Visualization and Quantification of Penetration/Mass Transfer of Acrylic Resin Retanning Agent in Leather using Fluorescent Tracing Technique

by

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Abstract

Acrylic resin is a widely used retanning agent in leather processing, and its retanning effectiveness is closely related to its penetration depth and filling parts in leather. But acrylic resin in leather cannot be visualized or quantified, and thus its application mainly depends on tanners' experience. In this study, 5-aminofluorescein-labeled poly(acrylic acid) (AF-PAA), as a model of fluorescent acrylic resins, was synthesized using a phase-transfer reaction and then purified using Sephadex gel filtration. AF-PAA was applied to retanning process and could be well visualized and quantified in retanned leathers using fluorescence microscopy and Image J software. Looser collagen fibers, increasing amount of acrylic resin or retanning together with some anionic retanning agents could enhance penetration/mass transfer of acrylic resin in leather. The fluorescent tracing technique is useful to investigate the mass transfer and reaction mechanism of acrylic resin in leather processing.

Introduction

Modern leather processing involves multiple mechanical, chemical and biological unit processes. During processing, retanning, as a post-tanning process, plays an important role because it can remarkably improve the physical and aesthetic properties of leather.[1-3] To meet customers' requirements, a wide variety of retanning agents are added to chrome tanned leathers in retanning process, such as mineral retanning agents[2], vegetable tannins[4], syntans[5-7], resins[2,8], polymers[9,10], and so on. Among them, acrylic resin is one of the most widely used retanning agents, mainly due to the fact that its abundant carboxyl groups can both react with chrome and collagen fibers in leather.[2,11,12] Since leathers have certain thicknesses with complex weaves of collagen fiber bundles, the effectiveness of acrylic resin retanning is closely related to the penetration depth and filling parts of acrylic resins in leather.[13] However, to the authors' knowledge, up to now the penetration/distribution of acrylic resins in leather cannot be visualized or quantified, which makes the application of acrylic resins in retanning process mainly depend on the experience of tanners. Therefore, the development of a method that is able to accurately detect acrylic resins in leather is vital for a thorough investigation of their penetration/mass transfer and reaction mechanism in leather, which is the scientific basis for a rational regulation of acrylic resin distribution in leather and for improving the effectiveness of acrylic resin retanning.

Fluorescent tracing technique makes it possible to accurately locate materials in cell or tissue, and recent reports documented that proteases labeled with fluorescein isothiocyanate could be exactly observed in cattle hides using a fluorescence microscope.[14-17] So it is reasonable to speculate that the fluorescent tracing technique can be used to detect acrylic resins in leather if they are endowed with fluorescent properties. Moreover, Dejugnat et al. synthesized a fluorescein-labeled poly(acrylic acid) (PAA) to visualize and quantify encapsulation of PAA in (PAH/PSS)n (n=6,8) hollow capsules.[18] The fluorescein-labeled PAA prepared by Déjugnat et al. had a high alkalinity (pH>9)[18] and may be not suitable for retanning, since the acrylic resin retanning of leather should be undertaken in the pH range of 4-7. But it is suggestive for us to prepare fluorescein-labeled acrylic resins that are applicable to retanning process and can be used to visualize acrylic resins in leather.

In this study, we chose poly(acrylic acid) (PAA) as a model of acrylic resin retanning agents. A fluorescent PAA (AF-PAA) was synthesized at pH 2.0-2.5 and then purified at pH 6.86. We next analyzed the effects of retanning conditions on the stability of AF-PAA. At last, chrome tanned pigskin leather was retanned with AF-PAA, and the distribution of AF-PAA in leathers was visualized and quantified using fluorescence microscopy and Image J software, respectively.

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Manuscript received May 31, 2016, accepted for publication August 17, 2016

Experimental

Materials

Conventional chrome tanned pigskin leather was used for retanning trials. Poly(acrylic acid) solution (PAA, number-average molecular weight (Mn) by GPC = 115,000, 35 wt.% in H_2O, pH in 1.0%(w/v) aqueous solution = 2.82) synthesized in our laboratory by radical polymerization of acrylic acid was employed as a model of acrylic resin retanning agent.

Dicyclohexylcarbodiimide (DCC) and 5-aminofluorescein (AF) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., and Sephadex G-50 (fine) was purchased from Sigma-Aldrich Co. LLC. All the chemicals used for leather processing were of commercial grade, and the chemicals used for the analyses were of analytical grade.

