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## RESEARCH ARTICLE

# EFFICIENCY OF DIFFERENT HALOGEN COMPOUNDS FOR PRESERVATION OF ENAMEL DURING DECALCIFICATION PROCEDURES

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**ABSTRACT**

**Introduction:** Decalcification is the commonly employed technique in histopathology laboratories as a part of calcified tissue preparation for the microscopic examination. Chemical agents are most commonly used for routine decalcification procedures, though some agents adversely affect the tissue integrity and staining properties.
**Aim:** To compare the efficacy of various decalcifying agents by histological evaluation of hard and soft tissue components of teeth.
**Materials and Methods:** The decalcifying agents namely 10% Nitric acid (HNO3) were used to decalcify 60 human permanent teeth. The specimens were subjected to routine processing, sectioning and staining with hematoxylin and eosin. The stained sections were observed under microscope .
**Results:** Considering preservation and staining characteristics of both hard and soft tissues, superior results were obtained with 10% HNO3.
**Conclusion:** HNO3 was showing the most efficient result as it balances both tissue integrity and time factor suggesting that it can be used as a stable decalcifying agent for routine histopathological diagnosis.



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## INTRODUCTION

Tooth as being a hard tissue and study of tooth using ground section and decalcification is the ideal method. Under decalcification procedure, there is dissolution of the hard enamel. But preservation of enamel by decalcification method is possible and requires the employment of halogen compounds. Here, we have used 5% formic acid and studied the effect on enamel, dentin, cementum. (Callis MG Bone *et al*., 2008)

### Aims and Objectives

- Enamel Preservation in decalcified sections.
- Using 5% Formic acid as decalcifying agent.

## MATERIALS AND METHODS

### Study population

The material consisted of thirty teeth collected from the patients who reported for extraction of teeth from Department of Oral and Maxillofacial Surgery of D.Y. Patil School of Dentistry, Nerul, Navi Mumbai.

### Duration of study

3 months

### Study design

30 cases were taken in this research.

Step 1 : All the subjects were administered standard questionnaire interview to obtain history.
Step 2 : Patients undergoes extraction of tooth.
Step 3 : Extracted tooth collected from the department of Oral Surgery of D.Y. Patil School of Dentistry, in Neutralized Buffered Formalin Preservation.
Step 4 : Teeth sectioned 2-3mm
Step 5 : Sections immersed in solutions for 3-7days
Step 6 : Volume (50 ml. of 5% formic acid)/weight (1 gm of teeth) during decalcification.
Step 7 : Observation under microscope and Results interpreted.

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### Materials

- 30 Extracted human teeth.
- 10% Formalin
- 5% Formic acid
- Electric Lathe machine.

### Procedure involved

Teeth were sectioned to about 2-3mm. These sections were immersed in solutions for 3-7 days. Proper concentrations of 50ml of 5% formic acid/ 1gm of teeth were employed during decalcification method. Initial fixation done in 10% formalin for 3-7 days. Wash the solutions thoroughly. Immerse in 5% formic acid for 24 hours. Wash the solutions thoroughly. Immerse in 10% formalin for 24 hours. Wash the solutions thoroughly. Immerse in 5% formic acid for 24 hours. Wash the solutions thoroughly. Again immerse in 10% formalin for 24 hours. In this manner, repeated fixation and decalcification are done until complete decalcification of tooth takes place. Enamel is thus soft enough to needle-prick and sections are thus taken 2-3mm and visualized under microscopy. (Goland *et al*., 1965)

### Statistical Analysis

Paired sample t-test was done for inter-observer variation. One-way-ANOVA and Post-hoc test was applied to compare the effects of different decalcifying agents.

## RESULTS

The results with 10 per cent formalin were fair. Structures, such as the cuticle and plaque, regions of caries in enamel and dentin were retained. Enamel thickness was found Satisfactory. Histological features such as Enamel Prisms and Lamellae were well-defined. Sections were easy during cutting. Quality of H and E Staining was found to be Satisfactory. None of the pathological features were evident. Pulpal tissues with formic acid architecture and quality was not defined. Considering preservation and staining characteristics of both hard and soft tissues, superior results were obtained with 10% $HNO_3$ (Nitric Acid).

