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# In vitro release studies of methylmethacrylate liberation from acrylic cement powder

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**Abstract**

Bone cement or polymethylmethacrylate (PMMA) is commonly used for anchoring cemented prosthesis to the bone. Cytotoxic effect of culture media exposed to PMMA powder may be related with long term problems associated with acrylic cement application, being the monomer (methylmethacrylate) one of the cement's component partly responsible for the cytotoxic effect. The present work reports the studies of monomer release from acrylic bone cement powder under different experimental conditions: setting time of PMMA (in solution and air) and different culture media composition. High-performance liquid chromatography was used for the determination of residual monomer. Mathematical models were applied to experimental dissolution data revealing that monomer release is lightly affected by the studied variables. The monomer release seems to be a surface phenomena, suggesting that the possible actions of monomer will mainly be due to the initial loss of non polymerized monomer rather than to further depolymerization of the already polymerized cement. 

*Keywords:* Poly(methylmethacrylate); Methylmethacrylate; Biomaterial; Bone cement; Release model

## 1. Introduction

Bone cement or polymethylmethacrylate (PMMA) is a synthetic biomaterial widely used in orthopaedic surgery for anchoring cemented prosthesis to the contiguous bone.

From a biomechanical point of view, PMMA has been a satisfactory material, however long-term problems have been repeatedly reported leading to loose prosthesis (Moreau et al., 1998).

In an attempt to understand a possible cause for prosthetic failure, the authors (Vale et al., 1997), have demonstrated the cytotoxic effect of culture media: phosphate buffer saline solution (PBS) and minimal essential medium (MEM) exposed to PMMA (powder) on human fibroblasts,

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which was probably due to some PMMA soluble components, like the monomer (methylmethacrylate).

Although there are studies referring to the monomer release from polymerized cement in water (Linder et al., 1976; Brauer et al., 1977; Schoenfeld et al., 1979) or in tissues adjacent to bone (Petty, 1980), the purpose of present investigation was to evaluate the influence of: (1) age of polymerized cement (time after setting) in solution and air; and (2) different media composition on monomer release rate in exactly the same conditions used in studied cells: same culture media (PBS and MEM) and same examined material (PMMA powder).

The release rate, during the first hour after incubation, was also examined in an attempt to elucidate the mechanism of monomer release from polymeric matrix in the surrounding media.

## 2. Materials and methods

### 2.1. Materials

CMW 1, an orthopaedic bone cement, was obtained from CMW Laboratories (Exeter, UK); acetonitrile, methylmethacrylate (MMA), $Na_2HPO_4$ and $KH_2PO_4$ were reagent grade (Merck, Darmstadt, Germany); PBS (without $CaCl_2$ and $MgCl_2$), foetal calf serum, penicillin and streptomycin were obtained from Cansera (Canada); TES and HEPES were from Sigma (USA); L-glutamine and MEM were from BiochromKG seromed.

### 2.2. Supplementation of MEM

Minimum essential medium used contained: Earle's salts, 20 mM glutamine, 20 mM of the buffer TES–NaOH, 20 mM HEPES–NaOH (both pH 7.4), 10% (w/v) of foetal calf serum and 100 U/ml penicillin and 100 U/ml streptomycin.

### 2.3. Preparation of the bone cement powder

Different batches of CMW1 were used to obtain the acrylic bone cement powder. For each batch, the liquid component was mixed with the powder at room temperature (24–25°C) in a glass mixing bowl and rolled out to obtain thin plates; these were mechanically grounded until a fine powder was obtained. The powder from all batches was then mixed in order to obtain an homogeneous sample.

### 2.4. Influence of different media composition (PBS and MEM) and aging effect (in solution and air) on monomer release rate

The acrylic powder previously prepared (Section 2.3) was divided in 24 aliquots (1.75 g each). Six of them were stored, in closed glass flasks, at 4°C, during 3 days, in order to study aging effect in air (Section 2.4.3). The other 18 were divided in two groups: nine for release studies in PBS (group No. 1) and the reminder (group No. 2) for release studies in MEM.

