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An in-needle extraction technique in determination of organic compounds released from dental tissue conditioners incubated in artificial saliva

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ABSTRACT

The use of an in-needle technique for direct isolation of analytes from real liquid samples is a new proposal. The in-needle technique has been relatively seldom used for direct sampling of liquid matrix through the needle. In this work the in-needle technique has been applied for the determination of compounds evolved to artificial saliva from dental prosthetic materials. It has been shown that results from the experiment with in-needle device were at least comparable with those obtained with using well known solid phase extraction (SPE). It is worth to mention that in-needle extraction offers some advantages: lower consumption of solvent, shorter step-preparation time and reduced costs. The compounds released from prosthetic materials may affect the stability of tissue conditioners and limit their long-term use in the oral cavity. Examined soft dental materials have been found to be stable as minor amount of various species have been emitted from them. Results of the stability tests of soft dental materials with the use of in-needle device on sample preparation step enable their quick evaluation and estimations of their quality.

1. Introduction

Sample preparation is an important analytical step. Isolation of components and their concentration to a level enabling the quantitative determination is a major problem in most of modern analytical procedures. Conventional sample preparation methods most often require large amount of organic solvents, are relatively complicated and time-consuming. Two sample preparation techniques were used in this work: solid phase extraction (SPE) and an in-needle technique. By proper designing the in-needle system it was possible to avoid the main drawbacks of SPE [1] and SPME [2], such as labor expense and the need of careful handling of expensive.

Solid-phase extraction was used successfully and widely for the preparation of liquid samples. SPE was applied in many studies because, in comparison with other conventional methods, it has a lot of advantages. However, some limitations and drawbacks of SPE caused that more effective methods of sample preparation are developed continuously. In-needle extraction has many advantages. Therefore, it is desired to apply this technique to the direct liquid sample preparation. First of all, in-needle device is much cheaper. Moreover, the amount of solvent can be reduced to less than 1 mL and an in-needle extraction device can be used on-site. Its mobility might be treated as another advantage.

The needle trap device (NTD) was described in the literature very thoroughly, see e.g. [3–11] but over the years the in-needle technique was used to prepare gaseous samples. Isolation of analytes from water samples was most often combined with head-space (HS) or purge-and trap- (P&T) techniques, probably due to high flow resistance produced by a sorbent layer. Moreover, only a portion of analytes was passed through the sorbent bed, leading to problems in quantitative analysis and a possible loss of the sample components.

The novelty of the proposed in-needle device lies in the reduction of solvent consumption, cost reduction and possibility of the prediction of device efficiency basing on its macroscopic properties.

1.1. Direct analysis of the liquid sample

The resistance resulting from the flow through porous material causes considerable prolongation of the extraction process. The efficacy of the in-needle extraction device may be increased by using smaller sorbent particles of packing. This will be, probably, related to better recovery of the analyte. However, smaller size of particles restricts the flow rate and limits the sampling speed.

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Therefore, parameters affecting the efficiency of extraction should be examined and optimized.

Saito et al. prepared the extraction device, with a bundle of the polymer-coated filaments as the sorbent material packed into a needle. The extraction was made by pumping the aqueous sample solution into the needle extraction device. The sample flow rate during the extraction was optimized and it was found that the flow rate should be about 16 μL/min, which is equivalent to 1 mL/h [12].

In 2012 Pietrzyńska et al. [13] proposed a direct use of the in-needle technique for the preparation of liquid samples by passing water samples directly through the needle filled with a sorbent. The effectiveness of the NTD system was studied based on experimental data and chemometric evaluation.

The quantitative criteria for selection of parameters of the NTD system were derived by Kaczmarek et al. [14]. The conditions were formulated for the force exerted on syringe, the volume of tested solution, for the time of test and contact time of solution with the sorbent. The last two conditions allowed to establish limits for combinations of fundamental geometrical and macroscopic structural characteristics of the system.

1.2. Prosthetic materials

Tissue conditioners belong to a group of prosthetic materials that are used during the treatment of patients wearing removable partial or complete dentures. They release to the oral cavity compounds that may, when occurring in certain amounts, have a negative influence on human health. The factors accelerating their release may include liquids such as saliva or disinfectants, which, in contact with the prosthetic material, can penetrate the polymer network. Amount of released potentially dangerous compounds should be determined to obtain accurate information regarding the harmful effects of prosthetic materials on the human body.

