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**Original Research Article**

# A Cost-Effective Novel Method of Preparing Plastinated Specimens of Brain

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## ABSTRACT

The human bodies and organs have traditionally been preserved using formalin solution, although it irritates the eyes, nose and throat. Plastination is an unique and expensive method for preserving biological samples that can be used in teaching and research. Therefore, the goal of the current study was to prepare a cost-effective solution using thermocol to plastinate the brain specimens as a supplement for teaching and research. Two human brains were used in this pilot research project. According to standard procedures, the brains were first fixed in 10% formaldehyde, sectioned horizontally, sagittally and coronally dehydrated in acetone, and then immersed in the plastination solution at room temperature and pressure. Then the specimens were air-dried at room temperature. A team of 62 Anatomists and Pathologists assessed the quality of the specimens using a self-developed grading scale. The grading was provided based on the specimens' clarity, stability, aesthetic look, colour and smell. Statistical analyses was performed using SPSS software, Kruskal Wallis test showed that the lowest mean score was 4.04 provided for colour and highest mean score was 5 provided for the smell with a statistically significant $p<0.001$. Thus our plastinated specimens were of good quality, durable and handle-friendly. Our study demonstrated that the cost-effective plastination solution (CEPS) procedure is an inexpensive and efficient way to create plastinated specimens that are appropriate for teaching neuroanatomy.

**KEY WORDS:** Brain, Thermocol, Plastination, Cost-Effective Plastination.

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## INTRODUCTION

Cadavers (deceased human bodies) are an essential learning tool in medical education. There is no substitute for using cadavers while teaching and learning anatomy [1]. Over the past few decades, anatomical researchers have been exploring for reliable and secure ways to preserve bodies [2].

The procedures employed include mummification, fixation, deparaffinization, epoxy-resin, formalin embalming and plastination [3].

Von Hagens first developed the method of plastination in 1977 as a means of preserving biological tissues. The plastination method

yields odourless, dry and long-lasting specimens [4, 5].

The process of plastination requires enormous time, a skilled Anatomist's team, chemicals and equipment [6]. Around 500 to 1000 hours of skilled dissection will take around a year to complete the process of whole-body plastination [7]. The organs and soft parts are treated with deep freezing and forced vacuum impregnation techniques to replace the water with acetone and acetone with resin, respectively [8]. In addition to being used to plastinate body components, epoxy resin can also be utilized to plastinate high-quality translucent slices with a thickness range of 2 to 5 mm [9]. Providing chemicals, deep freezers, forced vacuum impregnators and related products for plastination processes for more than 40 years, Biodur has established itself as a major participant in the market. According to the estimation of Biodur company products, a minimum of rupees 25 lakhs will be required to set up a basic plastination unit [10].

According to a study by Holladay et al., the chemicals used in the plastination process, such as silicone, epoxy and polyester are mildly harmful to extremely dangerous. Some of the compounds used in plastination may be dangerous when they are in liquid or gaseous form. Epoxy resins are well-known irritants of the skin, eyes and mucous membranes [11].

The silicone product ingredient poly-alkylsiloxane is known to irritate the skin, eyes, mucous membranes and has also been associated with allergic skin reactions [12].

The literature mentioned above indicates that most institutions are hesitant to implement plastination procedures due to the high budget and equipment costs. Some studies have been conducted to reduce the cost of the plastination process. P J Kapadnis created a low-cost resin solution [13].

Therefore, the present study was aimed to develop a custom-made low-cost plastination technique for teaching and research purpose.

## MATERIALS AND METHODS

The following materials were employed: 2 cadaveric brains, formalin, acetone, sodium chloride (NaCl), thermocol, benzene, petroleum jelly, a ph metre and dissection tools. The plastination method was carried into 4 steps: fixation, dehydration, impregnation and curing for a period of 53 days. This study used 2 human brains from middle-aged men who donated their bodies to our medical college. The Independent Ethical Committee of Symbiosis International University approved the study. Reference: Proposal No. SIU/IEC/179 dated 11/11/2020.