Preparation of AF-labeled PAA (AF-PAA)

In order to obtain AF-PAA (viz. fluorescent acrylic resin) in the pH range of 4-7, PAA was fluorescently labeled based on a phase-transfer reaction by using AF as fluorophore and DCC as activating agent according to the method described by Kibler and Bachmann with minor modification.[19] A PAA solution (14 wt.% in H_2O) was prepared by diluting PAA (35 wt.% in H_2O) with distilled water. The PAA solution and a solution of 500 mmol/L DCC in diethyl ether were settled at 25°C for 10 min, respectively. Then, 60 mL of the PAA solution was mixed with 15 mL of the DCC solution, and the mixture was stirred at 25°C for 5 min. Subsequently, the upper organic phase of the mixture was collected, and the lower phase was remixed with another 15 mL of DCC solution at 25°C for 5 min. The subsequent upper organic phase was also collected and combined with the preceding organic phase. After adding 0.4 mL of 100 mmol/L AF in dimethylformamide to the combined organic phase, the labeling reaction was performed by stirring in the dark at 25°C for 10 min, where PAA was labeled with AF. At last, the organic phase was extracted with 6 mL of 1 mmol/L sodium hydroxide solution, so that the AF-labeled PAA (AF-PAA) was re-extracted from the organic phase into the aqueous sodium hydroxide solution.

After labeling, approximately 6 mL of the aqueous sodium hydroxide extracts was concentrated to 0.5 mL using Amicon Ultra-15 centrifugal filter devices (10 kDa MWCO, Millipore). To remove unreacted AF, the concentrated solution was loaded onto a Sephadex G-50 gel-filtration column (1.6 x 35 cm). The column was equilibrated and eluted with phosphate buffer (25 mmol/L, pH 6.86) at a flow rate of 0.1 mL/min. The eluate was collected with an automatic fraction collector (4.0 mL per fraction), and the absorbance of each fraction was measured at 473 nm (the absorbance maximum of AF) using an ultraviolet-visible spectrophotometer (UV-Vis, UV-1800PC, Mapada, China). Then, 20 mL of the fractions containing AF-PAA without AF were collected and concentrated using Amicon Ultra-15 centrifugal filter devices (10 kDa MWCO). As a result, about 5 mL of purified AF-PAA (2.0 wt.% in H_2O, pH in 1.0%(w/v) aqueous solution = 6.83) was obtained.

Analysis of Fluorescence Emission Spectra

The fluorescence emission spectra of AF (50 mg/L), PAA (50 mg/L) and AF-PAA (50 mg/L) solutions were measured with an excitation wavelength of 486 nm (the excitation maximum of AF-PAA) using a fluorescence spectrophotometer (Cary Eclipse, Agilent, USA).

Determination of AF-PAA Stability

In order to evaluate whether AF-PAA is applicable to retanning process, the effects of temperature, pH and other retanning agents on the stability of AF-PAA were determined as follows.

Thermal Stability of AF-PAA

The fluorescence emission spectra of 50 mg/L AF-PAA solutions were measured with an excitation wavelength of 486 nm after heating at 25°C (control), 35°C, 45°C, 55°C or 65°C for 60 min.

pH Stability of AF-PAA

The pH values of 50 mg/L AF-PAA solutions were adjusted from 7.2 (control) to 6.9, 6.4, 5.8, 5.5, 5.0, 4.7 and 4.4, respectively, using 0.1 mol/L HCl. After incubating at 25°C for 60 min, the fluorescence emission spectra of these AF-PAA solutions were measured as described above.

Stability of AF-PAA in the Presence of Other Retanning Agents

A series of AF-PAA–X solutions were prepared, where X represents other typical retanning agents such as replacement syntan (phenolic condensate), auxiliary syntan (naphthalene sulphonic acid condensate), dicyanodiamide resin, melamine resin and mimosa (a typical vegetable tannin extract). The concentrations of AF-PAA and X were both 50 mg/L. After incubating at 25°C for 60 min, the fluorescence emission spectra of the AF-PAA–X solutions were measured as described above.

Analysis of Molecular Weight

PAA solution (3.5 mg/mL) and AF-PAA solution (10 mg/mL) filtered through 0.22 μm pore size membrane was subjected to molecular weight analysis by gel permeation chromatography (GPC). GPC was performed on a Viscoteck 270max™ system equipped with a TSK-gel GMPWXL column, a refractive index (RI) detector, a right angle light scattering detector (RALS) and a low angle light scattering detector (LALS). Aqueous sodium nitrate solution (0.1 mol/L) was used as the mobile phase at 40°C (flow rate: 0.60 mL/min). Chromatograms were processed using the OmniSEC 4.7 software.

