Anal Bioanal Chem (2005) 381: 1336–1346
DOI 10.1007/s00216-005-3059-x

SPECIAL ISSUE PAPER

**Afrodite Sofou · Irene Tsoupi · John Emmanouil
Miltiades Karayannis**

HPLC determination of residual monomers released from heat-cured acrylic resins

Received: 30 September 2004 / Revised: 10 January 2005 / Accepted: 17 January 2005 / Published online: 10 March 2005

Abstract HPLC was used to examine the leachability of three non-phthalic and four phthalic post-polymerized residual monomers from three commercially available heat-cured acrylic resins. Specimens of equal dimensions were constructed from each brand of material by following the standardized procedure and were stored under three different conditions, namely, distilled water, artificial saliva, and a binary mixture of ethanol/water. The resulting liquids provided samples for analysis by HPLC. Three different experiments were performed for each brand of acrylic and each storage condition in order to examine the effects of parameters, particularly time and temperature. The results obtained from this study suggest that a wide spectrum of residues diffuse out of the three examined acrylic resin materials. The non-phthalic compounds were leached at high concentrations, whereas all the phthalates examined exhibited different degrees of elusion commensurate with the storage condition, brand of material, and type of experiment. It seems that a significant quantity of non-phthalic and phthalic residues diffuse out of the acrylic resin materials examined. The main component extracted was methyl methacrylate, the level of which seems to be time-dependent and decreases for a period of up to 5 days when resins are stored in distilled water at room temperature.

Keywords Acrylic · Phthalic resins · Non-phthalic resins · Leachability

A. Sofou · J. Emmanouil
Department of Removable Prosthodontics,
School of Dentistry, Aristotle University of Thessaloniki,
54124 Thessaloniki, Greece

I. Tsoupi · M. Karayannis (✉)
Laboratory of Analytical Chemistry, Department of Chemistry,
University of Ioannina, 45110 Ioannina, Greece
E-mail: mkaragia@cc.uoi.gr
Tel.: +30-26510-98406
Fax: +30-26510-98793

Introduction

The residual monomer content of denture base resins (after processing procedure) has been suspected of being a contributing factor to chemical irritation, sensitization, or allergic reactions of the oral mucosa [1]. In addition, hypersensitivity reactions to heat-polymerized acrylic dentures, which had been worn for between 5 and 10 years, have been reported [2].

Some authors have claimed that monomers, by reacting with molecular oxygen, may produce formaldehyde, which is known to cause hypersensitivity reactions [3, 4]. Several investigators have suggested that true allergy is rare and that most mucosa reactions appear because of a variety of other reasons, for instance, trauma, fungal infection with candida species, poorly fitting dentures, unbalanced occlusions, and chemical irritation from monomers [5–6]. However, despite inconclusive clinical trials, some studies using cell culture techniques have provided strong evidence that the cured denture base resins have a direct cytotoxic effect on cells [7, 8].

The mechanical and physical properties of denture base materials depend on their composition about which very little information can be found in the literature. Modern acrylic resins contain additives that have been incompletely studied. For example, phthalates, interfused with high polymers, as plasticizers in order to increase flexibility, extensibility, and to enhance working properties, are potentially toxic compounds. 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 plasticizer to diffuse through the resin matrix to the attracting media [9]. In medical use, phthalates were detected in human plasma perfused through hemodialysis units and have been found in the tissues of diseased patients who had received transfusion [10, 11]. Phthalates have come under close investigation [12, 13]; however, some phthalates have shown low or no toxicity [14].

A literature search revealed that a number of papers [15, 16] have dealt with the leachability of plasticizers as a means of studying the physical or mechanical properties of tissue-conditioning materials; thus far, less attention has been given to the leachability of plasticizers from heat-cured acrylic resins. This may be due to their long history in clinical use without reports of major biological or toxicological problems. However, since denture base materials are to have long-term contact with tissue, it is important to be able to develop an analytical methodology to assess the presence of unreacted monomers remaining after the polymerization. Over the years several methods have been used for the determination of residual monomer content [17, 18] among which gas chromatography circumvented many of the technical problems [19–21]. 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 [22, 23].

The purpose of the present study was to identify and quantify several monomers leaching out of three commercially available products of heat-cured acrylic resin denture base materials after polymerization. HPLC was chosen as a sensitive and reliable analytical method.

Materials and methods

The following commercially available products were investigated: Triplex (Ivoclar AG, Schaan Liechtenstein), Paladon 65 (Kulzer & Co. GmbH, Germany), and Type 15 (Ivoclar AG, Schaan Liechtenstein). Molds were produced by fixing stainless steel blocks with dimensions of 10×10×2 mm in stone according to conventional dental flasking techniques. The dough was prepared in a polymer–monomer mixing ratio 3.5:1 (by volume). The mold separation, packing with two trial closures and clamping procedure, followed standard practice. The curing cycle ran overnight at 90°C for 10 h. Polishing procedures were not carried out in an attempt to avoid contamination.

The cured specimens were stored at room temperature (23 ± 2°C) in de-ionized water for 24 h before further investigation. The entire specimens were used in order to simulate a situation close to clinical use.

The release of seven monomers (listed in Table 1) was monitored; these were selected as probable or potential constituents of the materials under investigation.

HPLC was chosen as the analytical monitoring method. The apparatus used was a Shimadzu HPLC chromatograph Model LC-10AD. A Zorbax ODS reserved-phase partition chromatographic column (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 (Fig. 1).

Twenty-four hours after processing the specimens were rinsed and stored in three liquid environments: artificial saliva, fresh distilled water, and a binary mixture of 40% ethanol and 60% water. After treatment a sample suitable for analysis was injected into the chromatographic column and analyzed by HPLC.

