Pharmaceutical Regulatory Affairs: Open Access

Shintani, Pharmaceut Reg Affairs 2014, 3:3
DOI: 10.4172/2167-7689.1000123

Research Article Open Access

Serum or Saliva Extraction of Toxic Compounds from Methyl Methacrylate Dental Materials and HPLC Analysis Combined with SPE

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Abstract

Methyl methacrylate polymer (PolyMMA) is widely used as the composite resin for the dental plate. During the preparation process of PolyMMA by the polymerization reaction, benzoylperoxide (BPO) and N,N'-dimethyl*p*-toluidine (DMPT) are added as the initiator and the stimulator, respectively. These compounds exhibit toxicity as well as a residue potential, their use raises concerns regarding human safety. The degree of elution into serum or saliva was determined to evaluate risk to the user. Analysis was by HPLC combined with solid-phase extraction using a C-18 column. The eluted compounds were found to be in the order of 10 to 70 ppm.

Keywords: Solid phase extraction; HPLC analysis; Dental materials; Methyl methacrylate; Benzoylperoxide; N,N'-dimethyl *p*-toluidine; Toxic compounds

Introduction

Methyl methacrylate polymer (PolyMMA) was originally prepared in 1942 by Kulzer Co. Ltd. Since then it has been widely used as the composite resin for the dental plate [1]. In accordance with the current method of preparation, PolyMMA powder and MMA monomer (MMA) solution were mixed to prepare a composite resin. For the polymerization process, benzoylperoxide (BPO) was added to the PolyMMA powder as the initiator and N,N'-dimethyl *p*-toluidine (DMPT) was added as the stimulator for polymerization. Radicals produced by the oxidation-reduction reaction of BPO and DMPT proceeded the polymerization reaction.

If insufficiently hardened by polymerization, MMA, DMPT, and BPO exhibit a residue potentia1. Because these compounds are reportedly toxic [2], their use represents a degree of risk. The elution of several compounds from dental materials has been previously reported [2-10]. In order to evaluate the risk factor to the user by the elution of these compounds, the authors quantitatively analyzed residue in composite resins in serum and saliva using solid phase extraction (SPE).

Determination was by HPLC combined with SPE. The fact that BPO converts within a few seconds to benzoic acid (BA) when in contact with blood, serum, and saliva is the first finding and original data from the authors. Both BA and BPO are significantly cytotoxic. The originality of this research is the determination of residual amount of MMA, DMPT and BA (BPO) in serum and saliva eluted with HPLC combined with automated SPE. The chemical structures of these components are presented in Figure 1.

Experimental

PolyMMA samples

Two individual PolyMMA composite resins (Yunifast[R] and Acron[R], described samples A and B, respectively) were analyzed. Sample A was polymerized at room temperature and sample B at 100°C. DMPT is not used in the preparation of sample B. The average Rockwell rigidity of samples A and B was 60 and 84 (n=3), respectively, indicating that sample A was more pliant. Each sample (3×6×0.1 cm sheet of approximately 3 g) was immersed into 10 mL of serum or saliva at room temperature. Serum or saliva was replaced daily. The serum or saliva extract was subjected to solid phase extraction followed by HPLC analysis. For each sample, three different specimens were tested (n=3). The serum used was equine serum supplied by Flow Laboratories. Saliva is the author's saliva corrected before breakfast. Concerning serum constituent, there are no significant differences in major components between human serum and equine serum.

HPLC method of analysis

MMA, DMPT, and BPO determination by HPLC was as follows.

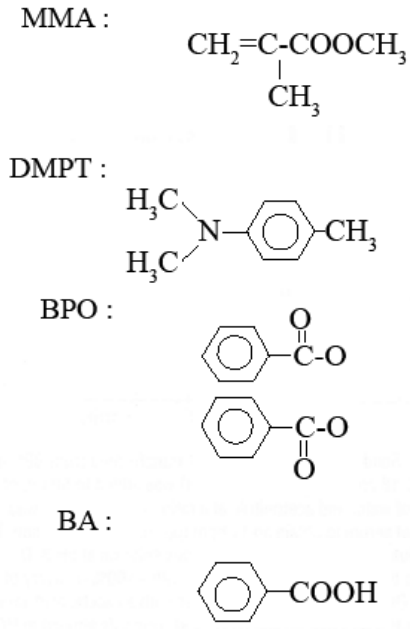

Figure 1: Chemical structure of methyl methacrylate (MMA), N,N'-dimethyl *p*-toluidine (DMPT), benzoylperoxide (BPO), and benzoic acid (BA).