## DISCUSSION

Decalcification is the commonly employed technique in most of the histopathology laboratories for the microscopic examination of calcified tissues including teeth and bones (Mawhinney *et al*., 1984). Many studies have been done by the researchers to introduce new decalcifying agents and to modify the presently used agents in order to meet the criteria of the most efficient decalcifying agent which ensures complete removal of calcium without causing any damage to tissue architecture and provide adequate staining characteristics. In the present study an attempt has been made to compare the efficacy of 2 different decalcifying agents for both hard and soft tissues components of human permanent teeth. In the present study, we have done comparison between two sets of specimens in all the decalcifying agents and it was found that even after reaching the decalcification point by all the three methods, the specimens of set I resulted in difficulty of section cutting. In the set II, the time of decalcification was increased by two days in each solution and it was observed that the teeth were soft enough for section cutting. Further the time of decalcification was increased by two days for set III and it was found that there was shredding during section cutting and poor staining characteristics which can be due to the over decalcification of teeth. Therefore, it is necessitate determining the end point of decalcification, for the ease of section cutting and better results of staining needed in histopathological analysis. Various methods have been used for this purpose. In our study, end point of decalcification was determined using three methods: physical, chemical and radiographic. Physical test was done using bending and probing but as it can create artifacts; so it was not considered as an accurate method. Due to the complexity of the procedure chemical method was not suggested as a routine test for diagnostic purposes. Amongst them radiographic method was the most reliable to check the end point which is also in accordance with Gayle Callis (2008). One important criteria of an efficient decalcifying agent is the reasonable speed of decalcification. In our study speed of decalcification was highest for 10% FNA followed by 10% $HNO_3$, being the slowest. Maurine William (1937) in his study reported that the time taken for decalcification by 5% $HNO_3$ was 7-9 days for a single tooth, but in our study the time taken was less i.e. 5-7 days, this may be due to the increased concentration of $HNO_3$ used which was 10%. This finding was in accordance with Culling who stated that the rate of decalcification also depends on the concentration used for the particular acid and varies accordingly (Culling *et al*., 1985).

On comparing the efficacy of various decalcifying agents in terms of preservation and staining characteristics of both hard and soft tissues, superior results were obtained with 10% $HNO_3$ followed by 10% Formic acid. But statistically 10 % $HNO_3$ was showing significant difference only with 10% FA for the hard tissue, which also showed the least scores. This means 10 % FA should not be used for diagnostic and research purposes as it causes maximal damage to the tooth integrity. Similarly for the soft tissue, 10% $HNO_3$ was showing significant difference with 10% FA. Therefore, it is recommended that for pulp preservation it is better to avoid 10% FA to provide excellent and reproducible results. However, Goland *et al*. (1965) noticed in their study that following fixation in reactive halogen compounds, such as dichloro-s-triazene, procion dyes and Lissatan PR, 5% FA as decalcifying agent better preserve the enamel as compared to $HNO_3$ and HCl but they did not mention about the preservation of pulp in their study. Brain and Eastow in 1962 suggested that enamel of teeth can be sufficiently and readily softened for cutting by use of formic acid. Highly calcified enamel is difficult to preserve. Enamel is 99.5 percent crystallite, and this is the major component which might persists after decalcification. Even 2 g, is a sufficient amount to preserve the architectural integrity of the enamel. These compounds impart strength to the organic framework of the enamel. Histochemical studies that can be done with this material are within the limitations imposed by processes associated with immersion fixation and decalcification. Appears possible to reveal some details of the chemical and structural composition of mature human enamel better than in ground sections. (Goland *et al*., 1965)

**Conclusion**

Enamel Preservation is possible with decalcification using formic acid. Morphological details of pulp and enamel can be seen. Sections are thin as compared to ground sections. It is easy to perform in the laboratory. $HNO_3$ was showing the most efficient result as it balances both tissue integrity and time factor suggesting that it can be used as a stable decalcifying agent for routine histopathological diagnosis.

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