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Monoolein–Water Liquid Crystalline Gels of Gentamicin as Bioresorbable Implants for the Local Treatment of Chronic Osteomyelitis: In Vitro Characterization

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To maximize the efficacy of chronic osteomyelitis antibiotherapy while reducing antibiotic systemic toxicity, as well as time and costs of hospitalizations, it has been thought that monoolein–water gels incorporating gentamicin sulfate could be used as local, bioresorbable, and sustained-release implants. For this purpose, four formulations were examined with regard to their physicochemical and in vitro drug release characteristics. Hot stage microscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and X-ray diffraction showed cubic liquid crystalline and eutectic structures. The more suitable formulation consisting of 80–15–5% wt/wt monoolein–water–gentamicin sulfate progressively released the antibiotic for a period of 3 weeks without burst effect. Moreover, the content and the release profile of gentamicin sulfate were not significantly changed after storage at 2–6°C for a period of 10 months.

Keywords monoolein; cubic phase gels; gentamicin implants; in vitro characterization; osteomyelitis

INTRODUCTION

Osteomyelitis is an infection of the bone and its marrow, mainly caused by pyrogenic microorganisms such as *Staphylococcus aureus*. It is characterized by the progressive inflammatory destruction and new apposition of bone (Lew & Waldvogel, 1997). Its treatment is currently carried out by surgical curettage of the infected regions followed by systemic and repetitive administrations of antibiotics such as gentamicin over a long period. Unfortunately, the relatively low blood flow in the bone, and the short half-life and systemic toxicity of the antibiotic agents do not always permit sufficient levels of drug concentration to be achieved at the site of infection using this method.

To overcome this difficulty, the use of sustained-release implants, which are able to deliver locally sufficient concentrations of antibiotic into the site of infection while maintaining low systemic levels, is more suitable (Désévaux, Dubreuil, & Lenaerts, 2002; Garvin et al., 1994; Scott, Rotschafer, & Behrens, 1988; Zhang, Wyss, Pichora, & Goosen, 1994). That is why nondegradable gentamicin poly(methylmethacrylate) beads have been clinically employed for preventing or treating osteomyelitis since the 1970s (Powles, Spencer, & Lovering, 1998; Trippel, 1986). To avoid the cost, pain, and other risks associated with the second surgical intervention necessary for extracting nonbiodegradable delivery systems (Yang, Zeng, Zhou, Huang, & Xu, 2003), biodegradable implants, using polyanhydride (Li, Deng, & Stephens, 2002; Nelson, Hickmon, & Skinner, 1997) or poly(D,L-lactide) or poly(D,L-lactide-co-glycolide) (Zhang et al., 1994) carriers have been investigated. Although these polymeric delivery systems allow prolongation of drug release, they are solid, nonbioadhesive, and often show marked burst effects due to the high proportion of nonencapsulated drug. For example, the initial release of gentamicin from these delivery systems can reach up to 50% wt/wt of the total drug load (Mauduit, Brukh, & Vert, 1993; Schmidt, Wenz, Nies, & Moll, 1995).

These limitations of the polymeric implants in osteomyelitis management led us to evaluate the potential of a nonpolymeric delivery system, consisting of monoolein (glyceryl monooleate)–water liquid crystals, incorporating gentamicin

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sulfate as an antibiotic drug. The monoolein–water mixtures, which are semisolid, bioresorbable, and bioadhesive (Nielsen, Schubert, & Hansen, 1998) and which form cubic phase gels in contact with aqueous body fluids at 37°C, could not only fill in the empty spaces in the curetted bone but also sustain the antibiotic release.

The aim of this work was to investigate the suitability of monoolein–water liquid crystal gels containing gentamicin sulfate for the management of chronic osteomyelitis. For this purpose, various formulations were prepared and their physicochemical and in vitro drug release properties were evaluated with a view to select the most appropriate controlled-release system.

MATERIALS AND METHODS

Preparation of the Implants

Four formulations of gentamicin, referred to as implants 1, 2, 3, and 4 (Table 1), weighing 200 g each, were prepared as follows: gentamicin sulfate (Id Indis, Aartselar, Belgium) and monoolein (Danisco Pharma, Brabrand, Denmark) were separately dissolved in 50 mL of deionized water and 50 mL of ethyl alcohol/ethyl ether 97.1/2.9 (Stella, Liège, Belgium), respectively. The solutions were placed together in a 500 mL glass flask that was then mounted on a Büchi rotary evaporator R-205 (Switzerland). The solvent mixture was then evaporated at 50°C, under speeds varying from 150 to 235 revolutions per minute (rpm) and pressures ranging from –0.70 to –0.92 bar, until the desired quantity of final product (200 g) was obtained. The products were immediately conditioned in brown glass bottles and kept in a refrigerator (2–8°C). Blank implants 1 and 2, without gentamicin sulfate (Table 1), were also prepared in the same way.