Table 1 The seven standard samples used in this study

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

The chromatograms shown in Fig. 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. A qualitative analysis was performed first 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

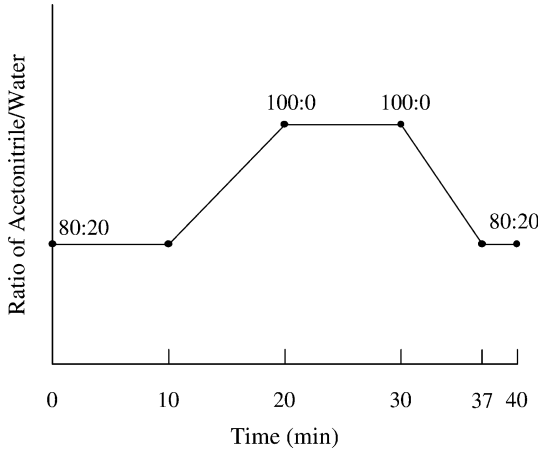

Fig. 1 Elution profile for acetonitrile/water mobile phase

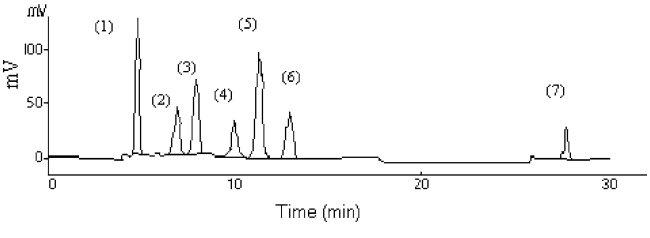

Fig. 2 Typical chromatogram of the seven standard monomers

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 μL^{-1}. The limit of detection for the different monomers lies in the range 0.02–0.10 ng μL^{-1}. The recorded chromatograms were integrated three times and the relative intensities of the lines were obtained as the mean value of the integration.

Three different experiments were performed for each acrylic resin material (Triplex, Paladon, Type 15) and each storage condition (artificial saliva, water, and ethanol/water) in order to investigate the effects of 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 h for 5 days at room temperature. Specimens of each material were kept under stirring in 8 mL of each storage 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 h. Specimens of each material were kept under stirring in 8 mL of each storage solution for 6 h at a constant temperature of 40°C. Every 2 h, 20-μL aliquots were analyzed.

In the third type of experiment, monitoring was performed at a temperature in the range from 10°C to 40°C. Specimens of each material were kept for 1 h at 10, 20, 30, and 40°C under stirring in 8 mL of each of the storage solution. 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 was considered as very significant. The standard deviations of the chromatographic measurements were 1.5% ($n = 5$).

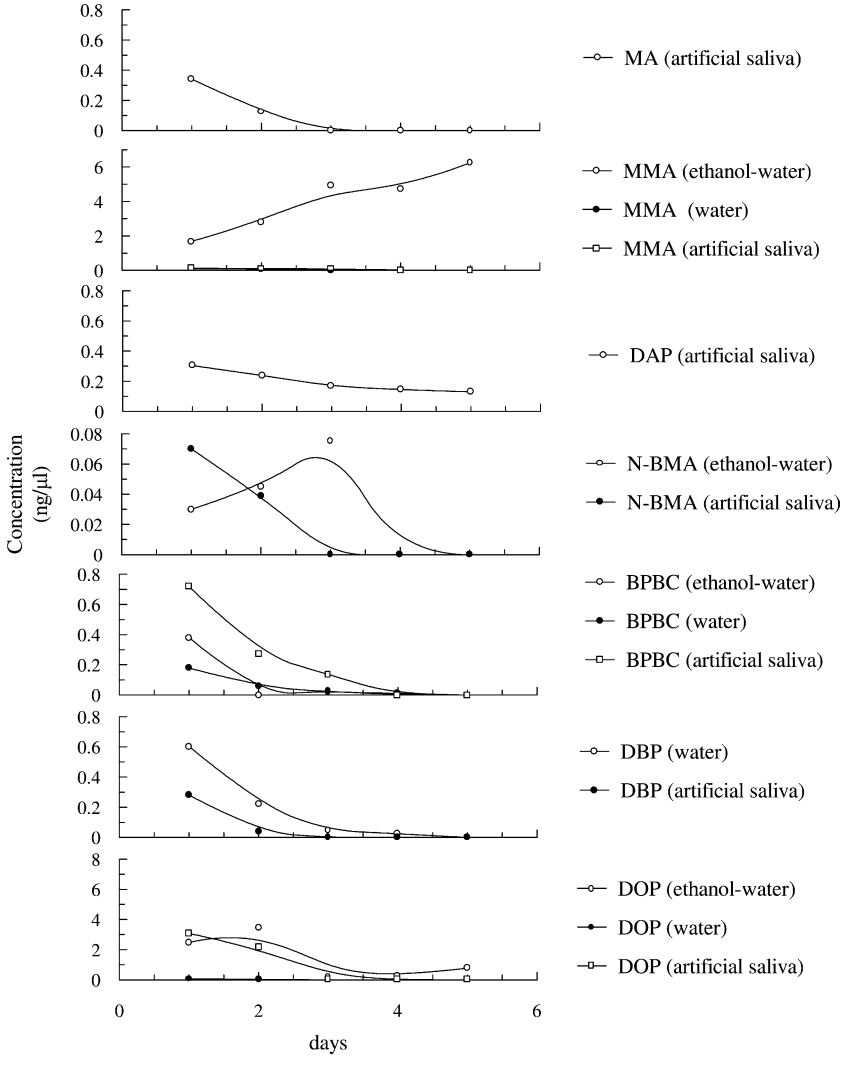

Fig. 3 Rate of elution of monomers leaching out of Triplex in artificial saliva, distilled water, and a binary mixture of 40% ethanol and 60% water during daily monitoring at room temperature

Results

Monitoring every 24 h for 5 days at room temperature

Methacrylic acid (MA) eluted from Triplex specimens stored in artificial saliva and Paladon stored in water decreased during the period up to 3 days. In contrast, MA leaching from Paladon specimens stored in ethanol/water increased, reaching a maximum concentration of 0.875 ng μL^{-1} on the 5th day.

The experiments showed that methyl methacrylate (MMA) leached out from all the acrylic resins examined. The various storage conditions influenced the rate of leaching of MMA. For the specimens stored in water and artificial saliva the elusion decreased with time for approximately 4–5 days, whereas in ethanol/water the rate of elusion was positive up to the 5th day.

Diallyl phthalate (DAP) leached out of all the examined acrylic resins but was not detected under all storage conditions. The rate of extraction decreased after the 2nd or 4th day with the exception of Type 15 specimens stored in artificial saliva. A quantitative determination showed that concentrations of 0.02–0.50 ng μL^{-1} remained even after 5 days.

n-Butyl methacrylate (N-BMA) was detectable at decreasing and very low levels only from Triplex specimens stored in artificial saliva and ethanol/water in this experiment. No chromatogram was obtained after the 4th day.

