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Original Article

Anatomy Section

Impact of Melamine on the Weight of Specimens in Different Stages of Plastination: A Cross-sectional Cadaveric Study

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ABSTRACT

Introduction: Due to the lack of cadavers, anatomists across the nation are facing trouble in teaching. Plastinated specimens can be used to get around this deficiency and reliance.

Aim: To assess the change in weight during plastination using melamine.

Materials and Methods: A cross-sectional cadaveric study was conducted in the Department of Anatomy at GMC Kota, Rajasthan, India, using nine specimens from cadavers obtained between June 2016 and June 2017. Freshly dissected specimens from a fresh cadaver were used for plastination. The weight reduction after dehydration using acetone (three changes of seven days each), degreasing using xylene (three changes of seven days each), impregnation with a melamine-xylene mixture (10 days in a vacuum), and curing was recorded. Weight was measured using a digital weighing balance and recorded after each step. All statistical analyses were performed using Analysis of Variance (ANOVA) in MedCalc software version 22.009.

Results: There was a gradual weight loss after each step except for impregnation. The percentage of mean weight after dehydration was 58.59±4.03%, after degreasing was 56.21±2.55%, after impregnation was 66.06±4.69%, and after curing was 48.26±5.39% and p-value was highly significant (<0.05).

Conclusion: Continuous reduction in weight without distortion of anatomy resulted in lightweight plastinates which were odor-free and aesthetically pleasing.

Keywords: Acetone, Anatomists, Cadavers, Dehydration, Plastinates, Weight reduction, Xylene

INTRODUCTION

There have been numerous uses mentioned for plastinated tissues, organs, and body parts that have undergone normal plastination procedures. They have also been acknowledged as ideal instruments for both direct and indirect instructions [1].

Plastination was first introduced in 1946 by Romaniak [2], which was updated in 1987 by Von Hagen who employed polymerising resins instead of unpolymerised resins. The technique was shown in detail in Jain A et al., and Satte M et al., [3,4]. Using more modern methods of processing specimens, such as light plastination, one can currently observe more significant impacts of plastination in education. In the original conventional techniques of plastination operations, the expense of resin and heavy weight of resultant plastinates are significant considerations. To overcome this, many new materials/substances are being tried all over the globe [2,3]. Melamyne was used as the impregnation material, which reduced the cost to a greater extent and reduced the weight as well.

Plastination polymers strike a balance between these fundamental needs and a number of secondary requirements, such as specimen stability, Ultraviolet (UV) light resistance, and specified light refraction index. It is possible to create better specimens for displays and instruction, including hands-on activities for students, by combining various polymers into one plastinate rather than doing it separately [5]. Melamyne has high transparency, excellent hardness, a high refractive index, heat resistance, and possesses waterproof, tracking resistance, and anti-fouling properties which make it suitable for plastination [6]. By using light plastination, anatomical specimens can be preserved without the usual issues that come with wet specimens, such as desiccation, mould, and special storage requirements [7]. Although there are still debates among anatomists about the utility of these instruments, papers emphasise the fundamental function and significance of the plastinated specimens [8]. Moreover, handling and mounting of heavy plastinated specimens can occasionally come with restrictions. Lightweight plastination, which produces lightweight, robust, high-quality, and long-lasting specimens, has made work in this field easier [9]. As the total number of students per class is increasing, it has become mandatory to demonstrate the topics in small groups, and for that, demonstrations have to be taken at different places. Lightweight plastinates are easy to carry compared to wet specimens. The objectives were to assess the mean weight of plastinates, to find the decrease in weight from the initial weight, and the weight after dehydration using acetone, the weight after degreasing using xylene, and the weight after impregnation using a melamine-xylene mixture and curing.

MATERIALS AND METHODS

A cross-sectional cadaveric study was conducted in the Department of Anatomy after obtaining ethical approval no. Acad/Ethical Clearance/2023/29 Dated: 14-06-2023. The process of plastination was carried out from June 2016 to June 2017. Convenient sampling was employed, so specimens were procured from cadavers in the department.

