ISSN : 0974-7419 Volume 11 Issue 3

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 11(3) 2012 [98-104]

Calcium hydroxide diffusion associated with five different vehicles: A high performance liquid chromatographic study

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Received: 19th November, 2011 ; Accepted: 19th December, 2011

ABSTRACT

Objective:

An intracanal medicament is used to: (i) eliminate any remaining bacteria after canal instrumentation; (ii) reduce inflammation of periapical tissues and pulp remnants; (iii) render canal contents inert and neutralize tissue debris; (iv) act as a barrier against leakage from the temporary filling; (v) help to dry persistently wet canals.

This study was undertaken to evaluate and identify which of the different vehicles used to deliver Ca (OH) 2 was effective in releasing more calcium and hydroxyl ions with less of other components used providing maximum benefit to the patients.

Materials and Methods:

Using high performance liquid chromatography (HPLC), small amounts of liquid samples in which 25 premolar human teeth were immersed were evaluated. Calcium hydroxide was kept in differenct vehicles:

Group 1 Polyethylene glycol and Colophony;

Group 2 glycerin, camphorated para-monochlorophenol (PMCC), PMCC and camphor;

Group 3 PMCC; group

Group 4 glycerin Tricresol formal (TCF – 19% formaldehyde, 10% cresol);

Group 5 Anesthetic solution Lignox.

Five polyethylene tubes were filled with each of these pastes and placed unsealed in similar bottles. At the end of this period, HPLC analyses of the aqueous medium related to each group were performed to detect the diffusion pattern of Ca(OH) 2 along with other substances that had diffused from pastes used in the canals of the teeth other than calcium and hydroxyl ions.

Results: Although the groups presented different maximum peaks when there was no barrier, they all showed higher values than when the tooth was present. At least maximum number of substances other than Calcium and hydroxyl ions were detected in the group 4.

Conclusion: Considerable quantities of other components of the pastes used to deliver Ca (OH) 2 diffused through the dentine and reached the external root surface along with calcium and hydroxyl ions. Further studies will be needed to understand whether there are any adverse effects of such diffusing components on periodontium.

KEYWORDS

Intra canald dressing;
Calcium hydroxide;
Periodontium.

INTRODUCTION

One of the major goals of root canal treatment is the elimination of micro organisms from the root canal system, which is normally achieved by instrumentation combined with various irrigating solutions as well as antibacterial dressing of the canal during inter-appointments. "Complete elimination of microorganisms from within root canal system and its effective obscuration are considered to be the major factors in the success of endodontic treatment"[7].

Endodontic instruments are used to shape the root canal system, while irrigants are used to clean and disinfect the canals. Bacteria may still be detected in the root canal after chemo mechanical preparation. It has been observed that bacteria, which survive instrumentation and irrigation, rapidly increase in the number in the empty canals in the period between appointments[8].

The use of bacterial medicaments has been recommended between appointments during root canal treatment of non-vital teeth. Intracanal medication denotes the application of an antiseptic agent to the walls of the pulp cavity with the objective of eliminating micro organisms still present after chemo mechanical preparation[11]. A number of materials have been advocated as intracanal medicaments. These include combination of steroids and antibacterial agent's phenol derivates, chlorhexidrine depots, iodine potassium iodide and calcium hydroxide[4].

The vehicle plays a most important role in the overall process because it determines the velocity of ionic dissociation causing the paste to be solubilized and resorbed at various rates by the periapical tissues and from within the root canal. According to FAVA, the ideal vehicle should:

1. Allow a gradual and slow, Ca^{++} and OH^- ionic release.
2. Allow slow diffusion in the tissue with low solubility in tissue fluid.
3. Have no adverse effects on the induction of hard tissue deposition[3].

Lipid-soluble substances may have difficulty reaching targets at a distance in the tissues. Amphipathic drugs may have particular benefits; it may not be coincidental that aldehydes and phenol derivatives have had clinical success. Thus aqueous solutions of paramonochlorophenol may penetrate further and have greater antimicrobial activity than the more concentrated lipid solute. The low but significant solubility in water of calcium hydroxide has the dual advantage of limiting its toxic effects while the depot of the compound in suspension at the same time provides continuous release of the agent. Vaporizing agents have been advocated on the premise that the vapor would be more permeating than liquids[12].