PHYSICOCHEMICAL CHARACTERIZATION

Assay of Gentamicin Sulfate

Based on the method of the *European Pharmacopoeia* (2002), gentamicin contents in the implants were determined in triplicate as follows: about 500 mg of sample was accurately weighed, introduced into a 100 mL volumetric flask, and manually dissolved in 20 mL methanol. After completion to the required volume with distilled water and stirring at 500 rpm for 2 h with a magnetic stirrer, the solution was decanted and the supernatant was filtered twice through membrane filters (Durapore, pore size 0.45 μm).

A volume of 10 mL of this filtered solution was placed into 25 mL volumetric flasks, and 800 μL of *O*-phthaldialdehyde reagent was then added. This reagent, the pH of which was adjusted to 10.4 with 45% wt/vol potassium hydroxide aqueous solution, was obtained by adding 400 mg of 1,2-phthalic dicarboxaldehyde (Acros Organics, Belgium), 2 mL of methanol, and 800 μL of mercaptoacetic acid 98% (Acros Organics) to 38 mL of sodium borate buffer.

The volumes were then completed with methanol to 25 mL, the flasks were manually stirred, and were then placed in a thermostated bath set at 60°C for 15 min. After cooling at room temperature for 25 min, the absorbencies of the solutions were measured at 325 nm, using a UV/VIS spectrophotometer (PU 8620 UV/VIS/NIR, Philips, UK). Solutions prepared in the same way with the corresponding blank implants were used as blanks. A calibration curve (absorbencies vs. concentrations), established using 10, 25, 50, 75, and 100 μg/mL gentamicin sulfate solutions, was finally used for calculating the drug content (%, wt/wt) of the implants.

Determination of Free Fatty Acid Content

The free fatty acids in the implants were extracted with hexane, esterified with 0.5 N chlorhydric acid in methanol (Supelco, USA), and then identified and assayed in triplicate using gas chromatography (Carlo Erba Instruments AUTO/HRGC/MS, Italy). Esterified fatty acids (methyl stearate, methyl palmitate, methyl linoleate, and methyl oleate) and methyl *cis*-10-heptadecenoate (Sigma-Aldrich, Germany) were used as external and internal standards, respectively. The GC column (Chrompack N.V., Belgium) used was WCOT 25 m × 0.32 ID coating FFAP CB (with 0.32-μm film thickness), the injection volume was 1 μL, the carrier gas was helium, and pressure was maintained at 0.59 bar. The temperature program of the oven was as follows: hold at 80°C for 1 min, then heat up to 230°C at a rate of 8°C/min

TABLE 1
Theoretical Qualitative and Quantitative (% wt/wt) Compositions of Gentamicin and Blank Implants

Formulations	Implant 1 (%)	Implant 2 (%)	Implant 3 (%)	Implant 4 (%)	Blank 1 (%)	Blank 2 (%)
Gentamicin sulfate	5.0	5.0	10.0	10.0	0.0	0.0
Deionized water	12.5	15.0	12.5	15.0	12.5	15.0
Glycerin monooleate	82.5	80.0	77.5	75.0	87.5	85.0

and, finally, hold at 230°C for 6 min. The temperatures of the flame ionization detector (FID) and injector were both at 250°C.

Rheological Studies

These were performed in triplicate with a digital Brookfield Model DV-V viscosimeter (Brookfield Engineering Laboratory, USA) mounted with a no. 3 spindle. About 8 mL of each sample, previously liquefied at 40°C, was introduced into the external cylinder of the viscosimeter and the temperature was maintained at 37°C, using a Haake K15 thermostat (Germany). At each speed of the spindle (varying from 1 to 12 rpm and then from 12 to 1 rpm), the viscosity (mPa.s) was recorded. The sample rheograms were then plotted, after converting the viscosity and the spindle speed values into shear stress (mPa) and shear rate (s^{-1}), respectively.

Hot Stage Microscopy

An Olympus BX 60 optical microscope (Japan) linked to a Linkam Hot Stage instrument (UK) and a JVC color video camera (Japan) was used to examine implant and monoolein samples at 37°C. The samples were successively observed, first, under nonpolarized light and then, under polarized light.

