Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytica Chimica Acta 565 (2006) 129–135
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Determination of low-aliphatic aldehyde derivatizatives in human saliva using polymer monolith microextraction coupled to high-performance liquid chromatography

Hui-Juan Zhang, Jing-Fang Huang, Hong Wang, Yu-Qi Feng *

Department of Chemistry, Wuhan University, Wuhan 430072, China

Received 24 January 2006; received in revised form 19 February 2006; accepted 21 February 2006
Available online 31 March 2006

Abstract

In this study, a polymer monolith microextraction (PMME) using a poly (methacrylic acid–ethylene glycol dimethacrylate) (MAA–EGDMA) monolith in conjunction with high-performance liquid chromatography (HPLC) was developed for the determination of 2,4-dinitrophenylhydrazine (DNPH) derivatives of several aldehydes in human saliva. The conditions for the labeling reactions of aldehydes with DNPH and followed extraction of the derivatives were optimized. The precision, recovery and detection limits were evaluated with spiked saliva. The limits of detection ranged from 0.43 to 1.40 μg/L. The inter-and intra-day relative standard deviations were less than 10%. The proposed method was successfully applied to the determination of aldehydes in saliva samples from a non-smoker, a passive smoker and a heavy smoker.

Keywords: Polymer monolith microextration; Monolithic capillary; Aldehydes; 2,4-Dinitrophenylhydrazine; HPLC

1. Introduction

Saliva is the first body fluid to encounter the exogenous materials and its complicated chemical components can reflect the development of some diseases [1–3]. Moreover, the collection and treatment of saliva is rather easier and quicker compared to other human samples, such as plasma, hair, tissues and cells. Therefore, monitoring the content of certain chemical components in saliva is of academic and practical importance.

Low-aliphatic aldehydes such as formaldehyde, acetaldehyde, acrolein, butyraldehyde are acknowledged to be harmful organic pollutants. Formaldehyde (FA) is classified as a human carcinogen and acetaldehyde (AA) can also cause cancers by damaging deoxyribonucleic acid (DNA) and preventing it from being repaired [4]. Acrolein (AC) and butyraldehyde (BA) act primarily as irritants to the eyes and respiratory tract [5]. Meanwhile, as a product induced by free radicals reacting with cellular lipids, aldehydes are considered as evidence that the free radical-induced reactions have happened to obtain the information of cancer [6–8].

It is well known that smoking leads to serious diseases such as heart disease, lung cancer and oral cancer. Large amount of aldehydes has been detected in the smoke of cigarette by GC and HPLC [9,10]. Furthermore, Nagler et al. found that aldehydes in the smoke of cigarette would interact with saliva and destruct biological macromolecules rapidly [11–13]. Consequently, the investigation of the relationship between smoking and aldehyde contents in saliva is helpful to evaluate the damage degree of oral diseases. At present, a few studies about this work have been presented [14,15].

Owing to the volatility and activity of aldehydes, it is difficult to analyze them directly and accurately by chromatographic techniques without appropriate pretreatment. Derivatizations prior to the detection are usually adopted, especially for low-aliphatic aldehydes. A variety of derivatizing reagents, such as, 2,4-dinitrophenylhydrazine (DNPH) [16,17], *O*-2,3,4,5,6-(pentaflurobenzyl)hydroxylamine (PFBHA) [18–20], dansyl-hydrazine (DNSH) [21,22], *N*-methyl-4-hydrazino-7-nitrobenzofurazan (MNBDH) [23], has been used for the analysis of aldehydes. The most commonly used reagent for UV detection in recent years is DNPH with a hydrazine group (–NH–NH_2) acting as the reactive site in derivatization [24–26].

* Corresponding author. Tel.: +86 27 87867564; fax: +86 27 68754067.
E-mail address: yqfeng@public.wh.hb.cn (Y.-Q. Feng).

0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2006.02.050

Solid phase microextraction (SPME) has become a popular pretreatment method due to some advantages such as simplicity of procedure, solvent-free characteristic and convenience of automation compared with solid-phase extraction (SPE) and liquid–liquid extraction (LLE) [27]. Moreover, lower detection limits and higher recoveries can usually be achieved by the combination of derivatization and SPME in the determination of trace analytes. For example, Martos and Pawliszyn found that this method could gain lower limits of detection by three or more orders of magnitude compared to direct SPME [28]. Similar method has also been applied to the determination of aldehydes in drinking water [29], beer [30], the product from heated vegetable oil [31] and so on. Recently, the derivatization-SPME modes have been reviewed in detail [32].

