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Determination of Residual Monomers Released from Soft Lining Materials with the use of HPLC

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Abstract

A study was carried out to examine the post polymerized leachability of three non phthalic and four phthalic residual monomers, from twelve commercially available soft lining materials, using HPLC. Specimens of equal dimensions were constructed from each brand of material following a standardized procedure and were stored in three different conditions of storage i.e. distilled water, artificial saliva and a binary mixture of ethanol-water, with the resulting liquids providing samples for analysis in the HPLC apparatus. Three different experiments were performed for each brand of material and each condition of storage, in order to examine the parameters time and temperature. The results obtained from this study suggest that a wide spectrum of residues is diffusing out of the twelve examined soft lining materials. The non phthalic compounds were leaching at high concentrations while all the phthalates examined exhibited different degrees of elusion commensurate with the storage condition, brand of material and type of experiment. The main non phthalic component extracted from all the materials was methyl methacrylate, while the mainly extracted phthalic compound was different from each material. The level of elusion seems to be increasing dependent on time, medium of storage, and temperature as well.

Keywords: *soft lining materials, phthalic monomers, non phthalic monomers, leachability*

Introduction

Edentulous patients with thin mucosa, sharp residual ridges, or bony undercuts, wear dentures bearing for short or long periods of time soft lining materials, for their viscoelastic properties, especially elasticity [1-3]. The residual monomer content of soft lining materials (after processing procedure) has been suspected of being a contributing factor to chemical irritation, sensitization or allergic reactions of the oral mucosa [4]. The cytotoxicity and estrogenic activity of phthalate esters for dental use as plasticizers was concerned as well [5-7]. Some authors have claimed that monomers, by reacting with molecular oxygen, may produce formaldehyde, which is known to cause hypersensitivity reactions [8, 9]. Despite the inconclusive clinical trials, some studies, by cell culture technique have provided strong evidence that the cured denture base resins have a direct cytotoxic effect on cells [10, 11]. The mechanical and physical properties of soft lining materials probably depend on factors inherent in their chemical composition, about which little information can be found in the literature, and contain additives that have been incompletely studied. For example, phthalates, inter-fused with high polymers, as plasticizers in order to increase flexibility, extensibility, and to enhance working properties, are potentially considered as toxic compounds [12-14]. It has been suggested that the loss of plasticizers change the viscoelastic properties of these materials [15-16]. When the environment has a very high affinity for the plasticizer it may migrate or be extracted from the polymer matrix at a rate dependent on the ability of the

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plasticizer to diffuse through the resin matrix to the attracting media [17]. In medical use, phthalates were detected in human plasma perfused through haemodialysis units and have been found in the tissues of diseased patients who had received transfusion [18, 19]. Phthalates have come under close investigation [20, 21] although some phthalates have shown low toxicity while no toxicity was reported for others [22]. A literature search revealed that a number of papers [16, 23] have dealt with the leachability of plasticizers as a means of studying the physical or mechanical properties of tissue conditioning materials, while thus far less attention has been given to the leachability of plasticizers from heat cured acrylic resins, may be due to their long history in clinical use without reports of major biological or toxicological problems [24]. Over the years several methods have been used for the determination of residual monomer content [25, 26] among which Gas Chromatography circumvented many of the technical problems [27-29].

