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# ETHANOL-BASED EPOXY SHEET PLASTINATION OF HUMAN KIDNEY: A NON-VACCUM IMPREGNATION METHOD FOR REDUCED SHRINKAGE AND COLOR PRESERVATION

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## ABSTRACT

Plastination offers a reliable way to create anatomical specimens that can be preserved for many years. However, achieving accurate color retention and reducing shrinkage remain ongoing challenges. Epoxy sheet plastination is commonly used to prepare thin, and durable tissue sections, yet traditional techniques often result in pigment fading, tissue distortion, and the need for expensive vacuum systems. In this study, we present a modified technique that uses ethanol-supported dehydration followed by passive epoxy infiltration carried out entirely under atmospheric pressure for human kidney slices. Thin tissue sections (2–5 mm) were gradually dehydrated through a series of ethanol and acetone concentrations, then embedded in epoxy resin without applying vacuum. The specimens prepared using this method retained their natural coloration and demonstrated noticeably reduced shrinkage compared to the levels typically associated with room-temperature alcohol dehydration. The final results were clear, structurally sound, and economical to produce. This approach provides a practical and high-quality alternative to conventional vacuum-dependent methods, increasing accessibility for institutions involved in anatomical teaching and research.

**KEYWORDS:** Epoxy sheet plastination, ethanol-assisted dehydration, passive impregnation, kidney, shrinkage, color preservation.

## I. INTRODUCTION

Plastination, introduced by Gunther von Hagens in 1977, revolutionized the preservation of anatomical specimens by enabling the production of dry, odorless, durable, and long-lasting models suitable for medical education and scientific research. The technique involves replacing water and fat within biological tissues with polymers that harden, allowing the specimen to maintain its structural integrity and resist decomposition.[1] As a result, plastinated specimens can be handled easily and safely without the need for special storage environments. These qualities have made plastination a significant tool in advanced anatomical teaching, comparative research, and in correlating gross anatomy with contemporary imaging techniques.

Among the available plastination methods, Epoxy Resin Sheet Plastination (E12) stands out for its ability to create thin, transparent sections of organs and anatomical regions. Typically prepared between 0.7–1.2 mm or 2–5 mm in thickness, these sections are well-suited for studying topographical relationships, sectional anatomy, and the fine internal organization of tissues.[2] For the human kidney, E12 plastinated slices provide exceptionally clear views of the cortex and medulla, vascular pathways, and internal segmentation, making them particularly valuable for both educational instruction and research applications.

Despite its broad adoption, conventional E12 plastination still faces several challenges that affect both the quality of the final specimen and its accessibility. One of the most significant issues is the preservation of natural color. During typical dehydration and resin infiltration, pigments may fade, leach, or chemically degrade, often resulting in discoloration or a yellowish tint over time.[3] Maintaining true organ coloration is especially important in educational settings, where clear visual distinction—such as between the cortical and medullary regions of the kidney—is essential for anatomical orientation and for recognizing structural or pathological variations.

Another major limitation is tissue shrinkage and distortion[4] that can occur during the dehydration phase. Traditionally, cold acetone—used at approximately −25 °C—has been employed to reduce dimensional changes,[4] but this strategy requires specialized freezers and meticulous handling. Although room-temperature solvents such as methanol or acetone are more convenient and cost-effective, they have been shown to produce considerably higher shrinkage, with reported rates ranging from 20% to 22.6% in previous studies.[5] Such shrinkage not only alters the physical appearance of specimens but also affects morphometric accuracy and reduces the reliability of topographical comparisons with radiological imaging.

In addition, the cost and technical demands of conventional E12 plastination can restrict its use, particularly in smaller laboratories or institutions with limited funding. Standard procedures rely on forced vacuum impregnation to replace the intermediary solvent with polymer and require low-temperature conditions during dehydration, necessitating deep freezers and specialized equipment. The investment in vacuum systems, cold storage units, and dedicated resin-handling infrastructure can be substantial, creating barriers to the broader adoption of high-quality sheet plastination.

