(REFEREED RESEARCH)

RESIN EMBEDDING MEDIA AND TECHNIQUES FOR INVESTIGATING 3D STRUCTURE OF WOVEN FABRICS

ÜÇ BOYUTLU DOKUMA KUMAŞ YAPILARININ İNCELENMESİNDE KULLANILAN REÇİNE KAPLAMA MALZEMELERİ VE TEKNİKLERİ

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ABSTRACT

Thin sectioning and microscopic investigation are widely applied methods in the structural analysis of textile materials. In this study, several types of resin embedding media and methods were used in order to evaluate thin sectioning as means of determining the internal structure of a woven fabric. However, various problems were encountered with these resin types, which resulted in distorted or incomplete sections when the sample blocks were microtomed. Several solutions were introduced in order to overcome these problems occurred during embedding and sectioning processes. It was observed that successfully microtomed slices of tight sett fabric samples were achieved withultra-low viscosity Epoxy resin.

Key Words: Thin sectioning, Resin embedding, Woven fabric structure, Microtome.

ÖZET

Kesit alma ve mikroskobik inceleme, tekstil materyallerinin yapısal analizlerinde geniş çapta kullanılan yöntemlerdir. Bu çalışmada, dokuma kumaşın içyapısını belirlemek amacıyla kullanılan kesit alma işlemini değerlendirmek için farklı reçine kaplama malzemeleri ve yöntemleri kullanılmıştır. Bununla birlikte, farklı reçine tiplerinin kullanımları sırasında çeşitli sorunlarla karşılaşılmış, örneklerin mikrotom ile kesimi sonucunda deformasyona veya bölgesel kayıba uğramış kesitler elde edilmiştir. Reçineye gömme ve kesit alma işlemleri sırasında meydana gelen bu sorunları giderebilmek için çeşitli çözümler üretilmiştir. Sıkı yapılı dokuma kumaşların incelenmesinde kesit kalitesi bakımından en iyi sonuçlara, ultra-düşük vizkoziteli epoksi reçine ile ulaşılmıştır.

Anahtar Kelimeler: Kesit alma, Reçine kaplama, Dokuma kumaş yapısı, Mikrotom.

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1. INTRODUCTION

There are various microscopic instruments and associated analysis facilities that have been routinely employed for many decades (1) to either obtain information regarding the surface features or the internal structure of any fibrous textile type sample and/or the components therein (including: fibres, yarns, fabrics, composite materials, *etc.*).

Thin sectioning and microscopic (optical microscopy and scanning electron microscopy) investigation are widely applied methods (1, 2, 3, 4) in the structural investigation of textile materials. The objective of sectioning is to obtain a thin section of a specimen that may be viewed either by transmitted illumination (optical microscopy) or by an electron beam (scanning or transmission electron microscopy), so as to provide information regarding the internal fine structure or anatomy of the sample.

Virtually all sectioning techniques that involve physically cutting the sample are invasive, which implies that a portion of the sample is actually destroyed during the sectioning process. From the development of the earliest microscopes, up to the 1880's, sections were typically cut by hand, by simply cutting thin slices of the sample using a steel razor. Around the 1920's, simple sectioning machines called "microtome" were produced. In order to cut thin slices on the microtome, the specimen must be embedded in a solid block using various resin compounds. There is a huge choice of proprietary resin embedding media available. Most embedding media used for light microscopy applications are water soluble, and based on either acrylic, polyester or methacrylate resin (glycol-, methyl- or butyl-). Using embedding, any type of tissue can be sectioned reproducibly at section thicknesses of 5-10 µm.

The first stage of embedding is to attach the sample into the embedding mould. This can be achieved by using a frame to hold the sample in stable position. Then the mould should be filled with resin polymer till it covers all surfaces of the sample. The sample must also be completely penetrated by the resin polymer, and it is this aspect that forms the most important initial step in the embedding process. The process of polymerisation, which is the next step, varies with different resin types, some mixtures are 'cold setting', where the hardening begins

immediately after addition of the hardener component, whilst other resins require additional steps, such as exposure to ultraviolet radiation or slight heat, to initiate the polymerisation process. Once polymerized, the blocks containing the specimens to be sampled are removed from their moulds, then mounted either directly on the microtome or stuck to metal stubs using adhesive. The stubs with sample are then mounted onto the microtome for sectioning.

