ORIGINAL ARTICLE

Ciprofloxacin monoolein water gels as implants for the treatment of chronic osteomyelitis: *In vitro* characterization

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J. Adv. Pharm. Technol. Res.

ABSTRACT

This work investigated the possibility of using the biodegradable gentamicin-monoolein-water gels as models, in order to obtain a similar sustained release of ciprofloxacin hydrochloride. Four gels containing antibiotics were prepared and were examined with regard to their physicochemical properties and *in vitro* drug release characteristics. Ciprofloxacin, unlike gentamicin, which was dissolved in the matrix, was in dispersed form. However, despite its insolubility, microscopic observation, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and X-ray diffraction showed that the developed gel was in the cubic liquid crystalline structure and have maintained their ability to progressively release ciprofloxacin. ciprofloxacin-monoolein-water (5:80:15% w/w), which released *in vitro* approximately 85% of ciprofloxacin after 16 days could possibly be considered as an alternative to a gentamicin-monoolein-water gel for the treatment of chronic osteomyelitis.

Key words: Ciprofloxacin, *in vitro* characterization, liquid crystalline, monoolein, sustained release

INTRODUCTION

Chronic osteomyelitis is a chronic infection of bone and its marrow, mainly caused by pyrogenic microorganisms such as *staphylococci, streptococci, pneumococci, enterobacteria* and *Pseudomonas aeruginosa*.[1] Due to the deep localization of the infection and the presence of vascular intra-osseous thrombosis, high doses of antibiotic are needed to reach effective concentration at the infection site. However, the commonly used antibiotics are characterized by important systemic side effects such as ototoxicity.[2]

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Access this article online	
Quick Response Code:	Website: www.japtr.org
	DOI: 10.4103/2231-4040.143029

In the past few years, many local delivery systems have been developed in order to avoid the risk of systemic toxicity. The Lautenbach method,[3] biodegradable implants of poly (L- lactide), poly (DL-lactide/glycolide), polyanhydrides, and collagen,[4,5] or non-biodegradable polymethylmethacrylate (PMMA) bone cement impregnated with antibiotics,[5-7] are extensively described in the literature. However, despite these significant advances, only a few implantable systems, clinically used for the treatment of chronic osteomyelitis, exist on the market. Moreover, none of them are characterized by bioadhesive properties.

Recently, Ouédraogo and co-workers developed a biodegradable gentamicin-glycerol monooleate (GMO)-based gel c.[8] The developed gel was bioadhesive and able to absorb water and maintained *in vitro* a sustained release of the drug for more than 20 days.[8] These suitable physico-chemical properties allowed to us envisage its use in the treatment of chronic osteomyelitis. Moreover, *in vivo* investigation of the biocompatibility performed in mice showed that the gel was biocompatible and well-tolerated.[9] It also presented a safety profile and an *in vivo* efficacy in a phase II clinical trial performed on 19 patients.[10]

Nevertheless, due to the plethora of microbial pathogens that are involved in osteomyelitis, the incorporation of

different antibiotics in the gel was investigated in order to overcome possible resistance which could appear during the treatment.

In this work, ciprofloxacin hydrochloride was chosen as a second model drug. This drug is a fluoroquinolone and is widely used in the treatment of bacterial bone infection.[11-13] Indeed, it has an action over most of the germ spectrum encountered in chronic osteomyelitis including aminoglycoside-resistant germs, low toxicity and suitable bone diffusion.[1,14,15]

This study aimed to investigate the possibility of using the biodegradable gentamicin-monoolein-water gels as models, in order to obtain a similar sustained release of ciprofloxacin hydrochloride.

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride and gentamicin sulfate were purchased from Dr. Reddy's Laboratories Ltd. (India) and Yantai Justaware Pharmaceutical (China), respectively. GMO (Rylo MG PHARMA 20) was purchased from Danisco (Denmark). Ethanol 96% denaturized with isopropanol was purchased from Fagron (Belgium) and deionized water was freshly prepared in the laboratory.

Formulation of the gels

Four gels containing antibiotics were prepared and their physicochemical properties were compared to a drug-free reference (REF) [Table 1].

All gels were prepared at 50°C, at low pressure as previously described by Ouédraogo.[8]

Microscopic observation, evaluation of residual water and the free fatty acids content, X-Ray Diffraction, and thermal analysis were determined as previously described by Ouédraogo.[8]

Assay of the ciprofloxacin and gentamicin contents of gels

Both gentamicin and ciprofloxacin were extracted from the gel using a liquid-liquid extraction method. Approximately 1500.0 mg of Gels 1 and 3 and 750.0 mg of Gels 2 and 4, containing 75.0 mg of active ingredient, were dissolved in 40.0 ml of chloroform (Sigma-Aldrich, Germany). Both gentamicin and ciprofloxacin were extracted three times with 5.0 ml of distilled water in order to obtain 5 mg/ml solution of the drug before being filtered through a 0.45 µm cellulosic membrane (VWR, Belgium). Then the assay of the active ingredients was performed on the extracted solutions.

