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Self-assembled nano-architecture liquid crystalline particles as a promising carrier for progesterone transdermal delivery

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ABSTRACT

The study aims to elaborate novel self-assembled liquid crystalline nanoparticles (LCNPs) for management of hormonal disturbances following non-invasive progesterone transdermal delivery.

Fabrication and optimization of progesteroneloaded LCNPs for transdermal delivery were assessed *via* a quality by design approach based on 2^3 full factorial design. The design includes the functional relationships between independent processing variables and dependent responses of particle size, polydispersity index, zeta potential, cumulative drug released after 24 h and *ex-vivo* transdermal steady flux. The developed nanocarrier was subjected to TEM (transmission electron microscope) for morphological elucidation and stability study within a period of three months at different storage temperatures.

The cubic phase of LCNPs was successfully prepared using glyceryl monooleate (GMO) *via* the emulsification technique. Based on the factorial design, the independent operating variables significantly affected the five dependent responses. The cubosomes hydrodynamic diameters were in the nanometric range (101–386 nm) with narrow particle size distribution, high negative zeta potential ≥ -30 mV and entrapment efficiency $\geq$94%. The LCNPs succeeded in sustaining progesterone release for almost 24 h, following a non-fickian transport of drug diffusion mechanism. *Ex-vivo* study revealed a significant enhancement up to 6 folds in the transdermal permeation of progesterone-loaded LCNPs compared to its aqueous suspension. The optimized LCNPs exhibited a high physical stability while retaining the cubic structure for at least three months.

Quality by design approach successfully accomplished a predictable mathematical model permitting the development of novel LCNPs for transdermal delivery of progesterone with the benefit of reducing its oral route side effects.

1. Introduction

Over the last decades, hormonal disturbances represent a major clinical issue for both pre-and postmenopausal women. It is well known that there is a close relationship between estrogen and progesterone hormones. Specifically, progesterone helps to balance and neutralize the powerful effects of excess estrogen (Schindler, 2009). Fluctuating levels of progesterone plays an important cause for a variety of vasomotor disturbances, which include hot flushes, night sweats, palpitations, headaches, bone pain, vaginal dryness and breast tenderness (Al-Safi and Santoro, 2014). Further, the long-term deficiency of progesterone can be associated with high risk of endometrial hyperplasia and finally, endometrial cancer (Schindler, 2009). Thus, reducing the burden of progesterone deprivation-related health problems receives recently a great attention. Since the 19th, several synthetic progestins, norethisterone and medroxyprogesterone acetate had been introduced as a replacement therapy, but their usage is not favoured because of their severe teratogenic, metabolic and vascular side effects (Knopp, 1988; Salem, 2010).

In this context, natural progesterone is still the only recommended treatment for these conditions. Unfortunately, progesterone clinical implementation faces massive obstacles as a result of its poor aqueous solubility resulted in low bioavailability following its oral administration (Salem, 2010). Moreover, orally administrated progesterone is proved to have a relatively short half-life attributed to intensive hepatic metabolism (Biruss and Valenta, 2006). In the meantime, large repeated oral doses (300 mg) are required to achieve effective drug serum level which may cause systemic side effects. Due to the aforementioned virtues,

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progesterone has been administrated *via* intramuscular injection assuring a reliable absorption, but with non-compliance patient attitude, it is painful and risky (Tavaniotou *et al.*, 2000). Thus, producing a feasible and safer alternative approach with the idea of sustaining its effect for a longer period and decreasing systemic side effects becomes increasingly of interest. Accordingly, a number of formulation attempts have been adopted overcoming the obstacles of progesterone *via* the preparation of nano-suspensions (Salem, 2010), liposomes (Biruss and Valenta, 2006), microspheres (Yang and Owusu-Ababio, 2000), nano-emulsions (Klang *et al.*, 2010) and magnetic nanoparticles (Ragab *et al.*, 2012).

Skin is characterized by advantageous criteria that make it attractive for both cutaneous and percutaneous drug delivery (Elgindy *et al.*, 2007; Mehanna *et al.*, 2014). Transdermal drug delivery has gained a considerable attention among other conventional routes as it can bypass hepatic first pass metabolism, maintain sustained drug plasma level and enable blood steady state profile with subsequent reducing the dose and systemic adverse effects resulting in the improvement of patient compliance (Elsayed *et al.*, 2006). Nevertheless, one of the main challenges of transdermal drug delivery is the barrier nature of stratum corneum, which restricts the penetration of the majority of drugs (Mehanna *et al.*, 2014). Several physical and chemical approaches have been used to enhance percutaneous penetration including iontophoresis, electroporation, sonophoresis, micro-needles, prodrugs, salt formation, ion pairs and cosolvents (Benson, 2005).