The enrichment ability and extraction selectivity of SPME depend mainly on the properties and thickness of coating materials. Although many coating materials are commercially available, the development of SPME was limited by the main disadvantages of the unprotected stationary phase coating on the outer surface of the fiber when extended through the syringe needle and the film thickness. Recently, the monolithic capillary column by in situ polymerization [33–35] has shown to be several attractive features: the in-tube configuration can protect the extraction material to avoid the outside breakage; compared to the coated capillaries, the monolithic column exhibits high extraction capacity; at the same time, the monolithic material has emerged as more popular alternatives to packed columns due to the simplicity of their preparation as well as the diverse surface chemistry; moreover, a convective mass transfer procedure offered by the porous structure can facilitate the extraction process [36]. In our previous study, poly (MAA–EGDMA) monolithic capillary column has been applied to in-tube SPME of several basic analytes [37,38]. Based on these investigations, a novel polymer monolith microextraction (PMME) coupled to capillary electrophoresis (CE) has been established to analyze several angiotensin II receptor antagonists in human urine [39]. In this study, PMME using a poly(MAA–EGDMA) monolithic capillary for 2,4-dinitrophenylhydrazine (DNPH) derivatized low-aliphatic aldehydes has been developed for HPLC determination of low-aliphatic aldehydes in saliva samples from a non-smoker, a passive smoker and a heavy smoker.

2. Material and methods

2.1. Chemicals and materials

Ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA), 2,2′-azobis (2-methylpropionitrile) (AIBN), dodecanol and toluene were obtained from Shanghai Chemical (Shanghai, China) and were of analytical reagent grade.

Formaldehyde standard solution (100 mg/L) was purchased from The State Environmental Protection Administration of China (Beijing, China). Acetaldehyde (99.5%) was purchased from Acros (Sweden). Acrolein, butyraldehyde, 2,4-dinitrophenyl-hydrazine, acetacetic ester and methanol were of analytical reagent grade and purchased from Shanghai General Chemical Reagent Factory (Shanghai, China). Each standard aldehyde stock solution (0.1 mg/mL) was prepared in methanol and stored at −20 °C. DNPH was further recrystallized twice in acetonitrile–water (1:5) solution before use. Double distilled water was used in all experiments.

2.2. Sample preparation

Saliva samples were collected from three volunteers. Two of them were healthy female nonsmokers, aged 25 and 30, the other was male (up to 10 cigarettes per day), aged 40. All saliva samples were collected 2 h after eating. To obtain the mixed saliva samples, each volunteer was told not to swallow the saliva but to store it in their mouths. After 6 min, saliva was collected in Eppendorf tubes and stored at −20 °C immediately. The saliva samples were centrifuged for 5 min at 13,000 rpm (4 °C) when they were used.

2.3. Derivatization procedure

Aldehyde stock solutions were mixed together and diluted to the desired concentration with phosphate buffer (0.8 mol/L, pH 3.8 ± 0.1). After that, 420 μL of DNPH solution (2 mg/mL) was added to the solution. The flask was capped, shaken for 1 min and then allowed to react 1 h at 40 °C in the oven.

2.4. Preparation of poly(MAA–EGDMA) monolithic capillary

The poly(MAA–EGDMA) monolith was synthesized inside a fused silica capillary (20 cm × 530 μm i.d., Yongnian, Hebei, China). The polymerization method was described in detail previously [38]. At first, the fused silica capillary was cleaned and activated by 1 mol/L NaOH for 30 min and then 1 mol/L HCl for 1 h. After rinsing with double distilled water until the pH value of the outlet solution was 7.0, it was dried with nitrogen at 160 °C for 10 h.

A 3-(triethoxysilyl)propyl methacrylate methanolic solution (50% v/v) was introduced to fill the activated capillary. After sealing the ends of the capillary with silicon rubbers, the reaction was allowed to run at 40 °C for 12 h. Then, the residual solution was driven out and the capillary was rinsed thoroughly with methanol. Finally, nitrogen was driven to flow through the capillary to dry the inner surface at room temperature prior to use.

The pre-polymerization mixture solution was consisted of monomer MAA 48 mg, crosslinker EGDMA 420 mg, porogenic solvent toluene 110 mg and dodecanol 860 mg, and initiator AIBN 4.5 mg. After purged with nitrogen to remove oxygen, the mixture solution was allowed to fill in the pretreated capillary. Immediately, the capillary was sealed with silicon rubber, and then the reaction was initiated at 60 °C for 16 h. Following polymerization, the capillary was washed with methanol to remove the unreacted component and porogenic solvent.