#### 2.4.1. Influence of different media composition on monomer release

*2.4.1.1. Study in PBS.* Each of the nine samples from group No. 1 were exposed to 25 ml of PBS (in closed plastic flasks). Six of them were incubated for 24 h at 37°C. Aliquots of supernatant were collected at 0, 0.5, 1, 2, 4, 6, 8 and 24 h for MMA content determination. The other three were incubated for only 1 h at 37°C (aliquots of supernatant were collected each 10 min for MMA quantification).

*2.4.1.2. Study in MEM.* For group No. 2 samples the same procedure was followed (Section 2.4.1.1), the only difference being the media composition (PBS was replaced by MEM).

#### 2.4.2. Influence of aging effect, with different temperatures, (in solution) on monomer release

*2.4.2.1. Study in PBS.* The six solutions of group No. 1, after incubation for 24 h at 37°C (Section 2.4.1.1), were divided in two groups of three samples each. One group was kept for 2 days at

37°C and the other for the same time at 4°C. After 48 h of incubation aliquots of supernatant were collected at 72, 72.5, 73, 74, 76, 78, 80 and 96 h, since PMMA setting, for MMA quantification.

*2.4.2.2. Study in MEM*. For the six solutions of group No. 2 (Section 2.4.1.2) it was followed the procedure previously described (Section 2.4.2.1).

### *2.4.3. Influence of aging effect (in air) on monomer release*

*2.4.3.1. Study in PBS*. The three samples of acrylic powder kept for 3 days, in contact with air at 4°C (Section 2.4), were after that time exposed to 25 ml of PBS (in closed plastic flasks) and incubated for 24 h at 37°C. Aliquots of the supernatant were collected at 0, 0.5, 1, 2, 4, 6, 8 and 24 h for MMA content determination.

*2.4.3.2. Study in MEM*. For the other three samples of acrylic powder the same procedure was followed (Section 2.4.3.1), the only difference being the media composition (PBS was replaced by MEM).

### *2.5. MMA content determination*

All the aliquots collected were centrifuged and the supernatant analyzed by high-performance liquid chromatography (HPLC), using a modification of a method described in National Formulary of the United States (1995). The HPLC procedure employed a 5 μm column (Lichrospher 100 RP-18, Merck, Darmstadt, Germany), and a mobile phase of phosphate buffer:acetonitrile (70:30), pH 3, flow rate:1 ml/min, and UV detection at 205 nm.

Concentrations of released MMA, in both media, were calculated from the areas under the curve. Analysis of blank PBS and MEM media showed no interfering peaks.

The amount of MMA released was calculated in % (cumulative monomer released per 100 g of PMMA powder).

### *2.6. Gas-chromatography/mass spectrometry*

The existence of MMA liberated from PMMA powder was confirmed by GC–MS (gas-chromatography/mass spectrometry), using a modification of a previously described method (Willert and Frech, 1973). The GC–MS procedure employed a gas-chromatograph (Hewlett Packard 6890) with a 5-phenylmethylsiloxane column (HP-5MS, column lengh: 30 m; internal diameter: 0.25 mm; $T = 60$°C), helium was used as the carrier gas (flow: 1.1 ml/min) and injection port temperature was 280°C. The detector ($T = 300$°C) was a mass selective detector (Hewlett Packard 5973); EI (electron impact).

## 3. Results and discussion

### *3.1. MMA content determination*

HPLC was used for determination of residual MMA without the need of a preliminar extration from the media, revealing to be a good method for MMA quantification.

Method specificity was determined by collecting a 'peak' from HPLC and subjecting it to independent analysis by GC–MS.

The detection by MS confirmed that the mass-spectrum of the supposed monomer, in the analyzed sample, clearly matched the mass-spectrum of the standard monomer (Fig. 1) and showed no co-eluating compound, ensuring the specificity of the HPLC method.

### *3.2. Influence of different media composition in monomer release*

#### *3.2.1. MMA release during the first 24 h of incubation in PBS and MEM*

The MMA release data into PBS and MEM reveals the same pattern and shows that the release process increases rapidly during the first hour of incubation (Fig. 2) and then remains essentially constant, with no significant further release of MMA.