To address these limitations, various alternative dehydration strategies have been explored. Ethanol, a less aggressive alcohol, presents several potential advantages in terms of color retention and controlling tissue shrinkage. With a polarity that lies between water and acetone, ethanol enables more gradual fluid exchange, which may reduce pigment loss and limit osmotic stress on tissue. Stepwise ethanol dehydration has the potential to preserve natural coloration, maintain structural integrity, and lessen shrinkage, positioning it as a viable substitute for methanol or acetone in room-temperature plastination methods.

The present study proposes a modified epoxy sheet plastination protocol for human kidney slices that combines ethanol-assisted dehydration with passive resin infiltration conducted under atmospheric pressure, effectively eliminating the need for costly vacuum equipment.

## II. MATERIALS AND METHODS

### 1. Specimen Collection and Preparation

A human kidney was procured from a legally donated cadaver, with all procedures conducted in accordance with approved ethical guidelines. The specimen was fixed in 10% neutral buffered formalin[6] for two weeks to ensure adequate cross-linking of tissue proteins and to prevent decomposition. Proper fixation is essential for preserving structural detail and preventing distortion during later stages of dehydration and resin infiltration.

After fixation, the kidney was manually sectioned into slices measuring 2–5 mm in thickness, appropriate for epoxy sheet plastination.[6] The slices were then rinsed under running tap water to remove any remaining fixative, reducing the likelihood of chemical interference during dehydration and subsequent resin impregnation.

### 2. Ethanol-Assisted Dehydration

Dehydration was carried out at room temperature using a graded ethanol series,[7] aimed at gradually removing water while preserving tissue pigments:

1. 50% Ethanol (v/v): Kidney slices were immersed until the solution turned turbid, indicating initial water removal. The ethanol was replaced as required to ensure continued dehydration.
2. 70% Ethanol (v/v): Tissue sections were then transferred to 70% ethanol, with the solvent refreshed whenever turbidity appeared to maintain effective dehydration.
3. 90% Ethanol (v/v): Finally, slices were placed in 90% ethanol, with periodic solvent replacement until the solution remained clear, signaling completion of the dehydration process.

After ethanol dehydration, the kidney slices were transferred to acetone[7] for final dehydration and defatting:

1. **90% Acetone (v/v):** This intermediate step continued the dehydration process while initiating lipid removal. The solvent was replaced whenever turbidity or color changes were observed.
2. **100% Acetone (v/v):** Complete dehydration and defatting were confirmed when the solvent remained clear for three consecutive days, indicating thorough removal of both water and lipids.
   This gradual, observation-based approach minimized tissue shrinkage and effectively preserved natural coloration by avoiding sudden changes in solvent polarity that could otherwise cause pigment loss

### 3. Passive Epoxy Impregnation

In contrast to traditional E12 plastination, which relies on vacuum-assisted resin infiltration, this study employed passive impregnation at atmospheric pressure to minimize equipment requirements and reduce costs.

#### a. Resin Preparation

The epoxy resin mixture (E12 polymer) with hardener and resin ratio (2:1) was prepared according to manufacturer instructions.

### b. Casting

The kidney slices were arranged on a flat glass plate, with 3–4 mm spacers placed around each section to form a uniform chamber for resin embedding using the sandwich method.[8] The epoxy resin mixture was carefully poured to fully cover the slices, ensuring thorough contact with the tissue. The chamber edges were then secured with clamps to prevent resin leakage during polymerization.(figure1)

