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Ciprofloxacin HCl Loaded Cubic Phase Gel for Periodontal Intrapocket Administration

Preeti K Suresh* and Mahendra Kumar Dewangan

University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, 492010, INDIA

ABSTRACT

The aim of the present work was to investigate the potential of cubic phase gel based drug delivery system for periodontal delivery of ciprofloxacin. The prepared gels were characterized for drug content, drug loading efficiency, gelation temperature, gel melting temperature, pH, bioadhesive force, viscosity, gel strength, swelling and drug release profile. The cubic phase gels were prepared with varying combinations of glyceryl monooleate(GMO), glyceryl monostearate (GMS), methyl cellulose, Span 80, propylene glycol, polyethylene glycol and glycerol. The drug content in the formulations was found to be high and in the range of 89.91-96.44%. All the formulations displayed the gelation temperature within the appropriate range of 30-36°C. C-2 had the highest gelation temperature, gel melting temperature, viscosity and mucoadhesive strength. The surface pH of gels was found in the range of 6.41 – 6.78 and gel strength from 60–75s. The drug release kinetic studies divulged the best fit with the Higuchi's equation, representing the release of ciprofloxacin HCl by diffusion.

Keywords: bioadhesion, drug delivery, glyceryl monooleate, glyceryl monostearate, liquid crystal

**Corresponding author*



INTRODUCTION

The oral cavity provides a diverse environment for colonization by a wide variety of microorganisms. Supragingival and subgingival plaque play an essential role in the causation of dental caries and periodontal disease, respectively [1]. Bacteriological studies show that most of the periodontal diseases are infections caused by the overgrowth of a limited number of typically Gram-negative anaerobic microorganisms. The bacterial composition of early plaque consists of mainly aerobic or facultatively anaerobic microorganisms, which later becomes colonized by strict anaerobes.

Control of bacterial plaque helps in slowing or arresting periodontal infections. Conventional therapy has long sought the use of mechanical plaque control procedures, which are time consuming, require highly trained personnel to carry them out and result in varying amounts of discomfort to the patients [2]. This warrants systemic antibiotic administration in eliminating pathogenic bacteria that invade gingival tissue and in helping control periodontal pathogens residing in various domains of the mouth from where they may translocate to periodontal sites. Systemically applied antimicrobials have been advocated for the treatment of severe forms of periodontal infections, which are also reported to give rise to a number of adverse reactions and should be administered only after proper patient evaluation [3, 4].

Multiple systemic doses of antibiotics have shown several drawbacks including inadequate antibiotic concentration at the site of the periodontal pocket as the drug gets diluted thousand fold leading to low benefit to high-risk ratio; a rapid decline of the plasma antibiotic concentration to subtherapeutic levels [5]; development of microbial resistance; and high peak-plasma antibiotic concentrations, which may be associated with side effects like hypersensitivity, gastrointestinal intolerance, depression, and tachycardia [6].

Due to disadvantages of the systemic administration, of late considerable attention has been focused on local periodontal delivery systems of antimicrobial agents. Various drug delivery systems like fiber [7, 8], film [9, 10], sponge [11], microparticles [12], and nanoparticles [13] have been investigated for periodontal drug delivery. These systems may be injected or inserted into periodontal pocket to achieve the controlled delivery of antibacterial.

Amphiphilic polar lipids like phospholipids when placed in water spontaneously rearrange to yield thermodynamically stable lipid bilayers, which can assume various geometric shapes and structures. Apart from liposomes, where amphiphilic lipids reorganize into closed circular lipid bilayers enclosing an aqueous phase, spontaneous reorganization of amphiphilic lipids in aqueous environment can also yield other three-dimensional structures such as the lamellar phase, the cubic phase, and transferosomes which have also found a number of drug delivery applications [14-16]. A group of fatty acids esters with a low molecular weight and capable of forming liquid crystals has been identified as potential bioadhesive substance [17, 18]. The cubic phase of GMO is reported to serve as delivery system for drugs with different physico-chemical properties [19, 20].

Cubic phase reveals a great flexibility, as drugs of varying polarities and size may be incorporated. However, the restraining factor is that the drug must not disrupt the formation of the cubic phase crystalline lattice structure [21, 22]. The cubic phase has been successfully used for sustained delivery of drugs with varied molecular weights and solubilities in water, such as aspirin, and vitamin E [20], propantheline bromide, oxybutynine hydrochloride [23], metronidazole [21], tetracycline [22], timolol maleate [24], chlorpheniramine maleate, propranolol HCl [25], melatonin, pindolol, propranolol, pyrimethamine [26], hemoglobin [27], cefazolin [28] and insulin [29].