This is in agreement with the in vitro studies of both Brauer et al. (1977) and Schoenfeld et al.

(1979) who demonstrated that most free monomer was released, into water, within 1 h after polymerization.

The results of cumulative amount of MMA released during the first 24 h of incubation (first hour included), were fitted by:

$$y = a\ (1 - e^{-k_1 t}) \quad (1)$$

(Donbrow, 1992) characterized by $y$ (amount of monomer release with time $t$), $a$ (maximum amount of monomer released in the medium) and $k_1$ (positive first order release rate constant). The results are presented (Table 1) by mean parameters ($a$ and $k_1$) $\pm \sigma$ (S.E. of estimate).

The maximum cumulative amount of monomer released in PBS and MEM (Table 1) was com-

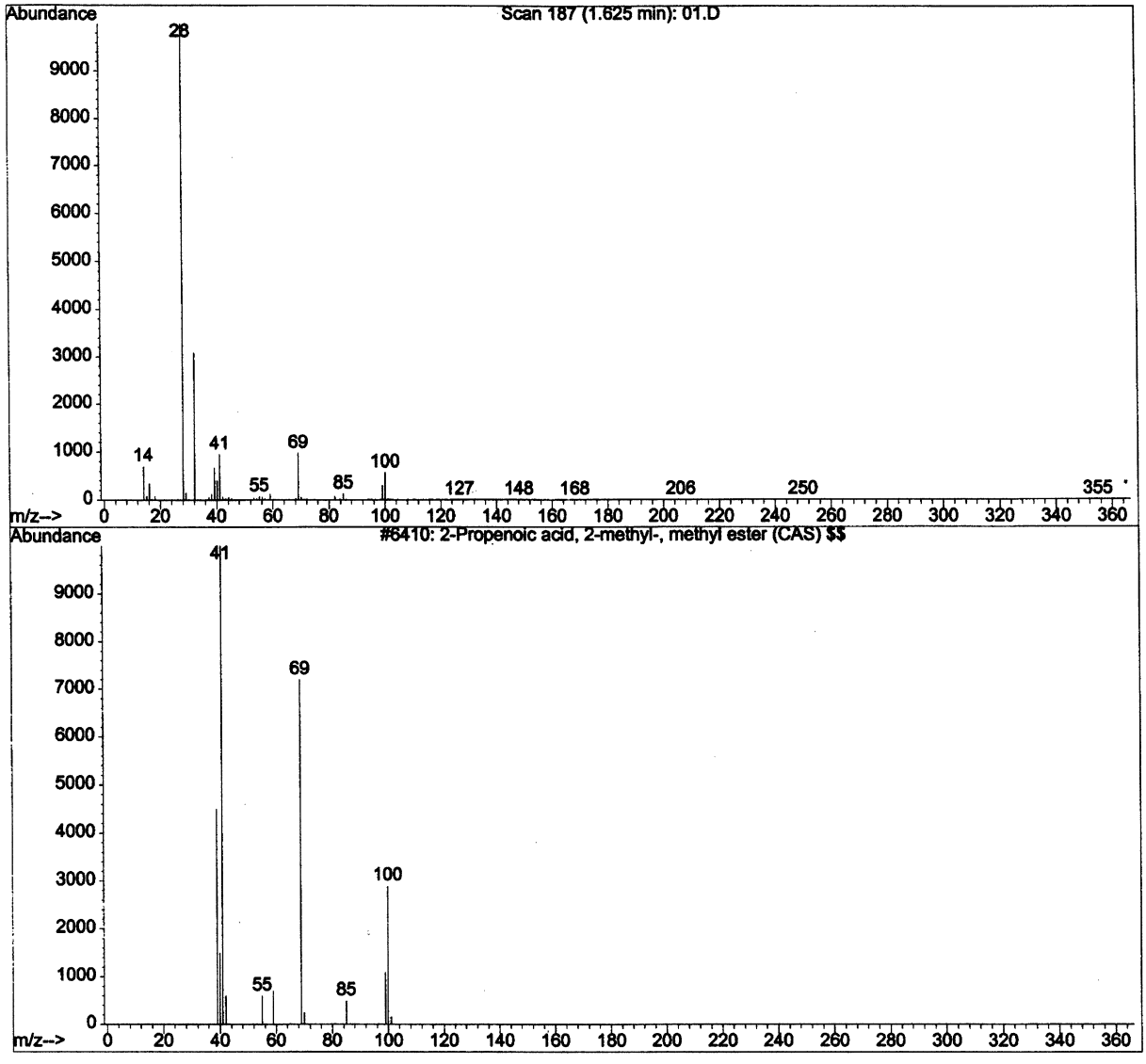



Fig. 1. GC–MS spectrum of standard MMA (below) and of the isolated 'peak' collected from HPLC of a PBS solution, after 4 h of incubation with acrylic powder.

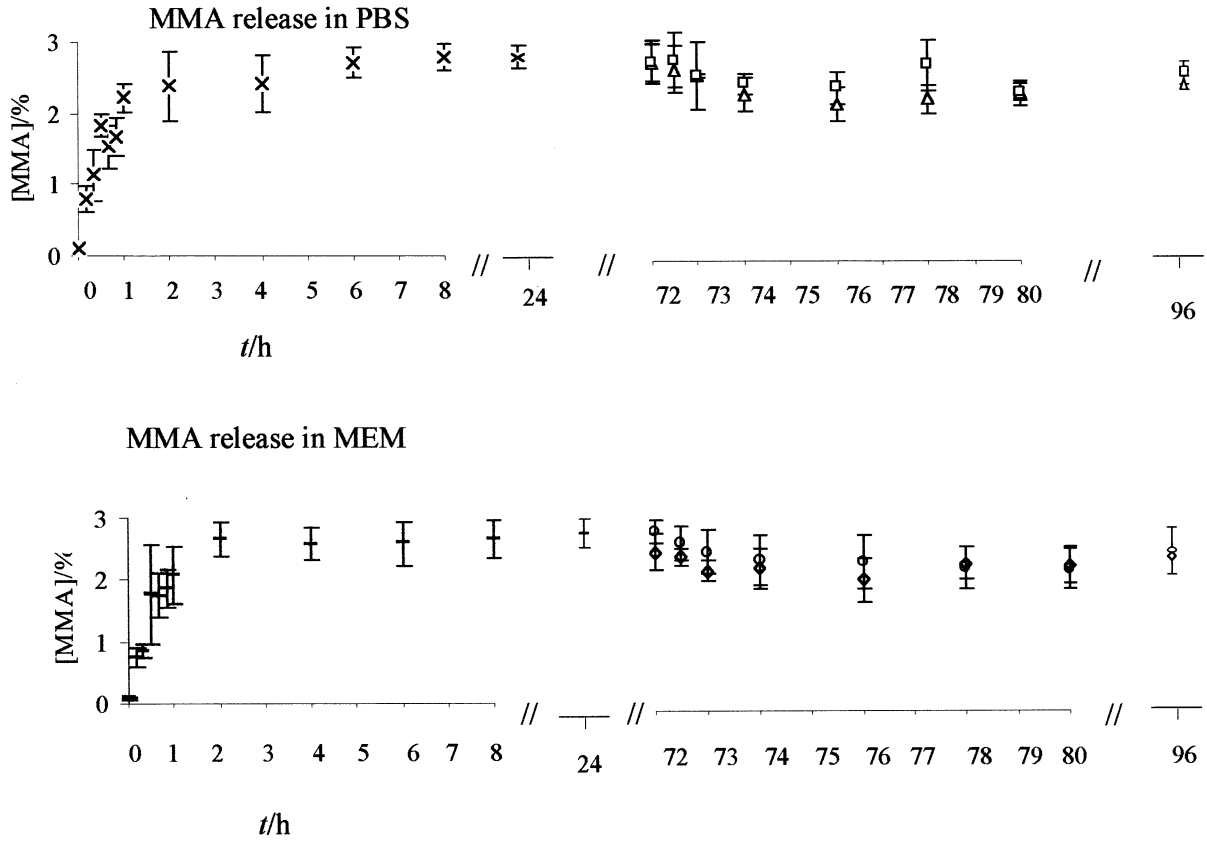



Fig. 2. Experimental dissolution data of monomer release ( ± S.D.) in PBS and MEM: ×, experimental data for PBS during the first day after polymerization; □, experimental data for PBS after storage at 37°C; △, experimental data for PBS after storage at 4°C; –, experimental data for MEM during the first day after polymerization; ○, experimental data for MEM after storage at 37°C; ◇, experimental data for MEM after storage at 4°C.

