

In vitro diffusion of hydroxyl ions from medicaments pastes based on calcium hydroxide

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ABSTRACT

Objective: Analyzing, *in vitro*, the pH of six endodontic pastes based on calcium hydroxide [Ca(OH)₂]. **Methods:** Six groups were formed (n = 5 pastes/group) and a control group (distilled water): GI – Ca(OH)₂, propylene glycol 400 (PEG 400) and camphorated paramonochlorophenol (PMCC); GII – Ca(OH)₂, iodoform 1:1, PEG 400 and PMCC; GIII – Ca(OH)₂, iodoform 4:1, PEG 400 and PMCC; GIV – Ca(OH)₂ and Otosporim®; GV – Ca(OH)₂ and olive oil; GVI – Ca(OH)₂ and chlorhexidine gel 2%. The pastes were previously placed in distilled water and stored at 37° C, and the pH of each sample was measured at seven different time

intervals. The assay was performed in two steps, whereas in the second stage the distilled water was replaced after each reading. **Results:** In both phases, there was no statistically significant difference between the pH values of GI, GII, GIII and GIV (p > 0.05) in the 7 time intervals evaluated. All groups showed higher pH compared to the GV and the control group (p < 0.05), which were statistically similar to each other (p > 0.05). **Conclusion:** The pastes presented alkaline pH, with variations according to their composition, having a greater dissociation when a viscous substance was present in the composition.

Keywords: Diffusion. Endodontics. Calcium hydroxide.

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Introduction

The prevention and control of pulpal and periapical infections are crucial in endodontic treatments. The results of the endodontic therapy depend on the reduction or elimination of microorganisms and pathogenicities from periapical lesions and, therefore, the chemomechanical preparation is considered a major step in the disinfection of the root canal system. However, the elimination of bacterial strains involved in the infection process is difficult to perform and thus the intracanal medication between clinic sessions plays a fundamental role in the control of pulpal diseases.^{1,2}

Calcium hydroxide [$\text{Ca}(\text{OH})_2$] has been used in Endodontics since 1920, when Hermann employed it for the first time on direct pulp capping, and later as intracanal¹ medication. Since then, it has been widely used in clinical dentistry due to its therapeutic properties, both on its own and in the composition of cement and medicinal pastes. The success of $\text{Ca}(\text{OH})_2$ as medication is mainly due to its ionic effect, caused by the chemical dissociation in calcium ions and hydroxyl.³

The hydroxyl ions diffuse through the dentin, raising the pH of the media and producing an alkaline environment, which is unfavorable for bacterial growth since it favors the lysis of the cell membranes and the inactivation of the enzymes present in the microorganisms. Such mechanisms may explain the antimicrobial activity of the $\text{Ca}(\text{OH})_2$.^{3,4} Furthermore, the hydroxyl ions activate the alkaline phosphatase, an essential enzyme in the bone repair process. Now calcium ions allow the reduction in the permeability of new capillaries present in the granulation tissue of devitalized teeth, reducing the amount of intercellular fluid and triggering the pyrophosphatase acceleration, which plays a role in the mineralization.^{2,5,6}

Different substances have been used together with $\text{Ca}(\text{OH})_2$ in intracanal medications, being that the ideal substances are those which modify as little as possible its original alkalinity.⁷ Among the substances in those combinations, we have camphorated paramonochlorophenol (PMCC), propylene glycol (PEG), iodoform, olive oil and chlorhexidine, which can circulate and enhance the beneficial effects of calcium hydroxide on periradicular tissues.¹ The aqueous, soluble vehicles and the viscous, water soluble vehicles have the ability to raise the pH to an ideal alkaline value.

The only difference lies in the fact that the aqueous vehicles provide a faster hydroxyl ion dissociation and diffusion than the viscous vehicles.⁶

Given the above, the objective of this study was to analyze the ionic dissociation of medicinal pastes based on $\text{Ca}(\text{OH})_2$, in combination with different substances routinely used in clinical endodontics, and check the alkalinity of the medium, so important for the success of endodontic treatments.