The aim of the present study was to develop a sustained drug delivery system of ciprofloxacin for localization at the periodontal intra-pockets.

MATERIALS AND METHODS

Materials

Ciprofloxacin was a kind gift from Cadila Pharmaceuticals, Ahmedabad, India. Methylcellulose, Span 80 and Tween 80 were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All reagents were of analytical grade and were used as received.

Preparation of gel base

The components were melted by heating to 45-60⁰ C to form a viscous liquid base. Insoluble ingredients were pulverized and only the portion with particle size <63 μm was dispersed throughout the viscous liquid base. The mixture was slowly cooled with constant stirring until congealed. The gel base so obtained was stored at 4⁰ C.

Preparation of drug containing cubic phase gel

The gel base was melted at 45⁰C over a water bath. Ciprofloxacin HCl was dissolved into the melt to yield 10% w/w of drug concentration in the base.

Drug content

Phosphate buffer (pH 6.8) (100 ml) was added to 50mg of cubic phase gel. The mixture was allowed to equilibrate for 24 hr. The extracted buffer solution was spectroscopically analysed following suitable dilution. The drug content was determined by:

Drug content = Amount of drug in known amount of formulation / Initial drug load x 100

Gelation and gel melting

Gelation and gel melting temperature of the samples were assessed using a modification of the Miller and Donovan technique [34]. Accordingly, a 5-mL aliquot of gel was

transferred to test tubes, immersed in a water bath at 4°C, and sealed with aluminum foil. The temperature of the water bath was increased in increments of 1°C and left to equilibrate for 1 minute at each new setting. The samples were then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90°. The gel melting temperature, the temperature at which the gel starts flowing upon tilting through 90°, was recorded.

Viscosity studies

The viscosity of gels was measured using Brookfield Viscometer DV- I + Version 5.1 programmable viscometer (Brookfield Engineering Laboratories Inc, Middleboro, MA). Viscosity was measured at different rpm with 1-minute equilibration time at each rpm.

Bioadhesion study

The bioadhesive force of cubic phase gel was determined by adapting the reported methods [35-37]. A measuring device fabricated in-house was employed. A section of goat buccal tissue was collected and secured with mucosal side out onto each glass vial using a rubber band. The vials with the buccal tissues were stored at 37±1°C for 10 min. Subsequently one vial with a section of tissue was connected to the balance and the other vial was placed on a height-adjustable pan. The gel was placed onto the buccal tissue and the height of the vial was adjusted so that the gel was located between the mucosal tissues of the two vials. The weights kept were raised till the two vials remained attached. Bioadhesive force, the detachment stress (g force), was determined from the minimal weights that detached two vials. The buccal tissue pieces were changed for each measurement.

Surface pH of cubic phase gel

The pH was measured by a digital pH meter (Digital pH meter ELICO LI 610 pH meter) by dipping the electrode completely into the gel so as to cover the electrode. The results are presented in Table 2.

Measurement of gel strength

The gel strength was measured by the method reported by Choi et al. [35], 1998. The gel (50g) was placed in a 100 ml-graduated cylinder and placed in a thermostat at 37±1°C. The apparatus for measuring gel strength was then placed over the gel. The gel strength (expressed as the viscosity of gel at physiological temperature) was determined by the time the apparatus took to sink 5 cm down through the gel.

Swelling study

An accurately weighed portion of the gel (1 g) was placed in a series of petri dishes as supporting containers and weighed. Each gel-containing dish was immersed in 30 ml of distilled

water at $37 \pm 1^\circ\text{C}$. After one hour, the dish with gel was blot-dried and reweighed. Water uptake was determined by:

$$\text{Degree of swelling (water uptake)} = \frac{\text{Wet weight} - \text{original dry weight}}{\text{Original dry weight}}$$

Drug release profile

Fifty milligrams of accurately weighed gel was used for drug release study in phosphate buffer (pH 6.8) using USP Type II Paddle dissolution apparatus. At specified time intervals aliquots were withdrawn and after sufficient dilution with phosphate buffer analyzed spectrophotometrically. The withdrawn amounts were replaced by equal volumes of fresh phosphate buffer. The results are graphically presented in Fig.1.

Kinetic analysis of dissolution data

The drug release data were analysed for the drug release mechanism from the cubic phase gels. Release kinetic study of all formulations was studied for different kinetic equation (zero order, first order, Higuchi equation and Korsmeyer's Peppas).