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Received June 07, 2014; **Accepted** June 23, 2014; **Published** June 28, 2014

Citation: Shintani H (2014) Serum or Saliva Extraction of Toxic Compounds from Methyl Methacrylate Dental Materials and HPLC Analysis Combined with SPE. Pharmaceut Reg Affairs 3: 123. doi:10.4172/2167-7689.1000123

A Spectra-Physics HPLC (SP-8750) combined with a Shimadzu UV detector (SPD-2A) was used. The column was Capcell Pak^R C-18 SG-120 from Shiseido Co. Ltd. The eluent was a mixed solution of water and acetonitrile at a ratio of l:1 (v/v). The flow rate was 1.2 mL/min. The detection wavelength was 235 nm. The retention time (t_R) of MMA, DMPT, BPO was 5, 15 and 24 min, respectively.

BPO is converted to benzoic acid (BA) when in contact with serum, saliva or blood; therefore BA analysis is required as follows. A Capcell Pak^R C-18 AG-120 was used with an eluent of a mixed solution of water and acetonitrile at a ratio of 4:1 (v/v). The detection wavelength was 250 nm. The t_R of BA was 5.8 min. The rest of the HPLC procedure was identical to MMA, DMPT, and BPO analysis.

Solid phase extraction of MMA, DMPT and BPO

The described compounds, with the exception of DMPT, are non-ionic. In order to successfully retain DMPT in a reverse phase column (C-18 column), it is necessary to depress ionization. For this purpose, the authors used a 50mM phosphate buffer at pH 7.5 for column conditioning. The column used for SPE was a Bond Elut^R C-18 with a resin weight of 100 mg supplied by Analytichem Co. Ltd.

The standard aqueous solution and the serum or saliva extracted sample were prepared as follows. One to 100 mg each of MMA and DMPT was added to 50 mL of a mixed solution of water and acetonitrile at a ratio of 20:1 (v/v). 0ne aliquot of 100 µL was added to 1900 µL water and to the serum or saliva. A BPO solution was separately prepared identical to this procedure. At this time BPO was converted to BA. This is a requisite procedure as DMPT and BPO were reacted when mixed, and the mixed solution became bluish in color due to a reaction [9,10]. BA and DMPT derivatives are reported to be included in the reaction products [9,10].

A C-18 column was conditioned with 2 mL of acetonitrile and 2 mL of a 50 mM phosphate buffer at pH 7.5. Thereafter, 1 mL of the standard aqueous solution and the serum or saliva sample containing one to 100 ppm of MMA, DMPT, and BPO were applied to the conditioned column, rinsed with 0.5 mL of a 50 mM phosphate buffer at pH 7.5, and eluted by l mL of acetonitrile. The drain was trapped and 20 µL applied to HPLC. These procedures were conducted under a vacuum system using a Vac Elut^R SPS24 supplied by Analytichem Co. Ltd. [11].

Solid phase extraction of BA

BA is an acidic compound with a terminal carboxylate. In order to successfully retain BA in a reversed phase column, it is necessary to depress ionization. For this purpose, an acetic acid aqueous solution at pH 3 was added to the sample solution at a ratio of 1:1 and mixed well. The column was a Bond Elut^R C-18 with a 100 mg resin weight. Both the standard aqueous solution and the serum or saliva sample were prepared as follows. From l to 100 mg of BA were added to 50 mL of a mixed solution of water and acetonitrile at a ratio of 20: 1 (v/v). 100 µL was added to 1900 µL of water and to the serum or saliva.

C-18 column was conditioned with 2 mL of acetonitrile and 2 mL of an acetic acid aqueous solution at pH 3. Thereafter, 1 mL of the standard aqueous solution and the serum or saliva sample containing l to 100 ppm of BA were applied to the conditioned column. These were two-fold diluted with an acetic acid aqueous solution at pH 3 prior to application to the C-18 column and applied to the conditioned column. Thereafter, both were rinsed with 0.5 mL of an aqueous solution of acetic acid at pH 3 and eluted by l mL of acetonitrile acidified with acetic acid to pH 2.5. The drain was trapped and 20 µL applied to HPLC. The above procedure was carried out under a vacuum system [11].

Results and Discussion

MMA, DMPT, BPO and BA analysis by HPLC

In order to successfully determine DMPT, free silanol in HPLC packing should be sufficiently endcapped to prevent DMPT tailing. Simultaneous analysis of MMA, DMPT, and BPO without DMPT tailing can be successfully attained using a Capcell Pak C-18 SG-120 column because SG-120 successfully prevents free silanol effect by silicone coating. Paired-ion reverse phase chromatography using an addition of alkylsulfonate (e.g., 10 mM octanesulfonate) to the eluent is an alternative method for DMPT analysis.

BA was successfully determined without tailing using a CapcelIPak C-18 AG-120 column. Ion-suppression chromatography using an acidified eluent to depress BA ionization is an alternative method. Unless depressed, BA showed tailing or delayed elution. It is necessary to adjust the eluent pH to 3 as the pKa of BA is 4.2.