2.5. Microextraction procedure

The device configuration was composed of extraction pinhead and syringe barrel as shown in Fig. 1. The extraction device

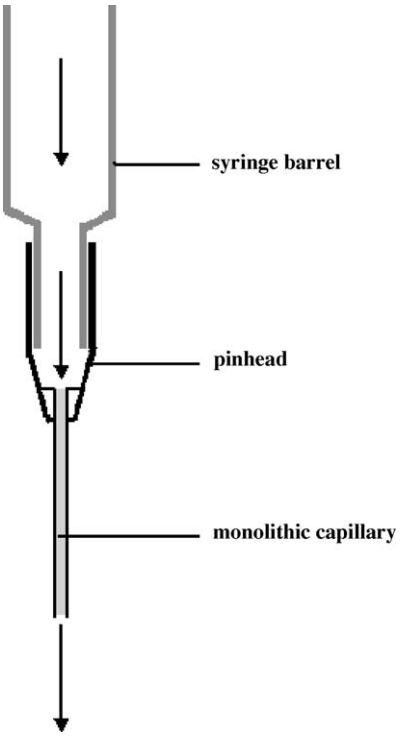

Fig. 1. A scheme of the novel PMME [39].

was prepared as follows [39]: the syringe barrel and the pinhead were produced out of uniform molds. The metallic needle of the pinhead was replaced by 3 cm long monolithic capillary. Due to the similar external diameter, the capillary column can fit for the pinhead as well as the metallic needle. The extraction device can be used after the capillary was fixed in the pinhead by the adhesive.

In this study, the mobile phase was driven through the monolithic capillary by a syringe infusion pump (CP 2000, Silugao high-technology development, Beijing, China) to process the whole extraction procedure including pretreatment, sorption, clean-up and desorption as shown in Fig. 2. For pretreatment, 0.3 mL methanol in the syringe was ejected via the monolithic capillary at 0.05 mL/min, and then 0.5 mL phosphate buffer (0.8 M, pH 3.8) was washed the monolithic capillary at 0.15 mL/min. For the sorption as a similar way, 1 mL sample solution was pushed through the capillary at 0.15 mL/min, and then 0.2 mL phosphate buffer (0.8 M, pH 3.8) was driven through at the same velocity to get rid of the residual matrix in order to avoid the interference for separation. And then, the residual phosphate buffer solution was expelled from the pinhead and monolithic capillary by air via a clean syringe. For the adsorption, 0.05 mL methanol was ejected via the monolithic capillary at 0.05 mL/min and the eluate was collected into a vial for the subsequent analysis by HPLC.

2.6. Chromatographic apparatus and operating conditions

The HPLC system consisted of P200 pump (Elite Analytical Instruments, Dalian, China), a Hypersil ODS column (150 mm × 4.6 mm i.d., 5 μm), and Model UV-1 detector (RAININ Instrument Co. Inc., USA). The mobile phase was methanol/acetacetic ester/water (60:4:36 v/v/v). The flow rate of mobile phase was set at 1 mL/min. The injection volume was 10 μL, and the detector was set at 360 nm.

3. Results and discussion

3.1. Optimization of derivatization

As a typical derivatizing reagent for carbonyl compounds, the derivatization reaction of DNPH with aldehydes has been studied in detail [40–42]. Considering the particularity in the derivatization-PMME mode, such as the co-enrichment of excess DNPH with aldehyde derivatives, the derivatization conditions should be investigated carefully to attain the extraction efficiency as high as possible. The labeling reaction was affected by various parameters, including the pH of the reaction solution, the concentration of the reagent, the concentration of the buffer solution and the reaction temperature. The derivatization conditions were optimized in terms of both the high derivatization yield and high extraction efficiency. The labeling reaction of carbonyl compounds with DNPH can be accelerated in the acidic medium to gain the corresponding hydrazones. Optimization

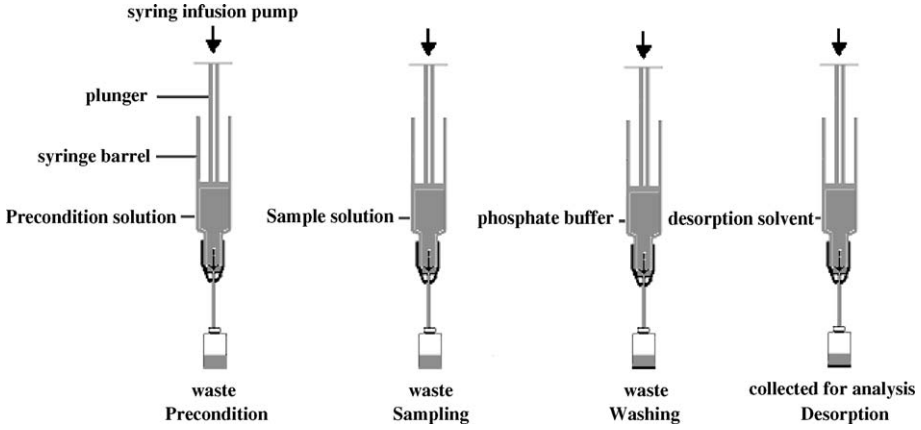

Fig. 2. A scheme of the PMME process [39].