Observation of AF-PAA in Retanned Leather

The belly and butt parts of conventional chrome tanned pigskin leather were degreased, washed and neutralized to pH 6.0-6.5 according to typical leather processing procedures. Then, they were washed and divided into five groups for the following retanning trials. Each group included approximately 3 g of belly (1.2 mm in thickness) and 3 g of butt (2.4 mm in thickness). Three of the five groups were retanned with 3% acrylic resin and 100% water (percentage was based on weight of neutralized leather) at 35°C for 30 and 90 min, respectively. The acrylic resin (35 wt.% in H_2O) used herein for retanning was a mixture of PAA and AF-PAA (MPAA), and the mass ratio of PAA to AF-PAA was 2:1. One of the five groups was retanned with 6% MPAA and 100% water at 35°C for 90 min. One of the five groups was retanned with 3% MPAA and 100% water at 35°C for 30 min, and then retanned with 3% auxiliary syntan, 2% melamine resin and 3% mimosa for 60 min. After retanning, the five retanned leathers were sampled and cut into vertical sections of 20 μm thickness using a freezing microtome (CM1950, Leica, Germany). Subsequently, the sections were observed using a fluorescence microscope (Ti-U, Nikon, Japan) to locate AF-PAA in the retanned leathers. Furthermore, the fluorescence micrographs were analyzed by using Image J software to obtain the relative content of AF-PAA in the leathers.[20] Additionally, the sections were stained with hematoxylin and eosin (HE) stain to identify collagen fibers[21] and then observed using a biologic microscope (CX41, Olympus, Japan).

Results and Discussion

Characteristics of AF-PAA

As mentioned previously, the purpose of this study was to develop a method of accurately detecting acrylic resins in leather. For this purpose, we first need to obtain fluorescent acrylic resins. Hence, the PAA, as a model of acrylic resins, was fluorescently labeled based on a phase-transfer reaction using DCC as an activating agent and AF as a fluorophore (see Figure 1).[19] At the last step of the labeling reaction, the AF-labeled PAA (AF-PAA) was extracted into the aqueous sodium hydroxide solution. Here it is worth noting that unreacted AF also existed in the sodium hydroxide solution extracts together with AF-PAA. The unreacted AF is very likely to adhere to leathers in retanning process, which can interfere with accurate fluorescence detection of AF-PAA in the retanned leathers. Therefore, after the labeling reaction, the sodium hydroxide solution extracts of AF-PAA was subjected to a Sephadex G-50 gel-filtration column for removal of the unreacted AF. As shown in Figure 2, the AF was well separated from AF-PAA using gel filtration chromatography, and AF-PAA with high purity was obtained. Moreover, as the pH of the eluent was 6.86, the pH of the purified AF-PAA solution was 6.83, which met the condition for acrylic resin retanning (pH between 4 and 7). The purified AF-PAA was then analyzed by fluorescence spectroscopy. As shown in Figure 3, while PAA had no emission following excitation at 486 nm, AF-PAA had an emission maximum at 513 nm, which was close to the emission maximum of AF at 520 nm. The emission spectra of PAA, AF and AF-PAA demonstrated that AF was successfully incorporated into PAA, providing AF-PAA with detectable fluorescence.

Tanners usually control the penetration rate and the fixation extent of retanning agents in leathers by adjusting temperature and pH of retanning float,[12] where the temperature and the pH are usually in the range of 30°C-55°C and 4.5-6.5, respectively. Therefore, to determine whether AF-PAA is applicable to the actual retanning processes, we first investigated the effects of temperature and pH on the fluorescence intensity of AF-PAA. The temperature range employed herein was from 25°C to 65°C, and the pH range was from 4.4 to 6.9. As can be seen in Figure 4(a), the increase in temperature only resulted in a slight decrease in the fluorescence intensity of AF-PAA, where the intensity of the emission maximum at 513 nm mearsured at 65°C remained about 94% compared with that of the control (25°C). It is evident that the decrease in pH exhibits a negative effect on the fluorescence intensity of AF-PAA as presented in Figure 4(b), which is consistent with previous studies.[18,22] This should be due to the fact that PAA was conjugated with a pH-sensitive fluorophore (AF). In spite of this, the emission intensity of AF-PAA in the pH range of 4.5-6.5 is still acceptable to an observation using a fluorescence microscope.

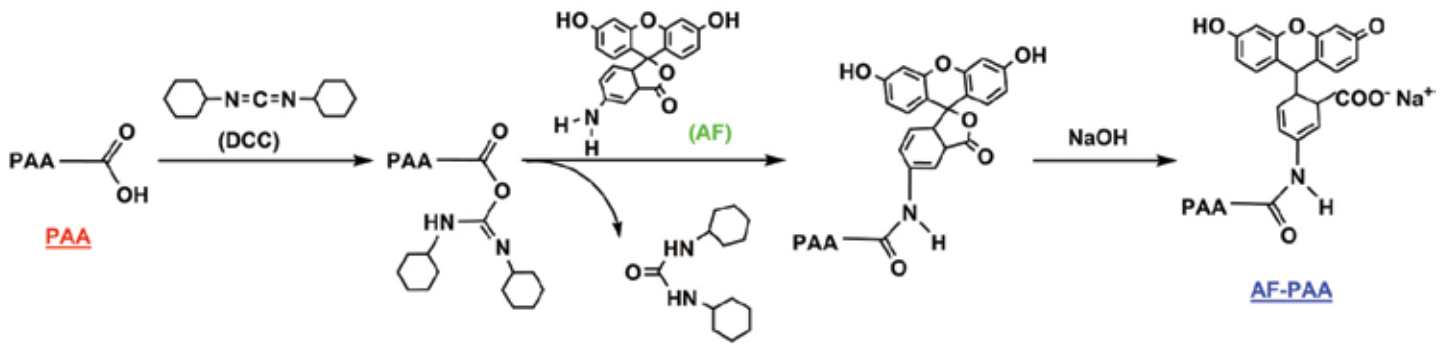

Figure 1. Schematic diagram of the labeling reaction of PAA with AF.