Thermal Analysis

The thermal properties were investigated using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

DSC was carried out using a DSC 7 equipped with a Controller TAC-7/DX, Intercooler-2-cooling, and Pyris software (Perkin Elmer Instruments, USA). The instrument, calibrated with indium and cyclohexane, was continuously purged with nitrogen at 20 mL/min. The 5–10 mg samples were placed in perforated aluminum sealed 50 µL pans and were thermally scanned, according to the following cycle of temperatures: equilibration at –10°C for 5 min, heating from –10 to 45°C at a rate of 2°C/min, cooling from 45 to –10°C at 40°C/min, and heating from –10 to 45°C at 2°C/min.

For TGA, an analytical balance TGA MTB 10-8 (Setaram, France) equipped with two ovens was used. Samples of about 10 mg were loaded on quartz pans suspended from the analytical balance and continuously purged with dry air to avoid atmospheric moisture uptake. They were then heated from 20 to 350°C at 2°C/min and the weight loss was recorded, using the Dasilab software.

Determination of Moisture Content

According to the Karl Fischer titration described in United States Pharmacopeia ([USP] 2000), the moisture content of implants was determined in triplicate on samples of about 5 mg, using a coulometer 756 KF (Metrohm, Switzerland). Hydranal coulomat CG and AG (Sigma-Aldrich France) were used as reagent and solvent, respectively.

X-Ray Diffraction

X-ray diffraction (XRD) patterns for each sample (implants, monoolein, or gentamicin sulfate) were recorded at 20°C, using a Siemens Diffractometer D5000 (Germany) with Cu Kα radiation of wavelength of 1.5418 Å. Standard runs using a 40 kV voltage, a 40 mA current, and a scanning rate of 0.02°C/min over a 2θ (scattering angle) range of 5–50° were used.

In Vitro Dissolution Testing

In vitro dissolution studies were conducted in triplicate at 37.0 ± 0.5°C, using the USP24 (2000) no. 2 (paddle) apparatus. The dissolution media consisted of 500 mL of pH 7.0 acetate–phosphate buffer containing Polysorbate 20 (Federa, Belgium). The paddle was positioned to 3.5 cm from the bottom of the dissolution vessel and its speed was set to 60 rpm. Topical dissolution cells (Distek, Netherlands) were filled with weighed quantities of implants (about 1.6 g) and then put into the vessels.

After 3, 6, 24, 48, 96, 168, 240, 336, and 480 h of the test, 15 mL of dissolution medium was withdrawn from each vessel and immediately replaced with fresh dissolution medium maintained at 37°C. After filtering through membrane filters (Durapore, pore size 0.45 µm) and appropriate dilution of the withdrawal solutions, 10 mL was used for assaying gentamicin sulfate, as described in the section on Assay of Gentamicin Sulfate. The mean cumulative percentages of drug released in the dissolution media were plotted as a function of time and the standard deviations were represented on graphs by error bars.

Elsewhere, to verify the stability of gentamicin sulfate during the dissolution, a standard vessel containing about 80 mg of gentamicin sulfate was also evaluated in parallel. Other standard vessels containing about 80 mg of gentamicin sulfate and 1.6 g of blank implant 1 or 2 were also evaluated to understand the decrease in drug concentration in the media during the dissolution tests on gentamicin implants (see Results and Discussion).

Finally, with a view to confirming the hypothesis that this decrease could be due to the complexation of gentamicin by the free fatty acids of monoolein, 10 mL of dissolution media containing gentamicin implants was withdrawn at the same predetermined time intervals and was acidified with 125 µL of sulfuric acid. Free fatty acids were then threefold extracted with chloroform and the aqueous phase, alkalinized with 45% wt/wt hydroxide potassium aqueous solution, was used for quantifying the total concentration of gentamicin sulfate contained in the dissolution media.

Study of Stability

To evaluate the stability of the formulations, implant samples were stored for 10 months in a refrigerated cabinet (2–8°C). Then, drug assay, DSC, XRD analysis, and in vitro dissolution testing were performed, as described above.

