

A Simple Embedding and Lining Method for the Sectioning of Wood and Wood-based Materials using an Alpha-Cyanoacrylate Resin.

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アルファー・シアノアクリレート樹脂による木材および木質材料の包埋と裏打ち法

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Résumé

A very simple embedding method and an effective lining method were devised using so-called "quick-setting adhesive resin (the trade name: Aron Alpha (Crazy Glue) 201)". The former is suitable for the dry and porous materials such as wood, bark and paper to obtain very thin sections of 0.5-5 μm for the light microscopic observation and the clear cut-surface for the scanning electron microscopy. The latter is applicable to the wet materials such as living tissue or wood blocks softened for the ordinary sliding-microtomy.

要 旨

一般に市販されているいわゆる瞬間接着剤のアロンアルファー 201 を用いて、非常に簡便かつ迅速な樹脂包埋法を開発した。また通常のマイクローム操作にも応用できる切片の「裏打ち」法を考案した。

包埋法は Fig 1 に示すように、(1)乾燥した試片を試料台の上に置く。(2)アロンアルファー 201 を滴下して試片中に樹脂を浸透させる。(3)余剰のアロンアルファーを濾紙で吸い取り約 1 時間放置する。この手順で完了し、この包埋試片から 0.5 ~ 5 μm 厚さの切片がガラスナイフを用いて容易に切り出せる。包埋樹脂はアセトンで除去されるので、切片は必要に応じて脱包埋できる。また切片作製後の試片をアセトンで脱包埋すると、走査電顕観察に適した断面が得られる。この包埋法は木材はもちろん樹皮、紙、合板などの乾燥した多孔性の試料に適用できる。

「裏打ち」法は湿潤試料に有効で、Fig 2 に示すように、(1)試片をスライディングマイクロームに装着し、通常のマイクローム操作で試料表面を平滑にする。(2)アロンアルファー 201 を滴下し余剰の液を濾紙で吸いとる。約 1 分後にはアロンアルファーは試料表面に固着する。(3) 5 ~ 20 μm 厚さの切片を切り出す。以後(2)(3)の操作をくり返す。切片はアロンアルファー膜で裏打ちされているので切削時の損傷が少なく、軟弱な組織や、バラバラになりやすい組織の薄切に有効である。なお、アロンアルファーの裏打ち膜は必要に応じてアセトンで除去することができる。

Introduction

In order to make the best use of the light microscope the tissues are desirable to be sectioned as thin as possible. However, the sections obtained by the ordinary sliding-microtomy are only 10 μ m in thickness and often accompanied by serious artifacts such as the damage of fine structure and the breakdown of section during the sectioning and following preparations. Although several embedding methods using plastics such as epoxy resin¹⁾, methacrylate resin²⁾ and so on are very effective to obtain very thin sections and to prevent such artifacts, the actual handling is very complicated and needs a laborious work.

A new method introduced in this paper is very simple and is widely applicable to wood and wood-based materials. An embedding resin used is ethyl-alpha-cyanoacrylate. The monomer is commercially available as a "quick-setting adhesive resin" by the trade name of "Aron Alpha (Crazy Glue) 201" from Toagosei Chemical Industry Co., Ltd., Japan, being preserved in a 2 g tube. It is a colorless, transparent liquid having very low viscosity (η : 2), and polymerizes and hardens quickly by the catalytic action of a very small amount of moisture contained in atmosphere or dry tissue. The shrinkage upon polymerization is only 15% in volume, and the polymer is soluble to acetone. If a drop of the monomer is placed on dry and porous tissue, it penetrates deeply into the tissue and hardens quickly in the tissue. Only by using this simple preparation method the tissue is embedded in a plastics having proper hardness for sectioning.

On the other hand, if a drop of the monomer is placed on wet tissue, it hardens immediately on the tissue surface without penetrating into the tissue. This phenomenon is also applicable for the lining of wet tissue as well as the lining method for the dry tissue using cellophane tape.³⁾ That is, if the tissue surface is covered with a firm resin membrane, the breakdown of thin section which often happens during the ordinary sliding-microtomy and the following preparations will be prevented.

Methods and Results

Embedding method of dry materials

The general procedure of the embedding is shown in Fig. 1.

- 1) Put a small piece of specimen block on a plastic holder.
- 1') Insert the tissue into the split of a plastic holder for the transverse section of thin specimens such as paper.
- 2) Drop the monomer on the specimen.
- 4) Remove the surplus monomer using a filter paper, and leave as it is. The resin hardens in about one hour and simultaneously the specimen block adheres to the holder.

Thin sections of 0.5-5 μ m thick can be sliced off from the trimmed tissue surface, using a glass knife set on a rotary microtome or an ultramicrotome. The "wet-cutting" is more appropriate to obtain the thinner sections under 2 μ m thick, while the "dry-cutting"

for the thicker ones. When the sections are wavy in the dry-cutting, they are smoothed by floating the section on water surface. The staining are also performed by floating sections on the dye solution. The sections are transferred on a glass-slide, mounted after the drying, and observed under a light microscope. The staining effect of tissue was very excellent, comparing with the case of epoxy sections, and the undesirable stains of the embedding resin occurred scarcely. If the embedding resin causes any obstacle during the procedures, it can be removed using acetone.