In dental prosthetics, tissue conditioners are used for short-term relining of removable dentures. With time, the mucosal surface of a relined denture changes its shape adapting to the configuration of the soft tissues of the prosthetic base, which can lead to faster healing of the soft tissues, for example after a surgery. Tissue conditioners may also be applied in taking functional impressions. Patient, using a relined denture for some time, shapes its surface under physiological conditions. Such materials exist as powders (e.g., ethyl polymethacrylate) and liquids (monomers, plasticizers, and alcohols). After mixing both ingredients, bridges between the polymer particles are made and a three-dimensional network is created. In the case of polymers of low molecular mass, the cross-linking proceeds to a small extent, and the resulting substance takes the form of gel. Alcohol accelerates the process. The elution of alcohol and plasticizers leads to a quick deterioration of the adhesiveness and elasticity of tissue conditioners. Over time they become as hard as the denture corpus, which may lead to irritation and damage of the soft tissues of the base. In certain concentration plasticizers may show hormonal activity towards the human body tissues, acting as xenoestrogens. They demonstrate an ability to interact with the endocrine system and to modulate its activity in a way characteristic of estrogens. Therefore, it seemed advisable to conduct laboratory studies in order to evaluate qualitatively and quantitatively the chemical compounds that are eluted from tissue conditioners.

1.3. The aim of this study

The aim of this study was to determine the possibility of using in-needle device for direct liquid samples preparation and to identify as well as quantitatively determine the compounds released from tissue conditioners incubated in artificial saliva.

2. Materials and methods

2.1. Materials

The ageing of two commonly used soft lining materials, Softone (Bosworth Comp.) and Visco-Gel (Dentsply DeTrey), was examined (Table 1).

The methanol p.a., and dichloromethane p.a. were obtained from Polskie Odczynniki Chemiczne S.A. (Gliwice, Poland). The water was purified by the membrane technique using a RO5max system for water deionization (Bichmitte, Poland). The components for SAGF, i.e. Gal-Fovet artificial saliva [15] listed in Table 2 were obtained from Chempur (Poland).

The stainless steel needles with an internal diameter of 2.7 mm and the 10 mL gas-tight syringe were products of Danlab, Poland. The styrene–divinylbenzene copolymer (SDB-1) (J.T. Baker, Deventer, Holland) was supplied by Witko, Łódź, Poland. The SPE columns with the SDB sorbent (200 mg) were manufactured by J.T. Baker, Deventer, Holland.

2.2. Methods

2.2.1. Needle preparation

The filling needle process consists of 3 stages. First stage concerns the introduction of the first supporting layer into needle. Then, needle was filled with sorbent material with the dry pack

Table 2
The components of SAGF (pH 6.8), buffer 0.1 N NaOH or 0.1 N HCl.

Component	Concentration (mg/L)
NaCl	125.6
$NaHCO_3$	630.8
KCl	963.9
KH_2PO_4	654.5
KSCN	189.2
$CO(NH_2)_2$	200
$CaCl_2 \cdot 2H_2O$	227.8
NA_2SO_4	763.2
NH_4Cl	178

Table 1
Prosthetic materials used as tissue conditioners.

Marketing name	Producer	Components (%, m/m)			
		Powder		Liquid	
Softone	Harry J. Bosworth Company (USA)	Poly (ethyl methacrylate)	N/A	Acetyl phthalate Ethanol	N/A N/A
Visco-Gel	Dentsply DeTrey (Germany)	Poly (ethyl methacrylate)	50–100%	Paraffin oils Ethanol	50–100% 2.5–10%

method. The last stage concerns the introduction of the last supporting layer also serving as a filter.

2.2.2. Samples storage

Batches of the prosthetic materials were placed in artificial saliva and in ethanol. The SAGF was prepared according to Table 2.

The samples were placed in 40 mL vials filled with SAGF. The vials were incubated at 37 °C for four weeks. After isolation of the compounds from the artificial saliva, the samples were immersed in another portion of the fluid for next four weeks (static samples).

The second batch of the samples was placed in vials with a closed circulating flow of the fluid. The volume of the liquid was 40 mL and its temperature was 37 °C (dynamic samples). The third batch was placed in 2 mL vials filled with ethanol. The analytes were isolated from the samples by two methods: in-needle extraction and SPE.