RESULTS AND DISCUSSION

The method adopted for manufacturing the implants permits us to obtain, in less than 3 h, homogeneous, clear, viscous gels. In contact with water, they become more solid and adhesive to the fingers because the increased hydratation of monoolein leads to better structured cubic phases, known to be solid-like, skin-, and mucoadhesive gels (Geraghty, Attwood, Collett, Sharma, & Dandiker, 1997; Kim et al., 2004; Nielsen, Schubert, & Hansen, 1998). The potential of monoolein cubic phase gel as a chemical stability enhancer of antibiotic drugs such as cefazolin and cefuroxime has been also reported (Sadhale & Shah, 1998). These properties, which could be very promising for local drug application in the curetted bone cavities, are not observed with the biodegradable polymeric systems already investigated by some authors (Li et al., 2002; Mauduit et al., 1993; Nelson et al., 1997; Schmidt et al., 1995; Zhang et al., 1994). That is why we have characterized in vitro, for the first time to our knowledge, the potential of gentamicin sulfate monoolein–water gels as implants for the local treatment of chronic osteomyelitis.

Physico-Chemical Characterization

Gentamicin sulfate contents found in the various formulations were identical to the theoretical values. The various implants also contained oleic acid (2.5% wt/wt) and small quantities of stearic, palmitic, and linoleic acids (Table 2). Their contents are similar to those of the monoolein used, according to the specifications of the supplier. As a result, no degradation of the raw materials occurred during the manufacturing process.

The rheological studies showed that all the formulations display a pseudoplastic behavior at 37°C. The shear stress measured increases with the increasing of the shear rate (Figure 1) and the "up" and "down" rheograms were superposable (not shown). Moreover, except for implant 3, the log–log rheograms' representation of all the blank and gentamicin implants showed linear curves (regression coefficient $r^2 \geq 0.87$) and were characterized by N values

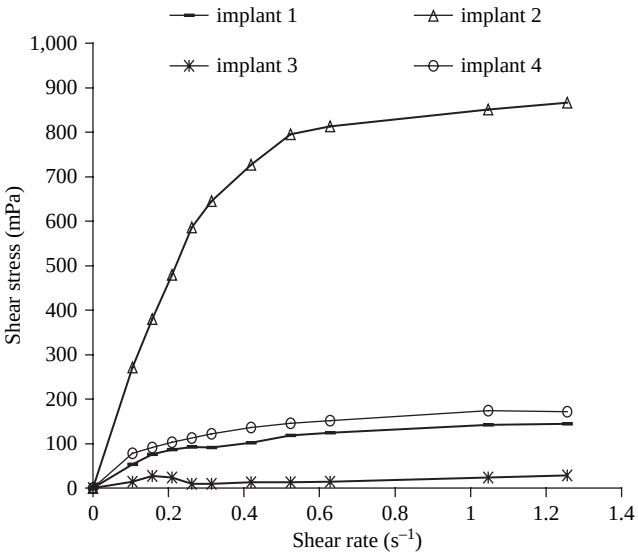

FIGURE 1. Rheograms of the various gentamicin implants and of the blank implants 1 and 2.

TABLE 2
Values for Some Physicochemical Characteristics of the Various Gentamicin Implants

Formulations	Implant 1	Implant 2	Implant 3	Implant 4	Implant 2, After 10 Months
Gentamicin sulfate content (% wt/wt, mean ± *SD*, *n* = 5)					
	99.7 ± 0.3	103.8 ± 0.4	101.8 ± 0.8	100.0 ± 0.4	100.6 ± 0.9
Water content (% wt/wt, mean ± *SD*, *n* = 3)					
	10.3 ± 0.4	13.0 ± 0.1	10.97 ± 0.06	12.7 ± 0.2	
Free fatty acids content (% wt/wt, mean ± *SD*, *n* = 3)					
Stearic acid	0.27 ± 0.14	0.56 ± 0.01	0.40 ± 0.09	0.32 ± 0.11	0.49 ± 0.04
Palmitic acid	0.13 ± 0.01	0.18 ± 0.00	0.16 ± 0.01	0.11 ± 0.03	0.18 ± 0.02
Linoleic acid	0.33 ± 0.01	0.52 ± 0.00	0.46 ± 0.01	0.32 ± 0.08	0.46 ± 0.36
Oleic acid	2.56 ± 0.07	3.89 ± 0.07	3.51 ± 0.10	2.42 ± 0.62	4.80 ± 0.29
Viscosity (mPa.s) at 1 s^{-1} Shear rate (m ± *SD*, *n* = 3)					
	135 ± 15	812 ± 28	22.49 ± 8	166 ± 18	

(shear thinning) of less than 1, according to the "power law." The higher the water content, the higher the viscosity value (implants 2 and 4) because monoolein forms a more structured liquid crystalline gel when it is more hydrated (Kim et al., 2004). Moreover, as has been observed by Caboi et al. (2001) with other biological relevance compounds, this monoolein–water crystalline gel structure is also modified by gentamicin sulfate as the viscosity of the implants significantly varied with the content of gentamicin sulfate (Table 2); the highest and the lowest viscosity values were observed with implant 2 (higher water content and lower drug content) and implant 3 (lower water content and higher drug content), respectively.