Acetone, xylene, melamine, hardener, paintbrush, acetone, diluted sulphuric acid, calcium chloride, glass jars, vacuum chamber, mortuary chamber, digital weighing scale with a minimum sensitivity of ±0.1 gm were used in this study.

Inclusion criteria: All freshly procured specimens from a freshly embalmed cadaver were selected for the study.

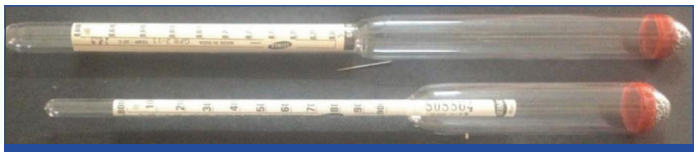
Exclusion criteria: Any decomposed or damaged specimens during the procedure were excluded from the study.

Procedure

The specimens selected from the cadavers were a transverse section of the wrist, forearm, transverse section of the neck at the C6 level, a coronal section of the face showing paranasal sinuses, transverse section of the brain showing the anterior horn of the lateral ventricle, horizontal section of the brain, cut section of the brain showing the internal capsule, and a section of the brainstem showing the floor of the fourth ventricle. All the specimens were procured by following

Cunningham's manual dissection using a band saw and hand saw by the authors and were fixed in 10% formalin. After fixing the specimens, the rest of the steps of plastination were followed [10].

Dehydration: The specimens were then transferred to acetone with a specific gravity of 0.8, which was measured by an acetonometer/alcoholmeter [Table/Fig-1]. Specimens were subjected to three changes of acetone. Each change comprised seven days of treatment. The acetone left after the last change was reused for another specimen until the specific gravity dropped to 0.6. Measurements of weight were taken after the last change, thrice on a digital weighing balance, and the mean of the three readings was recorded. The weighing balance was kept in the laboratory with an installed hygrometer to ensure the humidity level between 45% to 80%. After each weighing procedure, the balance was thoroughly wiped, and the instrument was re-calibrated. The specimen was then placed directly over the weighing balance after wiping it off from the blotting paper [11].



[Table/Fig-1]: Alcoholmeters.

Degreasing: Dehydrated specimens were then subjected to three changes of xylene, each change lasting seven days. Xylene acts as the volatile intermediary with the acid-curing polymer and is also a degreasing agent for lipid-rich specimens [4]. Measurements were taken similar to the first step after the last change.

Impregnation: An intermittent vacuum-assisted impregnation was done for the specimen, which comprised a solution of melamine and xylene mixed in a 1:1 proportion [Table/Fig-2]. A 7 mm Hg vacuum was applied until the bubbling stopped (averaging 10 days for each specimen). The twenty-minute timer included a five-minute break. The specimens were then removed from the vacuum chamber and cleaned of excess polymer. All measurements were taken once more. The above three steps were conducted at room temperature (35-40°C) [5,10].

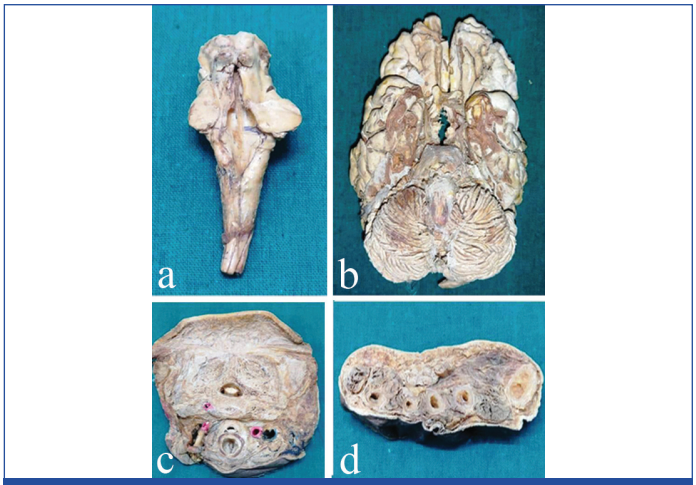
[Table/Fig-2]: Hoover chamber: impregnation with melamine.

