
Formaldehyde release from root-canal sealers: influence of method

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Abstract

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Aim To examine formaldehyde release from three different root-canal sealers: one phenol resin, one epoxy resin and one paraformaldehyde containing zinc oxide eugenol cement.

Methodology Formaldehyde was measured from freshly mixed material and from specimens allowed to set for 2 days or 2 weeks. Different mixing ratios and sample sizes were tested. Analysis was performed by reacting formaldehyde with acetylacetone and ammonia to form diacetyldihydrolutidine, which was revealed by spectrophotometry.

Results Analysis revealed that mixing ratio, time after mixing and surface:weight ratios of specimens had different influences on the formaldehyde release from different materials.

Conclusions From the results of this study it is difficult to define a standardized in-vitro test that can be applied universally for the definitive determination of formaldehyde release from endodontic sealers. Prediction of in-vivo formaldehyde release from endodontic sealers is difficult.

Keywords: analysis, formaldehyde, methenamin, paraformaldehyde, root canal sealer.

Introduction

Formaldehyde release from endodontic materials has been known for many years. Formaldehyde is reputed to act as a disinfectant. For this reason materials such as N2 (Indrag-Agsa, Bologna, Italy) and Riebler's paste (Amubarut; Wera Karl, Biesingen, Germany) contain paraformaldehyde (van der Burgt *et al.* 1986). Additionally, the setting reaction of Riebler's paste is a polycondensation of a phenol derivative (resocin) and formaldehyde (which is added as a 25% aqueous solution).

In the original publication of the AH26 method, this substance was also purported to contain a disinfectant (Schröder 1954). The disinfective agent in AH26 is methenamin, which is hydrolysed to ammonia and formaldehyde. However, the efficacy of long-term disinfection

by formaldehyde released from a root canal sealer appears to be low (Broisman *et al.* 1978, Tronstad *et al.* 1985). Moreover, there are case reports of adverse reactions such as paraesthesia of the inferior alveolar nerve attributed to the formaldehyde release from root canal sealers (Orlay 1966, Grossman 1978, Grossman & Tatoi 1978, Kaufman & Rosenberg 1980, Ørstavik *et al.* 1983, Allard 1986, Erisen *et al.* 1989, Gumru & Yalcin 1991). In addition, the systemic distribution of formaldehyde from different endodontic materials and medicaments has been shown in several studies (Myers *et al.* 1978, Block *et al.* 1980, Pashley *et al.* 1980, Wemes *et al.* 1982, Block *et al.* 1983, Hata *et al.* 1989, Araki *et al.* 1993). The potential for adverse systemic effects exist regarding the use of formaldehyde (Block *et al.* 1978). Following root canal treatment, there have been cases of allergy on account of systemic exposure to formaldehyde (Burri & Wüthrich 1985, Forman & Ord 1986, Fehr *et al.* 1992). Other effects of formaldehyde are carcinogenicity (Svenberg *et al.* 1980), or mutageni-

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city (Goldmacher & Thilly 1983). These effects have not been attributed to formaldehyde released from endodontic materials (Lewis & Chestner 1981, Ranly & García-Godoy 1991).

To elucidate possible risks, the *in vitro* release of formaldehyde from root canal sealers should be investigated. However, as the available materials show differences in their chemical and physical properties, the methods used might influence the results of such studies.

In this study three different materials (with different physico-chemical properties) were prepared and stored under different conditions, to establish subsequent formaldehyde release.

Materials and methods

Materials

One root canal sealer was epoxy resin-based (AH26, batch no. 930427; DeTrey/Dentsply, Konstanz, Germany), one was a phenol resin (Amubarut, batch no. 9891; Wera Karl), and one was a zinc oxide eugenol based cement, which contained paraformaldehyde (N2, batch no. 12010297; Indrag-Agsa).

Mixture and storage

An amount of approximately 0.5 g liquid (paste) was weighed out, then the powder was added up to the intended ratio (to an accuracy of 0.001 g). Care was

taken not to mix liquid A and B of Amubarut before weighing the powder.

The material was mixed for 60 s and then placed in a polypropylene vessel (8 mm in diameter and 5 mm deep). The weight of the mixed material was recorded. For each test, 10 samples were mixed.