Table 1: Theoretical qualitative and quantitative (% w/w) composition of the developed gels and of the reference

Formulations	Gel 1	Gel 2	Gel 3	Gel 4	REF
Ciprofloxacin hydrochloride	5.0	10.0	0.0	0.0	0.0
Gentamicin sulfate	0.0	0.0	5.0	10.0	0.0
Deionized water	15.0	15.0	15.0	15.0	15.0
GMO	80.0	75.0	80.0	75.0	85.0

GMO: Glycerol monooleate, REF: Reference

Absorbances of ciprofloxacin and gentamicin were measured using an HP 8453 UV-Visible spectrophometer (Hewlett Packard, Germany) with 1 cm quartz cells, at 276 nm and 325 nm respectively.

However, as gentamicin does not absorb in the UV domain, a derivatization step was previously performed at pH 10.4.[8,16]

Rheological studies

Rheological studies of the gels were performed in triplicate at 37.0 ± 0.1°C using a Brookfield viscometer LVDV-II + (Brookfield Engineering Laboratories, Inc., USA) mounted with a small sample adapter. An SC-25 spindle was used to evaluate the rheological properties of the gels. The viscosity (mPa.s) was recorded at rotational spindle speeds from 2-200 rpm after stabilization of values. The rheological properties of the developed gels were evaluated by drawing the rheograms representing the evolution of the viscosity as a function of the shear rates (s-1).

In vitro dissolution test

In vitro dissolution studies were conducted in triplicate at 37.0 ± 0.5°C, using USP24 (2 000) no. 2 (paddle) apparatus (60 rpm). The dissolution media consisted of pH 7.0 buffer phosphate (50 mM) supplemented with 0.05% w/v polysorbate 20 (Sigma Aldrich, USA) and 0.02% (w/v) of sodium azide (Sigma Aldrich, USA). Topical dissolution cells (Distek, Netherlands) were filled with about 1.5 weighed gels containing about 75.0 or 150.0 mg of active ingredients before being placed into the dissolution vessels. The dissolution baths were filled with 500.0 ml and 1 000.0 ml of buffered medium for gentamicin gel and ciprofloxacin gel, respectively. In order to preserve sink conditions, 15 ml and 150 ml of dissolution medium were withdrawn for gentamicin sulfate and ciprofloxacin hydrochloride respectively at 3, 6, 24, 48, 96, 168, 240, 336, 384, and 480 hours. Fresh dissolution medium maintained at 37 °C was added in the dissolution bath immediately after sampling. The samples were filtered through a 0.45 µm cellulosic membrane. The released amounts of ciprofloxacin and gentamicin were then measured at 276 and 325 nm, respectively. At the end of the dissolution test, the residual amounts of ciprofloxacin and gentamicin were also determined.

RESULTS

The developed gels were obtained within 3 hours. The gels were viscous [Table 2], homogeneous,

transparents (gentamicin and the reference placebo gel) and heterogeneous opaque with visible particles of ciprofloxacin.

The characterization of the structure of gel and the possible interactions between the components contained within the gels as a function of the temperature was described in Figures 1-5.

The release of ciprofloxacin and gentamicin was sustained with approximately 100% of released of gentamicin (Gel 3 and 4) and only about 80% of ciprofloxacin (Gel 1 and 2) after 480 hours [Figure 6].

Table 2 shows the results of some properties determined from the different gels.

DISCUSSION

Influence of the manufacturing process

Assay of ciprofloxacin and gentamicin content in the gels

The content of ciprofloxacin hydrochloride and gentamicin sulfate were similar to the theoretical in both Gels [Table 2]. Therefore, it seemed that the method of preparation developed allowed the incorporation of at least two different antibiotic molecules without degradation and loss of material.

