Leaching from denture base materials in vitro

Henning Lygre, Einar Solheim and Nils Roar Gjerdet
Department of Dental Biomaterials, School of Dentistry, and Department of Clinical Biology, Section of Oral Pharmacology, School of Medicine, University of Bergen, Bergen, Norway

Lygre H, Solheim E, Gjerdet NR. Leaching from denture base materials in vitro. Acta Odontol Scand 1995;53:75–80. Oslo. ISSN 0001-6357.

Specimens made from denture base materials were leached in Ringer solution and in ethanol. The specimens comprised a heat-cured product processed in two different ways and two cold-cured materials. The organic compounds leaching from the specimens to the solutions were separated, identified, and quantified by a combined gas-chromatography and gas-chromatography/mass-spectrometry technique. Additives and degradation products, possibly made by free radical reactions, were released from the denture base materials. In Ringer solution only phthalates could be quantified. In ethanol solvent, biphenyl, dibutyl phthalate, dicyclohexyl phthalate, phenyl benzoate, and phenyl salicylate were quantified. In addition, copper was found in the ethanol solvent from one of the denture base materials. The amount of leachable organic compounds varies among different materials. Processing temperature influences the initial amount of leachable compounds. ☐ *Acrylic resins; dental materials; denture bases; gas-chromatography/mass-spectrometry*

Henning Lygre, Department of Dental Biomaterials, School of Dentistry, Årstadveien 17, N-5009 Bergen, Norway

Polymeric biomaterials are subjected to mechanical and chemical influences and may exhibit degradation and leaching. Leaching is the reverse phenomenon of sorption and swelling, and the leaching compounds may include additives and non-additives (1). To evaluate biomaterials toxicologically, it has been recommended to identify and quantify leaching compounds (2).

Heat-cured poly(methyl methacrylate) (PMMA) is the commonest denture base polymer, and most complete dentures are made from this material (3, 4). Cured PMMA denture base materials are assumed to be multiphase systems (5), consisting of the remaining part of polymer beads from the original powder embedded in an interstitial matrix of newly formed polymer. Plasticizers, inhibitors, catalysts, and fillers are additives in these materials (6). Low-molecular-weight fractions, degradation products, and contaminants introduced during manufacturing and handling are non-additives in polymers (1). In a previous study the content of dibutyl phthalate in saliva, from subjects wearing new heat-cured dentures made of PMMA, was quantified (7). Organic additives and degradation products have also been shown to leach in vitro and in vivo from removable orthodontic appliances made of cold-cured PMMA (8–11). However, there is still little information about organic compounds leaching from denture base polymers made of PMMA.

The aim of the present study was to separate, identify, and quantify organic compounds leaching in vitro from specimens made of PMMA denture base materials. A combined gas-chromatography and gas-chromatography/mass-spectrometry technique was applied.

Materials and methods

Specimens

Three different denture base materials were used in this study, one heat-cured and two cold-cured (autopolymerized) products (Table 1). In vitro specimens were made in stainless steel molds with a diameter of 50 mm and a depth of 0.5 mm (12) and processed in accordance with the manufacturer's instructions (Table 1). In addition, specimens from the heat-cured product (Vertex 5 RS) were processed using a technique involving lower processing time and temperature than with the producer-recommended technique (Table 1).

Leaching from specimens in Ringer solution and ethanol

Specimens were placed in separate glass vessels and leached for 7 days in Ringer solution (1 l = 40.5 g NaCl, 89 g KCl, 1.125 g $CaCl_2$, distilled water, pH = 6.0) or 20 h in redistilled ethanol. The vessels were filled with 50 ml Ringer solution or ethanol. They were subjected to agitation (100 rpm) at 37 °C. At the end of the leaching period the specimens were removed, and 1 ml of distilled ethylacetate with an internal standard of diethylphthalate (2 µg/ml) was added.

Analytical procedure

Details of the extraction procedure have previously been described (11).