In this study, various resin types and sample preparation techniques were used to investigate the structure of woven fabrics. The problems encountered during application of sectioning procedure were observed and the efficiency of resin penetration through the fabric structures was examined with light microscopy and Scanning Electron Microscopy (SEM) analysis.

2. MATERIAL AND METHOD

100% Cotton and 100% Polyester woven fabrics with loose, medium and tight fabric sett values(5, 6, 7) were used to examine the embedding media. Four different resin types; TAAB Transmit LM Resin, JB-4 GMA Kit, TAAB Methacrylate Embedding Resin (M017) and TAAB Ultra-Low Viscosity Epoxy Resin were investigated as embedding media used in analysis of textile structures.

2.1. TAAB Transmit LM Resin

TAAB Transmit is a medium viscosity aliphatic epoxy resin plus reactive anhydride which allows the production of semi-thin sections for light microscopy. The monomer is extremely hydrophobic. The basic procedures for embedding are virtually the same for most types of polymer-based embedding media. The main objective is to totally encase the specimen in the block to give support, so that it can be sectioned without causing distortion to the sample. For this purpose, the three TAAB Transmit Resin components were initially mixed together, using a polythene container and magnetic stirrer.

In order to keep fabric samples aligned and to avoid distortion of the sample in the resin mixture during the infiltration and polymerisation process, a set of 25.0 mm x 25.0 mm ‘support frames’ were prepared using triple-ply 7.0 mm thick corrugated cardboard. The fabric samples were attached to the cardboard supports using two-pack rapid-setting epoxy adhesive, whilst ensuring that the fabric specimens were mounted horizontally straight across the top of the support frames, in order to facilitate sectioning of a correctly orientated sample when the trimmed block was subsequently mounted in the microtome. The support frames with attached fabric samples were then placed in 120 ml polythene embedding moulds, with ‘snap-fit’ caps. Moulds were then filled with the prepared resin mixture to a depth of 1.5 cm.

Once the samples were immersed in the resin, they were left at 4˚C for 24 hours in order to allow thorough infiltration of the resin mixture into the sample. Samples were then cured at 70˚C for 24 hours. The resultant blocks were allowed to cool to room temperature as polymerised TAAB Transmit is thermoplastic. After cooling, the hardened blocks were removed from the polythene moulds, then trimmed to the required size using a fine jewellers metal-cutting saw.

2.2. JB-4 GMA Kit

TAAB JB-4 is a hydrophilic glycol-methacrylate (GMA) resin system. These hydrophilic methacrylate-based resin embedding media were originally developed mainly as a means of enabling hydrated biological samples to be embedded and sectioned, without the requirement to pre-fix and then dehydrate using organic solvents. This process has the dual advantage of preventing the production of artefacts caused by both the conventional fixation and solvent exchange procedures, and allows wet samples to be embedded simply by using a graded series of the resin monomer as the dehydrating agent.

It was evident, that these resins would be ideal for embedding of hydrated cellulosic paper-making fibres, and this subject was thoroughly invested by Sherratt (8). This author also showed that hydrophilic methacrylate resins were ideal for thin-sectioning of 'dry' cellulosic paper-making fibres derived from wood pulps, since such fibres normally contain approximately 8.0% moisture in the air-dry state at room temperature, which causes difficulties when attempting to embed such fibres using the standard hydrophobic epoxy-based resin embedding media available at that time.

Subsequent work by Wilkins over the next 15 years (personnel communication) showed that JB4 resin proved to be ideal as a medium for embedding and sectioning of a wide range of natural plant-derived cellulosic fibres, including fibres derived from leaf, xylem, bast and seed-hairs.

