Aspects of the Exact Quantitative Determination of Formaldehyde in Tooth Tissue by TLC

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Key Words:

TLC
Formaldehyde
Formaldemethone
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Summary

Formaldehyde (HCHO) is a natural component of plant, animal, and human tissue. It has been proved that it is formed within cells by demethylation and methylation via demethylases and special peroxidases. Fundamental research work studying the role of formaldehyde in plants has been performed by *Tyihák* and coworkers. Current research concentrates on investigation of physiological processes in human cells. On the basis of *Tyihák's* work it was decided to investigate the physiological and pathological role of formaldehyde in human tooth tissue.

It was, however, shown that accurate determination of the levels of formaldehyde in the hard tissue of teeth is influenced by such factors as the amount of pulverization of the biological material studied, choice of the optimum concentration of extraction reagent (e.g. dimedone), and the technique and duration of extraction.

To determine the influence of these factors on the precision of HCHO analysis several trials have been performed with biological material characterized by different extents of pulverization. Relationships were determined between the results obtained and the size of the particles. Adsorption TLC was used to study the relationship between HCHO recovery from the hard tissue of teeth and the concentration of dimedone used to extract the formaldehyde.

It was proved that in the TLC determination of HCHO the analytical results were influenced by many factors.

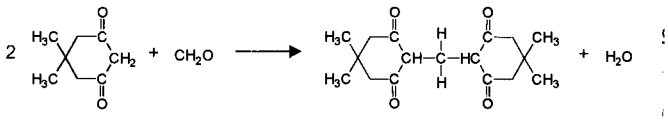
1 Introduction

Numerous studies have shown that the level of formaldehyde in plant, animal, and human tissues (in body fluids) is related to the physiological state of the organisms. In recognition of the intracellular occurrence of HCHO described by *Tyihák* [1,2] in numerous papers on plant tissues, it was decided to investigate the influence of the physiological state of teeth on the level of formaldehyde in tooth tissue.

During our preliminary work in this field it was found that the results obtained from the analysis of formaldehyde levels in the hard tissue of teeth (several tens of micrograms) is influenced by such factors as the amount of pulverization of the biological material studied, selection of the optimum concentration of extraction reagent (dimedone), and the technique and duration of the extraction process.

In our numerous papers in the field of HCHO determination in biological material (hard tissue of teeth) [3–8] and in *Tyihák's* work it was concluded that the accuracy of these determinations can be considerably increased by selection of a suitable concentration of the dimedone used to extract HCHO and by appropriate preparation of the sample for chromatographic analysis. Because these analytical guidelines have so far been approximate only (i.e. qualitative), it was resolved to study this problem very thoroughly.

The level of formaldehyde in the hard tissues of teeth is usually determined in the form of formaldemethone (a dimedone adduct of formaldehyde). The reaction between dimedone and formaldehyde is:



The accuracy of quantitative determination of this adduct is influenced by several factors of apparently considerable importance.

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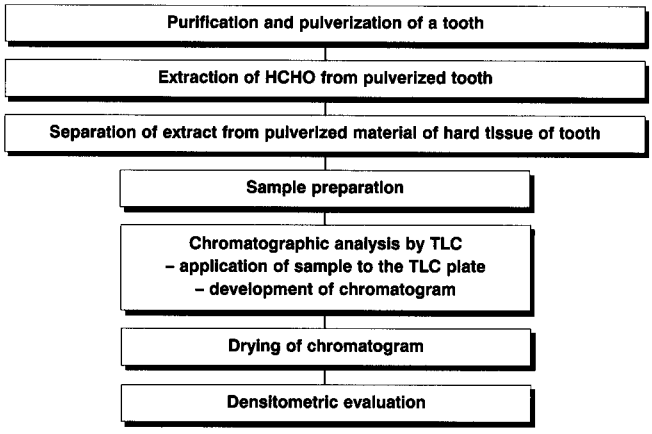

Figure 1

Schematic representation of the method used for the determination of formaldehyde.

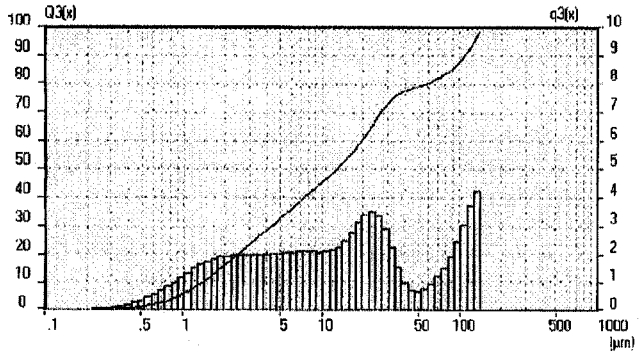

Figure 2

Particle-size distribution after 20 min milling.

