

The influence of aqueous and PEG 400 solvent vehicles on hydroxyl ion release from calcium hydroxide medicaments

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Abstract

Objectives: Calcium hydroxide pastes have been used in endodontics since 1947. Most current calcium hydroxide endodontic pastes use water as the vehicle, which limits the dissolution of calcium hydroxide which can be achieved and thereby the maximal hydroxyl ion release which can be achieved within the root canal system. **Methods and Materials:** This study compared the ionic properties of calcium hydroxide solutions in water or polyethylene glycol (PEG), then the ionic properties of commercial endodontic medicaments made using water or PEG, and finally hydroxyl ion release into radicular dentine in vitro using a new model with colour change in anthocyanin stained roots. **Results:** PEG 400 as a solvent gave a higher pH and more bio-available hydroxyl ions than water. Commercial pastes made using PEG 400 as the solvent showed a greater pH than those formulated with aqueous vehicles (water or saline). Hydroxyl ion penetration into roots under physiological conditions was greater when PEG was used as the medicament base than mixtures of PEG and water. **Clinical significance:** Enhanced availability and release of hydroxyl ions will enhance antimicrobial actions of calcium hydroxide when it is the active agent in endodontic medicaments.

Keywords: Calcium hydroxide, PEG 400, non-aqueous solvents, pH

Short Title: pH of calcium hydroxide medicaments

Introduction

Hydroxyl ion release from calcium hydroxide is responsible for the key attributes of broad spectrum antimicrobial activity, penetration into biofilms, inhibition of endotoxins, and dissolution of organic tissues.^{1,2} The maximum solubility of calcium hydroxide in water at 25°C is only 0.159 g/100mL (0.16%), and this reduces to 0.140 g/100 mL (0.14%) at 40°C, with an accompanying decrease of 0.033 pH unit/°C with increasing temperature.³ Calcium hydroxide is much less soluble in water than sodium hydroxide or potassium hydroxide, however both the latter too caustic to soft tissues for clinical use.⁴

To gain the greatest antimicrobial actions, release of hydroxyl ions from endodontic pastes must be optimized. Traditional water-based calcium hydroxide pastes have a long history of clinical use, with Pulpdent™ paste (Pulpdent Corporation, Watertown, Massachusetts, USA) having been on the market since 1947. In products with a water-based vehicle (e.g. distilled water or saline), the common ion effect operates, since water already contains some free hydroxyl ions. Most calcium hydroxide endodontic pastes which are water-based calcium hydroxide contain a large excess of calcium hydroxide, well beyond that which can be dissolved.² Placing calcium hydroxide into water in amounts above the solubility limit will not elevate the pH which can be achieved, since no further material can dissolve. The undissolved material will act as a filler or thickener in the paste, and as hydroxyl ions diffuse into the surrounding environment, it can also act as a reservoir.

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Table 1. Relationships between ion concentrations, pH, pOH and millivoltage

pH	H ⁺ concentration (mol/l)	pOH	OH ⁻ concentration (mol/l)	mV
0	1	14	1×10 ⁻¹⁴	+414.12
1	0.1	13	1×10 ⁻¹³	+354.96
4	1×10 ⁻⁴	10	1×10 ⁻¹⁰	+177.48
7	1×10 ⁻⁷	7	1×10 ⁻⁷	0
9	1×10 ⁻⁹	5	1×10 ⁻⁵	-118.32
12	1×10 ⁻¹²	2	0.01	-295.80
14	1×10 ⁻¹⁴	0	1	-414.12

Mathematical relationships seen in pure water at 25°C between pH, pOH, hydroxyl ion concentrations and millivoltage recordings for conventional pH electrodes with KCl electrolyte.

There is a long history of the use of non-aqueous liquids in endodontic pastes, going back to the mid-1970's when components such as polyethylene glycol (PEG) 400 were added to alter the viscosity to improve the clinical handling of the paste. As an example, the 1976 formulation of a calcium hydroxide paste (Calen) included both PEG 400 and colophony resin, with the latter being a thickening agent. Other viscosity modifiers used in calcium hydroxide pastes have included glycerol and propylene glycol.¹ It is now recognised that such non-aqueous liquids are not merely passive agents, but may actively contribute to the chemical and biological properties of the paste, by enhancing dissolution of calcium hydroxide and release of hydroxyl ions. PEG 400 is used in contemporary endodontic pastes which contain calcium hydroxide, calcium hydroxide plus ibuprofen, or antibiotics and corticosteroids.