Additionally, besides acrylic resin, some other retanning agents, such as syntan, amino resin and vegetable tannin, are also commonly used in retanning process to meet customers' requirements.[2,11] So it is necessary for AF-PAA to possess enough stability in the presence of other retanning agents. The fluorescence emission spectra of AF-PAA in the presence of some typical retanning agents are given in Figure 4(c). It was found that the emission intensity of AF-PAA was increased by adding replacement syntan, auxiliary syntan, dicyanodiamide resin and melamine resin, while it was decreased in the presence of vegetable tannin (mimosa). But all the changes in the intensity of the emission maximum at 513 nm were less than 13%. These results suggest that AF-PAA is detectable even used together with other retanning agents in retanning processes.

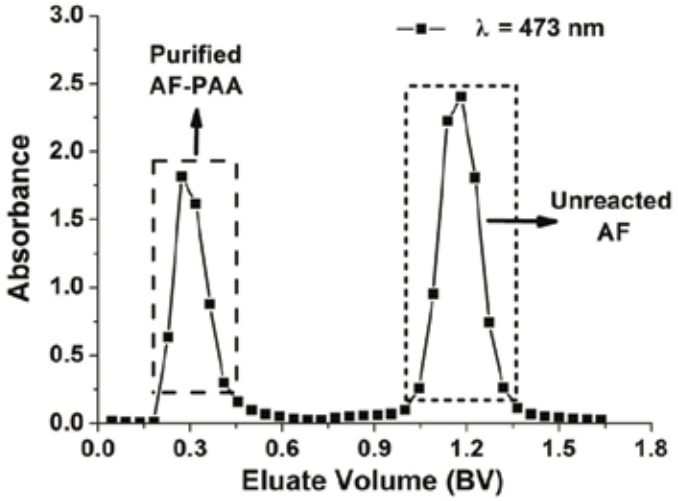

Figure 2. Chromatogram of AF-PAA on a Sephadex G-50 gel-filtration column (1.6 x 35 cm). The column was eluted with phosphate buffer (25 mmol/L, pH 6.86) at a flow rate of 0.1 mL/min.

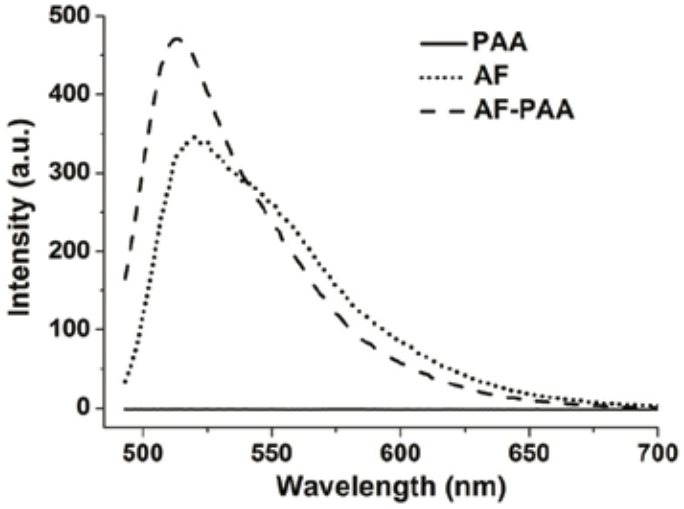

Figure 3. Fluorescence emission spectra of PAA, AF and AF-PAA obtained using an excitation wavelength of 486 nm (the excitation maximum of AF-PAA).

