Iranian Journal of Pharmaceutical Research (2005) 4: 227-232
Received: November 2004
Accepted: February 2005

Original Article

Determination of Residual Methylmethacrylate Monomer in Denture Base Resins by Gas Chromatography

Khosrou Abdi[a]*, Ali Mandegary[b,c], Mohsen Amini[a], Mozhgan Bagheri[a] and Farideh Gerami-Panah[d]

[a]*Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.* [b]*Department of Toxicology and Pharmacology Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.* [c]*Department of Toxicology and Pharmacology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman Iran.* [d]*Faculty of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.*

Abstract

Acrylic base resins are widely used in orthopedics and dental surgery. It is generally accepted that due to the incomplete conversion of methyl methacrylate (MMA) monomer to the polymer form during polymerization of the resin, some MMA monomers remain in the hardened material. MMA monomer has been reported to cause abnormalities or lesions in several organs of animals. Study of the literatures showed that there are no perfect and valid methods for analysis of MMA. The aim of this study was to develop a simple and valid method for determination of MMA residual monomer in the denture base resins.

We have developed and validated an analytical procedure employing gas chromatography with flame ionization detector (GC-FID), with temperature programming, and a close analogue internal standard for fast and repeatable analysis of MMA residual monomer contents in denture base polymers. For quantification of monomer, two calibration curves were used by two different methods of solution preparation and they were compared to each other. The assay was linear over the range of 0.03-0.6 mg/ml MMA with correlation coefficients (r^2) of greater than 0.99. Accuracy, intra-day (Error<9.1%) and inter-day (Error<6.6%) , precision (RSD<5.5% and RSD<6.5%, respectively) were in acceptable values over the examined concentration range. Limit of detection (LOD) and limit of Quantification (LOQ) for MMA were 0.01 and 0.02 mg/ml, respectively. In conclusion, the proposed method was a simple and fast assay for MMA residual monomer in denture base resins.

Keywords: Methylmethacrylate; Residual monomer; Denture Base Resins; GC-FID.

Introduction

Polymethylmethacrylate (PMMA)-based cements have been used in orthopedic surgery since 1960 (1). PMMA-based cements had been commonly used in the dental profession as bonding agents, filling materials, provisional crowns, bridges and dental prostheses in the past (2). In addition, PMMA has also been widely used in other biomedical fields, including dental surgery, ophthalmology (contact or intraocular lenses), nephrology (membrane for dialysis), and drug delivery (micro capsulation of drugs) (3). Moreover, removable dentures are made of almost 100% polymerized MMA.

* Corresponding author:
E-mail: khmabdi@yahoo.com

From a chemical point of view, cements are prepared by dissolving PMMA in the methymethacrylate monomer and adding both a polymerizing initiator and a polymerization accelerator (3), whilst surgical-grade PMMA is self-polymerizing and endothermic during curing (4). PMMA polymer powder and liquid monomer are mixed to obtain a paste and after 12-15 min, the dough "sets up" into a rigid mass. It is generally accepted that MMA monomer dose not reach complete conversion after the cure of the resin, and that a certain amount of residual monomer remains in the hardened material (4). The incomplete cure of the MMA monomer has two principle consequences that have been reported in the literature; Firstly, unreacted MMA monomer leaks from the cement into the surrounding tissues and secondly, its presence in the hardened resin influences the mechanical properties of the resin because it acts as a plasticizer (3).

There are some reports about the toxic effects of MMA monomer on cells and tissues, including sudden but transient cardiovascular collapse and irreversible cardiac arrest, increase in platelet aggregation coupled with fibrinogen synthesis (5), carcinogenicity (6), skeletal abnormalities and reduction of fetal weight (7), damage to lung, kidney, heart and liver after exposure to high concentration of MMA (8) in animals and a variety other skin reactions (9). Contradictory results have been reported concerning histopathological effects of MMA on internal organs (5). It has also been shown that MMA hydrolyses in serum (human and rat) is due to a serum nonspecific carboxyl esterase (5).

Gas chromatography (GC) is most often used for determination of MMA monomer hardened cements and few GC methods for this have been reported (10-12), but none of these methods have been validated for this purpose. In these articles, LOD, LOQ, inter-and intra-day, precision and accuracy of the method had not been mentioned. Also, no details of the standard and sample preparation in these articles have been given. An on-line coupled liquid chromatography-gas chromatography (LC-GC) was also used for determination of MMA monomer in process samples (13). This method needs some special equipments and circumstances which are not available everywhere. Regarding the lack of any practical and validated method for analysis of MMA residual monomer in acrylic base polymers in the literature, we described this GC-FID method which can be used simply in the labs. Also, two different methods of preparation of standard solutions were compared.