Butoxy carbonyl methyl butyl phthalate (BPBC) was detected leaching out of all the examined resins under all storage conditions. The rate of extraction decreased with time with the exception of Paladon specimens stored in ethanol/water and Type 15 in artificial saliva, for which even on the 5th day concentrations of 0.18 and 0.51 ng μL^{-1}, respectively, were measured.

Dibutyl phthalate (DBP) was released from all the resins under examination under all storage conditions except for the Triplex specimens stored in ethanol/water. Although the rate of release decreased continuously in most of the cases, small amounts of residue were measured even on the 4th day. The concentration measured for the Type 15 specimens, stored in ethanol/water, was 0.59 ng μL^{-1} even on the 5th day. Paladon specimens in

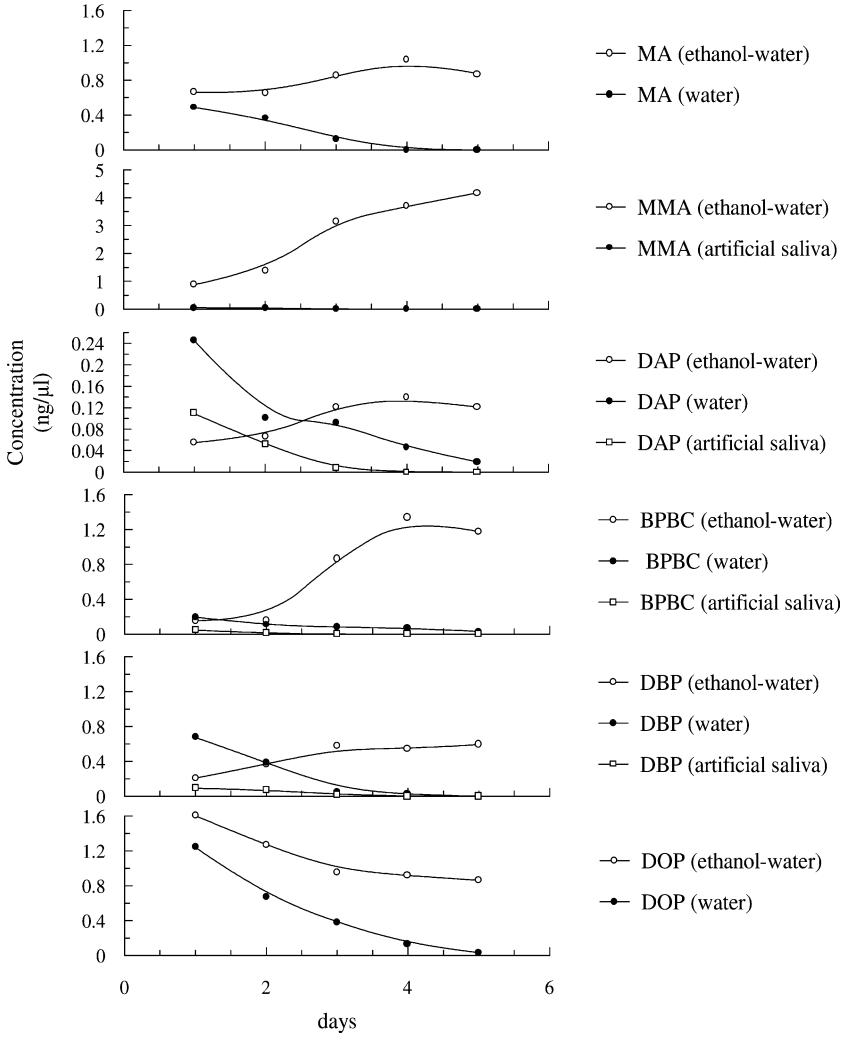

Fig. 4 Rate of elution of monomers leaching out of Paladon in artificial saliva, distilled water, and a binary mixture of 40% ethanol and 60% water during daily monitoring at room temperature

ethanol/water and Type 15 in artificial saliva eluted 0.59 and 0.41 ng μL^{-1}, respectively, on the 5th day indicating continued leaching.

Dioctyl phthalate (DOP) was detected leaching out of all the resins examined and under all storage conditions with the exception of Paladon specimens stored in artificial saliva. Although decreasing in most cases, small amounts of the residue were still detected on the 5th day. The rate of extraction from Triplex specimens stored in artificial saliva increased, reaching a concentration of 2.1 ng μL^{-1} on the 5th day.

Comparison of materials

The HPLC analysis of the three acrylic resins provided data about the release of almost all the examined monomers. Figures 3, 4, and 5 show the variations in residual monomers leaching out of the three resins under several storage conditions. The results of the elemental analysis demonstrated that the leaching monomers were similar in the three resins. A time-associated effect was found, as the residues extracted from all materials and under most storage conditions became noticeably smaller with the time. The main variations between resins occurred according to the 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.

Within 5 days all the monomers under examination had been leached out of Triplex resin except for DAP stored in artificial saliva and DOP stored in the ethanol/water mixture. Measurements on Paladon resin demonstrated that on the 5th day small amounts of DAP, BPBC, and DOP persisted when specimens were stored in water, while concentrations of 0.12–4.18 ng μL^{-1} of all the examined monomers were still leaching from specimens stored in the ethanol/water mixture. As regards the Type 15 resin, analysis on the 5th day revealed that no elusion was observed when specimens were stored in water, whereas MMA, DAP, BPBC, and DOP

