

Identification and quantification of leachable substances from polymer-based orthodontic base-plate materials

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SUMMARY The aim of this study was to analyse leachable monomers, additives, and degradation products from polymer-based orthodontic base-plate materials.

One heat-cured resin (Orthocryl), one light-cured (Triad VLC), and three thermoplastic materials (Biocryl C, Essix A+, and Essix Embrace) were investigated. Elution was performed in water at 37°C for 10 days. The extract medium was changed and analysed daily. Chromatographic methods were used to identify and quantify the leachables. In addition, the content of residual methyl methacrylate (MMA) was quantified in the poly(methyl methacrylate) (PMMA)-based materials. Statistical analysis of the quantitative results was performed using a *t*-test for comparison of two independent samples.

Monomers and additives leached from the materials polymerized *in situ* and from the thermoplastic PMMA-based material. No leachable substances were found in the extracts from the other thermoplastic materials. Accumulated over 10 days, a larger amount of MMA leached from the powder-and-liquid material, Orthocryl (42 µg/cm²), than from the thermoplastic material, Biocryl C (0.49 µg/cm²). The accumulated amounts of monomers leached from Triad VLC were 91 µg/cm² of urethane dimethacrylate and 2.2 µg/cm² of 2-hydroxyethyl methacrylate. Formaldehyde was found to leach from methacrylate-based materials: 3.2 µg/cm² from Orthocryl and 0.16 µg/cm² from Triad VLC. However, formaldehyde was not detectable in extracts from Biocryl C. Residual MMA was 5.4 wt % in Orthocryl and 0.4 wt % in Biocryl C. No phthalates were detected in the tested materials.

In this *in vitro* study, minimal leaching was found from the thermoplastic materials, while leaching of methacrylates and formaldehyde was observed from the powder-and-liquid type and the paste material. Within the limitations of this study, the results suggest that prefabricated thermoplastic plates should be preferred for patients with an allergy to methacrylates.

Introduction

Polymer-based biomaterials are widely used in dentistry. In orthodontic treatment, polymer-based materials are used both as base-plate materials and as adhesives for brackets. Incomplete polymerization of dental resins is the cause of residual monomers in the material (Ruyter and Oysa, 1982; Vallittu *et al.*, 1998). These unreacted monomers are free small molecules present in the polymer matrix. As such, they may leach out of the material both from the surface layer and, over longer time perspectives, from inside of the material through diffusion. Several reports are available on monomers leaching from orthodontic adhesives (Baker *et al.*, 1988; Eliades *et al.*, 1995; Gioka *et al.*, 2005), whereas information is scarce in the case of orthodontic base-plate materials (Stafford and Brooks, 1985; Rose *et al.*, 2000).

Concern has been raised about leaching of monomers and other constituents from polymer-based biomaterials into the surrounding biological environment. These leachables may cause adverse effects. Allergic responses as well as oestrogenic, carcinogenic, and cytotoxic effects from acrylate monomers, plasticizers, or degradation products have been discussed (Jacobsen and Hensten-Pettersen,

1989; Kanerva *et al.*, 1989; Tsuchiya *et al.*, 1994; Kedjarune *et al.*, 1999; Becher *et al.*, 2006; Gonçalves *et al.*, 2006; Eliades *et al.*, 2007). Both occupational health problems and patient reactions have been addressed. Some additives in dental materials have been reported to give adverse reactions, and the effect of the initiator benzoyl peroxide (BPO) has also been discussed (Hogan, 1991). However, the amount of BPO leaching from the material was found to be negligible in artificial saliva and distilled water (Boeckler *et al.*, 2008). Formaldehyde, which is considered to be carcinogenic (International Agency for Research on Cancer, 2006), may be released from methacrylate-based dental materials, such as composites and denture bases (Ruyter, 1980; Oysa *et al.*, 1988; Tsuchiya *et al.*, 1994). Release of formaldehyde from orthodontic base-plate materials has not yet been reported. Orthodontic base-plate materials are usually used for young patients, and exposure to possible xeno-hormone substances, such as phthalate plasticizers and other compounds in pubertal children is a concern (Scientific Committee on Emerging and Newly-Identified Health Risks, 2008). It is known that plasticizers may leach from soft dental materials, such as tissue conditioners and

lining materials (Lygre *et al.*, 1995; Munksgaard, 2004, 2005; Elsemann *et al.*, 2008).

