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Gel in core carbosomes as novel ophthalmic vehicles with enhanced corneal permeation and residence

Mona A. Moustafa[a], Wessam M. El-Refaie[a,*], Yosra S.R. Elnaggar[a,b], Ossama Y. Abdallah[b]

[a] Department of Pharmaceutics, Faculty of Pharmacy and Drug Manufacturing, Pharos University in Alexandria, Egypt
[b] Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, Egypt

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ABSTRACT

Carbopol is a good bio-adhesive polymer that increases the residence time in the eye. However, the effect of blinking and lacrimation still reduce the amount of polymer and the incorporated drug available for bioadhesion. Gel-core liposomes are advanced systems offering benefits making it a good tool for improved ocular drug delivery and residence time. Incorporation of carbopol in gel-core liposomes and their potential in ocular delivery have not so far been investigated. Fluconazole (FLZ) was selected as a challenging important ocular antifungal suffering from poor corneal permeation and short residence time. In this study, gel-core carbosomes have been elaborated as novel carbopol-based ophthalmic vehicles to solve ocular delivery obstacles of FLZ and to sustain its effect. Full in vitro appraisal was performed considering gel-core structure, entrapment efficiency, particle size and stability of the vesicles as quality attributes. Structure elucidation of the nanocarrier was performed using optical, polarizing and transmission electron microscopy before and after Triton-X100 addition. Ex-vivo ocular permeation and in vivo performance were investigated on male albino rabbits. Optimized formulation (CBS5) showed gel-core structure, nanosize (339.00 ± 5.50 nm) and not defined before (62.00% ± 1.73) entrapment efficiency. Cumulative amount of CBS5 permeated ex-vivo after 6 h, was 2.43 and 3.43 folds higher than that of conventional liposomes and FLZ suspension, respectively. In-vivo corneal permeation of CBS5 showed significantly higher AUC0-24 h (487.12 ± 74.80) compared to that of FLZ suspension (204.34 ± 7.46) with longer residence time in the eye lasts for more than 18 h. In conclusion, novel gel-core carbosomes could successfully be used as a promising delivery system for chronic ocular diseases.

1. Introduction

Carbopol is a cross-linked homopolymer of acrylic acid that has good bio-adhesive properties. At physiological pH, carbopol can interact with mucus and biological surfaces (Rajput et al., 2010) through hydrogen bonding of the ionized carbonyl functionalities (Tiwari et al., 2009a). This results in the formation of a strengthened gel network which allows the particles to remain adhesive for extended periods of time. Moreover, it was reported that eye drops containing 0.3% Carbopol reduced the severity of symptoms and extent of ocular surface staining in subjects with moderate dry eye (Johnson et al., 2008). There is a large number of commercial eye drops containing carbopol 940 for improved corneal retention and enhanced bioavailability (Ikada and Kishida, 2001). Up to our knowledge, there was no reported cytotoxic effect of carbopol on ocular tissues (Ahuja et al., 2008; Liu et al., 2016; Dubald et al., 2018). It was reported that nanoparticles which combine the PLGA with the mucoadhesive carbopol coating showed no cytotoxic effect (Aksungur et al., 2011). However, in ocular applications, the effect of blinking and lacrimation still reduce the amount of polymer and the incorporated drug available for bioadhesion and accordingly reduce the clinical efficacy. Therefore, incorporation of part of carbopol in the core of phospholipid vesicular system might reduce rapid polymer elimination and enhance corneal penetration.

Gel-core liposomes are the most advanced liposomal system bearing core of biocompatible polymer inside the lipid vesicle. The polymer in the core serves the function of skeleton and provides mechanical strength to vesicles (Elnaggar et al., 2014). Moreover, the phospholipid membrane can keep the polymer inside with slow partitioning to the external medium. These benefits might be a good tool for increasing the residence time in the eye and enhancing the corneal permeability for improved ocular drug delivery. However, the potential of gel-core liposomes in ocular route of delivery has not been widely investigated although utilized in other routes (Tiwari et al., 2009b; Tiwari et al., 2009a; El-Refaie et al., 2015a; El-Refaie et al., 2015b). Polymers exploited to prepare gel-core liposomes included hyaluronic acid (HA) (Moustafa et al., 2017), sodium alginate (Hong et al., 2008), polyacrylic

* Corresponding author.
E-mail address: wessamelrefaie@yahoo.com (W.M. El-Refaie).

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acid (Tiwari et al., 2009b) and heparin-Pluronic (Nguyen et al., 2015). Integration of carbopol in the core matrix of a phospholipid vesicular system has not so far been investigated for ocular delivery.

Fluconazole (FLZ) was selected as a challenging drug in this study. FLZ is an effective antifungal agent with a low molecular weight 306.27 g/mol, short half-life (15–30 min) and limited corneal permeation (Corrêa and Salgado, 2011). It is available as eye drops for the treatment of ocular mycoses. It was found that after the local application of eye drops, the concentration in the aqueous humor is lower than the minimal inhibitory concentration (MIC) of FLZ; 8 μg/ml (Pfaller et al., 2010; Liu et al., 2012). Among the different studies performed to improve FLZ bioavailability, only one study incorporated it in carbopol matrix but mixed it with a lot of ingredients namely; Tween80, poloxamer and benzalkonium chloride in borate buffer solution, to form a thermoresponsive ophthalmic FLZ in situ gels (Lihong et al., 2014). However, this thermoresponsive gel failed to keep the FLZ concentration above MIC.