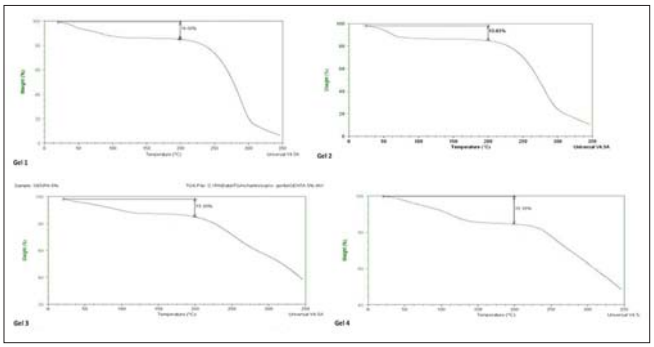

Figure 1: Thermogravimetric analysis thermograms of the ciprofloxacin (1 and 2), gentamicin (3 and 4) monoolein water gels

Water content

The residual amount of water in the produced gels was below about 13% w/w when evaluated by the Karl-Fisher method [Table 2]. This experimental result was lower than the theoretical value, which was set at 15% (w/w). Part of the water was probably bound to the GMO and could not be assayed by the Karl-Fisher method. TGA seems to confirm this hypothesis because the loss of weight from the developed gels as a function of the temperature was, in the first stage of degradation at 200°C, about 14% (w/w) [Figure 1]. This was probably due to the evaporation of both free water and water bound to the GMO. Indeed, 200°C was lower than the degradation temperature of the GMO (216°C), ciprofloxacin hydrochloride (318°C) and gentamicin sulfate (228°C).[17] The loss of weight

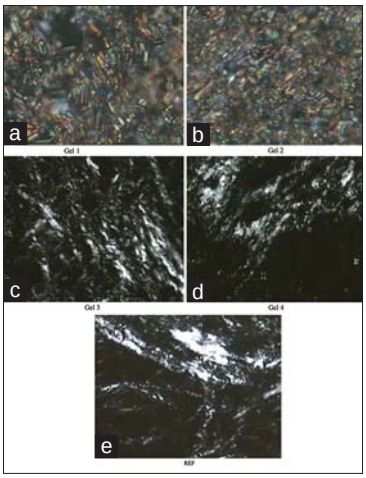

Figure 2: Pictures from the optical microscope at ×500 magnification with polarized light of the ciprofloxacin (1 and 2), gentamicin (3 and 4) and REF monoolein water gels

Table 2: Values of some physicochemical characteristics of the developed gels and of the GMO

Formulations	Gel 1	Gel 2	Gel 3	Gel 4	GMO	REF
Time of preparation (min)	140	150	130	130		
Ciprofloxacin hydrochloride content (% w/w, m±SD, *n*=3)	98.3±0.3	98.8±1				
Gentamicin sulfate content (% w/w, m±SD, *n*=3)			101.1±0,3	100.2±1		
Water content (% w/w, mean±SD, *n*=3)	12.4±0.1	11.6±0.3	10.5±0.7	13.3±0.4		
Drug content determined in the Residual gels at the end of dissolution test (% w/w, m±SD, *n*=3)	11.0±0.7	24.2±0.3	5.4±0.6	2.8±0.4		
Free fatty acid content (% w/w, mean±SD, *n*=3)						
Palmitic acid	0.34±0.00	0.29±0.00	0.33±0.00	0.27±0.00	0.44±0.00	
Stéaric acid	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.03±0.00	
Oléic acid	5.54±0.01	5.29±0.01	5.64±0.00	5.03±0.05	7.07±0.00	
Linoléic acid	0.52±0.00	0.50±0.00	0.53±0.00	0.48±0.00	0.67±0.00	
Viscosity (mPa.s) at shear rate 0.44 s -1 (mPa.s, m±SD, *n*=3)	23 421±477	23 355±327	30 300±387	35 560±602		23 321±735

GMO: Glycerol monooleate, REF: Reference, SD: Standard deviation

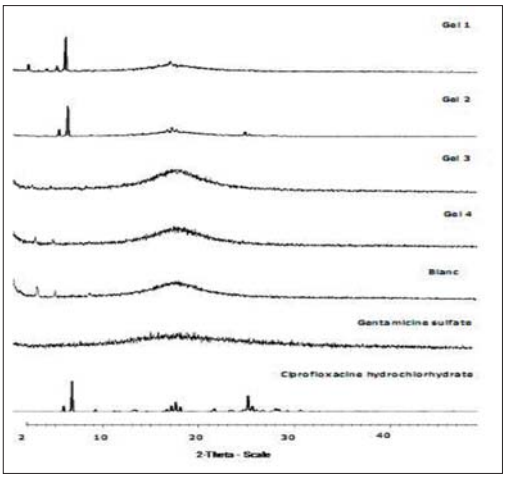

Figure 3: X-ray diffraction spectra of ciprofloxacin hydrochloride and gentamicin sulfate powders, ciprofloxacin (1 and 2) , gentamicin (3 and 4) and REF (Blanc) monoolein water gels