The aim of the present study was therefore to assess the chemical properties of PEG 400 when used as a non-aqueous solvent for calcium hydroxide, with a particular emphasis on the release of hydroxyl ions. Such release can be considered in terms of the pOH and pH scales. While originally theorized by Sorensen for water-based solutions, it is now well known that the pH scale can also be used as a measure of the acid-base properties of non-aqueous solutions.^{5,6} This is done typically using research-grade pH meters that display both pH (from -2.00 to +20.00) and millivolts, and which are fitted with either conventional electrodes or electrodes specifically designed for the

measurement of non - aqueous solutions, using reference electrolytes such as lithium chloride 2 mol/l in ethanol or tetraethylammonium bromide (TEABr) 0.4 mol/l in ethylene glycol), for acidic and alkaline materials, respectively. Using such meters and electrodes, assessments of hydroxyl ion concentration (pOH) and pH can be made for aqueous and non-aqueous materials. There are mathematical relationships between pOH, pH and millivoltage (Table 1). When considering the ionic properties of non-aqueous solvents, one has to consider dissociation of the solvent and the slower response time of non-aqueous electrodes, which require extended measurement times. Thus, the first part of the study assessed ionic properties of calcium hydroxide in different solvents, the second the ionic properties of calcium hydroxide medicaments with different compositions, and the third the diffusion of hydroxyl ions into roots of extracted teeth.

Materials and Methods

Part 1. Ionic properties of solutions

A research grade pH meter (model HI 4222, Hanna Instruments, Woodsocket, Rhode Island, USA) was used for all measurements. The meter was calibrated using four standard aqueous buffers at pH values of 4, 7, 10, and 13 (ACR Chemical Reagents, Moorooka, Queensland, Australia). Two different electrodes were used to measure the pH in medicaments and different calcium hydroxide/PEG 400 mixtures, the first being a conventional electrode (model IJ-44C, Ionode, Tennyson, Queensland, Australia), while the

Table 2. Components of commercial calcium hydroxide pastes

	Pulpdent	Calasept Plus	CalMix	Odontocide
Active ingredients	39-42% Ca(OH) ₂	41.07 % Ca(OH) ₂	37.5% Ca(OH) ₂	20% Ca(OH) ₂ 7% Ibuprofen
Vehicle	Water	Isotonic saline	45.5% PEG 400	61% PEG 400 5% Water
Thickener	Methyl cellulose	Nil	PEG 4000	2.3% PEG 3000
Radiopaque agent	Barium sulphate	8.33% Barium sulphate	14% Zirconium dioxide	2% Barium sulphate

Components are shown in percentages by weight. Information on product composition was obtained from material safety data sheets and manufacturer websites.

second was designed specifically for non-aqueous solvents (Solvotrode, Metrohm Instruments, Gladesville, NSW, Australia), and contained 2% lithium chloride in ethanol as the reference electrolyte. As instructed by the latter electrode manufacturer, for testing alkaline materials the lithium chloride in ethanol electrolyte was removed and replaced with a solution of TEABr in ethylene glycol prior to use. When the electrodes were not in use they were stored in their recommended storage solutions (2% KCl in water for the Ionode electrode, and TEABr in ethylene glycol for the Solvotrode electrode).

The pH of various concentrations of calcium hydroxide (analytical grade, Chem-Supply, Gillman, South Australia, Australia) placed in ultrapure water or polyethylene glycol 400 (Ace Chemical Company, Camden Park, South Australia, Australia) was measured, at final concentrations of 5%, 10%, 20% and 40%. Calcium hydroxide powder was added to PEG 400 in disposable plastic clear tubes 15 mm diameter and 95 mm high, and mixed vigorously using a metal spatula for 20 seconds to achieve a uniform mix. The Solvotrode electrode was inserted into this mixture and the pH recorded at room temperature (23°C) every 5 minutes until a stable measurement was reached.

The pH values of five separate samples for each of the different concentration of calcium hydroxide/PEG 400 mixtures were recorded. For all pH measurements, values were tracked over time until they became consistent. For the different mixtures of calcium hydroxide in PEG 400, between 60 and 140 minutes was required before a stable value was reached. Recorded values for pH from five independent

experiments were assessed for normality, and then differences analyzed using Student's t test, or analysis of variance with Bonferroni post-tests. Data sets met requirements for parametric statistical comparisons.