Under the umbrella of the aforementioned background and based on that carbopol 940 can be promising in treatment of ocular fungal infections (Sarkar et al., 2016; Tarff and Behrens, 2017), this study aimed to design and evaluate novel FLZ-loaded carbopol-integrated gel-core liposomal systems (Carbosomes). Simple and spontaneous thin film hydration method was used for preparation with reduced carbopol concentration and without addition of other viscosity enhancing polymers. FLZ-loaded carbosomes were evaluated using optical and transmission electron microscopical examination. Optimization and full in vitro characterization was performed for the novel carbosomes compared to conventional liposomes and carbopol gel. Ex-vivo permeation experiments were carried out using the excised cornea of New Zealand white rabbits. In-vivo permeation was assessed as well on rabbits after topical application FLZ-loaded gel core carbosomes. Draize test and histological examination of the cornea was studied to ensure the safety of the novel gel core formulations on ocular tissues. Stability of the selected formulations was studied for any changes in drug entrapment efficiency, particle size and zeta potential at 4 °C for a period of six months.

2. Materials and methods

2.1. Materials

Fluconazole was provided as a gift sample from Chempifine chemicals Ltd. (Mumbai, India). Carbopol 940 was obtained from BF Goodrich Company (Saginaw, Michigan, USA). Lipoid S100 (Phosphatidylcholine from soybean) was a kind gift from Lipoid GmbH (Ludwigshafen, Germany). Triton-X 100 was purchased from Sigma Chemicals Corporation, U.K. Transcutol HP, and Caproyl 90 were purchased from Gattefosse Co. (Lyon, France). All other chemicals used were of analytical grade.

2.2. Preparation and optimization of gel-core carbosomes

2.2.1. Preparation of carbosomes

Novel gel core carbosomes were prepared by simple lipid film hydration technique (El-Refaie et al., 2015a; El-Refaie et al., 2015b). In Brief, lipoid S100 (0.425 g), cholesterol (0.1 g) and FLZ were dissolved in solvent mixture (chloroform: methanol) in ratio (2:1 v/v). The solvent was removed under reduced pressure in a rotary evaporator to form a thin dried film. Evaporation was continued for 1.5 h after the formation of the dry film to ensure complete removal of the solvent. Then, carbopol 940 in different concentrations (0.3, 0.5, 0.7 g%) was added as a hydrating medium with 1 h hydration time. The prepared carbosomes were extruded through polycarbonate membranes with pore size of 200 nm. The formed dispersion was then sealed and stored at 4 °C. Moreover, other gel-core carbosomes were prepared by the same method using Tween 80 (0.075 g) or the previously mentioned oils (0.1 g) instead of cholesterol. Conventional liposomes were prepared under same conditions for comparison. Table 1 showed the prepared formulations with their detailed composition.

Table 1
Composition of the prepared gel-core carbosomes and conventional liposomes.

Formulation code	Composition*		
	Polymer used (w/v)**	Fluconazole concentration (w/v)	Additives in the lipid bilayer
CBS1	Carbopol (0.3%)	0.3%	Cholesterol
CBS2	Carbopol (0.3%)	0.3%	Tween 80
CBS3	Carbopol (0.3%)	0.3%	Transcutol HP
CBS4	Carbopol (0.3%)	0.3%	Caproyl 90
CBS5	Carbopol (0.3%)	0.9%	Cholesterol
CBS6	Carbopol (0.3%)	1.2%	Cholesterol
LS1	–	0.3%	Cholesterol
LS2	–	0.9%	Cholesterol

* Each formulation contains o.1 g additive and 0.4 g PL per 10 ml final dispersion.

** Other concentrations failed to spontaneously form the system.

2.2.2. Preliminary screening of vesicles composition

Different factors have been examined to optimize the formulation of novel gel-core carbosomes; namely, concentration of carbopol, the additives in the bilayer structure, and the initial FLZ concentration. Carbopol 940 was used in different concentrations (0.3, 0.5, 0.7 g%) respectively. Cholesterol and lipoid S100 were utilized in the bilayer structure of gel-core carbosomes in ratio (1:4 w/w) (El-Refaie et al., 2015a). The effect of addition of Tween 80 (0.075 g) and other oils namely; Transcutol HP, and Caproyl 90 (0.1 g) in the vesicles structure was studied. Furthermore, FLZ was used in different concentrations (0.3, 0.6, 0.9, and 1.2 g%) to obtain the most appropriate entrapment efficiency. Selection criteria of the optimum formulations were based on the ideal gel-core structure, entrapment efficiency, particle size and stability of the carbosomes.

2.2.3. Characterization of gel-core liposomes

2.2.3.1. Gel-core structure elucidation. The structure of prepared gel-core carbosomes was illustrated using microscopical investigation. The gel-core liposomal structure was confirmed by means of a projection microscope fitted camera. Briefly, Triton X-100 was added to the formulations and their phase behavior was examined before and after Triton X-100 treatment (Tiwari et al., 2009a). Furthermore, a polarized light microscope was utilized to show a complete structure of the prepared gel-core carbosomes without any dilution. Then undiluted samples were investigated for the presence of liquid crystals.

2.2.3.2. Morphological characterization. Morphology of the prepared gel-core carbosomes was examined by transmission electron microscopy (TEM) before and after treatment with Triton X-100. Samples were firstly diluted with distilled water and then dropped onto a carbon-coated copper grid and left for 1 min to allow the adhesion of vesicles on the carbon substrate. The excess dispersion was then collected with a filter paper. Then they were stained by negative staining using a 2% phosphotungstic acid solution (w/w) for 45 s. The air dried samples were immediately examined under the TEM (Freag et al., 2013).

2.2.3.3. Determination of apparent entrapment efficiency. The prepared novel gel-core carbosomes were diluted with PBS in appropriate ratio