Material and methods

Six types of medicinal pastes used in the treatment of various clinical situations in endodontics were manipulated, $\text{Ca}(\text{OH})_2$ being the most common component to all of them. PEG 400 and 2% chlorhexidine gel were manipulated at Cavallieri compounding pharmacy (Juiz de Fora, state of Minas Gerais, Brazil). The other components were commercially obtained: $\text{Ca}(\text{OH})_2$ P.A., iodoform and PMCC (Biodinâmica, Ibitiporã, state of Paraná, Brazil); Otosporim® – each mL containing: polymyxin B sulfate 10,000 IU, 5 mg neomycin sulfate and 10 mg hydrocortisone (Farmoquímica, Rio de Janeiro, state of Rio de Janeiro, Brazil); and the L&C paste, composed of $\text{Ca}(\text{OH})_2$ and olive oil (Dentsply, Petrópolis, state of Rio de Janeiro, Brazil).

Six experimental groups were formed, each group consisting of five samples of the same paste, namely: Group I – $\text{Ca}(\text{OH})_2$, PEG 400 and PMCC; Group II – $\text{Ca}(\text{OH})_2$ + iodoform 1:1, PEG 400 and PMCC; Group III – $\text{Ca}(\text{OH})_2$ + iodoform 4:1, PEG 400 and PMCC; Group IV – $\text{Ca}(\text{OH})_2$ and Otosporim®; Group V – $\text{Ca}(\text{OH})_2$ and olive oil; Group VI – $\text{Ca}(\text{OH})_2$ and 2% chlorhexidine gel.

The thick, toothpaste-like consistency type of medicinal paste is often recommended by professionals, being that the powder/liquid ratio varies widely.^{3,7} In order to determine the proportions of each component, the amount equivalent to a measuring spoon was used, whose volume corresponds to 0.13 cm³ to measure powdered substances. For liquid substances, the approximate measure of the volume of one drop (0.05 ml) was used. Five manipulations were performed, based on the desired consistency (toothpaste), and, at the end, the proportion of each substance used in the experimental manipulation was established.

Each of the five pastes in each group was manipulated and the amount equivalent to a measuring spoon – 0.13 cm³ – was separately placed in containers holding 15 ml of distilled and deionized water. One of the containers did not receive the paste, remaining with only 15 ml of distilled and deionized water, working as negative control. The samples were duly stored in closed flasks and kept at 37° C in order to remove the effects of the environment until all measurements were performed.²

In the first stage of the experiment, the pastes analyzed remained immersed in distilled water during all the periods of the analysis, and the readings were called GIA, GIIA, GIIIA, GIVA, GVA and GVIA for the respective groups. In the second stage of the assay, exchanges were performed between each reading of the distilled water in which the pastes were immersed, and the readings were called GIB, GIIB, GIIBB, GIVB, GVIB and GVB for the respective groups.⁸ For the analysis of the ionic dissociation of the medicinal pastes for each group, a digital pH meter (Model PH 710, state of São Paulo, Brazil) was used, being duly calibrated with standard buffer solutions with pH 7.0 ± 0.02 and pH 4.0 ± .02. Such apparatus consists of a glass electrode (EPC 70) connected to a digital display which allows reading the pH value. For the measurements, the calibrated micro-electrode was kept in contact with the solution for about 45 seconds until the pH reading was established.⁹

The measurements were done with 15 and 30 minutes, 1.24 and 48 hours, and 7 and 14 days after manipulation. The pH values found in function of the time intervals were duly entered in a Microsoft Excel® spreadsheet program, through which the average of the five pastes of each group was calculated. The data were analyzed by using the statistical program SPSS 15.0 for Windows (Chicago, USA). The variance analysis (ANOVA) was performed, followed by the Scheffé post-hoc test for comparisons between groups of medicinal pastes. The significance level was set at 5%.