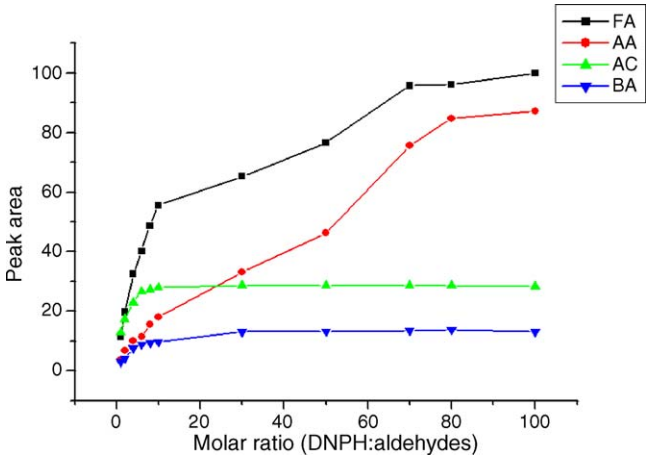

Fig. 3. Influence of DNPH concentration on the peak area of FA, AA, AC, BA hydrazones, spiking level was 4 μmol/L, the phosphate buffer was 0.8 M, pH 3.8.

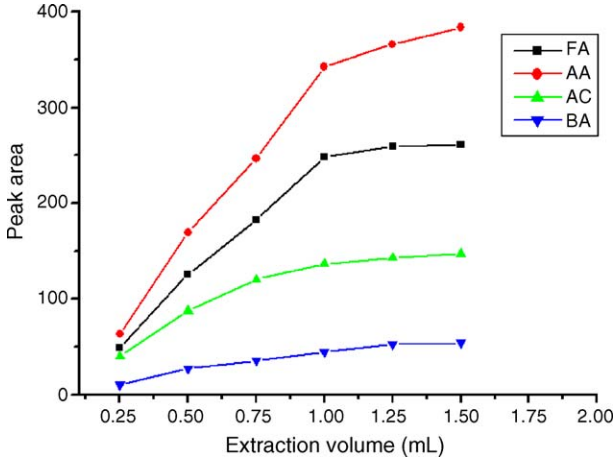

Fig. 4. The extracted sample volume profiles for FA, AA, AC, BA hydrazones using PMME, spiking level was 1.5 μmol/L. The optimal derivatization condition was used.

was performed in the pH range of 1.8–7.0 and the molar ratio of DNPH to aldehydes was 1:1. The derivatization yield, except for acrolein, did not change significantly over the pH range from 3 to 5 and had a maximum at about pH 4. In the case of the pH value being over 4, the sharp decrease in yield of acrolein derivative is probably relevant to the fact that acrolein is α,β-unsaturated aldehyde. Therefore, pH 3.8 of the phosphate buffer solution was suitably used as the reaction pH value for the derivatization.

With respect to the buffer concentration (pH 3.8), the yields of all aldehyde derivatives remained almost stable in the concentration range of 0.8–3 mol/L. Since the hydrazone derivatives can be salted out at high buffer concentrations, the buffer concentration was chosen as 0.8 mol/L.

The concentration of DNPH is critical for the labeling reaction and subsequent extraction. The effect of DNPH concentrations on the derivatization yields was investigated for aldehyde derivatives. The molar ratios of DNPH to total aldehydes were considered in the range from 1:1 to 100:1. As shown in Fig. 3, it was found that high reagent concentrations could give higher reaction yield. Constant derivatization yields of aroclein and butytaldehyde were achieved with the addition of 30-fold molar reagent excess to total molar aldehydes; however, the yield of formaldehyde and acetaldehyde were not constant until the 100 molar excess of the reagent was used. In views of the possible interference of excess DNPH to PMME and subsequent HPLC seperation, we could not choose the 100-fold molar reagent, but the use of 70-fold molar reagent was relatively appropriate for the derivatization. The effect of the reaction temperature was tested in the phosphate buffer solution (0.8 mol/L, pH 3.8) with the 70-fold molar DNPH excess to aldehydes. Between 25 and 40 °C, the reaction yield of the latter was slightly higher than the former after reacting one hour. Consequently, the experiments were processed at 40 °C for 1 h. The calculated yields of aldehyde derivatives were generally around 90% for formaldehyde and acetaldehyde, about 85% for acrolein and butyraldehyde under the optimized derivatization conditions employed. These aldehyde derivatives can be stable at room temperature for 12 h according to the experiment result.