The hot stage microscopy of monoolein and implants showed a homogenous aspect under the nonpolarized light. On the contrary, a black background was observed under the polarized light, showing the isotropic nature of the implants and the complete solubilization of gentamicin sulfate incorporated. The isotropic nature of monoolein–water mixtures, which characterizes the liquid crystalline cubic phases, has also been described by other authors when the water content is around 20–40% wt/wt (Chang & Bodmeier, 1997a; Geraghty, Attwood, Collett, & Dandiker, 1996; Nielsen et al., 1998; Sallam, Khalil, Ibrahim, & Freij, 2002,). The lower water contents necessary for obtaining the cubic phase with our formulations could be due to the manufacturing method that allows a better hydratation of monoolein, or to the presence of gentamicin sulfate, as has been observed in mixtures consisting of monoolein–water (10–13 to 35% wt/wt) and sodium decanoate (2–5% wt/wt) or amantadine hydrochloride (4–5% wt/wt) (Caboi et al., 2001).

The examination of DSC curves (Figure 2) obtained during the first heating shows one endothermic peak at about 33°C for monoolein. In contrast, all the gentamicin implants presented two endothermic peaks, like any eutectic system (Passerini, Albertini, Gonzalez-Rodriguez, Cavallari, & Rodriguez, 2002). The first peak, which appeared at lower temperatures (13–20°C), could correspond to the melting of the monoolein and gentamicin sulfate aqueous solution mixtures. According to the findings of Kim et al. (2004), the second peak, which is close to that of monoolein, corresponds to the melting of nonhydrated monoolein. The areas of this peak are lower than those of the first ones, indicating that the proportions of nonhydrated monoolein were smaller. It has been also noticed that DSC curves of gentamicin implants recorded during the first and the second heating were practically similar, attesting to the good stability of these eutectic systems, over temperatures ranging from –10 to 45°C. In contrast, the first and the second heating curves of monoolein were different, indicating its metastable nature.

The TGA shows three stages of weight loss for the all gentamicin implants. As examples, TGA thermograms of implants 1 and 2 are presented in Figure 3. The first, observed at between 20 and 110°C, probably corresponds to the