Experimental

Materials

All solvents and materials were of analytical reagent grade. Methylmethacrylate, acetone, methanol, hydroquinone, and n-butyl acetate were purchased from Merck, Germany. Denture hardened resin was prepared by a dentist.

Instruments and apparatus

Experiments were carried out using a Hewlett-Packard HP 6890 gas chromatograph equipped with a flame ionization detector (FID) system. Chromatographic separation was achieved on a fused-silica capillary column (30 m × 0.23 mm i.d. 0.25 μm ft.) coated with 5% phenylmethylp olysiloxane.

The injector was in the mode of split (10:1) and its temperature was maintained at 200ºC throughout the experiments. The column temperature was raised from 40ºC (hold 4 min) to 100ºC (hold 5 min) at 20ºC/min rate. The flow rate of carrier gas (N_2) was 1.2 ml/min. The detection was carried out by a flame ionization detector; with the temperature of 250ºC and the ratio of H_2/air at 45/450. The H_2 and air for FID were provided by a hydrogen generator (Packard) and zero- air generator (Texol), respectively.

The instrument control and data processing utilities included the use of Hewlett-Packard application software GC Chemstation (Agilent technology).

Methods

Methanol and acetone diluting solutions:

Hydroquinone (HQ) as stabilizer of MMA, was added to the methanol and acetone at a concentration of 20 ppm, and used for preparation of standard and sample solutions, respectively.

Internal standard (I.S.) solution:

In order to achieve an I.S. peak that

represents a concentration located in the middle of the calibration curve, 750 mg butyl acetate was weighed into a 25 ml volumetric glass flask and added methanol to a total volume of 25 ml. This volume is to ensure that there is enough I.S. volume for additional analysis. The concentration of the I.S. in the final solution would be approximately 4.5% mass fraction of the quantity of the specimen.

Calibration curves:

Direct weighing of MMA for each concentration: Six standard solutions with concentration of MMA at approximately 0.2, 0.6, 1.1, 2.3, 3.3, and 4.6% mass fraction of the quantity of the sample were made (approximately 0.03, 0.08, 0.15, 0.3, 0.45 and 0.6 mg/ml). Calibration solutions of MMA were made by separately weighing approximately 13, 39, 71, 150, 215, and 300 mg of MMA in 5 ml volumetric flasks. Then, 100 µl of each solution and 200 µl of the I.S. solution were transferred into 10 ml volumetric flasks and methanolic solution was added to the volume.

Dilution of stock solution of MMA: A stock solution of MMA was prepared by dissolving an adequate amount of the compound in methanol to give a final concentration of 2 mg/ml. Standard solutions with concentration of MMA between approximately 0.2, 0.6, 1.1, 2.3, 3.3, and 4.6% mass fraction of the quantity of the sample were made by adding appropriate volumes of the stock solution along with 200 µl of the I.S. solution to the 10 ml volumetric flasks and diluting with methanol to the volume.

Sample preparation

Three sample sizes of approximately 650 mg of each specimen disc were broken into pieces, small enough to pass through the neck of 10 ml volumetric flasks. From each disc, three sample solutions were prepared. Acetone diluting solution was added to the volume. Each solution was stirred using clean polytetrafluoroethylene (PTFE) coated magnetic stirring bar. For 72 ± 2 h at room temperature. Then, 200 µl of the I.S. solution and 2 ml of slurry were added to 10 ml volumetric flasks and reached up to volume by methanol diluting solution. Using separate pipettes, 5 ml of the mixture slurry from each of the 10 ml flasks transferred to glass centrifugation tubes. Each slurry was centrifuged at 3000 rpm for 15 min then, 1 µl of supernatant was injected to the GC.

Calibration curves

Triplicate 1 µl injection of each concentration for both methods of standard preparation, were made. The peak area ratio of MMA to the I.S. areas was plotted against the corresponding concentration to obtain the calibration curve.

Limit of detection (LOD) was defined as the analyte concentration giving a signal equal to the blank signal (Y_B) plus three standard deviations of the blank (S_B); namely $Y\text{-}Y_B=3S_B$ (13). Limit of quantification (LOQ) was also calculated according to Ref. 13. Briefly, it was the lower limit for precise quantitative measurement, and defined as follow: $Y\text{-}Y_B=10S_B$.