3.2. Optimization of conditions for PMME

To achieve the best extraction efficiency of the poly(MAA–EGDMA) monolithic capillary towards aldehydes derivatives, various conditions like the extraction equilibrium profiles, extraction flow rate, pH value and the influence of the reagent concentration for PMME need to be optimized. These optimization experiments were performed with the optimized derivatization condition and water samples spiked with 1.5 μmol/L of each aldehydes.

The extraction equilibrium profile was monitored by increasing the volume of the extracted sample from 0.25–1.5 mL at a constant rate. It can be seen from Fig. 4 that the yield of aldehyde hydrazones increased with the increasing volume of the extracted sample and the amount of DNPH-derivatives except formaldehyde were attained at 1.25 mL. For getting the sensitivity as high as possible, 1.25 mL was selected as the optimal volume of extracted analytes.

The flow rate of the sample solution was optimized in the range of 0.05–0.4 mL/min by 1.25 mL sample solution. The change of the flow rate had no obvious influence on the extraction efficiency. Therefore, the flow rate of 0.18 mL/min was selected considering the extraction time and the pressure of monolithic capillary column.

The effect of pH in range of 2–9 on the extraction efficiency was investigated. The result showed that the extraction efficiency decreased slightly in the alkaline conditions. This can be explained by the fact that the interaction between the analytes and the monolithic capillary was mainly based on the hydrophobic interaction. In the alkaline matrix, the amount of the ionized carboxylic groups of the polymer extraction phase increased, causing the decreased hydrophobic interaction between derivatives and the extraction phase, thus a slow decrease was observed in the high pH value range. As a result, the derivative solutions were kept at pH 3.8 in microextraction process.

According to the optimization results on derivatization, the derivatization yield of the formaldehyde and acetaldehyde were not constant even though the 100-fold molar reagent was used.

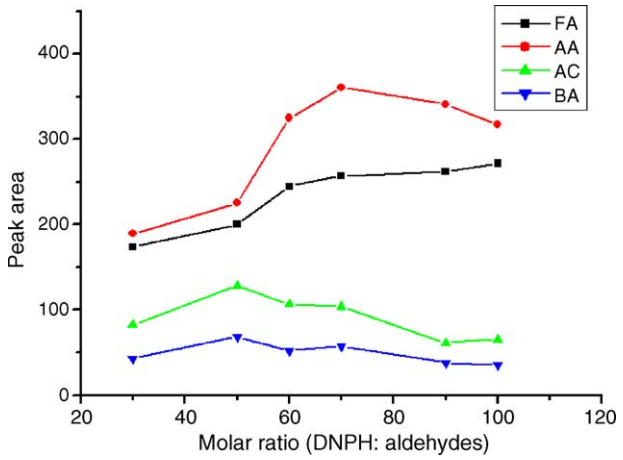

Fig. 5. Influence of DNPH concentration on the peak area of FA, AA, AC and BA hydrazones for the PMME, spiking level was 1.5 μmol/L. The optimal derivatization condition was used.

The monolithic capillary can also extract the excess DNPH because the derivatization was taken place in the sample matrix before sampling. The extraction efficiency of the derivatives may be affected for this reason. It was necessary to optimize the influence of DNPH concentration on PMME. The 1.25 mL volume of extracted analytes was chosen to make sure that the yield of all aldehyde derivatives was constant. It was indicated in Fig. 5 that overfull reagent could decrease the extracted yield of the aldehyde derivatives except formaldehyde. This exception was probably due to the fact that the yield of formaldehyde–DNPH derivative was increased with increasing the reagent even though the molar excess reached to 100. Consequently, the 70-fold molar DNPH was proved again to be suitable for derivatization and extraction.

The desorption procedure was optimized to achieve accurate quantification of the analytes. After sample extraction, 0.05 mL methanol was used to elute the analyte for three times, and then each 0.05 mL eluate was collected for detection. The result indicated that the first 0.05 mL methanol could elute more than 90% extracted aldehyde hydrazones from the monolithic capillary. Moreover, we optimized the flow rate of the desorption solution in the range of 0.025–0.1 mL/min, and the flow rate of 0.05 mL/min was used suitably in view of the pressure caused by the flow rate. The chromatograms obtained after PMME and direct injection were shown in Fig. 6, respectively. In comparison with the chromatogram of direct injection, it was observed that greater enhancement of the peak height of the derivatives was obtained for PMME. The calculated enrichment factors of the derivatives for formaldehyde, acetaldehyde, acrolein and butyraldehyde were found to be 17.6, 15.2, 13.4 and 14.2, respectively. Based on these optimal experiment conditions, the extraction yields of four aldehyde derivatives were 88.2%, 76.1%, 66.9% and 70.9%, respectively.

