AJMB
eISSN: 2600-8173
https://doi.org/10.37231/ajmb.2025.9.1.788
https://journal.unisza.edu.my/ajmb
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Universiti Sultan Zainal Abidin

Asian Journal of Medicine and Biomedicine

Decalcification Agents for Histological Analysis of Human Dental Tissues: A Review

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Received: 5th August 2024 **Accepted: 17th November 2024** **Published: 30th April 2025**

Abstract

Background: In histopathology laboratories, fixation and decalcification are essential steps that are carried out during the processing of mineralised tissues such as bone and teeth. While formalin and formaldehyde are universally accepted as the primary fixative solutions used in most laboratories, the lack of a standardized decalcifying agent for mineralized tissues remains a significant gap in histopathological processing protocols. Objective: The present review aimed to compare and discuss the various decalcifying agents used for the decalcification of human teeth with a focus on the effect of these agents on the overall structure and staining qualities of decalcified tissues. Study Design: A comprehensive literature search was conducted across PubMed, Science Direct, Scopus, and Web of Science databases, covering studies published from 2003 to 2023 using specific keywords. Published articles that studied decalcification of bones or non-human teeth were excluded from this study. Results: The most efficient decalcifying agent for rapid decalcification was found to be 5% formic acid, capable of maintaining most tissue structures suitable for histological examination. In contrast, EDTA is a better option when optimal tissue preservation is critical and time is not an issue, whereas Perenyi's fluid proved to be the least effective, as no studies reported favorable outcomes with its use. Conclusion: Despite the importance of decalcification for sectioning and staining of hard tissues, there is no consensus among histopathologists and researchers on a standard decalcifying solution. The choice of decalcifying agent is primarily influenced by staining characteristics and the rate of decalcification, which may vary according to the specific needs of the pathologist or researcher.

Keywords
Decalcification, decalcifying agent, histology, teeth, dental tissue

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Introduction

Decalcification is an important step involved in the initial processing of mineralised tissue samples such as bone or teeth before staining, immunohistochemistry (IHC) or immunofluorescence (IF) can be performed. These techniques are usually carried out in histopathology laboratories where histological examination of tissue sections is used for diagnosing and characterising various pathologies including cancer [1]. Additionally, these methods are also common experimental techniques used by researchers to investigate protein/antigen expression as well as to study how cells and tissues might respond to a particular drug or therapy [2].

In oral pathology laboratories, decalcification is particularly critical due to the mineralization of dental tissues. The highly mineralized dentin and enamel surrounding the soft dental pulp (Figure 1) pose unique challenges for tissue processing, as inadequate decalcification can lead to poor staining quality and compromised morphological analysis. Despite the critical role of decalcification in histological analysis of tooth samples, there is considerable variability in the methodologies and outcomes reported in the literature. Different decalcifying agents and protocols can lead to variable results in terms of tissue preservation and staining efficacy [3]. For example, some studies have shown that ethylenediaminetetraacetic acid (EDTA) decalcification significantly diminishes tissue antigenicity [4,5]. However, other research suggests that EDTA decalcification provides superior morphological preservation and staining outcomes [6,7]. These discrepancies introduce inconsistencies in diagnostic practices and research findings, making it challenging to achieve reliable and reproducible results. Therefore, the selection of an appropriate decalcifying agent is pivotal, as it influences the preservation of tissue structure and the efficacy of subsequent staining techniques. The aim of the present review was to evaluate and discuss the various decalcifying agents used for human tooth samples, focusing on the structural integrity and staining quality of decalcified samples. By providing a comprehensive comparison of these agents, this review seeks to identify the most optimal decalcifying reagent for improved histological analysis of dental tissues.