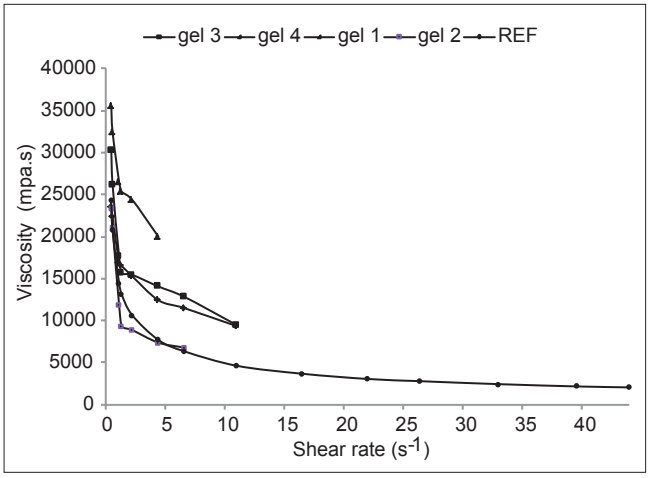

Figure 5: Rheograms of the ciprofloxacin (1 and 2), gentamicin (3 and 4) and REF monoolein water gels, measured at 37 °C with a SC4-25 spindle (m ± SD, *n* = 3)

observed by TGA increased abruptly after 200°C, probably due to the degradation of monoolein and active ingredients [Figure 1].

The TGA thermograms thus allowed water contents to be obtained that are closer to their theoretical value.

Free fatty acid content

The free fatty acids that are mainly present in the GMO are palmitic, stearic, oleic, and linoleic acids. The presence of free fatty acids other than oleic acid is due to the fact that the oleic acid used in the esterification process was not sufficiently pure.[18] Similar amounts of free fatty acid were found before and after manufacturing of the gels [Table 2]. Therefore, it could be concluded that the manufacturing process prevented the production of free fatty acids from GMO.

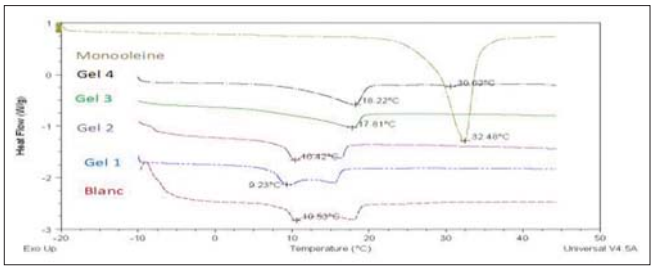

Figure 4: Differential scanning calorimetry heating curves for glycerol monooeleate (monooleine), ciprofloxacin (1 and 2), gentamicin (3 and 4) and REF (Blanc) monoolein water gels recorded during the first heating.

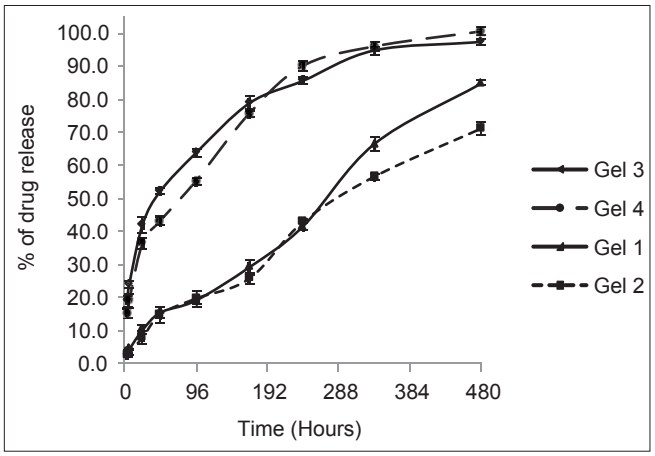

Figure 6: Dissolution profiles for the ciprofloxacin (1 and 2) and gentamicin (3 and 4) monoolein water gels (m ± SD, *n* = 3)

Physico-chemical properties

Microscopic observation

Microscopic observation under polarized lens at 25°C [Figure 2] showed that the developed Gels were characterized by a liquid crystalline structure which corresponded to previous observations made by different authors.[19-22] As expected, due to their high viscosity [Table 2] and their isotropic nature, the liquid crystals of the gels were in a cubic phase.[8,20] In addition, rod-shaped particles of undissolved ciprofloxacin hydrochloride were observed in Gels 1 and 2. However, it has been reported that the incorporation of insoluble drug in a cubic phase did not cause a change in the structure.[21]