**Fixation:** The brain was procured from the body carefully after a standard embalming procedure. The obtained brains were sectioned into 4 parts using the brain knife: cerebellum with brainstem, hemisection, coronal section and transverse section of the cerebral hemisphere. The sectioned brain specimens were submerged in 7 litres of 10% neutral buffer formalin fixative (a mixture of 6300 ml of water, 700 ml of formalin, 63 gms of sodium chloride) for 2 weeks [14].

**Dehydration:** After 5 hours of washing with running tap water, the tissue samples were dehydrated using 100% acetone (using tissue volume and acetone in 1:10 ratio) [15]. At room temperature, the specimens were treated with 3 changes of 100% pure acetone and the purity of the acetone was monitored using a ph-meter. The purity of the acetone declined to 98% when the specimens were treated with first acetone for 3 days. The specimens were then moved to a second acetone, where it showed 98% acetone purity level on day 7. The specimens were then treated with third acetone until day 9, at which point the purity level was 99.4%. The samples were dehydrated using 3 changes of acetone over the course of 19 days.

**Impregnation:** Dehydrated brain specimens were air dried for an hour. For the purpose of impregnating the specimens, 8 litres of cost-effective plastination solution (CEPS) was prepared. Fifteen gm of thermocol and 5 gm of petroleum jelly were dissolved in 100 ml of benzene to prepare a cost-effective plastination solution. Within an hour, the thermocol in benzene was dissolved entirely and the solution was put to use. For the

impregnation, the specimens were kept in the CEPS solution and the process was seen by observing the bubble formation on the surface of the solution. Since no bubbles were formed after 11 days, the impregnation procedure was complete [16].

**Curing:** Extra impregnation solution was wiped up and specimens were air-dried at room temperature. The specimens were completely dried in 9 days at room temperature [17].

