

Demineralization of root canal dentine with EDTA and citric acid in different concentrations, pH and application times

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Abstract

The aims of the study are:

- 1. Determination of the amount of extracted Ca ions from dentine using 17% and 10% EDTA, at pH 7 and pH 9.*
- 2. Determination of the amount of extracted Ca ions from dentine using citric acid 5% and 1%, at pH 7 and pH 9.*

Methodology

30 extracted, single rooted, human teeth were tested. Their crowns were sectioned at CEJ using diamond disks. The root canals were manually prepared with K-files #50-60. After each instrument 2.5 ml of 5.25% NaOCl was used and 0.9 NaCl as final irrigation. All teeth were longitudinally sectioned and 8 samples of dentine taken from each sample. EDTA 10% and 17% and citric acid 1% and 5% in neutral and alkaline pH were used. Each sample was immersed in acid and then exposure time was evaluated after 1, 5, 10, 15 and 25 min.

The release rate of calcium ions from root dentine was evaluated by atomic absorption spectrometer.

Results

There were significant differences in the amount of extracted Ca by citric acid 1% and 5% or EDTA 10% and 17% in human teeth.

Conclusions

It may be concluded that EDTA is a better chelating agent than citric acid. The decalcifying activity of these solutions is related to the duration of exposure, pH and their concentrations.

Key words: *demineralization, dentine, EDTA, citric acid.*

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Introduction

Primary goal in endodontic treatment is to achieve cleanness of the root canal. A root canal is clean only after complete and necessary removal of all noxious agents. The smear layer is a micro-layer in the dentine walls of the root canals that is formed during root canal instrumentation with adequate endodontic instruments.

The smear layer consists of organic and inorganic components¹⁶. The removal of organic components is done with sodium hypochlorite (NaOCl), while the inorganic components are removed with chelating agents⁴. The efficiency of these chelating agents depends on the root canal length, penetration capabilities of the agent, duration of exposure, dentinal strength, pH and concentration of the solution⁷.

Sodium hypochlorite (NaOCl) with concentration 1-5.25% is widely used for smear layer removal because of its antibacterial effect^{30,4}. Root dentine demineralization can be done with phosphoric acid, citric acid and EDTA²⁸. Smear layer removal with different concentrations of EDTA solution has been investigated¹. Root canal irrigation with 10 ml of EDTA 17% combined with 10ml of NaOCl 5.25% has been shown very efficient on smear layer removal.³⁰

Another agent used for smear layer removal is citric acid, investigated in different concentrations. It has been reported that citric acid is less cytotoxic for the tissues².

The aims of this study were:

1. Determination of calcium (Ca^{2+}) ions extracted from the root dentine with EDTA solution 17% and 10%, in pH 7 and pH 9, with exposure duration of 1', 5', 10', 15' and 25'.
2. Determination of calcium (Ca^{2+}) ions extracted from the root dentine with citric acid 1% and 5%, in pH 7 and pH 9, with exposure duration of 1', 5', 10', 15' and 25'.

Materials and methods

This study was performed at the Medical Faculty - Dentistry School in Prishtina.

Thirty extracted teeth for various indications were used as the material of the study. They were immersed in distilled water before use. The teeth belonged to the maxillary inter-canine sextant.

The crown was separated at the cement-enamel junction with diamond discs in continuous water irrigation. The pulp tissue was removed from the root canal with barbed broaches (EDENTA-E, SWISS) and the canal was irrigated with NaOCl 5.25% solution (ADD Vision Germany). Root canals were instrumented with manual step-back method. The initial instrumentation was done with K-

files #10, while the manual instrumentation was done with K-files #40-60, depending on the root canal volume.

Each canal was irrigated with NaOCl 5.25% for five times with 2.5 ml - total of 12.5 ml for each canal. After the instrumentation, sterile saline (NaCl 0.9%, Baxter, Espania) was used to preserve the working samples.

After the root canal instrumentation the cement was removed from the root with diamond discs under continuous water irrigation. Then, the root was sectioned longitudinally in eight equal segments, thus having 240 samples from 30 teeth. These segments were put in Petri-plates numbered 1-30, at temperature 37°C for two hours. Then, each dentinal sample was weighted in a scale (Sartorius, Analytic, Germany) and immersed in acidic solution.

Acidic solution preparation

EDTA solution (Komplekson III, Zorka, Šabac) has been prepared in concentrations 10% and 17%. The pH has been calibrated with pH-meter (Sentron Sensor Integrated Technology, Netherlands), and buffering in neutral and alkaline pH was done with addition of ammonia (NH₄).