3.3. Validation of the method

The coexistence of some carbonyl compounds and the biological matrix effect that may interfere with the separation of

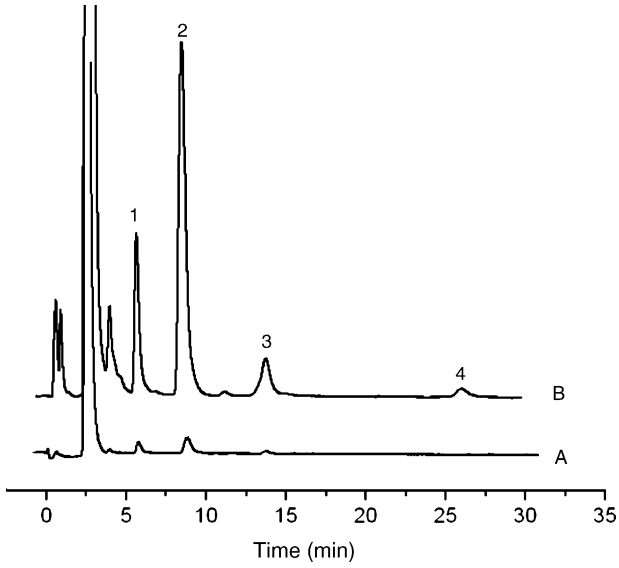

Fig. 6. Chromatograms of aldehyde hydrazones obtained by PMME (B) and direct injection (A). Peaks: (1) FA, (2) AA, (3) AC, (4) BA, spiking level was 1.5 μmol/L. The optimal derivatization and microextraction condition were used.

HPLC was investigated. Fortunately, the matrix effect produced by saliva, as shown in Fig. 7, did not influence the separation. Meanwhile, it was found that the derivatives of other carbonyl compounds could not affect the determination under the optimized separation conditions. The linearity of this method was obtained by constructing calibration curves in the corresponding concentrations. Due to its high viscosity, the saliva should be diluted with buffer solution prior to the extraction. The standard aldehyde solutions were added in the 1 mL saliva, and then the mixture was diluted to 5 mL with the phosphate buffer (0.8 mol/L, pH 3.8) to obtain the desired aldehyde concentration (in the range from 0.01 to 1.5 μmol/L). Subsequently, the

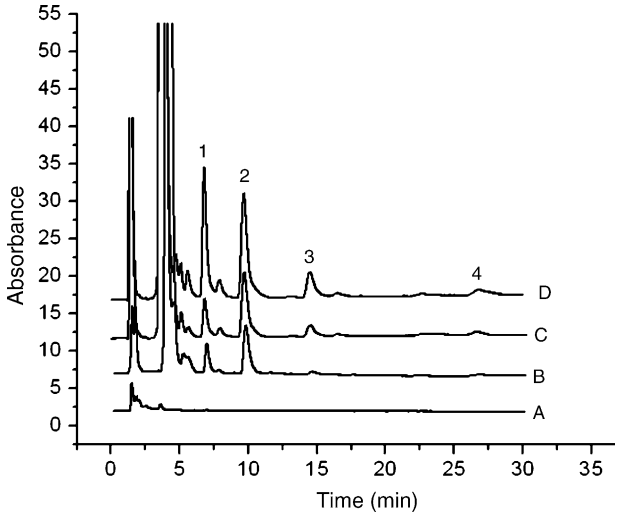

Fig. 7. HPLC profiles for PMME obtained from an underivatized saliva sample (A); saliva sample from a passive smoker (C); saliva sample from a nonsmoker before and after having smoked a single cigarette (B and D). Peaks: (1) FA, (2) AA, (3) AC, and (4) BA, respectively. The optimal derivatization and microextraction condition were used.

Table 1
The linear equation, detection limit of aldehydes for PMME from saliva samples

Compound	Formaldehyde	Acetaldehyde	Acrolein	Butyraldehyde
Linear equation[a]	$Y = 143X + 3.5$	$Y = 183.1X + 5.8$	$Y = 67.9X - 5.7$	$Y = 32.4X + 2.5$
R^2	0.9976	0.9924	0.9977	0.9986
Linear range (μg/L)	1.5–225	2.2–330	3.64–420	4.68–540
The limit of detection (μg/L)	0.43	0.71	0.99	1.40

[a] X is the concentration and Y is the peak area.

Table 2
Precision and recovery in saliva samples

Compound	Concentration (μmol/L)	Recovery (%)	Precision (R.S.D., %)	
			Intra-day ($n = 5$)	Inter-day ($n = 3$)
Formaldehyde	0.25[a]	75.6	7.3	9.1
	7.5[b]	81.4	6.1	6.4
Acetaldehyde	0.25	72.4	8.3	7.9
	7.5	84.8	3.1	7.1
Acrolein	0.25	77.9	7.1	8.2
	7.5	96.2	4.8	6.6
Butyraldehyde	0.25	85.6	8.6	7.4
	7.5	83.1	6.5	7.1

[a] Spiked concentration = 0.25 μmol/L for each aldehyde.
[b] Spiked concentration = 7.5 μmol/L for each aldehyde.

derivatization, extraction and separation were processed according to the methods described above. The data about linear range, linear equation, and detection limit were listed in Table 1. As seen from Table 1, good linearity can be observed with correlation coefficients more than 0.99 for the four aldehydes. The limits of detection (LODs) was studied with low concentration (0.05 μmol/L) and calculated with the signal-to-noise ratio of 3. The LODs of the four aldehydes were in the range of 0.43–1.40 μg/L.

