

# Formaldehyde levels in absorbent paper points through hplc

## *Níveis de formaldeído em pontas de papel absorvente através de hplc*

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### ABSTRACT

Formaldehyde tablets have been frequently used to keep absorbent paper points sterilized. However, the toxicity of this drug must be considered. The present study aims to determine rates of formaldehyde impregnation in absorbent paper points kept in environments containing formaldehyde tablets. Thus, an accurate analytical methodology requiring simple operations and providing the determination of low concentration levels (ppm) of the formaldehyde impregnated in absorbent paper points was used. The absorbent paper points which had been kept in environments containing formaldehyde tablets were placed in test tubes containing 2,4-dinitrophenylhydrazine and, following an ultrasound bath, were transferred to a microcolumn and washed with distilled water. The retained hydrazones were diluted in acetonitrile and the amount of 10ml was injected in the High Performance Liquid Chromatographer. The results were subjected to descriptive statistics encompassing the estimation of mean values, the standard deviation, and the confidence interval (95%) for mean values. This study showed a significant rate of formaldehyde impregnation in absorbent paper points of any size, especially in those kept in environments containing formaldehyde tablets over a seven-day period.

**Uniterms:** Formaldehyde. Root canal therapy.

### INTRODUCTION

The valuable usage of absorbent paper points in endodontics is beyond question, given their relevance in the drying stage of the root canal, especially before obturation<sup>1</sup>. The absorption properties of these points, together with the maintenance of the aseptic chain during treatment, have called the attention of several researchers. However, scientific information in the literature is still scarce.

The absorption properties of paper points have been reported to undergo alterations when subject to sterilization by heat<sup>2-5</sup>. This fact has led to the search for alternatives to maintain both the properties of the paper points and the aseptic chain during endodontic treatment.

Storage of absorbent paper points in environments containing formaldehyde tablets has

been advocated since it keeps the paper points sterilized and does not alter its absorption properties<sup>4,6-9</sup>. However, concern has arisen regarding the amount of formaldehyde that may be assimilated by the absorbent paper points kept in environments containing formaldehyde tablets, especially since live periapical tissues may be exposed to this drug during the drying stages of root canal treatments. This concern is harbored in the toxicity of formaldehyde, a primary irritant prone to cause a number of disorders in humans, ranging from mild irritations to death, depending on its concentration<sup>10-14</sup>.

This study seeks to analytically determine the rates of aldehyde impregnation in absorbent paper points kept in environments containing formaldehyde tablets for periods of 24 hours, 48 hours, 7 days, 14 days, 21 days, and 28 days through High Performance Liquid Chromatography. It also aims to determine

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whether the size of the absorbent paper points may play a role in the level of impregnation by formaldehyde.

## MATERIALS AND METHODS

For this study, absorbent paper points from the same origin were used (Odahecan Herpo Produtos Dentário Ltda, Petrópolis, Rio de Janeiro, Brazil) encompassing three different sizes, namely: absorbent paper points #15, absorbent paper points #40, and absorbent paper points #80.

In order to keep these paper points in environments containing formaldehyde tablets, six endodontic storage boxes (Inodon, São Paulo, São Paulo, Brazil), which presented internal chambers around a central area, were used. The absorbent paper points were stored in the chambers separately, according to their sizes, whereas the formaldehyde tablets (Miyako do Brasil Indústria e Comércio, Guarulhos, São Paulo, Brazil) were placed in the central area, amounting to 4 g<sup>o</sup>.

The absorbent paper points #15, #40, and #80 remained in the boxes containing formaldehyde for the following storage periods: box 1 – 24h, box 2 – 48h, box 3 – 7 days, box 4 – 14 days, box 5 – 21 days, and box 6 – 28 days.

The determination of formaldehyde rates in each sample was carried out in accordance with the methodology recommended by Andrade et al.<sup>15</sup> by making use of the devices and substances described below.

