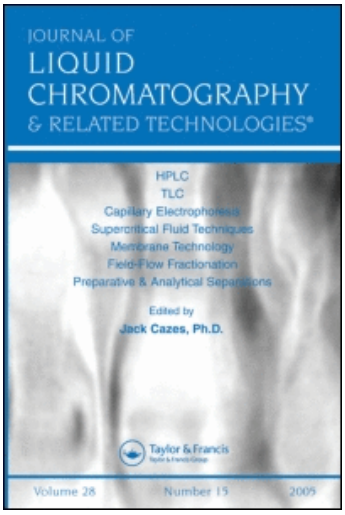


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC Analysis of Toxic Additives and Residual Monomer from Dental Plate

H. Shintani[a]
[a] National Institute of Health Sciences, Tokyo, Japan

To cite this Article Shintani, H.(1995) 'HPLC Analysis of Toxic Additives and Residual Monomer from Dental Plate', Journal of Liquid Chromatography & Related Technologies, 18: 3, 613 — 626
To link to this Article: DOI: 10.1080/10826079508009261
URL: http://dx.doi.org/10.1080/10826079508009261

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(3), 613-626 (1995)

HPLC ANALYSIS OF TOXIC ADDITIVES AND RESIDUAL MONOMER FROM DENTAL PLATE

H. SHINTANI
National Institute of Health Sciences
1-18-1, Kamiyoga, Setagaya, Tokyo, Japan 158

ABSTRACT

Methyl methacrylate polymer, polysulphone, polycarbonate and Cleafil^R are widely used as the dental plate. During fabrication, there is a potential for toxic compound residue of methyl methacrylate, N,N-dimethyl p-toluidine, benzoylperoxide, benzoic acid, bisphenol A, 4,4'-dichlorodiphenylsulfone, triethyleneglycol dimethacrylate and bis phenol A diglycidylmethacrylate. This raises a safety concern as these compounds exhibit carcinogenicity and mutagenicity. The elution of these compounds to water, organic solvents and serum is determined to evaluate a risk to the patients. Analysis is by HPLC using a newly developed and sufficiently endocapped ODS column combined with UV detection. The eluted amount is generally proportional to the hydrophobicity of the organic solvent. Serum extracts hydrophilic compounds as well as hydrophobic compounds. Risk to the patients exposed by these compounds is not so significant.

INTRODUCTION

The leaching of chemicals from various denture base resins into the solvents (water, methanol, acetone, tetrahydrofuran (THF) and serum) was investigated. Two types of polymethylmethacrylate (PMMA) resins, Acron^R (heat-curing) and Yunifast^R (chemically activated), and polysulfone (PS), polycarbonate (PC) resins and Cleafil^R were used in the study. Eluted methyl methacrylate (MMA), N,N-dimethyl p-toluidine (DMPT), benzoylperoxide (BPO), benzoic acid (BA), bisphenol A (bis A), 4,4'-dichlorodiphenylsulfone (DCDPS), triethyleneglycol dimethacrylate (TEGDMA, 3G) and bisphenol A diglycidylmethacrylate (bis GMA) in the solvent were determined. In serum as well as saliva extraction, BPO transformed immediately into BA due to

enzymes, therefore BPO exists as BA in serum. Thus BA was determined as BPO in serum extraction.

Residual monomer and several kinds of additives caused hazardous effects to the patient such as mutagenicity, carcinogenicity or cytotoxicity. Therefore, the author studied for the time course elution and total elution amount of these chemicals. A high performance liquid chromatography (HPLC) determination was carried out using a newly fabricated and completely endocapped C-18 column combined with UV detection. Additionally, the author studied for the procedure to prevent the elution of these compounds as well as the risk to the patients.

MATERIALS AND METHODS

Polymethylmethacrylate dental material (Yunifast^R and Acron^R), PS, PC and Cleafil^R were prepared at 1 mm thickness plate according to the instruction of the manufacturers. After fabrication, Yunifast^R and Acron^R were kept in air for one week at 37°C and thereafter kept into polyethylene bag at room temperature. These were used for the experiment. Other reagents used were HPLC use grade.