Many vehicles like camphorated para monochlorophenol, glycerine, anesthetic agents, are used with $Ca(OH)_2$ as intracanal dressings, and these chemical associations supply to the external region of the root canal with Ca^{2+} and OH^- ions. However, it is not known which other substances from these associations can permeate the dentine and what effect they may have on the periodontal ligament. HPLC is used as an analytical tool to determine the permeation of substances from Ca $(OH)_2$ associated with five different vehicles Polyethylene glycol 400, Glycerin, Camphorated para monochlorophenol, Tricresol formal and Anesthetic Lignox.

This study was undertaken to evaluate and identify which of the different vehicles used to deliver Ca (OH) 2 was effective in providing maximum benefit to the patients.

MATERIALS AND METHODS

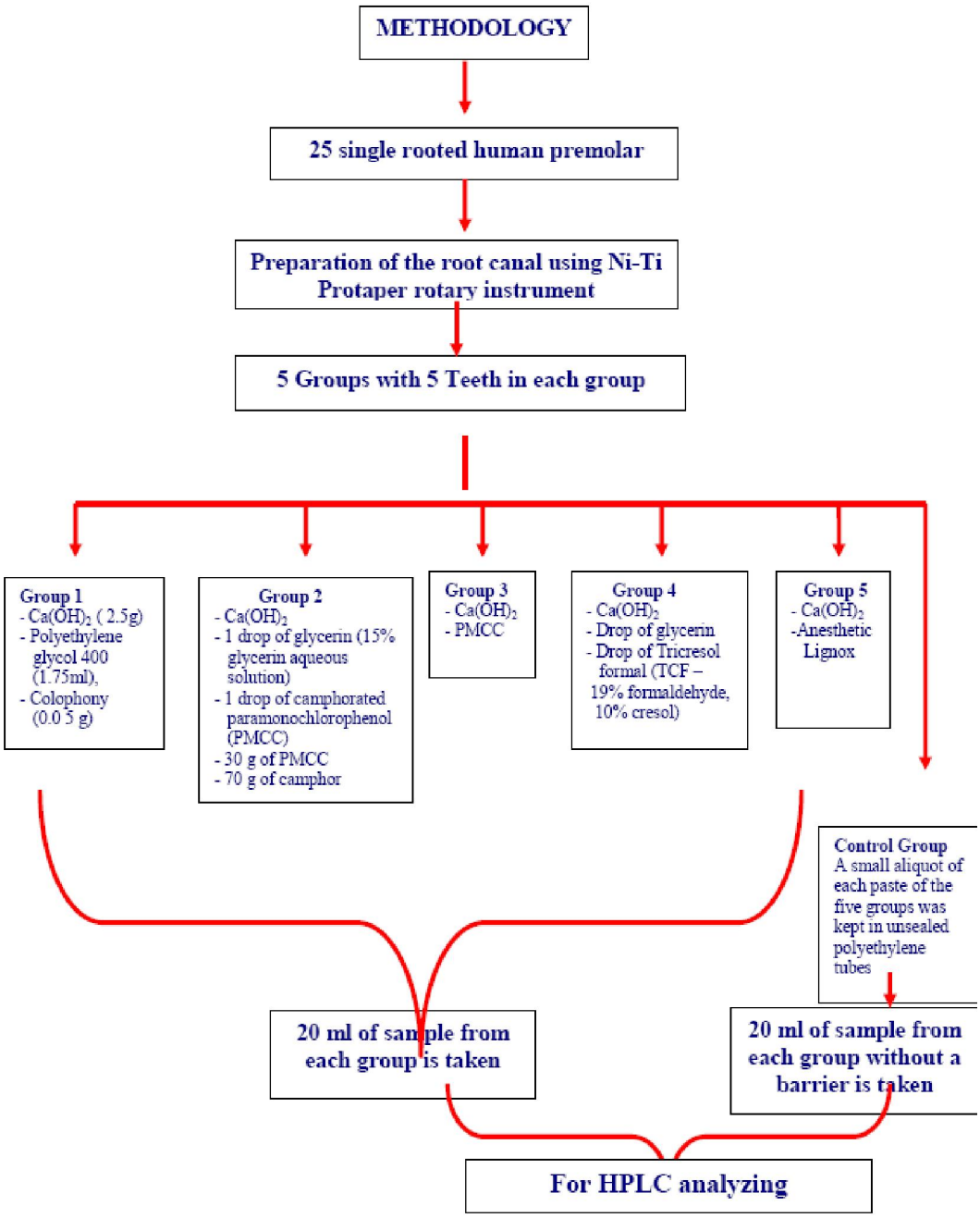
25 single rooted human premolar teeth, free of fractures and caries were preserved in 0.5% thymol aqueous solution before the experiment.