Part 2. Ionic properties of medicaments

The pH of four commercial calcium hydroxide medicaments was measured. Two of the commercial medicaments were water based (Pulpdent™ paste, Pulpdent Corporation, Watertown, Massachusetts, USA, and Calasept Plus™, Nordiska Dental, Angelholm, Sweden) and two used PEG 400 as their base (Odontocide™, Australian Dental Manufacturing, Kenmore Hills, Queensland, Australia, and Calmix™, Ozdent, Castle Hill, NSW, Australia). The composition of these medicaments is given in Table 2.

For the two PEG 400-based commercial medicaments (Calmix, Odontocide) between 60 and 140 minutes was required before a stable value was reached, whilst the water based medicaments (Pulpdent, Calasept plus) took only 10 minutes to reach a stable pH value. For Pulpdent and Calasept Plus (aqueous solvent), between measurements the electrode was washed by vigorous stirring in three separate lots of distilled water for 2 minutes each to remove any paste on the electrode, and to reduce the final pH registered on the electrode to approximately pH 7, to ensure a neutral reading at the electrode tip prior to further readings. For Calmix and Odontocide (PEG solvent), the same regimen was followed, but the electrodes were then placed in their relevant storage solutions for a minimum period of five minutes to replenish the electrode tip after which it was washed in distilled water.

Part 3. Hydroxyl ion release into radicular dentine

The experimental model involved tracking changes in the colour of anthocyanin dye in stained roots kept at physiological conditions. Anthocyanin is the pH indicator found in red cabbage, and it undergoes multiple colour changes with pH over the alkaline range, with corresponding increases in both the red and green channels as the colour changes. A series of buffers from pH 8.0 to 13.0 were prepared to serve as a reference for changes in pixel data (Fig. 1). Extracted human permanent teeth were collected from an oral surgery clinic with the approval of the institutional ethics committee. A total of 15 single-canal teeth free from intrinsic discolouration or translucency were selected, then the crowns removed at the cemento-enamel junction and the root face smoothed to form a flat coronal surface using abrasive discs. After patency of root canals was confirmed using a #8 K-file (Dentsply Maillefer, Switzerland), they were then prepared with NiTi rotary instruments (ProTaper Next, Dentsply Maillefer, Switzerland) to size X3 with variable taper and an apical preparation of ISO #30. The root canals were irrigated alternately with sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA) using syringes with side-vented needles. After a final irrigation step using EDTA for 2 minutes, the canals were dried with paper points, and the roots placed in saline at room temperature for 24 hours to neutralise any remnants of sodium hypochlorite.

To stain the roots, a 3% solution of anthocyanin (Red Cabbage Jiffy Juice™ powder) in water was prepared. The prepared roots were removed from the saline bath and dried with paper towels and paper points, and the canals then irrigated with dye solution before submerging the roots into the dye for 48 hours. This achieved a uniform purple stain throughout the root. The stained roots were dried with paper towels and mounted upright using sticky wax onto a holder so that the coronal surface of the root could be photographed using a digital camera under fixed conditions (constant lighting, distance and exposure settings) together with a colour reference card and a calibration ruler, to obtain a baseline image as a reference point from which subsequent changes could be measured. The roots were assigned randomly assigned to 5 groups of 3 each. One served as an untreated control in which no medicament was placed. The four treatment groups were PEG 400 as a vehicle control, 20% calcium hydroxide in PEG 400, Odontocide and Calmix. The materials were injected into the root canal under positive pressure until excess was seen to extrude apically. After removing excess material on the