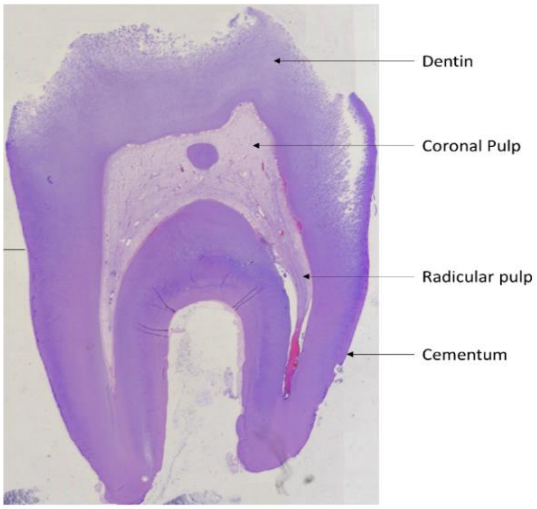

Figure 1: Hematoxylin and eosin-stained section of a human molar tooth decalcified in 5% nitric acid. Decalcification caused morphological changes to the hard tissues of the tooth. As seen at the top of the image, complete decalcification of the enamel has occurred, leaving only the dentin, cementum and the soft pulp tissue. Tissue shrinkage and poor soft tissue attachment can also be seen, especially at the radicular pulp

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Materials and methods

Studies evaluating the various methods of human tooth decalcification were searched in the following databases: PubMed, Science Direct, Scopus, and Web of Science databases. Keywords used to conduct the searches were 'decalcification', 'decalcifying agents', 'tooth', 'teeth' and 'human'. Articles were selected using the following inclusion criteria: (1) published from the year 2003 to 2023; (2) studied human tooth samples; (3) compared different types of decalcifying agents or conditions; (4) written in the English language.

The initial search on PubMed, Science Direct, Scopus and Web of Science databases yielded 85 references, of which 16 duplicate references were excluded before screening. Upon reviewing the title and abstract of the articles, 47 articles were excluded. The remaining 22 articles were included for full text evaluation and sought for retrieval; three articles could not be retrieved. The 19 retrieved articles were assessed for eligibility and eight articles were excluded based on the inclusion and exclusion criteria. A flowchart of the screening process is shown in Figure 2. After screening, 11 articles were included in this analysis. The general characteristics of the included studies are listed in Table 1.

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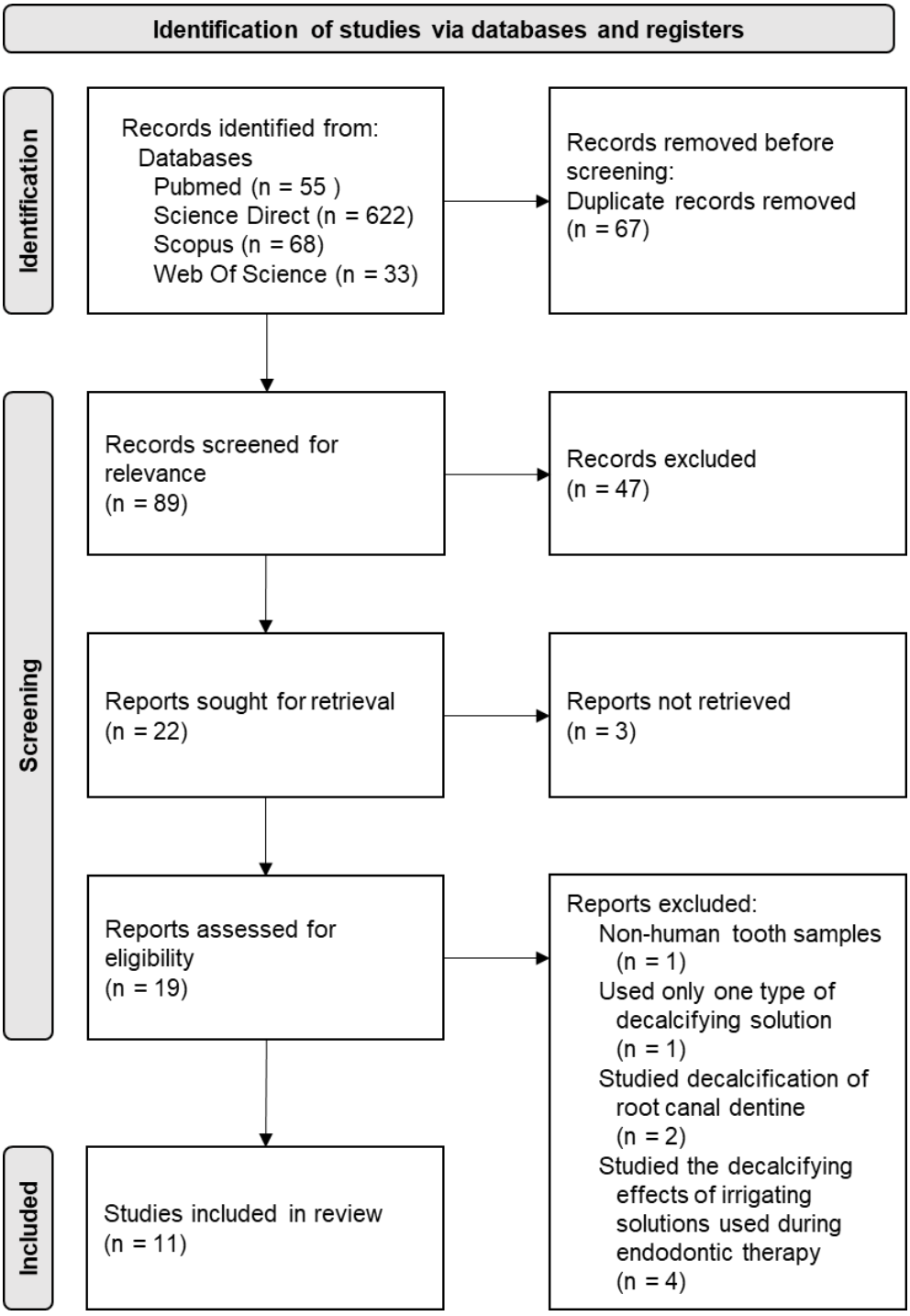