Ion-paired chromatography for DMPT analysis and ion-suppression chromatography for BA analysis were found to be inferior to the authors' current method due to an unstable baseline fluctuation in UV detection caused by impurities in the reagents and the irregular pumping of the HPLC system. These methods may in fact cause deterioration of the analytical column by reagent deposit and dissolution of silanol on the column [12].

Solid phase extraction of MMA, DMPT, BPO and BA

Initially, a liquid-liquid extraction procedure was employed by adding an identical volume of acetonitrile to serum or saliva to deproteinize and extract MMA and DMPT, as was carried out in our previous research for 4,4"-methylenedianiline analysis [12]. Because MMA peak showed insufficient separation from serum admixtures and insufficient recovery, therefore the alternative SPE was considered.

The SPE of BA in food has been reported [13,14]. However, there have been no reports of a satisfactory recovery rate in SPE of MMA, DMPT and BA in serum or in saliva. When citation 14 method was applied to serum BA recovery, the recovery rate was insufficient as a result of a weak eluent driving force (the eluent being a mixed solution of water and acetonitrile at a ratio of 1:1 (v/v) [14]. Subsequently, the use of acetonitrile, alkalized acetonitrile containing 50 mM sodium hydroxide, and acidified acetonitrile adjusted to pH 2.5 with acetic acid were examined to compare. Acetonitrile resulted in an insufficient recovery rate. Alkalized acetonitrile and acidified acetonitrile showed 85 and 100% BA recovery, respectively, (the average recovery of n=3 and CV being within 1.4%); therefore, acidified acetonitrile was selected.

Acidified aqueous solution was used for conditioning and rinsing; and thus, acidified acetonitrile was found to be superior. Acidification successfully suppresses dissociation of free silanol and ionization of BA. A satisfactory recovery of BA from C-18 column was attained using acidified acetonitrile. The authors speculated that, due to BA ionization, alkalized acetonitrile would be superior to acidified acetonitrile. The experimental result, however, was to the contrary, possibly due to dissociation of free silanol by alkalization and the retention of ionized BA by dissociated silanol, resulting in an unsatisfactory recovery using alkalized acetonitrile. Therefore, the dissociation of the compound of interest and the behavior of free silanol must be considered at the same time when considering the eluent condition of SPE.

When considering DMPT elution, alkalized acetonitrile was thought to be more effective than acetonitrile as indicated in the authors' previous paper describing a C-18 column solid phase extraction for an

aromatic amine compound [11]. Alkalized acetonitrile in fact showed a satisfactory recovery of serum DMPT. Acetonitrile also produced a satisfactory recovery for MMA and DMPT. Acidified acetonitrile is speculated to be inferior to alkalized acetonitrile, but this is not certain.

With respect to SPE of MMA, DMPT, and BPO, a 50 mM phosphate buffer at pH 7.5 was used for column conditioning. If water and more than a 50 mM phosphate buffer were used, a lower DMPT recovery was resulted due to insufficient depression of DMPT ionization and to unsatisfactory retention as water is acidified by dissolving atmospheric CO_2. When using more than a 50 mM phosphate buffer, salts may interfere with the retention of MMA and DMPT in the reversed-phase column.

Alkalized serum for depressing DNIPT ionization is an alternative choice and assists retention of DMPT in a C-18 column. Serum or saliva naturally indicates alkalinity (pH 7.4); thus, DMPT in serum or saliva is perceived to be successfully retained on a C-18 column without alkalizing serum or saliva. A phosphate buffer at pH 7.5 used for column conditioning also contributed to DMPT retention in the C-18 column.

Figure 2a and 2b show HPLC chromatograms of a standard sample solution, a serum sample containing 11 ppm of MMA and DMPT, and a blank serum, respectively. Saliva was almost identical to serum. Following treatment, MMA and DMPT were 100% (n=3) recovered from the C-18 column with no significant difference between the standard sample solution and the serum or saliva sample in the HPLC chromatogram. This indicates that there is no significant interference by serum or saliva admixtures with the elution and recovery of MMA and DMPT. The blank serum chromatogram in Figure 2c shows no elution of these compounds and no interference by serum admixtures. Chromatogram using saliva was almost identical to serum. The recovery rate is calculated by the comparison of the recovered peak area and the peak area of the standard sample solution.

Figure 3a shows that the standard sample solution of BPO at a concentration of 11 ppm was 100% recovered from a C-18 column. When BPO was added to serum or saliva at the concentration of 11 ppm, BPO was wholly converted to BA within a few seconds with no BPO elution and residue (Figure 3b). The blank serum or saliva chromatogram was identical to Figure 2c. BPO did not react with serum components such as amino acid, protein, and nucleic acid. BPO when contacting with serum or saliva, BPO immediately converts to 2 mol BA and no attach to serum or saliva constituent. It can be clarified from the 100% recovery of BA as mentioned in advance.

Figure 4a shows an HPLC chromatogram of serum sample containing 2.2 ppm of BPO, which was 100% converted to BA and 100% recovered from a C-18 column by serum or saliva BA solid phase extraction. Figure 4b shows a serum blank.