Curing and Hardening: The specimens were placed in a sealed chamber with calcium chloride as a hygroscopic element and diluted sulfuric acid in another beaker. The entire chamber was then placed in a freezer at a temperature of 8°C for one month. Following drying, the entire chamber was kept under room-temperature UV light.

The [Table/Fig-3a-d] shows plastinated specimens of the fourth ventricle, transverse longitudinal section of the brain, section of the specimen of the neck at level C6, transverse section of the forearm, and after curing, respectively.

STATISTICAL ANALYSIS

All specimens were given their final measurements before using MedCalc software version 22.0 Initial weight and weight at the end



[Table/Fig-3]: a) fourth ventricle; b) Transverse longitudinal section of brain; c) section of specimen of neck at level C6; d) Weighing of transverse section of forearm and after curing, respectively.

of each step of dehydration, degreasing, impregnation, and curing were recorded. There were limited specimens that greatly differed in their dimensions and weights. To avoid higher variations in the mean of the groups, the authors calculated the percentage weight reduction of each specimen after each step. The reduced weight percentage was then compared using one-way ANOVA. A p-value <0.05 was considered significant.

RESULTS

[Table/Fig-4] shows the actual weights of each specimen after each step. The weight reduced to almost half of the original weight in the specimen of the coronal section of the face. In the specimen of the fourth ventricle, the weight is reduced to 20 gm from 35 gm, i.e., 57.14% of its original weight.

S. No.	Specimens	weight (gm) before procedure (gm)	After Acetone (gm)	After Xylene (gm)	After Impregnation (gm)	after curing/ final weight (gm)
1	Face coronal section	300	150	160	170	110
2	4th ventricle	035	015	020	030	020
3	Brain (HS)	545	290	280	310	200
4	Brain (CS)	330	190	180	210	120
5	Brain (AHLV)	210	120	100	110	90
6	TS at C6 level	1260	600	590	610	390
7	TS of wrist	150	100	90	100	70
8	T.S. forearm (P)	190	150	130	160	140
9	TS forearm (D)	150	110	100	120	110

[Table/Fig-4]: Change in weight after each step.
HS: Horizontal section; CS: Coronal section; AHLV: Anterior Horn of lateral ventricle; P: Proximal; D: Distal; TS: Transverse section

[Table/Fig-5] shows the weight reduction of each specimen in percentage considering the initial weight to be 100%. Hence, after dehydration, the weight reduced to 50% of the original weight in the coronal section of the face. After degreasing, the weight reduced to 53.33% of the original weight in the coronal section of the face. After impregnation, the weight reduced to 56.66% of the original weight in the coronal section of the face, though there was a slight increase from the previous step.

[Table/Fig-6] shows the mean reduction of weight of each group. The acetone-subjected specimens reduced to 58.59±4.03 percent of the original weight, xylene-treated specimens reduced to 56.21±2.56 percent of the original weight, after impregnation with melamine, the weight became 66.06±4.70 percent of the original weight, and finally, after curing, the specimens' mean weight decreased to 48.26±5.40 percent of the original weight.