EXPERIMENTAL PROCEDURE

Each one gram of Yunifast^R and Acron^R was immersed in 20 ml aliquot of water, methanol, acetone, THF and serum in a glass-stoppered flask and allowed to stand for 24 hr at room temperature. The solvent was decanted and MMA, DMPT, BPO, BA, bis A, DCDPS, 3G and bis GMA in the solvent were determined by HPLC. Thereafter, to the identical flask were added 20 ml of the fresh solvent and extraction and HPLC analysis were repeated in the identical manner to attain the time course elution as well as the total elution amount.

In case of analysis, the solvents was not removed by vacuum evaporation by heating in order to prevent vaporization of MMA. Furthermore, the mixture solution of acetonitrile and water at the ratio of 1:1 was added to the extraction solvent at an identical volume in order to deposit polymer and oligomers. The supernatant was sampling, filtrating with the filter of pore size of 0.45 μm and 20 μl were applied to HPLC.

In case of PS, PC and Cleafil^R, bis A, DCDPS, 3G and bis GMA were determined in the identical manner mentioned above. Solvents were not removed by evaporation in order to prevent polymerization.

Chemical structure of MMA, DMPT, BPO, BA, bis A, DCDPS, 3G and bis GMA is presented in Figure 1.

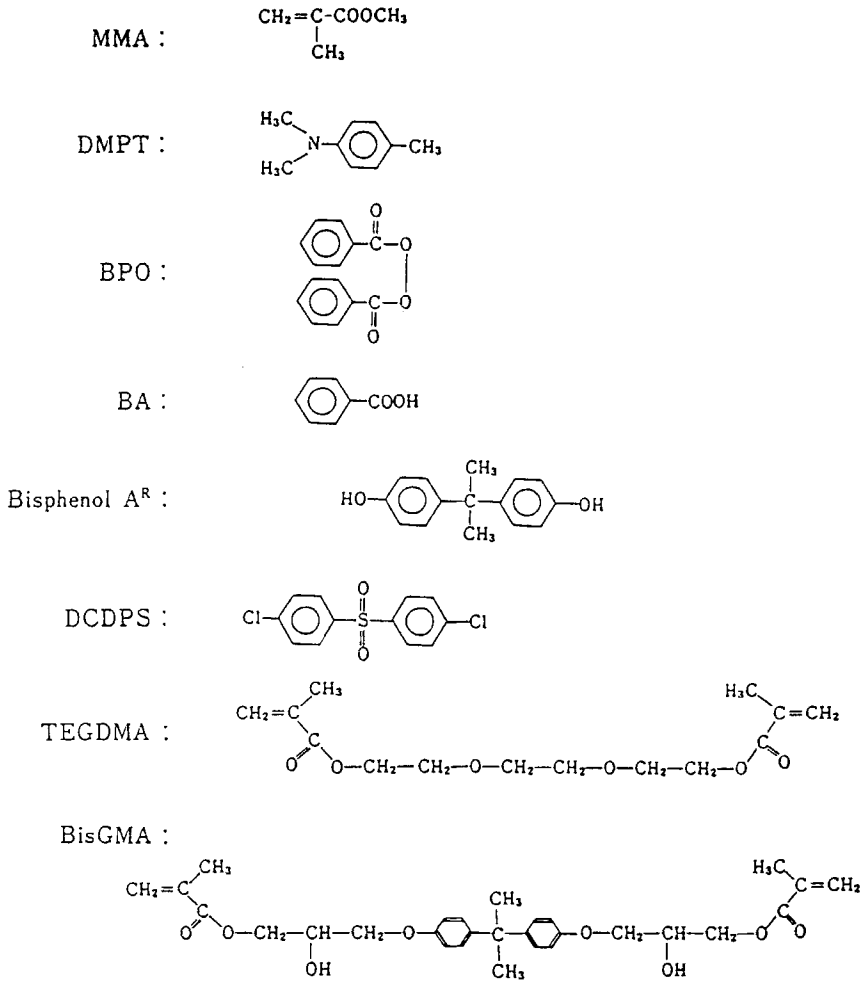


FIGURE 1 Chemical structure of compounds

ANALYTICAL PROCEDURE

HPLC analysis of MMA, DMPT and BPO

A newly developed and successfully fabricated C-18 column of Capcell Pak^R SG-120 (4.6X250 mm) from Shiseido Co. Ltd. was used. This column was successful for

preventing a residual silanol effect by silicone coating. In general fabrication procedure of Capcell Pak^R, silanol was coated with silicone and thereafter C-18 was linked onto silicone, therefore residual silanol effect was totally diminished. Weakness of this column is that the capacity was less than that of the conventional silanol linked C-18 column. The eluent was the aqueous mixture of water and acetonitrile at the ratio of 52/48, flow rate of 1 ml/min and detection at 235 nm at room temperature. Retention time of MMA, DMPT, BPO was 6.3, 17.3 and 29.6 min, respectively.