Materials and Methods

Table 1. **The commercial products examined in the study**

Type of material	Commercial products	Polymerization form	Manufacturers
Tissue conditioner	Coe Comfort	Autopolymerizing form	GC America Inc Chicago IL USA
Tissue conditioner	Kerr Fit	Autopolymerizing form	Kerr Europe AG Basel
Tissue conditioner	Visco Gel	Autopolymerizing form	Dentsply De Trey GmbH
Resilient liner	Myerson Soft Denture Liner	Heat-polymerizing form	Austenal, Inc Chicago, IL USA
Resilient liner	Molloplast B	Heat-polymerizing form	Detax GmbH & Co KG Ettingen Germany
Resilient liner	Soft Liner	Autopolymerizing form	GC Dental Prod. Corp Japan
Resilient liner	Ufi Gel C	Autopolymerizing form	VOCO Cuxhaven Germany
Resilient liner	Ufi Gel P	Autopolymerizing form	VOCO Cuxhaven Germany
Resilient liner	Vertex Soft	Heat-polymerizing form	Dentimex BV Zeist Netherlands
Resilient liner	Kooliner	Autopolymerizing form	GC America Inc Illinois USA
Resilient liner	Rebaron	Autopolymerizing form	GC Dental Products Corp. Japan
Resilient liner	Flexacryl Hard	Autopolymerizing form	Lang Dental Mfg Co. Inc. IL USA

In alternative approaches, High Performance Liquid Chromatography (HPLC) has offered a convenient method of determining various organic materials and evaluating low residual monomer values, which can be compared under identical conditions [30, 31].

The purpose of the present study was to identify and quantify several monomersleaching out of three tissue conditioning and nine resilient lining commercially available products, after curing. High Performance Liquid Chromatography (HPLC) was chosen as a sensitive and reliable analytical method.

Three tissue conditioners and nine resilient liners commercially available (listed in Table 1), were investigated. Samples of each product 10x10x2 mm in dimension were constructed following standard practice, in a polymer-monomer mixing ratio, mixing time and form of polymerization, according to manufacturers' recommendations.

After processing, the cured specimens were placed in three storing liquid environments, a) artificial saliva b) fresh distilled water and c) a binary mixture of 40% ethanol and 60% water. Aliquots of the storing media (for each material, storing medium and type of experiment), provided samples suitable for analysis. injected into the chromatographic column and analyzed with the HPLC apparatus.

The monitoring of the release of seven monomers (listed in Table 2), methacrylic acid (MA), methyl methacrylate (MMA), diallyl phthalate (DAP), n-butyl methacrylate (N-BMA), butoxy carbonyl methyl butyl phthalate (BPBC), dibutyl phthalate(DBP), and dioctyl phthalate (DOP), as probable or potential constituents of the materials under investigation, were studied.

HPLC was chosen as the analytical monitoring method. The apparatus used was HPLC Liquid Chromatograph Model LC-10AD Shimadzu Japan. A reserved-phase partition chromatographic column Zorbax ODS 25 cm long and 4.6 mm bore was used with a solvent system comprising of a combination of isocratic and gradient elusion. Acetonitrile and water were used as solvents (Figure 1).

The chromatograms shown in Figure 2 were run at a flow rate of 0.4 mL min^{-1} with an operating pressure of 80 bars. The samples were injected by means of a rotary valve injector equipped with a 20 µl sample loop. The UV detector (SPD-10AV, Shimadzu) was set at 210 nm. Firstly was performed a qualitative

analysis by comparing the chromatograms of the samples with those of the standards. For the quantitative determinations, the peak-areas of the chromatograms obtained from the leaching monomers were compared to a standard calibration curve obtained by plotting the peak areas against known concentrations of the seven monomer-standards. The peak areas were measured electronically with a computing integrator (C-R5A Chromatopac Shimadzu). By referring the peak areas from the HPLC analysis of the samples, to the standard calibration curve, the amount of each component was calculated in $ng\mu L^{-1}$. The limit of derection for the different monomers lies in the range 0.02-0.10 $ng\mu L^{-1}$. The recorded chromatograms were integrated three times and the relative intensities of the lines were obtained as the mean value of the integration.

Table 2. **The seven standard samples used in this study.**

Standard materials	Lot	Manufacturer
Methacrylic acid (MA)	2012044	Fluka Germany
Methyl methacrylate (MMA)	16304-077	Aldrich U.K.
Diallyl phthalate (DAP)		Aldrich U.K.
N-butyl methacrylate (N-BMA)	67H3453	Sigma Chemical Co. USA
Butoxy carbonyl methyl butyl phthalate (BPBC)		Aldrich U.K.
Dibutyl phthalate (DBP)	67H0327	Sigma Chemical Co. USA
Dioctyl phthalate (DOP)	24772-078	Aldrich U.K.