Citric acid solution (Limunska kiselina, Belgrade) has been prepared in solutions of 1% and 5%. The pH has been calibrated with pH-meter (Sentron Sensor Integrated Technology, Netherlands), and buffering in neutral and alkali pH was done with addition of ammonia (NH₄).

Each dentine sample was immersed in previously prepared acidic solution. The solutions were dispensed in plastic laboratory cups divided in four groups with 10 cups each.

Group I - five cups with 10ml of EDTA17%, pH 7 and exposure duration of 1', 5', 10', 15" and 25';

Group Ia - five cups with 10ml of EDTA17%, pH 9 and exposure duration of 1', 5', 10', 15" and 25';

Group II - five cups with 10ml of EDTA10%, pH 7 and exposure duration of 1', 5', 10', 15" and 25';

Group IIa - five cups with 10ml of EDTA10%, pH 9 and exposure duration of 1', 5', 10', 15" and 25';

Group III - five cups with 10ml of citric acid 5%, pH 7 and exposure duration of 1', 5', 10', 15" and 25';

Group IIIa - five cups with 10ml of citric acid 5%, pH 9 and exposure duration of 1', 5', 10', 15" and 25';

Group IV - five cups with 10ml of citric acid 1%, pH 7 and exposure duration of 1', 5', 10', 15" and 25';

Group IVa - five cups with 10ml of citric acid 1%, pH 9 and exposure duration of 1', 5', 10', 15" and 25'.

Duration of exposure to acid action on the immersed dentine samples was measured with a chronometer and after expiring time the content of each cup was put in glass tubes marked numbered 1-1200 and preserved until the readings. Before AAS reading of the glass tubes, 2ml of lantan oxide were added.

Lantan oxide was used as a buffer to avoid errors deriving from water solutions containing salt, potassium and magnesium. These elements can disturb the readings of calcium and other components in acetylene flame. After addition of the lantan oxide, the samples were centrifuged for five minutes at 3000 rpm (HETTICH, Germany).

After centrifugation, the reading of the extracted calcium ions from the dental samples was done with AAS - Atomic Absorption Spectrometer 1100 B (PERKIN ELMER, Germany). AAS was calibrated with standard solutions.

The amount of calcium ions released from each dentine sample was verified and the mean from three readings was considered.

Statistical analysis was done with One-Way-ANOVA and Tukey-s test.

Results

Table 1 presents the amount of Ca^{2+} ions extracted from the root dentine in pH 7 with EDTA and citric acid of different concentrations, measured in five duration periods (1', 5', 10', 15' and 25').

The values for each measurement are expressed with mean \pm standard deviation. One- Way ANOVA showed significant difference for the amount of Ca ions extracted in the solutions as follows:

- Citric acid 1% (F=3.07, p=0.018) Tukey's test showed difference between the amount extracted in the first minute and 25th, and between fifth and 25th minute;
- Citric acid 5% (F= 9.12, p<0.0001) Tukey's test showed difference between the amount extracted in the first minute with other periods (5th, 10th, 15th and 25th minute);
- EDTA 10% (F=57.74, p<0.0001) Tukey's test showed difference between the amount extracted in the first minute with other periods (5th, 10th, 15th and 25th minute);
- EDTA 17% (F=48.78, p<0.0001) Tukey's test showed difference between the amount extracted in the first minute with other periods (5th, 10th, 15th and 25th minute).

This shows that the amount of the Ca^{2+} ions extracted increased after longer period of time, but figure 1 shows that this increase was higher with EDTA solution.

One-Way ANOVA showed highly significant difference when comparing the solutions used for all of the duration periods (F=137.15, p<0.0001).

Tukey test showed that the highest amount of extracted ions was with EDTA 10% ($p < 0.001$).

Table 1. Comparison of the solvents with different concentrations and pH 7.