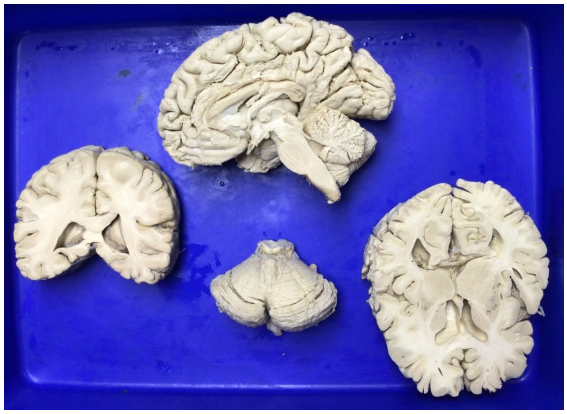

**Fig. 1:** Shows the parts and sections of the brain specimens.

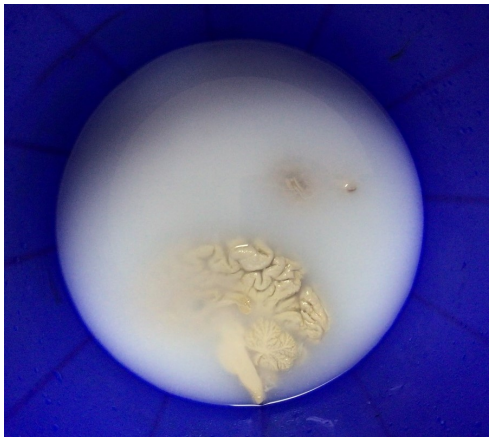

**Fig. 2:** shows the specimens being treated with CEPS solution.

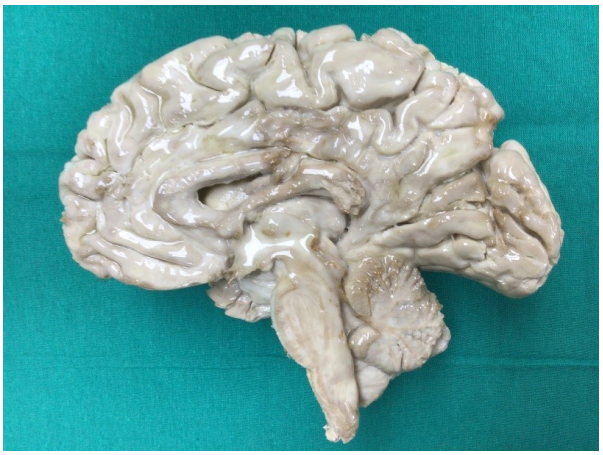

**Fig. 3:** shows the sagittal section of the brain after curing.

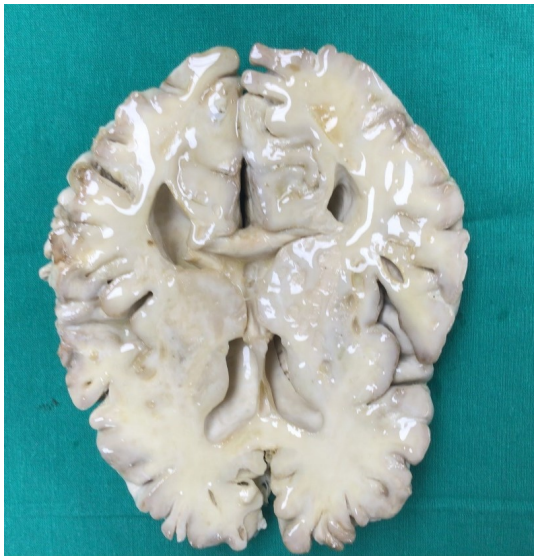

**Fig 4:** shows the horizontal section of the brain after

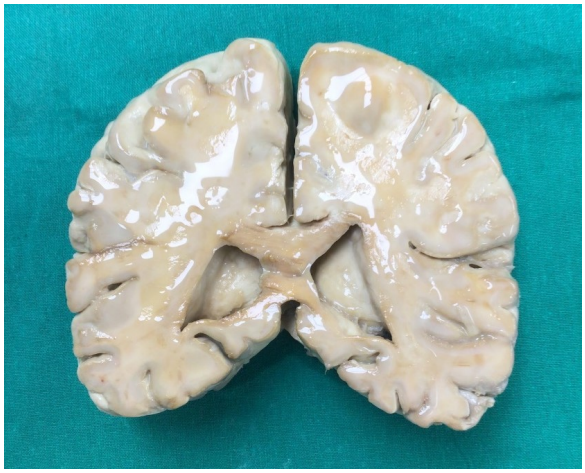

**Fig. 5:** shows the coronal section of the brain after curing.

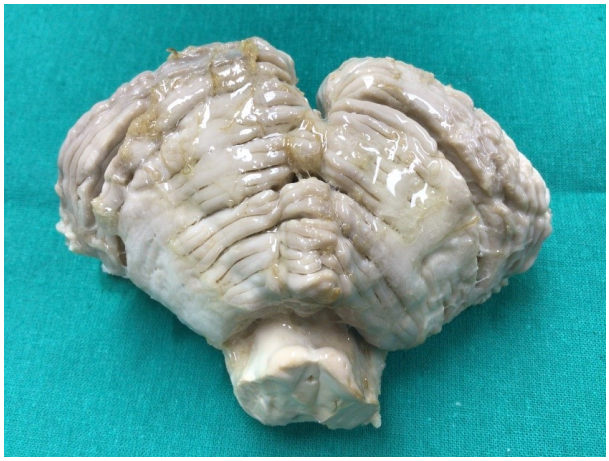

**Fig. 6:** shows the cerebellum with brainstem after curing

## OBSERVATIONS

The quality of the plastinated specimens was observed under the following criteria using a self-developed grading scale: clarity, stability, aesthetic appearance, colour and smell. A team of 62 anatomists and pathologists gave

the score based on the grading provided in the self-developed grading scale.

## RESULTS

SPSS software was used for the statistical analysis; the significant was set to $p<0.05$ with 95% confidence interval (CI). Each component received a grade ranging from poor (0) to excellent (5) based on the quality of the specimens used. Table 1 showed that assessors did not assign scores of 0 to 2 to any of the 5 components. This indicates that the specimens' quality was not that poor. Kruskal Wallis test was used to find the mean rank between all the components. It revealed that the lowest mean score was 4.04 provided for colour and highest mean score was 5 provided for the smell with a statistically significant $p<0.001$ as shown in the table 2.