2.3. TAAB Methacrylate Embedding Resin (M017)

Methacrylate M017 is a low viscosity resin that rapidly penetrates samples. The final desired hardness of block is adjusted by varying the proportions of methyl and butyl methacrylate. The greater the proportion of methyl methacrylate in the final mixture, then the harder the block will be. Three different proportions of methyl and butyl methacrylate were evaluated for fabric samples. The amount of methyl methacrylate was successively increased by 10% and 20% of recommended amount for the second and third resin mixtures, respectively.

The samples were placed in the various resin mixtures and the infiltration was carried out under vacuum for one hour. The resin was then cured at 55˚C for 24 hours. The hardness of the resin block obtained using a 20% increased amount of methyl methacrylate was found to be satisfactory for thin sectioning of fabric samples.

2.4. TAAB Ultra-Low Viscosity Epoxy Resin

TAAB Ultra-low viscosity resin has a very low viscosity (20 cps at 25˚C), which facilitates rapid and complete infiltration in minimum time. This resin is routinely used to embed hard biological samples such as bone.

The mixture was prepared by weighing and measuring each component into a 120 ml polythene container, then thoroughly mixing using a magnetic stirrer for 5-6 minutes. The samples were embedded in resin mixture and infiltration was then carried out under vacuum for 1.5 hours until all air bubbles were eliminated. The resin was then polymerised at 60˚C for 24 hours.

3. EMBEDDING AND SECTIONING PROCEDURES

The sample blocks containing fabric samples were mounted onto 2.4 cm diameter X 2.0 cm length steel microtome specimen holder stubs using cyanocrylate ‘superglue’. Once mounted on the stubs, the resin surrounding the sample was then

trimmed as required, in order to facilitate sectioning 6.0 mm width glass blades. A Reichert Jung 2050 general purpose microtome was then used to cut sections for optical microscopic analysis of the fabric samples. Sample blocks were sectioned on the microtome using freshly prepared 6.0 mm glass blades which are suitable for cutting sections for both optical and electron microscopy.

As each section was cut, it was carefully lifted away from the blade edge using fine forceps, then dropped onto the surface of a beaker of distilled water at approximately 40˚C, to relax the section and relieve stresses caused by the sectioning process. Sections were then lifted off the water surface onto glass slides, and dried on a warming plate at 40˚C. Once sections had been dried onto glass slides, they were effectively permanently bonded to the surface of the glass. Sections were then ready for examination by using either Light Microscope or SEM.

An “Olympus BH-2” optical research microscope was used in order to examine the cross-sections of fabric samples embedded in resin blocks. The internal photomicroscopy lens used has a magnification of 2.5X. Photomicrographic images were taken using a Fuji FinePix S2 Pro Digital 6.0 Mega pixel SLR camera.For SEM analysis of samples, a Hitachi S-3000-N variable pressure SEM (VPSEM) fitted with a back-scattered electron (BSE) detector facility was used. The VPSEM facility has a two-chambered vacuum column, with differential vacuum pumping system, and the capability of maintaining the specimen chamber at variable pressures. Uncoated samples can therefore be examined, but only using the BSE detector. Although the maximum magnification from this instrument under ideal (high vacuum and using secondary electron detector) is 100,000X, for uncoated samples examined in variable pressure using BSE, the magnification is limited to around 5000X.

4. RESULTS AND DISCUSSION

Fabric samples (cotton and polyester) were embedded in TAAB Transmit resin and sectioned at 5.0µm thickness on the microtome. Numerous embedding protocols were investigated, including varying section thickness (5µm, 10µm, and 15µm), varying infiltration times (24 hours, 48 hours) and vacuum infiltration. Several problems were encountered with this resin type, including poor section relaxation, section distortion, section separation and incomplete resin infiltration and/or lack of adhesion between the fibres and the resin.