The embedding and sectioning procedures are also useful for the scanning electron microscopy, because a very clear cut-surface of specimen block can be obtained using a glass knife without the distortion of fine structure and severe knife marks commonly associated with the ordinary microtomy and the razor blade cutting, and the embedding resin can be removed using acetone. Moreover, the three-dimensional structure observed under a scanning electron microscope are coordinated with the light microscopic observation of the neighboring section. An example is shown in Figs. 3 and 4.

Lining method of wet materials

The actual procedure of the lining method is as follows (Fig. 2) .

- 1) Set a wet material to a sliding microtome, and make the surface smooth, according to the ordinary microtome operations.
- 2) Drop the monomer on the smoothed tissue surface.
- 3) Remove the surplus monomer with a filter paper, and leave as it is.
- 4) After one minute, cut the tissue surface together with the lining resin membrane.
- 5) Repeat the same operations from the second description.

The simple operations described above are applicable directly to our ordinary sliding-microtomy, and are very effective to the feeble materials such as living tissue and bark because of the toughness of lined sections during the staining, dehydration and mounting preparations. The embedding resin can be also removed by soaking in acetone in case of need.

REFERENCES

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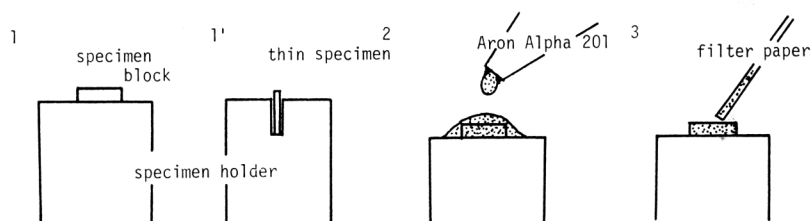


Fig. 1. Embedding procedure of a dry material.

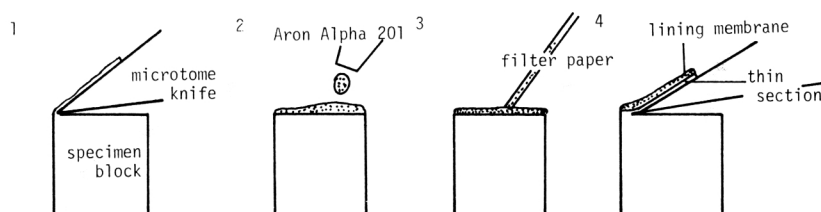


Fig. 2. Lining procedure of a wet material.

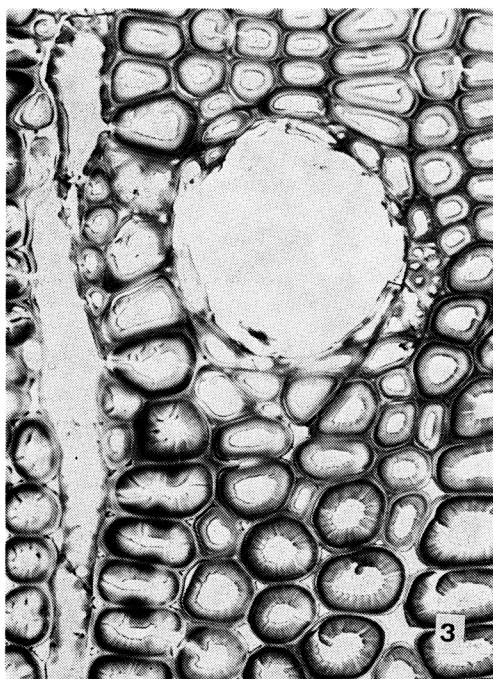


Fig. 3. Light micrograph of the compression wood of TOGASAWARA (*Pseudotsuga japonica* Beissn.). A $3\ \mu\text{m}$ section sliced transversely from the embedded tissue and stained with safranin. X800

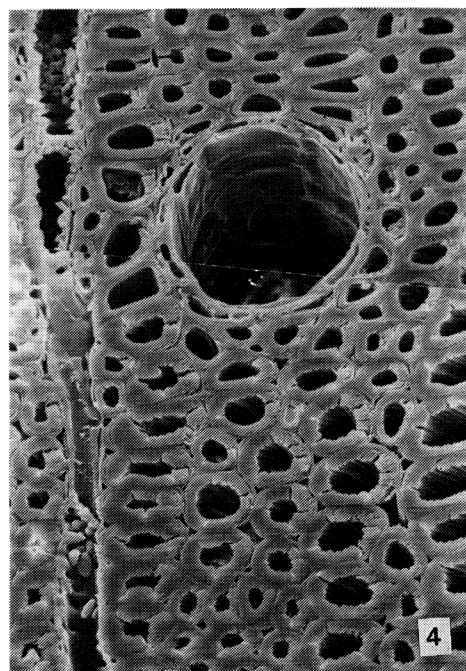


Fig. 4. Scanning electron micrograph of the same region as that of Fig. 3. A tissue surface after sectioning and removal of embedding resin. X720