HPLC analysis of bis A and DCDPS

Almost identical to the analysis of MMA, DMPT and BPO with the exception of eluent of aqueous mixture of water and acetonitrile at the ratio of 1/1 and the retention time of bis A and DCDPS was 6.2 and 21.9 min, respectively.

HPLC analysis of 3G and bis GMA

Almost identical to the analysis of MMA, DMPT and BPO with the exception of eluent of aqueous mixture of water and acetonitrile at the ratio of 45/55 and the retention time of 3G and bis GMA was 7.0 and 15.7 min, respectively.

HPLC analysis of BA

Almost identical to the analysis of MMA, DMPT and BPO with the exception of the use of the column of Capcell Pak^R C-18 AG-120 (4.6X250 mm) in place of SG-120. The eluent was the aqueous mixture of water and acetonitrile at the ratio of 4/1 at pH 3 with phosphoric acid. Retention time of BA was 5.2 min.

MMA, DMPT and BA from BPO in serum were recovered using a solid phase extraction (SPE) with C-18 column (resin weight 100 mg, void volume 120 μl) cited in reference 1.

RESULTS AND DISCUSSION

Selection of the most appropriate column for HPLC analysis

The author studied for several C-18 columns available in the market to attain the sufficient baseline separation and shorter elution time. As indicated in Figure 2, the

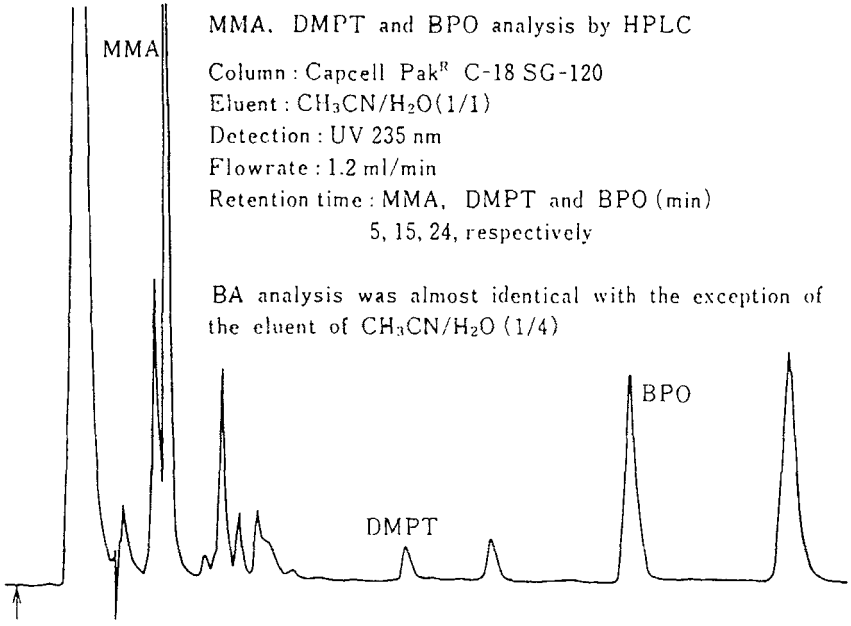


FIGURE 2 HPLC chromatogram of methanol extract of Yunifast^R

extract matrix is so complicated, therefore it was quite difficult to find out the appropriate column. Most of the C-18 column, even if they were endocapped, DMPT did not successfully elute or indicated a tailing phenomena.

