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A preliminary study of the release of quaternary ammonium antimicrobial compounds from acrylic bone cement

Manojgna Mathey · Vijaya Surana · Mark Edwards · John W. Nicholson

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Abstract The quaternary ammonium compounds benzalkonium chloride and cetyl pyridinium chloride have been studied as potential anti-microbial additives for incorporation into acrylic bone cement. Using the commercial bone cement Palacos K-40, each compound was added at 1% and 5% by mass. Setting time of the cement was 9.75 min. This did not change when benzalkonium chloride (5%) was present, but was shortened to 9.00 min by 5% cetyl pyridinium chloride. Release of additive was estimated by determining chloride release from set cement specimens using a chloride-ion selective electrode. This showed that release occurred but was a complicated process and did not follow simple diffusion-based kinetics. Release thus appeared to occur by a similar overall mechanism to that of gentamicin sulphate from bone cements. The results show that these anti-microbial compounds can be incorporated into acrylic bone cement and then released in a satisfactory manner and suggest that these compounds have the potential to replace gentamicin sulphate as active ingredients in orthopaedic cements.

1 Introduction

Acrylic bone cement is widely used in orthopaedic surgery to secure the prosthesis to the bone in both total hip and knee arthroplasty [1]. It was introduced in the 1960s by Sir John Charnley in his pioneering work on total hip arthroplasty [1, 2]. Soon afterwards, the technique of incorporating an antibiotic, gentamicin sulphate, into the bone cement to reduce the incidence of infection was developed and is now widely used [1, 2].

Acrylic bone cement comprises two components, namely a powder consisting of pre-polymerised polymethylmethacrylate (PMMA) and a liquid component, methyl methacrylate monomer (MMA). Typically, the ratio of PMMA powder to MMA monomer used is 2:1 w/w. The initiator is benzoyl peroxide, in association with *N*,*N*-dimethyl-p-toluidine as accelerator which brings about setting through free radical polymerisation [1–3]. As well as the main components, chlorophyll may be used as the colour for the cement [4].

Gentamicin sulphate may be incorporated into such a cement to address the problem of infection, caused principally by *Staphylococcus* sp. and *Propionibacterium acnes* [5]. Such infection may necessitate revision surgery, and in one study was shown to be the cause of failure in 22% of a sample of hip replacement operations [5]. Inclusion of gentamicin sulphate in the bone cement has been shown to significantly lower the risk for revision surgery [6, 7], and also to have no significant effect on the handling properties of the cement [8] as determined by the tests specified in ISO 5833 [9].

Gentamicin sulphate is a highly water soluble compound and also thermally stable [10, 11]. It also has low allergenicity. It is typically released from bone cement in a biphasic fashion, involving a high rate of release in the initial time period, and a later much reduced rate of release [8]. The short-term initial elution occurs through imperfections in the cement, and these gradually grow due to ingress of water and cause stress-cracking. This contributes to release of the antibiotic, and also to the development of numerous interlocking channels, through which the gentamicin sulphate escapes at more moderate rates over longer time periods [12].

M. Mathey · V. Surana · M. Edwards · J. W. Nicholson (✉)
School of Science, University of Greenwich, Medway Campus, Chatham, Kent ME4 4TB, UK
e-mail: J.W.Nicholson@gre.ac.uk

Although the release of gentamicin continues for some time, it does not continue to completion [13]. Experiments have shown that only about 20% of the total incorporated antibiotic is released after several months [13, 14]. Despite this retention, gentamicin sulphate is a successful additive to bone cements with incidence of infection reduced [15–17], as well as reduced need for revision surgery [6, 7].

However, there are difficulties with gentamicin-loaded bone cement, in particular that bacteria develop a resistance to the antibiotic with time. Resistant strains of *Staphylococcus sp.* have developed and, for example, one study reported that 88% infections in patients with primary arthroplasty fixed with gentamicin-loaded bone cement showed that at least one of the infecting strains of staphylococcus was resistant to gentamicin [18]. More recently, bacterial infections have been observed associated with acrylic bone cement in revision surgery, suggesting the development of antibiotic resistance is becoming a practical problem in the clinic [19].

This suggests that alternative antimicrobial compounds could usefully be considered. In the current work, two such antimicrobials are studied, namely benzalkonium chloride and cetyl pyridinium chloride. These are similar compounds, in that they are quaternary ammonium compounds with some surfactant activity [20, 21]. Their properties are given in Table 1, along with details of their application as antimicrobials.