Gas chromatography (GC). A gas chromatograph (Perkin Elmer Autosystem Gas Chromatograph, Perkin Elmer Corp., Norwalk, Conn., USA) was used. The instrument was equipped with a flame ionization detec-

Table 1. Denture base materials and processing conditions used

Material	Code	Batch no., powder	Batch no., liquid	Powder/liquid ratio (v/v)	Processing temperature (°C)	Processing pressure (MPa)	Time under pressure (min)	Manufacturer
Vertex 5RS	V	9203124	9111054	3:1	100	Compression molding	65	Dentimex, Zezit, Holland
Vertex 5RS, lower processing temperature and time	V_{low}	9303124	9111054	3:1	80	Compression molding	45	Dentimex, Zezit, Holland
Palpress vario, pink	P	0099	193	2:1	55	0.2	15	Kulzer & Co. GmbH, Germany
Swebond Compact	SC	019202	019202	2.5:1	40	0.2	10	Svedia Dental-Industri AB, Sweden

tor, a fused silica column with an inside diameter of 25 m × 0.32 mm, and a film (5% phenyl methyl) thickness of 0.52 µm (Hewlett-Packard Ultra 2 WCOT, Hewlett-Packard Co., Avondale, Pa., USA). Column temperature was taken from 150°C to 200°C at 5°C/min. The injector temperature was 200°C, and the detector temperature 250°C. Splitless injection was used. Peak areas and retention times were recorded by means of an integrator (PE Nelson, Model 1020 GC Plus Upgrade, S100-0310). The quantitative detection limits of dibutyl phthalate (DBP), dicyclohexyl phthalate (DCHP), phenyl benzoate (PB), phenyl salicylate (PS), and biphenyl (BP) were established by different concentrations in Ringer solution and found to be 0.1, 0.5, 0.5, 0.1, and 0.5 µg/ml, respectively. A recovery test was performed in Ringer solution with known added amounts of compounds, and was calculated to be 80–110%.

Gas chromatography/mass spectrometry (GC/MS). A gas-chromatography/mass-spectrometry system (Hewlett-Packard 5970 MSD) with an autosampler (Hewlett-Packard 7673) was used to verify the identity of the substances separated by the GC. Total ion monitoring and selected ion monitoring were used for the MS analyses. The columns used were the same as those described for gas chromatography.

High-performance liquid chromatography (HPLC) and high-performance liquid chromatography/mass spectrometry (HPLC/MS). Control analyses were performed on HPLC, HPLC/MS, and GC with various injector temperatures. Benzoyl peroxide and extracts from leached specimens were used to exclude the possibility that the compounds biphenyl, phenyl benzoate, and phenyl salicylate might be formed from the decomposition of benzoyl peroxide as an analytical artefact by the high temperature in the

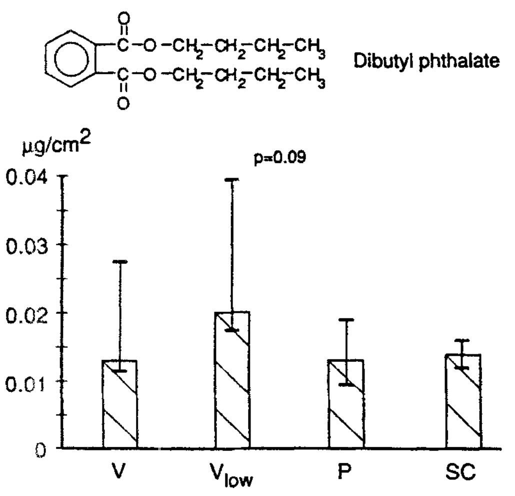

Fig. 1. The amount of dibutyl phthalate (DBP) leaching from denture base materials (see Table 1 for definition of codes) in Ringer solution. Median values (bars) and quartiles (vertical lines) are given.