Solution	pH	Total mgCa ²⁺ /g mean±SD	Time (min.)					ANOVA p-value
			1'	5'	10'	15'	25'	
Citric acid 1%	7	1.0157±0.795	0.8738±0.465	0.8331±0.370	0.9165±0.424	1.0121±0.513	1.4431±1.483	p=0.018
Citric acid 5%	7	1.4817±0.737	0.9899±0.784	1.2485±0.674	1.5970±0.667	1.6325±0.558	1.9406±0.637	p<0.001
EDTA 10%	7	6.0496±3.940	2.1305±0.546	4.1909±1.644	5.5204±2.182	7.1545±2.726	11.251±3.946	p<0.0001
EDTA 17%	7	5.1589±3.420	2.0133±0.743	3.4365±1.201	4.6395±2.351	6.1659±2.522	9.5389±3.425	p<0.0001
ANOVA p-value		p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	

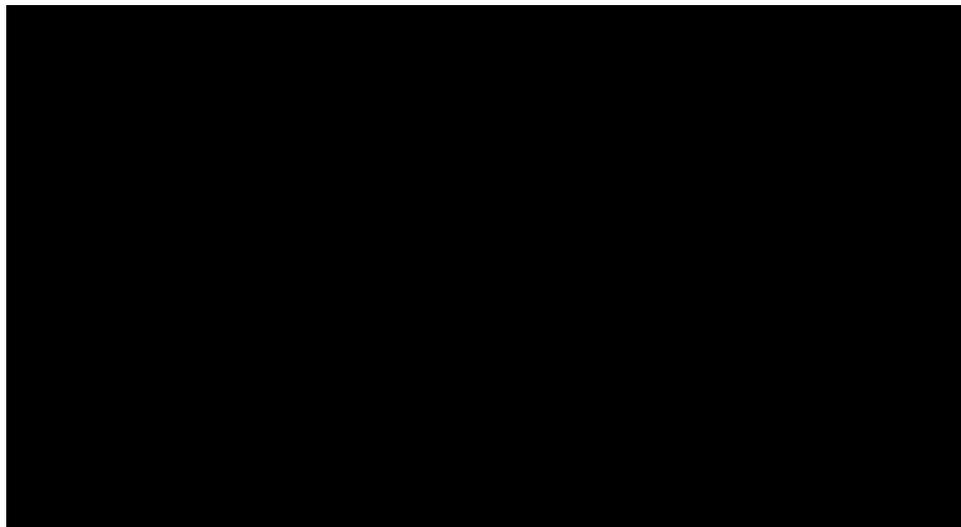


Figure 1. The amount of Ca²⁺ ions (mg/g) extracted with solvents of different concentrations in pH7 and compared by the duration periods.

Table 2 shows the One-Way ANOVA, that didn't show significant difference of the extracted ions amount by duration for citric acid 1% ($F=0.56$, $p=0.691$), but the difference was found for citric acid 5% ($F=3.77$, $p<0.0001$). Using Tukey's test the difference was found between the amounts extracted in the first minute and the 25th minute;

- EDTA 10% (F=33.16, p<0.0001), Tukey's test showed difference between the amount extracted in the first minute with the amount extracted after all other duration periods;
- EDTA 17% (F=20.04, p<0.0001) Tukey's test showed difference between the amount extracted in the first minute with the amount extracted after all other duration periods.

This shows that the amount extracted ions increased with increased duration, but with higher increase for EDTA. One-Way ANOVA showed high significant difference when comparing used solvents in all duration periods (F=182.06, p<0.0001). Tukey test showed that the highest amount of ions were extracted with EDTA 10% (p<0.001). According to pH, Ca²⁺ ions were extracted mostly with EDTA 10% (p<0.0001

Table 2. Comparison of solvents with different concentrations and pH 9.

Solution	pH	Total mgCa ²⁺ /g mean±SD	Time (min.)					ANOVA p-value
			1'	5'	10'	15'	25'	
Citric acid 1%	9	0.8197±0.425	0.7254±0.447	0.8533±0.438	0.8115±0.425	0.8790±0.418	0.8294±0.408	p=0.6914
Citric acid 5%	9	1.0537±0.429	0.9106±0.454	0.9424±0.346	1.0363±0.382	1.0988±0.436	1.2803±0.443	p=0.0059
EDTA 10%	9	3.8378±2.059	1.9932±0.662	3.1652±1.217	3.7112±1.079	3.9975±1.536	6.3221±2.436	p<0.0001
EDTA 17%	9	3.5562±1.958	1.8674±1.180	3.3691±1.600	3.5403±1.339	3.8215±1.424	5.1824±2.475	p<0.0001
ANOVA p-value		p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	