2.2.3. Needle trap experiment

Sample preparation with the in-needle device involved sorbent conditioning (washing with a series of solvents), sample passing, drying of the bed and eluting the analytes with an appropriate solvent (Fig. 1).

The conditions of in-needle extraction were as follows:

- 2.7 mm I.D. needles; sorbent mass 0.074 g; sorbent layer length 35 mm; sorbent grain size 80 μm;
- 5 and 20 mL glass syringes;
- hexane, ethanol, dichloromethane were used separately for desorption of the analytes from the needle trap sorbent layer; solvent volume 0.2 mL.

Two types of samples were prepared using the in-needle technique:

- the test sample, containing a dibutyl phthalate was used to determine the process parameters;
- the real liquid sample, which was artificial saliva containing evolved compounds from dental tissue conditioners.

2.2.4. SPE experiment

The sample preparation procedure with the use of the SPE technique was similar to that described in the previous section. Desorption of the analytes from the sorbent layer was carried out with the use of three solvents: hexane, ethanol and dichloromethane. The solvent volume was 2.0 mL

2.2.5. GC–MS analysis

A GC–MS system (PerkinElmer, USA) equipped Rtx-5 MS, 5% diphenyl, 95% dimethyl polysiloxane capillary column; 0.25 mm I.D.; 30 m length was used in the analysis of separated analytes. Injection volume 1.0 μL. Carrier gas – helium; flow rate 1 ml/min. Injector and detector temperature – 180 °C. Column temperature programmed: initial – 80 °C for 1 min; increase 15 °C/min to 240 °C; final temperature kept for 14 min. Mass range: 30–400 *m/z*, ionization method: electron ionization (EI) and ionization energy: 70 eV.

Quantitative analysis: Determination of the amount of compounds eluted from the examined materials was carried out with the use of an internal standard procedure. Cymene was used as the internal standard. The amount of cymene depended on the amount of analytes in the sample and ranged from 0.1 to 1 μg.

3. Results and discussion

3.1. Optimization of in-needle extraction

The results of initial experiments showed that the amount of the analytes separated from the static and dynamic samples were very similar. The selection of the desorbing solvent indicated that the best recoveries were achieved by using dichloromethane (Fig. 2). Therefore, the results of the ageing experiments are presented for the static samples and desorbed with the use of dichloromethane. The achieved recovery was close to 90%, and determined LOQ and LOD values were respectively 0.8 μg/mL and 0.2 μg/mL.

The time contact of sample with the sorbent material is crucial for the recovery level. Therefore, effect of sampling flow rate was examined (Fig. 3). To achieve high recovery, the flow rate should not be higher than 2 ml/min. The same high recovery was achieved at a flow rate between 0.5 ml/min and 2.0 ml/min.

3.2. The analysis of released compounds

The identified compounds released from Visco-Gel and Softone are listed in Table 3. Butanol, dodecanol, hexadecane as well as

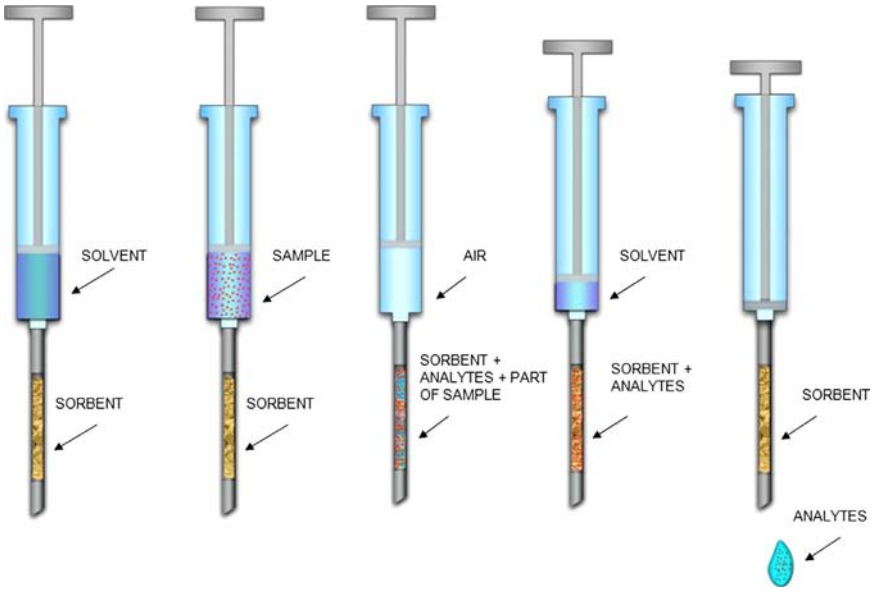

Fig. 1. Sample preparation with the in-needle device.

various esters (mainly phthalates) were isolated from SAGF solutions.