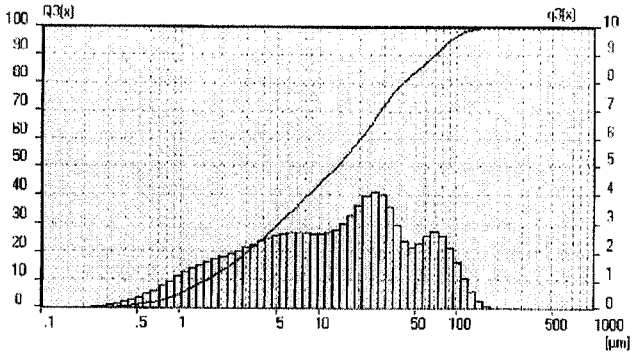

Figure 3

Particle-size distribution after 40 min milling.

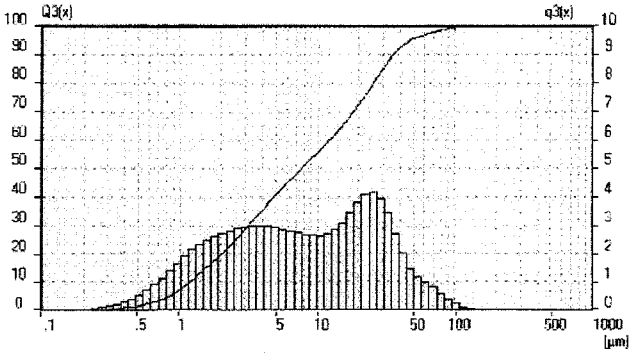

Figure 4

Particle size distribution after 120 min milling.

2 Experimental

The analysis of HCHO in hard tissues of teeth can be represented by the scheme shown in **Figure 1**.

2.1 Pulverization of Teeth

The teeth analyzed must be thoroughly cleaned of remnants of soft tissue, blood, and, when present, dental fillings. This is very important because the level of HCHO in the hard tissue of teeth is one twelfth that in soft tissues. The presence of fillings would make it impossible to pulverize the tooth homogeneously.

The teeth were pulverized in a Pulverisette 0 vibrating mill (Fritsch, Germany) with an agate mortar. After preliminary pulverization to approximately 0.5–1 mm, eight separate samples (0.6 g) were further milled in the vibrating mill for 5, 10, 20, 30, 40, 60, 90, 120, or 240 min. The influence of the particle-size distribution of the analyzed material was studied by means of an Analysette 22 laser analyzer (Fritsch, Germany). **Figures 2–4**, respectively, and **Table 1** show the particle-size distributions obtained for samples pulverized for 20, 40, and 120 min. It is clearly apparent that increasing the duration of pulverization reduces the percentage of the largest-sized particles. The results obtained enabled choice of the optimum amplitude of mortar vibrations (1.5 mm in the range 0–3 mm) and the optimum duration of pulverization – 120 min. Preparation of samples in this manner ensures optimum extraction of HCHO for TLC analysis.

2.2 HCHO Extraction

A methanol solution of dimedone was used to extract HCHO from the pulverized teeth. Samples (0.25 g) were extracted with solutions (400 µL) containing 0.050, 0.100, 0.250, 0.500, 0.750, 1.00, 1.500, 2.500, or 3.00 mg/mL^{-1} dimedone.

Figure 5 shows the relationship between the level of formaldemethone determined in the extract and in the initial concentration of the extractant. It is evident that the level of formaldemethone increases at first sharply and later slowly as the concentration of dimedone is increased. As well as increasing the amount of formaldemethone obtained, increasing the amount of dimedone also increases the amount of the so-called 'blind sample' (solution of pure extractant), which causes overloading of a TLC plate. The results obtained enabled determination of the optimum concentration – 1.00 mg mL^{-1} – of the solution used for extraction.

Table 1

Particle size distributions after 20, 40, and 120 min milling.