Regardless of fabric type, section relaxation problems resulting in the formation of severe creases were initially a major problem, and hindered the early stages of this work. This problem was eventually overcome for cotton fabrics by carefully dropping freshly cut sections onto the surface of very hot water (approx. 80-90˚C). The latter problems listed above were mainly occurred while using tight fabric sett values. These problems could encounter either as a result of the resin failing to penetrate between individual fibres within yarns, due to the physical compactness of the yarns and fabrics, or; adhesion problems caused by moisture within cotton fibres (natural cellulosic fibres typically have a moisture content of approximately 8% at normal room temperature and humidity), and the hydrophobic nature of the resin. A typical example of section separation and distortion can be seen in Figure 1.

In the case of polyester fibres, the problem of section distortion could simply be due to poor chemical adhesion. Polyester films are used as release substrates for commercial moulding with epoxy resins, and it is highly likely that the sections obtained were purely as a result of ‘chance’ physical support, with the sectioned fibre fragments being very loosely held within the resin section, and not adhesion; and it was noted that the integrity of the sections was very unstable, with fibre fragments readily ‘dropping out’ of the section (Figure 2).

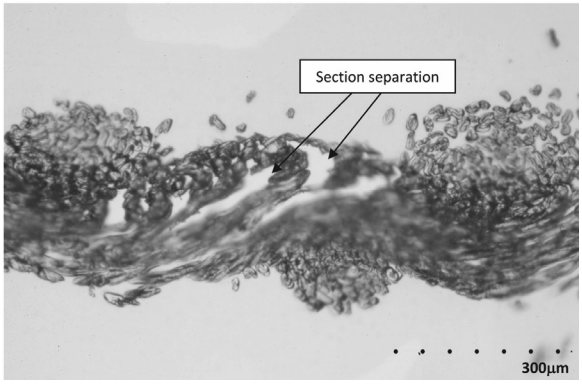

Figure 1. Example of section separation occurring in fabric sample embedded in TAAB Transmit resin

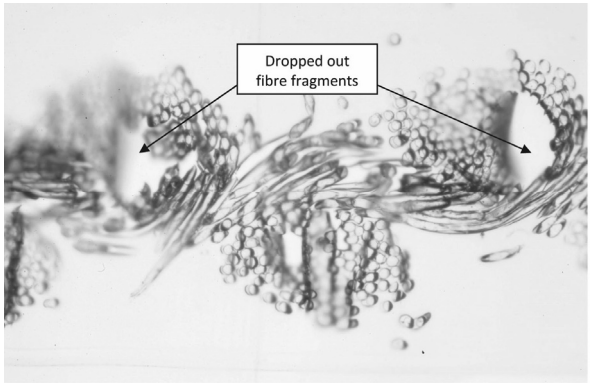

Figure 2. Example of poor chemical adhesion in the polyester fabrics embedded in TAAB Transmit resin