The recovery of DNPH-derivatives spiked at low and high concentrations was studied by comparing the extracted amounts of aldehydes from saliva sample with the total amounts added. As shown in Table 2, the recoveries are in the range from 72.4% to 96.2%.

The reproducibility was determined by the inter-day and intra-day precision. The reproducibility of the proposed method was expressed as the relative standard deviation (RSD). The intra-day and inter-day precisions of the peak areas were both less than 10%.

3.4. Quantitative analysis of aldehydes in human saliva

Derivatization-PMME-HPLC was applied to the analysis of the aldehydes in real samples from one non-smoker, one passive smoker and one heavy smoker. As shown in Fig. 7, not surprisingly, trace amount of aldehydes was found in the nonsmoker saliva. As previously reported, formaldehyde and acrolein was the product of lipid peroxidation [43], acetaldehyde was created from microbial oxidation of ethanol [44]. The height of analyte peaks in C and D was higher than B. As seen from Table 3, high level of aldehydes was detected in the saliva from passive smoker and heavy smoker. The amount of aldehydes in the saliva of passive smoker was higher than the nonsmoker and, what is more, the amount of acrolein was more 10 times than the nonsmoker. It is seen that the amount of aldehydes in the saliva of the heavy smoker was much higher than the nonsmoker. After one nonsmoker smoked a single cigarette, the amount of aldehydes in saliva is found to increase quickly.

Table 3
Concentration (μg/L, $\bar{x} \pm s$) of formaldehyde, acetaldehyde, acrolein, butyraldehyde in saliva of different smokers

Saliva from	Formaldehyde	Acetaldehyde	Acrolein	Butyraldehyde
Non-smoker[a]	4.20 ± 0.31	21.14 ± 1.25	7.28 ± 0.49	23.80 ± 1.50
Passive smoker	14.41 ± 0.85	44.49 ± 2.94	168.18 ± 7.91	33.17 ± 2.58
Single cigarette smoker[a]	177.18 ± 14.00	202.63 ± 6.28	263.48 ± 13.70	95.19 ± 4.76
Heavy smoker	204.21 ± 10.82	214.08 ± 12.63	318.42 ± 19.74	199.02 ± 12.74

[a] Saliva samples were both from the same man.

4. Conclusion

The proposed novel PMME using a poly (MAA–EGDMA) monolith with derivatization for the analysis of trace low-aliphatic aldehydes in human saliva is proved as a relatively simple, sensitive and reliable technique. The high sensitivity achieved using only 1 mL saliva sample makes this method attractive for the trace determination of aldehydes in different biologic matrices such as plasma, urine. In addition, the good permeability of the monolithic capillary can achieve the whole extraction procedure quickly compared to traditional LLE technique. Due to the limited number of individual investigated, we could not provide more precise information on the role of low aliphatic aldehydes in the pathogenesis of smoke-related oral disease. Nevertheless, the proposed method has shown favorable feasibility for studying the interaction between aldehydes in saliva and the oral disease. We could certainly obtain more useful information of them as possible biomarkers for oral cancer by the analysis in a greater number of samples.

Acknowledgements

The authors gratefully acknowledge the support of the National Nature Science Foundation of China (Grants: 20275027 and 20172040) and Excellent Teacher Program of MOE, China.