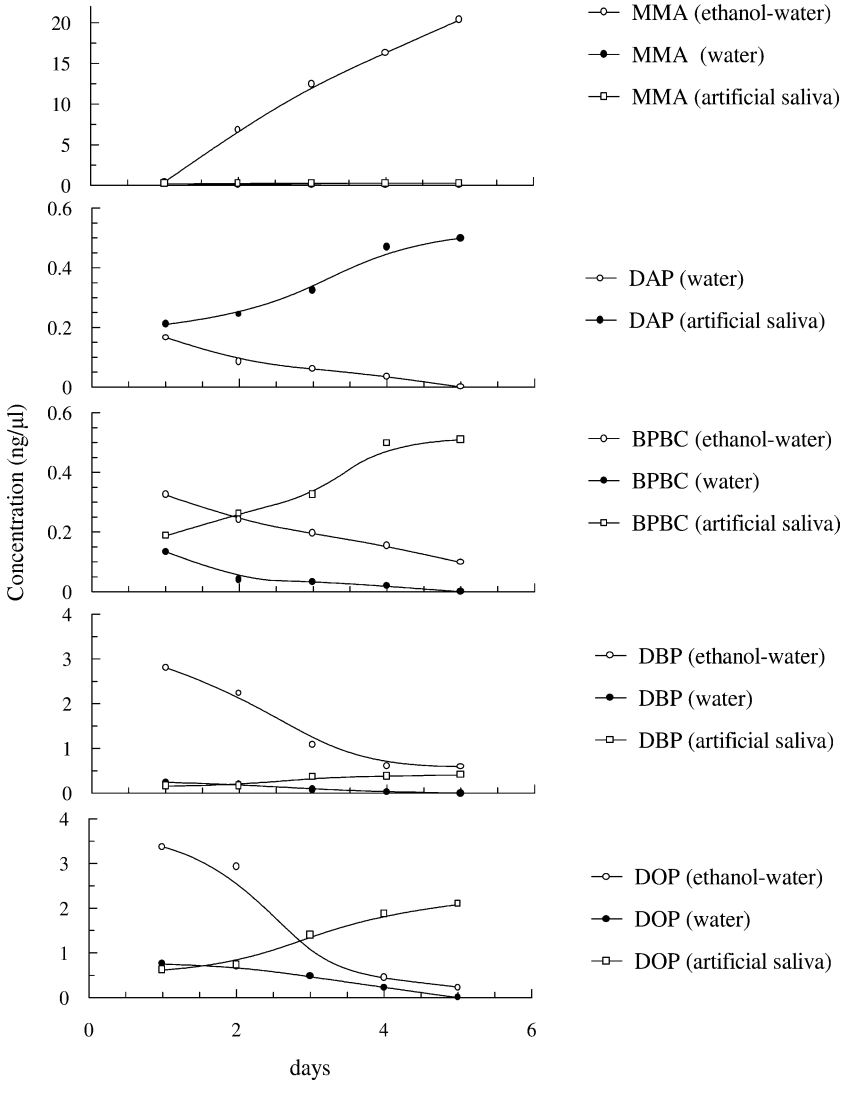

Fig. 5 Rate of elution of monomers leaching out of Type 15 in artificial saliva, distilled water, and a binary mixture of 40% ethanol and 60% water during daily monitoring at room temperature

were detected from specimens stored in artificial saliva, and MMA, BPBC, DBP, and DOP continued to be eluted even after the 5th day from specimens stored in ethanol/water mixtures.

Monitoring at constant temperature

Figures 6, 7, and 8 show the release of the monomers examined from the three resins under different storage conditions at constant temperature. The analysis revealed differences in monomer leaching compared to daily monitoring at room temperature. In most cases the leaching occurred rapidly at an increasing rate closely related to the surrounding storage condition. A quantitative determination of residual monomers showed that significant amounts ($p < 0.05$) still remained even after 6 h, demonstrating a continuing process of leaching. Completion of the leaching after the 4th hour was observed only for MMA and DBP in Triplex specimens stored in artificial saliva, and BPBC and DOP in Type 15 specimens stored in ethanol/water mixtures.

Monitoring at a temperature range from 10°C to 40°C

Temperature variations of 10–40°C produced no change in the leaching of monomers but did cause variation in the rate of the process as illustrated in Figs. 9, 10, and 11. When samples of the three brands were examined at a temperature of 10°C, only DOP was observed leaching out of Triplex specimens stored in ethanol/water, whereas peaks of the majority of the residues were obtained from Paladon and Type 15 resins. Generally, although the rate of extraction seemed to increase with temperature, variations in peak areas observed in the temperature range from 10°C to 40°C suggest a close relationship to both temperature and storage conditions ($p < 0.001$). However, in this experiment the levels of residual monomers were found to be higher at 40°C in most cases.

Discussion

The results obtained from this study suggest that a whole spectrum of residues diffuse out of the three ac-

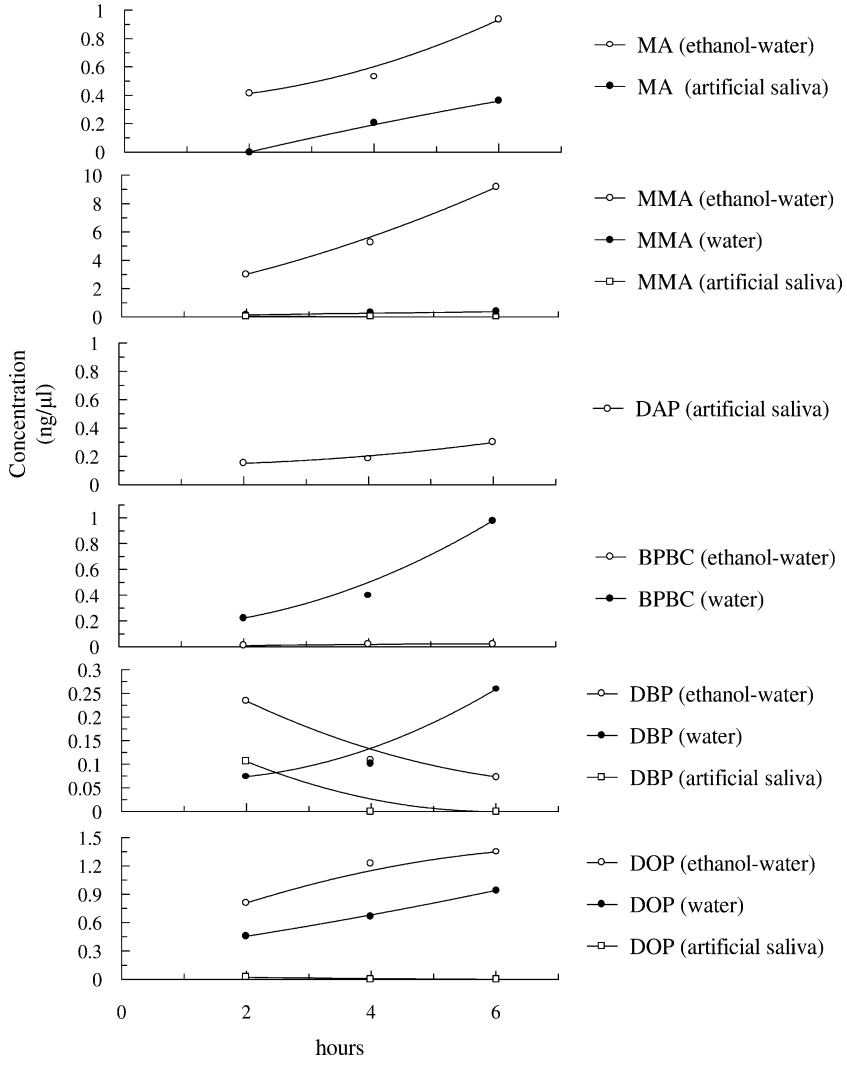

Fig. 6 Rate of elution of monomers leaching out of Triplex in artificial saliva, distilled water, and a binary mixture of 40% ethanol and 60% water at constant temperature (40°C) for 6 h