Figure 2. Flowchart of the screening process.

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Table 1: General characteristics of included studies.

Type of Teeth Used	Fixative Used	Dental Pulp Access	Decalcification Solutions	Decalcification Conditions	Reference
Incisors and canines	1. 4% Formaldehyde 2. 10% Formalin	No direct access	1. Ana Morse solution 2. 10% EDTA	1. Room temperature 2. Agitation	Grando et al., 2007
Incisors, canines, premolars, and molars	10% Formalin	No direct access	1. Formalin-Nitric acid 2. Neutral EDTA 3. Perenyi's Fluid 4. 5% Nitric acid 5. 5% Trichloroacetic acid 6. 10% Formic acid	Agitation	Sanjai et al., 2012
Premolar	10% NBF	No direct access	1. 5% Formic acid 2. 5% Nitric acid 3. 14% EDTA	1. Room temperature 2. Microwave	Sangeetha et al., 2013
Incisors, canines, premolars, and molars	10% Formalin	1/3rd of apical root was cut	1. Neutral EDTA 2. 8% Potassium formate + 8% Formic acid 3. 10% Formal nitric acid 4. 10% Nitric acid 5. 10% Formic acid	Agitation	Gupta et al., 2014
Premolars	10% Formalin	No direct access	1. Commercial decalcification solution (Osteomal) 2. 10% EDTA 3. 20% EDTA 4. 30% EDTA 5. 10% Formic acid 6. 20% Formic acid 7. 30% Formic acid 8. 10% Nitric acid 9. 20% Nitric acid	Room temperature	Priya et al., 2016

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			10. 30% Nitric acid		
Premolars	Not mentioned	No direct access	1. 5% Formic acid 2. 7% Formic acid 3. 5% Nitric acid 4. 7% Nitric acid 5. 5% Trichloroacetic acid 6. 7% Trichloroacetic acid	1. Room temperature 2. Microwave	Srinivasyaiah et al., 2016
Molars	10% NBF	2 mm of apical root was cut	1. Formalin-EDTA 2. Formal-nitric acid 3. Formic acid-formalin 4. Perenyi's Fluid 5. Von Ebner's solution	1. Room temperature 2. Electric current 3. Agitation 4. Heat	Choube et al., 2018
Premolars	10% Formalin	No direct access	1. EDTA 2. 10% Formic acid 3. 10% Nitric acid	Agitation	Bhat et al., 2019
Premolars and molars	4% Formaldehyde	1. Access drilled at a non-relevant area 2. Separation of the crown from the root	1. Morse's solution 2. 17% EDTA	Room temperature	Widbiller et al., 2021
Incisors, canines, premolars, and molars	10% Formalin	No direct access	1. Formalin-Nitric acid 2. Perenyi's Fluid 3. 5% Nitric acid 4. 5% Trichloroacetic acid 5. 8% Formic acid 6. 14% EDTA	Room temperature	Khangura et al., 2021