References

[1] C. Streckfus, L. Bigler, I. Tucci, Cancer Invest. 18 (2000) 101.
[2] M.A. Belazi, T. Galli, D. Drakoulakos, Int. J. Paediatr. Dent. 8 (1998) 29.
[3] S. Bodis, A. Haregewoin, Biochem. Biophys. Res. Commun. 194 (1993) 347.
[4] M.P. Salaspuro, Crit. Rev. Clin. Lab. Sci. 40 (2003) 183.
[5] National Research Council, Formaldehyde and Other Aldehydes, National Academic Press, Washington, DC, 1981.
[6] M. Yazanpanah, X.P. Luo, L.J. Fisher, D.C. Lehotay, Free Radic. Biol. Med. 23 (1997) 870.
[7] S. Kato, G.C. Post, V.M. Bierbaum, T.H. Koch, Anal. Biochem. 305 (2002) 251.
[8] C.H. Deng, W. Zhang, J. Zhang, X.M. Zhang, J. Chromatogr. B 805 (2004) 235.
[9] C.H. Risner, P. Martin, J. Chromatogr. Sci. 32 (1994) 76.
[10] K. Rustemeier, R. Stabbert, H.J. Haussmann, Food Chem. Toxicol. 40 (2002) 93.
[11] R. Nagler, S. Lischinsky, E. Diamond, Arch. Biochem. Biophys. 379 (2000) 229.
[12] R. Nagler, S. Lischinsky, E. Diamond, J. Lab. Clin. Med. 137 (2001) 363.
[13] O. Hershkovich, J. Oliva, R. Nagler, Eur. J. Cancer 40 (2004) 1760.
[14] L. Annovazzi, V. Cattaneo, S. Viglio, E. Perani, Electrophoresis 25 (2004) 1255.
[15] V. Salaspuro, M. Salaspuro, Int. J. Cancer 111 (2004) 480.
[16] S. Takeda, S. Wakita, M. Yamane, K. Higashi, Electrophoresis 15 (1994) 1332.
[17] A. Asthana, D. Bose, S. Kulshrestha, S.P. Pathak, S.K. Sanghi, W.T. Kok, Chromatographia 48 (1998) 807.
[18] B. Cancho, F. Ventura, M. Galceran, J. Chromatogr. A 943 (2001) 1.
[19] W. Wardencki, J. Orlita, J. Namieśnik, Fresenius J. Anal. Chem. 369 (2001) 661.
[20] N. Li, C.H. Deng, X.M. Zhang, Anal. Chim. Acta 540 (2005) 317.
[21] K. Bachmann, I. Haag, K.Y. Han, R.Q. Schimitzer, Fresenius J. Anal. Chem. 346 (1993) 786.
[22] N. klaening, H. Karst, Fresinius J. Anal. Chem. 362 (1998) 270.
[23] A. Buldt, R. Lindahl, J.O. Levin, J. Environ. Monit. 1 (1999) 39.
[24] P. Marcos, M.P. Lué-Merú, R. Ricardo, Talanta 64 (2004) 1299.
[25] S.M. Leeuwen, L. Hendriksen, U. Karst, J. Chromatogr. A 1058 (2004) 107.
[26] M. Emmrich, H. Floss, B. Zühlsdorf, J. Chromatogr. B 795 (2003) 363.
[27] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[28] P.A. Martos, J. Pawliszyn, Anal. Chem. 70 (1998) 2311.
[29] B. Cancho, F. Ventura, M. Galceran, J. Chromatogr. A 943 (2001) 1.
[30] P. Vesely, L. Lusk, G. Basarova, J. Seabrooks, D. Ryder, J. Agric. Food Chem. 51 (2003) 6941.
[31] E.E. Stashenko, M.A. Puertas, W. Salgar, W. Delgado, J.R. Martýnez, J. Chromatogr. A 886 (2000) 175.
[32] E.E. Stashenko, J.R. Martýnez, Trends Anal. Chem. 23 (2004) 553.
[33] E.F. Hilder, F. Svec, J.M.J. Frechet, J. Chromatogr. A 1044 (2004) 3.
[34] T. Ikegami, N. Tanaka, Curr. Opin. Chem. Biol. 8 (2004) 527.
[35] Y. Shintani, X. Zhou, M. Furuno, H. Minakuchi, K. Nakanishi, J. Chromatogr. A 985 (2003) 351.
[36] S.F. Xie, F. Svec, J.M.J. Frechet, Chem. Mater. 10 (1998) 4072.
[37] Y. Fan, Y.Q. Feng, S.L. Da, X.P. Gao, Analyst 129 (2004) 1065.
[38] Y. Fan, Y.Q. Feng, J.T. Zhang, S.L. Da, M. Zhang, J. Chromatogr. A 1074 (2005) 9.
[39] M. Zhang, W. Fang, Y.F. Zhang, J. Nie, Y.Q. Feng, J. Chromatogr. A 1102 (2006) 5294.
[40] M.K.L. Bicking, W. Marcus Cooke, F.K. Kawahara, I.E. Longbottom, J. Chromatogr. 455 (1988) 310.
[41] S. Selim, J. Chromatogr. 136 (1977) 271.
[42] F. Van Hoof, A. Wittcox, G. Van Buggenbout, J. Jonssens, Anal. Chim. Acta 169 (1985) 419.
[43] H. Esterbauer, R.J. Schaur, H. Zollner, Free Radic. Biol. Med. 11 (1991) 81.
[44] N. Homann, J. Tillonen, J.H. Meurman, H. Rintamaki, C. Lindqvist, M. Rautio, Carcinogenesis 21 (2000) 663.