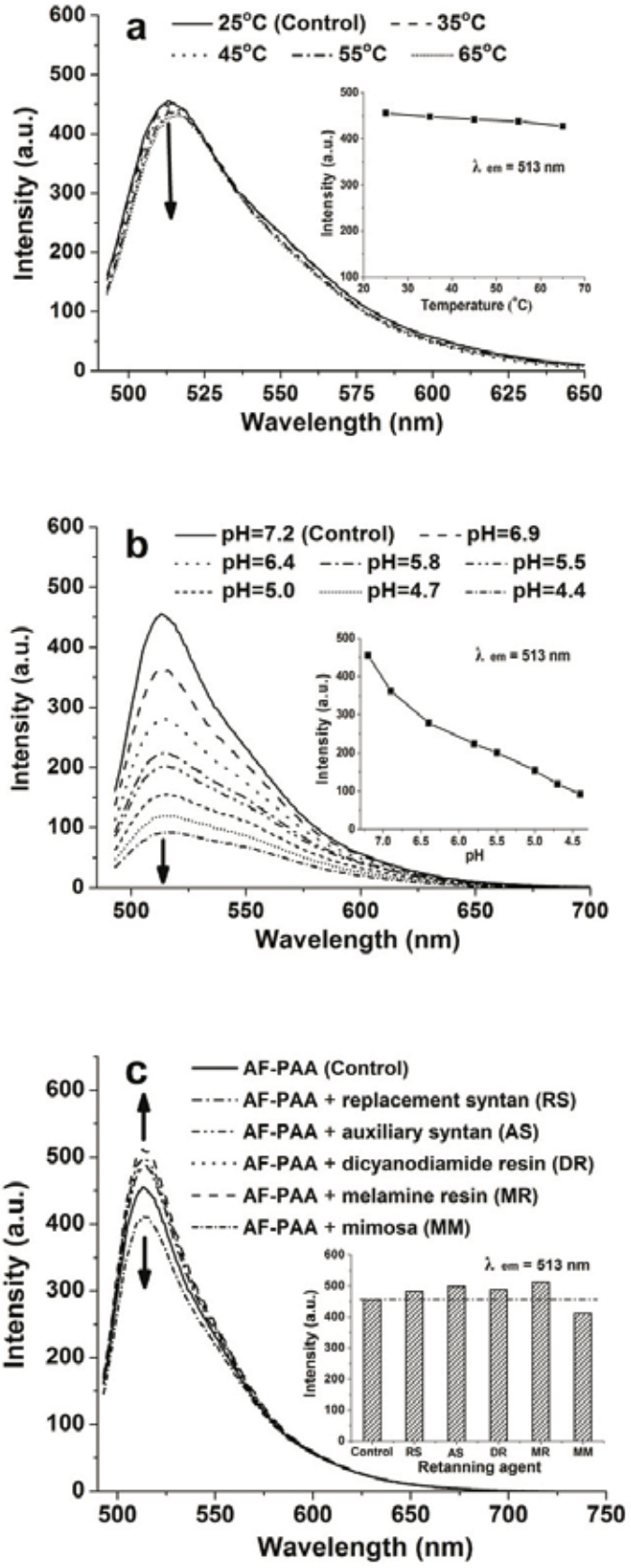

Figure 4. Fluorescence emission spectra of AF-PAA at different temperatures (a), pH (b) and in the presence of various retanning agents (c). Excitation wavelength was 486 nm.

As we know, the molecular size is an important factor influencing the penetration rate and the fixation extent of chemicals in leather. Therefore, the effect of labeling with AF on the molecular weight of PAA was investigated. The Mn of AF-PAA determined by GPC was 154 kDa, which was larger than that of PAA (115 kDa). This was probably because the ultrafiltration and Sephadex gel filtration for removal of AF from AF-PAA caused loss of PAA components with low molecular weight, whose GPC retention volume was around 9.5-11.0 mL (see Figure 5). Additionally, the larger molecular weight of AF-PAA was also partly due to the incorporation of AF into PAA. Despite this, we consider that the Mn of AF-PAA is in the rational range as an acrylic resin.

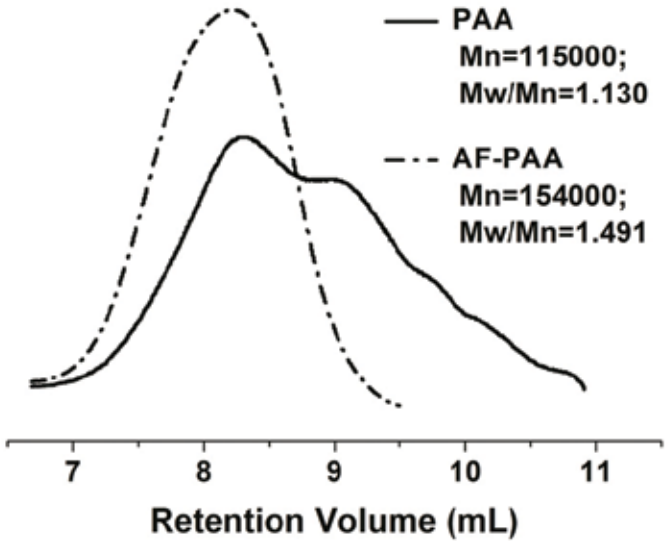

Figure 5. GPC traces of PAA and AF-PAA obtained with RI detection and 0.1mol/L sodium nitrate as the mobile phase.