pared by Student's $t$-test for unpaired observations. Results suggested no difference in the maximum amount of monomer released in both media, ($P$ values less than 0.05 were interpreted as statistically significant), meaning that monomer's liberation in studied conditions was not affected by different media composition.

### 3.2.2. MMA release during the first hour of incubation in PBS and MEM

Showing the release profiles obtained (Fig. 2), that most of residual monomer was released within 1 h of incubation, other mathematical studies were only carried out with first hour dissolution data.

Experimental data were fitted by:

$$m = m_0 \, e^{-k_2 t} \quad (2)$$

and

$$Q = k_3 t^{1/2} \quad (3)$$

(Brossard and Woessidjewe, 1990), illustrated in Fig. 3.

Eq. (2) (Wagner model) is characterized by $m$ (monomer remaining to be released at time $t$), $m_0$ (estimated initial amount of monomer) and $k_2$ (first order release rate constant). Eq. (3) (Higuchi model) is characterized by $Q$ (cumulative release per unit area after time $t$) and $k_3$ (release rate constant related with: diffusion coefficient of monomer in dissolution media, monomer's solubility, monomer's concentration in polymer ma-

Table 1
Parameters resulting from the application of mathematical models to experimental dissolution data, in PBS and MEM

| Equations | PBS | MEM |
|---|---|---|
| $y = a\ (1 - e^{k}1^{t})$ | | |
| $k_1 \pm \sigma(k_1)$ ($min^{-1}$) | $0.028 \pm 0.004$ | $0.027 \pm 0.004$ |
| $a \pm \sigma(a)$ (%) | $2.59 \pm 0.22$ | $2.66 \pm 0.22$ |
| $r$ | 0.98 | 0.99 |
| $m = m_0 e^{-k}2^{t}$ (Wagner model) | | |
| $k_2 \pm \sigma(k_2)$ ($min^{-1}$) | $0.018 \pm 0.001$ | $0.025 \pm 0.002$ |
| $m_0 \pm \sigma(m_0)$ (%) | $2.22 \pm 0.05$ | $2.56 \pm 0.06$ |
| $r$ | 0.98 | 0.99 |
| $Q = k_3.t^{1/2}$ (Higuchi model) | | |
| $k_3 \pm \sigma(k_3)$ (% $min^{-1/2}$) | $0.22 \pm 0.008$ | $0.27 \pm 0.03$ |
| $r$ | 0.99 | 0.98 |

trix, porosity and tortuosity). The kinetic models applied showed no difference in release rate constants obtained in different media (Table 1).

The extremely good fit of cumulative release data to the square root of time (Table 1), suggest that MMA is released by a simple diffusion mechanism and that the process exhibit a classic 'burst effect' (Knepp et al., 1987).

Although Eqs. (1) and (2) are essentially the same, describing a first order release process, they were applied with different aims. Eq. (1) was applied to 24 h release data, allowing to estimate the maximum cumulative amount of monomer released in both media tested.

The parameter ($a$) calculated from the aforementioned fitting procedure, and assuming that it corresponds to the initial amount of the monomer in the polymer matrix, allowed us to calculate the monomer quantities remaining to be released at different times and to apply Eq. (2) to experimental first hour dissolution data.

### 3.3. Influence of aging effect (in solution) on monomer release

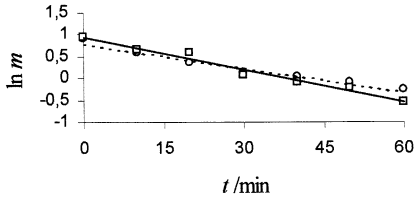
Our results suggest (Fig. 2) that there was no difference in monomer release with different storage temperatures and with different media composition, suggesting that after 96 h of incubation there was no further depolymerization of the cement, even in a medium with higher lipid content (MEM) and at 37°C.