The current study employed these compounds at levels of 1% and 5% in a commercial acrylic bone cement, and was aimed at determining both the effect of antimicrobial incorporation on the setting behaviour of the acrylic cement and the nature and extent of antimicrobial release.

2 Materials and methods

The bone cement used was Palacos-K40 (Schering-Plough Europe, Belgium). It was mixed in the ratio 2 g powder to 1 cm^3 liquid in a glass beaker using a spatula, with mixing taking approximately 1 min. The antimicrobial compounds used were benzalkonium chloride and cetyl pyridinium chloride [monohydrate] (Sigma-Aldrich, Poole, Dorset, UK). These were incorporated at levels of 1% and 5% by mass (i.e, 0.026 g or 0.144 g respectively for a 2 g/1 cm^3 mixture).

Setting behaviour was determined in a modified version of the ISO 5833 test [9] by mixing a sample of cement, and once it attained a dough-like consistency, transferring it to a silicone rubber mould of dimensions 1 × 1 × 1 cm. A thermocouple was placed at the centre of the mass, and the temperature recorded every 15 s. From this data, a graph of temperature vs time was plotted and used to determine the setting time. This was carried out for the cement itself, and cement with 5% of each antimicrobial compound.

Release of chloride was determined from the parent cement and from cement containing antimicrobial compounds at 1% and 5% by mass. Specimens were prepared by mixing samples of cement with and without the antimicrobial compounds, and for each cement type, preparing six cylindrical specimens (6 mm high × 4 mm diameter). These were formed by transferring the cement at the dough stage to split stainless steel moulds of the appropriate size. Cements were allowed to set at room temperature for 20 min, then removed from the moulds and placed in individual 5 cm^3 portions of deionised water in plastic storage bottles. The release of the chloride ions was detected using a chloride electrode (Model 7065, Electronic Instruments Ltd) at time intervals of 15, 30, 45, 60, 120, 180, 240 min and 2 weeks, and results for amounts released were recorded as means and standard deviations.

Release versus time was plotted for the 1% and 5% additive-loaded bone cements, in order to determine the nature of the release profiles of chloride from bone cement. In addition, release vs $(time)^{1/2}$ graphs were plotted as required by Fick's second law to test whether release was a diffusion process.

Data on release were tested for significance using the Neumann–Keuls test.

Table 1 Properties of the antimicrobial compounds used [16]

Property	Benzalkonium chloride	Cetyl pyridinium chloride
Description	White/pale yellow powder	White powder
Solubility	Soluble in water, ethanol and acetone	Soluble in water and ethanol
Anti-microbial action	Against both gram-positive and gram-negative bacteria	Against both gram-positive and gram-negative bacteria
Uses	Food hygiene products, per-operative disinfection, in lozenges for mouth and throat infections, and in creams for nappy rash	To treat halitosis and skin infections. Used in wound cleansing, and for disinfecting surgical utensils

3 Results

Table 2 shows results for the setting time determined for the bone cement along, and with the addition of 5% by mass of each anti-microbial compound. There was no difference in the presence of benzalkonium chloride, which gave 9.75 min, compared with the control, but in the presence of cetyl pyridinium chloride, the working time was shortened slightly to 9.00 min.

Release profiles for the first 4 h of release are shown in Figs. 1, 2, 3, and 4. The first two are for benzalkonium chloride at 1 and 5%, respectively; Figs. 3 and 4 are for cetyl pyridinium chloride, also at 1 and 5%, respectively.

All plots show the same general shape. They have a high initial release rate followed by a region that is very flat, with a slope approaching, though not quite equal to, zero. Each thus shows an approximately two-step pattern. In the plateau region, it is worth noting that the slight increases were not statistically significant. For example, for benzalkonium chloride at 1% concentration, the release level rose from 2.4 ppm (SD 1.1 ppm) at 120 min to 3.2 ppm (SD 1.3 ppm) at 240 min, and at 5%, the mean values of release rose between 60 and 240 min from 21.6 ppm (SD 7.8 ppm) to 26.0 ppm (SD 12.3 ppm).

Cetyl pyridinium chloride showed similar behaviour at both 1 and 5% levels, in that release rose to a plateau region, during which release levels rose by amounts that were small and not statistically significant. For the 1% level, this ranged from 2.5 ppm (SD 0.6) at 60 min to 2.9 ppm (SD 0.6 ppm) at 240 min. For 5%, it ranges from 25.3 ppm (SD 3.3 ppm) at 120 min to 26.6 ppm (SD 3.9 ppm).