Premolars	10% NBF	No direct access	1. 5% Trichloroacetic acid 2. 10% Formic acid	Agitation	Ali et al., 2021

Decalcification

Decalcification, defined as the process of removing calcium or other minerals from mineralised tissues such as bone or teeth, is a crucial procedure in histology laboratories. This process must be completed prior to the sectioning and staining of these tissues for microscopic analysis. The decalcification process is usually carried out by submerging fixed tissue in a decalcifying agent over a period of time [8].

The two types of decalcifying agents that are commonly used are acids and chelating agents, both with different mechanisms of action. Acidic decalcifying agents react with insoluble calcium in the tissues to form soluble calcium salts, while chelating agents bind to the calcium ions and form a complex. As the calcium content decreases, the tissues soften, facilitating easy sectioning with a microtome. Inadequate or incomplete decalcification can lead to difficulties during sectioning, causing rips and tears, and residual calcium can appear as artifacts during staining, resulting in unclear images and inaccurate analysis [9]. However, immersion in these chemicals can alter tissue structure and architecture during the decalcification process, potentially affecting staining characteristics and leading to inaccurate observations [10,11]. Therefore, choosing the appropriate decalcifying agent for each sample type is very critical. Using a harsh chemical may result in difficulty with sectioning, improper staining, tissue shrinkage, and changes in cellular organisation [12]. On the other hand, a mild decalcifying agent may prolong the decalcification process and exposing tissues to the chemical for a longer time may also lead to the problems mentioned earlier.

While complete decalcification is desired, it is imperative to prevent over-decalcification, which can also compromise sectioning, staining, tissue integrity and cellular organisation. Therefore, an ideal decalcifying agent should: (1) have little effect on tissue integrity and structure, (2) not affect the sectioning of the tissue, (3) allow for accurate staining, and (4) allow for complete decalcification in an appropriate amount of time.

Factors Affecting Decalcification

Several factors can affect the decalcification process including the fixation process prior to decalcification, the concentration and volume of decalcification solution used, frequency of decalcification solution changes and the decalcifying conditions. In histological studies, fixation is a crucial first step that is done after specimen acquisition to ensure that the tissues are maintained in a life-like state [13]. It strengthens the tissue, ensuring that the morphological and cellular details of the sample is well preserved throughout the entire staining process [14,15]. Poor fixation can lead to shrinkage of the pulp from the dentine, altering the pulpal architecture and cellular morphology of the tissues [16]. Once adequately fixed, the samples can then undergo decalcification. The decalcification solution should be changed frequently, based on the solution used, as the calcium that leaches out from the tissue will bind to the active agent in the decalcification solution, hindering further decalcification [16]. Regular changes ensure fresh solution is always in contact with the tissue, thereby enhancing the efficiency of decalcification, reducing the overall decalcification time and preventing unwanted effects of prolong exposure to the decalcifying agent. Altering the conditions under which decalcification occurs can also influence the process. Heating tissue samples can significantly facilitate the decalcification process by promoting the solubility of calcium salts and improving the activity of the decalcifying agent [17]. Agitation can further expedite decalcification by improving the circulation of

https://doi.org/10.37231/ajmb.2025.9.1.788
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the decalcifying solution, ensuring consistent exposure of the tissue to the agent [18]. While heating and agitation have been shown to be useful in decreasing the decalcification duration, they should be controlled carefully to avoid any tissue damage [19,20]. Therefore, optimizing these parameters is essential to balance efficiency and tissue preservation, often requiring careful monitoring to achieve desired outcomes without compromising histological quality.