Results

In the first stage of the experiment, the average value of GIA at t = 15' was pH = 10.40; with exponential growth until t = 24 h (pH = 12.16) and stabilization at t = 14 days (pH = 12, 31). Statistically similar pH readings (p > .05) occurred in the other groups analyzed: GIIA 15' (pH = 10.33), 24 h (pH = 12.17) and

14 days (pH = 12.24); GIIIA 15' (pH = 10.46), 24 h (pH = 12.21) and 14 days (pH = 12.32); GIVA 15' (pH = 10.62), 24h (pH = 12.21) and 14 days (pH = 12.31); GVIA 15' (pH = 9.62), 24h (pH = 12.24) and 14 days (pH = 12.33). GVA and control groups presented similar behavior (p > 0.05), with pH values lower than those found for the other groups (p < 0.05 for all the comparisons): GVA 15' (9.17), 24h (8.98) and 14 days (8.53) and the control group 15' (9.26), 24 h (9.09) and 14 days (8.77) (Fig 1).

In the second stage of the experiment, GIB showed, at t = 15', a pH = 10.38, with an increase up to t=24h (pH = 12.14) occurring, as in the first stage of the experiment, a stabilization at t=14 days (pH = 12.23). The other groups analyzed showed similar behavior: GIIB 15' (pH = 10.28), 24h (pH = 12.18) and 14 days (pH = 12.26); GIIBB 15' (pH = 10.42), 24h (pH = 12.18) and 14 days (pH = 12.26); GIVB 15' (pH = 10.56), 24h (pH = 12,20) and 14 days (pH = 12.30). GVIB showed pH = 9.63 at t=15', with a growth at t=30' (pH = 10.42) and stabilized until t=14 days (pH = 10.58). GVB and control groups showed pH values lower than the other groups (p < 0.05 for all the comparisons): GVB 15' (pH = 9.16), 24h (pH = 8.92) and 14 days (pH = 8.55) and control group 15' (pH = .17), 24h (pH = 9.21) and 14 days (pH = 9,18) (Fig 2).

In both phases of the experiment, there was no statistically significant difference between the pH values of GI, GII, GIII and GIV (p > 0.05 for all the comparisons). All groups showed higher pH compared to the GV and the control groups (p < 0.05 for all the comparisons), which were statistically similar to each other (p > 0.05 for all the comparisons). The control group remained with no significant changes in pH throughout all the periods.

GVI showed a particular behavior, with an initial pH statistically lower than the GI, GII, GIII, GIV and higher than the GV and the control groups (p < 0.05 for all the comparisons). In the first stage, when there was no water exchange, the pH values for GVIA showed initially to be lower but with exponential growth, following the behavior of the groups GIA, GIIA, GIIIA and GIVA. In the second stage of the experiment, in which medium water exchange was carried out, GVIB showed an alkaline pH, but with lower values throughout the periods compared to groups GIB, GIIB, and GIIBB GIVB (p < 0.05 for all comparisons).

