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Gentamicin bone cements: characterisation and release (in vitro and in vivo assays)

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Abstract

Due to the extended use of acrylic bone cements, its necessary to develop improved formulations in order to resolve their many drawbacks. The present work was conducted to make a physical–chemical characterisation of this kind of acrylic cement in order to introduce future changes in the formulations to: (1) improve or at least maintain their mechanical properties; (2) diminish their toxicity, and (3) control the drug release (rate and amount). From the dissolution method we can conclude that the preparation method (with or without pressure) of specimens is not responsible for the erratic release. The cumulative amount of gentamicin released was fitted to a semi-empirical equation to explain the possible release mechanism. The powder size, shape and distribution that could affect several properties of bone cement were studied with the aid of different techniques such as SEM, laser diffraction spectroscopy, and powder X-ray diffraction. From SEM micrographs, it was possible to observe that the surfaces of the specimens were very irregular with numerous small craters that may serve as conduits for eluting the antibiotic. An 'in vitro' drug diffusion model is proposed to elucidate the drug release mechanism. Finally an 'in vivo' study was performed to evaluate the antibiotic release to the neighbouring bone sites.

Keywords: PMMA bone cements; Gentamicin; 'In vitro' release; Physical–chemical characterisation; 'In vitro' drug diffusion

1. Introduction

Acrylic bone cement occupies a distinctive place in the hierarchy of synthetic biomaterials. Self-curing acrylic cements have been widely used in dentistry and orthopaedic surgery as filling agents, for the fixation of joint prostheses, and as spacers in a modified two-stage exchange arthroplasty, and even have been proposed for other uses like medical devices in the urinary tract (Tunney et al., 1997). However there are several disadvantages associated with the commercially available cements (Lewis, 1997). These include thermal necro-

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sis of bone, chemical necrosis due to unreacted monomer release, shrinkage of the cement during polymerization, poor cement distribution around the implant and property mismatch at the interfaces because the cement is orders of magnitude weaker than the bone or the implant. Many researchers have attempted to solve these problems by incorporating additional agents into the conventional ingredients of the acrylic bone cement (Pilliar, 1980; Ishihara et al., 1992; Smetana et al., 1992). Nevertheless, one of the first modifications of the poly(methylmethacrylate) is concerned with one of the more serious complications of orthopaedic surgery, the bacterial bone infections (osteomyelitis). Previous studies have made it clear that systemic administration of broad-spectrum antibiotics is insufficient to diminish the rate of infection and sustained high local concentrations of antibacterial compounds are required to minimize and even to cure osteomyelitis. In the 1970s, Buchholz and Engelbrecht (1970) introduced the concept of local antibacterial therapy using bone cements including antibiotics in their composition for the treatment of bone infections. Our research group centred its attention on this release of the antibiotic from the cement because of the publication of some 'in vitro' and 'in vivo' contradictory data. For example, data have been reported that suggested both, that the gentamicin (the aminoglycoside antibiotic usually employed) does (Holm and Vejlsgaard, 1976; Welch, 1978) and does not (Elson et al., 1977; Wahlig and Dingeldein, 1980) elute well from the same commercial cement. Such discrepancies have been attributed to differences in the experimental testing conditions (Trippel, 1986) or to the use of low sensitivity assay techniques (Penner et al., 1999). Therefore, the first step of our work was to design an 'in vitro' elution study. The bone cement selected was the CMW1 Radiopaque (De Puy International Ltd.) because it is one of the most widely used and is available also as gentamicin-loaded cement (CMW1 Gentamicin). Different sensitivity gentamicin assay methods have been employed such as Fluorescent polarization immunoassay, HPLC and a validated spectrophotometric method (Frutos et al., 2000b). Bone cements were formed by simulating the preparation procedure of the surgeon, obtaining spherical but not exact specimens. The large standard deviation observed between the six samples (see Section 3) conducted us to repeat the experiment but preparing well-defined specimens using a Teflon® mould and an hydraulic press (Frutos et al., 2000b). Unfortunately, this study also presented a low and erratic drug release. From these results and due to the extended use of the acrylic bone cements, it is necessary to develop improved formulations in order to overcome their many drawbacks referred to previously. However, it was not possible to obtain the necessary physical–chemical characterisation data from the commercial firms. Therefore the present work was conducted to make a physical–chemical characterisation of this kind of acrylic cement in order to assist future developments of formulations to: (1) improve or at least maintain their mechanical properties; (2) diminish their toxicity, and (3) control the drug release (rate and amount).

The physical–chemical properties studied included, structural characterisation, particle size, moisture content, pharmaco-technological studies such as 'in vitro' drug diffusion and also 'in vivo' drug release of a widely used acrylic bone cement. The powder size, shape and distribution could affect several properties of bone cement such as the rate of setting, the dissipation of the heat released during the polymerization process, the temperature of the setting mass and also the porosity of the cement (Park and Lakes, 1992; Pascual et al., 1996). These characteristics were studied with the aid of different techniques such as scanning electron microscopy (SEM), laser diffraction spectroscopy, and powder X-ray diffraction. The moisture content was determined because humidity is another factor that can affect the setting parameters (Pascual et al., 1996). The drug diffusion study can help to elucidate the drug release mechanism. In this work, an 'in vitro' drug diffusion model is proposed. Finally, an 'in vivo' release study was performed to evaluate the possible gentamicin release from the commercial formulation to the bone tissue.

2. Materials and methods

2.1. Materials

The selected bone cements were CMW1 Radiopaque and CMW1 Gentamicin (De Puy International Ltd.). Each cement unit pack consists of a two-component system (powder and liquid). The constituents of the cement are:

- Bone cement powder: gentamicin sulphate, polymethylmethacrylate, benzoyl peroxide and barium sulphate;
- Bone cement liquid: Methyl methacrylate, *N*,*N*-dimethyl-*p*-toluidine, ethanol, ascorbic acid and hydroquinona.