rylic resin materials examined. The error connected with the method was used 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 methacrylate, the level of which seems to be time-dependent and decreases for a period up to 5 days when resins are stored in distilled water at room temperature. These results, although not strictly comparable because of the different methods applied, seem to be in general agreement with qualitative and quantitative investigations previously described [2, 24]. However, the post-polymerized residual monomer levels in this study do not approach the limiting values obtained from other studies [25–27]. This may be due to the use of a long-term curing cycle of polymerization without a terminal boil, which reduces the residual monomer level significantly [28, 29]. Treatment with artificial saliva revealed a decreasing residual MMA extraction over 5 days of the experiments although the examined resins were cured at 90°C for 9 h. This result does not support a previous study [10] in which the in vivo release of monomer into saliva proved that residual MMA in saliva was not detected leaching out of appliances polymerized at 70°C for 3 h. Apart from the fact that the results in vivo may differ significantly from those in vitro, this discrepancy could be also explained by: (1) the different brands of the materials and thus the different compositional compounds of the acrylic resin materials, (2) the probable difference in polarity between artificial and natural saliva, and (3) the potentially different sensitivities of the analytical methods.

This study also confirms that another non-phthalic leachable compound, methacrylic acid, is extracted in high concentrations in all experiments as well. Unreacted residual MA is known to be present in acrylic resins after curing; however, it has been found negative in sensitizing tests [30].

The HPLC 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 phthalate esters were detected. The extraction

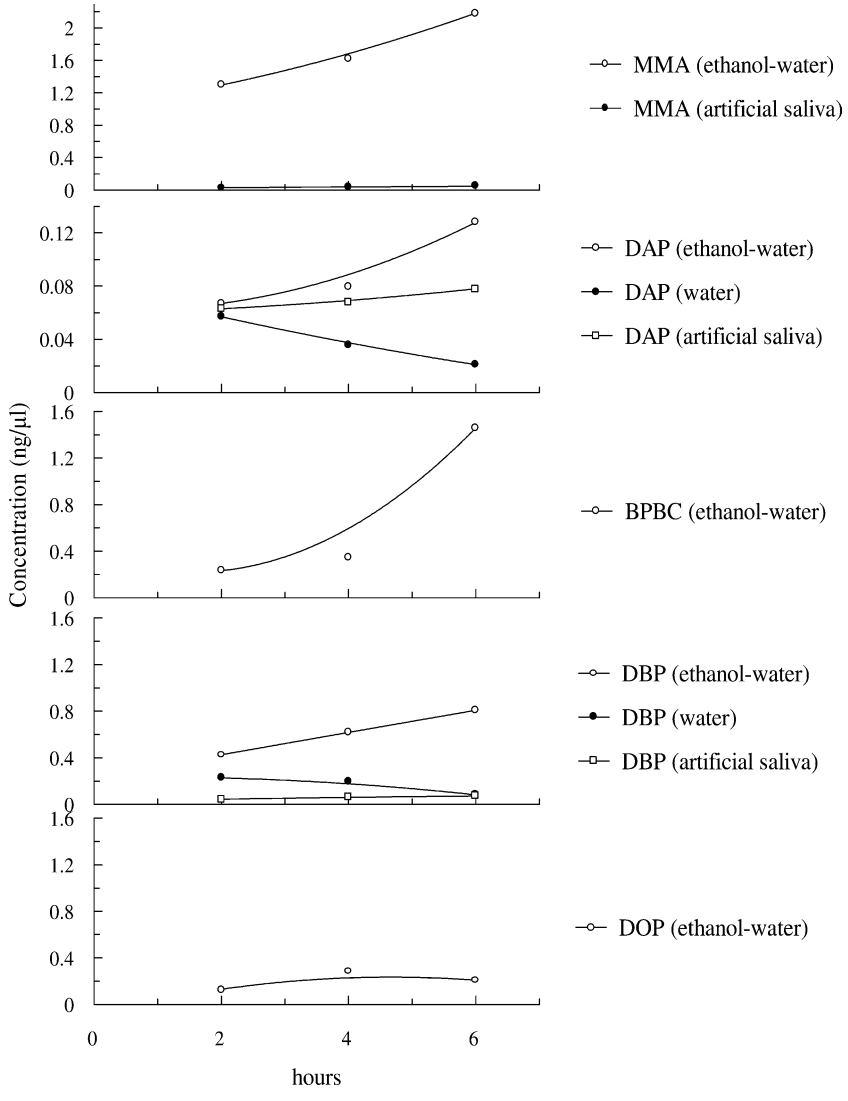

Fig. 7 Rate of elution of monomers leaching out of Paladon in artificial saliva, distilled water, and a binary mixture of 40% ethanol and 60% water at constant temperature (40°C) for 6 h

of phthalate esters from thermopolymerized resins has rarely been investigated [31, 32]. The extraction of dibutyl phthalate and dicyclohexyl phthalate has been detected (in vivo) in saliva from patients with new dentures, but not with old ones [31].

Once the presence of phthalate esters was known it was of interest to establish their concentration. In contrast to the relatively large amounts of the non-phthalic monomers, phthalates leach in smaller amounts at a rate dependent upon their ability to diffuse through the resin matrix into the storage medium. As the analysis revealed, the examined phthalates exhibited different degrees of release related to the storage condition, brand of material, and type of experiment ($p < 0.05$). A general observation could be that the extraction rate is directly dependent upon temperature: the higher the temperature, the higher the rate of extraction. This observation supports results of earlier investigations by other workers [9].

As regards the effect of ambient temperature, the results obtained when the temperature was kept constant at 40°C indicated small differences in levels of residual monomers with leaching increasing continually up to the 6th hour. Variations of the temperature from 10°C to 40°C produced no change in the weights of residues, while leaching did not occur very rapidly. However, the level of residual monomers was always higher in the range between 30°C and 40°C.