Recently Capcell Pak^R of SG and AG-120 for basic and acid compounds, respectively, were available and the author tried to use them for the experiment. They indicated no tailing of DMPT and successful separation of desired compounds from admixtures. Therefore, this procedure was adopted to an official procedure of Japan dental material examination. Since then, several innovated columns other than SG and AG-120 columns such as UG-120 from Shiseido, L-column^R from Kagakuhin kensa kyohkai, Pegasil^R from Senshu kagaku were circulated in the market. UG-120 is the column combined the superiority of SG and AG-120, thus neutral, acidic and basic compounds can be separated using a single column. L-column is specially endocapped, thus almost or sometimes superior to Capcell Pak^R columns. However, due to specific endocapping procedure, it is difficult to fabricate the large volume size column for collection. Pegasil^R is also newly fabricated and the superior characteristics of this column is used the completely purified silanol totally free from residual heavy metals, therefore no effect due to residual heavy

metal was observed and basic compound as well as a chelating agent or clathrate compound containing metal or compound in the molecular were successfully eluted.

According to the advancement and innovation of the column fabrication technique, the author needs to revise the official method using one of these columns in place of SG or AG-120 for dental material examination currently performed.

Due to the use of Capcell Pak^R column in the current study, no salts addition into the elution for attaining the common ion elution effect was unnecessary and basic compound was successfully determined without any tailing phenomena. If insufficiently endocapped C-18 column was used, significant tailing or no elution of basic compounds in the worst case was observed.

Analysis of compounds from Yunifast^R and Acron^R

Typical chromatogram of methanol extract of Yunifast^R is presented in Figure 2. Total elution amount is presented in Table 1. Elution of every compound from Yunifast^R was observed excepting DMPT and BPO from water extract. Elution of every compound from Acron^R was also observed excepting MMA and BPO from water extract and BPO from serum extract. DMPT was not used for fabrication in Acron^R (Table 1).

Concerning the elution from Yunifast^R, BPO elution significantly increased with increasing hydrophobicity of organic solvents. DMPT elution from Yunifast^R also somewhat increased with increasing hydrophobicity of organic solvents, however no significant difference among organic solvents (Table 1). MMA was eluted into methanol in the greatest amount, indicating that more pliant material of Yunifast^R has no significant difference of swelling among organic solvents. It may be the reason the most hydrophilic MMA could be extracted in the greatest amount with the most hydrophilic solvent of methanol. Time course elution of MMA, DMPT and BPO is presented in Figures 3, 4 and 5.

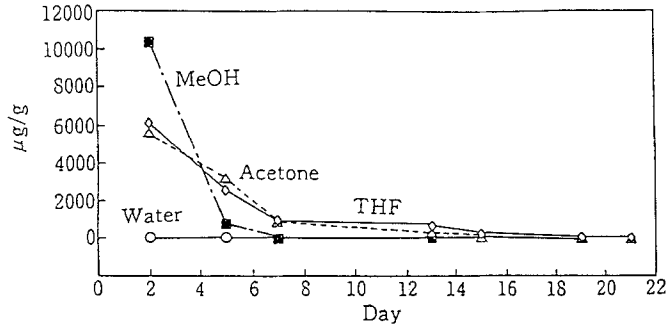
On the contrary, elution amount was parallel to swelling capacity of the solvents if the material is comparatively rigid and Acron^R was more rigid than Yunifast^R. Acetone indicated the greatest elution amount in MMA and BPO from Acron^R due to the greatest swelling capacity. A swelling capacity has an important factor to evaluate the elution amount if the material is rigid. An average swelling capacity (%) and Rockwell hardness (rigidity) of Yunifast^R and Acron^R were 798, 468 and 60, 84 (n=3), respectively, indicating Yunifast^R was more pliant.

In serum extraction, every compound was detected excepting BPO from Acron^R (Table 1). BPO was transformed into BA immediately when contacting with serum or saliva, therefore BPO was determined as BA. This is the first finding by the author.

