Materials and Techniques of the “Chun Rong Xuan Mao” Birthday Inscribed Plaque in the Bashu Area of China during the Reign of Tongzhi Emperor of the Qing Dynasty

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Abstract
In ancient China, people presented inscribed plaques as gifts to the elderly on their birthdays to wish them longevity. Under long-term weathering conditions, the plaques, often hung on the lintel of a door, gradually deform, the words fade, and the decorative parts deteriorate. Whenever possible, original materials and techniques should be used to restore such cultural relics. Therefore, it is important to analyze the materials and techniques used for the production of the inscribed plaques. However, no study has been carried out on the materials and techniques used to create inscribed plaques. In this study, multi-analytical scientific approach, including optical microscopy (OM), scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS), X-ray diffraction (XRD), micro-attenuated total reflection Fourier transform infrared spectroscopy (μ-ATR-FTIR), and micro-Raman spectroscopy (μ-Raman), were used to explore the materials and techniques utilized to create the “Chun Rong Xuan Mao” birthday inscribed plaque in the Bashu area of China produced during the fifth year of the reign of Tongzhi Emperor of the Qing Dynasty. The results showed that the plaque was made of cypress wood and decorative parts consisting of the surface lacquer layers, plaster lacquer layers and primer lacquer layers. Chinese lacquer was the principal material used in the surface lacquer layers; gypsum and Chinese lacquer were the materials used in the plaster lacquer layers; and the primer lacquer layers was composed of Chinese lacquer, calcite, and mixed pigments by cinnabar and minium. The surface lacquer layers of the inscribed plaque were lacquered black. Gypsum lacquer plaster has been commonly used in the ground layer of lacquerware in modern history. This study confirmed the existence of technology to make lacquerware using gypsum lacquer plaster in the Bashu area during the late Qing Dynasty. Moreover, this study not only provides new findings regarding the traditional production of inscribed plaques and offers technical support for the protection and restoration of such plaques but also has great significance to exploring the history of ancient techniques of lacquering and decorating lacquerware.

Keywords: Inscribed plaque, μ-FTIR, μ-Raman, XRD, SEM-EDS, Materials, Techniques, Bashu area

Introduction
Inscribed plaques, hung on the lintel of a door, are integral parts of ancient Chinese architectures and unique cultural symbols of China. As an artistic form integrating language, calligraphy, lettering, architecture and sculpture, inscribed plaques contain rich information about historical culture and traditional craftsmanship. Birthday inscribed plaques were gifts to the elderly on their birthdays to wish them longevity, and the custom of sending birthday plaques has been popular in China ever since the Tang Dynasty, reflecting the traditional virtues of the Chinese nation. The Bashu area, a region currently under administration of Sichuan Province and Chongqing Municipality, has a large number of inscribed plaques and is one of the areas in China with the

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largest number of famous inscribed plaques. In the Northern Song Dynasty, Li Xie documented the dimensions of different inscribed plaques in his book *Ying Zao Fa Shi* (营造法式) [1], but he did not mention the materials. Based on the variation in the standard length of Chinese rulers throughout the ages, the average dimension of inscribed plaques in the Song Dynasty was 61-215 centimeters high and 45-172 centimeters wide, indicating that the plaques were presented mostly in a vertical alignment. During the Ming and Qing Dynasties, as the development of inscribed plaques peaked, their sizes became larger, and the plaques were presented mainly in a horizontal alignment. The techniques used in producing an inscribed plaque generally included woodworking, carving, creating the ground layer, lacquering, screening, and tracing words. As the only existing monograph about lacquering in ancient China, *Xiu Shi Lu* (髹饰录), written by Huang Cheng [2], explains the raw materials and techniques of lacquering in great detail, providing a reliable basis for the naming and classification of ancient lacquerware. To show respect for the elderly, inscribed plaques used at birthday ceremonies were made of exquisitely selected materials with excellent craftsmanship. These inscribed plaques were fine works of lacquering at that time.