In both types of experiments the rate of release was closely related to both ambient temperature and storage condition ($p < 0.05$).

In comparing the polarity of the different storage media it should be noted that the release of monomers in distilled water was less than in artificial saliva, whereas in the less polar binary water/ethanol mixture the level of residual monomers was always higher ($p < 0.001$).

The time for completion of leaching of the monomers in distilled water was estimated to be approximately 4–5 days for the examined materials, although in a previous study significant amounts of monomers continued to leach from the acrylic dentures for a longer time [33].

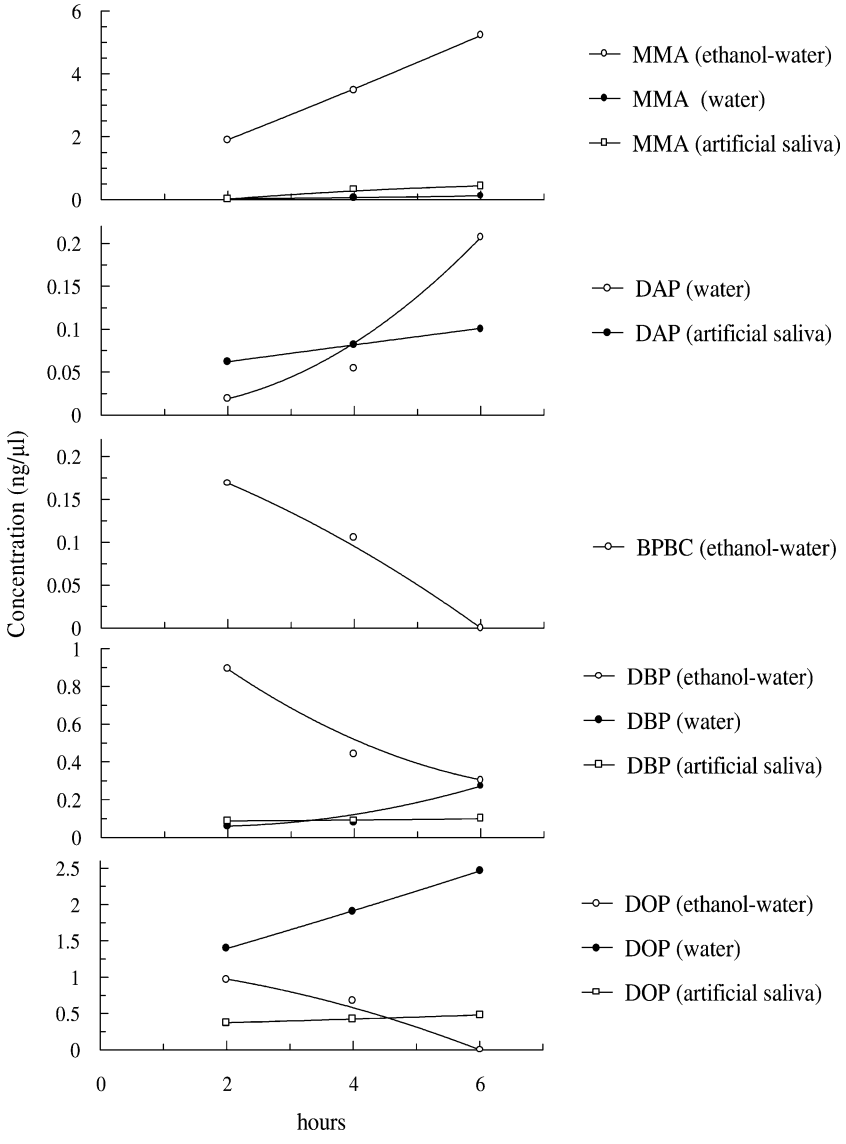

Fig. 8 Rate of elution of monomers leaching out of Type 15 in artificial saliva, distilled water, and a binary mixture of 40% ethanol and 60% water at constant temperature (40°C) for 6 h

The potential toxic effects of the monomers analyzed are beyond the scope of this study. However the release of plasticizers during use is an important consideration. Although there have been no observations of clinical problems attributable to the use of those compounds, there is experimental evidence of subtle toxicity [14].

It must be pointed out that the level of residues in this study were determined 24 h after processing, with the specimens stored in distilled water for that period, according to commonly followed practice.

Acrylic resins have shown a high degree of safety over many years of clinical use. In the past, emphasis has been given to physical or mechanical properties with less attention placed on their biological evaluation. Because of the increased concern about dental toxicology [33, 34] several articles have dealt with the leachability of monomers as a means of studying the toxicity of dental items [35, 36].

While most of the monomers under investigation were detected leaching out of the resins examined, under several storage conditions, it seems probable that several products contain a number of undetected compounds. The analysis showed that the three commercial products differed with respect to the leaching of additives. These results are reasonably consistent with the chemical formulation of the products. Therefore, further consideration must be given to the individual chemical constituents of dental materials and the manufacturers should make specific composition details available.

Conclusions

In summing up the results of the various analyses carried out in this study, the following conclusions are 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 acrylic resin materials examined.
3. The main component extracted was methyl methacrylate, the level of which seems to be time-dependent, decreasing over a period of up to 5 days when resins are stored in distilled water at room temperature.