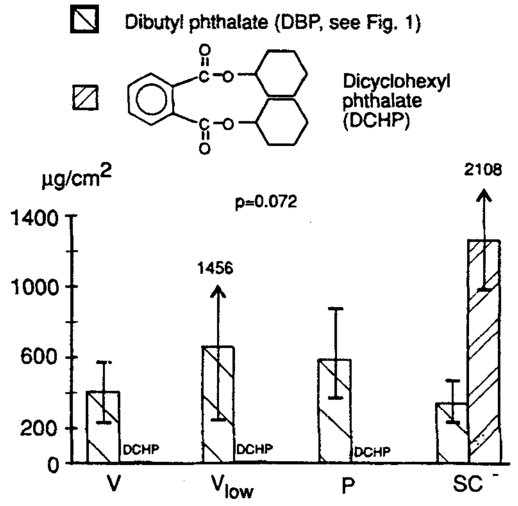

Fig. 2. The amount of dicyclohexyl phthalate (DCHP) and dibutyl phthalate (DBP) leaching from denture base materials (see Table 1 for definition of codes) in ethanol. *P* value referes to DBP. See also legend to Fig. 1.

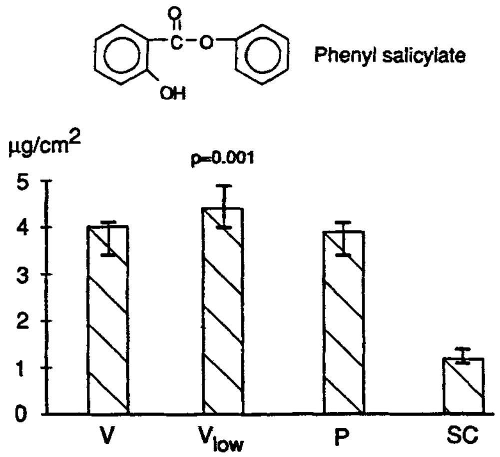

Fig. 3. The amount of phenyl salicylate (PS) leaching from denture base materials (see Table 1 for definition of codes) in ethanol. See also legend to Fig. 1.

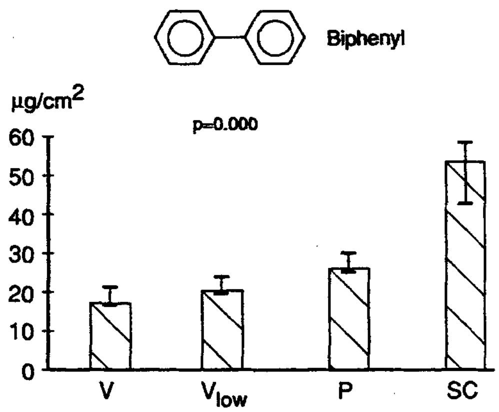

Fig. 5. The amount of biphenyl (BP) leaching from denture base materials (see Table 1 for definition of codes) in ethanol. See also legend to Fig. 1.

GC injector. Powder from the denture base materials was analyzed with the HPLC system to confirm the presence of benzoyl peroxide. The HPLC system (Hewlett-Packard 1050) had a diode-array detector (HP 1040A). A thermospray HPLC/MS system (Vestec Model 201) was applied. The column (10 cm × 4.6 mm inside diameter) was packed with 3-μm C-18 particles. The mobile phase was 70% methanol in 0.1 M ammonium acetate, and the flow rate was 1 ml/min.

Electrothermal atomic absorption spectrophotometry. Electrothermal atomic absorption spectrophotometry (Perkin-Elmer 372, Perkin-Elmer Corp., USA, and HGA 76B Graphite Furnace, Bodenseewerk Perkin Elmer & Co. GmbH, Germany) was used to determine the amount of copper and cadmium in the solutions. Unspecific absorption was corrected for by a deuterium background corrector. The analytical settings were as recommended for the analysis of the respective elements (13).