Type of Tooth Sample Used

None of the reviewed studies discussed the differences in the staining properties between different types of teeth. However, Widbiller et al. [21] reported that multi rooted teeth required twice as long to decalcify compared to single rooted teeth. They reported that single rooted teeth decalcified in Morse solution and EDTA took 7 and 14 days respectively, while multi rooted teeth took 14 and 28 days. As most studies reported the average time taken for complete decalcification of all the samples, based on the number of different types of teeth used by the researcher, the differences in decalcification duration may notably vary even when using the same decalcifying agent and condition.

Effect of Dental Pulp Access

While most studies proceeded with fixation and decalcification of the whole teeth, three studies cut the tooth samples in different ways to provide better access to the dental pulp. Gupta et al. [22] and Choube et al. [23] removed 1/3rd and 2 mm of the apical root, respectively. These modification to the samples did not significantly affect the time for complete decalcification. Comparing the study by Gupta et al. [22] , where the tooth was modified, and Bhat et al. [24], where there was no modification, both studies reported that complete decalcification took 29 days in EDTA and 6 days in 10% nitric acid. A three-day difference was observed with the samples in 10% formic acid, where the whole tooth took 14 days while the tooth with the dental pulp access took 11 days. However, discrepancies were observed in the staining qualities of the tissue that had pulp access as compared to whole tooth samples. Gupta et al. [22] reported that 10% nitric acid produced the best staining followed by 10% formic acid and EDTA, while Bhat et al. [24] reported that EDTA produced better results followed by 10% formic acid and 10% nitric acid. study, Widbiller et al. [21] also described two methods to expose the pulp. One involved drilling through enamel and dentine, while the other involved partially separating the crown from the root. However, the study did not further discuss the impacts of these techniques.

Effect of Fixative

All the studies included in this review used one of three fixatives: 10% formalin, 10% NBF or 4% formaldehyde. These fixatives contain formaldehyde as the active component, which may explain the insignificant difference observed in the quality of staining of each of these fixatives [25]. A 10% formalin solution is made of 4% formaldehyde in water with 1% methanol, while 10% NBF is a 4% formaldehyde solution diluted using phosphate buffer at a neutral pH. Formaldehyde is the most commonly used fixative in histopathological labs due to its reliability and convenience [26]. It easily penetrates tissues and binds to various amino acids forming cross links and preventing tissue degradation. Another reason that formaldehyde is preferred is that upon processing of the tissue samples, the undesired effects of crosslinking such as the masking of epitopes can be easily reversed using heat-based or enzymatic antigen retrieval methods. This reversibility is possible because formaldehyde-induced cross-links do not affect the secondary and tertiary structures of proteins, which is crucial for maintaining the immunoreactivity of tissue samples towards target-specific antibodies.

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Effect of Decalcifying Solutions

Ethylenediaminetetraacetic acid

EDTA was the only non-acidic, chelating agent type of decalcifying agent that was compared in all 11 papers. Chelating agents function by binding to metallic ions such as calcium that present in the tissue and remove them. While this is a gentler process and causes minimal damage to the samples, it does take longer for complete decalcification to occur. EDTA is preferred for research purposes as the negative effects of acid decalcification, such as poor nuclear staining and swelling of cells and tissues, can be avoided, thus allowing for better staining and accurate results [27]. It is also the preferred decalcifying agent for immunolocalization studies due to its ability to preserve the tissue antigenicity [28]. All papers that used EDTA reported that decalcification in EDTA took the longest time. Although microwaving reduced the time required, it still took longer than samples that were microwaved in other decalcifying agents [29].