Reference gentamicin employed for assay was kindly provided as a gift from Lab Normon (Spain); all other chemicals were of reagent grade or better.

2.2. Preparation of device bone cements

A batch of device bone cements was prepared using CMW1 Gentamicin, employing the same preparation conditions as the surgeon would use.

According to the manufacturer's instructions, the bone cement was prepared by mixing all the liquid with all the powder. The cement was mixed carefully to minimise the entrapment of air for approximately 1 min. When the dough was formed, the cement was taken into gloved hands and kneaded for 1 min. Afterwards the bone cement samples were formed by hand, simulating the procedure of the surgeon. Spherical specimens for the dissolution studies and tablet-form specimens for the in vivo studies were individually prepared. During this production stage, a surgical suture was introduced in the tablet-form specimens to allow the device system to be fixed in the existing space between the femur and the tibia of the experimental animals (Fig. 1).

Once the production was finished, the samples were maintained at room temperature for 24 h in a cabinet with flowing air in order to assure the total evaporation of the liquid monomers that were employed.

2.3. Dissolution studies

For drug release testing, the USP 23 apparatus 1 (United States Pharmacopeia, 1995) (Van-Kel) with a rotation speed of 150 rev./min was used. A 150-ml dissolution medium of pH 7.4 phosphate-buffered saline (PBS) was accurately measured and allowed to reach thermal equilibrium at 37 ± 0.1°C. The spherical bone cement specimens were each immersed into the solution, then the motor and the timer were simultaneously started. During the dissolution assay over 8 weeks, samples (2 ml) were withdrawn by pipette at suitable intervals and filtered with a Whatman filter (0.45 μm). All gentamicin concentrations were determined by fluorescent polarization immunoassay (TDx: Abbott Laboratories). TDx calibrators were used according to the gentamicin concentration of the different samples. Five independent experiments were carried out. The average weight of the spherical bone cement specimens assayed was 8 g.

Different mathematical models of mixing mechanisms of diffusion described below, were applied to evaluate their suitability in describing gentamicin release from bone cements.

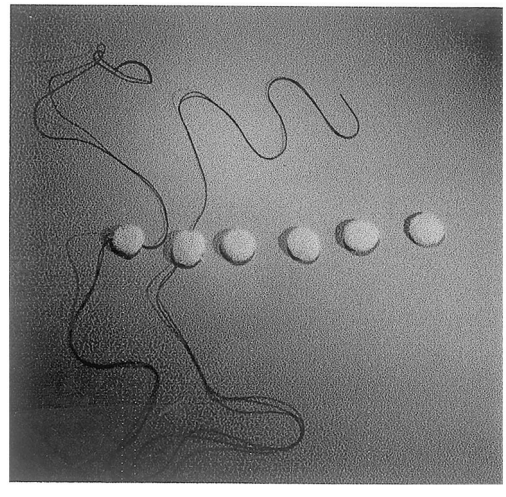

Fig. 1. Device systems for the in vivo studies.

2.4. Mathematical models

According to the standard reaction kinetics theory for dissolution of a solid, the drug content within a matrix is assumed to decline exponentially (Su et al., 1994; Dredan et al., 1996). The rate of drug release is proportional to the residual amount; that release can be modelled by the equation

$$M_t = M_\infty(1 - e^{kt}) \quad (1)$$

where M_∞ is the maximal amount of the released drug at infinite time or the initial amount of drug in the dosage form. The square root of time model was developed by Higuchi (1963) for a suspension planar matrix and it is a widely used model for describing empirical drug release from matrices; the model can be expressed according to the following equation

$$M_t/M_\infty = kt^{1/2} \quad (2)$$

The Higuchi equation is a mathematical relationship governing the rate of release of solid drugs randomly dispersed in solid matrices. Higuchi showed that the total amount of the drug released is a function of the square root of time as a result of the increasing diffusion pathway with the progression of the dissolution front through the porous matrix.

Ritger and Peppas (1987) introduced a semi-empirical equation to express the general drug release behavior from polymers, by coupling of Fickian and non-Fickian mechanism

$$\frac{M_t}{M_\infty} = k_1 t^{1/2} + k_2 t \quad (3)$$

where k_1 is a constant describing the diffusion-controlled release process and k_2 is a constant rate release (Case II transport). By means of this equation, the fraction of drug released at time t from several complicated systems can be represented. A generalized expression, previously proposed by Korsmeyer et al. (1983) and Peppas, (1985) was

$$M_t/M_\infty = kt^n \quad (4)$$

where k is a constant that incorporates characteristics of the macromolecular network system and the drug, and n is the diffusion exponent that determines the mechanism of release. To describe the drug release Lindner and Lippold (1995) proposed an expanded Korsmeyer equation, by adding a constant term, b, which characterizes the burst effect

$$M_t/M_\infty = kt^n + b \quad (5)$$

Kuhn and Wilson (1985) proposed an approximation for the complete release process, represented by the following equation

$$y = \text{const} + at^{1/2} - b\ \exp(-nt) \quad (6)$$

where y is the cumulative amount of drug released.

2.5. Scanning electron microscopy (SEM)

Particle morphology, size and shape of the CMW1 Radiopaque, CMWI Gentamicin, and reference gentamicin were analysed by scanning electron microscopy (SEM) on a Jeol 6400 electron microscope. All micrographs were the product of a secondary electron imaging used for surface morphology identification at different magnifications. The conductivity was increased by coating the sample surfaces with a thin layer of gold.

2.6. Particle size analysis by laser diffraction spectroscopy

The particle size was also assayed by laser diffraction spectroscopy. The samples were suspended in a non-reactive liquid (mineral oil) then a Galai-cis Laser Diffraction Spectroscopy was used to determine the particle size of the reference gentamicin and the CMW1 Radiopaque cement.