**Table 1:** shows the provided score based on the quality of the plastinated specimens.

| | Score | | | | | N = 62 |
|---|---|---|---|---|---|---|
| **Components** | **0** | **1** | **2** | **3** | **4** | **5** |
| **Clarity** | - | - | - | 1 | 23 | 38 |
| **Stability** | - | - | - | - | 9 | 53 |
| **Aesthetic Look** | - | - | - | 3 | 14 | 45 |
| **Colour** | - | - | - | 13 | 33 | 16 |
| **Smell** | - | - | - | 0 | 0 | 62 |

**Table 2:** shows Kruskal Wallis test for showing mean score in all 5 components.

| N = 62 | | | | |
|---|---|---|---|---|
| **Components** | **Score out of 310** | **Mean Score** | **Kruskal Wallis value** | **p-value** |
| **Clarity** | 285 | 4.59 | 96.07 | <0.001* |
| **Stability** | 301 | 4.85 | | |
| **Aesthetic Look** | 290 | 4.67 | | |
| **Colour** | 251 | 4.04 | | |
| **Smell** | 310 | 5 | | |

## DISCUSSION

In 1987, Von Hagens et al. employed the S-10 technique to plastinate brain sections with a thickness of 1.0 to 2.0 cm in order to prepare half and whole brain specimens that were used in neuroanatomy courses [5]. In 1992, Weber and Henry developed the sheet plastination of the brain, which resulted in specimens with a 4 mm thickness that were more accurate, robust and manageable [7]. P40 plastinated brain slices were made by thinly slicing fresh brain from post-mortem cadavers into pieces of 4, 6 or 8 mm. These slices showed a strong contrast between the white and grey matter [18]. In 1996, Weiglein constructed very thin (1 mm or less) band-saw-cut brain slice and compared the S-10 and P35 procedures [19]. Using the P40 approach, Barnet [20] made coronal and horizontal brain slices and compared them with those produced using the P35 technique from embalmed cadavers. All the above mentioned studies used vacuum impregnation with epoxy or polyster resin or silicon.

The CEPS for plastination method is less time-consuming, less expensive and less tedious. It was carried out using a basic setup without expensive tools like vacuum chambers, deep freezers, resin or silicon. This procedure was performed at standard room temperature and atmospheric pressure. The plastinated specimens without vacuum chambers, deep freezers and CEPS obtained from the present study were handler-friendly and non-sticky. They didn't even require gloves for research or teaching purpose. The plastinated specimens were dry to touch, odourless, non-toxic, clean and non-fragile as per the assessor scoring as shown in table 1 and 2. All the specimens retained their original forms and unaltered appearances and were portable as shown in figures 3, 4, 5, & 6. Ramkrishna V

[21] plastinated some human limbs and animal organs using a resin solution made by dissolving thermocol and plastic tea cups in chloroform. Moreover, it has been reported that the samples obtained using this technique are inexpensive and do not irritate the skin or eyes. When we compared the current investigation to the Ramkrishna V study, we prepared the plastination solution by dissolving the thermocol in benzene. Only brain samples were used in the current investigation and the plastinated specimens obtained were of high quality. Similar to study of Ramkrishna V, we also did not observe any unpleasant resin odour or eye or skin irritation.

## CONCLUSION

The usage of thermocol polymer for plastination in the present study yielded notable results and provided an alternate polymer for plastination. Thermocol comprises polystyrene, a crucial thermoplastic chemical made from styrene or phenylethene. Phenylethene and polyethene share the same chemical characteristics. Being a hazardous pollutant and laboratory waste, thermocol reacts slowly to bacterial breakdown in the soil, rendering it infertile. On burning, it also emits harmful chemicals that may result in respiratory issues. The use of thermocol for plastination helps maintain our environment pollution-free by reducing the dangerous pollution that these wastes produce.

## Author Contributions

**Dr.K.Vijayakumar:** Concept and execution of the study, Manuscript drafting

**Dr. Mandar Ambike:** Collection of the results and interpretation

**Dr.Daksha Dixit:** Interpretation of the data.

## Conflicts of Interests: None

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