Conventional endodontic access cavities were done with high speed hand piece in all 25 teeth. If the initial instrumentation to the apical foramen could not be performed with a size 10 k-file then teeth were excluded from the study. Pulp extirpation was done with barbed broaches. A small amount of wax was placed on the tip of the each root to prevent irrigating solution from passing through the apical foramen.

Canal instrumentation

Cleaning and shaping of the canal were performed with Ni – Ti rotary protaper instruments.

The root canals were irrigated with sodium hypochlorite between each procedure and kept flooded



during instrumentation phase. All the teeth were irrigated with a combination of 17% EDTA and 3% NaOCl. First, 3 ml of 17% EDTA was irrigated for a period of 1 min followed by 3 ml of 3% NaOCl for a period of 2 min. A 27-gauge blunt tip needle was used for irrigation in a corono-apical direction.

Then apically all 25 teeth were sealed with Araldite and the canals are filled with Ca $(OH)_2$ (Extra Pure) mixed with 5 vehicles using lentulo spiral:

Group 1

All the 5 teeth in this group were coated with Ca $(OH)_2$ mixed with the vehicle Polyethylene glycol 400 (1.75 ml), Colophony (0.0 5 g).

Group 2

All the 5 teeth in this group were coated with Ca $(OH)_2$ l mixed with the following vehicles 1 drop of glycerin (15% glycerin aqueous solution), 1 drop of camphorated para-monochlorophenol (PMCC), 30 g of PMCC and 70 g of camphor.

Group 3

All the 5 teeth in this group were coated with Ca

$(OH)_2$ mixed with the vehicle PMCC.

Group 4

All the 5 teeth in this group were coated with $Ca(OH)_2$ l mixed with the following vehicles 1 drop of glycerin and 1 drop of Tricresol formal (TCF – 19% formaldehyde, 10% cresol)

Group 5

All the 5 teeth in this group were coated with Ca $(OH)_2$ mixed with the vehicle Anesthetic solution Lignox (Lignocine hydrochloride 24.64mg, Adrenaline 0.0125mg; Excipients: Methylparaben 1mg and Water).

A small aliquot of each paste of the five groups were placed individually in 5 polyethylene tubes.

Then all the 25 teeth were subsequently sealed cervically with a cotton pellet in the pulp chamber and Araldite. The teeth were then placed individually in 25 individual glass bottle containing 800ml of ultra-pure deionized water. The bottles were kept in an Incubator at a temperature of 37 ± 2^0 C throughout experiment for 70 days.

A small aliquot of each paste that was placed in 5 polyethylene tubes were placed in bottles containing 800 ml of ultra-pure deionized water and incubated with the other 25 bottles.

Sample preparation

The teeth were removed from the flasks and examined to verify if the apical and cervical sealings were intact. Twenty milliliters were removed from each group (4ml per flask), 70 days after filling the canals for chromatographic analyses.

In this phase of the experiment, each group of the five groups was analyzed along control samples incubated without the teeth. 20 ml of solution from each flask containing unsealed polyethylene tubes was also removed. All solutions were filtered in a disposable, 0.45 μm pore filter to remove solid impurities.

Methodology for measurement of HPLC

A Schmidazu 10AVP with 254-nm UV-visible detector, 10 μl loop with a 1nm/min constant flow was used. A C-18 stationary column was used. The mobile stage contained 50% acetonitrile and 50% water. The solution was filtered and placed in a degassing ultra-

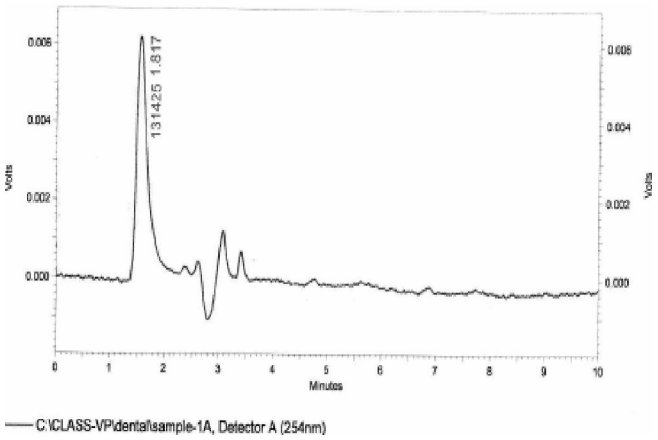


Figure 1 : HPLC graph of the open polyethylene tube with Group 1. There is a chromatographic peak area higher than the same group with teeth reaching at 131425 at 1.817 min, because of the absence of a diffusion barrier.