2.7. Powder X-ray diffraction studies

The structural characterisation of the CMW1 Radiopaque, CMW1 Gentamicin and reference gentamicin included conventional θ–2θ powder X-ray diffraction (Philips X'Pert-MPD) (CAI Diffraction rays X, Pharmacy, UCM) for all the samples under study. The powder samples were irradiated with monochromatized Cu Kα radiation. Measurements were carried out with $2\theta = 5$–40°, using a step size of 0.04° (2θ) and 1-s time per step.

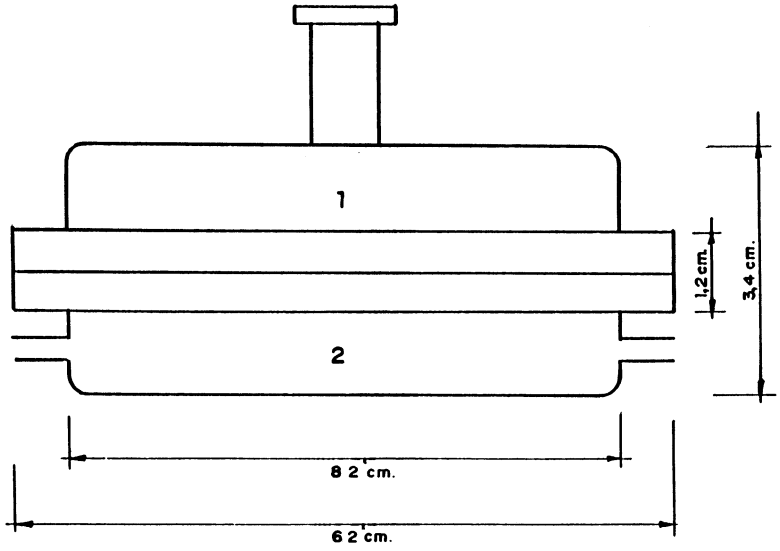

Fig. 2. Diffusion cells (1, donor cell and 2, receptor cell).

2.8. Moisture

The moisture measurements of the CMW1 Radiopaque, CMW1 Gentamicin and reference gentamicin were carried out using a Mettler LP 16 infrared dessicator attached to a Mettler PM 100 balance. The samples were heated at 105°C till they reached a constant weight. The loss of weight on drying was calculated and expressed as percentage. Each value represented an average of three runs.

2.9. Analytical methods

Two analytical methods were used: a separation method (HPLC) for the in vivo samples and a non-separation method for the in vitro samples (fluorescent polarization immunoassay).

1. The HPLC chromatographic method selected for the present work is the one described in the gentamicin sulphate monograph of the British Pharmacopoeia (1998) and also used previously by the authors (Frutos et al., 2000a). The HPLC system comprised a Gilson (Middleton, WI, USA) 305 pump and a Gilson 231 XL automatic sampler attached to a Rheodyne injection valve (100-μl sample loop). Detection of the analytes was accomplished using a Gilson 116 variable-wavelength UV detector. Data were recorded on a Spectra-Physics SP 4270 Integrator (San José, CA, USA).
2. Gentamicin levels of the dissolution and diffusion studies samples were assayed by fluorescent polarization immunoassay (TDx: Abbott Laboratories).

2.10. Diffusion assay

Diffusion of the antibiotic from the acrylic cement was studied in vitro. A CMW1 plate (10 cm diameter and 2 mm thickness) was prepared with a Teflon® mould. This plate was introduced into the middle of a diffusion cell (Fig. 2) and sealed with wax. The donor cell contained a saturated gentamicin solution in a buffer of pH 7.4. The receptor cell with a capacity of 10 ml was filled with the pH 7.4 buffer and connected to a buffer flask reservoir with a total volume of 150

ml in continuous replacement with the aid of a pump. Special care was taken to avoid the presence of air bubbles at the receptor cell, which might alter the diffusion process. The system was maintained thermostatically at 37°C. All the buffer was removed weekly, assayed for the antibiotic by the fluorescent polarization immunoassay (TDx: Abbott Laboratories), and replaced by fresh medium.

2.11. In vivo release studies

Twelve New Zealand White rabbits, weighing 2–2.5 kg were used. Overall phases of this study were performed in accordance with the recommendations of the Institutional Animal Care and Use Committee. The animals were sedated with 4% Floutane and 2 l of oxygen then they were anaesthetized with Combelem 1 ml/kg. An incision was made in the anterior surface of the back-limb exposing the femur and tibia. Under sterile conditions, a polymerised bone cement sample with a total weight of 500 mg containing 46.5 mg of gentamicin was inserted into the space between the tibia and the femur and fixed to the femur with a surgical suture. After closing the wound, the animals were observed till externally recumbent and then returned to animal housing. Postoperative analgesia consisted of administering an i.m. injection of Nolotil 1/2 ampoule and Nolotil s.c. injection of 1/2 ampoule. Six rabbits were sacrificed each time at intervals of 1 and 2 weeks after surgery by injecting pentobarbital (Dolethal). Two centimetres of the tibia and femur were cut 3 cm distal to the joint. The device system and the crushed bone segments were introduced into a recipient of 10 ml of phosphate buffer solution (pH 7.4) at 37°C for 24 h, and afterwards an aliquot of the supernatant was taken and assayed by the HPLC method (Frutos et al., 2000a).