X-ray diffraction

X-ray diffraction (XRD) spectra of ciprofloxacin hydrochloride and gentamicin sulfate powders showed crystalline peaks only for ciprofloxacin hydrochloride [Figure 3]. Ciprofloxacin hydrochloride was therefore in the crystalline state while gentamicin sulfate was in amorphous state. Furthermore, the DRX spectra of the developed gels also showed peaks [Figure 3]. Those of the REF and Gels 3 and 4 were superposable, indicating that the gentamicin sulfate was completely dissolved in the matrix. On the other hand,

other peaks were found in gels containing ciprofloxacin hydrochloride (Gels 1 and 2) and were consistent with those obtained with the powder of ciprofloxacin hydrochloride. This observation showed that it was the undissolved ciprofloxacin hydrochloride in crystalline state that was effectively found in gels.

Differential scanning calorimetry (DSC) analysis

The DSC thermogram representing the first heating cycle of GMO showed an endortherm peak at about 33°C [Figure 4], which corresponded to the melting point of GMO.[8,23,24] The REF showed an endotherm peak at about 10°C [Figure 4]. All the developed gels were characterized by a melting endotherm peak ranged between 9 and 18°C [Figure 4], like any eutectic system. It seemed that GMO interacted with other components to form crystalline structures. Similar observations have been reported by other authors.[22,25] However, the solubility profile of the active ingredients has an influence on the melting endotherm. Indeed, the melting endotherm of both Gels 3 and 4 containing gentamicin sulfate (17.8°C and 18.2°C respectively) was higher than that of gels containing ciprofloxacin hydrochloride (9.2°C and 10.4°C respectively). The thermograms of gels with ciprofloxacin hydrochloride were similar to the REF; consequently, the insoluble ciprofloxacin hydrochloride did not seem to influence the melting point of the gel. A similar observation was reported by Semdé after the dispersion of hydroxyapatite in monoolein-water-gentamicin gels.[16] It was also noticed that DSC curves of the developed gels recorded during the first and the second heating were practically similar, attesting the good stability of these eutectic systems.

Rheological studies

All the developed gels showed a non-Newtonian rheological behavior of the pseudoplastic type [Figure 5], similar to gels based on a biphasic mixture of GMO and water as previously reported by other authors.[8,24] However, the solubility profile of the active ingredient in the matrix influenced both the viscosity and the rheological behavior of the preparation. The solubilization of gentamicin sulfate into the matrix resulted in an increase of the viscosity, probably due to a reorganization of the internal structure of the gel. In contrast, the dispersion of ciprofloxacin in the matrix did not affect the viscosity of Gels 1 or 2.

In vitro drug release

The release of ciprofloxacin and gentamicin was similar to those previously reported by other authors for monoleine-water gels.[8,16,24] The incomplete release of ciprofloxacin was not related to its solubility in the dissolution medium because the dissolution was conducted under sink conditions. This suggests that part of ciprofloxacin was bound to the GMO.[21] Since gentamicin was soluble in the gel, its release was done mainly by diffusion. In the case of ciprofloxacin, it was partially insoluble in the gel, so its release was done partially by erosion and took more time as a consequence. Erosion of the gel was evidenced by finding fragments of gel in the dissolution medium. The determination of ciprofloxacin from the gel particles found in the dissolution medium gave residual contents of about 10%.

The drug release profile is typical of a matrix-type delivery system, and means that it follows a Fickian diffusion.[26] A similar release mechanism has also been reported from gentamicin[8,16] and clonidine[24] monoolein water gels respectively.

CONCLUSION

The developed gels were viscous, homogeneous (for gentamicin sulfate) and opaque (for ciprofloxacin hydrochloride). Ciprofloxacin, unlike gentamicin, which was dissolved in the matrix, was in dispersed form. However, its insolubility in the matrix did not cause a change in the crystalline structure of the gel. Gel 1, ciprofloxacin-GMO-water (5:80:15% w/w), which released *in vitro* approximately 85% of ciprofloxacin after 16 days could possibly be considered as an alternative to a gentamicin-GMO-water gel for the treatment of chronic osteomyelitis.

ACKNOWLEDGEMENT

The authors thank the University Cooperation for Development and the General Commissariat for International Relations of the French Community of Belgium

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How to cite this article: Sombié BC, Yameogo JG, Semdé R, Henschel V, Amighi K, Goole J. Ciprofloxacin monoolein water gels as implants for the treatment of chronic osteomyelitis: *In vitro* characterization. J Adv Pharm Technol Res 2014;5:158-63.

Source of Support: University Cooperation for Development and the General Commissariat for International Relations of the French Community of Belgium, **Conflict of Interest:** Nil.