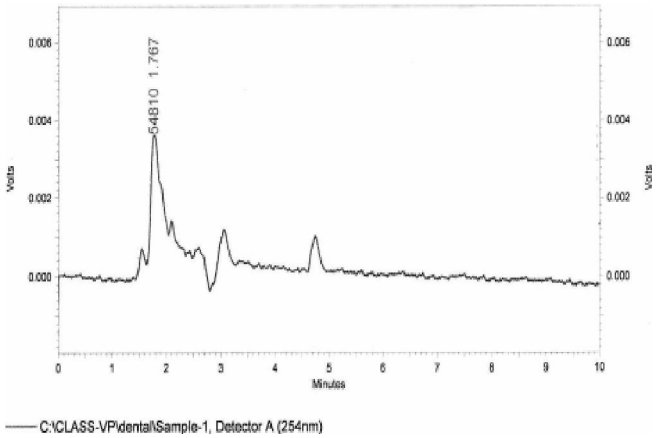


Figure 2 : HPLC graph of Group 1 in which the sealed teeth were immersed. There was only one significant chromatographic peak area 54810 at 1.767, which was inferior to the peak area of the polyethylene tube of group 1 because of the diffusion barrier effect of the tooth.

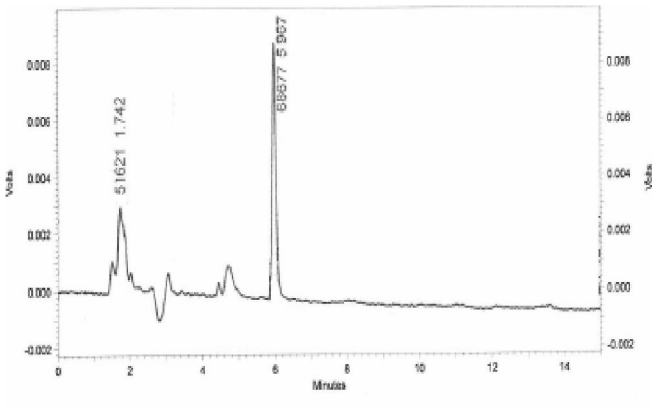


Figure 3 : HPLC graph of the open polyethylene tube with Ca (OH) 2, glycerin and PMCC (Group 2). There are chromatographic peak areas higher at 51621 at 1.742 and 68677 at 5.967 than the same group with teeth acting as a diffusion barrier.

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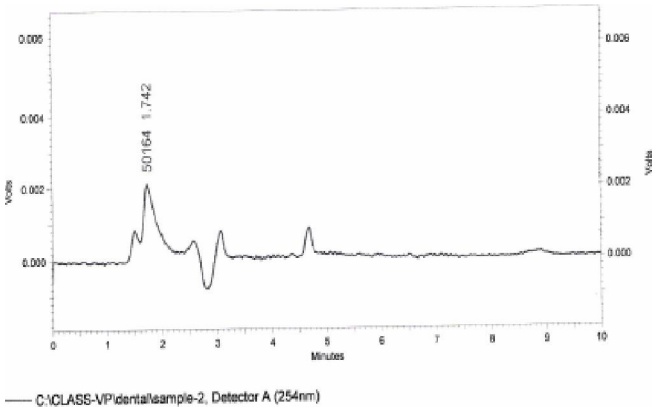

Figure 4 : HPLC graph of Group 2 in which the sealed teeth were immersed. There was only one significant chromatographic peak area 50164 at 1.742 min, which was inferior to the peak area of the open polyethylene tube of group 2 because of the diffusion barrier effect of the tooth.