Furthermore, lipid-based particulate systems are considered to be a promising candidate for transdermal drug delivery due to their distinctive natures (Kwon *et al.*, 2012). Lipids are amphiphilic molecules that can be spontaneously self-assembled in the aqueous solution feature into different liquid crystalline nanoparticles phases including bicontinuous cubic and inverse hexagonal phases (Barauskas *et al.*, 2010). Nowadays, cubosomes or cubic phase nanoparticles offer outstanding scopes for application as controlled drug delivery vehicles (Dian *et al.*, 2013). The most characterized system of bicontinuous cubic phase forming lipid is the monoglyceride/water system (Sagalowicz *et al.*, 2006). Monoglycerides are defined as non-toxic, biodegradable and biocompatible materials that can self-associate depending on the temperature and aqueous content forming a reversed micellar (L2), a lamellar (Lα), or a bicontinuous cubic phase (C). The bicontinuous cubic phase is classified into three different phases; the double diamond cD, the gyroid cG and the primitive cP phase (Sagalowicz *et al.*, 2006).

Structurally, the cubic phase particles are characterized by a unique nano-architecture, being consisted of two non-intercrossing aqueous domains separated by a highly twisted monoglyceride bilayer (Rizwan *et al.*, 2013). Compared to other lipid-based carriers, cubosomes have a flexibility to incorporate different guest molecules, either hydrophilic, amphiphilic or lipophilic one, within its complex structure (Sherif *et al.*, 2014). Cubosomes are also expected to offer a significant protection against the harsh conditions together with a controlled release of the incorporated molecules (Kwon *et al.*, 2012). Moreover, they were reported to be localized in body cavities, on the skin or different mucosal surfaces (Sherif *et al.*, 2014). However, the interesting property of cubosomes as innovative drug carriers is that they are thermodynamically unstable dispersions (Dian *et al.*, 2013). For processing uniform dispersion vehicles a high energy input *via* homogenization or ultrasonication is required (Spicer *et al.*, 2001). Non-ionic block co-polymer (Poloxamer® 407), polyvinyl alcohol (PVA) and polypeptides were evaluated to stabilize cubosome dispersions (Dian *et al.*, 2013).

The physicochemical features of cubosomes offer an attractive strategy for drug delivery, especially dermal/transdermal system. They were reported to enhance the skin permeation and percutaneous absorption of poorly soluble drugs as indomethacin and triclosan with increasing their effects (Esposito *et al.*, 2005; Kwon *et al.*, 2012). Rattanapak *et al.* (2012), reported the potential of cubosomes in increasing the penetration of a peptide vaccine through stillborn piglet skin in comparison to the others lipid vesicular systems. This feature was attributed to the bioadhesive characteristic and permeation enhancement of their building units (Jin *et al.*, 2013). Furthermore, cubosomes structural organization is the same to that of skin biomembrane, making them superior for dermal/transdermal drug delivery of both hydrophilic and lipophilic ones (Esposito *et al.*, 2005; Sherif *et al.*, 2014). Nevertheless, no published article have emerged the influence of cubosomes in the skin permeation of progesterone so far.

In the view of the above mentioned merits, the appraisal of progesterone- loaded self-assembled liquid crystalline nanoparticles for transdermal application becomes a substantial undertaking. Accordingly, the objective of our study is to fabricate a novel progesterone bearing cubosomes *via* simple processing methods namely; emulsification and solvent precursor dilution. The impact of the selected formulation variables and their interaction on cubosomes properties were explored *via* a 2^3 full factorial design. *In-vitro* characterizations of the prepared self-assembled liquid crystalline nanoparticles were carried out together with *ex-vivo* transdermal permeation using a rabbit ear skin. Furthermore, the physical shelf stability of the nanocarrier over three months was assessed.

2. Materials and methods

2.1. Materials

Progesterone was kindly donated by Pharco Pharmaceuticals Company, Egypt. Peceol® (glyceryl monooleate, GMO), was a kind gift from Gattefosse (Lyon, France). Poloxamer® 407 (PLX) was obtained from BASF chemical company (Ludwigshafen, Germany). Polyvinyl alcohol (PVA) and Propylene glycol were purchased from El-Nasr Pharmaceutical (Cairo, Egypt). All other chemicals were of analytical grade.

2.2. HPLC analysis of progesterone

A validated HPLC method was utilized for quantifying the concentration of progesterone (Maliwal *et al.*, 2009). The HPLC analysis was performed using an Agilent 1200 series (Agilent Technologies, Santa Clara, California, USA) equipped with a reverse phase C18 column (Agilent 5HC-C18 (2) 250 × 4.6 mm) and a photodiode array detector. The isocratic mobile phase, consisting of a mixture of methanol and water (80:20 v/v), was eluted at a flow rate 1 mL/min and the column effluent was detected at a wavelength 243 nm. Under these conditions, the retention time was approximately 7.5 min. The calibration curve of peak area versus concentration was linearly correlated (R^2=0.9996) over the progesterone concentrations (1–70 μg/mL).

2.3. Preliminary investigation

2.3.1. Preparation of progesterone loaded self-assembled liquid crystalline nanoparticles

In our preliminary investigation, two techniques were carried out for the development of progesterone-loaded liquid crystalline nanoparticles (LCNPs), namely; emulsification and solvent precursor dilution methods.