The results of quantitative analysis of released compounds from Visco-Gel and Softone are listed in Tables 4 and 5, respectively. Three types of sample preparation technique were used: the in-needle technique and also samples from pure ethanol were compared to classical SPE. Almost 5 mg of BPBG was released from 1 g of Visco-Gel into the artificial saliva.

A higher content of the analytes for both materials was found by using the in-needle technique. The exception was the content of hexadecane in the eluate from Visco-Gel and dodecanol in the eluate from Softone. The concentrations of the analytes were the highest in the samples aged in ethanol. It should be emphasized that there was a partial dissolution and deformation of the soft prosthetic material.

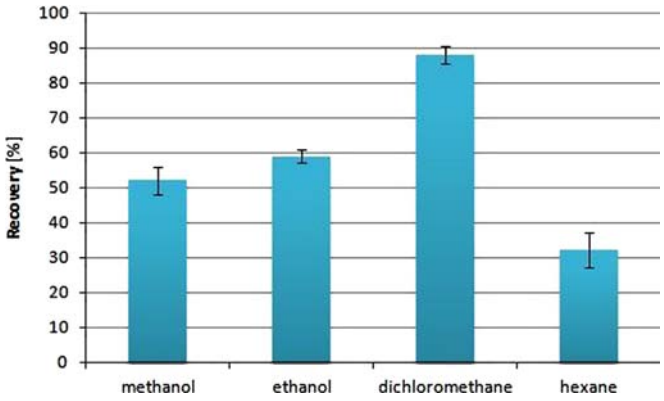

Fig. 2. Influence of the solvents on recovery of dibutyl phthalate.

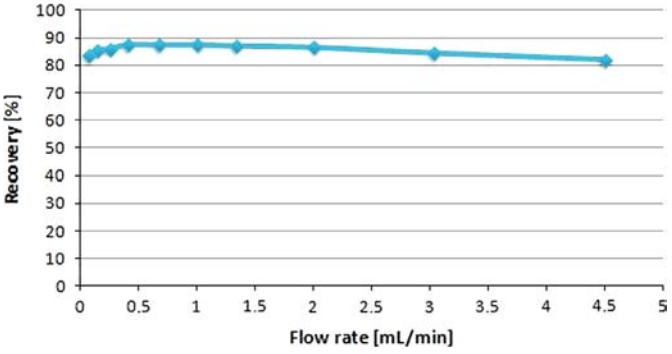

Fig. 3. The effect of sampling flow rate on dibutyl phthalate recovery.

The volume of solvent required to elute analytes from the in-needle device was determined (Fig. 4). The first 400 μL of solvent was sufficient to completely elute the analytes from the sorbent.

The same material sample was placed in another portion of SAGF (for further four weeks) to check if any compounds were additionally released during the prolonged time of storing in the artificial saliva. Butyl carbobutoxymethyl phthalate was again extensively eluted from the Visco-Gel. Dibutyl phthalate and bis (2-ethylhexyl) adipate were eluted in a small amount (Fig. 5). Hexadecane and diethyl phthalate were extensively eluted from the Softone while only slight amounts of dibutyl phthalate were found (Fig. 6).

Phthalate esters are potentially harmful when exposed to humans. Though most phthalate esters have low acute toxicity, the reproductive system is particularly sensitive to them which may possibly lead to abnormal sexual development and birth defects. Animal studies suggested that despite the effects of phthalates on the liver and kidneys, the primary effects are on reproduction and development [16].

The eluted compounds belong to solvents (e.g., butanol) and plasticizers (e.g., dibutyl phthalate). The tested materials released chemical compounds in the environment of the artificial saliva. It means that prosthetic materials are not unaffected by the conditions found in the oral cavity.