The aim of this study was to evaluate the release of possibly harmful compounds including monomers, phthalate additives, and the degradation product formaldehyde from orthodontic base-plate materials and, in addition, to investigate the content of residual monomer in methyl methacrylate (MMA)-based materials.

Materials and methods

Five commonly used resins for orthodontic appliances were investigated (Table 1). Orthocryl is a traditional powder-and-liquid system based on poly(methyl methacrylate) (PMMA). Triad VLC is a paste material with a urethane dimethacrylate (UDMA) as the main monomer. The three thermoplastic materials are delivered as pre-polymerized plates based on different types of polymers. Biocryl C is PMMA based, while Essix A+ and Essix Embrace are based on a copolyester and poly(vinyl chloride) (PVC), respectively. Reference monomers and other chemicals were used as received: acetonitrile (pro analysis grade; Merck KGaA, Darmstadt, Germany), ammonium acetate (98 per cent, VWR International Ltd, Poole, Dorset, UK), 2,4-dinitro-phenylhydrazine (DNPH) (pro analysis grade; Fluka, Buchs, Switzerland), distilled water (grade 2, ISO 3696, International Organization for Standardization, 1988), 2-hydroxyethyl methacrylate (2-HEMA; more than 99 per cent, Fluka), methanol (more than 99.9 per cent; Merck KGaA), MMA (99 per cent, Aldrich, St Louis, Missouri, USA), and UDMA (mixture of isomers; Aldrich).

Test specimen preparation and leaching studies

Triad VLC was delivered as unpolymerized paste in sheets with a thickness of 2 mm. Circular specimens, 35 mm in diameter, were cut from the sheets and then polymerized *in situ* with a Triad 2000 visible light curing unit (Dentsply International, York, Pennsylvania, USA) according to the manufacturer's instructions: 4 minutes initial curing followed by an additional 4 minutes after applying the Triad air barrier coating. For preparation of the Orthocryl specimens, a circular stainless steel mould, 35 mm in diameter and 2.0 mm in depth, was mounted in gypsum in a pressure vessel. The material

was polymerized *in situ* according to the manufacturer's instructions at 2.2 atmosphere at 46°C for 25 minutes. Specimens of the three thermoplastic plate materials (Biocryl C, Essix A+, and Essix Embrace) were formed in an Essix™ Machine (Raintree Essix) by means of heat and vacuum. A circular stainless steel plate, 35 mm in diameter, was used to press specimens of the desired size from the thermoplastic materials. Three specimens of each material were prepared. After preparation, all specimens were stored dry and in the dark at room temperature for 24 hours. All specimens were then polished with dental polishing chalk to create a smooth surface and rinsed thoroughly with water.

Three specimens of each material were placed individually on stainless steel grates in three glass containers with a Teflon-coated magnetic stir bar and 15 ml of distilled water was added. The sealed containers with the specimens were placed in an oven at 37°C with continuous stirring. The containers were taken out of the oven after 24 hours and the solutions were removed for analysis. Fifteen millilitres of water was again added to the containers with the specimens, and they were replaced in the oven for a further 24 hours. The sampling procedure was repeated every 24 hours for 10 days. For the two thermoplastic materials, for which no released compounds could be identified, the experiment was terminated after 7 days.

Test specimen preparation and formaldehyde release

The amount of formaldehyde released from the methacrylate-based materials (Biocryl C, Orthocryl, and Triad VLC) was assessed using an established method (Ruyter, 1980; Oysaet *et al.*, 1988). Specimens (diameter = 15 mm and height = 1.2 mm) were prepared according to the manufacturer's instructions as described above and stored dry and in the dark for 1 hour at 37°C. Three specimens were individually immersed in separate containers holding 4 ml of water for 72 hours at 37°C. Derivatization of formaldehyde with a DNPH solution (0.125 mg DNPH/ml acetonitrile) was undertaken before chromatographic analysis.