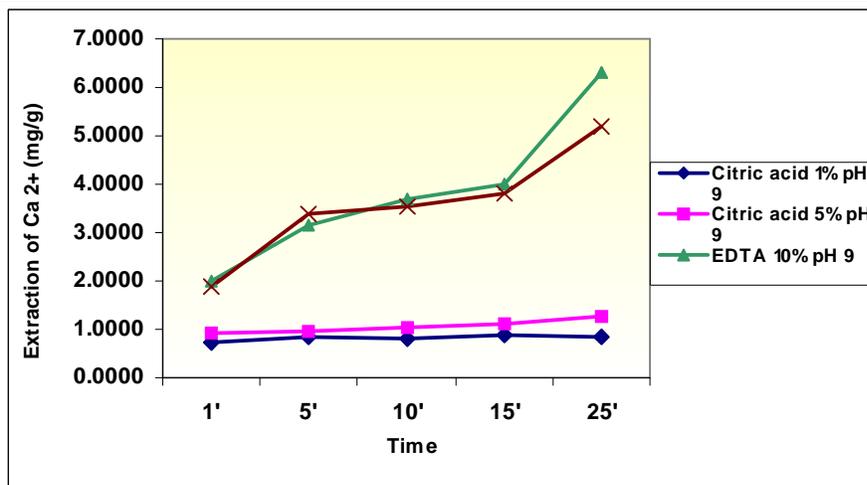


Figure 2. The amount of Ca²⁺ ions (mg/g) extracted with solvents of different concentrations in pH7 and compared by the duration periods.

Discussion

Now days, there are many methods for root canal instrumentation in order to achieve better and higher cleanness of the endodontium. The mechanical-chemical methods of root canal instrumentation are the mostly used resulting with better cleanness of intra-canalicular root canal dentine.

Many authors have investigated the duration of exposure of the root canal dentine to EDTA and citric acid solutions and the influence of their pH and concentration.

Ostby¹⁹ in 1957 was the first to use EDTA for root canal irrigation for dentine demineralization, and the best results were with EDTA 15%, in pH 7.3. Our results of dentine demineralization were the highest with EDTA 10% in neutral pH. According to Seidberg and Schilder 1974²¹ dentine demineralization is not dependent on the pH of the solvent. The pH 7.5 was more efficient in dentine demineralization than pH 9, according to Serper and Çalt, 2002²². Nikiforuk, Sreenby and Obst¹⁷ reported that chelating action is most efficient in pH 6-10. If pH is 10.3, the chelating effect of EDTA solution is higher in the proportion of ionized and non-ionized molecules and vice versa, and if pH is more acidic, then the chelating process is less efficient resulting with decrease of ionized molecules. EDTA solution in neutral and alkaline pH has the most optimal action on dentine demineralization (Nikiforuk dhe Sreebny 1953¹⁷, Rubin et al. 1979²⁰, Serper and Çalt 2002²²). On the other hand, it has been reported that the optimal pH for dentine demineralization is 5.0 to 6.0 (Cury et al. 1981⁷).

Serper dhe Çalt (2002)²² also found that demineralization of dentine is more efficient in neutral pH, than in acidic or alkali pH. Çalt et al. (2000)⁹, O'Connell et al. (2000)¹⁸ reported that EDTA solution in alkali pH is less efficient on smear layer removal, than the same solution in neutral pH.

The duration of exposure to the chelating agent also is an important factor on the smear layer removal. Calcine caused large loss of mineral components after exposure of nine minutes. Serper and Çalt (2002)²² reported that EDTA 17% has better demineralization effect than EDTA 10%. In the contrary, in our study, the removal of smear layer is more efficient with EDTA 10% than with 17% solution of EDTA.

After three, ten and fifteen minutes of exposure with EDTA 17% and citric acid 10% solutions, there was no significant difference on the calcium ions extraction (Scelza et al. 2003)²³. There is no exact established optimal exposure duration to chelating agent after the period of 15 minutes. Goldberg and Spielberg 1982¹⁰, McComb and Smith¹⁶ 1975 reported the best chelating effect after exposure of 14 hours. Cergneux et al. (1987)⁶ reported for efficient action of EDTA 15% with exposure duration of four minutes. In the quantitative aspect, smear layer removal depends on pH and the exposure duration to the chelating agent (Morgan dhe Baumgartner 1997)¹⁵, which was found also in our investig-

ation. Many studies showed that the loss of mineral components, as well as the cleanness of the root canal walls depends on the duration of exposure to the chelating agent (Nygaard-Ostby 1957¹⁹, Hülsmann and Heckendorff 2002¹³, Serper and Çalt 2002)²². Also, it has been reported that EDTA, either in solution as well as in paste form, has better effect after one and five minutes of exposure (Yamada et al. 1983³⁰, Cergneux et al. 1987⁶, Çalt and Serper 2000⁹, 2002, Hülsmann and Heckendorff 2002¹³, Scelza et al. 2003²³).