Under long-term weathering conditions, a considerable number of inscribed plaques deform, the words fade, and the decorative parts deteriorate. To help preserve these cultural relics, it is of great importance to study the material composition, the production techniques, and the scientific cognition. Based on the principle of minimum intervention, the original materials and techniques should be used for cultural relic restoration. Although there has been an increasing number of studies about the materials and techniques used in ancient wall paintings [3,4], colored paintings [5,6], and lacquerware [7-9], no study has been carried out on the materials and techniques used to create inscribed plaques. Therefore, in this study, we used a number of multi-analytical scientific technologies, including optical microscopy (OM), scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS), micro-attenuated total reflection Fourier transform infrared spectroscopy (μ-ATR-FTIR), X-ray diffraction (XRD), and micro-Raman spectroscopy (μ-Raman) to analyze microsamples that fell off of the “Chun Rong Xuan Mao” birthday inscribed plaque in the Bashu area during the reign of Tongzhi Emperor of the Qing Dynasty. The study provides new evidence for the traditional production techniques utilized to create inscribed plaques and offers technical support for the protection and restoration of inscribed plaques. Thus, this study is of great significance to exploring the history of ancient techniques for lacquering and decorating lacquerware.

**Experimental**

**The Inscribed Plaque and Samples**

Fig. 1a shows the “Chun Rong Xuan Mao” birthday inscribed plaque produced in the Bashu area of China in the 5th year during the reign of Tongzhi Emperor of the Qing Dynasty (A.D. 1866).
With a size of 215 × 70 × 4 cm, the inscribed plaque is well preserved as a whole, with localized damage in several places. It has a visually black surface with no linen or cloth in the ground layer. Four Chinese characters, “椿荣萱茂” (“Chun Rong Xuan Mao”), were inscribed on the plaque. “Chun” refers to the Chinese toon. “Xuan” is a day lily, believed by ancient Chinese people to be a plant that helps one forget about sad things. In ancient China, a father was referred to as “Chunting” (椿庭), and a mother was called “Xuantang” (萱堂). Thus, “Chun Rong Xuan Mao” means prosperity of the Chinese toon and flourishing of the day lily, which is a metaphor for the longevity of parents. According to the inscriptions, it is known that the inscribed plaque was presented by an individual as a gift to his parents at their 60th birthday. The 4 Chinese characters were inscribed by an official of second-grade rank in the feudal regimes.

The microsamples were fragments that fell off the plaque. With irregular shapes, the microsamples had a maximum size of 1.2 × 1.0 × 0.1 cm and a minimum size of 0.9 × 0.4 × 0.1 cm. The colour of the front of the microsample is black (Fig. 1b), and that of the back of the microsample is dark red with a white layer (Fig. 1c).

**Equipment and Experimental Conditions**

**Optical microscopy**

OM was used to observe the microsamples and identify the wood species of the base materials. Specifically, the surface and microlayer were analyzed with a 3D ultra-depth-of-field VHX-6000 microscopic system (Keyence Corporation, Japan) that has a 20-200× VH-Z20T ultra-miniature zoom lens, a 1 μm automatic resolution in the Z axis, and an electric XY table. To identify the wood species of the base materials, a BX51 microscope (Olympus Corporation, Japan) was used to observe the structure of wood cells.

**Scanning electron microscopy with energy dispersive X-ray spectroscopy**

For the SEM-EDS analysis, an FEI Quanta 450 FEG scanning electron microscope and X-MaxN50 energy dispersive X-ray spectroscopy (FEI Company, USA) were used to analyze the morphology and elements of the microsamples.

**Micro-attenuated total reflection Fourier transform infrared spectroscopy**

For the μ-FTIR analysis, a LUMOS microscope (Bruker, Germany) was used to analyze organic and inorganic substances in the microsamples. The instrument had a spectral range of 4000-600 cm⁻¹ and a spectral resolution of 4 cm⁻¹. With 16 times scanning and 8× magnification, the measurement micro-area was adjusted between 100-10 μm using a 100 μm (diameter) ATR crystal (germanium) tip.
**X-ray diffraction**

A Smartlab rotating target X-ray diffractometer equipped with micro-area optical components (Rigaku Corporation, Japan) was used for the XRD analysis to analyze the phase composition of the microsamples. It has a maximum power of 9 kW and an XY sample table, and metallic copper was used as the rotating target.

**Micro-Raman spectroscopy**

For the μ-Raman analysis, an inVia micro-Raman spectrometer (Renishaw, UK) was used to identify the composition of samples in the micro-area. It has a wavenumber range of 100 - 2000 cm\(^{-1}\) and a spot size of 1 μm. A 785 nm laser source was used for irradiation (3-8×) for a total duration of 10 s.