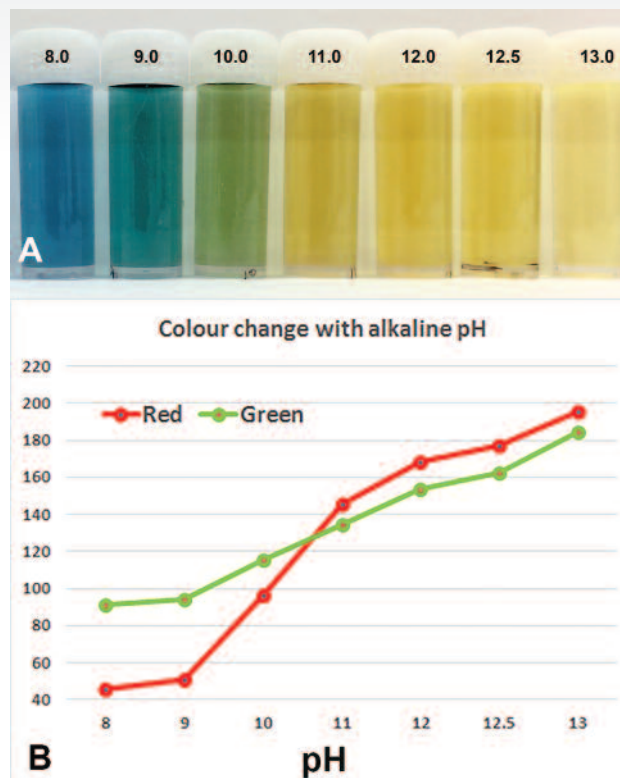


Figure 1: Colour changes in anthocyanin dye according to pH, using a series of buffers with pH values from 8 to 13. The upper panel (A) shows the visual appearance of a solution of anthocyanin dye as pH alters. Each vial has the same concentration of dye. The lower panel (B) shows the digital pixel data for red and green channels for the pH standards. There is a consistent increase in both parameters as the pH rises.

coronal and outer surface of each root, the apex was sealed with molten wax, and each root then placed into an individual Eppendorf tube which was filled with anthocyanin dye solution to a level just below the coronal surface of the tooth. The samples were then maintained at 37°C in an incubator for 3 weeks.

Digital photographs of the coronal surface of the roots were taken after 1, 2, 3, 5, 7, 14, and 21 days. Image analysis was undertaken using Adobe Photoshop™ CS6 Extended software to track changes in each root over time, and calculate the colour change in red and green channels from the baseline using a repeated measures assessment. The selected area of interest was located 500 microns from the root canal walls, as this was considered the greatest depth into the tubules that microorganisms would likely be found. The selected area (10 X 10 pixels) was identical on sequential images of each root. Pixel information was

Table 3. The pH of calcium hydroxide in PEG 400

Ca(OH) ₂ concentration	5%	10%	20 %	40%
Ionode electrode	12.918 d (0.115)	13.344 c (0.117)	13.938 b (0.066)	14.75 a (0.089)
Solvotrode electrode	13.260 d (0.083)	13.598 c (0.063)	14.298 b (0.078)	14.940 a (0.118)

Data show the mean and SD from five independent experiments. Letters indicate statistical groupings across each row, ranked from highest pH (a) to lowest pH (d).

collected using the histogram tool, which gave red and green values for the selected area on a scale with 256 points. The extent of change from baseline was calculated for the three replicates for each group, and analysed using GraphPad Prism™ software applying non-parametric analyses for repeated samples as well as intra-group comparisons.

Results

Ionic properties of solutions

When the pH of 5%, 10%, 20% and 40% solutions of calcium hydroxide in water or PEG400 was measured every 5 minutes for 2 hours using both a conventional electrode with KCl electrolyte (Ionode), and an electrode designed specifically for alkaline non- aqueous solvents (Solvotrode) containing TEABr in ethylene glycol, PEG 400 as a solvent for calcium hydroxide gave a higher pH than water. Water solutions gave pH values which reached a plateau at 12.7. For solutions of calcium hydroxide in PEG 400, the pH increased with the concentration of calcium hydroxide, and there was a significant positive association with concentration ($P<0.001$) (Table 3). With the Ionode electrode, the pH differential between 5% and 40% mixtures was 1.87, and 1.74 with the Solvotrode electrode. Differences in the readings between the two electrodes were consistent, and were statistically significant at each concentration ($P<0.02$). The Solvotrode electrode recorded higher pH values by 0.19-0.36 pH units for all mixtures. The non-aqueous electrode consistently recorded significantly higher pH values by 0.19-0.36 pH units for all mixtures and medicaments ($P<0.02$). pH increased by 1.74-1.87 pH units between the 5% and 40% calcium hydroxide mixtures in PEG 400 ($P<0.001$) with the KCl and TEABr electrodes respectively.