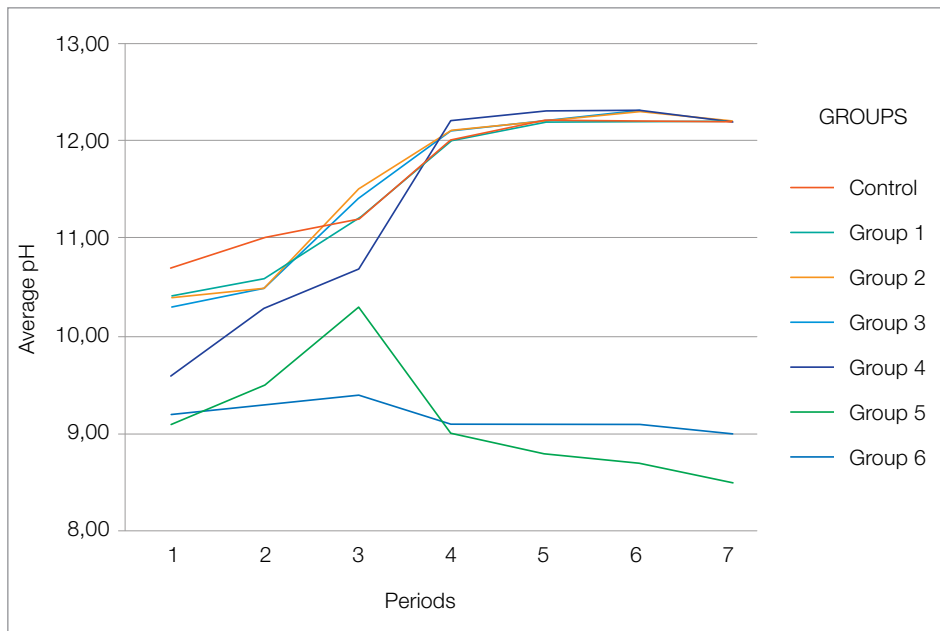


Figure 1. Alteration of the average pH against time, produced by the medicinal pastes in the first stage of the assay.

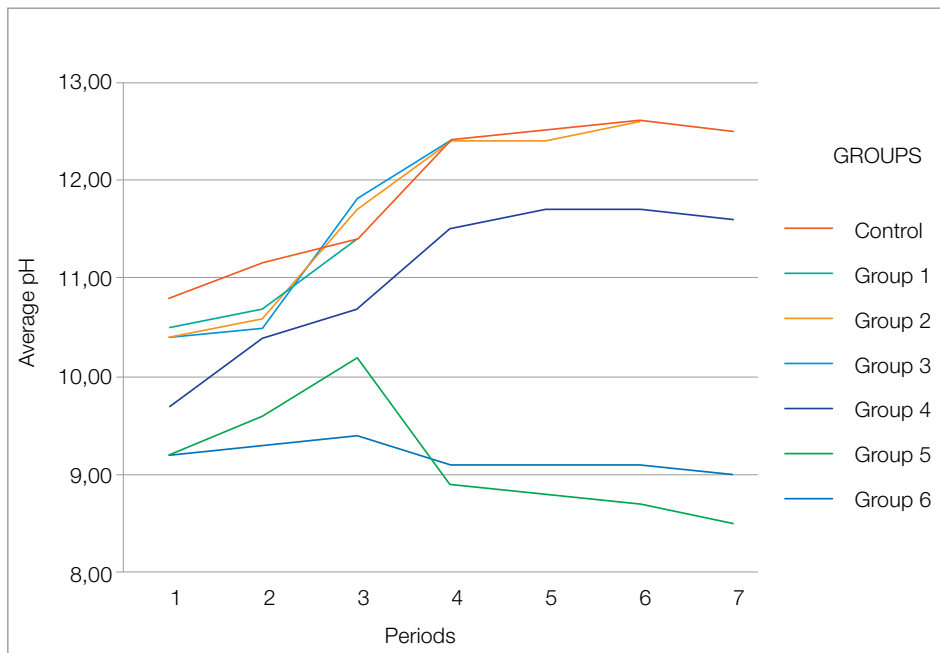


Figure 2. Alteration of the average pH against time, produced by the medicinal pastes in the second stage of the assay.

When comparing the two stages of the assay, it was observed that in the second stage (with periodic exchange of water where the pastes were inserted) pH values were lower, however, such difference decreased over the periods. Such difference was not statistically significant ($p = 0.709$).

Discussion

The combination of various substances to the $\text{Ca}(\text{OH})_2$ has been proposed as a way to enhance its beneficial effects to periapical tissues.⁷ Among those, we highlight the importance of the alkalization promoted by $\text{Ca}(\text{OH})_2$ -based pastes in the success

of endodontic treatment in various common clinical situations, like in cases of pulp necrosis, especially with periapical lesions, root resorption, and during the preservation of dental trauma.