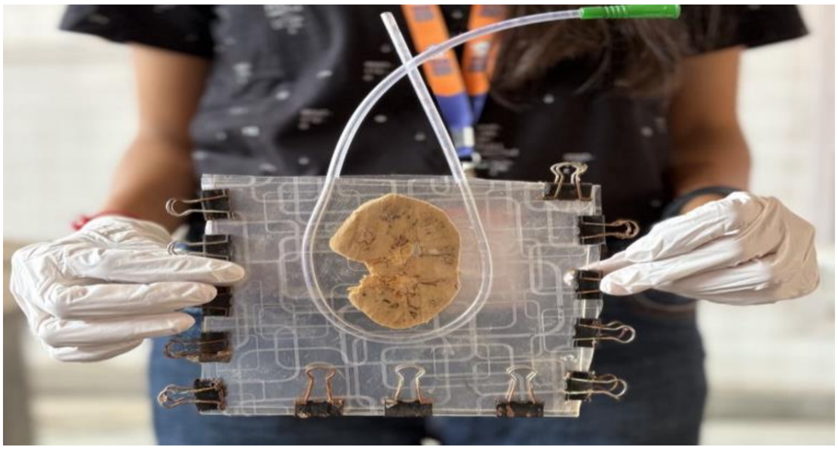


**Figure 1.**

### c. Passive Infiltration

The resin infiltrated the tissue through capillary action over several days at room temperature. Unlike vacuum-assisted methods, this approach relies on the gradual diffusion of resin into the tissue, minimizing equipment needs while ensuring adequate penetration for thin sections (2–5 mm).

### 4. Curing

Curing was carried out entirely at room temperature. The specimens were kept in a controlled, well-ventilated environment until complete polymerization was achieved, ensuring uniform hardening of both the surface and interior and resulting in durable, optically clear plastinated sheets.

### 5. Shrinkage and Dimensional Assessment

To quantify tissue shrinkage, linear dimensions and weight were recorded before dehydration and after full dehydration and resin curing:

- Length and width were measured at three points: upper pole, mid-region, and lower pole of each slice.
- Weight was recorded using a precision balance.
- Linear shrinkage (%) was calculated using the formula:

Shrinkage (%) = initial dimension-final dimension /initial dimension*100

This assessment allowed evaluation of dimensional changes across the slice and identification of regions with maximal shrinkage.

### 6. Structural and Color Assessment

Post-embedding slices were visually inspected for:

- Structural integrity: absence of tears, fissures, or gross distortions.
- Color preservation: comparison of cortical and medullary pigmentation before and after plastination.

This evaluation confirmed the effectiveness of ethanol-assisted dehydration in maintaining natural coloration and anatomical fidelity.

## III. RESULTS

### 1. Dehydration and Tissue Preparation

The stepwise ethanol-assisted dehydration followed by acetone treatment successfully prepared human kidney slices for passive epoxy infiltration. Visual assessment during the dehydration phase indicated gradual solvent replacement and defatting, with the 100% acetone bath remaining clear for three consecutive days, confirming complete water and lipid removal. The slices retained structural integrity throughout, with no visible tearing or fissures observed.

### 2. Dimensional Changes and Shrinkage

Weight and linear dimensions were measured before dehydration and after dehydration of the slice. The kidney slices exhibited a weight reduction of 16.14%, reflecting effective removal of tissue water and lipids.

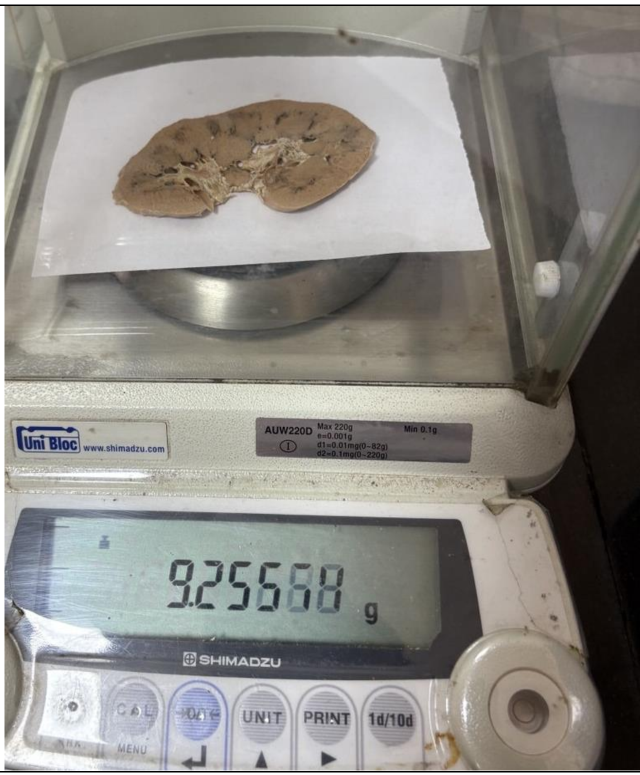
Linear shrinkage was assessed at three points along the kidney slice (upper pole, middle region, lower pole):