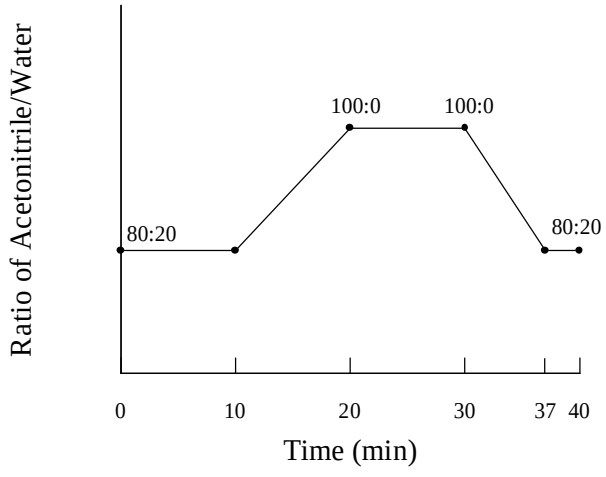

Figure 1. **Acetonitrile concentration in the mobile phase**

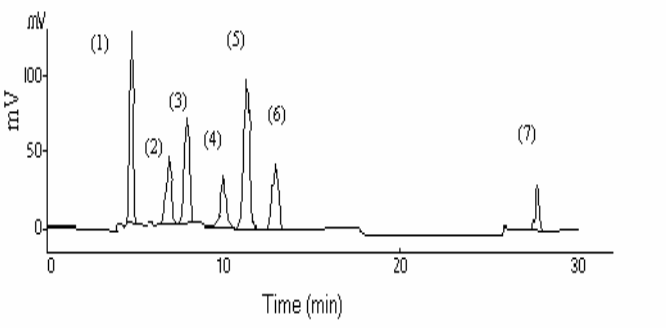

Figure 2. **Typical chromatogram of the seven standard monomers**

(1):methacrylic acid (4.6 sec), (2):methyl methacrylate (6.7 sec), (3):diallyl phthalate (7.6 sec), (4):n-butyl methacrylate (9.7 sec), (5):butoxy carbonyl methyl butyl phthalate (10.9 sec), (6):dibutyl phthalate (12.6 sec), (7):dioctyl phthalate (26.9 sec).

Three different experiments were performed for each material and each storage condition (artificial saliva, water, ethanol-water), in order to investigate the effect of the parameters time and temperature, on the extent of release of monomers from the polymerized materials. In the first type of experiment, monitoring was performed every 24 hours for 5 days at room temperature. Specimens of each material were kept under stirring in 8ml of each storing solution at room temperature 23±2°C. Aliquots of 20 μL were analyzed every day for a period of 5 days.

In the second type of experiment, monitoring was performed at constant temperature up to 6 hours. Specimens of each material were kept under stirring in 8mL of each storing solution for 6h at a constant temperature of 40°C. Every two hours 20 μL aliquots were analyzed.

In the third type of experiment, monitoring was performed at a temperature range from 10°C to 40°C. Specimens of each material were kept for one hour at temperatures 10°C, 20°C, 30°C and 40°C under stirring in 8ml of each of the storing solutions. At the end of every hour 20 μL aliquots were analyzed.

Statistical analysis was conducted with the S-plus 2000 (MathSoft Inc., MA, USA) package. The statistical methods used were the one-way and multiple-way ANOVA with Tukey's post-hoc tests and the paired *t* test. The *p* values were considered as statistically significant whenever they were less than 0.05. For *p* values smaller than 0.001 the difference were considered as very significant. The standard deviations of the chromatographic measurements were 1.5% (*n*=5).

Results

The HPLC analysis of the twelve soft lining materials provided data about the release of almost all the examined monomers Figures 3-5 show the variations in residual monomers leaching out of one indicative commercial product under several storing conditions and three types of experiments. The representative material is the *Coe comfort*, a tissue conditioning material of autopolymerizing type.