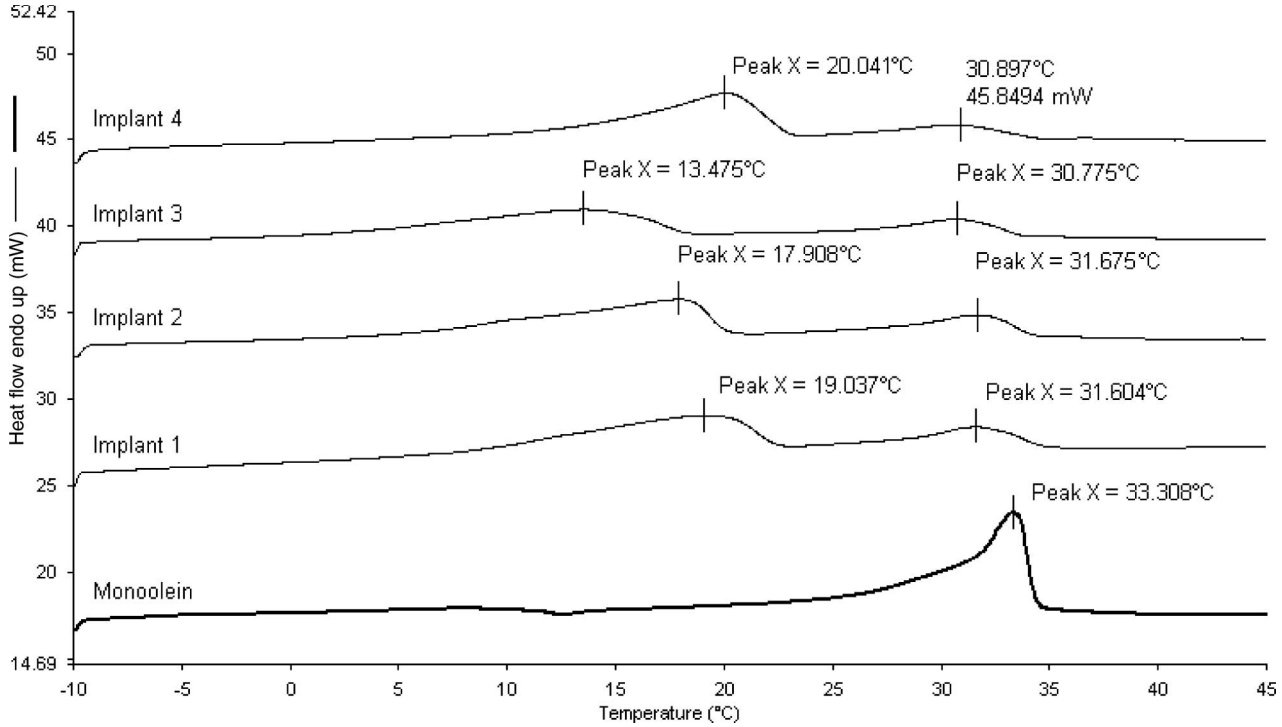

FIGURE 2. Differential scanning calorimetry (DSC) heating curves for monoolein and gentamicin implants recorded during the first heating.

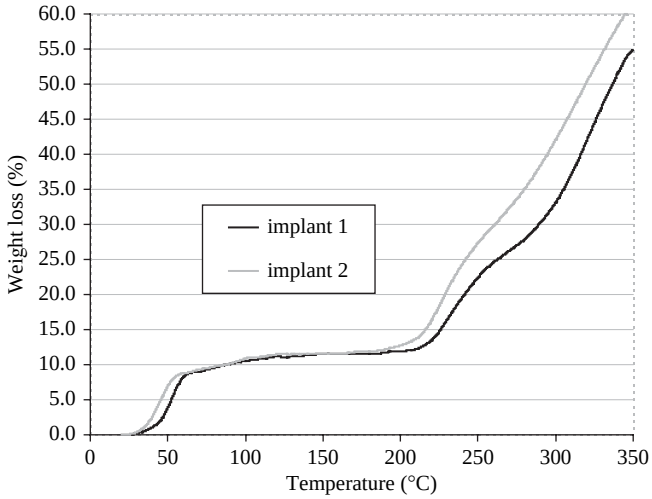

FIGURE 3. Thermograms obtained from thermogravimetric analysis (TGA) of gentamicin implants 1 and 2 of whose theoretical water contents were 12.5% wt/wt and 15.0% wt/wt, respectively.

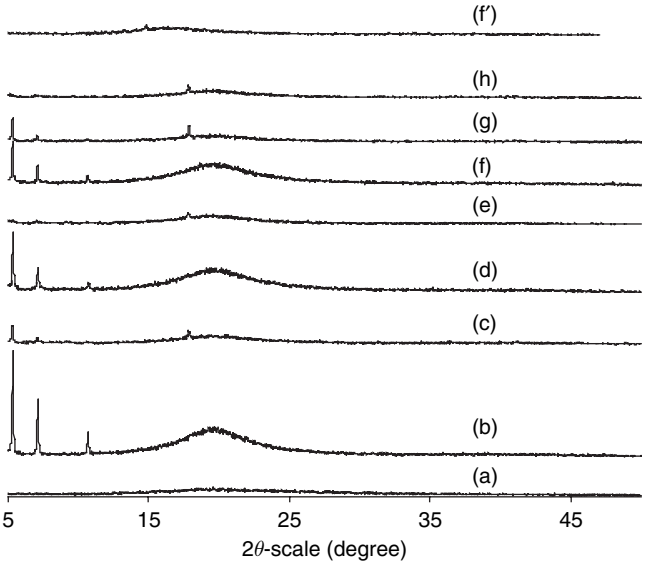

FIGURE 4. X-ray diffraction spectra of gentamicin sulfate powder (a), monoolein (b), blank implant 1 (c), blank implant 2 (d), gentamicin implants 1 (e), 2 (f), 3 (g), and 4 (h). A spectrum of gentamicin implant 2, recorded after 10 months of storage (2–8°C), is also given (f′).

evaporation of free water. The second stage (from 110 to 210°C) could correspond to the evaporation of water from hydrated monoolein (the amphiphilic molecules of monoolein can indeed bind to the hydroxyl groups of water [Engström, 1990]), confirming the eutectic nature of the implants. The weight loss abruptly increased from about 210°C (third stage), probably due to the degradation of gentamicin sulfate and monoolein. The cumulative weight losses of the various implants due to evaporation, calculated at the inflexion points (210–220°C) of TGA thermograms, are much closer to their theoretical contents of water. Consequently, the manufacturing process permitted complete elimination of the ethyl alcohol/ethyl ether (97.1/2.9) mixture used for dissolving monoolein.