3. Results and discussion

3.1. Dissolution studies

Fig. 3 shows the cumulative amount of gentamicin released versus time and its S.D. Each point in the plot represents the mean of five independent experiments. There is a great dispersion of the data which agrees with the literature for these

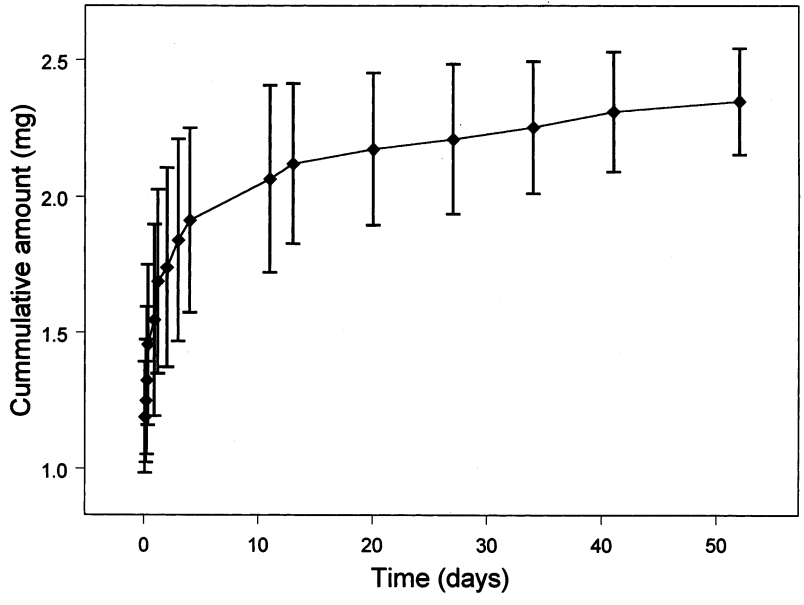

Fig. 3. Media experimental cumulative amount of gentamicin released from the cement and S.D. versus time.

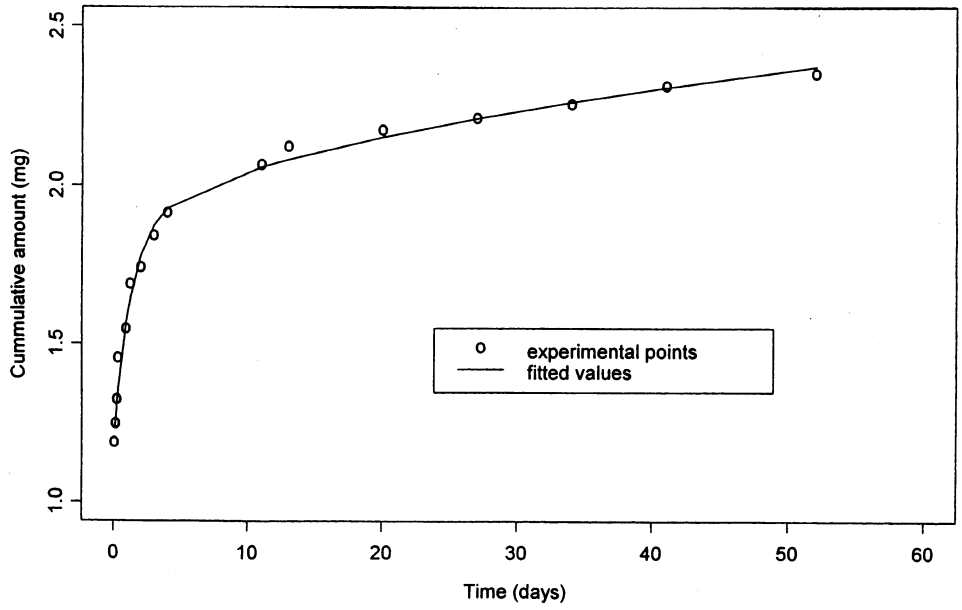

Fig. 4. Experimental cumulative amount of gentamicin released and the fitted values versus time.

kind of acrylic cements (Baker and Greenham, 1988; Klekamp et al., 1999; Penner et al., 1999; Scott et al., 1999). Likewise, when the experiment was conducted using well-defined shape specimens obtained with a Teflon® mould and a hydraulic press, the antibiotic release was also low and erratic. From these results we can conclude that the method of producing the specimens is not responsible for the erratic release. This conclusion agrees with the results obtained by Klekamp et al. (1999) using another commercial acrylic cement who observed that vacuum mixing of the cement did not alter the antibiotic elution. It may be due to the fact that only gentamicin molecules located in the superficial layers of the device system are accessible through voids and cracks to the dissolution medium, since as will be discussed later, gentamicin cannot cross the polymeric matrix because the aqueous dissolution medium is not able to diffuse in the hydrophobic matrix.

Several relationships were found to hold for the data under investigation in the present study. However, from the statistical point of view, the best fit was obtained when experimental data were fitted to the model of Khun and Wilson: $y = \text{const} + at^{1/2} - b\exp(-nt)$. The parameters in the model were estimated by minimizing the sum of squared residuals. All computations were made using a statistical software package, S-PLUS 2000 (MathSoft, 2000). The *nls*, an S-PLUS fitting function for non-linear models is available for solving the special minimisation problems of non-linear least squares.

For analysing release curves by mathematical models, several criteria were considered:

1. A graphical representation of predicted values against observed data. In Fig. 4, a good agreement can be observed between experimental and fitted points.
2. The estimated parameters and their S.E. Table 1 shows the parameters of this model and their S.E.
3. The degree of fit was quantified by the residual S.E. (0.040), the determination coefficient r^2 (0.9915) and the F-value (468.7).
4. The physical relevance of the estimated parameters.

According to these criteria, the Khun and Wilson model was chosen. The value of the determination coefficient and the F-value were the

Table 1
Statistical parameters and S.E. for the Khun and Wilson model

Constant value	S.E.	*a*-value	S.E.	*b*-value	S.E.	*n*-value	S.E.
1.7904	0.0487	0.0807	0.0095	0.6209	0.0480	0.7986	0.1453

highest and the S.E. was the lowest when the Khun and Wilson equation was used, but the physical meaning of the parameters was not so clear. The Khun and Wilson equation can be rewritten in a different form

$$M_t = M_{\text{burst}} + M_{\infty(\text{diss})}(1 - e^{-nt}) + kt^{1/2} \quad (7)$$

From the above expression, it could be postulated that there are three mechanisms implicated in the release behaviour of gentamicin from bone cement. The first term on the right-hand side, M_{burst}, is associated with the initial burst effect, the second term $M_{\infty(diss)}(1 - e^{-nt})$ is associated with the kinetics of a dissolution process and the third term could be associated with a Fickian diffusion of a solid particle (gentamicin particle) in its diluted solution.