Replotting these data as release versus $(\text{time})^{1/2}$ graphs did not give straight lines in any case, showing that release is not diffusion based for these compounds from acrylic cements.

Total release figures for each anti-microbial and each concentration were determined after 2 weeks of release, and these are shown in Table 3. Values for total release of anti-microbial compounds were determined taking the molar masses of benzalkonium chloride and cetyl pyridinium chloride monohydrate as 340 and 358, respectively.

Table 2 Working times determined from setting exotherms for cement with and without anti-microbial compound

Cement system	Setting time/min
Cement only	9.75
Cement + 5% benzalkonium chloride	9.75
Cement + 5% cetyl pyridinium chloride	9.00

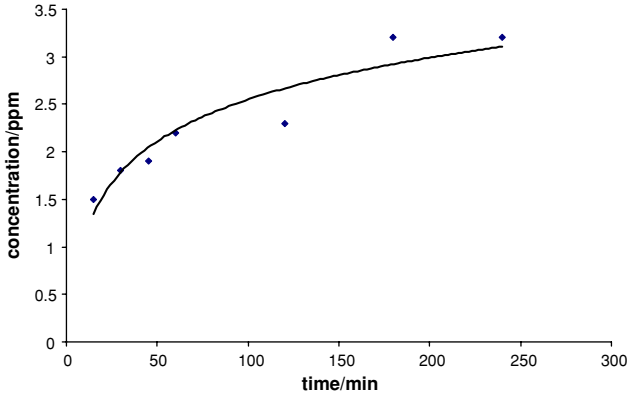

Fig. 1 Release profile for benzalkonium chloride (1%) from acrylic bone cement

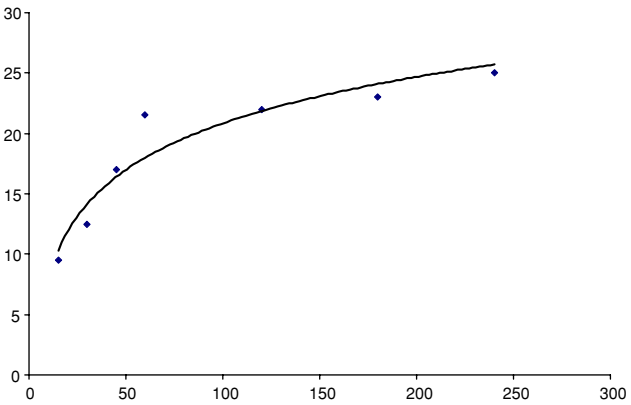

Fig. 2 Release profile for benzalkonium chloride (5%) from acrylic bone cement

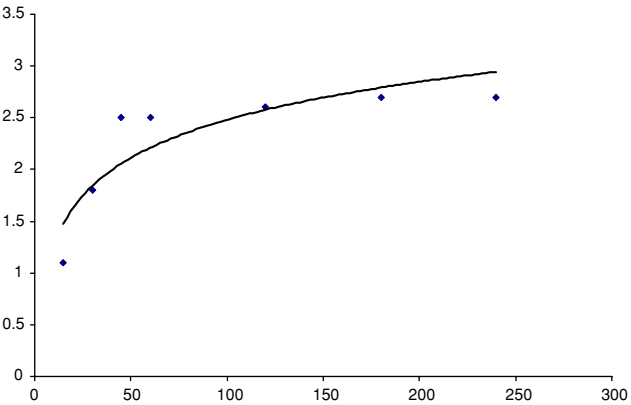

Fig. 3 Release profile for cetyl pyridinium chloride (1%) from acrylic bone cement

4 Discussion

As with gentamicin sulphate [8], inclusion of the candidate anti-microbial reagents had little or no effect on the setting of the acrylic bone cement. The setting of methyl

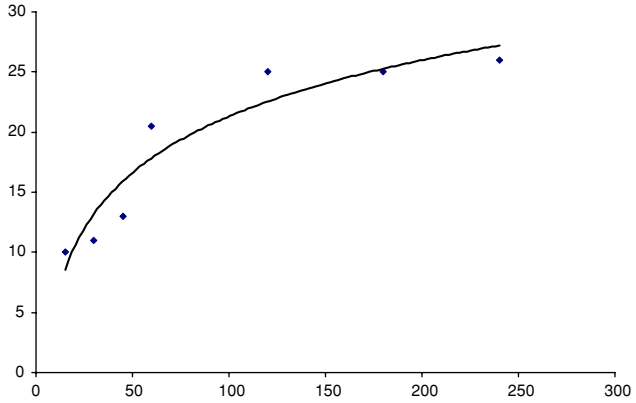

Fig. 4 Release profile for cetyl pyridinium chloride (5%) from acrylic bone cement