The results of the elemental analysis demonstrated that the leaching monomers were similar in the examined materials. A time-associated effect was found, as the residues extracted increasingly from most materials, experiments, and under most storage conditions. Some differences were observed between brands with respect to the amounts of the additives present that were leached, probably due to the differing chemical formulations of the products.

Specifically the analysis revealed the following:

Methacrylic acid (MA). The experiments showed that methacrylic acid (MA) leached out increasingly from all the examined materials, all the experiments and all storing media, with an exception of material Coe comfort in artificial saliva at the second experiment. A significant difference between 0.11ng/μL and 420.51 ng/μL (p value ranged between 0.7 and <0.001) in concentration levels was observed. Methacrylic acid (MA) eluted in low concentration levels from tissue conditioners (Coe comford, Kerr fit, Visco gel) and silicon rubber based resilient liners (Ufi gel C, Ufi gel P), while higher levels of concentration were detected leaching out of Flexacryl hard and Molloplast B materials, under all experiments and storage media

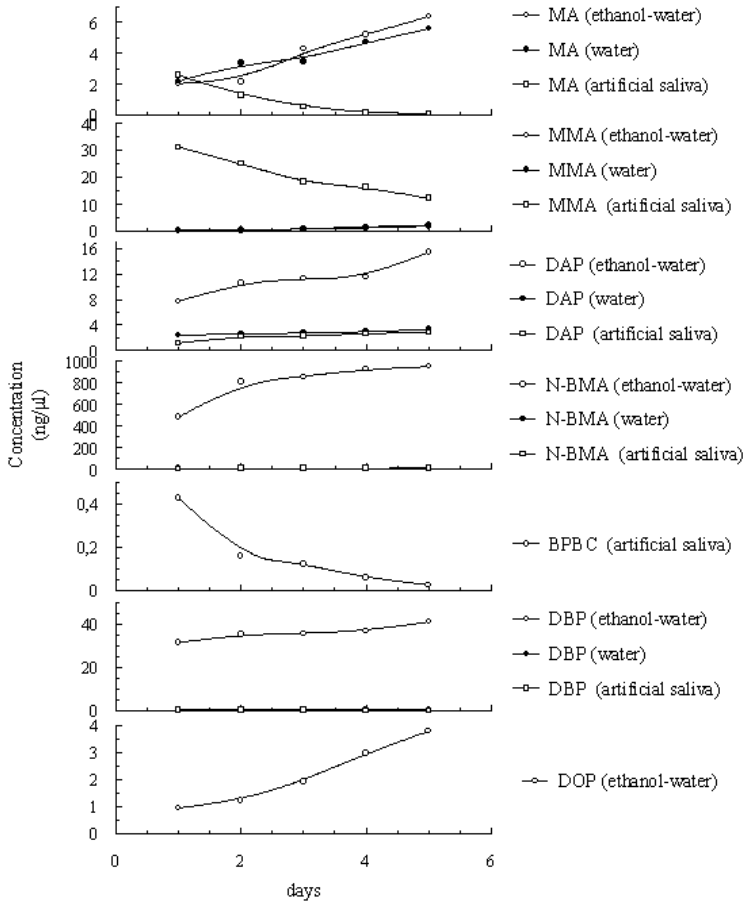

Figure 3. **Rate of elution of monomers leaching out of Coe Comfort in artificial saliva, distilled water and binary mixture ethanol: water 40%:60% at every day monitoring at room temperature**