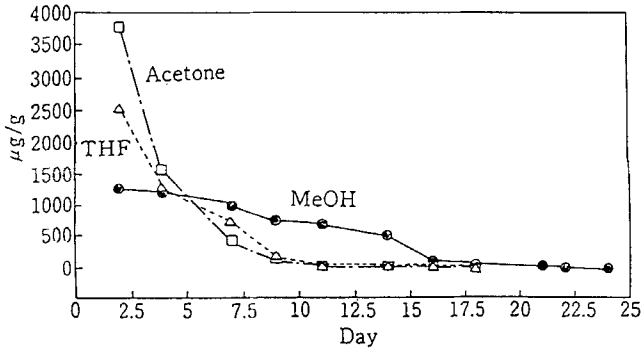
TABLE 1 MMA, DMPT and BPO from polyMMA Yunifast^{R} and Acron^{R}

Sample	Compound	Water	MeOH	Acetone	THF	Serum
Yunifast	MMA	84	11200	10400	10900	32
	DMPT	N.D.	347	373	396	67
	BPO	N.D.	38	796	1460	2
Acron	MMA	N.D.	5520	5920	4860	10
	DMPT	N.D. in all solvent				
	BPO	N.D.	4740	5470	4850	N.D.

Amounts (μg/g) shown are the average of three individual samples. All data refers to more than one week immersion excepting for three days serum immersion.

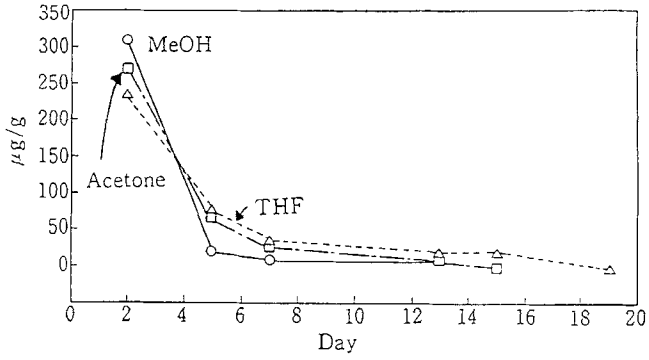


Elution of MMA from Yunifast



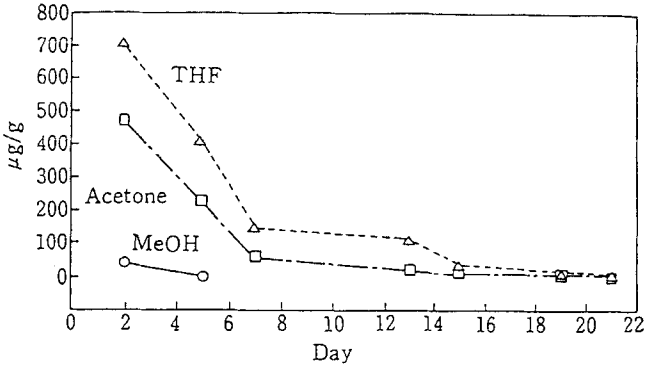
Elution of MMA from Acron

FIGURE 3 Elution time course of MMA from Yunifast^{R} and Acron^{R}

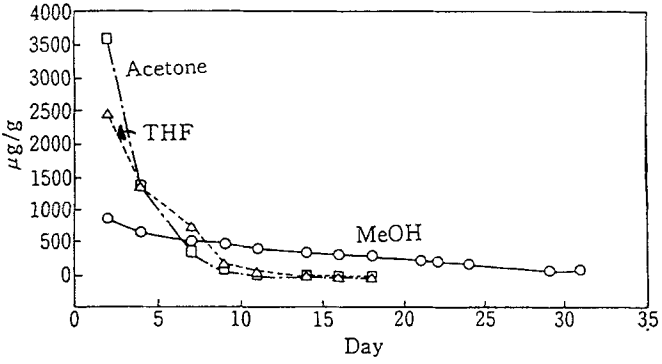


Elution of DMPT from Yunifast

FIGURE 4 Elution time course of DMPT from Yunifast^{R}



Elution of BPO from Yunifast



Elution of BPO from Acron

FIGURE 5 Elution time course of BPO from Yunifast^{R} and Acron^{R}

According to the time course elution of MMA and DMPT to serum, it is interesting that more hydrophobic compound of DMPT eluted greater than less hydrophobic compound of MMA with serum (refer to Table 1, Yunifast^{R} column). This is because serum contains wide range of polarity components from water (hydrophilic) to lipid (hydrophobic), so this phenomena was not surprising, but should be remembered.

Readers should keep in mind that serum was different from water or saline solution in the physical character as well as elution behavior or capacity when evaluating and considering the elution amount with blood or serum.