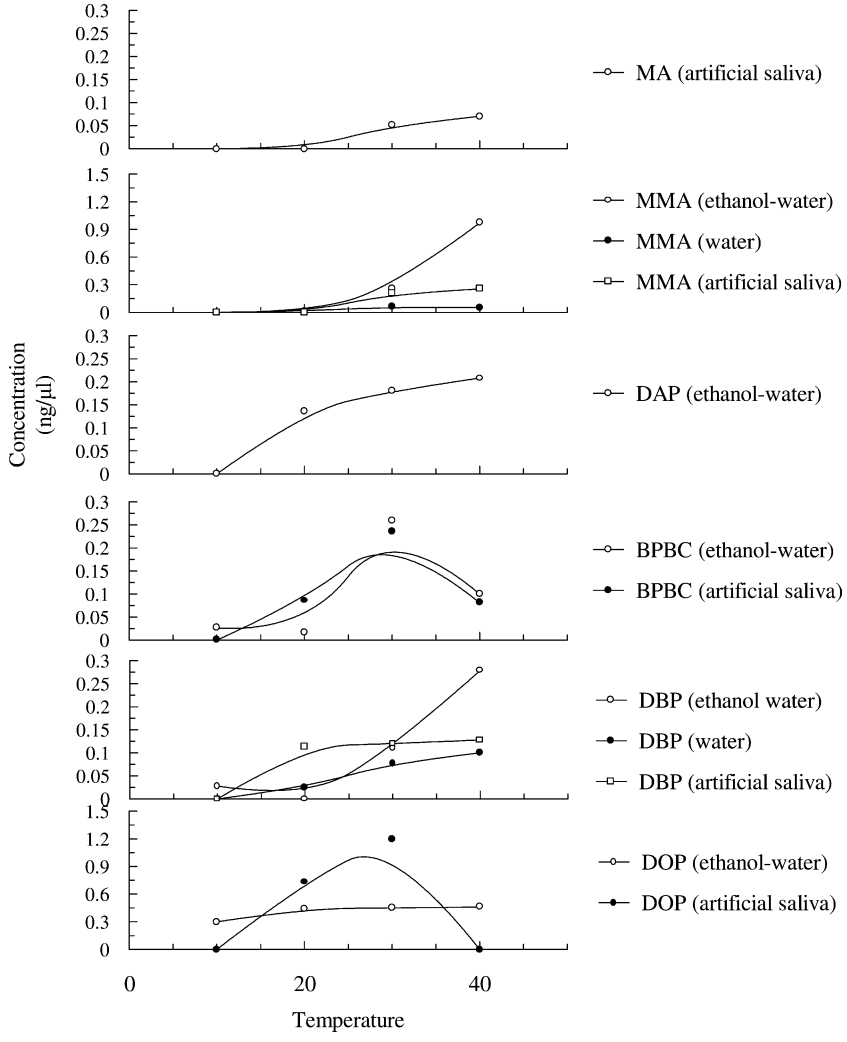

Fig. 9 Rate of elution of monomers leaching out of Triplex in artificial saliva, distilled water, and a binary mixture of 40% ethanol and 60% water after stepwise elevation of the temperature from 10°C to 40°C

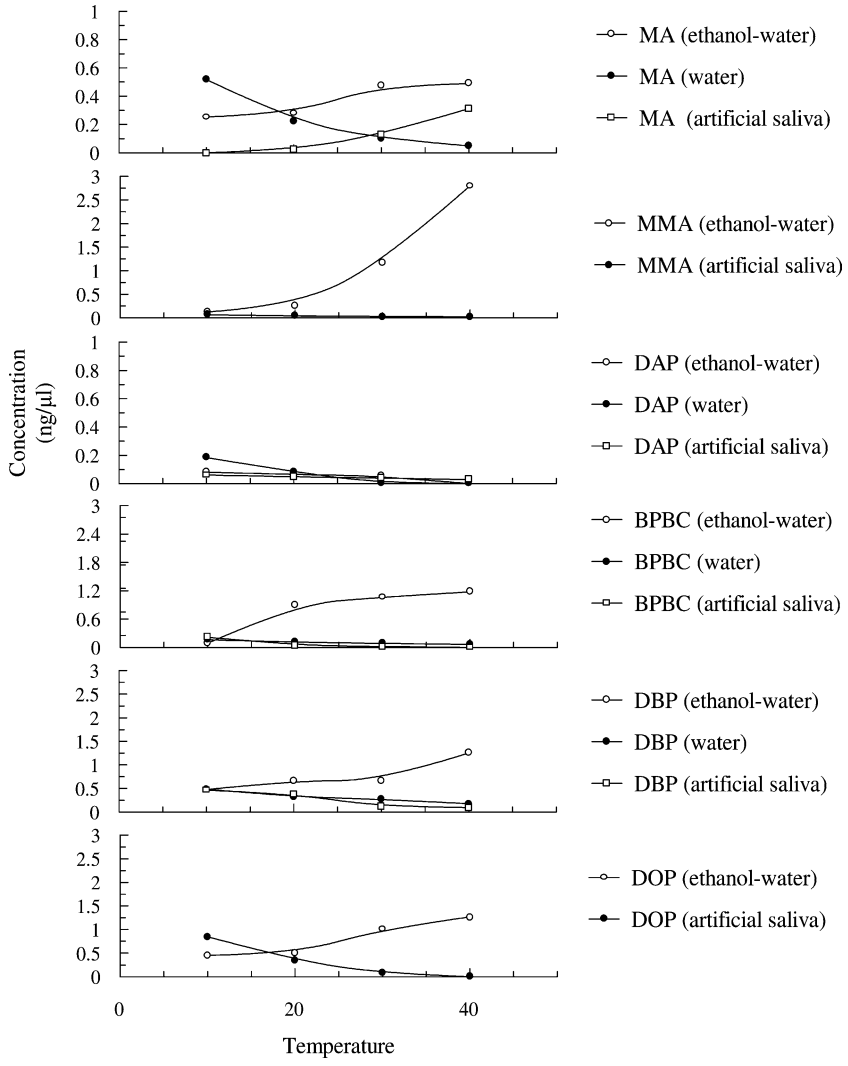

Fig. 10 Rate of elution of monomers leaching out of Paladon in artificial saliva, distilled water, and a binary mixture of 40% ethanol and 60% water after stepwise elevation of the temperature from 10°C to 40°C

Thus a period longer than 5 days immersion of acrylic dentures in water is suggested.

4. Although in smaller amounts, a large number of phthalates were detected leaching out of examined resins at a rate dependent upon both the storage medium and ambient temperature.
5. The release of monomers in distilled water is less than in artificial saliva, whereas in the binary water/ethanol mixture (60% and 40%) the level of leaching monomers is generally higher.
6. Further consideration must be given to the chemical ingredients of dental materials and the manufacturers should make specific formulation details available.

