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Release of Formaldehyde from Endodontic Sealers

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Periapical alterations or irritations resulting from root canal therapy may be caused by over-instrumentation, infection or adverse effects from substances liberated from root canal filling materials. Chemotherapeutic agents containing formaldehyde are commonly used in the treatment of dental disease. Formaldehyde is known to local irritation and skin sensitization following acute and subacute exposure. The objective of this study was to evaluate AH 26, endomethasone, N2, AH plus, diaket, forfenan and roeko seal automix (RSA) for the presence of the formaldehyde. Sealer's powder and liquid were analyzed before and after mixing formaldehyde analysis was done using high-performance liquid chromatography with thermo-separation products spectra system and photodiode assay. Analysis showed that the AH 26, endomethasone, N2 powders and forfenan liquid contained formaldehyde and and their mixture were releasing formaldehyde. It could not decetcted any formaldehyde in AH plus, diaket and RSA samples. Because endodontic sealers can get into contact with surrounding soft and hard tissues, they should have an acceptable biocompatibility. Sealers with inferior biocompatibility, such as formaldehyde-releasing materials, should no longer be applicable in practice because safer alternatives are available.

Key Words: Formaldehyde, HPLC analysis, Root canal sealers.

INTRODUCTION

There is growing concern about environmental health hazards to man. Within dentistry, this concern is twofold: the first concern is the long-and short-term effects of the medication on the patient and the second concern is the effects of the medication on the dentists and auxiliary personnel exposed to it. It is difficult to test formaldehyde in the gasseous phase and assess its long-term damage. However, effective techniques are available[1]. There is a need for investigation of formaldehyde in the vapour phase as this shows how the patient and dentist are exposed[1].

In terms of serious risks, formaldehyde poses problems to systemic health *via* ingestion routes, interaction in air with other aldehydes (outside the dental office, as in car exhaust) and in final breakdown products of formalin in the body[1].

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Dental materials, especially root canal filling materials, usually remain in close contact with living periapical tissues over a long period of time. Various studies have revealed that elutable substances or degradation or corrosion products from root canal fillings may gain access to surrounding tissues (periodontal ligament alveolar bone) through numerous connections, *e.g.*, dentinal tubules, accessory and lateral canals and apical foramina[2,3]. The ideal root canal sealer should have excellent physicochemical properties and biological compatibility[4]. Many parameters characterize the biocompatibility of an endodontic material such as genotoxicity/mutagenicity/carcinogenicity, cytotoxicity, histocompatibility or microbial effects. Today, numerous root canal sealers are available, based on various formulae and containing a variety of different and partly mutagenic components[5].

Chemotherapeutic agents containing formaldehyde are commonly used in the treatment of dental disease. The presence of formaldehyde in some endodontic sealers and its release after manipulation have been studied[6-8].

Much research has been conducted on mutagenic[9-11], genotoxic[12-14], cytotoxic[15-19] and alergic[20] effects of formaldehyde released from chemicals used in endodontics. It has been shown that these chemicals slowed down apical treatment[21] and spread formaldehyde into system after endodontic applications[22-26].

The objective of this study was to evaluate AH 26, endomethasone, N2, AH plus, diaket, forfenan and roeko seal automix (RSA) for the presence of the formaldehyde. In this study, we made the chemical analyses of 6 root canal filling materials of a different chemical structure used in endodonti to find out if there is formaldehyde in their structures. In this study, we aimed to draw the dentists' attention to the possible toxicity of formaldehyde, thus warn them about their choice of this material. Formaldehyde has a known toxic mutagenic and carcinogenic potential. There is a need to revaluate the use of formaldehyde in dentistry.

EXPERIMENTAL

Table-1 shows the root canal sealers included in this study. Sealers were mixed in accordance with the manufacturers' instructions.

TABLE-1
SEALER INCLUDED IN THIS STUDY

Type of sealer (chemical group)	Sealer	Manufacturer
Epoxy resins	AH 26	Dentsply, DeTrey GmbH, Konstanz, Germany
Epoxy resins	AH Plus	Dentsply DeTrey GmbH D-78467, Konstanz, Germany
Silicone	RSA	Lot #2110841; Roeko, Langenau, Germany
Polyketone	Diaket	3M ESPE AG, Seefeld, Germany
Zinc oxide-eugenol	Endométhasone	Septodont, Paris, France
Zinc oxide-eugenol	N2	Dr Sargenti
Polimethilciloxan	Forfénan	Septodont, St-Maur, France

Preparation of 2,4-dinitrophenylhydrozine (DNPH) derivatization solution: 1 mL H_3PO_4 was added into 0.14 g 2,4-DNPH and then solubilized in asetonitrile and volume adjusted to 100 mL adding acetonitrile.