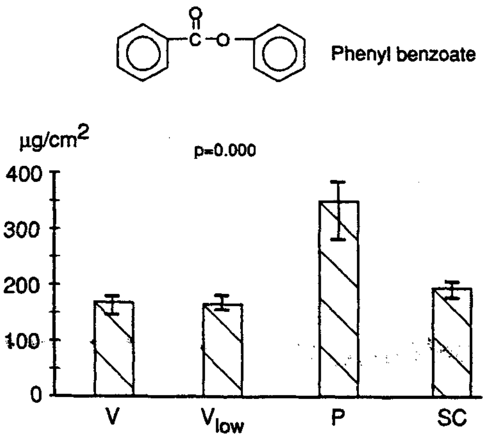

Fig. 4. The amount of phenyl benzoate (PB) leaching from denture base materials (see Table 1 for definition of codes) in ethanol. See also legend to Fig. 1.

Calibration curves were made from standard solutions prepared with the same reagents as those used for the samples. Each sample was analyzed twice.

Light microscopy

Samples from the specimens were embedded in epoxy resin and examined by incident light microscopy.

Presentation of results and statistics

The amounts of BP, DBP, DCHP, PB, and PS leaching from denture base specimens was calculated in μg/cm², on the basis of the mean of duplicate analyses and related to standard solutions. The number of peaks in the chromatograms was counted by the chromatography software, indicating the number of organic compounds that could be separated by the GC. The term 'total quantity of leaching compounds' used here refers to the calculated total amount of identified organic substances. The Mann–Whitney two-sample test and the Kruskal–Wallis one-way analysis of variance were used to test for statistical significance. A significance level of $p < 0.05$ was chosen.

Results

DBP leaching from specimens ($n = 5$) in Ringer solution was quantified (Fig. 1). No significant difference could be detected, either between the denture base materials or between the two processing methods for the heat-cured product (V and V_{low})($p = 0.09$). BP, DCHP, and PB were detected, but the values were below the quantitative detection limit.

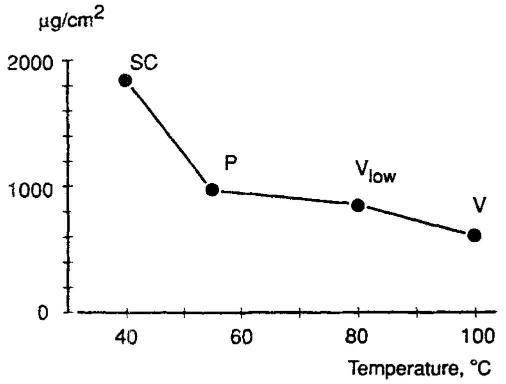

Fig. 6. Relationship between the total amount of identified leaching substances and the processing temperature (see Table 1 for definition of codes).

The amount of DBP leaching from the product Swebond Compact (SC) specimens ($n = 7$) in ethanol was about four orders of magnitude higher than that found in Ringer solution (Fig. 2). There was a significant difference between the denture base materials with regard to the amount in ethanol of DCHP (Fig. 2; $p = 0.001$), PS (Fig. 3), PB (Fig. 4), and BP (Fig. 5) but not for DBP (Fig. 2). PS was found to leach the smallest and DCHP the highest amount (Figs. 2 and 3).

An inverse relationship was found between the amount of leaching substances and the processing temperature (Fig. 6). The total amount of leaching organic compounds (in percentage of specimen weight) from the various denture base materials in ethanol was calculated to be 2.2% (V), 3.1% (V_{low}), 3.6% (P), and 6.8% (SC), respectively (Fig. 6).

Analyses using HPLC from powder of the products showed the presence of benzoyl peroxide. Control analyses could not show the presence of benzoyl peroxide in extracts from cured denture base materials.

The number of peaks in the chromatograms from the various denture base materials leaching in both Ringer solution and in ethanol differed significantly ($p = 0.012$, $p = 0.000$). Specimens from Swebond Compact showed the highest number of peaks and Vertex 5RS (standard processing) the lowest.