Stability study

Stability studies were performed to determine the effect of temperature and humidity on the formulations. The stability study was performed according to ICH guidelines at $4^\circ\text{C}/75\% \text{RH}$, $25^\circ\text{C}/60\% \text{RH}$ and $40^\circ\text{C}/75\% \text{RH}$. Samples were withdrawn at 7, 15, and 30 days. The physical appearance of the gels after the period of storage, such as color change and phase separation was compared to the freshly prepared gels.

RESULTS AND DISCUSSION

In the present study glycerylmonooleate (GMO) was used as a main principle in the gel base formulation since it is a viscous injectable liquid that can be changed to a liquid crystalline phase when in contact with water [30]. This crystalline phase possesses a desirable adhesive property [31, 32]. Moreover, it is biodegradable as it could be converted to oleic acid and glycerol by lysosomal enzyme liberated by neutrophils and some bacteria present in the pocket [33]. Glyceryl monostearate (GMS), a monoglyceride of saturated fatty acid having the same number of carbon atoms as GMO was employed to improve the base stiffness. Methylcellulose was used to promote swelling and impart adhesiveness. The composition of various aqueous and nonaqueous based gel bases are presented in Table 1.

Table 2 reports the characterization of various ciprofloxacin loaded cubic phase gels. The drug content in the formulation was found to be high in the range of 89.91-95.85 % with a small deviation indicating that the drug was uniformly distributed in the gel and there was minimum loss of drug by this method of preparation. Ideally, the gels must have a gelation

temperature in the range, 30–36°C, so that they are in a liquid form at room temperature but form a gel phase in the periodontal pocket. Results show that all formulations fell within this range.

Table 1: Composition of various cubic phase gel bases

Ingredients	C1	C2	C3	C4	C5
Glyceryl monooleate	40	70	60	60	60
Glyceryl monostearate	10	10	10	10	10
Methyl cellulose	10	10	10	10	10
Span 80	10	10	10	10	10
Propylene Glycol	-	-	10	-	-
Polyethylene Glycol	-	-	-	10	-
Glycerol	-	-	-	-	10
Water	30	-	-	-	-

Table 2: Characterization of various ciprofloxacin loaded gels

Formulation	Drug content (%)	Gelation temperature (°C)	Gel melting temperature (°C)	Bioadhesive force (g force)	pH
C1	95.85 ± 0.33	32.8 ± 0.9	72.3 ± 0.1	47.30 ± 1.21	6.59 ± 0.03
C2	92.40 ± 0.45	35.5 ± 0.7	74.5 ± 0.3	50.10 ± 1.06	6.41 ± 0.04
C3	89.91 ± 0.40	34.9 ± 0.7	73.9 ± 0.8	46.50 ± 2.10	6.51 ± 0.12
C4	96.44 ± 0.05	33.7 ± 0.8	73.1 ± 0.7	45.60 ± 1.10	6.63 ± 0.21
C5	95.26 ± 0.55	33.1 ± 0.5	72.9 ± 0.4	45.20 ± 1.30	6.78 ± 0.14

Values represent mean ± std. deviation (n=3)

The gel melting temperature of the formulation was in the range 72.3 – 74.5°C. The gel melting temperature is helpful in determination of gel stability at higher temperature. The gel structure remained unaltered with temperature until an excessively high temperature caused the destruction of the gel structure. At higher temperatures, the gel underwent dehydration. Viscosity study revealed that formulation containing polyethylene glycol and glycerol showed lower viscosity as compared to other formulations (Table 3). The mucoadhesive strength measurement showed that formulation C2 achieved the highest bioadhesivity and C5 lowest bioadhesive property. The surface pH of gel was found in the neutral range making it suitable for periodontal application without any discomfort and irritation.

Table 3: Viscosity of various cubic phase gels

Formulation	Viscosity (cps)	
	50 rpm	100 rpm
C1	621.55 ± 1.22	811.68 ± 1.48
C2	690.25 ± 2.49	987.34 ± 1.59
C3	678.34 ± 2.54	968.56 ± 1.36
C4	596.23 ± 1.79	764.57 ± 1.08
C5	565.54 ± 1.43	735.24 ± 1.14

Value represent mean ± std. deviation (n=3)

Gel strength of cubic phase gel is reported in Table 4 and it was illustrated that formulation C2 had highest gel strength and formulation C5 lowest strength. In the development of gel, the gel strength is important in finding the condition, which allows the easy insertion of the gel and no leakage from the periodontal pocket. In the cubic phase gels the range was 60–75 s.