The authors are currently studying the simultaneous SPE of MMA, DMPT, BPO, and BA (acidic, basic and non-ionic compounds) in serum or saliva using a combination of a cation exchange column (SCX column) and a reverse phase column (C-18 column), or using a single column, such as Bond Elut Certify^R and an acidified serum. The simultaneous method may be less complicated.

Eluted amount of MMA, DMPT, and BA from BPO to serum or saliva from two different PolyMMA dental materials

Two different samples of Yunifast and Acron, described as samples A and B respectively, with an almost identical weight were immersed in 10 mL of serum or saliva. The eluted amount was determined by

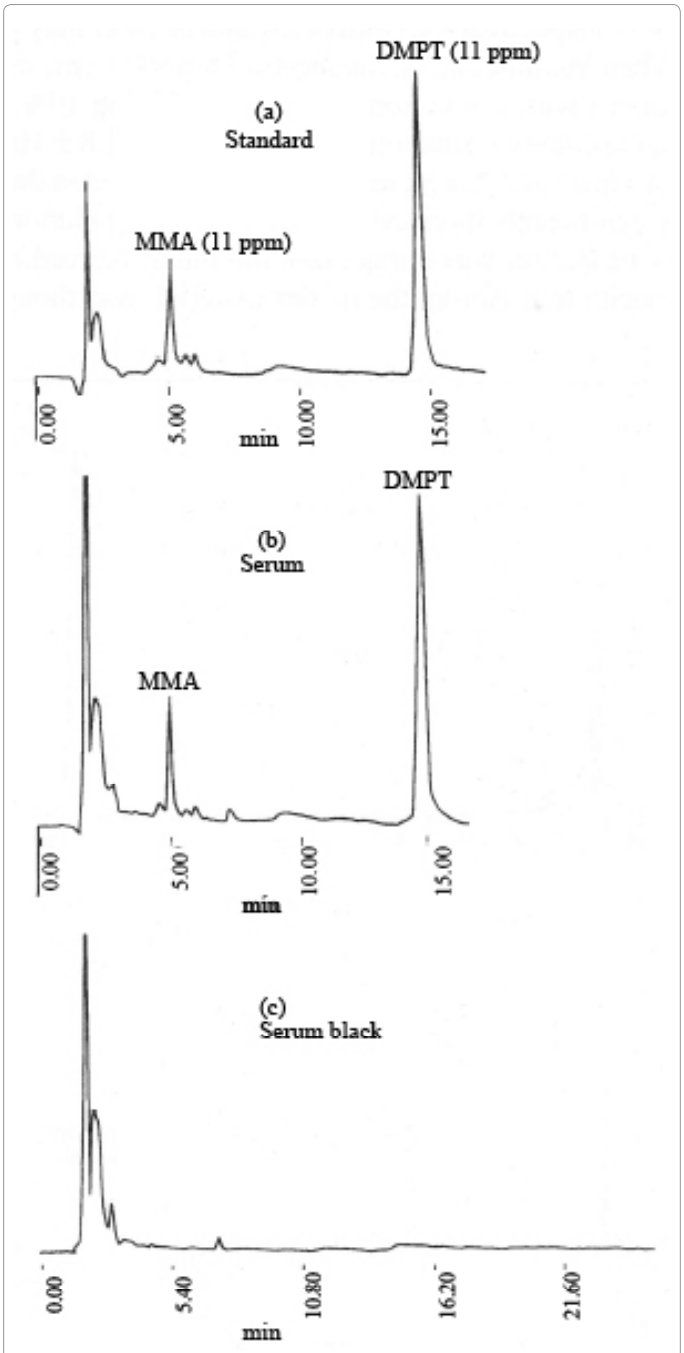

Figure 2: Solid phase extraction of MMA and DMPT from a C-1 8 column. (a) 11 mg of MMA and DMPT were added to 50 mL of a mixed solution of water and acetonitrile at a ratio of 20:1 (v/v). 100 µL was added to 1900 µL of water to obtain l l ppm (µg/mL) solution. 0ne mL was applied to the conditioned C-18 column with a 100% recovery. (b) Identical to (a) excepting that 100 µL of solution was added to 1900 µL of serum in place of water to obtain 11 ppm (µg/mL) serum solution. 0ne mL was applied to the conditioned C-18 column with a 100% recovery, (c) 0ne mL of a native serum was treated, recovered and applied to HPLC. The result indicated no elution of MMA and DMPT and no interference by serum admixtures.