2,4-Dinitrophenylhydrazone derivatization[27,28]: Common methods for formaldehyde determination are based on hydrazone formation with such as 2,4-dinitrophenylhydrazine (DNPH), chromotropic acid, 2,4-pentanedione or dimedone, giving chromophores (1). A frequently used method is derivatization with DNPH and subsequent analysis with HPLC (2). DNPH is used due to its rapid reaction with formaldehyde and the stability of the formaldehyde 2,4-dinitrophenylhydrazone (further designated formaldehyde hydrazone) is thus formed.

The formaldehyde measurement is based on the 2,4-dinitrophenylhydrazine (2,4-DNPH) derivatization technique which consists of two basic steps: (1) Enrichment of the aldehydes by derivatization with 2,4-DNPH, reacting to the specific 2,4-dinitrophenylhydrazones (2,4-DNPH). (2) Analysis of the 2,4-dinitrophenylhydrazone by high performance liquid chromatography (HPLC).

Preparation of standards and 2,4-DNPH derivatization: Formaldehyde (37 %) solution was diluted in distiled water and acetonitrile, with ratio of 1:1 then 12:5, 25, 50 and 100 ppm of standard formaldehyde solusions were prepared. Each of prepared standard solutions was mixed with solution of 2,4-DNPH derivatives (1:1 v/v) and shaken 5 min, filtered with 0.45 µL disposable filter and then taken into vial.

Preparation of samples and 2,4-DNPH: 0.2 g of pasta, powders and their mixture (1:1) from each samples were taken and 20 mL acetonitrile:distilled water (1:1) was added to each. Then, prepared samples (1/100) stored overnight at 37 ºC. Each of prepared standard solutions was mixed with solution of 2,4-DNPH derivatives (1:1) and shaken 5 min, filtered with 0.45 µL disposable filter and then taken into vial. On the other hand, paste and powder of forfénan were diluted 1/1000 ratio only to provide required standardization.

Roeko seal automix (RSA) was not dissolved in solvents used for other samples. Therefore, Kloroform was used to dissolve RSA and formaldehyde was not determined.

HPLC analysis formaldehyde analysis was performed by the use of thermoseparation products spectra system HPLC and photodiode assay (TSP UV 6000 LP). Standard and samples were run into Luna C18 colum (Phenomenex) 50:50 acetonitrile:distilled water mobile phase for 1 mL/min speed and determined at 365 nm.

RESULTS AND DISCUSSION

To determine formaldehyde with HPLC, it is necessary to form the derivative with 2,4-DNPH. In formaldehyde condition, after derivatization of 2,4-DNPH, peak given by residual 2,4-DNPH was seen 6.1 min later. Because the amount of residual 2,4-DNPH after derivatization was decreasing with inceasing the amount

of formaldehyde, 2,4-DNPH peak got smaller with increasing the amount of formaldehyde. Peaks of formaldehyde (10 ppm) standard and residual 2,4-DNPH after derivatization were shown in chromatogram (Fig. 1).

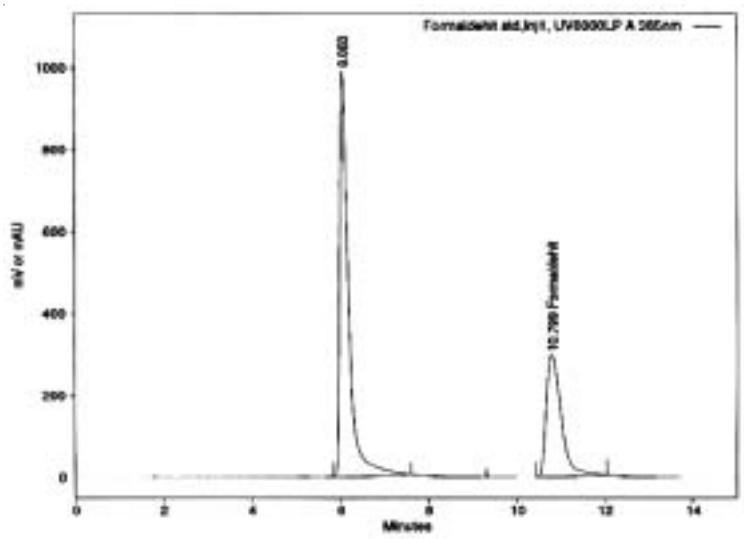

Fig. 1. HPLC chromatogram of formaldehyde derived with 2,4-DNPH

A 0.40 % formaldehyde was determined in N2 powder, however, it was not detected in N2 liquid formaldehyde. When N2 powder was mixed with equal amount of N2 Liquid, a half of the amount of formaldehyde in N2 powder was determined (Fig. 2).