Each material was analysed after being freshly mixed, 48 h after mixing, and 2 weeks after mixing. For each experiment new material was mixed to a 'medium viscosity' of 1:1 (weight of powder:weight of liquid) (for AH26 and Amubarut) and 1:2 (N2).

Two additional powder:liquid ratios were used as shown in Table 1.

Surface:weight ratio

In addition to the specimen size described above, one group was prepared with a small amount of material (approximately 25 mg) that just covered the bottom of the polypropylene vessel, resulting in a comparable surface (see Table 1). One group was prepared with a different polypropylene vessel that gave a free surface of approximately 0.5 cm^2 and a decreased surface:weight ratio (see Table 1).

Formaldehyde sampling and analysis

Specimens were immersed in 30 mL distilled water at 37°C for 12 h. As there is no standardized method for

Table 1 Methods used in this study

Variables	Method
Mixing ratio	AH 26: (powder:paste) 1:2, 1:1, 2:1 Amubarut (powder:liquids) 1:2, 1:1, 2:1 N2 (powder:liquid) 1:3, 1:2, 1:1
Setting (data available for median surface/volume ratio and median mixing ratio)	for each material: immersion of specimens of freshly mixed material, after 48 h of setting, after 2 weeks of setting
Surface/weight ratio (for this examination, freshly mixed materials with a median powder/liquid ratio were used)	Amubarut 0.50 cm^2 for approximately 0.44 g 0.50 cm^2 for 0.037–0.040 g 0.55 cm^2 for 4.10 g AH 26: 0.50 cm^2 for approximately 0.50 g 0.50 cm^2 for 0.043–0.050 g 0.55 cm^2 for 4.70 g N2: 0.50 cm^2 for approximately 0.44 g 0.50 cm^2 for 0.037–0.040 g 0.55 cm^2 for 4.10 g

analysis of formaldehyde release from dental materials, the formaldehyde concentration of the water was analysed by the Hantzsch reaction, and spectrophotometry at 412 nm (Fregert *et al.* 1984, Deutsches Institut für Normung 1988). Before analysis, the absorbance of each sample was tested to exclude a non-specific staining.

Formaldehyde was processed with ammonia and acetylacetone to diacetyldihydrolutidine. Calibration solutions were prepared after analysis of the stock solution by iodometric titration. As negative controls, one solution was prepared without root canal sealer for each material and each different method of storage, mixture, and volume:surface ratio.

The specificity of the formaldehyde reaction was verified by an HPLC using a Merk/Hitachi L6200 HPLC device (obtained from Merck, Darmstadt, Germany) with a 125×4 mm column filled with Licrosphere RP-18 column material (Merck) and a water-methanole (1:2) eluent. A UV/VIS 114200 detector (Merck) was used. Diacetyldihydrolutidine was processed from a diluted formalin solution and used as a standard. All agents used for analysis were obtained from Merck.

Statistics

The influence of different methods on formaldehyde release was tested for each material with a Kruskal-Wallis test for non-parametric data using the SPSS program (6.7; SPSS Software, Munich, Germany).

Descriptive *P*-values were calculated with a Wilcoxon test (using SPSS).

Results

For all materials, the influence of different methods on values of formaldehyde release was highly significant ($P < 0.0001$). All materials showed the highest release in freshly mixed samples.

Amubarut (Fig. 1) showed a release up to 96 mg per g in 24 h for freshly mixed material with a medium powder:liquid ratio and a high surface:weight ratio. Storage for 48 h reduced the release by a factor of three; further storage for another 12 days did not reduce the release. A change in the volume:surface ratio led to differences by a factor of two. The highest release was observed with the medium mixing ratio (Fig. 1).