## Reagents and Standards

All organic solvents were HPLC grade (Merck, São Paulo, São Paulo, Brazil) and were distilled in the presence of 2,4-dinitrophenylhydrazine (DNFH). The standard of 2,4-dinitrophenylhydrazine (DNFH<sub>o</sub>) was precipitated by means of DNFH, HPLC grade, twice recrystallized.

The DNFH solution was obtained by diluting 20 ml of concentrated DNFH solution (100 ml in phosphoric acid – acetonitrile at 1% per volume) in two liters of deionized water and phosphoric acid at 1%. The solution was purified by three successive extractions with carbon tetrachloride (CCl<sub>4</sub>) and stored in a two-liter glass vessel containing 200 ml of CCl<sub>4</sub> and a teflon-covered magnetic bar.

## Devices

A Variant Liquid Chromatographer (model 2510, USA), equipped with a Rheodyne injector and a UV/Vis variable wavelength detector (model 2550) was used. Absorbance detection was carried out at

350 nm (0.04 AUFS – Absorbance Unit Full Scale).

The analytical column used was Econosphere C<sub>18</sub> 5 mm, 250 mm X 4.6 mm of internal diameter (Altech, USA). The mobile phase was made up of acetonitrile, water (57:43 v/v) at a flow rate of 1.0 ml/min. In all analytical assays, a 10 ml aliquot was used.

A Microsonic ultrasound bath (model SX 10, USA) was employed both to speed the reaction between the formaldehyde and the DNFH as well as to enhance mass transport in the reaction milieu.

## Experiment

For each storage period a sample of each absorbent paper point was placed in a test tube containing 1 ml of DNFH and ultrasound bathed for five minutes. The material was subsequently transferred, by means of a syringe, to a microcolumn, and subsequently washed with distilled water. The retained hydrazones in the microcolumn were diluted in acetonitrile and transferred to a 5-ml volumetric balloon. A 10 ml aliquot from the resulting solution was injected into the High Performance Liquid Chromatographer. The hydrazones thus formed were separated by HPLC, the detector of which is programmed to change the wavelength and sensitiveness according to the retention time of each hydrazone. The hydrazone from formaldehyde is analyzed by using a wavelength of 354 nm together with a 0.04 AUFS sensitiveness detector.

The amount of formaldehyde measured was calculated by comparing the calibration curves of hydrazone standards and comparing the peak height of the sample length to the standards.

The descriptive statistical study of the results showed: averages, standard deviations as well as the respective confidence intervals (95%), when comparing groups.

## RESULTS

Results from this experiment show that absorbent paper points kept in environments containing formaldehyde tablets become impregnated by this drug.

The lowest formaldehyde impregnation rates in the absorbent paper points lie in those stored for 24 hours. As the storage period increases to 48 hours, the formaldehyde impregnation rates increase for paper points #15, #40 and #80.

This formaldehyde impregnation rate continues to increase and reached the highest levels on the seventh day of storage. The paper points stored for 14 days, however, showed a decrease in

formaldehyde impregnation rates. For those stored in the next two periods, the formaldehyde impregnation rates were kept within the same range. These results are shown in Table 1, separated by size.

When comparing paper point #15 with paper point # 40 for the storage periods of 24 hours, 48 hours, 7 day and 21 day no difference can be found. For both the 14 day and the 28 day periods, the results

presented small differences.

When the results for paper point #15 are compared with paper point #80 after 24 hours and further compared after 48 hours of storage differences arise, with the paper points size #80 presenting higher impregnation. However, after 7 and 14 days of storage, impregnation was higher in paper points #15.

**Table 1-** Formaldehyde impregnation rates in ppm in absorbent paper points #15, #40, and #80, stored for different time periods.