**Experimental Methods**

Each sample was placed on the sample table for SEM-EDS, μ-FTIR, XRD, and μ-Raman analyses. To perform microstratigraphic analysis, the sample (0.4 × 0.4 × 0.1 cm) was cross-sectioned. Specifically, the sample was first vertically embedded in AB glue made from epoxy resin (Shanghai Naibo Co., Ltd., China). After solidifying at 25 ℃ for 3-4 h, the epoxy resin was polished with 200-, 800-, 3000-, and 7000-grit sandpaper imported from Germany and 12000-grit sandpaper imported from the US. The cross-sections were observed under a microscope to obtain the microstructure characteristics and to identify the wood species.

**Results and discussion**

**Observation of the cross-section**

Fig. 2a shows a SEM image of the surface layer of the plaque. The particles are fine and glossy, and there is a large number of irregular cracks, through which the ground layer could be seen. The appearance of cracks on the surface layer indicates deterioration of the decorative parts. Fig. 2b,c show the SEM and OM images of cross-section, in which 8 microstratigraphic sequences are observed (L1-L8) (Table 1). In terms of color, as shown in Fig. 2c, L1 is white, L2 is red, L3-L7 are varying shades of black, and L8 (i.e., the surface layer) is deep red-brown. In terms of thickness (Table 1), the lower 3 layers (L1, L2, and L3) and the upper 2 layers (L7 and L8) are thin and uniform, with a thickness of approximately 10-50 μm, whereas the middle layers (L4, L5, and L6) are thick and uneven, with a thickness of approximately 80-165 μm. In terms of particle size (Fig. 2b), L4 and L5 are rough, and the particles contained within are large (maximum: 100 μm); L6 contains particles of a smaller size, with the maximum being approximately 60 μm; and the particles in the remaining layers are finer. In addition, interestingly, the surface layer (L8) appears glossy black to the naked eye; however, when observed under the 200×
ultra-depth-of-field microscope, the surface layer (L8) appears deep red-brown and has a delicate micromorphology (Fig. 2c). In L2, the red pigment is mainly dark red and comprises fine particles, with a small amount of orange-red pigment, comprising larger and more nonuniform particles than those composing the dark red pigment.

Decorative Materials

SEM-EDS Analysis

The results of the SEM-EDS analysis are shown in Table 1. There is a large amount of C and O in each layer. Thus, it is speculated that every layer probably contains organic substances. There is a large amount of C, O, and Ca in the white substances in L1, the total content of which exceeded 97%. L2 contains Hg and Pb, which might be the chromogenic elements of the red pigment. In L3 and L7, there is a large amount of C and O (97%), while the content of other elements does not exceed 2.9%. Moreover, L7 may only contain C and O, with no other inorganic substances. In L4, L5, and L6, where the particle size was large, Ca and S contents are relatively high. In L8, in addition to a large amount of C and O, elements such as Si, Al, K, Ca, Fe and Ba are also found, indicating that the surface is exposed to dust in the air.

XRD Analysis

XRD analysis was carried out to reveal the inorganic components of the inscribed plaque. Fig. 3 shows the XRD diagrams of the surface layer, gypsum, and quartz. The strong diffraction peaks of sample at 2θ of 11.6, 20.7 and 29.1 are consistent with the diffraction peaks of gypsum (CaSO₄·2H₂O, JCPD:33-0311), and the diffraction peaks of sample at 2θ of 20.8 and 26.7 are consistent with those of quartz (SiO₂, JCPD:33-1161), indicating that there are gypsum and quartz in the surface layer. Moreover, quartz features weaker diffraction peaks, suggesting that the amount of quartz is lower than that of gypsum. As shown in Fig. 2a, there is a large number of cracks on the surface layer. Based on the EDS analysis (Table 1), layers L4-L6 contain a large amount of Ca and S, possibly indicating the origin of gypsum. As shown in Table 1, the surface layer (L8) contained Si, Al, Fe and K, which are common elements found in the soil. It is inferred that there was some dust on the surface of the inscribed plaque and that the quartz originated from the dust. The sample has a mountain-shaped diffraction peak at 2θ of approximately 20, which means that the sample contains organic substances.

