Journal of Molecular Liquids 218 (2016) 219–232

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

Self-nanoemulsifying drug delivery system (SNEDDS) with enhanced solubilization of nystatin for treatment of oral candidiasis: Design, optimization, *in vitro* and *in vivo* evaluation

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ARTICLE INFO

Article history:
Received 25 August 2015
Accepted 16 February 2016
Available online xxxx

Keywords:
Nystatin
Self-nanoemulsifying drug delivery system (SNEDDS)
Poorly water soluble drug
Pseudo-ternary phase diagram
Immunosuppressed mouse-model
Oral candidiasis

ABSTRACT

The aim of the present study is to develop and optimize self-nanoemulsifying drug delivery systems (SNEDDSs) to improve the per-oral bioavailability of poorly soluble polyene antifungal drug, nystatin (NYS), and to evaluate its *in vitro* and *in vivo* performance. Solubility of NYS was estimated in various vehicles to select proper components combinations. Oleic acid (oil), Tween® 20 (Tw20) and Tween® 40 (Tw40) (surfactants) as well as dimethyl sulfoxide (DMSO) and propylene glycol (PG) (co-surfactants) were employed to construct pseudo-ternary phase diagrams. Thermodynamic stability, dispersibility and robustness to dilution tests were performed to optimize formulations from phase diagram. Five optimized formulations composed of oleic acid, Tw20 and DMSO or PG at Smix ratios (1:1, 2:1 or 3:1) were selected. They were spherical in shape of mean droplet size <100 nm with negatively charged zeta potential <-15 mV. The *in vitro* release profile of NYS-SNEDDSs was found significant in comparison to the plain NYS suspension. *In vitro* and *in vivo* evaluations against *Candida albicans* depicted promoted antifungal efficacy of selected NYS-SNEDDS formulations compared to marketed and plain NYS suspensions. The results indicate that NYS loaded SNEDDS, with enhanced solubilization and nanosizing, has potential to improve the absorption of drug and increase its oral antifungal efficacy.

1. Introduction

Gastrointestinal candidiasis (GIC) is one of the most common mucosal infections which may become pathogenic in patients with human immunodeficiency virus (HIV) or acquired immune deficiency syndrome (AIDS) [1,2]. Oropharyngeal candidiasis (OPC) is a common human fungal infection characterized by an overgrowth of *Candida* species in the superficial epithelium of the oral mucosa [3,4]. The vast majority of these infections are caused by *Candida albicans* [5]. Immunocompromised patients, including those with HIV infection or cancer, are at an enhanced risk of OPC [4,6]. In addition, OPC can be triggered in healthy patients by transient risk factors such as antibiotic, corticosteroid treatment or dental prosthesis [7,8]. The infection can be complicated by esophageal candidiasis and, in the worst cases, fungal septicemia [3]. OPC is not a lethal disease but must be treated to avoid chronicity, other tissue invasions or systemic infection [9]. Although it is infrequent, disseminated candidiasis has a mortality rate of 47% [6,10].

Nystatin (NYS) and fluconazole are the most widely employed antifungal agents in the treatment of OPC [5]. NYS is a polyene antifungal antibiotic, one of the oldest antifungal drugs, produced by *Streptomyces noursei* strains [8,11], commonly used for the prophylaxis and treatment of candidiasis. It acts by interfering with the fungal cell membrane of the antibiotic-sensitive organism by binding to sterols, chiefly ergosterol, and the formation of barrel-like membrane spanning channels [12]. NYS possesses a broad antifungal spectrum; it has been reported to be effective against azole-resistant strains of *Candida* and, in some cases, amphotericin B-resistant strains of *C. albicans* [13]. NYS is a yellow or slightly brownish hygroscopic substance [1,14], practically insoluble in water and alcohol, slightly soluble in methanol, and freely soluble in dimethyl formamide and dimethyl sulfoxide [14]. NYS is poorly absorbed from the gastrointestinal tract, and detectable blood concentrations are not obtained after usual doses [15]. Following oral administration, NYS is excreted almost entirely in feces as an unchanged drug [15]. It is not absorbed through the skin or mucous membranes when applied topically [15,16]. The chemical structure of NYS reveals formulation challenges, because it is characterized by the presence of a large lactone ring containing several double bonds conferring an amphiphilic and amphoteric nature [17,18], which in turn contributes to its low solubility in aqueous media and poor bioavailability. Special care needs to be taken for the delivery of this drug as it cannot be simply introduced into an aqueous solution because it forms aggregates that lead to formulation challenges [19]. NYS aggregates formed in aqueous media are non-selective and able to disrupt the integrity of both fungal and mammalian cell membranes, inducing toxicity and host cell death