	# 15			# 40			# 80		
	X	Sd	CI	X	Sd	CI	X	Sd	CI
24 h	0.21	0.02	[0.16 – 0.25]	0.18	0.01	[0.15 - 0.20]	0.57	0.00	[0.57 - 0.57]
48 h	4.41	0.06	[4.26 – 4.56]	4.50	0.17	[4.08 - 4.92]	4.68	0.02	[4.64 - 4.72]
01 wk	7.83	0.09	[7.60 – 8.06]	7.85	0.16	[7.46 - 8.24]	7.43	0.03	[7.34 - 7.52]
02 wk	1.73	0.02	[1.69 – 1.77]	0.94	0.01	[0.91 - 0.96]	0.76	0.01	[0.74 - 0.77]
03 wk	2.14	0.15	[1.76 – 2.52]	2.28	0.08	[2.08 - 2.48]	2.19	0.03	[2.13 - 2.25]
04 wk	2.76	0.07	[2.60 – 2.92]	1.36	0.02	[1.32 -1.40]	2.32	0.13	[2.00 - 2.64]

X = Means value; Sd = Standard deviation; CI = Confidence interval 95%

When were compared absorbent paper points #40 with those of #80 after 24 hours, 14 days and 28 days results showed higher impregnation in absorbent paper points #80 after 24 hours and 28 days of exposure to formaldehyde and in absorbent paper points #40 after 14 days of storage. After 48 hours, 7 days and 21 of storage, there were no difference.

The analysis regarding paper point #15 in all storage periods shows the highest formaldehyde impregnation rates on the 48 hour period and especially on the 7 day period, when compared to the remaining storage periods. The results for paper points #40 and #80 were similar to those for paper points #15, that is, the formaldehyde impregnation rate for the 48 hours period was high and reached its peak after 7 days.

## DISCUSSION

In this work, formaldehyde impregnation rates were analytically assessed in absorbent paper points. The paper point brand (Odashcan) was chosen due to its comparatively better absorption properties<sup>7</sup>.

The experiment was carried out through High Performance Liquid Chromatography (HPLC), as proposed by Andrade et al.<sup>15</sup>, as it is an easily operated, accurate, and precise analytical method which allows for the determination of formaldehyde

impregnation rates in absorbent paper points kept in environments containing formaldehyde tablets for different storage periods.

Within the reagents that could selectively be combined with formaldehyde, 2,4-dinitrophenylhydrazine was the reagent of choice since it presents, according to Pinheiro<sup>16</sup>, a high reaction speed in an aqueous milieu and/or when absorbed on a solid support, besides allowing the products to be easily separated and quantified by HPLC.

In this method, hydrazones from the formaldehyde are formed through the reaction with 2,4-dinitrophenylhydrazine:

This reaction is a result of a nucleophilic addition in the carbonyl followed by the 1.2 elimination of water and the formation of 2,4-dinitrophenylhydrazone. Because 2,4-dinitrophenylhydrazone is a weak nucleophile, the additional reaction occurs in the presence of a moderate amount of acid, that is, it is catalyzed by the acid that promotes the protonation of the carbonyl, which is rapidly processed at room temperature in an aqueous milieu<sup>16</sup>.

The usage of HPLC, a fast and highly sensitive technique with a short time delay for analysis, encompasses three stages for each experiment<sup>15</sup>: Stage 1 - Absorption of the formaldehyde from the absorbent paper point in a

solution of 2,4-dinitrophenylhydrazine, thus forming the 2,4-dinitrophenylhydrazone; Stage 2 - Separation of 2,4-dinitrophenylhydrazone in a C18 column reversed phase; and Stage 3 - Quantification through UV/Vis spectrophotometry.

Most of the existing absorbent paper points in the market are numbered according to their sizes. In this study, three different sizes were used: size #15, because it is one of the smallest; size #40, because it is average sized; and size #80, because it is the biggest and most commonly used. Hence, the role of the size in the amount of formaldehyde impregnated in the paper points could also be assessed.

The 24 hour period was chosen as the shortest storage time due to the findings by several authors who reported that within this time range formaldehyde acts efficiently in fighting various microbial strains<sup>17</sup>. On the other hand, considering that the storage of absorbent paper points in environments containing formaldehyde tablets favors the maintenance of the paper point sterilization, a maximum period of 28 days was chosen for this experiment.