20 min		40 min		120 min	
54.56%	> 10.00 μm	56.47%	> 10.00 μm	44.15%	> 10.00 μm
39.02%	> 20.00 μm	40.32%	> 20.00 μm	27.51%	> 20.00 μm
20.62%	> 50.00 μm	16.08%	> 50.00 μm	4.38%	> 50.00 μm
16.30%	> 80.00 μm	6.78%	> 80.00 μm	0.97%	> 80.00 μm
6.98%	> 120.00 μm	1.12%	> 120.00 μm	0.06%	> 120.00 μm
0%	> 150.00 μm	0.20%	> 150.00 μm	0.00%	> 150.00 μm
		0%	> 180 μm		
5.00%	< 0.93 μm	5.00%	< 1.01 μm	5.00%	< 0.84 μm
10.00%	< 1.38 μm	10.00%	< 1.58 μm	10.00%	< 1.21 μm
15.00%	< 1.88 μm	15.00%	< 2.27 μm	15.00%	< 1.60 μm
20.00%	< 2.50 μm	20.00%	< 3.09 μm	20.00%	< 2.04 μm
25.00%	< 3.31 μm	25.00%	< 4.07 μm	25.00%	< 2.56 μm
30.00%	< 4.37 μm	30.00%	< 5.23 μm	30.00%	< 3.17 μm
40.00%	< 7.49 μm	40.00%	< 8.44 μm	40.00%	< 4.85 μm
45.00%	< 9.77 μm	45.00%	< 10.75 μm	45.00%	< 6.03 μm
50.00%	< 12.69 μm	50.00%	< 13.61 μm	50.00%	< 7.58 μm
55.00%	< 16.03 μm	55.00%	< 16.84 μm	55.00%	< 9.61 μm
60.00%	< 19.35 μm	60.00%	< 20.22 μm	60.00%	< 12.19 μm
65.00%	< 22.78 μm	65.00%	< 23.75 μm	65.00%	< 15.20 μm
70.00%	< 26.75 μm	70.00%	< 27.74 μm	70.00%	< 18.37 μm
75.00%	< 32.77 μm	75.00%	< 32.71 μm	75.00%	< 21.68 μm
80.00%	< 55.24 μm	80.00%	< 40.10 μm	80.00%	< 25.27 μm
85.00%	< 86.78 μm	85.00%	< 53.22 μm	85.00%	< 29.50 μm
90.00%	< 108.75 μm	90.00%	< 68.58 μm	90.00%	< 35.33 μm
95.00%	< 127.05 μm	95.00%	< 87.95 μm	95.00%	< 47.44 μm

2.3 Separation of the Extract – Sample Preparation

The relationship between the extraction time the amount of formaldemethone obtained from the hard tissue of teeth was also examined. Samples (0.25 g) were extracted for different times (5, 10, 15, 30, 45, 60, 90, 120, 180, or 240 min) with a solution of dimedone in methanol (1.000 mg mL^{-1}, 400 μL). After extraction the suspension was centrifuged for 7 min.

The dependence on extraction time of the concentration of formaldemethone determined is presented in **Figure 6.** It can be concluded that results are improved by increasing the extraction time, but that the influence of extrac-

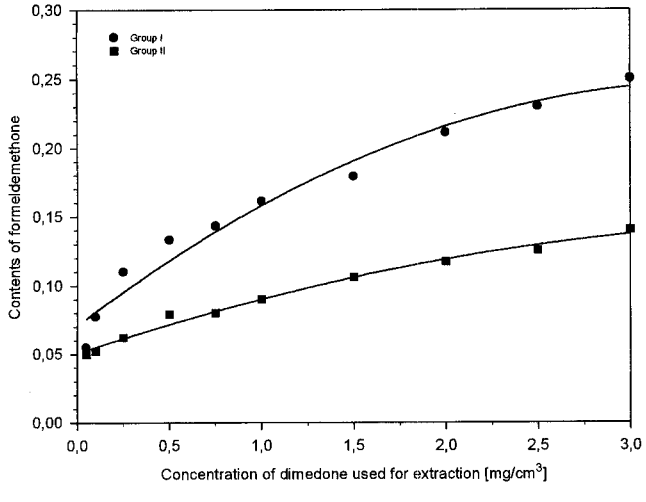

Figure 5

Relationship between the concentration of the extractant and the level of formaldemethone determined for two different groups of teeth.

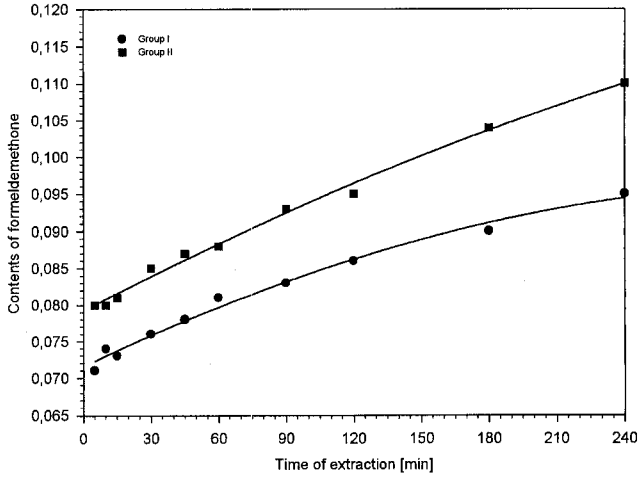

Figure 6

Relationship between the extraction time and the level of formaldemethone determined for two different groups of teeth.