SPE followed by HPLC. The serum or saliva was replaced daily. The amount of MMA and DMPT was accumulated to evaluate the extractable quantity. Figure 5 shows the HPLC chromatogram of MNIA and DMPT from PolyMMA sample A extracted with serum. Saliva extraction was almost identical to serum, therefore identical to

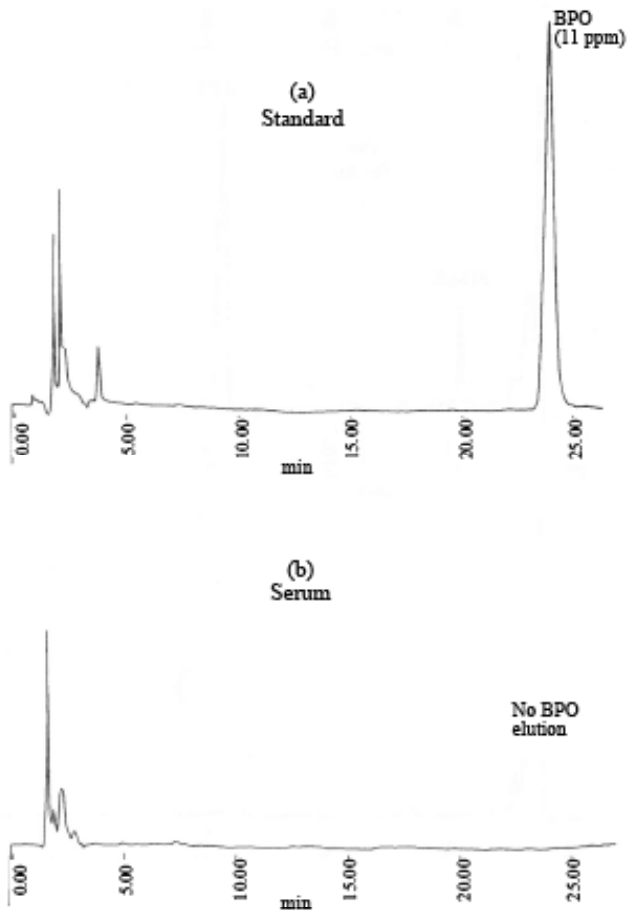

Figure 3: Solid phase extraction of BPO from a C-18 colum. (a) 11 mg of BPO was added to 50 mL of a mixed solution of water and acetonitrile at a ratio of 20:1 (v/v). 100 µL was added to 1900 µL of water to obtain an 11 ppm (µg/mL) solution. 0ne mL was applied to the conditioned C-18 column with a 100% recovery, (b) Identical to (a) excepting that 100 µL of solution was added to 1900 µL of serum in place of water to obtain an 11 ppm (µg/mL) serum solution. 0ne mL was applied to the conditioned C-18 column with no BPO recovery. This was due to the conversion of BPO to BA when in contact with serum. The blank serum was identical to Figure 2c.

Figure 5. The blank serum or saliva chromatogram is almost identical to Figure 2c, indicating no interference with determination of these compounds.

MMA and DMPT, eluted to serum or saliva during consecutive three days of immersion were 32.04 µg/g and 66.44 µg/g for sample A (Yunifast), and 10.32 µg/g and zero for sample B (Acron), respectively. Saliva's result was almost identical order to serum or somewhat less in Samples A and B. DMPT was not detected in sample B because it was not used during preparation of PMMA. A greater amount of MMA and DMPT was extracted from sample A, the softer material, than from sample B because serum or saliva can more easily penetrate the interior of the softer sample. The elution time course of serum is presented in Figure 6. Saliva presents also identical time course. The individual plot was the average amount of three different specimens (n=3).

The extract from sample A indicated more cytotoxicity. The degree of cytotoxicity of dental materials is proportional to the extractable quantity [15,16]. The order of cytotoxicity of the components of BPO, DMPT, and MMA was as follows: BPO ≫ DMPT > MMA [15]. The cytotoxicity data of BA in the data book is thought to equal that of BPO as BPO converted to BA immediately when in contact with cell culture medium containing 10% of blood serum. Cytotoxicity data of IC50 (µg/mL) of BPO, BA, DMPT, and MMA using Balb 3T3 cell were 22, 28.7, 1500 and 4400, respectively (Table 1). Cytotoxicity data of BPO may be due to BA. When Yunifast and Acron sheets (3×6×0.1 cm) were in direct contact with cell culture medium containing 10% of blood serum, the colony formation rate was 0% and 81.8 10.2% (n=3) for Yunifast and Acron, respectively; indicating that sample A was significantly more cytotoxic than sample B, mostly due to greater elution and therefore colony formation was completely inhibited. According to the cytotoxicity test, Acron, the harder material, was thought to be more appropriate material because of less elution. Less elution of cytotoxicity compounds is thought to be much safer.