Based on the results, the size does not seem to play a role in the amount of formaldehyde impregnated in absorbent paper points since a higher impregnation rate is sometimes observed in either the bigger or smaller sizes. When the differently sized paper points were compared in relation to different storage periods, some of them showed no differences, whereas others did; nevertheless the differences were small. Such findings demonstrate that, regardless of the size of the paper points kept in environments containing formaldehyde tablets, impregnation by this drug increases with storage time, from the very beginning (24 hours) to the 7 day period. After 7 days, the impregnation rates decrease (14 days), kept within the same range until the end of the experiment on the twenty-eighth day. It is also worth noting that from the fourteenth day on the impregnation rates are lower than those for 48 hours and slightly higher than those for 24 hours.

Since the tablets release the formaldehyde in a slow fashion, the level of this drug in the environment until the seventh day most likely increased. The existence of a higher concentration in the box produced a higher saturation in the paper points. As the days passed, due to the volatilization

of the formaldehyde, the impregnation rates decreased, and the impregnation detected is the one remaining in the paper point fibers.

Given the cytotoxic effects of formaldehyde for the organism<sup>10-14</sup>, as well as the fact that absorbent paper points present a significant rate of impregnation when kept in environments containing formaldehyde tablets, especially those kept for 7 days, the harmful effects of their usage in root canal treatment for human health are worrisome.

Notwithstanding the findings by Holland et al.<sup>8</sup> and Holland et al.<sup>4</sup> demonstrating the low level expressiveness of the influence of absorbent paper points kept in environments containing formaldehyde tablets in inflammatory reactions, it is beyond doubt that further studies should be carried out to determine the amount of formaldehyde residues that may remain in root canals dried by means of such paper points. The rate of formaldehyde associated with the atmospheric particulate material inside a dental office in which absorbent paper points and gutta percha points are kept in environments containing formaldehyde tablets should also be studied in order to search for other toxic effects of formaldehyde in the organism, thus promoting an increased biosafety for both patients and dental professionals.

## CONCLUSIONS

The results of this study have led to the following conclusions: 1. absorbent paper points present high formaldehyde impregnation rates when kept in environments containing formaldehyde tablets; 2. formaldehyde impregnation rates in absorbent paper points increase in the first week of exposure to formaldehyde, but decrease on the fourteenth day and fall within a stable range until the twenty-eighth day; 3. the size of the absorbent paper points has practically no influence on formaldehyde impregnation.

## RESUMO

O formaldeído em tabletes tem sido frequentemente utilizado para conservar esterilizadas as pontas de papel absorvente. Todavia, a toxicidade desta substância deve ser considerada. O presente estudo objetivou determinar os níveis de formaldeído impregnado em pontas de papel absorvente conservadas em caixas contendo tabletes de formaldeído. A metodologia analítica precisa requer simplicidade de operação das amos-

tras e exatidão na determinação de baixas concentrações (ppm) de formaldeído impregnado em pontas de papel absorvente. As pontas que tinham sido conservadas em caixas contendo tabletes de formaldeído foram colocadas em tubos teste contendo 2,4 - dinitrofenilhidrazina, e após o banho de ultra-som foram transferidas para uma microcoluna e lavadas com água destilada. As hidrazonas retidas foram diluídas em acrilonitrila, seguindo-se da coleta de alíquotas de 10uL injetadas no Cromatógrafo Líquido de Alta Eficiência. Os resultados obtidos foram submetidos à estatística descritiva e estimadas as médias, desvios padrões e intervalos de confiança (95%) dos valores médios. A partir do presente estudo observaram-se significativas concentrações de formaldeído impregnado nas pontas de papel absorvente de todos os tamanhos, especialmente naquelas que foram conservadas em ambiente contendo tabletes formaldeído pelo período de sete dias.

**Descritores:** Formaldeído. Tratamento do canal radicular.

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