S. No.	Specimens	Initial (%)	After acetone (%)	After xylene (%)	After impregnation (%)	After curing %
1	Face coronal section	100	50	53.33333	56.66666667	36.66666667
2	4th ventricle	100	42.85714	57.14286	85.71428571	57.14285714
3	Brain (HS)	100	53.21101	51.37615	56.88073394	36.69724771
4	Brain (CS)	100	57.57576	54.54545	63.63636364	36.36363636
5	Brain (AHLV)	100	57.14286	47.61905	52.38095238	42.85714286
6	TS at C6 level	100	47.61905	46.8254	48.41269841	30.95238095
7	TS. of wrist	100	66.66667	60	66.66666667	46.66666667
8	TS forearm (P)	100	78.94737	68.42105	84.21052632	73.68421053
9	TS forearm (D)	100	73.33333	66.66667	80	73.33333333

[Table/Fig-5]: Specimens showing weight reduction in percentage.

Factor	Mean	Standard error	95%CI
Initial	100	0	100.0000 to 100.0000
After acetone	58.5948	4.0324	49.2960 to 67.8936
After xylene	56.2144	2.5549	50.3229 to 62.1060
After impregnation	66.0632	4.6954	55.2357 to 76.8907
After plastination	48.2627	5.394	35.8240 to 60.7013

[Table/Fig-6]: Mean Weight in percentage after each step.

[Table/Fig-7] clearly reveals that acetone was responsible for a 41.5% reduction in weight. After the specimens were subjected to xylene, further weight reduction was 43.78±2.56%. After impregnation, a mean reduction of less than 33.94±4.70% was found. In the final step, the mean reduction of weight for the specimens was 51.73±5.4%. All the findings were significant as the p-value was <0.001.

Factors	Mean difference (%)	Std. Error	p*	95% CI[a]
After acetone	41.405	4.032	**0.0001**	25.951 to 56.860
After xylene	43.786	2.555	**<0.0001**	33.994 to 53.577
After impregnation	33.937	4.695	**0.0009**	15.942 to 51.932
After plastination	51.737	5.394	**0.0001**	31.065 to 72.410

[Table/Fig-7]: Mean reduction in weight, *p<0.05 was considered significant.

DISCUSSION

By substituting the water and lipids in biological tissues with curable polymers that are then hardened, a tissue preservation technique known as plastination produces dry, odorless, and enduring specimens. The weight of the individual samples changed at various plastination phases. Previous research demonstrated that the primary causes of tissue shrinkage, which were only 10-17% [3], and weight loss during the plastination process is approximately 51%, both attributed to dehydration, degreasing, and impregnation. Satte M et al., compared relatively expensive silicones plastinates from preserved biological tissues in a gum Arabic solution, which appeared semi-original and reasonably maintained their shape and size with a mean reduction in weight of 53% to 61.8% and shrinkage of 13% to 28%, similar to the present study [4]. Latorre R and Henry RW used P-40 resin for the preservation of brain slices at room temperature (5° at their place) and obtained durable, semi-transparent, easy-to-orient sections showing a good difference in white and gray matter [8].

Overall, plastination is an excellent technique for the long-term preservation of the most priceless specimens and can be used as an additional way to show anatomical variations [12]. Plastinates are also necessary to augment conventional dissection courses and improve the training of postgraduates and doctors [13]. Observations made by Ameko E et al., in Tilapia, catfish, and bone tongue revealed that each specimen experienced mean weight reductions at the conclusion of plastination of -35.2%, -34.5%, and -28.2%, respectively [14]. This was consistent with their prior study's findings, which involved room temperature plastination of whole guinea pigs in which their weights decreased gradually from dehydration onward, with a mean weight loss of 58.2% after curing [15]. Vijayakumar K et al., tried the Cost-Effective Plastination Solution (CEPS) for a plastination approach that required less effort, less money, and less time. It was completed with a simple set-up and without the need for pricey equipment like vacuum chambers, deep freezers, resin, or silicon [16], similar to the current study. With the standard S-10 technique of plastination established by Von Hagen, the main problems faced were the high cost of materials, heavy weight of specimens, and the procedure having to be conducted at very low temperatures [16]. A basic plastination machine will cost atleast Rs. 25 lakhs to set-up, according to the Biodur Company's estimation [16]. To overcome this, new trials are being conducted all over the globe. Since then, researchers have been using different polymers. Steinke H et al., observed a mean reduction of one-seventh of the original weight of the plastinated pikes [9]. In the present study, the mean reduction in weight of the plastinated specimens was 48.3% of the original weight. The colour of the specimens was not much affected by the plastination employed in the current study. Hence, the resultant plastinates were easy to tag or flag, and the normal architecture and relationships of the structures were well maintained. Mehra S et al., used quick fix and amyl acetate in equal parts as impregnates at room temperature [17].