Table 3 Total release data for anti-microbial compounds as determined after 2 weeks

Anti-microbial compound/level	Chloride release/ppm	Equivalent substance release/ppm
Benzalkonium chloride/1%	5.3 (SD 3.5)	50.8 (SD 34.2)
/5%	121.7 (SD 16.0)	1165.6 (SD 136.3)
Cetyl pyridinium chloride/1%	9.2 (1.2)	88.1 (SD 11.5)
/5%	46.1 (8.6)	464.9 (SD 86.7)

methacrylate occurs by an addition polymerization, typically initiated by benzoyl peroxide and accelerated by the presence of an amine [3]. The antimicrobials are both quaternary ammonium salts, so might possibly contribute to the accelerating effect. In fact, only cetyl chloride showed any such effect, and then only to a small extent, shortening the setting time by a mere 45 s at the 5% level.

Both additives were shown to be released readily over periods of time of up to 2 weeks. The initial levels were studied for possible diffusion release, i.e., by plotting release against $(\text{time})^{1/2}$. This follows from the mathematical form of Fick's 2nd law of diffusion, namely

$$M_t/M_\infty = 1 - \left(8/\pi^2\right)\Sigma 1(2n+1) \times \exp\left[-\pi^2 D/4\mathrm{l}^2(2n+1)t\right]$$

For disc-shaped specimens, as used in these experiments, edge effects can be neglected, and sorption/desorption follows the form of the so-called Stefan approximation, i.e., $M_t/M_\infty = 2(Dt/\pi l^2)^{1/2}$ where M_t is the mass uptake at time t, M_∞ is the equilibrium uptake, $2l$ is the thickness of the specimen and D is the diffusion coefficient [21]. From this equation, it follows that, where Fick's law is obeyed, plotting M_t/M_∞ against $t^{1/2}$ should give a straight line. However, for our data these plots were not linear, showing that release was not a simple diffusion process in any of the four cases.

This is similar to the release of gentamicin sulphate from these cements. For this substance, release has been shown to continue for at least 5 years [22], and to comprise three contributing mechanisms [23]. They are: (i) short-term initial elution through imperfections in the cement, (ii) stress-cracking due to ingress by aqueous medium, with resulting release of antibiotic, and (iii) permeation by surrounding aqueous medium, thus creating a series of interlocking channels through which the gentamicin sulphate is able to escape. This mechanism gives rise [24] to a kinetic equation of the form:

$$M_t/M_\infty = b + k[1 - \exp(-nt)]$$

Data for release of gentamicin sulphate have been shown to follow this equation with varying degrees of fit, depending on the initial concentration in the cement, with higher loadings giving better correlation coefficients [24].

Our experiments have been carried out over much shorter timescales than those which have been used for gentamicin sulphate, and they show much greater scatter. This means that curve fitting to an exponential type equation is not appropriate with this preliminary data. However, the findings demonstrate some similarities with gentamicin sulphate. First, there is measurable release of anti-microbial from the earliest exposure of the cement to the storage medium. Second, release is more complicated than simple diffusion kinetics, and shows some evidence of a two-step release mechanism. These are consistent with steps (ii) and/or (iii) in the above mechanism for gentamicin release, and suggest that the release of benzalkonium chloride and cetyl pyridinium chloride may occur by similar processes. This might be expected on the basis of their similar chemical nature, all three compounds being salts of relatively bulky organic molecules.

Overall, more work is needed to fully characterise the release mechanisms for the experimental systems we have studied. In addition, they need to be tested for efficacy against micro-organisms of concern in orthopaedic surgery, and in patients, to confirm absence of adverse reaction to the in situ release of these compounds. However, preliminary results suggest that these compounds may be worth further investigation as alternatives to the well-established gentamicin sulphate.

5 Conclusions

It has been demonstrated that both benzalkonium chloride and cetyl pyridinium chloride can be incorporated into commercial acrylic bone cement with little or no effect on the setting time. Once set, specimens have been shown by chloride-ion selective electrode to release chloride ions,

from which it is concluded that the quaternary ammonium chloride salt itself is released.

Release was not a simple diffusion process, but followed more complicated kinetics, showing at least two possible steps. This is similar to the release processes for gentamicin sulphate from acrylic bone cements.

These antimicrobial compounds have the potential be studied further as possible replacements for gentamicin sulphate in practical bone cements for clinical application.

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