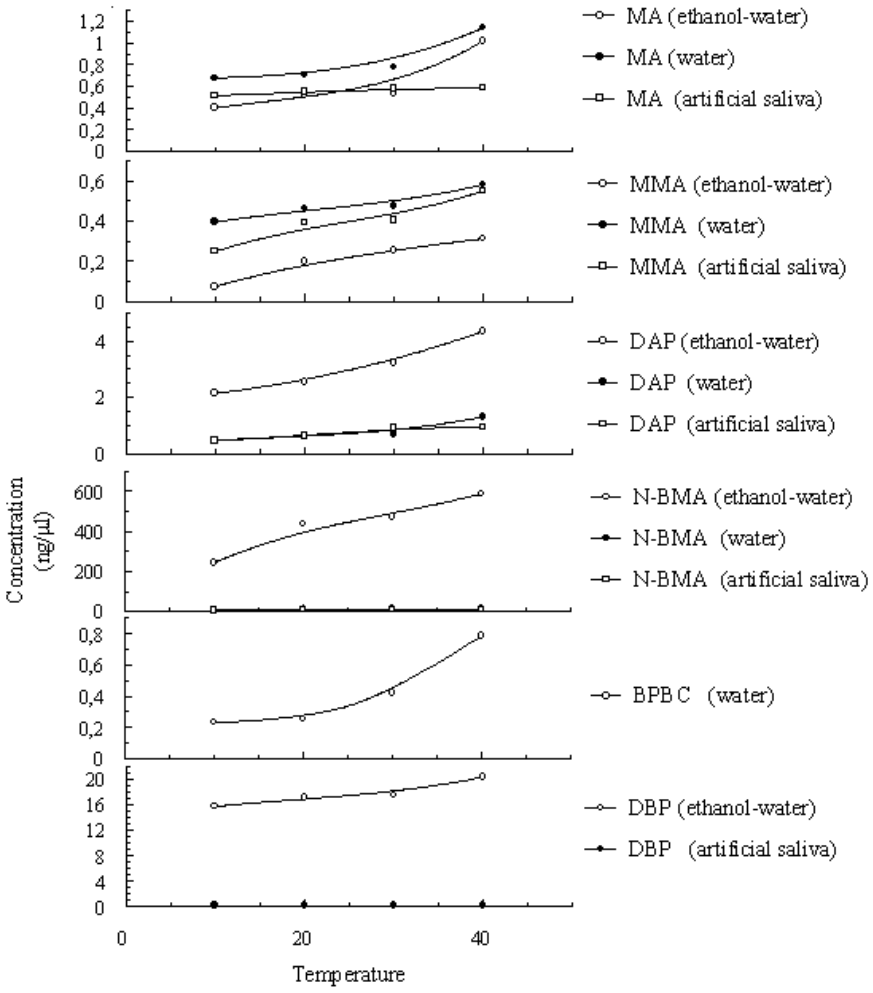

Figure 4. **Rate of elution of monomers leaching out of Coe Comfort in artificial saliva, distilled water and binary mixture ethanol: water 40%:60% by elevating temperature 10 °C to 40 °C**

Temperature of 40^{0}C during the second experiment, and the rising of temperature during the third experiment, produced decreasing of MA elusion from all the examined materials under all storing media (p <0.05) with the exception of materials Visco gel, Soft liner, Kooliner and Rebaron stored in ethanol/water (p=0.98, 0.2, 1, 0.16 respectively).

Methyl methacrylate (MMA) leached out increasingly from nine out of the twelve examined materials. No elusion of MMA was observed from Molloplast B and Ufi gel C in all experiments and storing conditions, while small amounts were detected leaching out from Ufi gel P in ethanol/water.

A significant difference between 0.09ng/µL and 1422.87 ng/µL (p value ranged between 0. and <0.001) in concentration levels was observed. The lower concentrations of Methyl methacrylates (MMA) were detected leaching out from Myerson soft and soft liner and the higher from Vertex soft and Rebaron.

Temperature elevation generally produced decrease of MMA elusion from most of the examined materials and storing media, with the exception of Myerson soft in ethanol water at the second and third experiment, and Rebaron under all storing conditions, where the highest concentration of 1422.87 ng/µL (p <0.001) was observed.