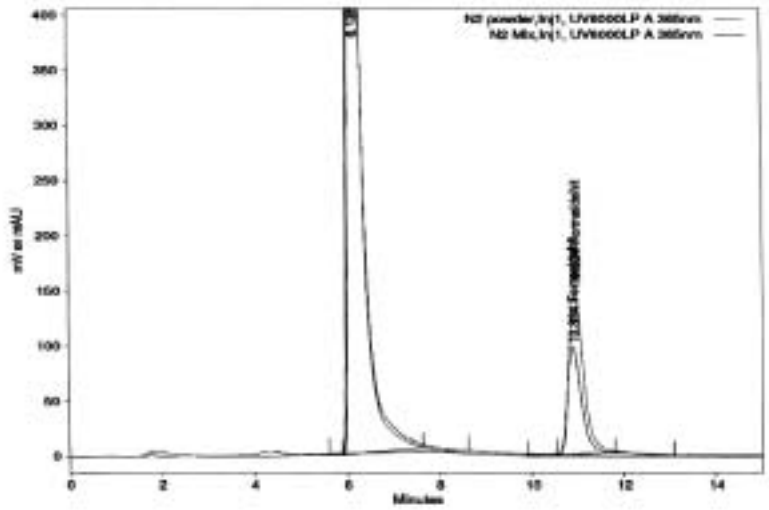

Fig. 2. HPLC chromatogram of N2 powder and mixture

While a 1.50 % formaldehyde was found in AH 26 solid, formaldehyde was not determined in AH 26 liquid. However; when AH 26 solid and liquid were mixed in equal amount, high amount of formaldehyde (3.23 %) was determined compared to AH 26 solid (Fig. 3).

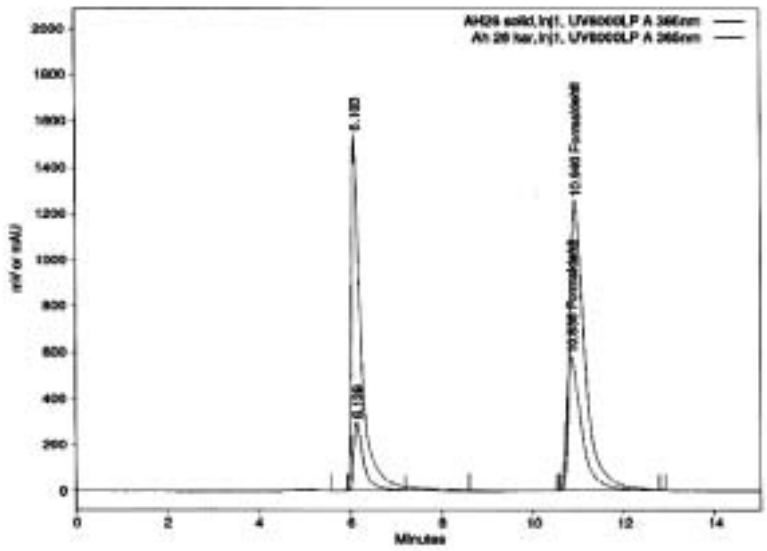

Fig. 3. HPLC chromatogram of AH26 powder and mixture

32 % Formaldehyde was found in forfenan liquid. On the other hand, formaldehyde was not detected in Forfenan powder and hardening agent (Fig. 4).

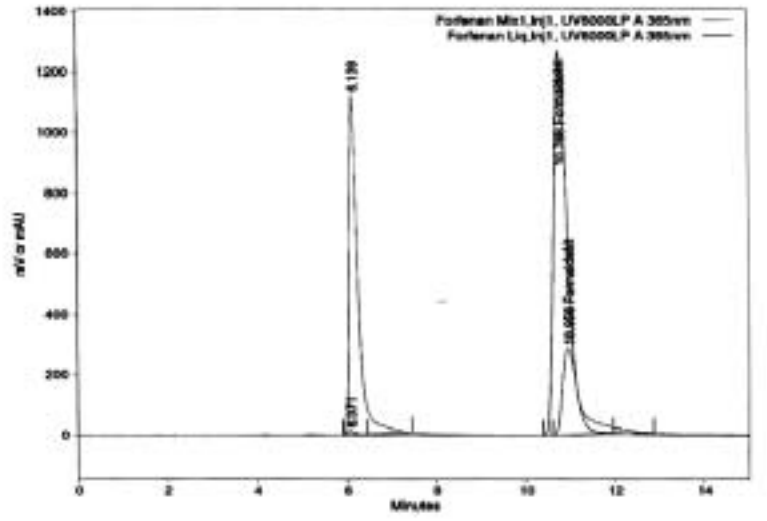

Fig. 4. HPLC chromatogram forfenan liquid and mixture

It was determined that a 2.36 % formaldehyde was found in endomethasone powder, but it was not exist in endomethasone liquid. However; when endomethasone powder and liquid were mixed in equal amount, the amount of formaldehyde was decreased to the half (1.38 %) of the amount determined in formaldehyde powder (Fig. 5).