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http://dx.doi.org/10.1016/j.molliq.2016.02.081

[20]. Therefore, it is proposed that NYS delivery in an un-aggregated form may improve its therapeutic index [19]. For this reason, the controlled-release delivery of this drug is a complex task. To overcome this problem, several approaches have been reported [11]. NYS has been formulated in micellar gels [18], mucoadhesive devices for topical use [21,22], liposomes [23], niosomes [24], nanoemulsions [19,25], lipid intravenous emulsions [11], intralipids [26], nanosuspensions [5], microparticles [27], pellets [1], solid lipid nanoparticles [28] and nanostructured lipid carriers [29].

The use of lipid formulations such as conventional microemulsion and self micro/nano emulsifying drug delivery systems (SMEDDSs/SNEDDSs) have generated much academic and industrial interest as potential formulations for improving the oral bioavailability of drugs [30] and representing a unique solution to delivery of poorly soluble compounds [31]. The advantages of SNEDDSs over the conventional emulsions or other lipid carriers are the significantly reduced energy required for their preparation, their physical stability upon storage [32] and easier to manufacture in a large scale [33]. The SNEDDSs can reduce the limitation of slow and incomplete dissolution of poorly water soluble drugs and facilitate the formation of solubilized phases from which absorption might take place [34]. Hydrophobic drugs can be dissolved in these systems, enabling them to be administered as a unit dosage form for per-oral administration. This leads to *in situ* solubilization of drug that can subsequently be absorbed by lymphatic pathways, by passing the hepatic first-pass effect [35]. The rationale to use SNEDDSs for the delivery of poorly soluble drugs is that, they are presented in the form of pre-concentrated solution. Hence, the dissolution step required for solid crystalline compounds shall be avoided [33,36]. In addition, the formation of a variety of colloidal species on dispersion and subsequent digestion of SNEDDSs facilitates drug absorption [37]. SNEDDSs have been described in the literatures as homogenous (transparent) complex systems consisting of oils, surfactants, co-solvents and water, which are thermodynamically stable [34]. SNEDDSs provide ultra low interfacial tensions and large o/w interfacial areas. Therefore, SNEDDSs have the advantages in possessing higher solubilization capacity than simple micellar solutions, leading to the incorporation of poor water-soluble pharmaceutical inside the oil phase [38]. They rapidly form oil in water (o/w) nanoemulsion when exposed to aqueous media upon gentle agitation or digestive motility of GI tract [35,39]. When a SNEDDS is introduced into the body, it is rapidly dispersed to form droplets of approximately nano-size range (<200 nm) [40]. Furthermore, these particulate delivery systems show a prolonged residence time on mucosal membranes [41] and they could reach greater mucosal surface areas, resulting in a comparatively higher drug uptake [40,42]. Because of the small droplet size, nanoemulsions keep the transparency after aqueous dilution [34].

On the basis of these considerations, the major aim of the present study is the development, optimization, characterization and evaluation of NYS-SNEDDS, with objectives of enhanced solubilization of the poorly soluble drug, NYS, for the treatment of oral fungal infections.