Light micrographs from the denture base materials showed numerous polymer beads embedded in a matrix for all the materials, most pronounced for SC (Fig. 7B). Vertex 5RS processed by the producer-recommended technique appeared homogeneous, with very few visible polymer beads (Fig. 7A).

Atomic absorption spectroscopy showed that specimens made of Palapress vario (P) released 0.02 µg/cm² of copper in the ethanol solution. The monomer liquid from this denture base material had a Cu content of 12.1 µg/ml. Cadmium was not detected either from test specimens in ethanol or in Ringer solution.

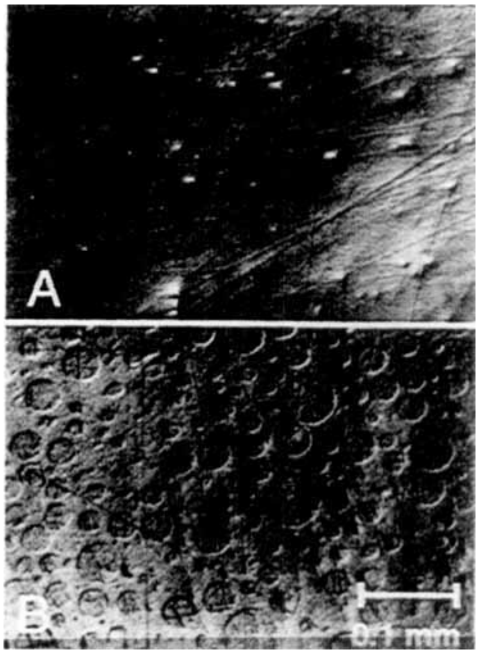

Fig. 7. Light micrographs from denture base materials: 7A) from Vertex and 7B) from Swebond Compact.

Discussion

In vitro leaching has been studied in various dental polymers, such as denture base materials (14, 15), orthodontic bonding resins (16), and dental resin-based composites (17, 18). Denture base materials based on PMMA have been reported to release methyl methacrylate monomer, methacrylic acid, benzoic acid, DBP, PB, BP, and formaldehyde (7, 19, 20, 21, 22). Hensten-Pettersen & Wictorin (23) have shown that denture base materials were cytotoxic to human epithelial cells in vitro. PMMA materials for orthopedic applications (bone cements) were also cytotoxic to fibroblasts and osteoblasts (24). Moreover, exposure of macrophages to PMMA particles free from unreacted monomer inhibited DNA synthesis and impaired their phagocytic ability (25). However, the single compound or compounds responsible for the cytotoxic effects have not been identified.

We suppose that the compounds PB and BP originate in reactions involving radicals from benzoyl peroxide used in the polymerization process of PMMA. A mechanism for formation of PB and BP is proposed (Fig. 8). The calculated total amount of leaching PB and BP was higher in the cold-cured product (Swebond Compact) than in the heat-cured one (Vertex). This is to be expected, since studies have shown a lower degree of conversion (26) and the presence of remaining polymer beads in cold-cured PMMA materials (27).

PS, which was detected in small quantities, could possibly originate from free radicals or from pollution

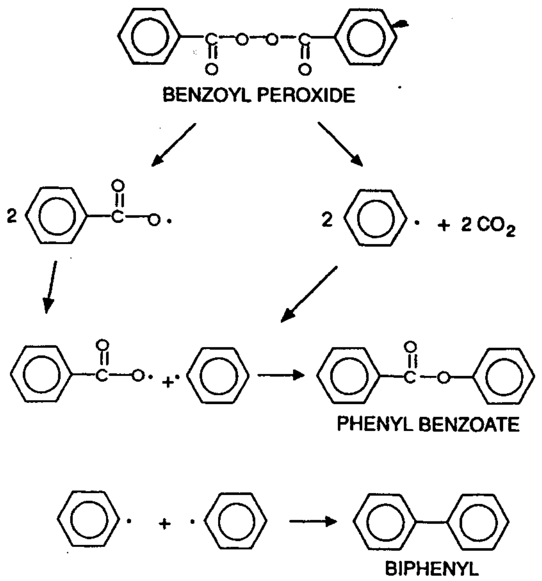

Fig. 8. Proposed mechanism for the formation of phenyl benzoate and biphenyl from benzoyl peroxide.

during the manufacturing process. The low-level leaching of copper in ethanol from the other cold-cured product (Palapress vario) was most likely associated with the initiator system based on barbituric acid (28).