These results also show that TGA or the oven-drying method (at about 105–110°C) could be used to determine the total water contents of the implants (Vyas, Pradhan, Pavaskar, & Lachke, 2004). However, the values obtained from TGA were higher than those recorded from the more selective Karl Fischer titration (Table 2): the latter probably only quantified the free water. Assuming that, the amount of water bound to monoolein in the implants (1.8–2.3% wt/wt) can be calculated as the difference between the results obtained from TGA and those from Karl Fischer titrations.

The XRD patterns (Figure 4) did not show any peak for gentamicin sulfate powder, confirming its complete solubilization in the implants and thus the absence of drug crystals (Budavari, O'Neil, Smith, Heckelman, & Kinneary, 1996). On the contrary, monoolein and gentamicin implants showed prominent peaks in the range of 5–20°C, confirming their crystalline state (Shah, Sadhale, & Chilukuri, 2001). Compared to the other formulations, implant 2, which has a higher water content (15% wt/wt) and lower gentamicin sulfate content (5% wt/wt), presented the highest diffraction peak and, therefore, the highest degree of crystallinity.

In Vitro Dissolution Testing

The ability of the crystalline cubic phase gel structure to sustain the release of water-soluble drugs such as gentamicin sulfate is well known (Rowe, Sheskey, & Weller, 2003; Wyatt & Dorschel, 1992). As indeed expected, implantable dosage forms based on such structures swelled in the dissolution media, becoming more viscous and progressively releasing gentamicin sulfate (Figure 5).

However, during the dissolution tests, a decrease in gentamicin sulfate concentrations was observed after the 14th day for implants 1 and 3 and after the 20th day for implants 2 and 4. It has been assumed that these decreases are due to either the degradation of gentamicin or the interactions between the amino groups of gentamicin and the carboxylic groups of the free fatty acids found in the various formulations (Table 2). Such interactions, which can result in the formation of insoluble complexes, have been also observed at pH 7.4 between oleic acid and propranol hydrochloride, a cationic drug like gentamicin sulfate (Chang & Bodmeier, 1997b). To verify these hypotheses, about 80 mg of gentamicin sulfate was put into vessels containing dissolution media in the absence and in the presence of blank implant samples, respectively.

As can be seen in Figure 5, gentamicin sulfate was not degraded during the test as its concentration in the dissolution

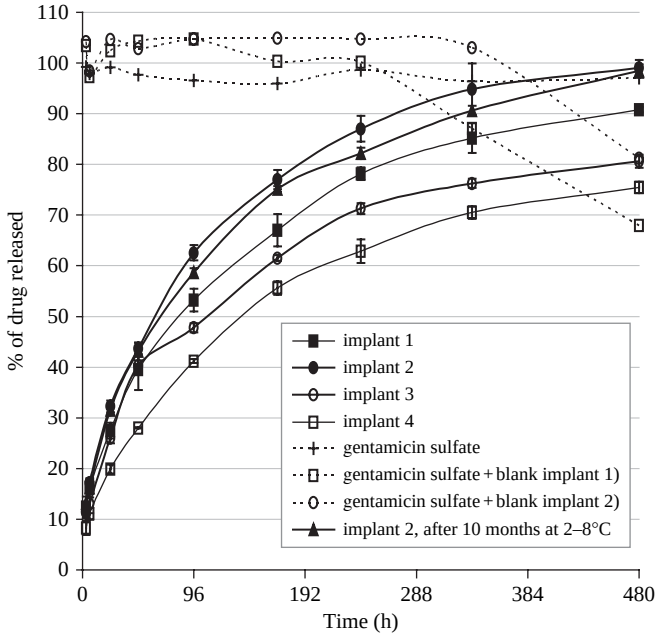

FIGURE 5. Dissolution profiles for gentamicin implants 1, 2, 3, and 4. The percentages of gentamicin sulfate in the standard vessels (dissolution media containing gentamicin sulfate alone, gentamicin sulfate + blank implant 1, and gentamicin sulfate + blank implant 2) as a function of time are also shown.

media without implant did not decrease. In contrast, gentamicin concentrations decreased in the dissolution media containing blank implants 1 (standard for implants 1 and 3) and 2 (standard for implants 2 and 4) after days 14 and 20, respectively, showing the occurrence of interactions between gentamicin sulfate and monoolein. After acidification and extraction of free fatty acids with chloroform, the total drug concentrations found in the dissolution media did not decrease with time, confirming the complexation of gentamicin by the free fatty acids, rather than the degradation of drug. Taking this observation into account, the drug concentrations in the dissolution media containing gentamicin implants were corrected by those found in the media containing the corresponding standards. The percentages of dissolution of the various implants presented in Figure 5 therefore correspond to the corrected values, considering the complexation phenomenon.