Serum extracted a greater amount from more pliant material of Yunifast^{R} than more rigid material of Acron^{R} (Table 1). Therefore the rigidity of the material, swelling capacity of solvents and hydrophobicity of the compounds of interest were the important factors to determine the elution amount. In case of BPO elution with serum, BPO in the surface of Yunifast^{R} was changed to BA when contacting with serum and BA was eluted. Due to the rigidity of Acron^{R}, serum supposed not to be penetrated into the interior of the material to extract BPO, therefore no elution was attained.

For international harmonization of the standards existing in individual country, there exists an International Standardization for Organization (ISO)/ Technical Committee (TC) 194, Biological evaluation of medical devices. In this document of part 9, Draft for International Standard (DIS) 10993-9, entitled "Degradation of materials related to biological testing", the extraction solvent *in vitro* from polymers recommends a phosphate buffer solution at pH 7.4 at 50 ^{o}C or 80 ^{o}C (2). Phosphate buffer at pH 7.4 is not an appropriate and representative solvent for evaluating the elution amount from polymer when contacting body fluids with polymers. So, elution with blood or serum was quite different from that with water, saline or phosphate buffer.

As presented herein, only MMA in Yunifast^{R} was extracted with water. This was quite different from serum elution because serum could extract MMA, DMPT and BPO as BA. The eluted amount with serum was greater than that with water with the exception of MMA with water from Yunifast^{R}. The author has no definite idea why MMA amount eluted with water was greater than that with serum in case of Yunifast^{R}. In case of Acron^{R}, serum extraction was greater than water extraction.

When comparing total amount used for polymer fabrication with eluted amount, MMA indicated the greatest elution amount, but eluted ratio compared with the added amount for fabrication was the least and most of MMA was eluted in the initial period in time course elution (Fig. 3). This is because MMA used for fabrication was almost completely polymerized and the residual MMA monomer was around 1 to 2 % used for fabrication.

On the contrary, ratio of DMPT and BPO elution was greater compared with the addition amount used for fabrication. This is because MMA with a lower boiling point

could be easily vaporized in the short period and did not remain in the polymer. DMPT and BPO with a higher boiling point could be difficult to be vaporized in the short period, thus remained in the long period in the polymer and could be extractable with solvents.

Water with less swelling capacity could not penetrate into the interior of the material and could not extract interior MMA. Only MMA remaining in the polymer surface was extractable.

From this result, the author considers the storage condition for materials after fabrication, especially for preventing vaporization of components. Otherwise easily vaporizable compounds could not be determined accurately and sometimes gave a comparatively less data. In one day after fabrication of Acron^{R}, MMA can also be extractable with water. The data in Table 1 is from one week storage at 37 ^{o}C after fabrication. Therefore, the elution data soon after fabrication will be greater than that in Table 1, indicating the storage condition after fabrication is an important factor to determine accurately.

On the other hands, MMA and other higher boiling point compounds can be diminishable by gently heating for a long period before shipping. As an alternative method to diminish the residual MMA before application to patients, it is recommended to immerse dental plate into a hot water to decrease the residual MMA and other hazardous compounds.

Analysis of compounds from PS and PC

Total elution amount is presented in Table 2. Total elution amount as well as elution time course from dental plate with several organic solvents were studied. Elution time course of bis A and DCDPS from PS and PC is presented in Figures 6 and 7.

In PS, both compounds could not be extracted with water and a few ppm of bis A was extracted with methanol and no elution of DCDPS was observed. In acetone and THF elution, elution of both compounds were observed and THF elution was greater than acetone elution, which was parallel to hydrophobicity of solvents.

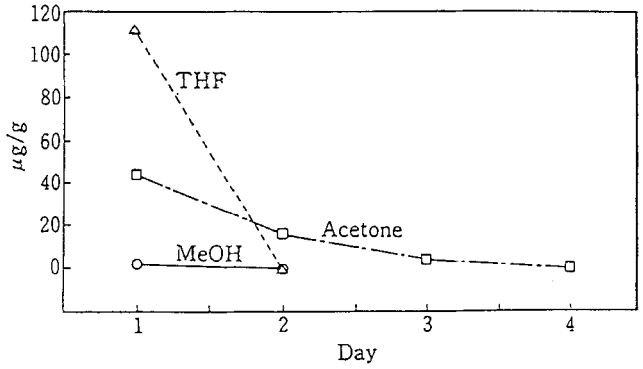
DCDPS was not used for fabrication of PC. In PC, no elution with water was observed. A few ppm elution with methanol was observed. Others were identical to PS. Total elution amount of PS was greater than that from PC, indicating PS was more pliant than PC.

