability Mean Difference = 0
Damaged Regions	1461	1069	392	0.0246
Adjacent Undamaged Regions	412	376	36	0.7979

G.M. Roomans: Would the use of peak-to-background ratios rather than the characteristic counts only have improved the statistics of your data?

Authors: We performed the Paired "t" Test using peak-to-background ratios and the t-statistics were nearly the same. This apparently occurred because we were extremely careful to maintain a constant angle between the fiber region analyzed and the detector in an effort to maintain constant background counts.

J.D. Fairing: Do you think that in view of the statistical uncertainty in your data, the method proposed in this paper can be used in practice? Is there any way that you could get a more convincing difference between the two samples?

Authors: The difference between the two samples seems convincing to us. The "t" test indicated that there is a 98% probability that the damaged fiber regions are different after treatment and there is only a 20% probability that the undamaged regions are different after treatment.