| **Measurements** | **Initial value (Pre-Fixation/Dehydration) (figure 2)** | **Final Value (Post-Dehydration/Pre-Impregnation) (figure3)** | **Percentage Reduction (Shrinkage/Loss)** |
|---|---|---|---|
| Weight | 11.03gm | 9.25gm | 16.14% |
| Longitudinal length | 9.5cm | 9.0cm | 5.26% |
| Width(upper pole) | 4.5cm | 4.4cm | 2.2% |
| width (middle region) | 3.8cm | 3.5cm | 7.89% |
| Width (lower pole) | 5.5cm | 5.3cm | 3.64% |

The average linear shrinkage across all measured dimensions was 4.92%**,** indicating minimal dimensional change. Notably, the middle region of the slice experienced the highest linear distortion (7.89%), while the upper pole demonstrated the least shrinkage (2.22%).

**Pre dehydration(figure 2)**

**Post dehydration(figure3)**




### 3. Comparative Analysis

The shrinkage achieved with the ethanol-assisted dehydration and passive impregnation protocol was markedly lower than that reported for conventional room-temperature alcohol dehydration methods:

- Room-temperature methanol: ≈22.6%[4]
- Room-temperature acetone: ≈20.2%[4]
- Cold acetone freeze substitution: ≈14.5%[4]

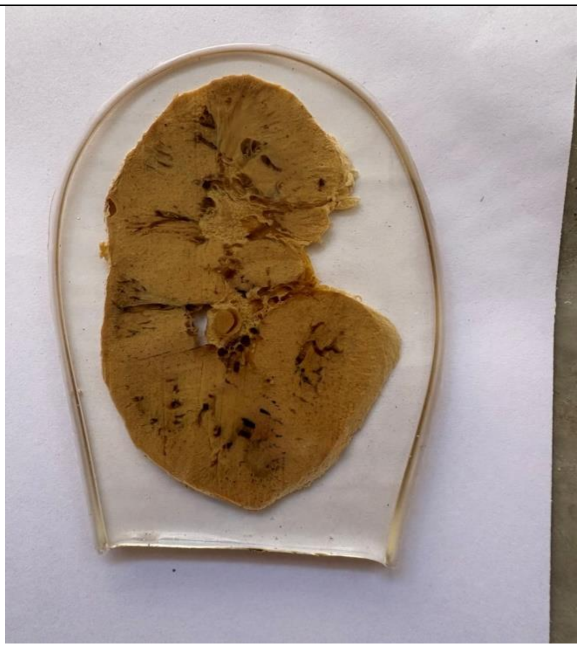
These results demonstrate that sequential ethanol dehydration, followed by acetone defatting, substantially reduces dimensional distortion.

### 4. Structural and Color Preservation

Visual inspection of the plastinated kidney slices revealed that cortical and medullary structures remained clearly distinguishable. The natural coloration of the tissue was effectively preserved, with minimal pigment loss or yellowing. This indicates that the graded ethanol series successfully stabilized tissue chromophores during dehydration, addressing a common limitation of conventional epoxy sheet plastination.