The rigidity of PS and PC was greater than that of Yunifast^{R} and Acron^{R}, therefore the total elution amount of the former was less than that of the latter. Elution amount was parallel to the order of hydrophobicity of organic solvents and the swelling capacity of solvents was insufficient to penetrate into the interior of the

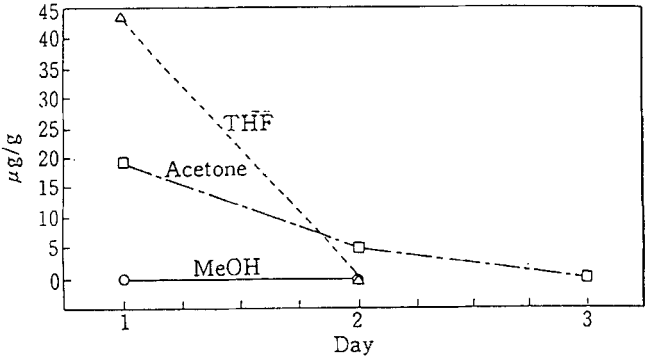
TABLE 2 Bis A and DCDPS from PS and PC

Sample	Compound	Water	MeOH	Acetone	THF
PS	bis A	N.D.	1.9	64.4	112.0
	DCDPS	N.D.	N.D.	24.1	43.5
PC	bis A	N.D.	5.6	22.5	37.0
	DCDPS	N.D. in all solvent			

Amounts (μg/g) shown are the average of three individual samples.



Elution of Bisphenol A from Polysulfone



Elution of 4,4′-dichlorodiphenylsulfone from Polysulfone

FIGURE 6 Elution time course of bis A and DCDPS from PS

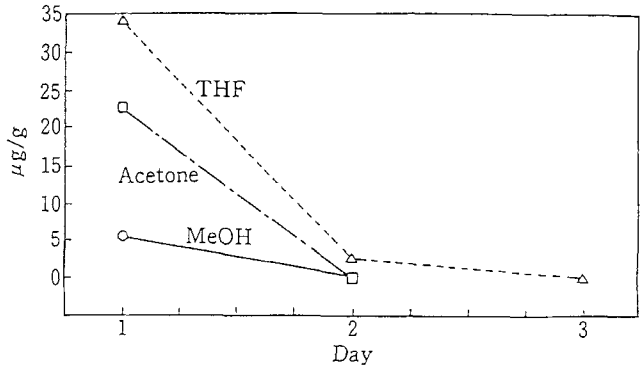


FIGURE 7 Elution time course of bis A from PC

polymer due to rigidity of PC and PS, therefore only residual compounds close to the surface was thought to be extracted in case of PS and PC.

On the contrary, Yunifast^R and Acron^R were more pliant than PS and PC, so the elution amount was greater. The more pliant material of Yunifast^R indicated a greater elution than that from more rigid material of Acron^R. If the material is comparatively pliant, the elution was mostly depend on hydrophobicity of solvents as well as swelling capacity of solvents. The latter was significant in the case of elution from Acron^R.

Analysis of compounds of 3G and Bis GMA from Cleafil^R

Total elution amount is presented in Table 3. Total elution amount as well as elution time course from dental plate with several organic solvents were studied.

In Cleafil^R, both compounds could not be extracted with water and extracted with methanol, acetone and THF. The extracted amount is parallel to the order of hydrophobicity of organic solvents. The elution time course was almost identical to bis A from PC presented in Figure 7.

Hazardous effect of MMA to human being

As MMA elution indicated the greatest amount, therefore it was studied the mutagenicity of MMA (Ames test with and without S9mix in Table 4). According to these results mutagenicity of MMA was not so significant.

TABLE 3 3G and Bis GMA from Cleafil^R

Sample	Compound	MeOH	Acetone	THF
Cleafil	3G	34.1	54.1	55.2
	Bis GMA	17.6	25.9	26.3

Amounts (μg/g) shown are the average of three individual samples.