Test specimen preparation and residual monomer

The content of residual MMA monomer in the PMMA-based materials (Biocryl C and Orthocryl) was determined according to ISO 20795-1 (International Organization for

Table 1 Orthodontic materials included in the study.

Product name	Manufacturer	Type	Lot number
Biocryl C	Scheu Dental, Iserlohn, Germany	Thermoplastic plate	3102A
Essix A+	Raintree Essix, Sarasota, Florida, USA	Thermoplastic plate	303-1
Essix Embrace	Raintree Essix	Thermoplastic plate	301-1
Orthocryl	Dentaurum, Ispringen, Germany	Powder-and-liquid cold-cure	100493(p), 090451(l)
Triad TranSheet VLC	Dentsply International, York, Pennsylvania, USA	Light-curing paste	030904A

Standardization, 2008). The materials were cured or processed (pre-polymerized plate) according to the manufacturer's instructions as described above to prepare the specimens (diameter = 50 mm and height = 3.0 mm). For each material, nine sample solutions were prepared.

Chemical analysis

Qualitative and quantitative determination of substances released from the materials were performed by gas chromatography/mass spectrometry (GC/MS: TurboMass Autosystem XL; Perkin Elmer, Waltham, Massachusetts, USA, with a DB-5 column) and liquid chromatography/mass spectrometry (LC/MS: Agilent 1100 Series LC, UV/DAD, MSD Trap XCT; Agilent Technologies, Santa Clara, California, USA, with a Zorbax Eclipse XDB-C8 5 μ m column). The LC system was connected to the mass spectrometer via an electrospray ionization interface. The solvent system was methanol/water (80/20 v/v) with 10 mM ammonium acetate. Identification of the substances was undertaken by comparison of retention time and ultraviolet and/or MS-spectra with those of the reference monomers. The quantities of monomers released were calculated relative to the surface area of the specimens.

Statistical analysis of the quantitative results was performed using a *t*-test for comparison of two independent samples. The confidence level was set at 99 per cent.

Results

Qualitative analysis of leachables

Both monomers and additives were found in aqueous extracts from the materials polymerized *in situ*. In the extracts from the Triad VLC specimens, the initiator camphorquinone and the monomers UDMA and 2-HEMA were detected. MMA and traces of ethyl methacrylate were found in the extracts from the Orthocryl specimens, and MMA was detected in the extracts from the PMMA-based thermoplastic material, Biocryl C. Neither monomers nor other substances were found to have leached into the aqueous solution from the non-acrylic thermoplastic plate materials (Essix Embrace and Essix A+). Phthalates were not found to have leached from any of the investigated materials.

Quantitative determination of leachables

The mean values of the amounts of leaching monomers from the three test solutions at each time investigated are presented in Figures 1–4, showing the day-by-day as well as the accumulated values. The amount of MMA leaching from the PMMA-based thermoplastic material (Biocryl C) was 0.49 (SD 0.05) μ g/cm² accumulated for the 10 day period. Significantly higher values ($P < 0.01$) of MMA were found leaching from the PMMA-based powder-and-liquid material (Orthocryl) where the accumulated amount was