The XRD diagram of the red pigment in L2 is consistent with that of cinnabar (Fig. 4). Thus, the red pigment is probably cinnabar (HgS, 2θ at 26.5 and 31.2, JCPD:99-0031). The strong diffraction peaks indicates that the cinnabar features a good crystal form, high content, and few impurities in the red pigment. Based on the EDS analysis, the cinnabar comprises 3.06% of Pb; however, the chromogenic phase of Pb was not detected in the XRD analysis. The possible reason
is a very low Pb content; therefore, the peak is masked by the strong diffraction peaks of cinnabar, which contains 14.35% of Hg.

Because there was a tiny amount of white substance in L1, μ-XRD analysis was performed. The results show that the white substances are mainly calcite (CaCO₃, 2θ at 29.4 and 47.6, JCPD:05-0586) and quartz (2θ at 20.8 and 26.7, JCPD:33-1161), as shown in Fig.5. The calcite has strong diffraction peaks, and the quartz has low diffraction peaks, suggesting a low quartz content.

μ-Raman Analysis
To reveal the Pb composition in the red pigment in L2 that was not detected by XRD analysis, μ-Raman analysis was performed on the samples. Fig. 6a,b show the microscopic image of the red pigment and Ramen spectra of the dark red and orange-red pigments in L2 of the ground layer of the inscribed plaque. As seen, the Raman peaks of the dark red pigment at 253(vs) and 343(m) cm⁻¹ are basically consistent with the Raman peaks of cinnabar reference, while the Raman peaks of the orange-red pigment at 151(s), 224(m), 313(m), 390(s) and 549(vs) cm⁻¹ are consistent with those of the minium reference. Based on the SEM-EDS analysis (Table 1), it is known that the red pigment in L2 is a mixture of cinnabar and minium, with a high cinnabar content and a low minium content.

μ-FTIR Analysis
To reveal the organic and inorganic substances in the decorative materials, μ-FTIR analysis was performed on the samples. Fig. 7 shows the μ-FTIR spectra of the ground and surface layers of the inscribed plaque as well as the reference FTIR spectra of lacquer film from the Qin Terracotta Army and gypsum reference. In the FTIR spectra of the lacquer film from the Qin Terracotta Army and gypsum reference the broad absorption band near 3430 cm⁻¹ is the stretching vibration peak of OH [10]; at 2926 and 2855 cm⁻¹, the absorption bands are the antisymmetric and symmetric stretching vibration peaks of CH₂ [11]; the weak absorption band at 1684 cm⁻¹ is the characteristic absorption peak of SO₄²⁻ [12]; the absorption band at 1625 cm⁻¹ is the stretching vibration peak of C=O[13] and the characteristic absorption peak of SO₄²⁻; the absorption band at 1407 cm⁻¹ is the stretching vibration band of C-O [14]; the strong absorption band at 1105 cm⁻¹ is the characteristic absorption peak of SO₄²⁻; the absorption band at 1033 cm⁻¹ is the stretching vibration peak of C-O and C-C; the weak absorption band at 797 cm⁻¹ is the out-of-plane bending vibration peak of C-H on the benzene ring [15]; and the absorption bands at 669 cm⁻¹ and 598 cm⁻¹ are the characteristic absorption peaks of SO₄²⁻.

Based on the characteristic infrared absorption peaks and the infrared spectrum of the surface layer, there are absorption peaks of Chinese lacquer at 3430, 2926, 2855, 1625, 1407, 1033 and 797 cm⁻¹, indicating that the raw material is raw lacquer. The absorption peaks at 3533, 3403, 1105
and 669 cm\(^{-1}\) are characteristic absorption peaks of gypsum. The low intensity of these absorption peaks implies a low gypsum content in the sample. Because there were a large number of cracks in the surface layer of the sample, as shown in Figure 2(a), the components of the ground layer were detected. Based on the SEM-EDS results (Table 1), it can be concluded that gypsum was from the ground layer.

The infrared spectra of L1-L6 of the ground layer shows that there are characteristic absorption peaks of Chinese lacquer at 3430, 2926, 2855, 1625, and 797 cm\(^{-1}\), indicating that the organic cement is Chinese lacquer. The absorption peaks at 3538, 3403, 1684, 1625, 1105, and 668 cm\(^{-1}\) are basically consistent with the characteristic absorption peaks of gypsum, confirming that gypsum is the inorganic substance in the ground layer. Based on the strong absorption peak at 1105 cm\(^{-1}\) and the disappearance of the absorption peak at 1033 cm\(^{-1}\), the ground layer has a lower Chinese lacquer content and a higher gypsum content than the surface layer.