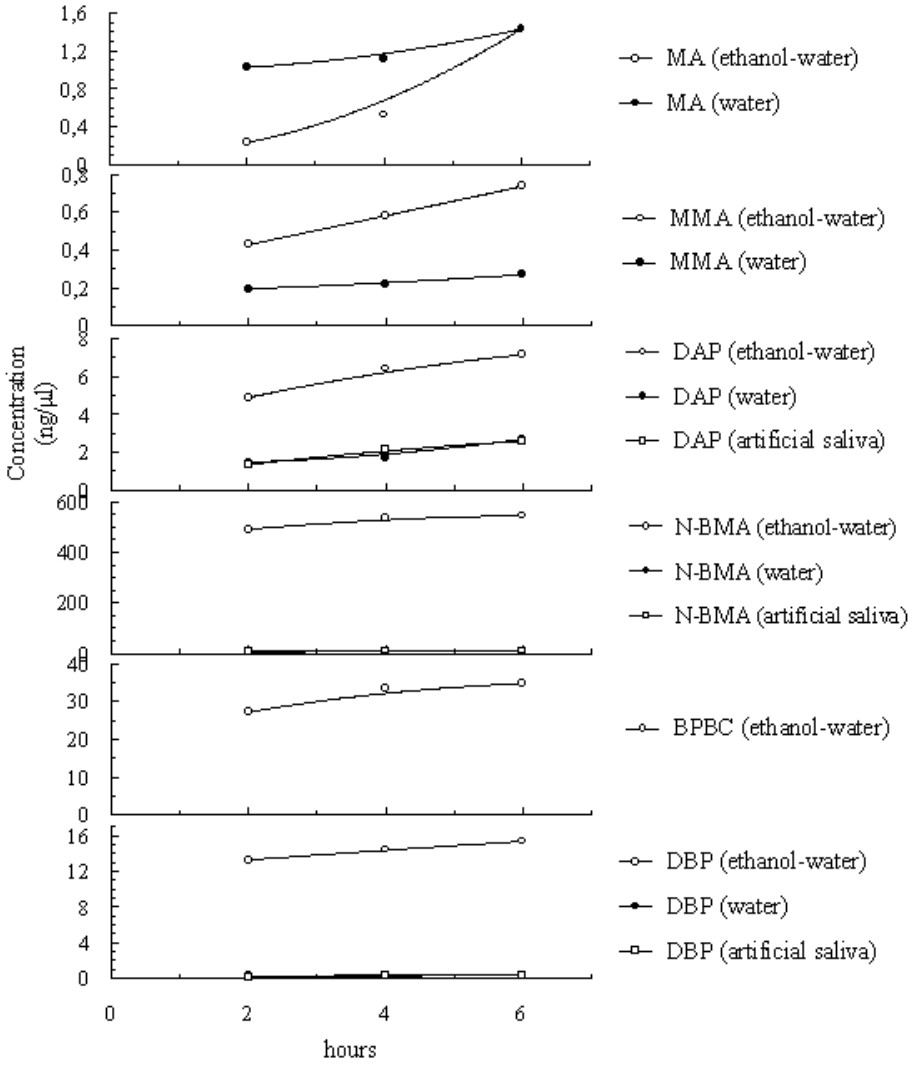

Figure 5. **Rate of elution of monomers leaching out of Coe Comfort in artificial saliva, distilled water and binary mixture ethanol: water 40%:60% at constant temperature 40 °C for six hours**

Diallyl phthalate (DAP) was detected leaching out increasingly from seven materials at all experiments and storing conditions, at concentration levels ranging between 0.02 ng/μL and 198.50 ng/μL.

No elusion of DAP was observed from Vertex soft and Myerson soft in all experiments and storing conditions, as well as from Flexacryl hard during the first and third experiments, although was detected leaching out from Flexacryl hard during the second experiment. The storing condition where Diallyl phthalate significantly eluted was the binary mixture ethanol-water 40%: 60% (p>0.05 in few cases).