2. Materials and methods

2.1. Materials

NYS was kindly donated by GlaxoSmithKline Co., Cairo, Egypt. Oleic acid (extra-pure, 99%) was purchased from Alpha Chemika, India. Tween® 20 (Tw20) (extra-pure, 99%) was obtained from VWR International, France. Tween® 40 (Tw40) (extra-pure, 99%) and Tween® 60 (Tw60) (extra-pure, 99%) were bought from Sisco Research Laboratories (SRL) PVT Ltd., India. Tween® 80 (Tw80) (extra-pure, 99%) was procured from Riedel-de Haën, Italy. Dimethyl sulfoxide (DMSO) (HPLC grade, 99.5%) was purchased from Tedia, USA. Dimethyl formamide (DMF) (99.5%) was obtained from S.D. Fine-Chem Ltd., India. Propylene glycol (PG) (99.5%) was bought from BDH Laboratory, UK. Methanol (HPLC grade, 99.7%) was procured from Fisher Scientific, UK. All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Solubility study

The solubility of NYS in various oils (Isopropyl myristate, Isopropyl palmitate, Oleic acid, Methyl laurate and Miglyol), surfactants (Tw20, Tw40, Tw60, Tw80, Diacetin, Captex 200 and Span® 80) and co-surfactants (PG, DMSO, Polyethylene glycol (PEG) 400 and Ethanol) was determined. An excess amount of NYS (≈50 mg) was added to 2 g of each component in screw-capped glass vials. The obtained mixtures were mixed continuously for 2 min using vortex mixer (Julabo, Paramix II, Germany) to facilitate proper mixing of NYS with the vehicles. The mixtures were shaken (100 rpm) for 72 h at 25 ± 0.5 °C in a thermostatically controlled shaking water bath (Memmert, SV 1422, Germany) followed by equilibrium for 24 h [31,43]. The equilibrated samples were removed and centrifuged at 5000 rpm for 30 min. The supernatant solution was taken and filtered through a Millipore membrane filter (0.45 μm) and then suitably diluted with methanol. The concentration of NYS was determined spectrophotometrically using UV–Visible recording spectrophotometer (Shimadzu, UV-2401 PC, Japan) at 304 nm using methanol as a blank [14,24]. The experiment was repeated in triplicates.

2.2.2. Preliminary screening of surfactants for their emulsification ability

Different surfactants for the per-oral use were screened for emulsification ability according to the method described by Date and Nagarsenker [44]. Briefly, 300 mg of each selected surfactant (Tw20, Tw40, Tw60 or Tw80) was added to 300 mg of the chosen oily phase (oleic acid). The mixtures were gently heated at 50 °C for homogenization of the components. Fifty mg of each mixture was then diluted with double distilled water to 50 ml in a stoppered volumetric flask to yield fine emulsion. Ease of emulsification was judged by the number of flask inversions required to yield homogenous emulsion. The resulting emulsions were allowed to stand for 2 h and their % transmittance was evaluated spectrophotometrically at 638 nm using double distilled water as a blank. Emulsions were furthermore observed visually for any turbidity or phase separation [45,46].

2.2.3. Construction of pseudo-ternary phase diagrams

Self-nanoemulsifying systems form fine o/w emulsions when introduced into aqueous media with gentle agitation. Surfactant and co-surfactant get preferentially adsorbed at the interface, reduce the tension, and provide mechanical barrier to prevent the globules from coalescence. The decrease in free energy required for emulsion formation consequently improves the thermodynamic stability [47]. On the basis of the solubility studies (Section 2.2.1) and preliminary screening of surfactants (Section 2.2.2), oleic acid was selected as the oil phase, Tw20 and Tw40 as the surfactants and DMSO and PG as the co-surfactants. Double distilled water was used as the aqueous phase for construction of phase diagrams. Pseudo-ternary phase diagrams of mixed surfactant and co-surfactant (Smix), oil, and water but without drug incorporation were plotted, and each of them represents a side of the triangle. For any mixture, the total of surfactant, co-surfactant and oil concentrations always added to 100% [46]. Ternary mixtures with varying compositions of surfactant, co-surfactant, and oil were prepared resulting in a total amount of 1 g. Surfactant and co-surfactant were mixed in three ratios, namely; 1:1, 2:1 and 3:1 (Smix, w/w). For each phase diagram, oil and specific Smix ratio was mixed thoroughly in nine different weight ratios from 1:9 to 9:1 in different glass vials. The nine different combinations of oil and Smix; 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1, were made so that maximum ratios were covered for the study to delineate the boundaries of phase precisely formed in the phase diagrams. Pseudo-ternary phase diagrams were developed using the aqueous titration method. Slow titration with