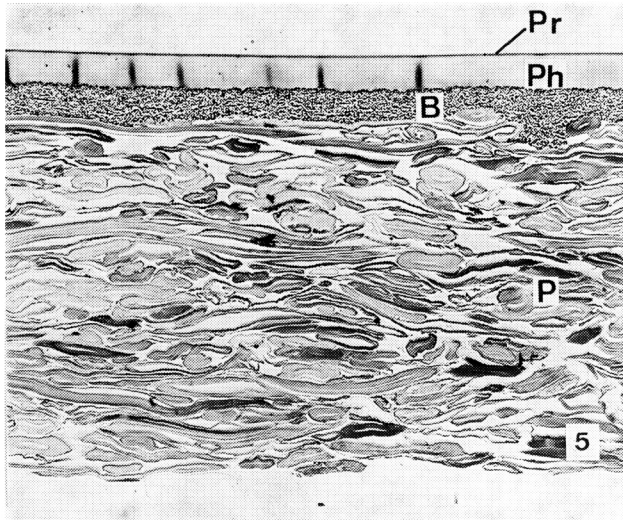


Fig. 5. Transverse section of a photographic paper. A $3\ \mu\text{m}$ section sliced from the embedded material, and stained with toluidine blue-O. Pr: protective layer. Ph: photographic emulsion layer. B: baryta layer. P: base paper. X270.

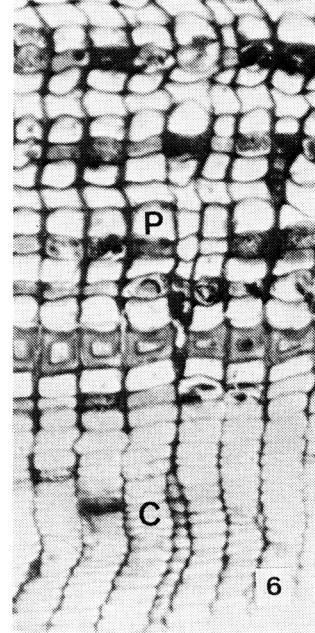


Fig. 6. Transverse section of cambial zone (C) and phloem (P) of SUGI (*Cryptomeria japonica* D. Don.). A $10\ \mu\text{m}$ section sliced from a glutaraldehyde-fixed specimen block by the lining method and stained with safranin. X200

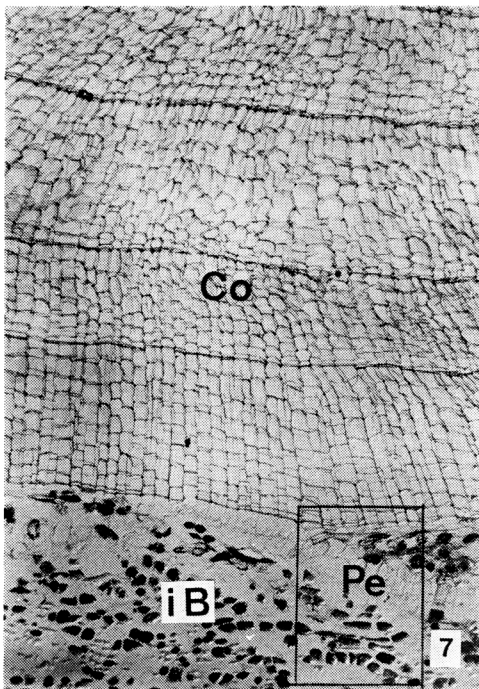


Fig. 7. Transverse section of a developed cork tissue (Co), a just forming periderm (Pe) and inner bark (iB) of ICHO (*Ginkgo biloba* L.). A $20\ \mu\text{m}$ section sliced from a glutaraldehyde-fixed specimen block by the lining method and stained with sudan-IV. X30.

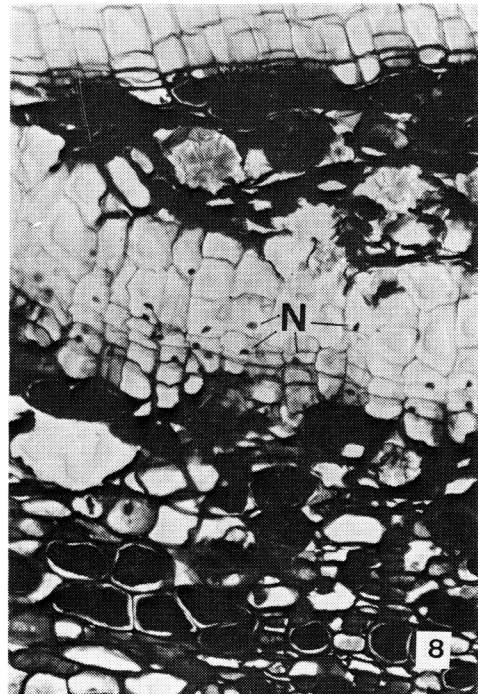


Fig. 8. Enlarged light micrograph of the enclosed region shown in Fig. 7. A $10\ \mu\text{m}$ section neighboring on that of Fig. 7 stained with safranin. Cork cambium is occurring in the inner bark. N: nuclei. X100.