Table 4: Gel strength and degree of swelling of various formulations

Formulation	Gel strength (Time in sec)	Swelling (%)
C1	65.21 ± 1.57	16.44 ± 0.69
C2	75.23 ± 1.33	24.17 ± 0.48
C3	68.54 ± 0.85	22.33 ± 0.42
C4	65.32 ± 1.66	19.32 ± 0.11
C5	60.16 ± 1.88	19.11 ± 0.26

Values represent mean ± std. deviation (n=3)

Table 5: Kinetic analysis of dissolution data from various cubic phase gels

Formulation	Zero order	First order	Higuchi	Korsmeyer's Peppas
C1	0.848	0.860	0.985	0.966
C2	0.834	0.898	0.977	0.959
C3	0.834	0.855	0.981	0.958
C4	0.835	0.894	0.982	0.971
C5	0.805	0.782	0.974	0.962

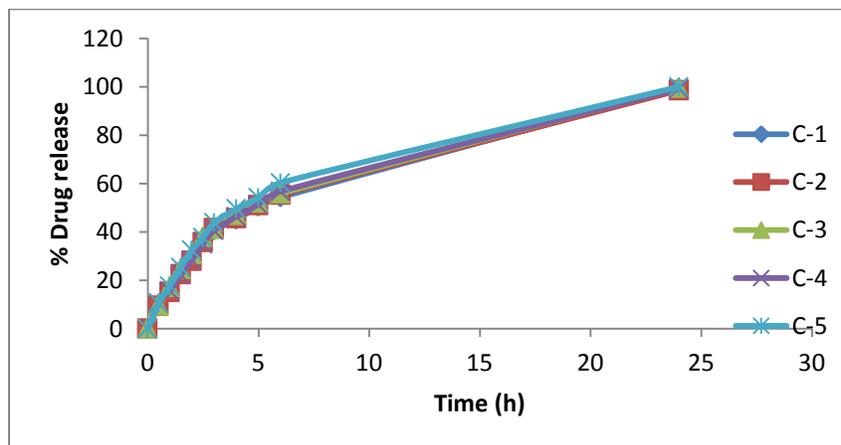


Fig. 1: Percent cumulative drug release from various cubic phase gels

The release of Ciprofloxacin HCl from gel base formula which contained no polyols, was relatively slow (Fig. 1). When the mixture of gel base and mainly GMO came into contact with water, Ciprofloxacin HCl from the surface zone immediately leaked into the surrounding liquid. As the drug slowly cleared and hydration progressed, the remaining gel base turned into a condensed liquid crystalline state. This did not occur instantaneously, depending on the thickness of the gel. The drug-cleared area of the liquid crystalline gel regulated the release of Ciprofloxacin HCl from the inner core, which slowly became hydrated. Different polyols, e.g.,

propylene glycol (PG), polyethylene glycol 400 (PEG 400), and glycerol, were added to the gel base formula C-3, C-4 and C-5 respectively in order to evaluate their effect on drug release profile. The results indicate that the drug release from the gel bases containing each of the polyols significantly increased. This can be attributed to the fact that the polyols increased the hydrophilicity of the cubic liquid crystalline phase of the gel. The addition of polyols to the base also demonstrated a decrease in viscosity of the gel. Increment in hydrophilicity and decrease in viscosity led to the increased rate of aqueous dissolution fluid entry into the gels with the resultant enhancement in drug release. The cumulative amount of drug released from glycerol containing gel was significantly higher than that from the other gels. Among the polyols used, glycerol was the best enhancer, as it exhibited the maximum enhancing power on Ciprofloxacin HCl release from the base.

Release kinetic study of formulations was performed and it was found that the best fit with higher correlation ($r^2 > 0.97$) was found with the Higuchi's equation for all the formulations, which means that release of ciprofloxacin HCl from the matrix system was due to diffusion.

CONCLUSION

The potential advantages of a localized controlled release drug delivery system for insertion into and/or around the periodontal pocket are manifold. They include increased local drug concentration for extended periods to maintain an effective concentration of antibiotic and a decrease in superfluous distribution of the drug to other body organs with a subsequent decrease in side effects. In the present study, the glyceryl monooleate was selected as gel base due to its cubic phase forming property by absorbing water, biodegradability and biocompatibility. Cubic phase is spontaneously formed as it comes in contact with water at body temperature and thus, can assist in retention of the formulation in periodontal pocket and provide the controlled release of drug. The findings showed that the formulated cubic phase gels had requisite gelation temperature, gel strength, pH, viscosity, swelling degree and release profile to endow them with features for successful periodontal delivery of ciprofloxacin.

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