DCHP in addition to DBP was leaching in ethanol from specimens made of one cold-cured product (Swebond Compact), and the total amount of phthalates released was comparable to the previously reported content of DBP from this material (29). This difference could probably be explained by the manufacturer's change of phthalate composition in this material.

The amount of DBP from denture base materials in Ringer solution was comparable to the amount of MMA and DBP leaching from new dentures to the saliva (7, 10). DBP has been shown to be present as an impurity in the liquid part of denture base materials, and it is the commonest plasticizer in dental soft polymers (30).

The finding that there was an inverse relationship between the amount of leaching substances and the processing temperature (Fig. 6) could be expected from the correlation between the degree of conversion and processing temperature in PMMA materials, as shown by Ruyter & Øysæd (26). A similar relationship has been established with resin-based composite (31).

Thermodynamic factors have been shown to influence the leaching of polymer additives and are related to the initial content of organic compounds, their solubility, equilibrium partitioning, and diffusion (31). We suppose that leachable compounds in polymerized denture base PMMA materials consist of by-products from the free radical polymerization reaction, in addition to additives (7). The powder part of PMMA materials usually has a benzoyl peroxide content ranging from 0.5% to 1.5% (33). Benzoyloxy and phenyl radicals, which are considered to be unstable compounds (34), are made from the decomposition of benzoyl peroxide (35).

The possibility that oral diseases of unknown etiology, such as denture stomatitis and burning mouth syndrome, could be associated with compounds leaching from dentures (22, 36) makes it desirable to identify and quantify any leachable substance from denture base materials.

In conclusion, our in vitro study has shown that organic compounds, additives and non-additives, leach from denture base polymers. Biphenyl and phenyl benzoate seems to be formed in PMMA denture base materials from the free radical reactions by the decomposition of benzoyl peroxide in the initiator system.

Acknowledgements.—This work was supported by Tannlege Einar Bergersens legat. We are grateful to Grete Moe for her technical assistance.

References

1. Bruck SD. Leachable components in polymeric medical implants and disposables: a brief critical review. Med Prog Technol 1990; 16:131–43.
2. Leusncher J. Legal requirements for the preclinical toxicological evaluation of biomaterials. Clin Mater 1992;10:51–7.
3. Stafford GD, Bates JF, Huggett R, Handley RW. A review of the properties of some denture base polymers. J Dent 1980;8:292–306.
4. Harrison A, Huggett R. Effect of the curing cycle on residual monomer levels of acrylic resin denture base polymers. J Dent 1992;20:370–4.
5. Øysæd H, Ruyter IE. Creep studies of multiphase acrylic systems. J Biomed Mater Res 1989;23:719–23.
6. Winkler S. Denture base resins. Dent Clin North Am 1984;28:287–97.
7. Lygre H, Solheim E, Gjerdet NR, Berg E. Leaching of organic additives from dentures in vivo. Acta Odontol Scand 1993;51:45–51.
8. Stafford GD, Bates JF, Huggett R. A review of the properties of some orthodontic base polymers. J Dent 1983;11:294–305.
9. Stafford GD, Brooks SC. The loss of residual monomer from acrylic orthodontic resins. Dent Mater 1985;1:135–8.
10. Baker S, Brooks SC, Walker DM. The release of residual monomeric methyl methacrylate from acrylic appliances in the human mouth: an assay for monomer in saliva. J Dent Res 1988;67:1295–9.
11. Lygre H, Klepp KN, Solheim E, Gjerdet NR. Leaching of additives and degradation products from cold-cured orthodontic resins. Acta Odontol Scand 1994;52:150–6.
12. International Standard. ISO 1567:1988 (E). Denture base polymers. The International Organization for Standardization, 1988.
13. Techniques in graphite furnace atomic absorption spectrophotometry. Norwalk (CT): Perkin-Elmer Corp., 1985.
14. Austin AT, Basker RM. The level of residual monomer in acrylic denture base materials. Br Dent J 1980;149:281–6.
15. Austin AT, Basker Rm. Residual monomer levels in denture base. Br Dent J 1982;153:424–6.
16. Thompson LR, Miller EG, Bowles WH. Leaching of unpolymerized materials from orthodontic boding resin. J Dent Res 1982;61:989–92.
17. Ferracane JL, Condon JR. Rate of elution of leachable components from composite. Dent Mater 1990;6:282–7.