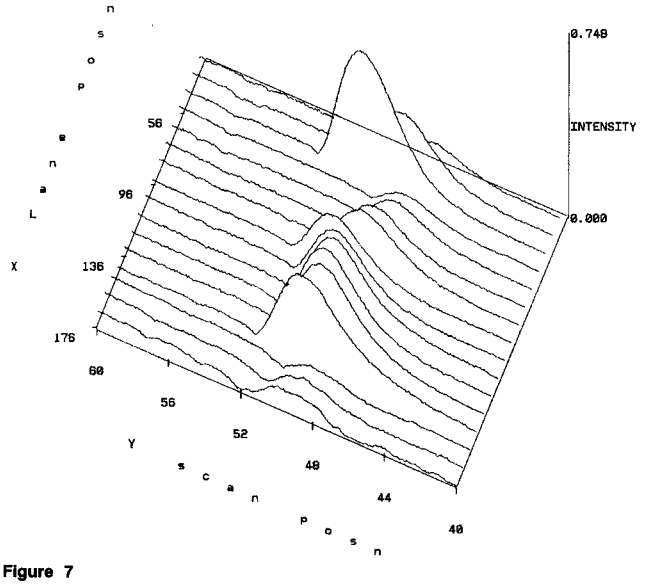

Figure 7

Densitograms from chromatograms of tooth extracts obtained by use of different concentrations of extractant.

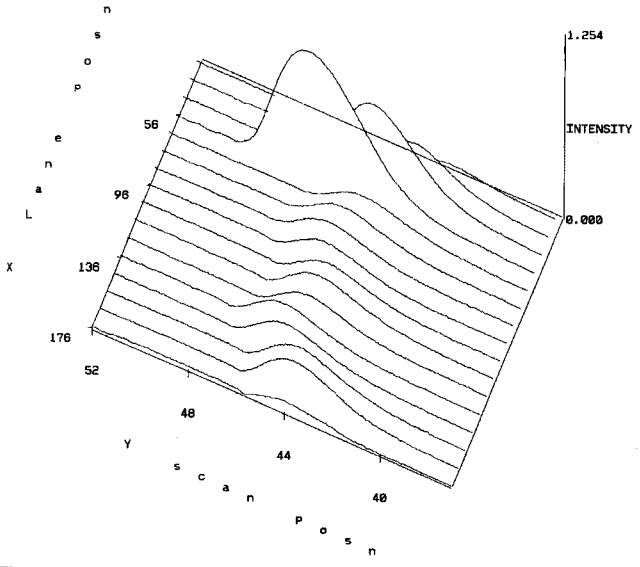

Figure 8

Densitograms from chromatograms of tooth extracts obtained by use of different extraction times.

tion time decreases the more it is increased. It can be assumed that the optimum extraction time is in the range of 60–90 min.

2.4 TLC Analysis of Extracts

Application of the HCHO extract (20 μL) to the chromatographic plates was performed by use of a Desaga AS 30 TLC applicator under pure nitrogen. Because of the high affinity of HCHO for dimedone it was necessary to minimize contact with laboratory environment, which always contains some free HCHO which can bind with dimedone. To determine and eliminate HCHO contamination from reagents and HCHO derived from the laboratory atmosphere, the so-called 'blind sample' (i.e. pure dimedone solution at the concentration used for extraction) was also applied on the plate.

2.5 Development and Drying of Chromatograms

All chromatograms were developed to a distance of 100 mm in Camag twin-trough chambers. Chloroform–dichloromethane, 1 + 3 (*v/v*), was used as mobile phase. The developed chromatograms were dried by standing for 15 min at room temperature.

2.6 Densitometric Evaluation

Densitometric analysis of chromatograms was performed with a Shimadzu (Japan) CS9001 PC densitometer immediately after drying of the TLC plates. The optimum wavelength was 275 nm. If necessary the chromatograms should be stored in a freezer and without contact with the laboratory environment.

Sample densitograms derived from chromatograms of tooth extracts obtained by use of different concentrations of extractant and different extraction times are presented in **Figures 7** and **8.**

The procedure described for preparation of samples of biological material for TLC analysis enables accurate determination of HCHO levels despite the small amounts present in these samples. The procedure could also be used for quantitative TLC determinations of HCHO precursors in cell metabolism in biological material.

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