Both extraction methods (SPE and in-needle) were effective in the qualitative analysis of the prosthetic materials used in treatment with partial or complete removable dentures. The difference between two extractive techniques: in-needle and SPE, used in the preparation of samples is not significant, although noticeable. For example, in the case of dibutyl phthalate the difference in

Table 4
Results of quantitative analysis for Visco-Gel after four weeks of ageing.

Compound	Sample preparation		
	Ethanol [mg/g of material] (RSD)	In-needle [mg/g of material] (RSD)	SPE [mg/g of material] (RSD)
Butanol	14.9 (1.3)	1.55 (2.5)	1.54 (3.3)
Dodecanol	0.28 (3.2)	0.03 (4.7)	0.02 (3.4)
Hexadecane	0.37 (3.3)	0.01 (4.9)	0.01 (3.9)
Dibutyl phthalate	5.38 (2.1)	0.09 (4.0)	0.06 (3.4)
Butyl carbobutoxymethyl phthalate	183.1 (4.5)	4.58 (2.7)	4.12 (3.3)
Bis (2-ethylhexyl) adipate	2.26 (1.8)	0.11 (2.2)	0.10 (1.7)
Diisooctyl phthalate	0.37 (1.4)	0.00	0.00
Butoxyethyl butyl phthalate	1.2 (1.0)	0.00	0.00

Table 3
Identified compounds eluted from the soft prosthetic materials.

Material	Compound	Abbreviation	CAS number	Retention time (min)	Molecular formula
Visco-Gel	Butanol		71-36-3	2.3	$C_4H_{10}O$
	Dodecanol		112-53-8	6.5	$C_{12}H26O$
	Hexadecane		544-76-3	7.4	$C_{16}H_{34}$
	Dibutyl phthalate	DBP	84-74-2	10.1	$C_{16}H_{22}O_4$
	Butyl carbobutoxymethyl phthalate	BPBG	85-70-1	12.7	$C_{18}H_{24}O_6$
	Bis(2-ethylhexyl) adipate	DEHA	103-23-1	13.2	$C_{22}H_{42}O_4$
	Diisooctyl phthalate	DIOP	27554-26-3	15.3	$C_{24}H_{38}O_4$
	Butoxyethyl butyl phthalate	BEBP	33374-28-6	17.7	$C_{18}H_{26}O_5$
Softone	Butanol		71-36-3	2.3	$C_4H_{10}O$
	Dodecanol		112-53-8	6.5	$C_{12}H26O$
	hexadecane		544-76-3	7.4	$C_{16}H_{34}$
	Diethyl phthalate	DEP	84-66-2	7.6	$C_{12}H_{14}O_4$
	Dibutyl phthalate	DBP	84-74-2	10.1	$C_{16}H_{22}O_4$
	Butyl carbobutoxymethyl phthalate	BPBG	85-70-1	12.7	$C_{18}H_{24}O_6$

Table 5
Results of quantitative analysis for Softone after four weeks of ageing.

Compound	Sample preparation		
	Ethanol [mg/g of material] (RSD)	In-needle [mg/g of material] (RSD)	SPE [mg/g of material] (RSD)
Butanol	5.08 (1.2)	0.38 (2.2)	0.30 (0.5)
Dodecanol	0.37 (2.8)	0.00	0.03 (1.2)
Hexadecane	33.45 (3.7)	1.09 (2.1)	0.83 (1.0)
Diethyl phthalate	79.6 (3.1)	2.04 (2.1)	1.91 (4.2)
Dibutyl phthalate	169.5 (5.0)	0.04 (3.0)	0.03 (3.9)
Butyl carbobutoxymethyl phthalate	2.1 (2.3)	0.02 (4.1)	0.01 (2.9)

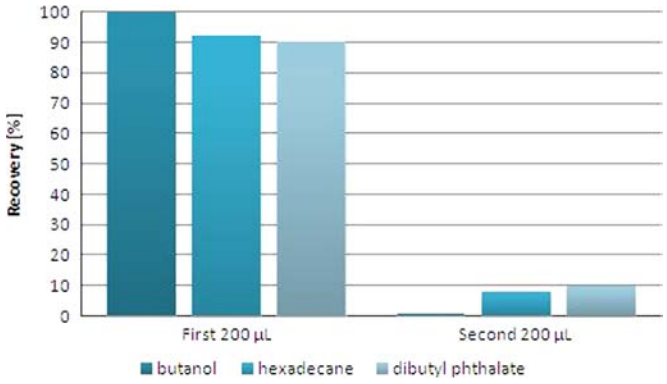

Fig. 4. Influence of volume of desorbing solvent on recovery of several components from Visco-Gel (desorption was carried out twice with 200 µL).