Generally, the absorbance in infrared spectra measured by the KBr pellet method is significantly higher than that measured by ATR-FTIR [16], and ATR-FTIR absorption peaks decline remarkably within the range of 3600-3000 cm\(^{-1}\) [17]. The infrared spectrum of the lacquer film from the Qin Terracotta Army was obtained by the KBr pellet method [18], while that of the inscribed plaque was obtained by the \(\mu\)-ATR-FTIR method; therefore, the absorption peak of the sample close to 3430 cm\(^{-1}\) is low.

**Wood Species of the Base**

Fig. 8 shows the microstructures of the wooden base of the inscribed plaque. The cross section is shown in Fig. 8a, with obvious growth rings and slow variation in the early and late wood features. The wood cells are tracheids with axial parenchyma cells; the tracheids of early wood have square and polygonal in the cross sections, and the tracheids of late wood show rectangular and polygonal in the cross sections. Fig. 8b shows the radial section of the wooden base; there are tracheid wall pits and cross field pits, and 2-4 small cypress cross field pits between ray parenchyma cells and early wood tracheids. Moreover, there is 1 row of round wall pits and scattered axial parenchyma cells; end wall nodular thickening of parenchyma cells containing dark resin is not obvious. Fig. 8c shows the tangential section; there was a single row of rays with a height of 1-14 cells. All the ray cells are parenchyma cells without ray tracheid, and the ray parenchyma cells have few or insignificant horizontal wall pits, with unobvious end wall nodular thickening and obvious dents.

According to the above microstructure characteristics of wood as well as existing data and spectra [19], it is determined that the wood species of the samples is a *Cupressus* species in the *Cupressaceae* family. Based on the distribution of *Cupressus* species in the Yangtze River basin in Sichuan Province, the wood should be *Cupressus funebris*.
Lacquering Technique

Based on the results of the OM, SEM-EDS, XRD, µ-FTIR and µ-Raman analyses, the microstratigraphic sequences of decorative parts of the inscribed plaque consists of 8 microlayers. According to the hue, thickness, particle size, and material composition of each layer, the decorative parts were classified into 3 sections, i.e., the surface lacquer layers, plaster lacquer layers and primer lacquer layers. The surface lacquer, with Chinese lacquer as the main material, was varnished twice (L7 and L8). The layers are thin and even. The plaster lacquer applied 3 times (L4, L5, and L6), with gypsum and Chinese lacquer as the raw material; the particles are coarse and uneven. The primer lacquer was applied 3 times (L1, L2 and L3). Calcite, the mixed pigments of cinnabar and minium and Chinese lacquer served as the main material to smooth the original surface of the wood. The ground layers include plaster lacquer and primer lacquer layers. The wooden-cored inscribed plaque is made of cypress.

The materials used the inscribed plaque are all common decorative materials with a long history of application in ancient China. Cinnabar and calcite particles are fine, while minium particles are large and not uniform. Cinnabar and Chinese lacquer were used on the red wooden bowl unearthed at the Hemudu site in China, dating to approximately 7000 years ago [20], and exhibited outstanding durability. Minium was used in the Han Dynasty. The mixture of cinnabar and minium was used as the red pigment in Dunhuang wall paintings, and studies have shown that the mixture of cinnabar and minium makes the pigment more stable [21]. Because cinnabar and minium contain the heavy metal elements of Hg and Pb, the mixture has bactericidal and anticorrosive. Gypsum is not only a white pigment commonly used in ancient wall paintings and polychrome cultural relics [22,23] but also a gel material that was used for architectural decoration in ancient China [24,25].