N2 showed an increase in formaldehyde release proportional to an increased powder content. Setting for 48 h decreased the formaldehyde release, yet there was no further decrease after storage for 2 weeks. The sur-

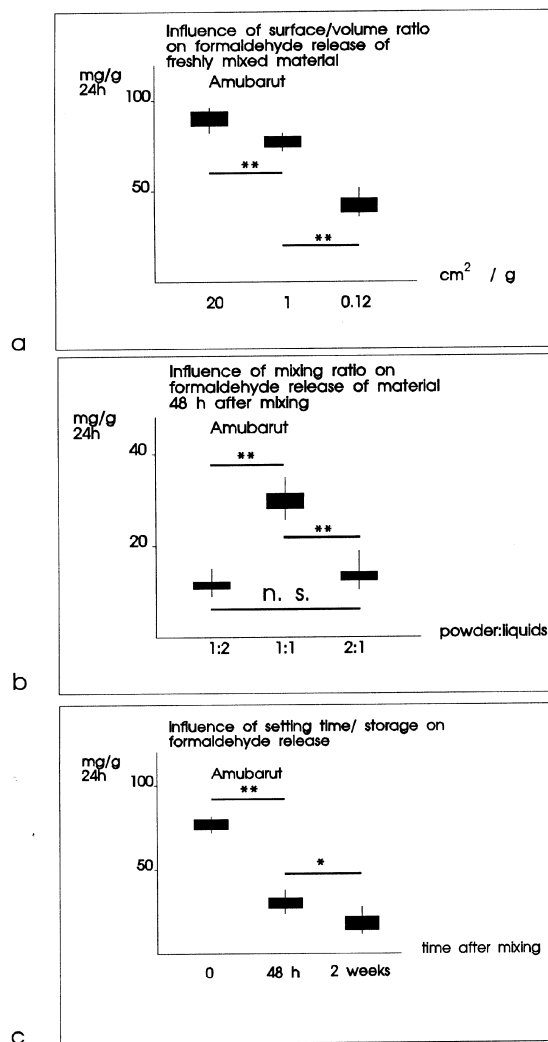


Figure 1 Influence of different methods on formaldehyde release of Amubarut. Note the different scales.

face:weight ratio showed a lower influence than in AH26 (Fig. 2).

AH26 did not release significant amounts of formaldehyde after a storage time of two weeks. A decrease of the weight with a constant surface area apparently led to a linear increase in relative formaldehyde release for freshly mixed material. There was some increase in release with increased powder content (Fig. 3).

Discussion

The materials examined differ considerably in their properties. It is therefore easy to demonstrate that changes in method exhibit different influences on the materials.