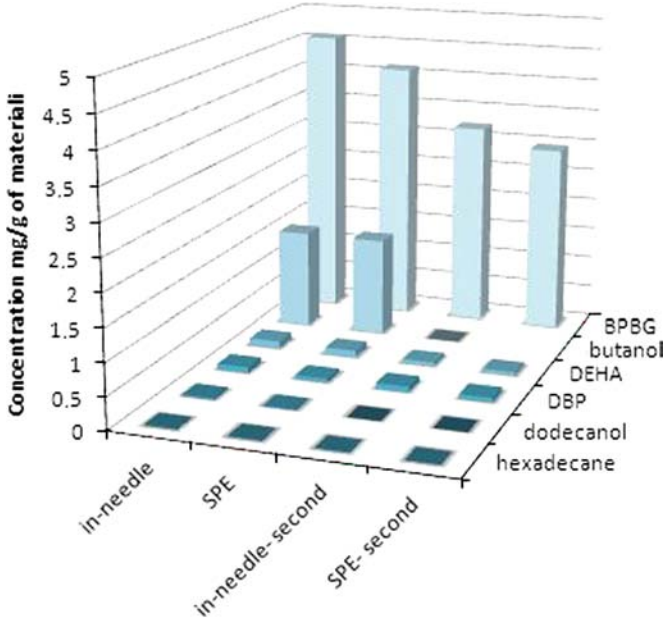

Fig. 5. Concentration of analytes in eluates from Visco-Gel for different extraction procedures (ageing in first and second portions of SAGF; 37 °C; each step four weeks).

the amount of analyte reaches tens of micrograms. Advantage of in-needle technique over SPE is probably due to influence of the amount of eluent used in the desorption process. Too large or small volume of solvent used for elution step is associated with a loss of analytes in the case of SPE [17–19]. Additionally, the vacuum generated by the pump chamber (used in the SPE) can cause loss (sucking) of the prepared sample.

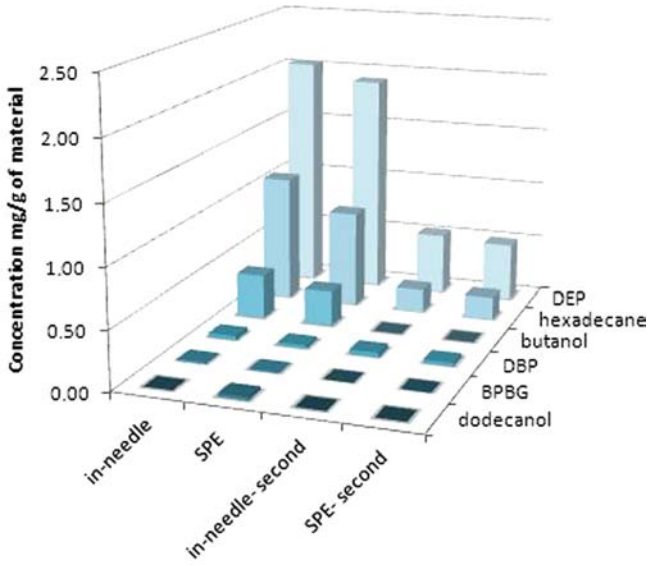

Fig. 6. Concentration of the analytes in eluates from Softone for two extraction procedures; (ageing in first and second portions of SAGF;37 °C; each step four weeks).

4. Conclusions

Various chemical compounds, classified as solvents and plasticizers, are released from relining prosthetic materials such as Visco-Gel and Softone exposed to the environment of the oral cavity. The compounds that released may affect the stability of tissue conditioners and limit their long-term use in the oral cavity.

The in-needle and SPE techniques are useful tools for isolation of chemical species released from soft dental materials. Higher extraction efficiency (lower loss of analytes) and lower consumption of the eluent are the main advantages of in-needle technique.

Acknowledgments

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