Despite being considered a milder decalcifying agent that preserves tissue morphology and cell structures, only five of the ten studies reported such results [22–24,30,31]. The ease of sectioning of EDTA decalcified samples was another parameter that was discussed by the authors. Sanjai et al. [30], Gupta et al. [22] and Bhat et al. [24] reported that EDTA decalcification did not interfere with tissue sectioning and produced good staining, maintaining adequate tissue structure and integrity. However, Choube et al. [23] and Khangura et al. [31] reported difficulties in sectioning despite good staining quality. The findings of Sangeetha et al. [29] contradict these results, as pulp shrinkage, cell structure damage, extracellular matrix degradation and patchy staining were reported. Similar results were noted with formic acid and nitric acid, suggesting no significant differences between the decalcifying agents used. The unsatisfactory and statistically insignificant results across all three agents in the study by Sangeetha et al. may be attributed to technical issues such as inadequate fixation or unsuitable processing techniques [29].

Formic acid

All papers reported that formic acid requires less time for complete decalcification compared to EDTA but more time than nitric acid. As a weak organic acid, formic acid is gentler and slower than stronger acids like hydrochloric or nitric acid. Although nitric acid decalcifies more quickly, formic acid is preferred in diagnostic labs for routine surgical specimens, especially when immunohistochemical staining is necessary, due to its minimal damage to tissue structures and antigens.

Four papers reported that formic acid decalcification resulted in good pulp organisation, minimal soft tissue shrinkage and preservation of tissue architecture such as the odontoblast layer [23,30–32]. Additionally, samples were also easy to section and showed good and adequate staining. However, two studies, Srinivasyaiah et al. [33] and Bhat et al. [24], reported fair staining characteristics but significant tearing and shrinkage, and poor soft tissue attachment, respectively. Contradictory findings were reported by Sangeetha et al. [29] and Gupta et al. [22] who reported that 5% and 10% formic acid decalcification resulted in poor tissue preservation, pulp shrinkage and patchy staining. Gupta et al. [22] also investigated a solution comprising 8% potassium formate and 8% formic acid, which showed limited hard and soft tissue preservation and compromised staining quality. The addition of potassium formate to formic acid resulted in the formation of an acidic buffered solution. Buffering of acids is usually done to counteract the undesired effects of the acid. However, Gupta et al. [22] reported that there was no significant difference between 10% formic acid and the 8% buffered formic acid solution.

Nitric acid

A common observation made by all the studies using nitric acid as a decalcifying agent was the significant yellowing of the samples after decalcification. As a strong acid, nitric acid was the fastest decalcifying solution but did not produce satisfactory results in terms of sectioning, tissue architecture and staining quality. Sanjai et al. [30], Sangeetha et al. [29], Bhat et al. [24] and Khangura et al. [31] all observed tissue shrinkage, poor tissue attachment, disorganised pulpal arrangement, loss of tissue architecture and poor overall histological appearance. While Khangura et al. [31] noted that the samples were easy to section, Sanjai et al. [30] and Bhat et al. [24] had difficulties in sectioning. On the other hand, Gupta et al. [22] reported that nitric acid produced excellent staining and based on the scoring system that was used by the authors, samples decalcified in nitric acid scored the highest for both hard and soft tissue preservation. This contradictory result might be attributed to the better fixation of the pulp due to the removal of the apical third of the samples. Better infiltration of fixative into the tooth perhaps resulted in improved preservation of the tissues. Gupta et al. [22] also reported that the addition of 10% formalin to the nitric acid solution provided similar hard and soft tissue preservation and produced good staining. When comparing between 5% and 7% nitric acid, 5% nitric acid was found to preserve good structural details and had good staining while 7% caused tearing and shrinkage in few samples and only fair structural details were observed [33]. Inconsistencies were also seen in the samples that were decalcified in the formalin-nitric acid solutions. Sanjai et al. [30], Choube et al. [23] and Khangura et al. [31] all reported tissue shrinkage, altered pulp organisation, loss of tissue architecture and poor staining, with only Sanjai et al. [30] having difficulties in sectioning.

Trichloroacetic acid

Sanjai et al. [30] and Khangura et al. [31] reported positive results when using trichloroacetic acid as a decalcifying agent. Samples were reported to be easy to section, maintained good pulpal organisation, and exhibited minimal tissue shrinkage and loss of tissue integrity. In this regard, it was even scored the second highest after EDTA by Sanjai et al. [30]. When comparing between 5% and 7% trichloroacetic acid, despite both concentrations resulted in tearing and shrinking of samples, 5% trichloroacetic acid provided good staining while 7% only showed fair staining [33]. Conversely, Ali et al. [32] reported completely different outcomes, indicating that 5% trichloroacetic acid decalcification resulted in friable and difficult to section samples with patchy staining. Additional issues such as damage to the odontoblast layer, retraction of the pulp, and fraying of dentinal tubules were also observed.