Results and Discussion

GC analysis

Optimal condition for the determination of MMA was investigated. According to the condition mentioned in Experimental, gas chromatograms of standard solution and sample are shown in Figure 1. Under the described conditions, the MMA and I.S. were well resolved. Retention times of MMA and I.S. (mean of three trials) were 4.6 and 5.6 min, respectively.

GC-FID method validation

Chromatographic method validation consisting of linearity, precision and accuracy of the method was assessed in order to demonstrate the suitability of the analytical method for the determination of MMA in solutions.

Table 1. Retention times, linear equations and regression coefficient of quantification for the MMA. The procedures of preparing two different standard solution are described in Experimental.

	t_R(min)	Linear equation	Regression coefficient (R^2)
[1]MMA	4.6	Y=2.31X-0.013	0.9996
		*Y=2.20X+0.0005	0.9967
[2]MMA	4.6	Y=1.44X+0.0043	0.9985
		*Y=1.48X-0.0089	0.9987

[1] Direct weighing of MMA to prepare each concentration.
[2] Dilution of the MMA stock solution to prepare each concentration.
* The linear equation of MMA analysis after one month.

Table 2. Intra-day accuracy and precision for the analysis of MMA in methanol.

	Concentration added (mg/ml)	Mean (n=3) concentration determined (mg/ml)	Error (%) (Accuracy)	R.S.D (%) (Precision)
[1]MMA	0.03	0.033	9.1	5.5
	0.08	0.084	4.7	4.7
	0.15	0.148	-1.3	6.5
	0.30	0.280	-6.6	1.9
	0.45	0.470	4.2	3.2
	0.60	0.595	-0.8	6.0
[2]MMA	0.03	0.035	16.6	4.1
	0.08	0.077	-3.9	3.7
	0.15	0.148	-1.3	5.9
	0.30	0.298	-0.6	5.3
	0.45	0.462	2.6	4.9
	0.60	0.593	-1.2	2.9

[1]Direct weighing of MMA to prepare each concentration.
[2] Dilution of the MMA stock solution to prepare each concentration.

Response linearity: As shown in Table 1, linear relationship between peak area ratio of MMA to I.S. versus the corresponding concentration was obtained over the range of approximately 0.2, 0.6, 1.1, 2.3, 3.3, and 4.6% of the target values. Data were mean of three injections of each solution. Regression analysis showed a good linear relationship ($r^2 > 0.99$) for MMA (Table 1).

Accuracy: Inter- and intra-day accuracy was expressed as percentage deviation from the spiked value using the following equation:

$$\text{Accuracy: Error \%} = [(C_{\text{mean cal.}} - C_{\text{spiked}}) / C_{\text{spiked}}] \times 100$$

Where, $C_{\text{mean calc.}}$ is the mean calculated concentration for each solution and C_{spiked} is the spiked theoretical concentration. Inter- and intra-day precision of the method was expressed as the relative standard deviation (RSD %) of the mean calculated concentration for each solution.

The limit of detection (LOD) and limit of quantification (LOQ) for MMA were 0.01 mg/ml and 0.02 mg/ml, respectively.

Comparison of two calibration curves

The two calibration curves were compared by comparing the slopes and Y intercepts of the two regression lines, using Student́ s t-test (15). If two lines are not concluded to have different slopes, then the two lines are parallel and common (or weighted) regression coefficient can be obtained. In this case, determination of the equality of Y intercepts is also done. If the

Table 3. Inter-day accuracy and precision for the analysis of MMA in methanol.

	Concentration added(mg/ml)	Mean (n=3) concentration determined (mg/ml)	Error (%) (Accuracy)	R.S.D (%) (Precision)
[1]MMA	0.03	0.029	-6.6	2.8
	0.08	0.775	-3.7	3.8
	0.15	0.143	-6.6	4.5
	0.30	0.319	6.6	5.5
	0.45	0.432	-5.3	3.3
	0.60	0.588	-1.6	0.7
[2]MMA	0.03	0.033	10.0	1.6
	0.08	0.080	0.0	7.3
	0.15	0.146	-2.6	1.6
	0.30	0.303	1.0	4.1
	0.45	0.449	-0.2	0.3
	0.60	0.601	0.2	0.9

[1]Direct weighing of MMA to prepare each concentration.
[2] Dilution of the MMA stock solution to prepare each concentration.