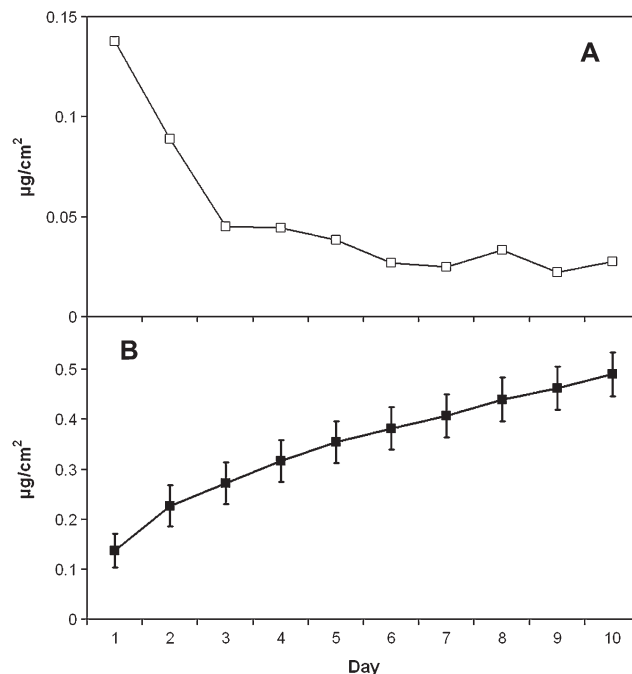


Figure 1 Leaching of methyl methacrylate from Biocryl C. (A) On a day-by-day basis and (B) accumulated values, in micrograms per square centimetre.

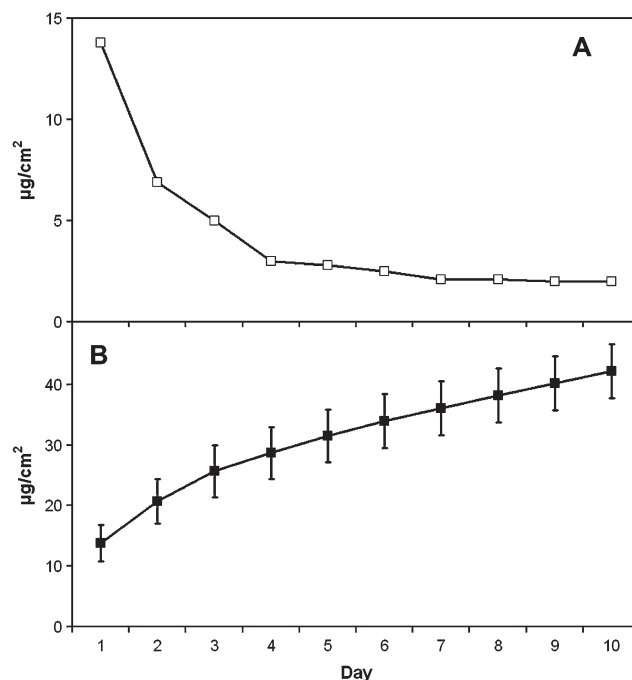


Figure 2 Leaching of methyl methacrylate from Orthocryl. (A) On a day-by-day basis and (B) accumulated values, in micrograms per square centimetre.

42 (SD 4) μ g/cm². The accumulated amounts of leached monomers from the light-cured paste material (Triad VLC) were calculated to be 91 (SD 4) μ g/cm² of UDMA and 2.2 (SD 0.2) μ g/cm² of 2-HEMA.

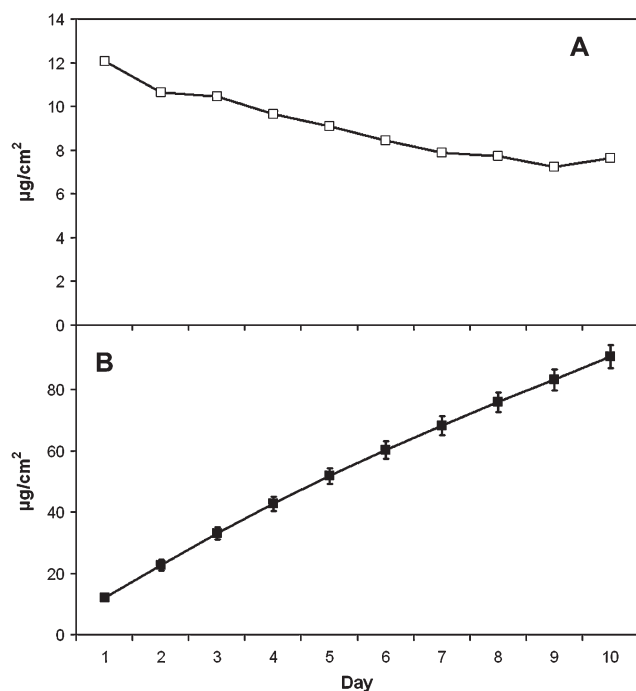


Figure 3 Leaching of urethane dimethacrylate from Triad VLC. (A) On a day-by-day basis and (B) accumulated values, in micrograms per square centimetre.