Perenyi's fluid

Perenyi's fluid is a decalcifying solution that comprises 10% nitric acid, 0.5% chromic acid and absolute alcohol. All three studies using this agent consistently reported difficulties in sectioning, significant tissue shrinkage, loss of tissue attachment, poor staining and overall histological appearance [23,30,31]. Choube et al. [23] also noted that 70% of the sections had artifacts such as tissue separation, tears and folds. These consistent findings across studies indicate that Perenyi's fluid is unsuitable for histological studies, likely due to its high acid content.

Morse solution and Von Ebner's solution

The decalcifying effect of Morse solution, which is an acid solution comprising of formic acid and sodium citrate, was briefly discussed by Grando et al. [25] where they reported that decalcification with Morse solution seemed to produce better preservation of cell structure, and the extracellular matrix as compared to 10% EDTA. The addition of sodium citrate to the widely used formic acid seems to have a positive impact on preserving the tissues during the decalcification process. While Widbiller et al. [21] did not report on the difference between staining qualities of samples decalcified in Morse solution and EDTA, they did report

https://doi.org/10.37231/ajmb.2025.9.1.788
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that samples in Morse's solution were completely decalcified seven days earlier than those in EDTA. Von Ebner's solution was only used by Choube et al. [23] and samples decalcified with this solution were easy to section and provided adequate staining. However, despite 60% of the samples having proper pulpal organisation, 70% of the samples had issues of tissue separation and tearing. This problem of tissue separation and tearing might be due to the concentrated hydrochloric acid present in Von Ebner's solution.

Effect of Decalcification Conditions

Heating during decalcification

Heating enhances the kinetics of reactant molecules, thereby accelerating various chemical reactions, including decalcification. Three studies compared the decalcification time of samples processed at room temperature versus those subjected to microwave heating [23,29,33]. Sangeetha et al. [29] subjected samples in 5% nitric acid and 5% formic acid for 8 seconds, and samples in 14% EDTA for 10 seconds, every hour, eight times a day, while Srinivasyaiah et al. [33] irradiated the samples 2 minutes every hour, eight times a day. In both studies, the decalcifying solution was changed every day and a significant reduction in decalcification time was observed. Sangeetha et al. [29] found that room temperature decalcification in 5% nitric acid, 5% formic acid and 14% EDTA took 35, 42 and 85 days, while samples subjected to microwave heating only took 4, 9 and 20 days, respectively. Additionally, it was also noted that only 20% of samples decalcified with nitric acid using microwave heating showed a damaged odontoblastic layer, compared to 80% of samples decalcified at room temperature. The microwave-heated samples also exhibited better staining quality, with only 20% showing patchy staining compared to 100% of the room temperature samples. This improvement in staining quality may be attributed to the reduced immersion time in the decalcifying agent. Similar results were observed by Srinivasyaiah et al. [33], where samples decalcified at room temperature in 5% and 7% formic acid, 5% and 7% nitric acid, and 5% and 7% trichloroacetic acid took 42, 40, 15, 121, 39 and 16 days while the microwaved samples only took 21, 20, 2, 2, 18, 13 days respectively. Choube et al. [23] reported that samples decalcified at room temperature in formic acid-formalin took an average of 55.4 days, whereas heating reduced this to 20.4 days. It was also noted that samples that were heated during decalcification were difficult to section, likely due to the additional tissue damage that was caused by the heat.