Temperature elevation generally produced decrease of DAP elusion from most of the examined materials and storing media, with the exception of Rebaron in ethanol-water at the second and third experiment, where the higher concentration levels of 1952.00 ng/μL and 1581.93 ng/μL (p <0.001) respectively were observed.

***n*-Butyl methacrylate (N-BMA)** was detected leaching out increasingly, from nine out of the twelve examined materials, in most storing media during all the experiments, at levels of concentration ranging between 0.01ng/μL to 2.562.91 ng/μL.

No elusion of N-BMA was detected under all storing media and experiments from Myerson soft, Ufi gel and Vertex soft during the first experiment. N-BMA was observed leaching out from these materials only when samples were stored in ethanol-water in association with elevation of the temperature (from Myerson soft during the second experiment, from Vertex soft and Ufi gel C during the third experiment) Generally, during all the experiments, the higher levels of N-BMA concentration were observed when the samples from most materials were stored in ethanol-water storing condition (p <0.001).

Butoxy carbonyl methyl butyl phthalate (BPBC) was released at an increasing rate of elusion under all storage conditions and experiments, from five of the examined materials (Soft liner, Ufi gel C, Ufi gel P, Kerr fit, Visco gel).

No elusion was observed from material Molloplast under all storage conditions and experiments, while from Myerson soft in distilled water, leached out at low levels of concentration, during the third experiment.

Dibutyl phthalate (DBP) was detected leaching out in low levels of concentration increasingly, from ten out of the twelve examined materials, under all storing conditions and during all experiments.

No elusion was observed from one material Molloplast B under all storage conditions and experiments, while from Flexacryl hard leached out during the second and third experiment.

Storing in ethanol-water condition increased the elution of BPBC (p <0.05), except for Visco gel, Ufi gel C and Rebaron, during the third experiment respectively p=0.11, 0.64, 0.97, in association with storing in artificial saliva). Storing of Kerr fit and Myerson soft at room temperature in ethanol-water, produced significantly high concentration levels of 997.20ng/μL and 311.6 ng/μL, respectively.

Temperature of 40^0C during the second experiment, generally decreased the elusion of (DBP). Increased leaching was detected from Kooliner and Visco gel stored in distilled water, from Coe comfort stored in artificial saliva and ethanol-water binary mixture, and from Kerr fit stored in ethanol-water, where the highest concentration of DBP at the level of 1440.85ng/μL was observed.

Dioctyl phthalate(DOP) was detected leaching out of only one material (Ufi gel C) under all storage conditions and experiments.

Under all storage conditions and experiments, no elusion was observed from materials Visco gel, Molloplast B, Soft liner and Flexacryl hard.

DOP leached from the other examined materials, under storing conditions and experiments deferring to each material. Generally the elusion of DOP was detected at low levels of concentration ranging from 0.19ng/μL to 13.78 ng/μL.

Neither storing in ethanol-water nor elevation of temperature produced increasing of elusion (p>0.05).

Discussion

The results obtained from this study suggest that a whole spectrum of residues diffuse out of the twelve soft lining materials examined. The statistical error in the method applied was about 8-10%, and the variations of the calculated results indicate trends rather than accurate values of concentrations.

All the experiments revealed that the organic component extracted consists primarily of Methacrylic acid (MA), which was detected leaching out from all the examined materials except for Coe comfort stored in artificial saliva at constant temperature of 40^0C.

A significant difference at the levels of concentration between the examined materials was observed. Unexpected low levels of concentration, detected leaching out from methacrylic tissue conditioning materials, while, also unexpectedly, MA leached out, at low levels of concentration, even from silicon rubber based resilient liners.

Methacrylic acid (MA), eluted at low levels of concentration from the silicon rubber based material Myerson soft, as was expected, but the material Molloplast B, although silicon rubber basedl, showed unexpected high levels of concentration.

Methyl methacrylate (MMA), leached out during fewer (than MA) experiments, but exhibited

higher levels of concentration under different storing conditions. No elusion was detected from silicon rubber based materials Ufi gel C, Ufi gel P, and Molloplast B, as expected.