Gonzalez et al. (2006)¹¹ concluded that the demineralization effect of all solvents depends on the duration of exposure to the chelating agent. Scelza et al. (2003)²³ did not find any significant difference of the release of the calcium ions in the first three minutes when comparing citric acid 10% and EDTA 17%. There was no significant difference on the calcium ions release after first and 15th minute, but citric acid was more efficient on calcium ions extraction than EDTA-T solution. In our results there was no significant difference between EDTA and citric acid in calcium ions extraction in 10-15 minutes of exposure.

Choudary and Roopa (2000)⁸ in their study showed that if EDTA solution is used alone, the smear layer is removed completely after 30 minutes. If NaOCl 5.25% is used alone in 30 minutes, there is uncontrollable effect on smear layer removal. But, the combination of these two solutions removes the smear layer completely after 20 minutes. Our investigation showed that EDTA 17% and 10% in neutral pH remove most efficiently the smear layer in 25 minutes.

Also, the efficiency of citric acid has been investigated, regarding the concentration, pH and exposure duration.

Sousa and Silva (2005)²⁵ in their results found that citric acid 1% in pH 7.4 has extracted more calcium ions from the root dentine compared with other chelating agents. Also our results showed that citric acid 1% in pH 7 has extracted more calcium ions from the root dentine.

Meryon et al. (1983)¹⁴ reported that the smear layer is completely removed with EDTA 10%.

Wajman et al. (1983)²⁸ concluded that the best results on smear layer removal are achieved with use of citric acid 10% and NaOCl 2.5%. Tidmarsh(1978)²⁷ reported that citric acid 50% was more efficient in smear layer removal.

Haznedaroglu (2003)¹² has studied the effect of different pH values of citric acid. He reported that citric acid with concentrations 5%, 10% and 50%, in pH 1.1-1.9 was more efficient than in pH 6.0. Our results showed contrary values, whereas citric acid 5% in neutral pH was more efficient for smear layer removal.

Sterret et al. (1993)²⁶ reported that only in 10% of cases the demineralization is not dependent on the duration of exposure. Dentine demineralization with citric acid 10% depends on the exposure duration during the first 10 minutes of action. Our study revealed that root dentine demineralization with citric acid

1% and 5% depends on the duration of exposure. According to Yamaguchi et al. (1996)²⁹ pH of citric acid has been shown as more important factor on dentine demineralization than the solvents' concentration.

Silveiro, Lopez and Rodriguez (2004)²⁴ in their results showed that citric acid 1% and 10% is more efficient than EDTA 17%. Citric acid 10% is more efficient on smear layer removal than the same solution with concentration 1%. According to their results, the efficiency of these solvents decreased with increased exposure duration. On the other hand, our results showed that citric acid 5% is more efficient on smear layer removal than citric acid 1%.

Our results showed that EDTA 10% solution was more efficient on smear layer than citric acid 1% and 5%.

According to Morgan and Baumgartner (1997)¹⁵, smear layer removal depends on the pH of the chelating agent, same as in our study, since the release of calcium ions was higher in neutral pH than in alkalic pH.

According to Bhatnagar, Kumar and Shivana⁵, maximal release of calcium ions was recorded after exposure to citric acid 10%, compared to EDTA-T that also showed satisfying results, but with lower efficiency than the citric acid. But, in our results EDTA showed better results on smear layer removal than the citric acid, regardless of concentration, pH and exposure duration.

Sterret, Bankey and Murphy²⁶ found that root dentine demineralization depends on the duration of exposure to the acidic solvent. Our study also revealed that with the increase of exposure duration of the chelating agent increases the efficiency of the root dentine demineralization.

Conclusions

Based on the results of our investigation we have come to these conclusions:

- Root dentine demineralization is the most efficient with EDTA 10% solution in pH 7;

- Citric acid with concentration 5% and pH 7 showed efficiency on root dentine demineralization, but in significantly lower level compared to EDTA solution;
 - neutral pH has influenced in higher calcium ions extraction for the both investigated solutions;

- With the increase of exposure duration to the acid, increases the calcium ions extraction from the root dentine.

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