Agitation and electric current during decalcification

Only one paper studied the effect of agitation and electric current on decalcification time [23]. While it took an average of 55.4 days for complete decalcification of samples at room temperature in formic acid-formalin, subjecting samples to continuous agitation and a 6V electric current decreased the duration to 22.7 and 26.5 days, respectively. The reason for the significant decrease in the decalcification duration is that 1) agitation increases the interaction between the sample and the decalcifying agent and 2) when under the influence of an electric field, the calcium ions liberated by the decalcifying agent will be rapidly removed [34]. Despite the reduced decalcification time with electric current, 90% of the samples showed dentin destruction, and 60% exhibited cementum destruction. The samples were also difficult to section, had higher number of artifacts and exhibited significantly poorer pulpal organisation [23]. They also reported that the carbon rods that were used as the electrodes caused carbon to precipitate on surface of the sample, which were seen on the sections of the superficial tissues. On the other hand, samples subjected to agitation during decalcification retained staining properties equivalent to non-agitated samples, suggesting that agitation is an effective method to significantly reduce decalcification time without compromising histology and staining quality.

AJMB
eISSN: 2600-8173
https://doi.org/10.37231/ajmb.2025.9.1.788
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Table 2: Summary of the advantages and disadvantages of different decalcifying agents and conditions.

	Advantages	Disadvantages
Decalcifying Agent		
EDTA	Gentler process of decalcification compared to acid decalcification. Good preservation of tissue morphology, cell structures and antigenicity.	Long duration for complete decalcification to occur.
Formic Acid	Faster decalcification compared to EDTA. Easy to section. Minimal damage to tissue structures and antigens.	Tearing of sections. Poor soft tissue attachment. Pulp shrinkage.
Nitric Acid	Fastest decalcifying solution.	Significant yellowing of the samples after decalcification. Difficulties in sectioning. Poor tissue attachment. Poor pulpal organisation. Loss of tissue architecture. Tissue shrinkage.
Trichloroacetic Acid	Easy to section. Minimal loss of tissue integrity. Good pulpal organisation.	Tearing and shrinking of sections.
Perenyi's Fluid	-	Difficulties in sectioning. Significant tissue shrinkage. Loss of tissue attachment. Poor staining.
Morse Solution	Good preservation of cell structure and extracellular matrix.	-
Von Ebner's Solution	Easy to section. Good pulpal organisation. Adequate staining.	Tearing of sections. Tissue separation.
Decalcification Conditions		
Heating	Decreases time taken for complete decalcification.	Difficulties in sectioning.
Agitation	Decreases time taken for complete decalcification.	-
Electric Current	Decreases time taken for complete decalcification.	Difficulties in sectioning. Destruction of dentin and cementum. Poor pulpal organisation. Higher number of artifacts.

Conclusion

In this review, we have summarized the findings of 11 studies that have tested, compared, and discussed the efficacy of various decalcifying agents and conditions used for the complete decalcification of human teeth. We have highlighted the advantages and disadvantages of the different decalcifying agents available as well as the different conditions in which decalcification can be carried out (Table 2). Although one specific solution encompassing all the advantages cannot be concluded, researchers and histopathologists can choose the suitable decalcifying agent based on several factors such as the type of tissue, acceptable duration of decalcification and the degree of preservation of morphological detail required. From the studies included in this article, 5% formic acid seems to the best decalcifying agent to use when rapid decalcification is required. Samples decalcified in this solution tend to retain most tissue structures which is sufficient for histological analysis. However, if better preservation of tissue structure is required and time constraints are not a factor, EDTA may be a more suitable option. On the other hand, Perenyi's fluid seems to be most unfavourable decalcifying agent as none of the studies that include this solution report positive outcomes. Further research focusing on exploring alternative decalcifying agents or optimizing the existing ones to address the limitations and challenges associated with current decalcification methods is needed in order to facilitate more accurate histological analysis of tooth samples.

Funding

This work was supported by Ministry of Education (MOE), Malaysia through the Fundamental Research Grant Scheme (FRGS/1/2021/SKK0/USM/03/4).

Conflict of interest

The authors declare no competing interests.

Ethics declarations

Not applicable.

Author contributions

Diveyaa Sivakumar: Writing – original draft, Visualisation. Nurhafizah Ghani: Writing – review and editing. Mogana Das Murtey: Writing – review and editing. Rosmaliza Ramli: Conceptualisation, Writing – review and editing, Funding acquisition.

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