18. Rathbun MA, Craig RC, Hanks CT, Filisko FE. Cytotoxicity of a BIS-GMA dental composite before and after leaching in organic solvents. J Biomed Mater Res 1991;25:443–57.
19. Koda T, Tsuchiya H, Yamauchi M, Hoshino Y, Takagi N, Kawano J. High-performance liquid chromatographic estimation of eluates from denture base polymers. J Dent 1989;17:84–9.
20. Koda T, Tsuchiya H, Yamauchi M, Ohtani S, Takagi N, Kawago J. Leachability of denture-base acrylic resins in artificial saliva. Dent Mater 1990;6:13–6.
21. Ruyter IE. Release of formaldehyde from denture base polymers. Acta Odontol Scand 1980;38:17–27.
22. Tsuchiya H, Hoshino Y, Kato H, Takagi N. Flow injection analysis of formaldehyde leached from denture-base acrylic resins. J Dent 1993;21:240–3.
23. Hensten-Pettersen A, Wictorin L. The cytotoxic effect of denture base polymers. Acta Odontol Scand 1981;39:101–6.
24. Perry M, Frondoza C, Jones L, Hungerford DS. The response of macrophages, fibroblasts and osteoblasts to PMMA and metal particles in tissue culture. Trans Orthop Res Soc 1990;15:486.
25. Horowitz SM, Frondoza CG, Lennox DW. Effects of polymethylmethacrylate exposure upon macrophages. J Orthop Res 1988;6:827–32.
26. Ruyter IE, Øysæd H. Conversion in denture base polymers. J Biomed Mater Res 1982;16:741–54.
27. Øysæd H, Ruyter IE. Formation and growth of crazes in multiphase acrylic systems. J Mater Sci 1987;22:3373–8.
28. Gross A. Is the use of cold curing polymers justifiable in the production of definitive dentures. Quintessence Dent Technol 1976;1:1.
29. Ruyter IE, Svendsen SA. Flexural properties of denture base polymers. J Prosthet Dent 1980;43:95–104.
30. Jones DW, Sutow EJ, Hall GC, Tobin WM, Graham BS. Dental soft polymers: plasticizer composition and leachability. Dent Mater 1988;4:1–7.
31. Pham DC, Ferracane JL. Leaching from light-cured composites with variable degrees of conversion. J Dent Res 1988;67:225.
32. Sanchez IC, Chang SS, Smith LE. Migration models for polymers additives. Polymer News 1980;6:249–56.
33. In: Craig RG, editor. Restorative dental materials. St. Louis (MO): The C.V. Mosby Co., 1989:510.
34. Bevington JC. The nature of the initiating reaction in the polymerization of styrene sensitized by benzoyl peroxide. Proc R Soc Lond [Biol] 1957;239:420–32.
35. Barson CA, Bevington JC. A tracer study of the benzoyloxy radical. Tetrahedron 1958;4:147–56.
36. Dutree-Meulenberg ROGM, Kozel MMA, van Joost T. Burning mouth syndrome: a possible etiologic role for local contact hypersensitivity. J Am Acad Dermatol 1992;26:935–40.

Received for publication 31 March 1994
Accepted 5 September 1994