The parameters obtained from the fitted values are showed in the next equation:

$$M_t = 1.1695 + 0.6209(1 - e^{-0.7985\cdot\text{time}}) + 0.0807\sqrt{\text{time}}$$

3.2. Scanning electron microscopy

According to Park and Lakes (1992), it may be considered that the powder size, shape and distribution could seriously affect the properties of bone cements. Scanning electron microscopy was used to study the shape of the CMW1 Radiopaque, reference gentamicin, and CMW1 Gentamicin particles and also of the polymerized bone cement.

Fig. 5 shows the micrograph of the CMW1 Radiopaque powder. The powder of the cement is a mixture formed by different constituents. The larger and prevailing structures observed are spherical granules corresponding to pre-polymerized PMMA with a characteristic appearance probably due to an emulsion obtaining method, the rest of the structures observed are more or less polyhedral and correspond to the other cement constituents (mainly barium sulphate).

Gentamicin particles have been shown to have a spherical form with one or several rounded clefts that may indicate that they were obtained by a spray-drying method (Fig. 6).

Fig. 7 shows a micrograph of the surface of the cured specimens. In this figure it is possible to observe that the surface device system is very irregular with numerous small craters which may serve as conduits for eluting the antibiotic. This micrograph also shows that the liquid polymer was hardened over the powder cement particles entrapping gentamicin particles, although some of them, which stayed on the surface, were partially recovered. All these factors can significantly affect the amount of drug released.

3.3. Particle size analysis by laser diffraction spectroscopy

It is well known that the distribution of drug particle size in a powdered material can affect the bioavailability of certain active drugs. On the

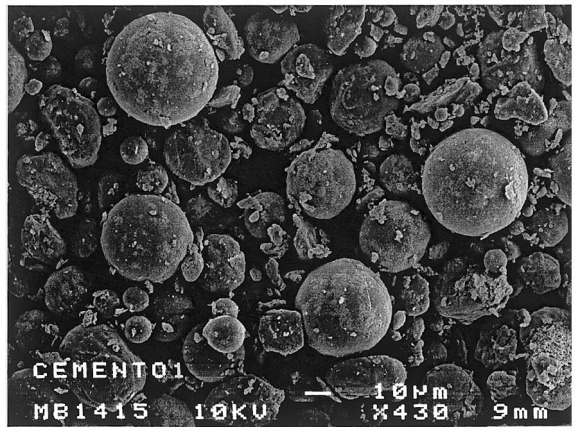

Fig. 5. CMW1 Radiopaque cement (× 430).

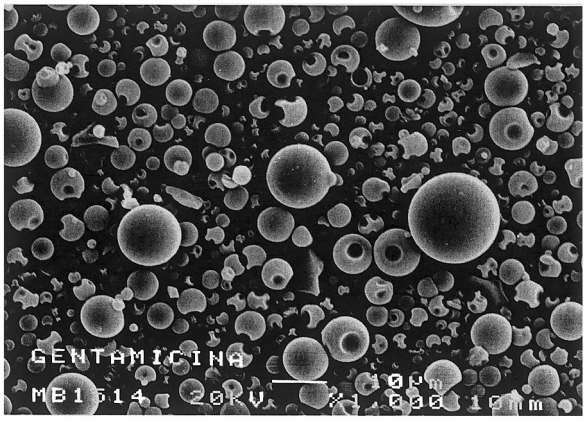

Fig. 6. Gentamicin (× 1000).

other hand, the size and size distribution of the PMMA beads have a strong effect on the temperature profile observed during the setting process. Other authors have observed that as the average size of PMMA beads increases, the maximum temperature reached during the polymerization process decreases (Pascual et al., 1996).

The PMMA particle size distribution is also a very important parameter because the greater specific surface PMMA particles will be better dissolved in the monomer than the lower specific surface ones. Therefore, the PMMA beads with a larger size will maintain their spherical form in the cured system and that will be responsible for the cement surface morphology (Fig. 7). So the

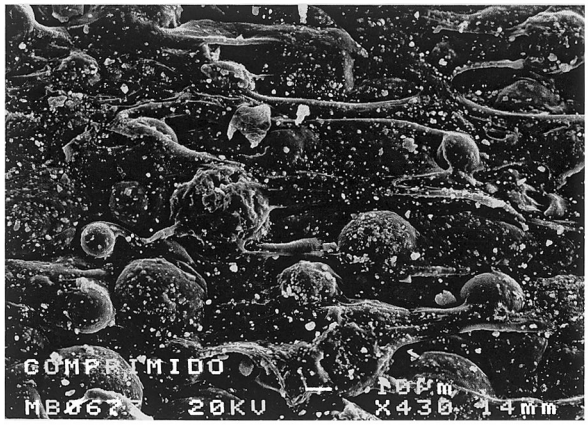

Fig. 7. Surface device system (× 430).

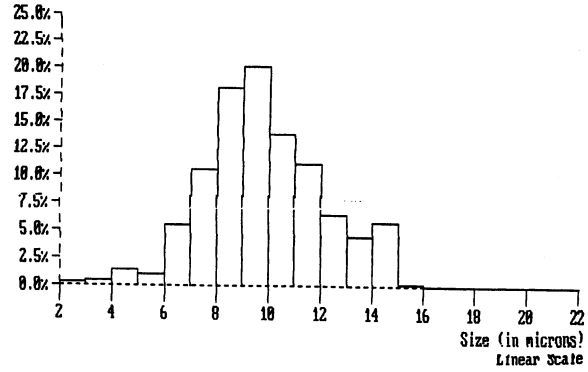

Fig. 8. Percentages of gentamicin particles versus size in microns.