Visualization and Quantification of AF-PAA in Retanned Leather

To evaluate whether the fluorescent tracing technique is useful for an accurate detection of acrylic resins in leather, the belly and butt parts of chrome tanned pigskin leather, which have significant differences in weave pattern and compactness of collagen fiber bundles, were retanned by a mixture of PAA and AF-PAA (MPAA).[23] Then, the AF-PAA in the retanned leathers was observed using a fluorescence microscope. Finally, the fluorescence micrographs of AF-PAA were processed with Image J software to quantify the relative content and the distribution of AF-PAA in leather.

The vertical section of the belly retanned for 90 min (HE stain) is shown in Figure 6(a), where the collagen fibers are red. The fluorescence micrographs of AF-PAA (green) in the belly of retanned leathers are presented in Figures 6(b)-6(e). Comparing Figures 6(b) and 6(c), we found that the fluorescence intensity of the middle dermic layer was improved with increasing retanning time, suggesting that AF-PAA gradually penetrated into the belly. After processing Figures 6(b) and 6(c) with Image J software, the quantitative distribution of AF-PAA in the belly was obtained as shown in Figure 7. The relative content of AF-PAA in the lower dermic layer was higher than that in the upper dermic layer, indicating that compared with the upper dermic layer (grain layer), AF-PAA penetrated faster into the lower dermic layer. This is because collagen fiber bundles in the lower dermic layer interweave more loosely (see Figure 6(a)), which is beneficial to the penetration of acrylic resins. However, it should be noted that when only 3% MPAA was used in retanning process, AF-PAA could not penetrate through the whole belly part in 90 min (Figure 6(c)). As seen in Figure 6(d) and Figure 7, by using 6% MPAA, the penetration depth of

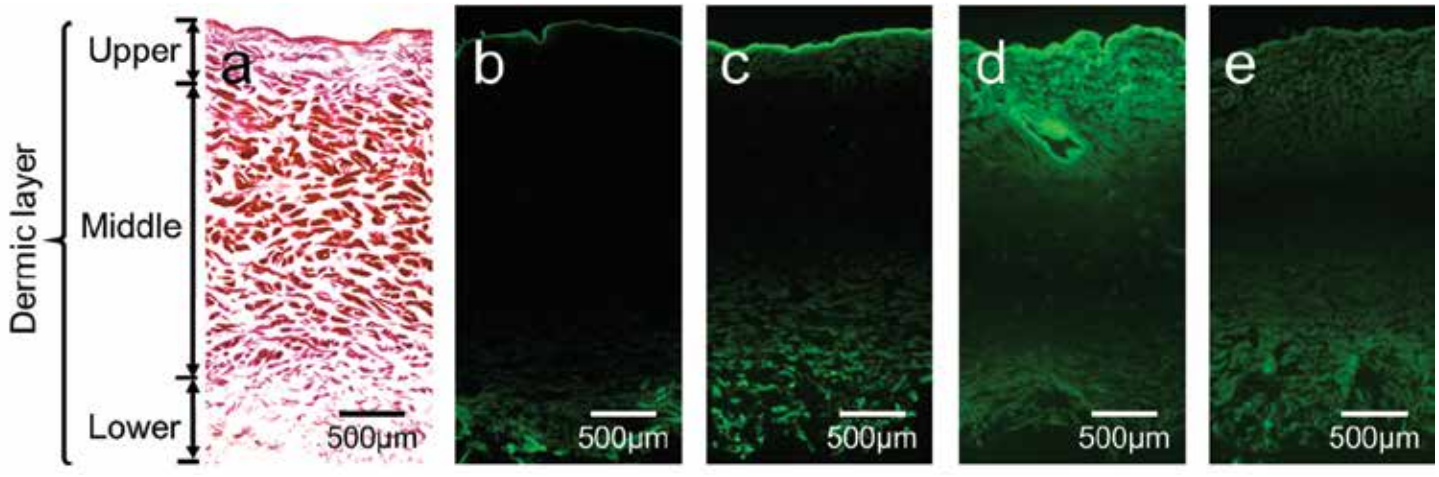

Figure 6. Vertical sections cut from belly of the leathers retanned with a mixture of PAA and AF-PAA (MPAA). (a) Vertical section (HE stain) observed using a biologic microscope; (b)-(e) Fluorescence micrographs of AF-PAA (green) in the vertical sections of the retanned leathers, (b) retanning with 3% MPAA for 30 min, (c) retanning with 3% MPAA for 90 min, (d) retanning with 6% MPAA for 90 min, (e) retanning with 3% MPAA for 30 min,then retanning with 3% auxiliary syntan, 2% melamine resin and 3% mimosa for 60 min.