Ionic properties of medicaments

Medicaments based on PEG400 had a more alkaline pH (Table 4). Differences in the readings between the two electrodes were consistent, with the TEABr electrode always being higher by 0.05-0.16 pH units. The ranking of medicaments from most alkaline to least alkaline was Calmix, Odontocide, Pulpdent and then Calasept Plus, with both electrodes. Measured pH differences between materials were statistically significant ($P<0.001$). With the Ionode electrode, all measured pH differences between materials were statistically significant ($P<0.001$), except for Pulpdent versus Calasept Plus. In contrast, with the Solvotrode electrode, all differences between Calmix and other materials were statistically significant ($P<0.001$), but there was no significant difference between the other three pastes. Differences in the readings between the two electrodes were consistent, with the Solvotrode electrode always being higher by 0.05-0.16 pH units. Differences in the readings between the two electrodes were statistically significant for all materials ($P<0.04$) other than for Odontocide, where the trend was the same ($P=0.6065$).

The measured pH values for Odontocide, which contains 20% calcium hydroxide in PEG 400 as well as ibuprofen, were not significantly different from those of the 5% calcium hydroxide in PEG 400 mixture. In contrast, the pH values for Calmix, which contains 37.5% calcium hydroxide in PEG 400, were significantly higher than those obtained for 40% calcium hydroxide in PEG 400 ($P<0.05$).

Hydroxyl ion release into roots

Tracking the extent of colour change over 3 weeks in the stained roots revealed that there were no changes in untreated roots with open canals or in canals treated with

PEG 400 only (Fig. 2). There were obvious changes in the stained roots over the 3 week period of the experiment, with the characteristic change from a deep purple-blue baseline colour to a yellow colour for the dentine adjacent to the root canal. From gross observation, such changes were most apparent with Calmix (Fig. 3). Quantitative analysis of changes in colour revealed positive changes in both green and red pixel data at 500 microns from the canal wall with each of the three calcium hydroxide materials, indicating that elevations in the pH of the root dentine had occurred progressively over the first 7 days and had then begun to stabilize. The groups were ranked in terms of greatest change to least as Calmix > Odontocide > 20% calcium hydroxide in PEG. Differences between treatments were statistically significant at 7 days and beyond for green and red channel data, with Calmix superior to other groups. After 7 days, the colour change continued to rise slowly for Calmix but began to decline for Odontocide, indicative of buffering of hydroxyl ions by dentine.

Discussion

The results of this study provide several insights into the behaviour of calcium hydroxide in different solvents. The results of the first part of the study showed that compared to water, polyethylene glycol 400 when used as a solvent allows high pH values to be achieved, which are above

pH 12.4, the nominal limit for aqueous pastes. While PEG 400 was first used as a viscosity modifier in the endodontic paste Calen in the 1970's, its ability to alter hydroxyl ion release is a novel finding. The present results indicate that PEG 400 is a suitable solvent for calcium hydroxide. Chemically, this is due to the large number of ethylene oxide groups along its backbone, which allows PEG to form complexes with metal cations, including calcium ions.⁷ Such binding of calcium ions drives the dissociation of calcium hydroxide, thus releasing more free hydroxyl ions. This notion is supported by the quantitative results of the root dentine experiment, which showed greater levels of available ions at the same location in the dentine.

PEG 400 is a colourless water soluble and hygroscopic polymer that is miscible with water in all proportions, which explains why PEG 400 can be used as a solvent in its own right with or without the addition of water. PEG is classified as "Generally Recognized as Safe" and has high biocompatibility.⁷ It has a suitable viscosity for delivery through a fine tip. When used without any added water, as in the case of Calmix, the material will not dry out over time.

Calcium hydroxide pastes produce a high alkaline pH when measured directly with pH electrodes, as shown in the second part of this study, but under clinical conditions the released hydroxyl ions have to diffuse throughout the dentine, during which some buffering will occur from both inorganic and organic components. A saturated aqueous solution of

Table 4. The pH of commercial endodontic pastes measured using two different electrodes

	Pulpdent	Calasept Plus	Odontocide	Calmix
Ionode electrode	12.706 c (0.006)	12.662 c (0.017)	13.170 b (0.025)	14.996 a (0.010)
Solvotrode electrode	12.862 b (0.008)	12.710 b (0.012)	13.062 b (0.450)	15.162 a (0.108)
pH value stated by manufacturer	> 12.0	12.4	12.5	>13.5

Data show the mean and SD from five independent experiments. Letters indicate statistical groupings across each row, ranked from highest pH (a) to lowest pH (c). Information on product pH stated by the manufacturer was obtained from material safety data sheets and manufacturer websites.