PMMA size distribution will affect the gentamicin release which is directly related to the existence of imperfections such as holes, fissures and pores in the surface layer. Figs. 8 and 9 show the granulometric graphics of the gentamicin and CMW1 Radiopaque cement, respectively. The volume moment diameter values are showed in Table 2.

3.4. Powder X-ray diffraction studies

The CMW1 Radiopaque cement powder spectrum (Fig. 10a) shows an amorphous halo centered on $2\theta = 14°$ and several sharp peaks characteristic of a crystalline structure. The more outstanding peaks are centered at $2\theta = 32.8$, 31.5, 28.7, 26.8, 25.8 and 24.8°. The amorphous halo may be attributed to the PMMA polymer (CAI

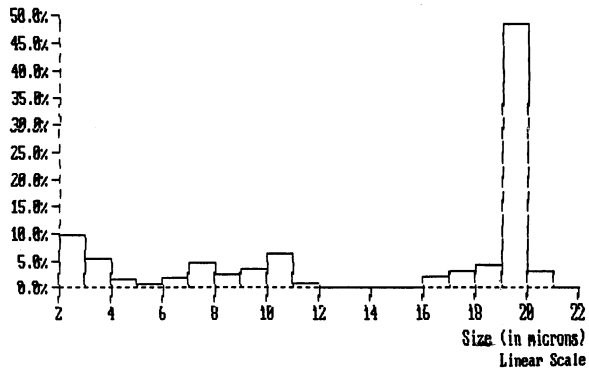

Fig. 9. Percentages of CMW1 Radiopaque cement versus size in microns.

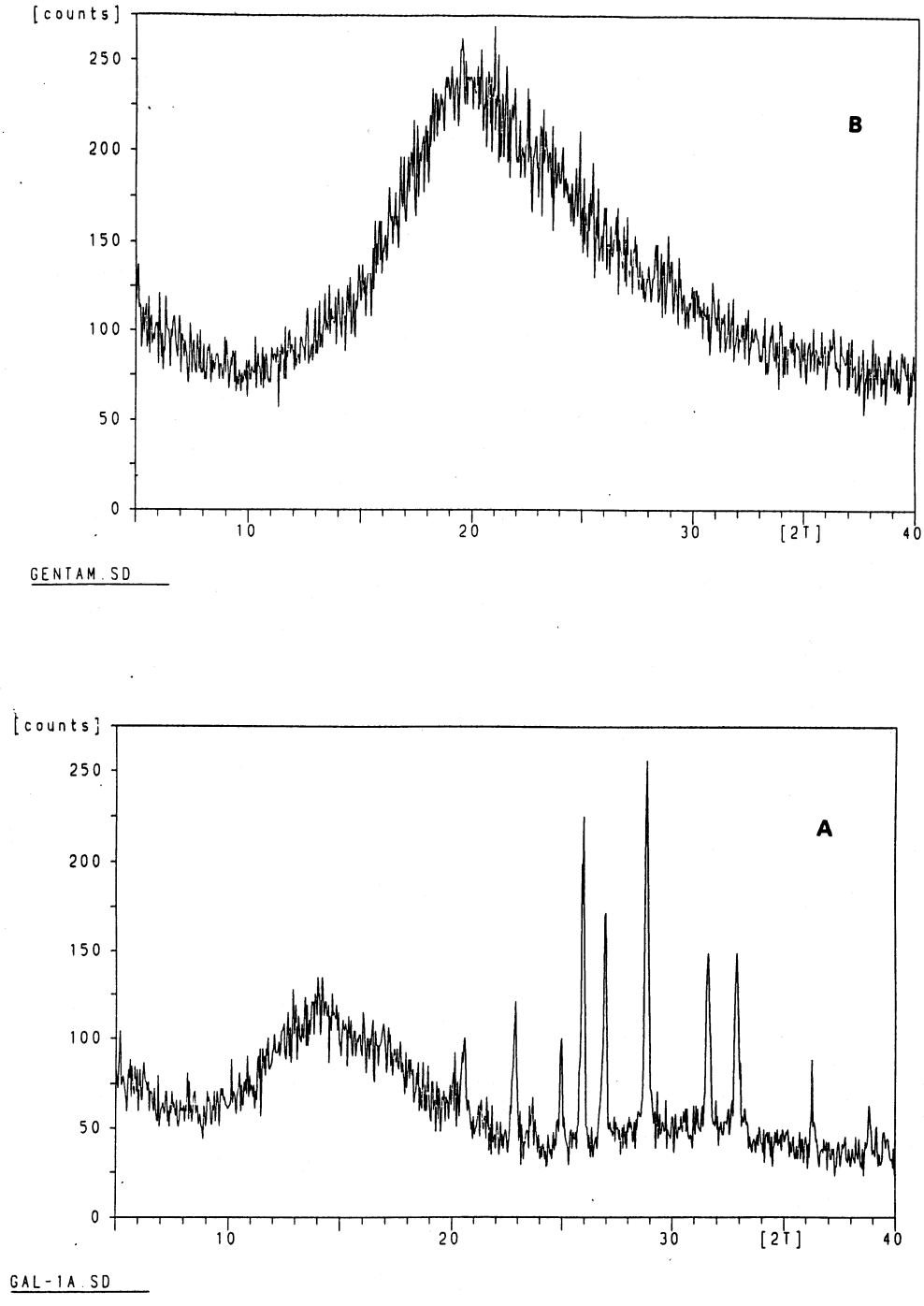

Fig. 10. CMW1 Radiopaque cement spectrum (A) and gentamicin spectrum (B).