AF-PAA in leather became much greater, meaning that an increase in the amount of acrylic resin can enhance mass transfer in leather. This is consistent with the typical effect of concentration on mass transfer. But, in consideration of the cost and the fading effect caused by acrylic resin, the amount of acrylic resin is usually limited.[12] Hence, a true understanding of how to increase the penetration depth of acrylic resin in leather when using a desired small amount is of great theoretical and practical importance. As can be seen in Figure 6(e) and Figure 7, it is exciting to observe that when MPAA was used together with other retanning agents such as auxiliary syntan, melamine resin and mimosa, the penetration depth of AF-PAA in the belly was markedly increased. This might be attributed to the fact that, the addition of some anionic retanning agents possessing moderate reactivity with leather collagen could result in a better dispersion of collagen fibers, an increase in interfiber distance and a decrease in positive charge of chromed tanned leather.[24,25] This result implies that a rational control of retanning conditions can enhance mass transfer of acrylic resin in leather. Therefore, our future work is planned to systematically investigate the effects of other retanning agents, pH, temperature and mechanical action on mass transfer of acrylic resin.

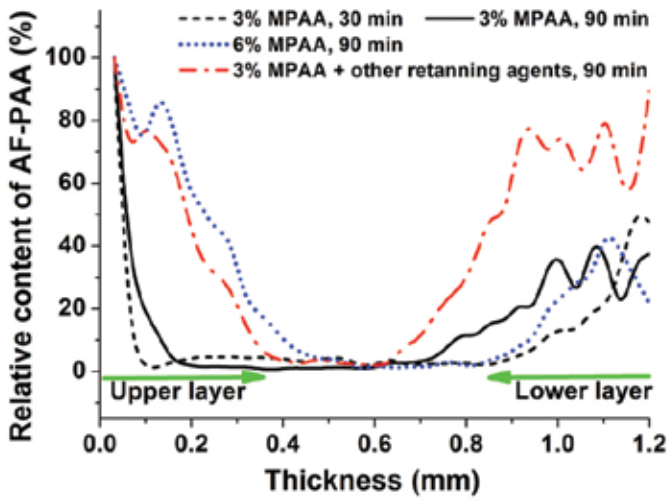

Figure 7. Distribution of AF-PAA in the belly of the leathers retanned with a mixture of PAA and AF-PAA (MPAA). The relative content of AF-PAA in the belly was quantified by analysis of the fluorescence micrographs in Figures 6(d)-6(e) respectively using Image J software.

From Figures 8(a)-8(c) and Figure 9, we found that after retanning with AF-PAA, the surfaces of the upper and lower layers of the butt exhibited bright green fluorescence, while the middle layer was black even after retanning for 90 min. These results indicated that when 3% MPAA was used for retanning, AF-PAA could hardly penetrate into the butt of pigskin leather that had coarse collagen fiber bundles and a quite compact weave with a high weave angle. Fortunately, the increase in the amount of MPAA (Figure 8(d)) and the addition of other retanning agents (Figure 8(e)) could increase the penetration depth of AF-PAA in butt. These trends are in line with the previous results obtained in belly.