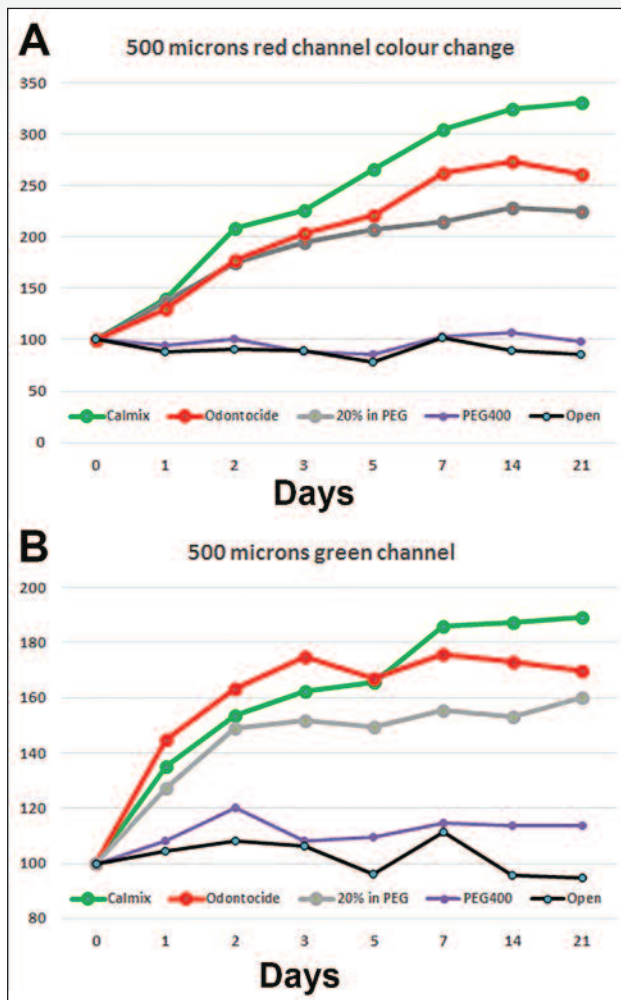


Figure 2: Quantitative assessment of changes from baseline in pixel data for the red (upper) and green (lower) channels for a sample area located 500 microns from the canal wall in the radicular dentine. The effects of two pastes containing PEG 400 (Calmix in green and Odontocide in red) are compared to 20% calcium hydroxide in PEG (grey). PEG 400 alone (purple) and an empty canal (black). Data are the mean of three independent experiments.

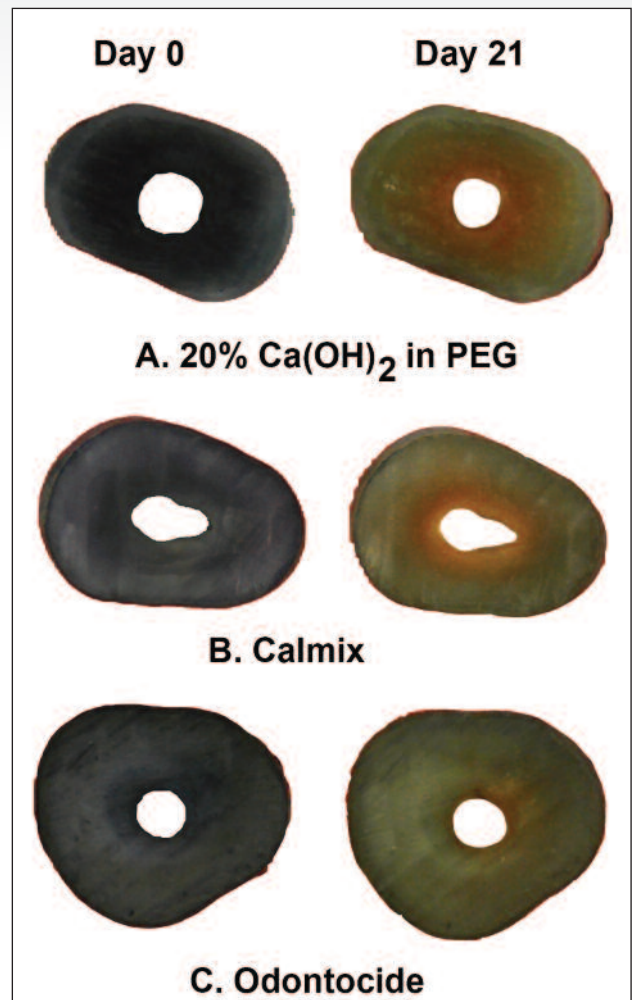


Figure 3: Typical examples of anthocyanin-stained roots showing colour changes due to hydroxyl ion release from medicaments over time, with the colour shifting from purple-blue to green and then yellow as pH rises from the canal walls through the dentine.