Table 2
Volume moment diameter (μm) of gentamicin and CMW1 Radiopaque cement

Sample	Volume moment diameter (μm)
Gentamicin	9.82 ± 2.30
CMW1 Radiopaque cement	14.37 ± 6.64

Diffraction rays X, Pharmacy, UCM) while the previously described peaks correspond to the barium sulphate (CAI Diffraction rays X, Pharmacy, UCM). Fig. 10b shows the gentamicin spectrum. As can be observed from this figure, gentamicin is in an amorphous state with a halo approximately centred at $2\theta = 20°$, which supports our interpretation based on the scanning electron microscopy. According to both assay methods, gentamicin particles were obtained using a Spray-Drying process as this technique usually produces drug particles in an amorphous state. Fig. 11 shows the spectra of the CMW1 Radiopaque cement powder (A) and of the CMW1 Gentamicin cement powder (B). In Spectrum (B), we can observe a displacement to wider angles of the amorphous halo ($2\theta = 14°$), due to superposition of the gentamicin amorphous halo. Moreover in this spectrum, we can also observe a broadening and splitting of the characteristic diffraction maximum of the barium sulphate probably because of decreasing mean crystal size during the mixing process of the cement with the gentamicin.

3.5. Moisture assay

The loss of weight expressed as a percentage was 1% for the CMW1 Radiopaque Cement, 2% for the CMW1 Gentamicin Cement and 3.91% for the gentamicin.

3.6. Diffusion study

Although our diffusion experiments showed detectable gentamicin concentration values (close to 0.5 μg/ml) in some of the samples, we consider

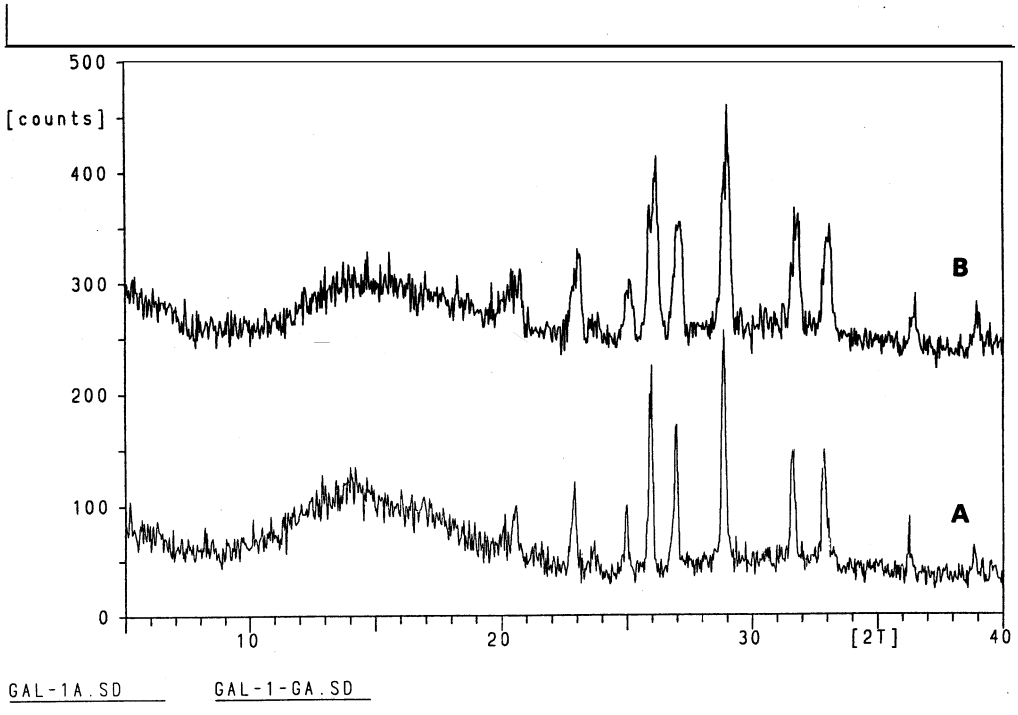

Fig. 11. CMW1 Radiopaque cement spectrum (A) and CMW1 Gentamicin cement spectrum (B).

that these low values, compared to the enormous concentration of the saturated gentamicin solution of the donor cell, cannot be related to a real and significant diffusion process. Moreover, these low values are too close to the sensitivity (lower limit) of the analyzing method (0.27 μg/ml). On the other hand, the assay of the same sample sometimes led to a non-detectable value, and at other times to value of 0.55 μg/ml. These differences are probably due to the dissolution medium used in our study (pH 7.4 buffer) which was different from the usual dissolution medium (serum) used when the method was validated. Therefore, we consider that the matrix of poly(-methylmethacrylate) bone cement is impermeable to gentamicin and that the antibiotic is released through an interconnecting series of voids and cracks in the cement, rather than through diffusion, which agrees with other studies (Baker and Greenham, 1988).

3.7. In vivo release studies

This study confirmed that the device system previously inserted in the rabbits was able to release gentamicin. The specimens after being implanted during a period of 7 and 14 days, were introduced into a recipient with 10 ml of phosphate buffer solution (pH 7.4) at 37°C for 24 h, and afterwards an aliquot of the supernatant was taken and assayed by HPLC. The amount of gentamicin released was 1% of the total theoretical amount.

The tibia and femur segments, both from the healthy paws and subjected to the surgical procedures were assayed for their gentamicin content. Samples were crushed and introduced into a recipient with 10 ml of phosphate buffer solution (pH 7.4) at 37°C for 24 h, and afterwards an aliquot of the supernatant was taken and assayed using the same procedure used for the cement specimen. No gentamicin was detected in the samples of the tibia and femur of the healthy paws. Unlike the assay of the paw, bones subjected to the implantation of the device system showed that the antibiotic was released from the specimen to the proximal bone sites in sufficiently large amounts to reach the minimum inhibitory concentration of the antibiotic (2 μg/ml) (Sande and Mandel, 1990). However, the amount of gentamicin released was very low when compared to the gentamicin retained by the device system. Therefore, the formulation of acrylic cements needs to be improved to achieve a more controlled release and in order to obtain reproducible clinical results.