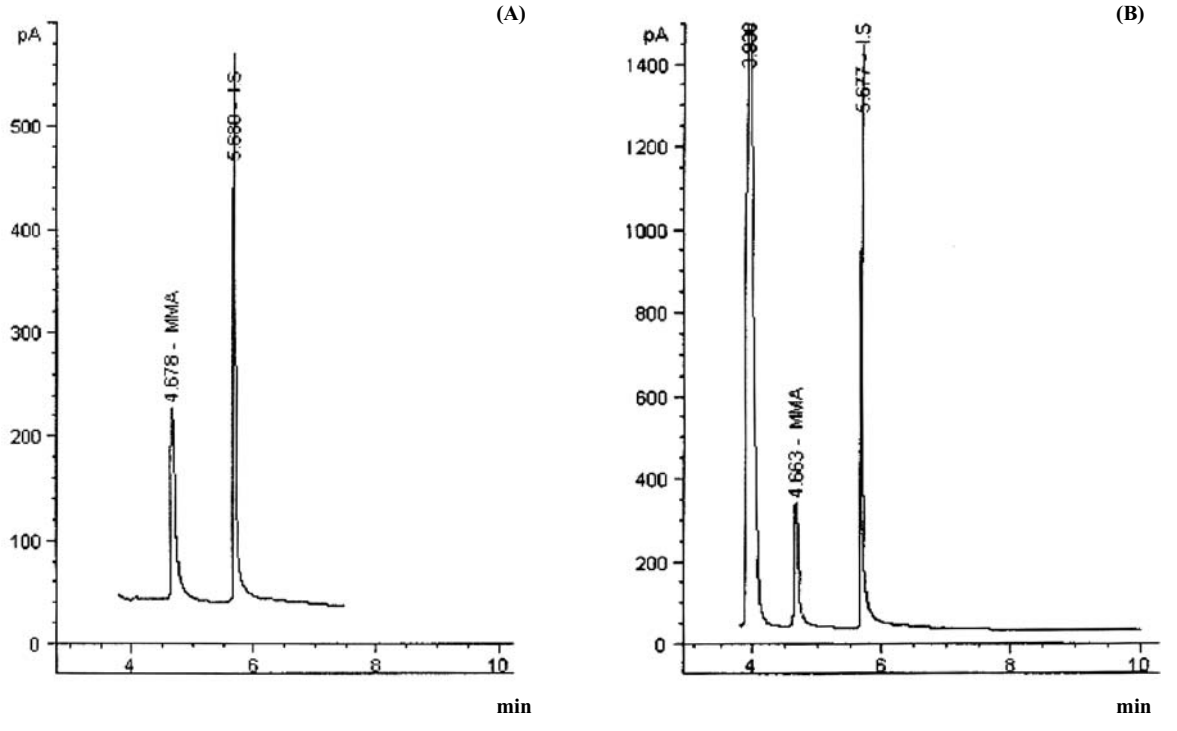

Figure 1. Typical chromatograms of MMA using n-butyl acetate as internal standard. (A) a standard solution (0.45 mg/ml MMA) and, (B) a sample solution of MMA. Analytical conditions are described in Experimental.

equality of slopes is rejected, intersection of the two lines can be calculated.

The results of this study showed that the slopes of the two calibration curves were significantly different ($p<0.05$) (intersection point was 0.005 mg/ml).

Results and Discussion

From a toxicological point of view, determination of MMA monomer in MMA-base polymers is very important, because some toxic effects of MMA monomer are reported in the literatures (5-9). Reviewing of literatures showed that analysis of MMA monomer is limited to some work by GC (10-12) and on-line coupled LC-GC (13). However, none of the GC methods had discussed sample preparation, preparation of standard solution, precision, accuracy and linear range of their methods. As validation of an analytical method is an important part of a quantitative analysis, we assess these parameters in our work. In the on-line coupled LC-GC, coupling of LC to GC requires special equipments which may not available in every laboratory. Moreover, inter-day precision, LOD and LOQ of the method have not been reported, although the method had a good linearity, intra-day precision and sensitivity to MMA (13). Our new method, showed a good separation of the MMA and I.S. with acceptable linearity, accuracy, inter- and intra-day precision (16). Furthermore, sample preparation did not require any extraction and MMA could be separated and determined in less than 6 min. The linear range of calibration curve satisfactvily covered the threshold concentration of MMA residual monomer for the rejection or pass of the product.