The inflammatory and infectious processes promote tissue pH acidification, which is ideal for the growth of microorganisms. The alkaline atmosphere created by the $\text{Ca}(\text{OH})_2$ often prevents the development of those processes, and such effect is directly proportional to its alkalinizing potential. The alkaline pH promoted by such medications are effective in stopping the growth or eliminating pathogenic strains present in persistent endodontic infections, such as *Enterococcus faecalis*.^{1,10,11}

The effects of the dissociation of hydroxyl ions, through the reading of pH values, and the release of calcium ions from different vehicles added to the $\text{Ca}(\text{OH})_2$ were studied by several authors.^{6,8,9,12-16} In all those experiments, we found higher pH values for pastes with viscous vehicle, which, theoretically, provide a faster ionic dissociation to hydroxyl ions than oily vehicles. That statement is consistent with the results of the present study, in which the vehicles with viscous pastes (GI, GII, GIII, GIV and GVI) had higher pH values regarding the combination containing olive oil (GV), an oily vehicle.

The use of a pH meter with high impedance increases the accuracy of the results, as well as provides numeric data which can be analyzed. Although other methods may be used, such as the paper strips with pH indicators, these have lower precision and can hinder the accurate interpretation of the results.¹⁷

The pH measurement periods must comply with the time required for the manipulation of the pastes, their insertion in flasks containing distilled water and the beginning of the analyses. In the present study, the first measurements were performed after 15 minutes, since, according to the methodology applied, lower periods would be impossible due to the number of flasks to be measured in each group by the same operator. We observed that in some studies, however, pH measures with similar methodologies were carried out in shorter periods of time.^{1,9,15,16}

The results of the present study demonstrated that in the first stage of the experiment, most groups have kept alkaline initial pH with exponential growth up to the period of 24 hours. After that period we observed equalization and stabilization of the pH reading. Such behavior, however, was not observed for GV – $\text{Ca}(\text{OH})_2$ and for olive oil – as well as for the control group: besides presenting a lower pH than the other groups, they also showed an inverse behavior, with a pH decrease during the periods observed. Such pH behavior is consistent with the results found by Pacios et al,¹⁴ Ferreira et al.¹⁵ and Nunes and Rocha.¹⁸

The regular exchange of the water where the pastes to be tested were immersed in the second stage of the experiment was performed to avoid saturation of the medium, since it would not present ion exchange, as it occurs in the clinical situation of the intracanal medication.⁸ Those authors showed that the pH values of the medicinal pastes they studied were different for most of the groups only in periods prior to 24 hours. From that range, such groups showed no pH changes between each other, indicating no interference of such variable. They concluded that all pastes presented similar pH behavior in all periods analyzed. Such findings are in agreement with the results in the present study for the majority of the pastes analyzed (GI, GII, GIII and GIV).

Calcium hydroxide has been currently the most widely used intracanal medication. According to Herrera et al,¹⁹ calcium hydroxide is a suitable material to be used as delay dressing in teeth with periapical lesion, since the long-term assessment demonstrates satisfactory clinical results after the endodontic treatment. Most likely, its mineralizer and antimicrobial effect is due to its chemical dissociation in calcium ions and hydroxyl.²⁰

The addition of some substances to the $\text{Ca}(\text{OH})_2$ for the formulation of a clinically viable medicinal paste must preserve its main properties, such as chemical dissociation in calcium and hydroxyl ions, alkaline pH and tissue biocompatibility. It is believed that most of the combinations proposed in this study preserved the ionic characteristics desirable for an endodontic drug.

Conclusion

The medicinal pastes examined had alkaline pH values, whereas the pastes with viscous vehicles (PEG, PMCC, Otosporim® and chlorhexidine) showed high pH values compared to the ones found for the combination containing olive oil, an oily vehicle. The water exchanges in the media were the medicinal

pastes were immersed did not interfere significantly in the ionic dissociation of the combinations.

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