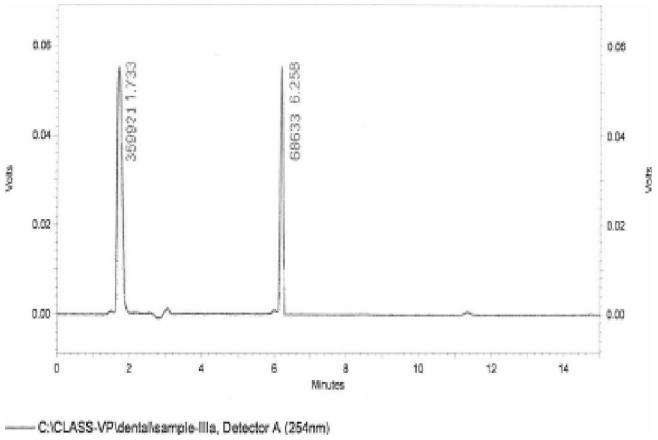

Figure 5 : HPLC graph of the open polyethylene tube with Ca (OH) 2 and PMCC (Group 3). There are chromatographic peak areas higher (359921 at 1.733 min and 68633 at 6.258 min) than the same group with teeth acting as a diffusion barrier.

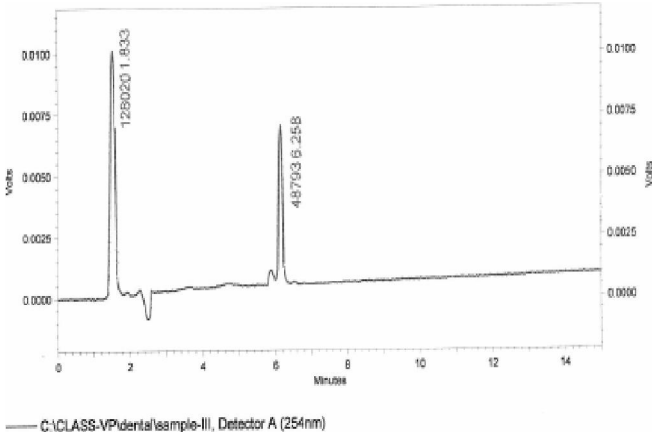

Figure 6 : HPLC graph of Group 3 in which the sealed teeth were immersed. The chromatographic peak areas (128020 at 1.833 min and 48793 at 6.258 min) were inferior to the peak areas of the open polyethylene tube of group 3 because of the diffusion barrier effect of the tooth.

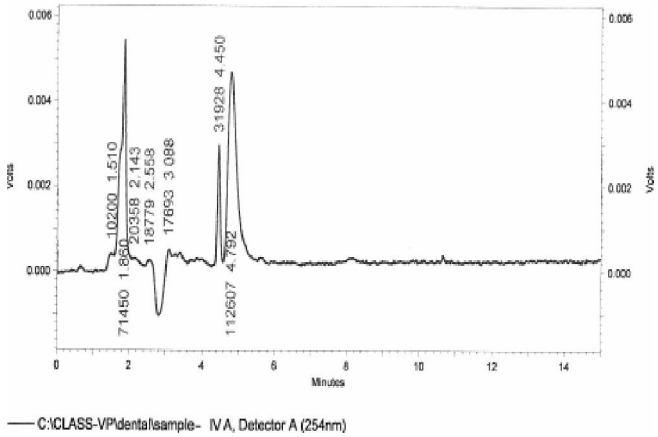

Figure 7 : HPLC graph of the open polyethylene tube with Ca (OH) 2, glycerin and TCF (Group 4). There are various chromatographic peak areas higher than the same group with teeth acting as a diffusion barrier.

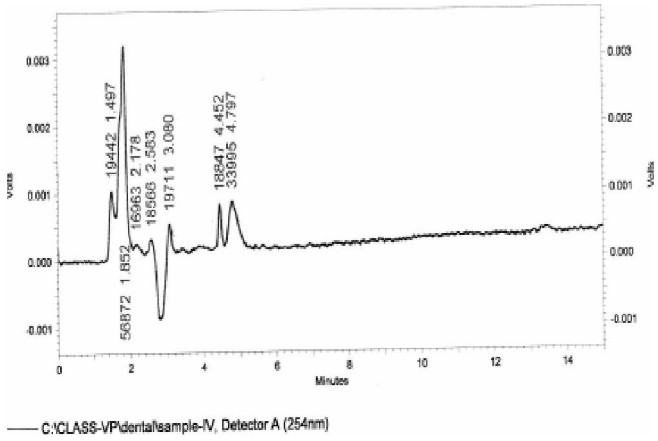

Figure 8 : HPLC graph of Group 4 in which the sealed teeth were immersed. There were various chromatographic peaks lower than those of the open polyethylene tube of Group 4 because of the diffusion barrier effect of the tooth.