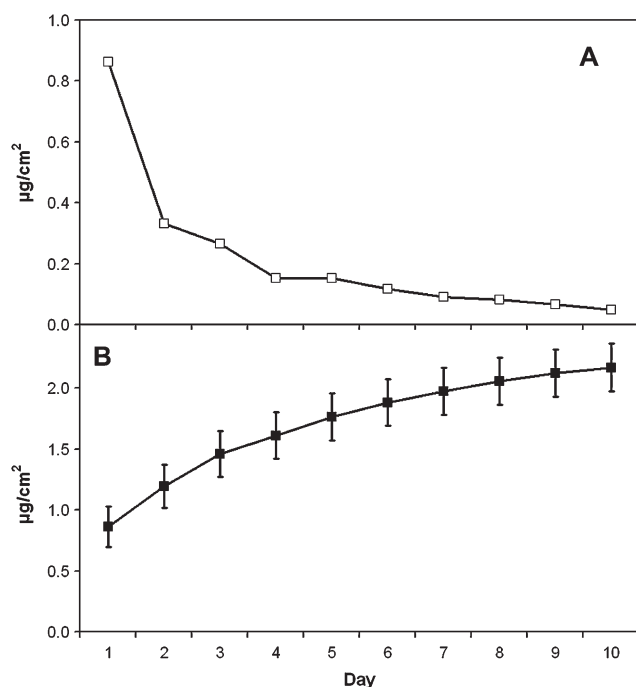


Figure 4 Leaching of 2-hydroxyethyl methacrylate from Triad VLC. (A) On a day-by-day basis and (B) accumulated values, in micrograms per square centimetre.

Formaldehyde release from the methacrylate-based materials averaged for the three individual specimens and analyses was found to be 3.2 (SD 0.2) $\mu\text{g}/\text{cm}^2$ for Orthocryl and 0.16 (SD 0.06) $\mu\text{g}/\text{cm}^2$ for Triad VLC, significantly

lower than for Orthocryl ($P < 0.01$). Although a possible signal from formaldehyde was seen for Biocryl C, it was below both quantification and detection limits, and no positive detection could be carried out (limit of detection: 0.02 $\mu\text{g}/\text{cm}^2$). The results (average of nine sample solutions) of the analyses for residual MMA monomer in the PMMA-based materials were 5.4 (SD 0.2) wt % for Orthocryl and 0.4 (SD 0.04) for Biocryl C, significantly lower than for Orthocryl ($P < 0.01$).

Discussion

The results of analyses of leachable substances (monomers, additives, and degradation products) from dental polymer-based materials may be influenced by the type of extraction media, the time and temperature of the extraction procedure, as well as the degree of curing and composition of the material (Baker *et al.*, 1988; Vallittu *et al.*, 1998; Santerre *et al.*, 2001). It is known that methacrylates may degrade hydrolytically in aqueous environments (Santerre *et al.*, 2001; Salz *et al.*, 2005). Analysing monomers collected after several days of leaching into aqueous media may give erroneous results as monomers may degrade during the time of the experiment. In this study, leaching was analysed on a day-to-day basis. In this way, detailed information was available on the release of substances into the extraction media. Changing and analysing the extraction medium every 24 hours ensured that the hydrolytic degradation of released monomers was controlled as long-term leaching was evaluated. Quantitative determination was only carried out for monomers as these were the main components that leached out of the materials.

The day-by-day amounts of MMA that leached out of Biocryl C and Orthocryl (Figures 1A and 2A) showed that the largest amount was leached during the first 24 hours for both materials. There was a 100-fold increase both in the amount of initially leached MMA and in the total amount of leached monomer, from the pre-polymerized plate to the cold-cured material. After 3–4 days in the aqueous solution, the amount of leached MMA-monomer levelled off for both materials. This high initial release has been documented previously for these materials (Stafford and Brooks, 1985; Tsuchiya *et al.*, 1994; Rose *et al.*, 2000).