Interestingly, the hydrophilic portion of acetone extract from Yunifast indicated more cytotoxicity than the hydrophobic portion, which is the first finding and the original data from the authors. BA was the major component and -toluidine from DMPT was the minor component contained in the hydrophilic portion. There is a possibility that unidentified cytotoxic components are contained in the

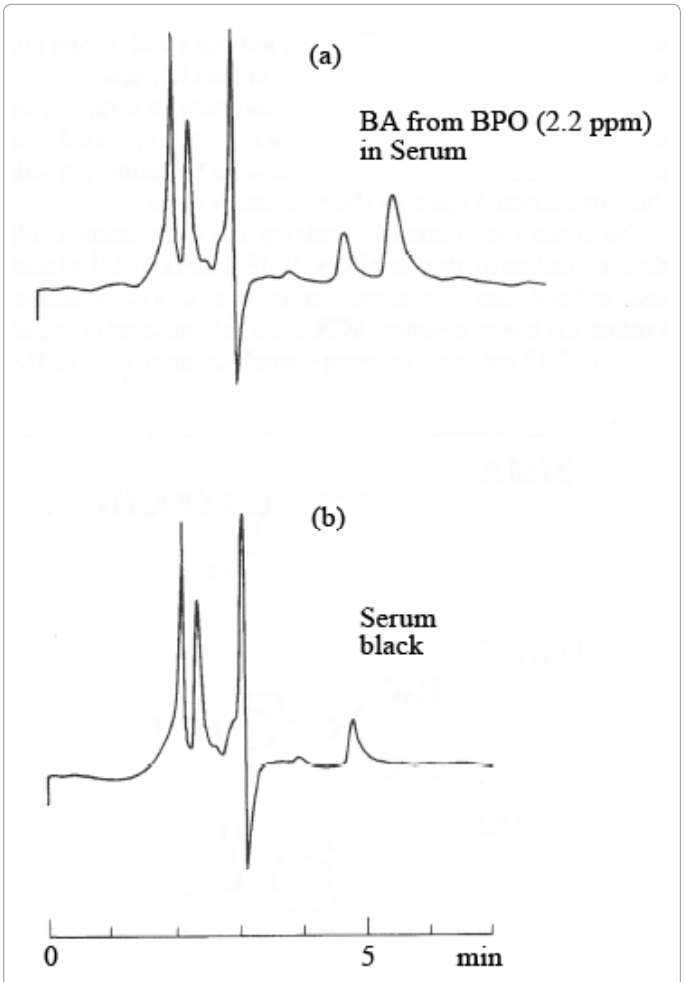

Figure 4: Solid phase extraction of BA transformed from BPO in serum using a C-18 column. (a) 11 mg of BPO was added to 50 mL of a mixed solution of water and acetonitrile at a ratio of 20:1 (v/v). 100 µL was added to 1900 µL of serum to obtain an 11 ppm (µg/mL) serum solution, This was 5-fold diluted with an acetic acid aqueous solution at pH 3. 0ne mL was applied to the conditioned C-18 column with a 100% recovery of BA from BPO, (b) One mL of 5-fold diluted serum with an acetic acid aqueous solution at pH 3 was treated and recovered. This was applied to HPLC. The result indicates no elution of BA and no interference by serum admixtures.

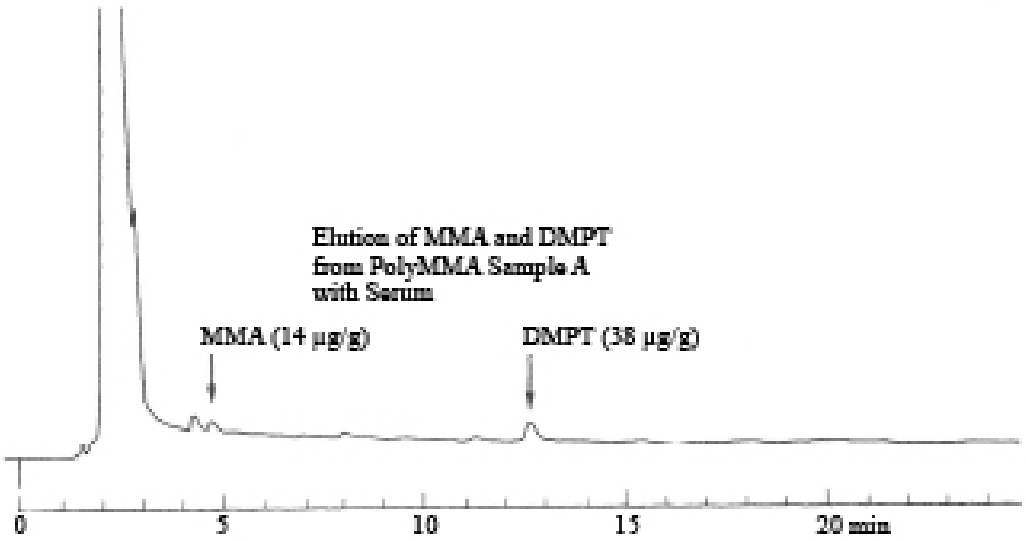

Figure 5: HPLC chromatogram of MMA and DMPT from PolyMMA sample A Yunifast with serum elution. The unit of MMA and DMPT amount is µg/sample g.