References

1. Kaaber S (1990) Int Dent J 40:359–365
2. Douglas WH, Bates JF (1978) J Mater Sci 13:2600–2604
3. Ruyter IE (1980) Acta Odontol Scand 38:17–27
4. Tsuchiya H, Hoshino Y, Kato H, Takagi N (1993) J Dent 21:240–243
5. Koutis D, Freeman S (2001) Australas J Dermatol 42:203–206
6. Svvensson P, Kaaber S (1995) J Oral Rehabil 22:887–895
7. Huang FM, Tai KW, Hu CC, Chang YC (2001) Int J Prosthodont 14:439–443
8. Houstveit G, Torheim B, Fystro D, Eidem T, Sandvik M (1984) Biomaterials 5:75–80
9. Graham PR (1973) Environ Health Perspect Exp Issue 3:3–12
10. Ganning AE, Brunk U, Dallner G (1984) Hepatology 4:541–547
11. Pollack GM, Buchanan JF, Slaughter RL, Kahli RK, Shen DD (1985) Toxicol Appl Pharmacol 79:257–267
12. Scott RC, Dugard PH, Ramsey JD, Rhodes C (1987) Environ Health Perspect 74:223–227
13. Kawai K (1998) Biol Pharm Bull 21:579–582
14. Gollamudi R, Lawrence WH, Rao RH, Autian J (1985) J Appl Toxicol 5:368–371
15. Graham BS, Jones DW, Sutow EJ (1991) J Dent Res 70:870–873
16. Kawano F, Dootz ER, Koran A, Graig RG (1994) J Prosthet Dent 72:393–398
17. Lamb DJ, Ellis B, Priestley D (1982) Biomaterials 3:155–159
18. Bartiloni JA, Murchison DF, Wofford DT, Sarkar NK (2000) J Oral Rehabil 27:488–493

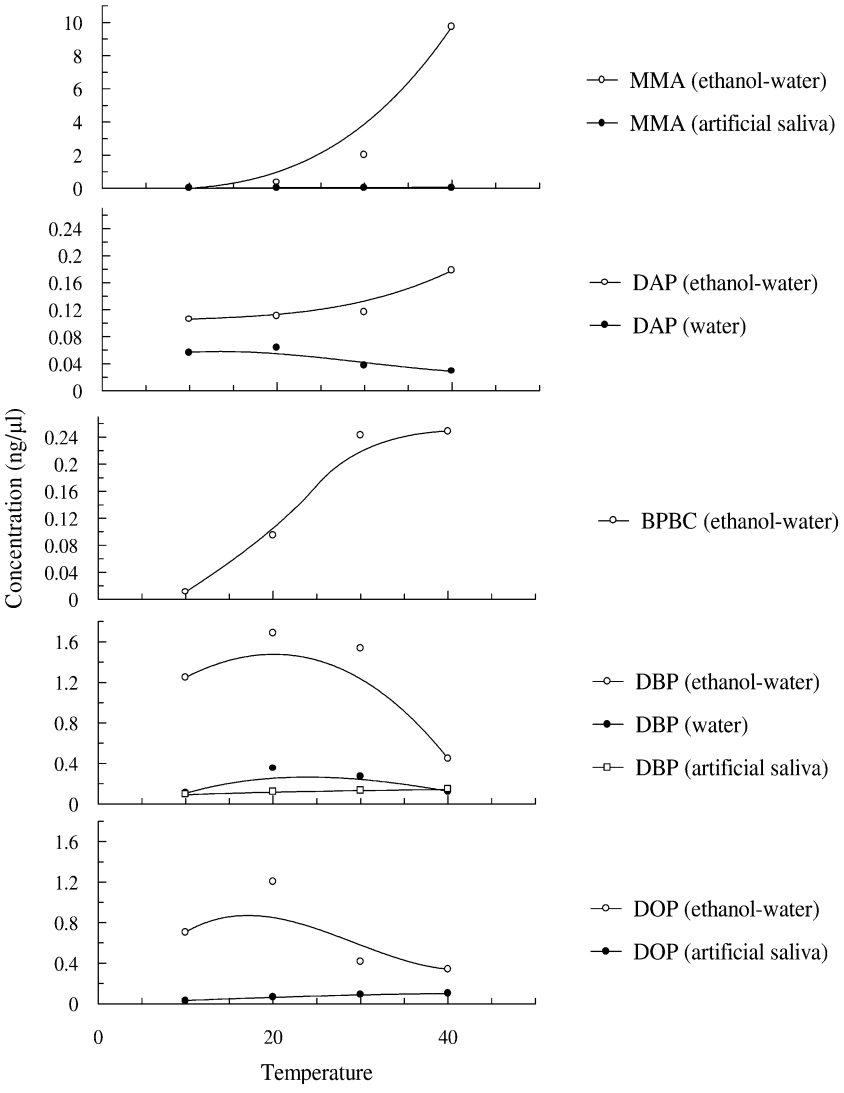

Fig. 11 Rate of elution of monomers leaching out of Type 15 in artificial saliva, distilled water, and a binary mixture of 40% ethanol and 60% water after stepwise elevation of the temperature from 10°C to 40°C

19. Brooks SC, Bates JF (1985) J Mater Sci 20:3890–3896
20. Baker S, Brooks SC, Walker DM (1988) J Dent Res 67:1295–1299
21. Haken JK, McKay TR (1973) Anal Chem 45:1251–1257
22. Shim JS, Watts DC (1999) Dent Mater 15:296–300
23. Ruyter IE, Oysaed H (1988) CRC Crit Rev Biocompat 4:247–279
24. Vallittu PK, Ruyter IE, Buykuilmaz S (1998) Eur J Oral Sci 106:588–593
25. Jagger RG (1978) J Oral Rehab 5:151–157
26. Jerolimov V, Huggett R, Brooks SC, Bates JF (1985) Quintess Dent Technol 9:431–434
27. Baker S, Brooks SC, Walker DM (1988) J Dent Res 67:1295–1299
28. Koda T, Tsuchiya H, Yamauchi M, Hoshino Y, Takagi N, Kawano J (1989) J Dent 17:84–89
29. Harrison A, Hugett R (1992) J Dent 20:370–374
30. Bjorkner B, Niklesson B, Persson K (1984) Contact Dermatitis 10:286–304
31. Lygre H, Solheim E, Gjerdet NR, Berg E (1993) Acta Odontal Scand 51:45–51
32. Lygre H, Solheim E, Gjerdet NR (1995) Acta Odontal Scand 53:75–80
33. Kawahara T, Nomura Y, Tanaka N, Teshima W, Okazaki M, Shinta H (2004) J Dent 32:277–283
34. Leggat PA, Kedjarune U (2003) Int Dent J 53:126–131
35. Munksgaard EC (2004) Eur J Oral Sci 112:101–104
36. Winberg LD, Badr MZ (1995) Toxicol Lett 76:63–69