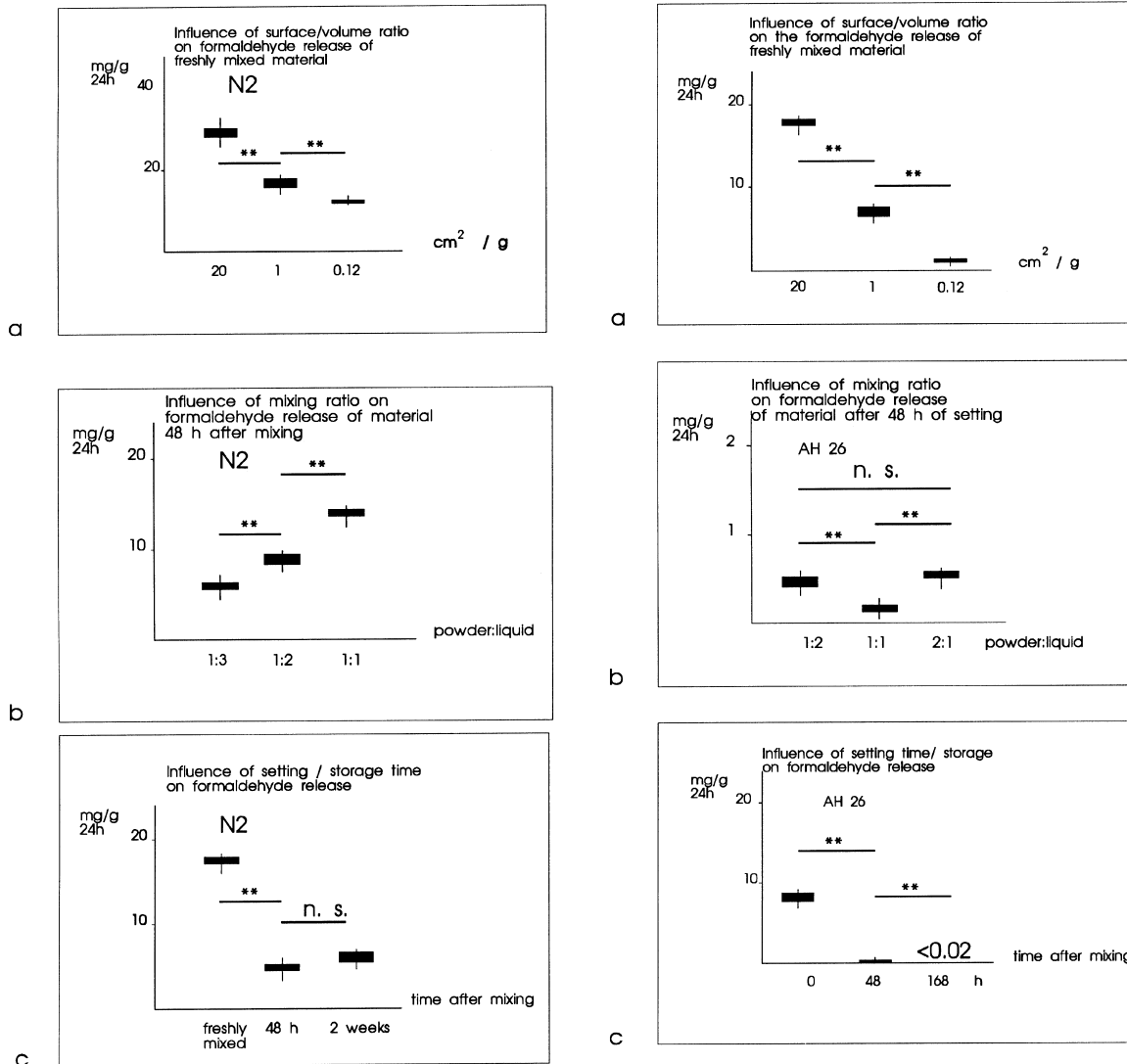


Figure 2 Influence of different methods on formaldehyde release of N2. Note that the mixing ratios differ from those in Fig. 1 and 3. Note also the different scales.

Mixture

It is a concern, that for the endodontic materials used in this study, no exact dosage scheme nor reliable dosage aids for mixing exist, at least for materials sold in Germany. The mixing properties used here probably represent a realistic range used by dental practitioners.

AH26 releases formaldehyde because of the presence of hexamethylenetetramine (methenamine) in the powder (hexamethylenetetramine accounted for 25% of the powder used in this batch). This agent is hydrolysed to

Figure 3 Influence of different methods on formaldehyde release of AH 26. Note the different scales.

ammonia and formaldehyde, 1 g hexamethylenetetramine with water can process an amount of approximately 1.2 g formaldehyde. A higher proportion of powder provides more material to process formaldehyde. The calculatory maximum of formaldehyde released from AH26 (with a powder:paste ratio 2:1) would be as much as 20% of the weight of freshly mixed material. It should not be concluded, however, that a lower powder content increases biocompatibility, because the setting time might be prolonged or other properties might be influenced. It has been shown, that the *in vitro* mutagenicity of AH26 is due mainly to epoxy resin and not to formaldehyde release (Schweikl *et al.* 1995).

Amubarut, however, contains most of the formaldehyde in one of the two solutions (32.6%). The calculatory maximum of formaldehyde release would be approximately 12% of the weight of freshly mixed material (using the data from a declared composition).

A higher powder proportion reduces the total formaldehyde content. The decrease of formaldehyde release by increasing the liquid proportion was unexpected. A potential explanation might be that the polycondensation of formaldehyde and resocine is an exothermic reaction. The heat might lead to higher formaldehyde-utilization and/or evaporation of remaining formaldehyde.

N2 contains paraformaldehyde in the powder (7% paraformaldehyde). As there is no given mixing ratio, a comparison using the same ratios for all materials might also be considered. In this case, for N2 a higher formaldehyde release could be expected. However, the theoretical maximum of formaldehyde release from N2 would be less than 5% of freshly mixed material.