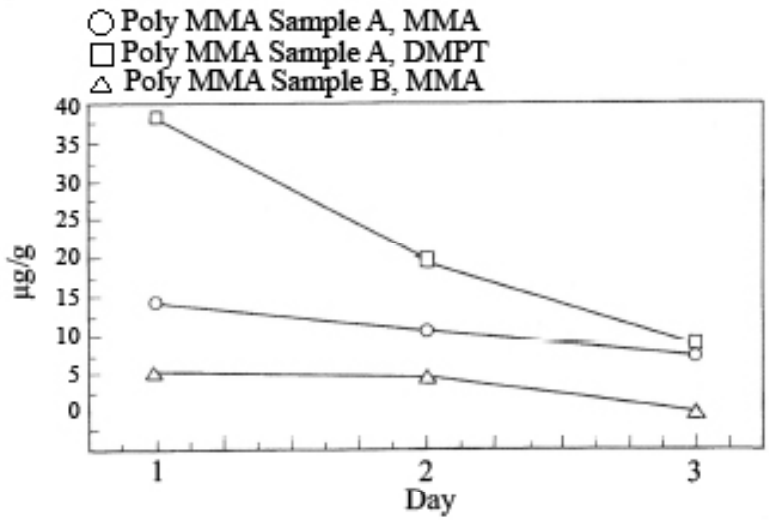

Figure 6: Elution of MMA and DMPT from PolyMMA samples A (Yunifast) and B (Acrom) into serum. The unit of MMA and DMPT amount is µg/sample g. No elution of DMPT was recognized in sample B Acron due to no use of DMPT for preparation.

Dental component	IC50 (µg/mL)
BPO	22
BA	28.7
DMPT	1500
MMA	4400

Table 1: Cytotoxicity data, IC50, of dental material IC50 data is the average of n=3.

hydrophilic portion. This study is now under progress. In the acetone extract from Yunifast and Acron, the average amount of BA eluted was 33 17 and 108 µg/g (n=3), respectively, indicating more BA, as well as MMA and DMFT, was eluted from sample A. BA and DMPT derivatives were reported to be produced when BPO and DMPT reacted [9,10]. Therefore, immersion of both PolyMMA materials in hot water before use is recommendable to prevent the elution of hydrophilic cytotoxic compounds such as BA and *p*-toluidine from PolyMMA dental materials [5].

BPO elution from PolyMMA to serum or saliva was negligible (a few µg/g and 0 µg/g for samples A and B, respectively). This is speculated to be due to the excessive hydrophobicity of BPO. A greater BPO quantity was eluted from the softer material of sample A. BPO elution to serum or saliva showed a dissimilar pattern because BPO was continuously converted to BA in serum or saliva, hence the driving force of elution shifts continuously to BPO elution. Therefore, extractable BPO amount may be greater than that by the normal elution status.

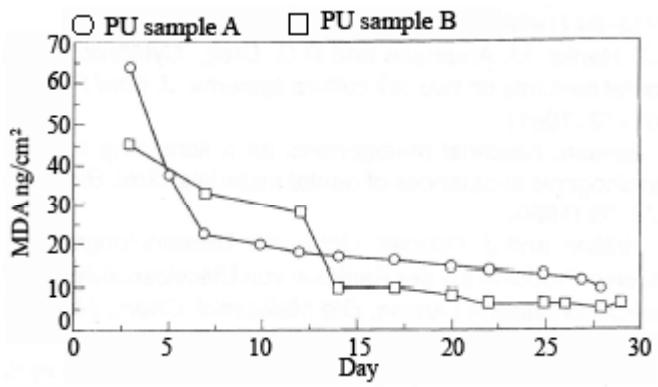

Figure 7: Elution of 4,4'-methylenedianiline (MDA) from two different thermosetting polyurethane (PU) potting material samples A and B into serum, The unit of MDA amount is ng/sample cm^2.

When considering the hydrophobicity of MMA, DMPT, and BPO, BPO shows the greatest hydrophobicity. Eluted DMPT of an ionic compound was greater than MMA of a non-ionic compound, indicating that serum or saliva extracts both hydrophobic and hydrophilic compounds [17]. The elution of hydrophobic compounds to serum or saliva is demonstrated by the presence of lipoprotein in serum [17].

Previously, the authors conducted experimental work with the elution time course of 4,4'-methylenedianiline (MDA) using equine serum from thermosetting polyurethane (PU) potting material samples A and B (Figure 7) [17]. PU sample A is more pliant than PU sample B. The average Barcol rigidity of PU samples A and B was 92 (n=3) and 98 (n=5), respectively [17,18], indicating that PU was more pliant than PolyMMA (Barcol rigidity of PolyMMA, at more than 100, was greater than that of PU).