Gentamicin release from the implants was sustained for a 3-week period and depended on the crystallinity of the system and the drug loading. Indeed, at the same theoretical water content (12.5 or 15% wt/wt), increased drug content corresponded to lower drug release, probably due to an alteration of the dissolution of gentamicin sulfate in the matrices (implants). On the contrary, implant 2, which had the highest crystallinity, allowed a more rapid and complete release of gentamicin, compared with the other implants. It released 50 and 75% of gentamicin sulfate within 60 h and 1 week, respectively. This dissolution profile could be advantageous for the management of osteomyelitis as the efficacy of antibiotics requires initially high drug concentrations at the site of infection (Changez, Burugapalli, Koul, & Choudhary, 2003). Moreover, the rest of the antibiotic, which is slowly released during the 2 weeks following application, could serve to maintain the local drug concentration at a high enough level for treatment of the chronic infection. Compared with gentamicin sulfate poly(L-lactic acid) implants (Mauduit et al., 1993; Schmidt et al., 1995) and calcium phosphate–gelatin impregnated with gentamicin (Yaylaoglu, Korkusuz, Ors, Korkusuz, & Hasirci, 1999), which present marked burst effects, implant 2 also seems to be more promising. Besides, only about 60% wt/wt of gentamicin sulfate are released from the poly(L-lactic acid) implants for a period of 4 months (Meyer et al., 1998).

Finally, the drug release profile from implant 2 is typical of a matrix-type delivery system, which means that it follows a Fickian diffusion (Stephens et al., 2000). The plot of the percentages of gentamicin sulfate released as a function of the square root of time was linear, with a regression coefficient (r^2) of .993 (not shown). A similar release mechanism of bupivacaine from monoolein cubic phase gels has been also described by Shah et al. (2001).

Stability

The stability of implant 2, which had the more promising release profile, was examined after storing it in a refrigerating cabinet (2–8°C) for a period of 10 months. The study was not carried out at room temperature because phase separations were observed after less than 1 week of storage for all implants.

The drug contents (Table 2) and the heating curves from DSC analysis were similar to those for the freshly prepared implants. The X-ray diffraction spectra showed that the intensities and the positions of peaks were lower, indicating some modifications in the implant physical structure during storage. Moreover, the oleic acid concentration slightly increased from 2.5 to 4.8% wt/wt (Table 2), probably by hydrolysis of monoolein to oleic acid and glycerol. Such a degradation of monoolein, which can modify the cubic phase structure and the interactions between gentamicin and the formulations, has already been observed by ^{13}C NMR (Stephens et al., 2000). However, these modifications did not significantly affect the profile of the in vitro release of gentamicin sulfate during the 10 months of storage (see Figure 5). A good stability of propranolol or pyrimethamine release characteristics from monoolein–water systems, stored at 4°C in dark conditions for up to 6 months, has been also reported by Burrows, Collett, and Attwood (1994).

It has also been brought to one's attention that incorporation of buffer substances into the formulations could be envisaged to decrease the monoolein hydrolysis, which is probably favored by the acidic environment resulting from the presence of gentamicin sulfate.

CONCLUSION

The in vitro characterization of the monoolein–water liquid crystal gel formulations containing gentamicin sulfate shows their real potential as bioresorbable implants for the local management of chronic osteomyelitis. In this respect, the gentamicin–monoolein–water (5–80–15% wt/wt) cubic phase gel referred to as implant 2 appears to be the most suitable delivery system. Indeed, it is stable for at least 10 months at 2–8°C and releases the antibiotic over 3 weeks. Moreover, its homogeneous, clear, and semisolid characteristics will facilitate the drug application in the whole bone cavity after surgical curettage and its swelling, hardening, and adhesive properties in contact with water are expected to allow it to fill in the bone cavity, thus avoiding further formation of haematoma. The in vitro biocompatibility of implant 2 has been already demonstrated and its clinical evaluation is in progress. The promising results obtained from these studies will be presented in a further paper.

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