The current study revealed that the weight of the specimens reduced and was significantly affected by the chemicals applied at each stage without distorting the normal anatomy of the specimens. Although all the studies attempted so far, none has emphasised the reduction of weight after each step, the current study on variations in weight at each step of the plastination process will invoke anatomists to further experiment with the use of not only different dehydrating and degreasing agents but also various cost-effective and amicable plastinating polymers. Less space was needed in the anatomy laboratory for the plastinated specimens compared to the formalin-preserved ones, which required containers. Not every medical college in our nation practices the plastination procedure. Although the method is continuously evolving as per the availability of materials and trained personnel, as well as the personal interests of anatomists in plastination, not much information is available about the effects of different chemicals or polymers on the plastinated specimens in terms of their weights or dimensions for comparison [18]. Hassan AR and Sawad AA concluded in their study that numerous scientific advantages result from the application of solid and flexible procedures in plastination technology, including the production of anatomical samples suitable for study in museums [19]. In 2016, Elnady FA innovated the Elnady Technique and used chemicals that were readily available locally to preserve tissue in an economical and environmentally responsible manner [20].

The studies on weight variation are depicted in [Table/Fig-8] [2-4,9,10,14,16,17,19,20].

Limitation(s)

Due to the lack of a fully equipped plastination laboratory and the unavailability of silicone polymer (S10), the gold standard technique

Author	Year	Place	Sample size	Polymer used	Weight reduction (%)	Shrinkage (%)
Mehra S et al., [17]	2003	New Delhi, India	6	Quick Fix In Amyle Acetate	-	-
Steinke H et al., [9]	2007	Germany	6	Silicone	14.28	-
Ameko E et al., [14]	2013	Ghana, Africa	4	Silicone Polymer	28.2-35.2	-
Chandel CS et al., [10]	2013	Jaipur, India	3	Melamyne	-	-
Pandit S et al., [2]	2015	Pune, India	24	Orthocryl And Silicone	-	-
Elnady FA [20]	2016	Egypt	13	Glycerine	-	-
Jain A et al., [3]	2017	Kota India	9	Melamyne	-	12-16
Satte M et al., [4]	2019	Saudi Arabia	12	Gum Arabic	53-61.8	13-28
Hassan AR et al., [19]	2021	Iraq	10	Glycerine And Polymer	-	Nil
Vijaykumar K et al., [16]	2023	Pune , India	1	CEPS Cost Effective Plastination Solution	-	-
Makhija K et al.,	Present study	Kota, India	9	Melamyne	35.8 -60.71	-

[Table/Fig-8]: Compilation of published studies on weights and shrinkage of polymers during plastination [2-4,9,10,14,16,17,19,20].

could not be attempted, and the results achieved by adopting the melamyne method for plastination could not be compared with the gold standard procedure [19]. A major constraint of the study was the small sample size, as bodies available for dissection were scarce. The fluctuation in environmental temperature also affected the final curing process, as better results are typically achieved when the plastination procedure is carried out in winter, but the present study was conducted in dry weather. Sun drying is mostly affected during the rainy season because of humidity, which may also affect the weight of the specimens.

CONCLUSION

Plastination has revolutionised how anatomy is seen and portrayed to students and academics, making it a powerful preservation technique. Melamyne was used as the impregnation material, reducing costs significantly and making the specimens portable.

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