The maximum acceptable concentration of MMA residual monomer in denture resins for pass for pass is 2.2% sample mass fraction (17). The product tested was failed when the calibration curve established by serial dilution of stock solution, whereas it was passed when the calibration curve established by direct weighing of MMA was applied. In this case, it is essential to select reliable curve for judgment about the product's residual monomer content. As in the weighing method, MMA sample was prepared by similar procedure as the sample, the errors for standard solutions and sample are similar to large extent. Therefore, preparation of standard

solutions by the direct weighing of MMA is recommended.

A GC-FID method for the analysis of MMA has successfully been developed. This new method is simple, rapid and precise, for detecting residual MMA monomer in denture based polymer. For precise quantification of MMA residual monomer, preparation of standard solutions by the direct weighing of MMA is recommended. This method can fulfill the requirements for analysis of MMA residual monomer in industry and quality control labs for analyzing this compound

References

(1) Charnley J. Anchorage of the femoral head prosthesis to the shaft of the femur. *J. Bone Joint surg*. (1960) 428: 28-32

(2) Andreason H, Boman A, Johnsson S, karlsson S and Barregard L. On permeability of methyl methacrylate, 2-hydroxy ethyl methacrylate and triethylleneglycol dimethylcrylate through protective gloves in density. *Eur. J. Oral Sci*. (2003) 111: 259-235

(3) Moreau MF, Chappard D, Lesourd M, Montheard JP and Basle MF. Free radicals and side products released during methylmethacrylate polymerization are cytotoxic for osteoblast cells. *J. Biomed. Mater. Res.* (1998) 40: 124-131

(4) Vallo CI. Residual monomer content in bone cements based on poly (methylmethacrylate). *Polym. Int.* (2000) 49: 831-838

(5) Bereznowski Z. *In vitro* assessment of methylmethacrylate metabolism and toxicity. *Int. J. Biochem. Cell Biol.*(1995) 27: 1311-1316

(6) Oppenheimer BS, Oppenheimer ET, Danishefsky AP, Stout AP and Erich FR. Further studies of polymers as carcinogenic agents in animals. *Cancer Res*. (1955) 15: 333-340

(7) Singh AR, Lawrence WH and Autian J. Embryonic fetal toxicity and teratogenic effects of a group of metacrylate esters in rats. *J. Dent. Res*. (1972) 51: 1632-1638

(8) Pozzani UL, Weil CS and Carpenter CP. Subacute vapor toxicity and range-finding dada for ethylacrylate. *J. Ind. Hyg. Tox.* (1949) 31: 311-316

(9) Kindt-Larsen T, smith D and Jensen JS. Innovations in acrylic bone cement and application equipment. *J. Appl. Biomater*. (1995) 6: 75-83

(10) Vallo CI, Montermartin PE and Cuadrado TR. Effect of residual monomer content on some properties of a poly (methylmethacrylate)-based bone cement. *J. Appl. Polym. Sci.* (1998) 69: 1367-1383

(11) Vallittu PK, Ruyter IE and Buykuilmaz S. Effect of polymerization temperature and time on the residual monomer content of denture base-polymers. *Eur. J. Oral Sci*. (1998) 106: 588-593

(12) Vallo CI, Montemartin PE, Fanovich MA, Porto lopez JM and Cuadrado TR. Poly methylmethacrylate-based bone cement mod ified with hydroxyapatite. *J. Biomed. Mater. Res.* (1999) 48: 150-158

(13) Andersson T, Hyötyläinen T and Riekkola ML. On-line coupled liquid chromatography-gas chromatography in the analysis of process samples. *J. High Resol. Chromatogr.* (1999) 22: 261-264

(14) Miller JC and Miller JN. *Statistics for Analytical Chemistry.* Ellis Horwood Limited, Chichester (1988) 115-117

(15) Zar JH. *Biostatistical Analysis*. 4th ed. Prentice Hall International, Inc. NJ (1999) 360-376

(16) Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilverary IJ, McKay G, Miller KJ, Patnaik RN, Powell ML, Tonelli A, Viswanathan CT and Yacobi A. Bioanalytical method validation- a revisit with a decade of progress. *Pharm. Res.* (2000) 17: 1551-1557

(17) ISO 7405: Dentistry- Preclinical Evaluation of Biocompatibility of Medical Devices Used in Dentistry- Test Methods for Dental Materials. DIN Dsutsches Fur Normung Germany (1997) 17-21

This article is available online at http://www.ijpr-online.com