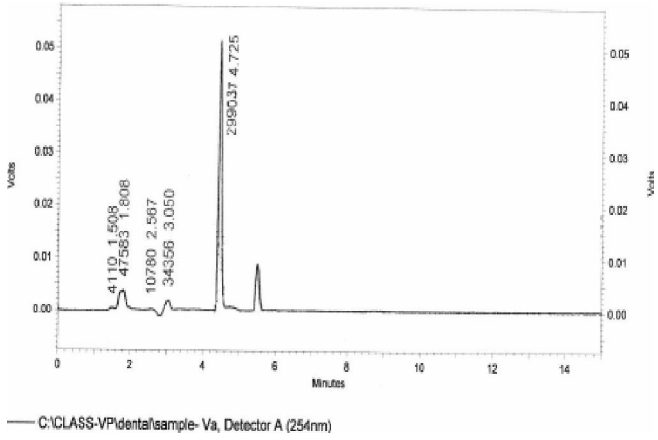

Figure 9 : HPLC graph of the open polyethylene tube with Ca (OH) 2 and Lignox (Group 5). There are various chromatographic peak areas higher than the same Group with teeth acting as a diffusion barrier.

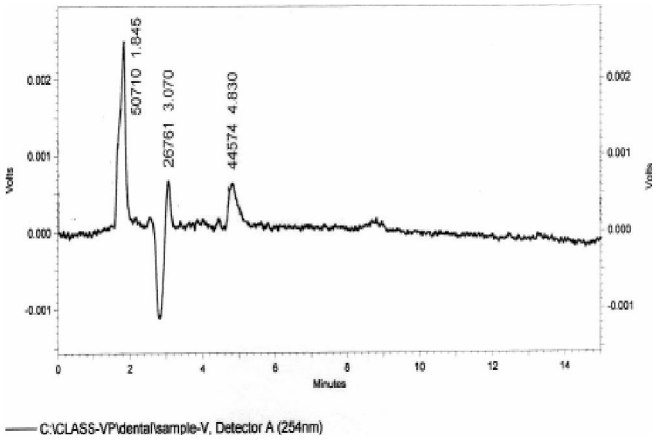

Figure 10 : HPLC graph of Group 5 in which the sealed teeth were immersed. There were various chromatographic peak areas much lower than those of the polyethylene tube of Group 4 because of the diffusion barrier effect of the teeth.

sound unit for 15 minutes.

For sample injection, a gas tight 710/20-µl Hamilton syringe was washed three times with acetonitrile and three times with sample. The loop washed with 3 times with 20 µl of acetonitrile and 3 times with sample. 20 ml of the solution was injected and chromatograph to be observed for 15 minutes. The syringe was cleaned between each sample, and the results and chromatography graphs were recorded using a computer.

RESULTS

The chromatography graphs showed peak areas, and higher the peak area the greater the substance concentration. The maximum peak areas registered retention times in minutes and showed when the sample started to be analyzed. The ordinate axis of the chromatograph is in arbitrary units (volts).

DISCUSSION

Within the limits of the study it is not possible to identify which ions have permeated, so further studies with other vehicles should be undertaken for studying the permeability of ions with or without the barrier.

The results of the present study indicate that the sample of Group 4 with sealed teeth showed substances clearly diffused more than in other groups. The reason for the substances to diffuse much more than others might be that the vehicle used had a vaporizing agent indicating the vapors would be more permeating than liquids[12]. The drug is obviously more effective when deliberately confined to a small space. This could be due to formocresol having dual effects- the production of fumes and coagulation of proteins[10]. Formaldehyde is a hapten (an incomplete allergen) that must undergo a chemical reaction with another substance (mainly proteins)[6].

Diffusion of formocresol may be more in younger patients than in elder patients. Dentinal tubules, lateral canals, ramifications and the apical foramen are smaller in elder patients than younger patients[9].

TCF is frequently used as an intracanal dressing because of its strong bactericide effect[2]. The use of TCF is questionable because of its immunogenic, carcinogenic, toxic, and mutagenic effects because once placed in the root canal it is absorbed systemically[5]. Thus, its use is not indicated in teeth with periodontal disease[1, 2].

The results of the present study indicates that the tooth can act as a barrier for medication diffusion and does not block it completely, because not only Ca^{2+} and OH^{-} [13]can diffuse through dentine and reach the external root surface but other components of the vehicle used can also diffuse and reach the external surface. Each medicament presents different diffusion characteristics, which will be directly related to its interaction with the tooth structure. Thus, further research is needed to determine which components of vehicles permeate the dentine, in what quantity they permeate, and whether these components have any effect on the periodontium.

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