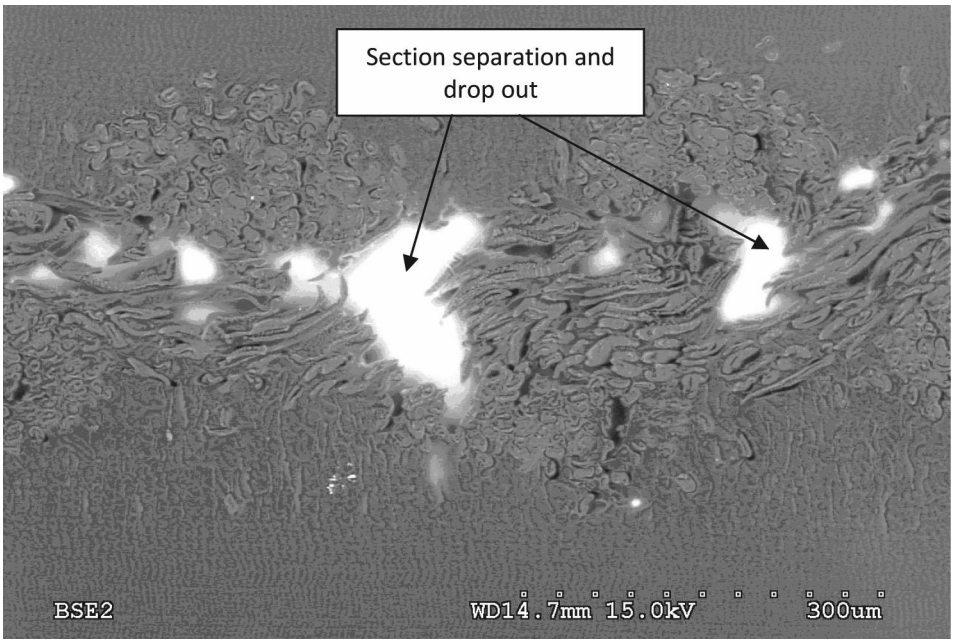

Figure 3. VPSEM image of section of cotton fabric (section thickness = 5.0 µm)

In view of the above concerns, a second, completely different resin type, TAAB JB4, was tried. The main reason for evaluating this resin type was due to the fact that it is relatively straightforward to achieve 'stress-free' sections with methacrylate polymers. It was also assumed that this resin type would be especially amenable to embedding of cellulosic fibres, due to the hydrophilic nature of the main monomer component. However, in this study, this particular resin kit was shown to give totally unsatisfactory results with fabric samples, with no successful sections obtained with polyester fabrics, and fairly unsatisfactory results with tight sett cotton fabrics. These problems were believed to be attributable to incomplete infiltration, as a result of the physical compaction of the component fibres within the yarns.

A second methacrylate-based resin, TAAB M017 Methyl Methacrylate kit, was then tried. This resin could be prepared to give any desired hardness of polymerised block. However, several problems were also encountered with this resin type, including poor structural integrity, mainly resulting in fibre 'drop out' problems, once the sections had been obtained. Use of this resin also caused section separation and section distortion problems as seen previously in the trials made using TAAB Transmit epoxy resin (Figure 3, Figure 4). In the case of polyester fabric samples, there was also failure of the sections to fully relax and relieve stresses after sectioning, which caused similar distorted 'wavy looking' sections to those encountered previously with other resin types.

The SEM analysis proved that the poor adhesion between the embedding medium and fibres within the fabric samples and the physical compactness of the fabrics caused imperfect block surfaces during the sectioning process.

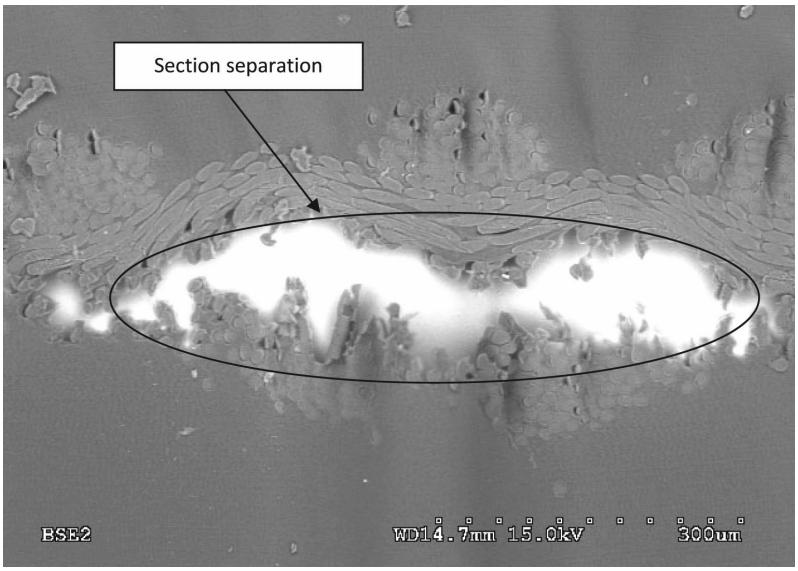

Figure 4. VPSEM image of section of polyester fabric (section thickness = 5.0 µm)