calcium hydroxide is easily buffered by dentine⁸ as has been seen when dentine powder is added to saturated calcium hydroxide solutions. This emphasizes the rationale behind third part of the study, which measured alkalinizing effects in radicular dentine using anthocyanin dye. This dye was chosen because colour changes correspond to pH changes in a predictable manner giving a gradual change between colours, from purple-blue to green and then to yellow as pH increases into the highly alkaline range.

The antimicrobial activity of calcium hydroxide is based

on effective release of hydroxide ions, which destroy phospholipids of microbial cell membranes as well as bacterial DNA^{9,10}. It is therefore important that hydroxide ions penetrate into the dentine of the root in sufficient concentration to overwhelm the buffering effects of dentine and cause a highly alkaline pH environment to be sustained both within the root canal system and the adjacent dentine, in order to suppress growth of microorganisms, particularly resistant species such as *Enterococcus faecalis*, which are able to survive and form biofilms in a pH¹⁰ environment. *E.*

faecalis is however inactivated by alkaline environments at pH values around 11.5 to 11.9^{11,12}. The rationale for using a non-water solvent such as PEG 400 is to help achieve a sufficiently high pH at the canal walls and into the inner radicular dentine to reach this objective.

Conclusions

While the concepts of ionic equilibria and pH in non-aqueous solvents have been well documented in the literature for many years¹³, the chemical properties of such materials as vehicles for calcium hydroxide in dentistry has not hitherto been explored. This study has shown that PEG 400 is a suitable vehicle for calcium hydroxide, and gives higher pH values than can be achieved in aqueous solutions. Commercial medicaments which use PEG without water show higher measured pH and greater pH change in radicular dentine than those which contain PEG mixed with water, or water alone. The results of the present study also show the potential of anthocyanin staining as a method which can show pH fluctuations in root dentine caused by endodontic medicaments. Such experimental approaches may be useful to inform the development of products which can achieve the desired pH changes in the inner dentine for effective disinfection.

Conflict of interest statement

Two of the authors (BA and LW) are co-inventors of Calmix paste.

References

1. Fava LR, Saunders WP. Calcium hydroxide pastes: classification and clinical indications. *Int Endod J*. 1999;32(4):257-82.
2. Athanassiadis B, Abbott PV, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. *Aust Dent J*. 2007;52(1 Suppl):S64-82.
3. Bates RG, Bower VE, Smith ER. Calcium hydroxide as a highly alkaline pH standard. *J Res Nat Bureau Stds* 1956;56(6):305-12.
4. Sweetman S (ed). *Martindale: the complete drug reference*. 36th edition. London: Pharmaceutical Press, 2009: p. 2272.
5. Tremillon B. *Chemistry in non-aqueous solvents*. Netherlands: Springer, 1974.
6. Quitmeyer J. pH Measurement in aqueous and non-aqueous solutions: When used in combination with titration, pH measurement is a simple method to monitor bath concentration. *Metal Finishing* 2008;106(10):21-24.
7. Chen J, Spear SK, Huddleston JG, Rogers RD. Polyethylene glycol and solutions of polyethylene glycol as green reaction media. *Green Chem* 2005;8:64-82.
8. Athanassiadis B, Abbott PV, George N, Walsh LJ. An vitro study of the inactivation by dentine of some endodontic medicaments and their bases. *Aust Dent J*. 2010;55(3):298-305.
9. Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. *Lab Invest* 1982; 47:412-26.
10. Ran S, Liu B, Jiang W, Sun Z, Liang J. Transcriptome analysis of *Enterococcus faecalis* in response to alkaline stress. *Front Microbiol*. 2015;6 (795):1-11.
11. Flahaut S, Hartke A, Giard JC, Auffray Y. Alkaline stress response in *Enterococcus faecalis*: adaptation, cross-protection, and changes in protein synthesis. *Appl Environ Microbiol* 1997;63:812-4.
12. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. *Int Endod J* 2002; 35:221-8.
13. Roses M. Ionic equilibria in non-aqueous solvents Part 1. General equations for calculation of pH, dissociation constants and reference potentials from potentiometric data. *Anal Chim Acta* 1993;276:211-21.