The accumulated amount of leached UDMA from the Triad VLC (Figure 3B) was the highest of the monomers studied. UDMA (Figure 3A) did not show the same relative decrease in the daily amounts of leached monomer as MMA or 2-HEMA (Figures 2A and 4A, respectively). This indicates that leaching of UDMA may persist at a higher level for a longer period of time. Rose *et al.* (2000) studied the cytotoxicity and elution of MMA and UDMA from cold-(MMA) and light-(UDMA) cured acrylics. They found the same pattern as in the present study, with the elution of MMA levelling off after a few days, and the elution of UDMA persisting at a high level over a longer time period.

None of the materials tested claimed to comply with ISO 20795-1 (International Organization for Standardization, 2008), but a comparison may still be meaningful. Residual MMA monomer in Orthocryl fails to comply with the requirement set in the standard, which is a maximum of 4.5 wt % for auto-polymerizable materials. For thermoplastic materials, the requirement is set to a maximum of 2.2 wt % and the amount of residual monomer in Biocryl C complied with this requirement. Although a very small amount of residual MMA was seen in Biocryl C, the results from this investigation indicate that the claim of the manufacturer that there is 'no residual MMA' in Biocryl C is not correct. Reports of residual MMA in other heat- and cold-cured dental PMMA materials (orthodontic and denture bases) range from approximately 0.1–6 wt %, with values of the heat-cured materials at the lower end of the range (Baker *et al.*, 1988; Stafford and Brooks, 1985; Vallittu *et al.*, 1998; Kedjarune *et al.*, 1999; Rose *et al.*, 2000). The absence of monomers leaching from the PVC material, Essix Embrace, and the copolyester material, Essix A+, combined with the very low leaching from the PMMA-based prefabricated material, Biocryl C (Figure 1B), suggest that prefabricated orthodontic base materials should be the material of choice if allergic patient reactions may be expected.

Formaldehyde release from dental materials may occur after the reaction of methacrylate groups with oxygen or from the degradation of oxygen-rich methacrylate polymer (the oxygen inhibition layer) to formaldehyde at temperatures at or above 37°C (Oysaet *et al.*, 1988). Reports of formaldehyde release have predominantly concerned PMMA-based denture base materials, where a higher release of formaldehyde is seen in auto-polymerized materials than in heat-polymerized materials (Ruyter, 1980; Tsuchiya *et al.*, 1994; Mikai *et al.*, 2006). Release of formaldehyde has not previously been reported from polymer-based orthodontic base-plate materials. In this study, it was shown that formaldehyde is released from a MMA-based orthodontic material of the powder-and-liquid type, as well as from a UDMA-based light-cured paste material. No confirmed detection of formaldehyde was made in the solutions from the thermoplastic PMMA material. This indicates that this prefabricated material is not subject to any significant formaldehyde formation due to degradation.

Phthalates have been found leaching from denture base and denture soft-lining materials (Lygre *et al.*, 1995; Munksgaard, 2004, 2005; Elsemann *et al.*, 2008). Leaching of phthalates from polymer-based orthodontic materials has been suspected, although evidence of such leaching has not been published. In agreement with this, the findings of this study did not detect phthalates leached from any of the materials. As the health risk of phthalates is still not resolved (Scientific Committee on Emerging and Newly-Identified Health Risks, 2008), it is reassuring that these plasticizers were not found in the tested orthodontic materials. As a

control, the initial compositions of the materials in the study were analysed. That investigation also showed no signs of phthalates.

Conclusions

Within the limitations of this *in vitro* study, it was found that the amounts of leaching methacrylate monomers from prefabricated thermoplastic plates were lower than those of monomer leaching from the powder-and-liquid-based material and from the paste material. Based on these results, orthodontic materials from prefabricated thermoplastic plates should be preferred for patients with an allergy to methacrylates.

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