Acknowledgements

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References

Baker, A.S., Greenham, L.W., 1988. Release of gentamicin from acrylic bone cement. J. Bone Joint Surg. 70-A, 1551–1557.

British Pharmacopoeia, 1998. Vol. 1, HMSO, London, pp. 302.

Buchholz, H.W., Engelbrecht, H., 1970. Uber die Depotwirkung einiger Antibiotica bei Vermischung mit dem Kunstharz Palacos. Chirurg 41, 511–515.

Dredan, J., Antal, I., Racz, I., 1996. Evaluation of mathematical models describing drug release from lipophilic matrices. Int. J. Pharm. 145, 61–64.

Elson, R.A., Jephcott, A.E., McGeghie, D.B., Verettas, D., 1977. Antibiotic-loaded acrylic cement. J. Bone Joint Surg. 59, 200–205.

Frutos, P., Torrado, S., Pérez-Lorenzo, M.E., Frutos, G., 2000a. A validated quantitative colorimetric assay for gentamicin. J. Pharm. Biomed. Anal. 21, 1149–1159.

Frutos, P., Diez, E., Barrales-Rienda, J.M., Frutos, G., 2000b Validation and 'in vitro' characterization of antibiotic-loaded bone cement release, Int. J. Pharm. 209, 15–26.

Higuchi, T., 1963. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drug dispersed in solid matrices. J. Pharm. Sci. 52, 1145–1149.

Holm, N.J., Vejlsgaard, R., 1976. The *in vitro* elution of gentamicin sulphate from methylmetacrylate bone. A comparative study. Acta Orthop. Scand. 47, 144–148.

Ishihara, K., Arai, H., Morita, S., Furuya, K., Nakabayashi, N., 1992. Adhesive bone cement containing hydroxyapatite particles as bone compatible filler. J. Biomed. Mater. Res. 26, 937.

Klekamp, J., Dawson, J.M., Haas, D.W., DeBoer, D., Christie, M., 1999. The use of vancomycin and tobramycin in acrylic bone cement. J. Arthroplasty 14, 339–346.

Korsmeyer, R.W., Gurny, R., Doelker, E., Buri, P., Peppas, N.A., 1983. Mechanisms of solute release from porous hydrophilic polymers. Int. J. Pharm. 15, 25–35.

Kuhn, A.T., Wilson, A.D., 1985. The dissolution mechanism of silicate and glass ionomer cements. Biomaterials 6, 378–382.

Lewis, G., 1997. Properties of acrylic bone cement: state of the art review. J. Biomed. Mater. Res. 38, 155–182.

Lindner, W.D., Lippold, B.C., 1995. Drug release from hydrocolloid embedding with high or low susceptibility to hydrodynamic stress. Pharm. Res. 12, 1781–1785.

MathSoft, 2000. S-PLUS 2000. Data Analysis Products Division, MathSoft, Seattle, WA.

Park, J.B., Lakes, R.S., 1992. Biomaterials: An Introduction, 2nd edn. Plenum, New York, pp. 29–62.

Pascual, B., Vazquez, B., Gurruchaga, M., Goñi, I., Ginebra, M.P., Gil, F.J., Planell, J.A., Levenfeld, B., San Román, J., 1996. New aspects of the effect of size and size distribution on the setting parameters and mechanical properties of acrylic bone cements. Biomaterials 17, 509–516.

Penner, M.J., Duncan, C.P., Bassam, A.M., 1999. The *in vitro* elution characteristics of antibiotic-loaded CMW and Palacos-R bone cements. J. Arthroplasty 14, 209–214.

Peppas, N.A., 1985. Analysis of Fickian and non-Fickian drug release from polymers. Pharm. Acta Helv. 60, 110–114.

Pilliar, R.M., 1980. Carbon reinforced acrylic cement. Orthop. Rev. 9, 67–72.

Ritger, P.L, Peppas, N.A., 1987. A simple equation for description of solute release 1. Fickian and non-Fickian release from non-swellable device in the form of slabs, spheres, cylinders or discs. J. Controlled Release 5, 23–36.

Sande, M.A., Mandel, G.L., 1990. Antimicrobial agents. In: Goodman, A., Gilman, Rall, T.W., Nies, A.S., Taylor, P. (Eds.), The Pharmacological Basis of Therapeutics, 8th edn. Pergamon, New York, pp. 1108–1110.

Scott, C.P., Higham, P.A., Dumbleton, J.H., 1999. Effectiveness of bone cement containing tobramycin. An in vitro susceptibility study of 99 organism found in infected joint arthroplasty. J. Bone Joint Surg. 81-B, 440–443.

Smetana, K., Stol, M., Jr, Korbelar, P., Novak, M., Adam, M., 1992. Biomaterials 13, 639–642.

Su, X.I., Al-Kassas, K., Li Wan Po, A., 1994. Statistical modeling of ibuprofen release from spherical matrices. Eur. J. Pharm. Biopharm. 40, 73–76.

Trippel, S.B., 1986. Current concepts review antibiotic-impregnated cement in total joint arthroplasty. J. Bone Joint Surg. 68, 1297–1302.

Tunney, M.M., Jones, D.S., Gorman, S.P., 1997. Methacrylate polymers and copolymers as urinary tract biomaterials: resistance to encrustation and microbial adhesion. Int. J. Pharm. 151, 121–126.

United States Pharmacopeia, 1995. United States Pharmacopeia 23, NF 18. US Pharmacopeial Convention, Rockville, MD, pp. 1791–1792.

Wahlig, H., Dingeldein, E., 1980. Antibiotics and bone cements. Experimental and clinical long-term observations. Acta Orthop. Scand. 51, 49–56.

Welch, A.B., 1978. Antibiotics in acrylic bone cement. *In vitro* studies. J. Biomed. Mater. Res. 12, 679–700.