TABLE 4 Mutagenicity of MMA

Method	note	result	reference
Ames test	5 strains, + or -S9mix S9mix (rat, hamster)	-	3
SCE assay	human lymphocyte	-	4
Ames test	5 strains, + or -S9mix	-	5

Additionally the followings were confirmed. Acute toxicity to human being, chronic oral toxicity to human being, carcinogenicity risk to human being, embryotoxicity and fetotoxicity to human being exposed with MMA were not problematic (6-9).

As a conclusion, acute toxicity, chronic toxicity, carcinogenicity, neurotoxicity, embryotoxicity and fetotoxicity were found not to be problematic. Potential to oral mucosa irritation was small (10-14). MMA, BPO and DMPT were reported to be allergen (15,16).

CONCLUSION

Yunifast^R and Acron^R of polymethylmethacrylate (polyMMA) dental plate were extracted with water, methanol, acetone, THF and serum. The extracted MMA, DMPT, BA, BPO, bis A, DCDPS, 3G and bis GMA were determined using HPLC. Elution of BPO from more pliant material of Yunifast^R increased with increasing hydrophobicity of solvent, indicating THF elution was the greatest. No significant difference of eluted amount of MMA and DMPT was observed excepting water and serum extraction. In the case of more rigid material of Acron^R, eluted amount with acetone indicating the greatest swelling capacity was the greatest, however there

was no significant difference among methanol, acetone and THF from Acron^R excepting water and serum elution. In water elution, only MMA from Yunifast^R was observed. In serum elution, elution of every compound was observed excepting BPO from Acron^R. As BPO was immediately transformed into BA, so BPO was determined as BA. Tiny amount of elution of bis A and DCDPS from PS and PC, and 3G and bis GMA from Cleafil^R of dental plate was observed and the elution order of these compounds was almost parallel to hydrophobicity of solvents.

Hazardous effect to the patients exposed by MMA, the greatest elution compound, was found not to be problematic.

REFERENCES

1. H. Shintani, Special Issue on Clinical Analysis. J. Liq. Chromatogr., 15: 1315-1335 (1992)
2. Draft International Standard ISO TC/194, DIS 10993-9, Biological evaluation of medical devices- part 9: degradation of materials related to biological testing.
3. W. Lijinsky, A.W. Andrews, Teratogenesis Carcinog. Mutagen., 1: 259-267 (1980)
4. M. Cannas, P. Bigatti, E. Rossi, P. Rossi, Ital. J. Orthop. Traumatol., 13: 387-391 (1987)
5. T.H. Waegemakers, M.P. Bensink, Mutat. Res., 137: 95-102 (1984)
6. S. Baker, S.C. Brooks, D.M. Walker, J. Dent. Res., 67: 1295-1299 (1988)
7. J.F. Borzelleca, P.S. Larson, G.R. Hennigar, E.G. Huf, E.M. Crawford, R.B. Smith, Toxicol. Appl. Pharm., 6: 29-36 (1964)
8. B.S. Oppenheimer, E.T. Oppenheimer, A.P. Stout, F.R. Eirich, Cancer Res., 15: 333-340 (1955)
9. C.A. Nicholas, W.H. Lawrence, J. Autian, Toxicol. Appl. Pharmacol., 50: 451-458 (1979)
10. B. Meding, A. Ringdahl, Ear Hear., 13: 122-124 (1992)
11. S. Kaaber, Int. Dent. J., 40: 359-365 (1990)
12. S. Kaaber, H. Thulin, E. Nielsen, Contact Dermatitis 5: 90-96 (1979)
13. T. Kanzaki, Y. Kabasawa, T. Jinno, K. Isayama, Contact Dermatitis 20: 146-148 (1989)
14. I. Pevny, A. Binzenhofer, Z. Hautkr., 59: 245-251 (1984)
15. M. Jager, B.R. Balda, Arch. Orthop. Traum. Surg., 94: 175-178 (1979)
16. R. Rajaniemi, S. Tola, Scan. J. Work Environ. Health 11: 281-286 (1985)

Received: July 17, 1994
Accepted: July 29, 1994