The results indicated that eluted amount of MDA from more pliant PU sample A was greater than that from more rigid PU sample B; therefore the rigidity of the material is a factor in the estimation of an extractable quantity. The penetration capacity of the solvent for the material is an another factor in estimating the extractable quantity. MDA in serum or saliva was determined by a reversed-phase HPLC method combined with SPE using Bond Elut C-18 Phenyl or Cyclohexyl columns [11].

Conclusion

MMA, DMPT, BPO and BA in serum or saliva were successfully determined by HPLC combined with SPE using a C-18 column. MMA and DMPT were eluted to serum or saliva in the order of 10 to 100 ppm. Almost negligible amounts of BA from BPO were eluted to serum or saliva. A greater amount of MMA, DMPT, and BA from BPO was extracted from Yunifast, a more pliant material. Cytotoxicity of dental materials is proportional to the extractable quantity. The rigidity of materials and the solvents capacity for penetration to the interior of the material are critical factors in estimating the extractable quantity. It is interesting to note that DMPT showed a greater elution to serum or saliva than MMA. The hydrophilic portion of Yunifast extract indicated greater cytotoxicity than the hydrophobic portion. The hydrophilic portion contained BA and *p*-toluidine, as major and minor components, respectively. Other unidentified components may also be contained in the hydrophilic portion. Therefore, it is recommended that both Yunifast and Acron are immersed in hot water before use in order to prevent the elution of hydrophilic toxic compounds from PolyMMA dental materials. The last warning is for the dentist for maintaining patient's benefit. Extraction with serum was almost identical to saliva or somewhat greater than that with saliva.

References

1. Tsuruta T, Ed. Medical Material. Japan Polymer Society, Tokyo, 1990.
2. Corkill JA, Lloyd EJ, Hoyle P, Crout DHG, Ling RSM, et al. (1976) Toxicology of methyl methacrylate: The rate of disappearance of methyl methacrylate in human blood in vitro. Clin Chim Acta 68: 141-146.
3. Thompson LR, Miller EG, Bowles WH (1982) Leaching of unpolymerized materials from orthodontic bonding resin. J Dent Res 61: 989-992.
4. Stafford GD, Brooks SC (1985) The loss of residual monomer from acrylic orthodontic resins. Dent Mater 1: 135-138.
5. Baker S, Brooks SC, Walker DM (1988) The release of residual monomeric methyl methacrylate from acrylic appliances in the human mouth: an assay for monomer in saliva. J Dent Res 67: 1295-1299.
6. Oysaed H, Ruyter IE, Sjøvik Kleven IJ (1988) Release of formaldehyde from dental composites. J Dent Res 67: 1289-1294.
7. Hanks CT, Anderson M, Craig RG (1981) Cytotoxic effects of dental cements on two cell culture systems. J Oral Pathol 10: 101-112.
8. Jonsen J (1980) Bacterial mutagenesis as a screening method for carcinogenic substances of dental materials. Adv Biomater I: 333-339.
9. Hrabak F, Coupek J (1971) Ueber die Umwandlungen der primaeren Produkte bei der Reaktion von Diacylperoxiden mit N,N'-dialkylaromatischen Amine. Die Makromol Chem 145: 289-298.
10. Hrabak F (1960) Initiation of polymerization by means of the systems peroxide-tertiaryamine. Collect Czech Chem Commun 25: 829-832.
11. Shintani H (1991) Solid-phase extraction of a carcinogen, 4,4'-methylenedianiline, in serum. J Anal Toxicol 15: 198-201.
12. Shintani H, Nakamura A (1989) Analysis of a carcinogen, 4,4'-methylenedianiline, from thermosetting polyurethane during sterilization. J Anal Toxicol 13: 354-357.
13. Shintani H (1989) The analysis of carcinogen, 4,4'-methylenedianiline (MDA) in gamma-ray and in autoclave-sterilized thermoplastic polyurethane. Z Anal Chem 333: 637-642.
14. Terada H, Sakabe Y (1985) Studies on the analysis of food additives by high-performance liquid chromatography. V. Simultaneous determination of preservatives and saccharin in foods by ion-pair chromatography. J Chromatogr 346: 333-340.
15. Personal communication with Dr. T. Tsuchiya at The National Institute of Hygienic Sciences, Tokyo, Japan, 1992.
16. Caughman WF, Caughman GB, Shiflett RA, Rueggeberg F, Schuster GS (1991) Correlation of cytotoxicity, filler loading and curing time of dental composites. Biomaterials 12: 737-740.
17. Shintani H, Nakamura A (1991) Formation of 4,4'-methylenedianiline in polyurethane potting materials by either gamma-ray or autoclave sterilization. J Biomed Mater Res 25: 1275-1286.
18. Washburn LR, Somerson NL (1979) Lipoproteins as substitutes for serum in Mycoplasma culture medium. J Clin Microbiol 10: 586-589.