The third non phthalic monomer, *n*-Butyl methacrylate (N-BMA), was observed leaching out in most storing media during all the experiments, but, in association with the other non phthalic monomers, at low levels of concentration.

The phthalic monomers, despite their- water non soluble- nature, leached out from most examined materials, during most experiments and storing media, except for Dioctyl phthalate(DOP), which eluted in few cases.

The release of phthalic monomers exhibited low levels of concentration, but it was observed, that from each material a different phthalate monomer was eluted, approaching the high levels of the non phthalic monomers concentration. This is probably due to the chemical composition of each material.

In this study, phthalic and non-phthalic monomers, eluted at a consistently increasing rate, indicating further elusion

Storing of soft lining materials in ethanol-water, did not increase the levels of concentration, contrastingly to the elusion of denture base materials in that storage condition [*24*].

Water absorption and monomer elusion from soft liners stored in aqueous solutions, has been revealed in earlier studies [3*2-35*].

Leaching of monomers from soft lining materials was detected at previous studies, concluding that higher levels of concentration eluted in artificial saliva than in distilled water [36,37].

These results are in partial agreement to the results obtained from the present study, as some monomers leach out at higher levels in artificial saliva, while other monomers in distilled water.

This discrepancy could be explained if the different brands of the materials and the potentially different sensitivities of the analytical methods are taken into account.

Jones et al (1988) revealed that different bands of the materials exhibit different compositional compounds [1*2*].

Pthalic esters as plasticizers in chemical composition of soft liners, have been investigated in several studies, and the higher loss of plasticizers from soft liners was observed during function [*38-40*].

Monitoring every 24h ay room temperature, demonstrated a continuing process of leaching, within the 5 days of the first experiment.

These results support a previous study carried out by Graham et al (1991), in which the in vitro and in vivo release of monomers was observed for a period up to 14-30 days [41].

The results of the elemental analysis demonstrated differences between materials with respect to the amounts of the additives present that were leached, probably due to the different chemical formulation of the products.

The non phthalic monomers examined eluted at low levels from silicon rubber based materials, as was expected, and the elusion of the phthalic monomers ranged at low levels as well, especially from Ufi gel C and Ufi gel P.

This observation supports a previous study in which water absorption and residue release from soft liners polymerized at room temperature, ranges at low levels[*35*].

The HPLC analysis used in this study offers a convenient method of evaluating various monomers at very low levels. As a result of this technique a relatively large number of almost all the examined monomers were detected.

The extraction of Dibutyl phthalate (DBP) among other residues has been detected in earlier investigation as well [*12*]. Therefore, after the liquid powder is mixed, a whole spectrum of monomers leach out from soft lining materials, placed in the mouth of the patient and they remain in a liquid environment afterwards. Moreover the tissue conditioners are replaced within 7-15 days.

The amounts of the residues that a patient swallows are unknown, but the penetration of Dibutyl phthalate (DBP) across the mucosa has experimentally been proven [42].

In a study carried out by Graham et al (1991), was observed that the in vivo leaching of plasticizers is higher than the in vitro detected [16].

The biological evaluation of the monomers analyzed is beyond the scope of this study, but the potential toxicity of dental items has come under increased concern [43-46].

Conclusions

In summing up the results of the various analyses carried out in the present study, the following conclusions can be drawn.

1. HPLC offers a convenient method of evaluating various monomers at low concentrations, which can then be studied and compared under the same conditions.
2. A lot of non phthalic and phthalic residues diffuse out of the soft lining materials examined, at an increasing rate of extraction.
3. The non phthalic monomers detected leaching at high levels of concentration.
4. Although in smaller amounts, a large number of phthalates were detected leaching out of the examined materials.
5. The level of leaching phthalic and non phthalic monomers from silicon rubber based materials is generally low.
6. Further consideration must be given to the chemical ingredients of dental materials and the manufacturers should make specific formulation details available.

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