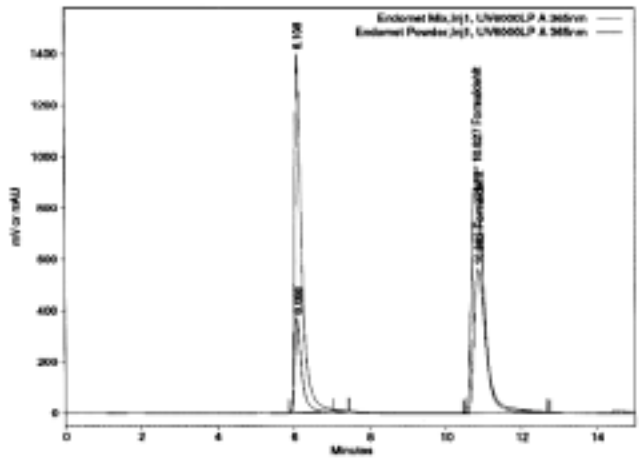

Fig. 5. HPLC chromatogram of endomethasone powder and mixture

In this study, it was shown that AH plus, diaket abd RSA materials and their mixtures did not release formaldehyde.

Since most dental materials release small amounts of various substances into their physiological environment, the potential genotoxicity, mutagenicity and carcinogenicity of dental materials must be determined[29]. In endodontics, formaldehyde can diffused out the apical opening and lateral canals and can injure the periodontal ligament and surrounding tissues.

In this study, AH 26, endomethasone, forfenan, AH plus, diaket, RSA and N2 were investigated for formaldehyde release. All of the materials used in this study were prepared according to producer companies procedures.

This study indicated that AH 26 powder contains formaldehyde and AH 26 mixture released formaldehyde. Similar results have been reported previously[6-8]. The powder of AH 26 contains hexamethylenetetramine (HMT), which is synthesized from formaldehyde and ammonia. Hexamethylenetetramine also decomposes in acid environment, yielding ammonia and formaldehyde. Such decomposition can also occur in water solution. The formation of formaldehyde from this sealer is attributed to the chemical reaction that occurs between bisphenol A resin and HMT[6]. Even though present study indicated that AH 26 liquid did not contain formaldehyde. It could cause more formaldehyde release from AH 26 mixture compared to AH 26 powder. Previous studies reported that N2 released higher amount of formaldehyde[6,8], which support present findings.

It has been shown that endomethasone powder used in this study included formaldehyde and endomethasone mixture released formaldehyde. Leonardo *et al.*[7] reported that endomethasone released formaldehyde. Based on present results, forfenan solusion contained formaldehyde and its mixture released formaldehyde. Manufacturer also reported that forfenan solution contains formaldehyde.

According to the manufacturers, AH plus is biocompatible and does not release formaldehyde and similar results were obtained in this study. However; Leonardo *et al.*[7] found that AH plus released formaldehyde in minimal concentration. The present study showed that diaket and RSA did not release formaldehyde.

Humans beings exposed to formaldehyde through dermal contact, inhalation, ingestion or dental absorption. When considering the high rate of exposure and tolerance of mammals to formaldehyde, the added formaldehyde load of some milligrams of formaldehyde in a root canal sealer is negligible from a toxicological point of view. Therefore, the undesirable effect of formaldehyde in an endodontic sealer should not be discussed as a general toxicity problem as this low exposure to formaldehyde is rather insignificant[6]. Schwarse *et al.*[30] indicated that the amount of sealer used in root canal filling is less than the amount of sealer used in *in vitro* studies, therefore cytotoxic component consantration is less than that *in vitro* studies. In addition, they have indicated that because of the higher amount of sealer usage, the usage of N2 with single cone technique is more cytotoxic than the usage of N2 with lateral condensation technique. *In vivo*, however, direct contact between a root canal filling and the surrounding tissues or indirect interactions due to diffusion or perfusion are limited to apical foramina, accessory canals or dentin tubules. Thus, the quantity of leachable substances is significantly reduced *in vivo* compared to *in vitro* assays. However; Myers *et al.*[31] conducted a study on dogs and they reported that formocresol absorbed from pulpotomy area caused changes in tissue chracteristics of kidney and liver.

It can easily be argued that dental absorption amounts are negligible, but it must strongly be emphasized that the possible interaction with other mutagenetic substances-in the air, clothes textiles and make up always present in environment[32].

Due to extremely serious and life-threatening consequences, mutagenicity and carcinogenicity are gaining increasing public interest. There is only scanty information about the mutagenicity of endodontic filling materials[33]. Because formaldehyde has mutagenetic and carcinogenetic properties, its continued use in dentistry has been questioned[32] and formaldehyde-releasing root canal sealers are no longer recommended[14].

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