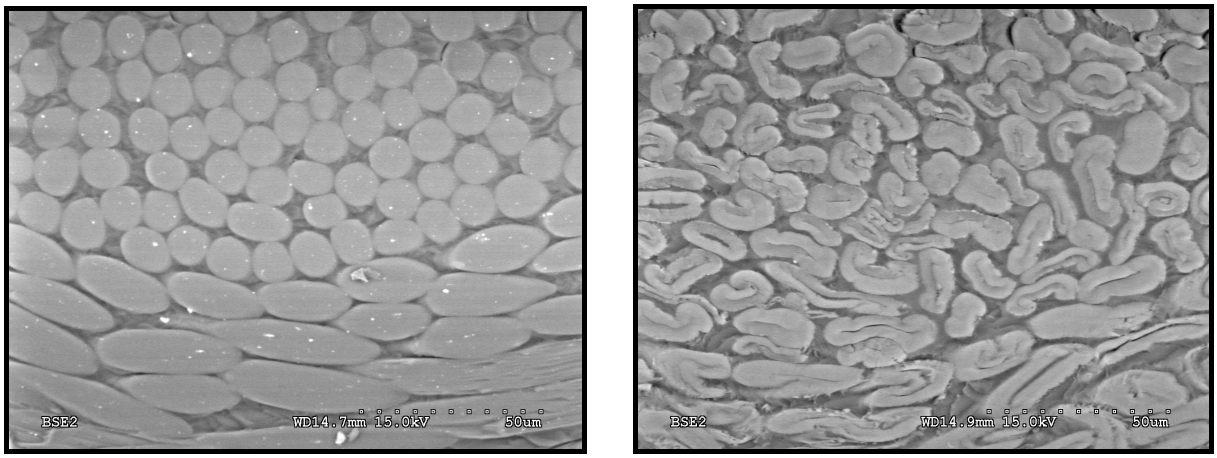

Figure 5. VPSEMimages of cut face of resin block containing PES and cotton fabrics

As a result of concerns regarding poor resin infiltration and lack of adhesion with the above discussed resins, an ultra low viscosity epoxy resin was investigated. This resin is mainly used for transmission electron microscopy, and it was reported by Chakrabarty, et. al. that this resin type was used successfully for sectioning polyester fibres (9). Sections mounted on glass slides and the freshly cut surface of the block containing the embedded fabric sample was examined using the VPSEM. The efficiency of resin penetration in-between the component fibres of the yarns was determined precisely by using 'atomic number contrast', obtained using backscatter electron detector (BSE) imaging and an electron beam accelerating voltage of 20Kv. This technique gave good (atomic number) contrast between the resin and the fibres. This method gave more successful results than the previous techniques attempted. Resin penetration was achieved by using ultra low viscosity epoxy resin (Figure 5).

5. CONCLUSIONS

For the microtome thin sectioning technique, several types of resin embedding media and methods were used in order to evaluate thin sectioning as a means of determining the internal structure of a woven fabric. The resin types used were TAAB Transmit LM,TAAB JB-4 GMA kit, TAAB Methacrylate and TAAB Ultra-Low Viscosity Epoxy Resin. Loose, medium and tight sett fabric samples were embedded in these resins. Sections were then examined by using either Light Microscope or SEM. Various problems like poor section relaxation, section distortion, section separation and incomplete resin infiltration and/or lack of adhesion between the fibres and the resin were encountered during embedding and microtome sectioning processes. Application parameters were adjusted in order to overcome these problems.

Section relaxation problems observed with TAAB Transmit LM were overcome for cotton fabrics by carefully dropping freshly cut sections onto the surface of very hot water.In the case of polyester fibres, the problem of section distortion due to poor chemical adhesion showed that TAAB Transmit LM resin was not suitable for embedding and sectioning of 100% polyester fabric structures.

Two methacrylate-based resin embedding media; TAAB JB-4 GMA kit, TAAB Methacrylate Resin were studied. Use of this resin also caused section separation and section distortion problems as seen previously in the trials made using TAAB Transmit epoxy resin.

As a result of concerns regarding poor resin infiltration and lack of adhesion with the first three resins, an ultra low viscosity epoxy resin was investigated. This resin type gave more successful results for both cotton and polyester fabric samples than the other resin embedding techniques attempted.

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