This fact is clinically relevant and is in accordance with Davy and Braden (1991), and Petty (1980) suggesting that possible monomer side effects are mainly important in the first h after cement insertion or being the result of cytotoxic role of residual MMA.

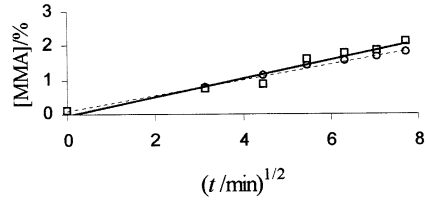
### 3.4. Influence of aging effect (in air) on monomer release

Mean results of MMA content (%) versus time for acrylic powder, after storage in contact with air, revealed the same pattern as those from the first day after polymerization (Fig. 4). Although there is a tendency for a decrease in maximum monomer release as the material ages there was no statistical difference ($P$ values obtained always higher than 0.05) between monomer released from freshly prepared acrylic cement (day 1) or after storage for 3 days, in both media tested.

These findings suggest that residual monomer has the tendency to be trapped in the scarfold of the polymer being released mainly by dissolution in aqueous solutions.




[2]




[3]

Fig. 3. Application of the mathematical models: Eq. (2) Wagner, and Eq. (3) Higuchi to experimental first hour dissolution data: □, experimental data for PBS; —, linear fitting for PBS; ○, experimental data for MEM; ---, linear fitting for MEM.

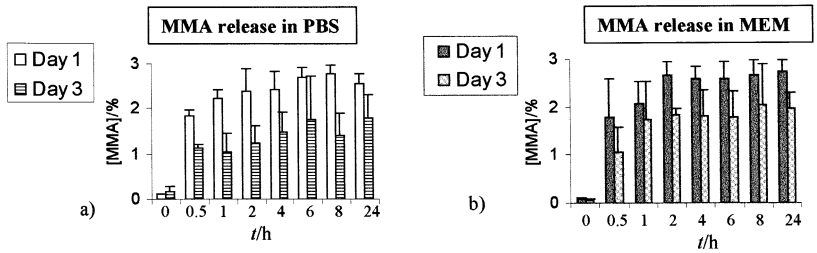



Fig. 4. Mean cumulative amount content of MMA ( ± S.D.) in PBS (a) and MEM (b), during 24 h of incubation at 37°C, for day 1 and day 3 after polymerization. There was no statistical difference ($P > 0.05$), between monomer release during day 1 versus day 3, in PBS and MEM.

## 4. Conclusions

The present study shows that the pattern and extent of monomer release was lightly affected by variables studied, revealing that it is more probably a surface phenomenon, with an initial fast release of residual monomer, followed by a much slower and steady release.

Acrylic powder used not being the best model to mimic in vivo conditions, allowed us to confirm that the monomer is one of cement's component released in studied culture media.

Methylmethacrylate is known to have a solubility in water or saline on the order of 1.5 g per 100 ml, with some variability, depending on the temperature (Petty, 1980). No measured concentration approached this maximum solubility, assuring the release of all residual monomer into the media and that no saturated monomer atmosphere was established in the flasks.

Our results are in accordance with Linder et al. (1976), who says that the medium outside the cement is unimportant as long as the leached out monomer can be transported away at a higher rate than the internal migration to the cement surface. Since in our protocol powder was used, the limiting factor was not the internal migration of the monomer but the solubility of the monomer that was the same in both media, as shown by equal release rate constants in both media (Table 1). Probably in vivo where the cement forms a mantle and is not a powder as such, the monomer will be trapped and will take more time to be released.

Although our studies do not replicate yet the complex clinical situation, they suggest that possible actions of monomer will mainly be due to the initial loss of non polymerized monomer rather than to further depolymerization of the already polymerized cement.

Nevertheless, the initial release of the compound, even in small quantities, requires further investigation in order to study the involvement of methylmethacrylate monomer in biocompatibility problems associated with acrylic cement application.

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