The slices were transparent, mechanically stable, and aesthetically intact**,** confirming that passive epoxy infiltration at atmospheric pressure is sufficient for thin (2–5 mm) sections. The specimens are thus suitable for anatomical education, morphometric analysis, and radiological correlation.(figure 4)

For visual comparison, a specimen previously processed using room-temperature acetone dehydration alone (author's image) demonstrated dark brown discoloration and increased stiffness**,** contrasting with the preserved natural coloration and flexibility observed in this ethanol-assisted protocol.(figure 5)

| **Sheet plastination seen from both sides (Ethanol assisted protocol)(figure 4)** | |
|---|---|
|  |  |
| **Sheet plastination (acetone assisted protocol)(figure 5)** | |
|  |  |

## IV. DISCUSSION

This study evaluated a modified epoxy sheet plastination protocol for human kidney slices, combining ethanol-assisted dehydration with passive epoxy impregnation at atmospheric pressure. The results demonstrate that this approach achieves minimal tissue shrinkage, preserves natural coloration, and reduces the need for costly vacuum-based equipment.

### 1. Shrinkage Reduction

A primary aim of the modified protocol was to reduce tissue shrinkage during dehydration and resin embedding. The average linear shrinkage of 4.92% observed in this study is considerably lower than values reported for conventional room-temperature alcohol dehydration methods (methanol: ≈22.6%; acetone: ≈20.2%) and even lower than optimized cold acetone freeze substitution (≈14.5%).[4]

The low shrinkage can be attributed to the graded ethanol series, which gradually replaces tissue water without causing abrupt osmotic stress. Ethanol's intermediate polarity likely stabilizes tissue proteins and prevents excessive contraction, while the subsequent acetone defatting ensures complete lipid removal without further dimensional distortion. The observation-based solvent replacement, performed until turbidity cleared, further minimized stress on the tissue and prevented over-dehydration, a factor often responsible for structural shrinkage.

**2. Color Preservation**

Maintaining natural tissue coloration is a critical challenge in epoxy sheet plastination. Conventional methods frequently cause pigment leaching, yellowing, or discoloration, which can reduce the educational and aesthetic value of specimens. In this study, kidney slices retained clear cortical and medullary differentiation and vivid natural pigmentation after dehydration and resin embedding.

The success in color preservation is likely due to the mild dehydration conditions provided by ethanol, which reduces pigment solubilization compared to methanol or acetone alone. Ethanol's less aggressive solvent properties may prevent abrupt polarity shifts that can extract endogenous chromophores, thereby stabilizing tissue coloration prior to epoxy infiltration. This approach ensures that specimens remain visually accurate for both teaching and research purposes.

The contrast observed with a specimen previously processed using room-temperature acetone dehydration alone, which demonstrated dark brown discoloration and increased stiffness, supports the potential advantages of a staged ethanol-assisted approach. Collectively, these findings suggest that this modified protocol may offer a practical, lower-cost alternative for institutions seeking improved color fidelity and structural preservation during sheet plastination.

**3. Passive Impregnation and Cost-Effectiveness**

A significant innovation of this protocol is the use of passive epoxy infiltration at atmospheric pressure, eliminating the need for forced vacuum equipment traditionally required in E12 plastination. Conventional vacuum impregnation is essential for deep solvent replacement with polymer in thicker sections but requires substantial capital investment, including vacuum chambers and deep freezers.

The success of passive impregnation for thin kidney slices (2–5 mm) demonstrates that capillary action alone is sufficient for adequate resin infiltration in thin sections. The resultant specimens were mechanically stable, transparent, and free of internal voids, confirming that vacuum-free methods can produce high-quality sheet plastinates while significantly reducing operational costs. This improvement has practical implications for institutions with limited resources, broadening accessibility to advanced anatomical specimens.

**4. Limitations and Future Perspectives**

While this protocol demonstrates clear advantages for thin kidney slices, its applicability to thicker specimens or other organ systems requires further investigation. Passive impregnation may be insufficient for sections exceeding 5 mm in thickness, where vacuum-assisted polymer infiltration may still be necessary. Additionally, long-term studies are needed to monitor polymerization stability, color retention, and mechanical integrity over extended periods.

Future research could explore the optimization of ethanol concentration sequences, curing temperatures, and resin formulations to further enhance specimen quality, reduce shrinkage, and preserve pigmentation across diverse tissues.

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