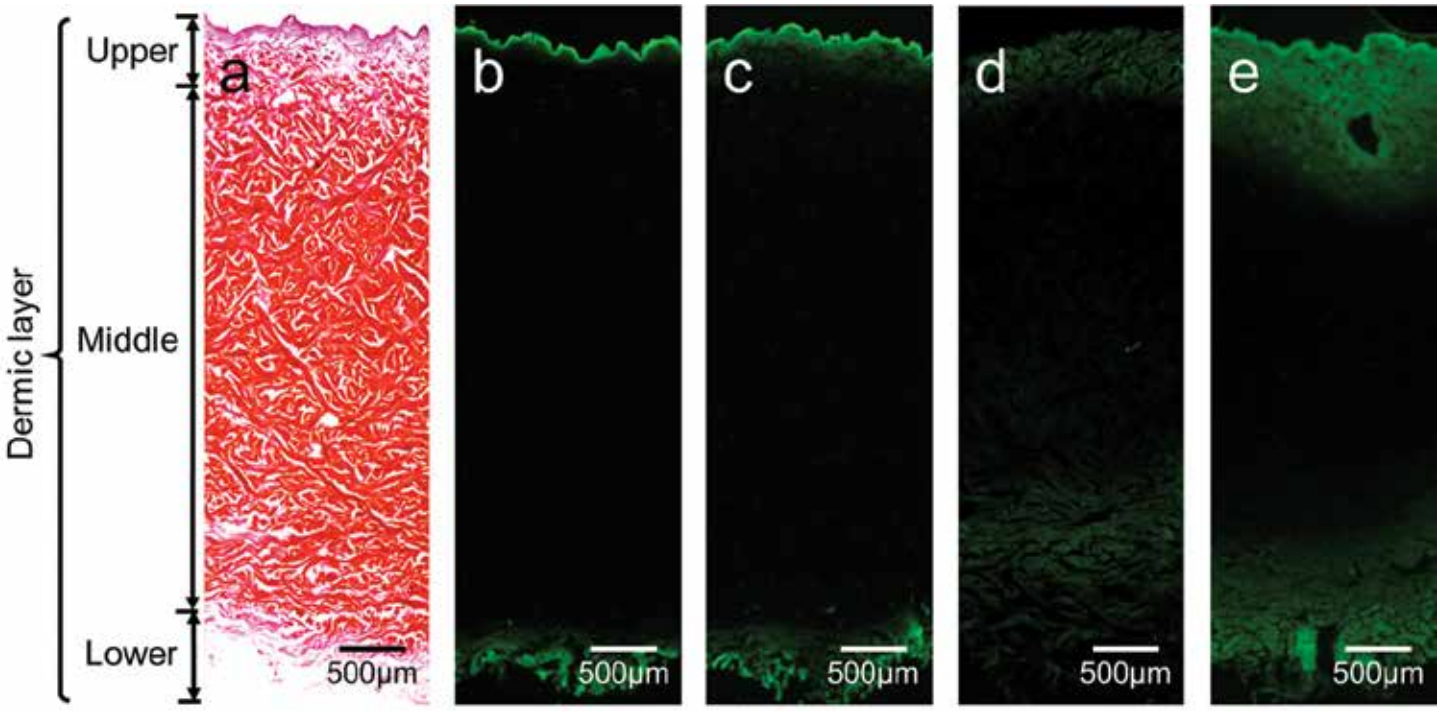

Figure 8. Vertical sections cut from butt of the leathers retanned with a mixture of PAA and AF-PAA (MPAA). (a) Vertical section (HE stain) observed using a biologic microscope; (b)-(e) Fluorescence micrographs of AF-PAA (green) in the vertical sections of the retanned leathers, (b) retanning with 3% MPAA for 30 min, (c) retanning with 3% MPAA for 90 min, (d) retanning with 6% MPAA for 90 min, (e) retanning with 3% MPAA for 30 min,then retanning with 3% auxiliary syntan, 2% melamine resin and 3% mimosa for 60 min.

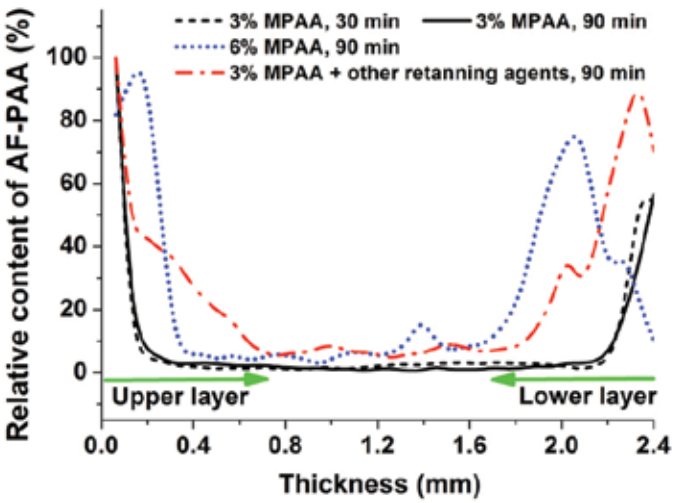

Figure 9. Distribution of AF-PAA in the butt of the leathers retanned with a mixture of PAA and AF-PAA (MPAA). The relative content of AF-PAA in the butt was quantified by analysis of the fluorescence micrographs in Figures 8(d)-8(e) respectively using Image J software.

Comparing Figures 6 and 8, as well as Figures 7 and 9, it is interesting to observe that the penetration rate and the relative content of AF-PAA in the belly are much higher than those in the butt. This should be due to the fact that the weave of collagen fiber bundles with a low weave angle in the belly is looser than that in the butt, where the weave with a high weave angle is very compact (see Figure 6(a) and 8(a)).[23] These results suggest that the compactness of collagen fiber bundles is an important factor influencing the penetration rate of acrylic resins, which is consistent with previous literature[26]. These phenomena also confirm that acrylic resins are predominantly filled into the looser parts of the leather and are capable of reducing the difference in the compactness of collagen fiber bundles between belly and butt parts. To the knowledge of the authors, these fluorescence micrographs provide the first visual evidence that acrylic resins have selective filling property and can increase the homogeneity of resultant leather.

Above all, acrylic resins in leather can be well visualized and quantified by using fluorescent tracing technique together with image processing technique, which is useful for a systematic investigation of the penetration/mass transfer and reaction mechanism of acrylic resins in leather.

Conclusions

The fluorescent tracing technique is effective in detecting the penetration and distribution of acrylic resins in leather, by which we can investigate the mass transfer and reaction mechanism of acrylic resins in leather processing. Obviously, this technique is useful for us to optimize the application conditions of acrylic resin retanning agent, which we plan to investigate in future work. Meanwhile, the information provided by this technique certainly favors the optimal design of molecular structure and size for acrylic resin retanning agent.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21406145), the Fundamental Research Funds for the Central Universities (SCU2015D008) and the South Wisdom Valley Innovative Research Team Program.

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