The surface of the inscribed plaque was lacquered black, which was the most traditional technique for making lacquerware in ancient China. The surface of the lacquer film appears glossy black to the naked eye but appears deep red-brown in OM images. An early record of the production of black lacquer is found in Nan Cun Chuo Geng Lu - Xiu Qi (南村辍耕录•髹器) written by Tao Zongyi in the Yuan Dynasty [26]: Chinese lacquer was boiled, and then the waste iron filings were soaked in rice vinegar to produce the black lacquer. The process may involve the following reactions:

\[
\begin{align*}
\text{CH}_3\text{COOH} + \text{Fe}_2\text{O}_3 &\rightarrow \text{Fe(OH)} (\text{CH}_3\text{COO})_2 + \text{H}_2\text{O} \\
\text{Fe(OH)} (\text{CH}_3\text{COO})_2 + \text{H}_2\text{O} &\rightarrow \text{Fe(OH)}_2 \downarrow \text{(white)} + \text{CH}_3\text{COOH} \\
\text{Fe(OH)}_2 + \text{O}_2 + \text{H}_2\text{O} &\rightarrow \text{Fe(OH)}_3 \downarrow \text{(bluish green)}
\end{align*}
\]

Coated onto a glass sheet, the black lacquer dyed with iron filings turns deep red-brown under bright light [27], which is consistent with the surface color of the cross-section of the inscribed plaque observed under OM.
The primer of the inscribed plaque is likely gypsum lacquer plaster (lacquer mixed with gypsum), which has lasted for more than 150 years without serious falling off, indicating excellent performance of this technique. As recorded in *Xiu Shi Lu*（髹饰录） by Huang Cheng in the Ming Dynasty, the ground layer was usually made from materials such as horn ash, bone ash, clam ash, brick ash, pig blood, and tung oil plaster [2]; gypsum was not mentioned at all. Records regarding gypsum lacquer plaster can be found in *Essentials about Lacquerware Techniques*（漆器工艺技法撷要） by Shen and Li [28]. Adding Chinese lacquer into gypsum can accelerate the drying of lacquer plaster, and the mixed material is more solid than gypsum mixed with pig blood. With the ability to gelate, gypsum may biomineralize with substances in Chinese lacquer such as urushiol and laccase, but the scientific principles remain to be further explored. Gypsum lacquer plaster is a commonly used material for making the ground layer of lacquerware in modern times. This study confirmed that the technique of making lacquerware with gypsum lacquer plaster existed in the Bashu area during the late Qing Dynasty.

**Conclusions**

In this study, we used a number of technologies, including OM, SEM-EDS, XRD, μ-Raman and μ-FTIR, to analyze the materials and techniques for the production of the “Chun Rong Xuan Mao” birthday inscribed plaque in the Bashu area of China in the 5th year during the reign of Tongzhi Emperor of the Qing Dynasty (A.D. 1866). We found that (1) cypress wood was the base material for the inscribed plaque, which symbolizes the gift of presenter’s best wishes for the elderly, i.e., nobility and longevity; (2) the decorative parts on the inscribed plaque have 3 sections, i.e., the surface lacquer layers, plaster lacquer layers and primer lacquer layers. Black lacquering techniques were used in the surface lacquer layers, and a gypsum lacquer plaster technique was used in the ground layer. The surface lacquer was varnished twice, with Chinese lacquer as the main raw material; the 2 layers were delicate, thin and even. The plaster lacquer was applied 3 times, with gypsum and Chinese lacquer as the raw material; the particles were coarse and uneven. The primer lacquer was applied 3 times, and calcite, the mixed pigments of cinnabar and minium and Chinese lacquer were the main materials used to smooth the original surface of the wooden base. Gypsum lacquer plaster is a commonly used material to make the ground layer of lacquerware in modern times. This study confirmed the existence of technology to make lacquerware using gypsum lacquer plaster in the Bashu area during the late Qing Dynasty. By providing new evidence for traditional techniques of producing inscribed plaques and offering technical support for the protection and restoration of inscribed plaques, the current study is of great significance to exploring the history of ancient techniques for lacquering and decorating lacquerware.
Abbreviations
OM: Optical microscopy. SEM-EDX: Scanning electron microscopy with energy dispersive X-ray spectroscopy. XRD: X-ray diffraction. μ-Raman: micro-Raman spectroscopy. μ-ATR-FTIR: micro-Attenuated total reflection Fourier transform Infrared spectroscopy.

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Authors’ contributions
LZ and LW performed the data analysis and were major contributors in writing the manuscript. XZ provided the data processing method of infrared spectrum. JY and MZ made the cross-section sample. YW provided processing method of microscopic image. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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