Storage after mixing

During a storage time after mixing a root canal sealer, different changes occur: (i) there is a setting reaction, which changes physico-chemical properties of the material (such as permeability); (ii) formaldehyde, which was added to the material, might evaporate, be used up, or entrapped in the material. The precise time formaldehyde is sampled for analysis after mixing the root canal sealer, is therefore of prime importance; (iii) AH26 does not release significant amounts of formaldehyde after complete setting. This is compatible with findings of cytotoxicity testing; (iv) with Amubarut, there appears to be some evaporation of formaldehyde after 2 weeks. However, there is also paraformaldehyde in the powder of Amubarut, the origin of formaldehyde released after 2 weeks cannot be determined in this study; (v) N2 shows a decrease in release after 2 days of storage before sampling (compared to the release in freshly mixed material). The release after a storage of 2 weeks is not lower than after 2 days. However, the long-term release *in vivo* cannot be determined from this *in vitro* study.

Surface:weight ratio

AH26 is a very hydrophobic material, and formaldehyde is processed only by hydrolytic cleavage of hexamethylenetetramine. The surface of freshly mixed

material in contact with water apparently determines the amount of formaldehyde released.

For N2, there is an increase in formaldehyde by increasing the amount of material (with a constant surface), and this is also seen in Amubarut. Therefore, the surface:weight ratio appears to have somewhat less influence on the formaldehyde release in these materials, compared with AH26.

This study gives a small sample of potential methodological variations. There might be even more variability by using different temperatures during setting, different analysis procedures and different sampling.

The methods were selected to show that the materials exhibit different properties, none of the methods represent a clinically ideal model. However, there might be even more variation of formaldehyde release *in vivo*.

It is very difficult to provide advice to the practitioner from these *in vitro* results. In the past four decades, N2 has been condemned because of its formaldehyde release. AH26 has been used by many practitioners without awareness of formaldehyde release. Yet, AH26 also releases formaldehyde under some conditions: there must be contact with water at a surface of AH26 before setting can occur. Significant overfilling of a root canal might distribute AH26 into trabecular bone structures, resulting in a high surface area of AH26, which will come into contact with water.

Most methods used in this study reveal a higher formaldehyde release (of the root canal sealers examined) compared to studies on formaldehyde release from dental materials, such as denture polymers (Ruyter 1980) dental composites (Oysaed *et al.* 1988) or denture adhesives (Ekstrand *et al.* 1993). On the other hand, comparison of results from different studies on different materials appears even more difficult than comparison of the materials examined in this study.

Formaldehyde is a *hapten* (an incomplete allergen) that must undergo a chemical reaction with an other substance (mainly proteins). Dependency of allergy manifestation is linked to the type of formaldehyde. It has been shown, that inhalative exposure of formaldehyde is not correlated to the presence of antibodies against formaldehyde-albumin (Dykewicz *et al.* 1991). It is difficult to assess the risk of allergy as a result of the endodontic application of formaldehyde.

The manufacturer of AH26 now has a new material (AH Plus, DeTrey/Dentsply) on the market,

which does not exhibit formaldehyde release using the methods mentioned above (M.J.K. 1996, unpublished data).

In a study by Spångberg *et al.* in 1993, N2 and AH26 were compared regarding formaldehyde release. A very different sampling method was used (evaporation of formaldehyde by heat in a dry environment), which is ideal in revealing formaldehyde from N2, and which certainly underestimates the potential of formaldehyde release from AH26. It is therefore appropriate to consider that other methods might reveal very different results.

In conclusion, it is difficult to compare the formaldehyde release of different endodontic materials by using only one *in vitro* method. Therefore, a final conclusion regarding the material with the highest formaldehyde release cannot be deduced from this study. However, there is evidence that Amubarut releases more formaldehyde than N2, and N2 more than AH26 under most conditions tested. This high release level is easy to explain, as the setting reaction requires the use of an aqueous formalin solution. The sampling by water extraction used in this study is therefore very effective in revealing formaldehyde from Amubarut.

Formaldehyde release itself must not be the only consideration for judgement of endodontic materials. Other root canal filling materials might release other substances with adverse effects.

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