Preparation and *in vitro* Characterization of Ketoconazole Loaded Solid Lipid Nanoparticles using Temperature Modulated Solidification Technique

Abid MehmoodYousaf*[1],Mehvish Sana[2],Yasser Shahzad[1],Talib Hussain[1], Tariq Mahmood[2] ,Muhammad Jamshaid

[1] Department of Pharmacy, COMSATS University Islamabad, Lahore Campus, 54000, Pakistan.

[2] Faculty of Pharmacy, University of Central Punjab, 1-Khayaban-e-Jinnah, Johar Town, Lahore, 54000, Pakistan.

*Corresponding Author Email: Abid MehmoodYousaf

*E-mail: abid.ucp@hotmail.com

Abstract

The intention of this research was to fabricate and characterize solid lipid nanoparticles (SLNs) laden with poorly water-soluble ketoconazole for oral or topical preparations. All the formulations were fabricated with different weight ratios of ketoconazole, glycerylmonostearate (GMS) and palmitic acid via the temperature modulated solidification technique. The effect of agitator speed and composition on the droplet-size was tested. The release rate of ketoconazole from SLNs was investigated in comparison with the plain drug powder. Other characterization was acquired using XRD, DSC, SEM, TEM and FTIR techniques. With increasing agitator speed or GMS concentration, the droplet-size was reduced. In particular, an SLN formulation containing ketoconazole, GMS and palmitic acid at the weight ratio of 0.2/1.0/1.0 resulted in the mean droplet-size of 326.5 ± 30 nm at 26000 rpm. The release of the drug from theformulation was about 87% in 15 min. The drug existed in the amorphous form in the smooth-surfaced spherical SLNs with no covalent interaction with other constituents. Thus, the SLN formulation might be a potential candidate to be used in oral or topical preparations for improved delivery of ketoconazole.

Keywords

Antifungal, GMS, ketoconazole, poorly water-soluble, release rate, SLNs

1. Introduction

Fungal infections exist across the globe (Brown et al., 2012). Several drugs are available to treat fungal infections; however, azole derivatives are considered the best to combat fungal infections invading skin (Fromtling, 1988). Ketoconazole, a synthetic imidazole, is very effective in the management of

dermatophytosis and mycosis (Dismukes et al., 1983). It was synthesized in 1977 for the first time (Phillips and Rosen, 2001) and presented at Janssen Pharmaceutical (Heeres et al., 1979) to be used as the first azole antifungal substance for the oral administration (Finkel et al., 2009) at the increment of 200-400 mg daily (Becker, 2001). It can be used both via the oral route (Brass et al., 1982, Van der Pas et al., 1983, Borelli et al., 1979) and topical route (Faergemann et al., 2007, Jain et al., 2010, Souto and Müller, 2005). It acts against several species of invading pathogenic fungi such as *Candida* species and *Malassezia furfur* (Huang et al., 1986).Ketoconazole shampoo is useful in the management of seborrheic dermatitis of scalp and numerous body areas (Reider and Fritsch, 2012, Rossi, 2013). Ketoconazole shampoo is also effective in treating androgenic alopecia when given concomitantly with oral 5α-reductase blocker (McElwee and Shapiro, 2012). It may be used either alone or in conjunction with other antifungal drugs in the treatment of alopecia (Khandpur et al., 2002, Piérard-Franchimont et al., 2002). Ketoconazolehas been very effective in eradicating fungal invasions such as athlete's foot, candidiasis, ringworm, jock itch (Phillips and Rosen, 2001), and other systemic infections caused by *Candida*, *Histoplasma*, *Coccidioides* and *Blastomyces*as (Finkel et al., 2009). Ketoconazole also blocks transformation of cholesterol to steroidal hormones such as sexocorticoids, glucocorticoids and mineralocorticoids (Jain et al., 2008, Finkel et al., 2009); thus, it has been successfully employed in the management of progressive prostatic carcinoma (Zelefsky et al., 2011).Furthermore, it has been prescribed in the management of Cushing's syndrome (Loli et al., 1986). It is also helpful in resolving hirsutism problem (Becker, 2001). Ketoconazole binds avidly to albumin and red blood cells by about 83.7% and 15.3%, respectively; therefore, only 1% of the free drug is available for producing pharmacological action (Daneshmend and Warnock, 1988). Ketoconazolebelong tobiopharmaceutical classification system (BCS) class II which has low solubility (<1 mg/ml) in anaqueous media (Tsume et al., 2013). It is a highly lipophilic drug having about 84-99% protein binding property. Moreover, it is a weak base possessing a permeability coefficient of 0.0012; thus, cannot accommodate a therapeutic concentration in the epidermis of skin (Huang et al., 2005). The aqueous solubility and dissolution rate of ketoconazole have been successfully improved using solid dispersion technique (Kanaujia et al., 2011) and inclusion complex formation (Balata et al., 2010). Moreover, self-emulsifying drug delivery system (Bhattacharyya and Bajpai, 2013) and microencapsulation techniques have also been employed for bioavailability enhancement of ketoconazole (Aziz et al., 2007). Nanoemulsions, microemulsions, nanoemulgels and microsponge gels have also been utilized for effective delivery of ketoconazole (Mahtab et al., 2016, Patel et al., 2013, Badawi et al., 2012, Saboji et al., 2011). Conventional preparations of antifungal agents, such as creams and lotions, have demonstrated less effectiveness in the management of fungal invasions. Furthermore, need of time and again application of these preparations to the skin may lead to patient non-compliance (Huang et al., 2005). Therefore, novel approaches for the expedited pervasion of antifungal drugs are desirable.

Nanoparticles (<1 µm diameter) promote the aqueous solubility, release rate and permeation of APIs. Solid lipid nanoparticles (SLNs) are a heterogeneous system in which the solid lipid is disseminated in water with one or more surfactants (Mehnert and Mäder, 2001). SLNs are in the solid form at room temperature (Muller and Lucks, 1997). They have been magnificently utilized in improving bioavailability of various drugs through the oral route (Harde et al., 2011, Kakkar et al., 2011, Severino et al., 2011, Hu et al., 2010). As compared to conventional drug delivery systems, SLNs offer more efficacy, less toxicity and ameliorated biocompatibility (Souto and Müller, 2010).Solid lipid nanoparticles (SLNs), prepared using GMS and Tween 80, have been very successful in improving release rate and oral bioavailability of various drugs such asefavirenz (Gaur et al., 2014), cryptotanshinone (Hu et al., 2010) andvinpocetine (Luo et al., 2006). SLNs protect the drugs from premature degradation in the GIT and ensure greater stability. This ultimately leads to improved bioavailability of the drug when administered via the oral route (Harde et al., 2011). Patel loaded *amphotericin B* in SLNs for enhancing its oral bioavailability (Patel and Patravale, 2011). Moreover, in topical preparations, SLNs possess remarkable capability to pervade through the stratum corneum of the skin; thus, enhancing bioavailability (Jenning et al., 2000b, Wissing and Müller, 2003, Vyas and Khar, 2002, Požnjak, 2011).SLNs have also been extensively utilized for the delivery of several drugs, such as vitamin-A (Jenning et al., 2000a, Jenning et al., 2000b), glucocorticoids (Maia et al., 2000),tretinoin (Shah et al., 2007) and isotretinoin (Liu et al., 2007b), via the topical route. Muller elucidated the use of SLNs in cosmetics and other dermatological preparations (Müller et al., 2002).

SLNs promote drug permeation across the skin (Jenning et al., 2000a, Wissing and Müller, 2003), provide protection from ultraviolet radiations (Wissing and Müller, 2003) and mitigate irritation (Sivaramakrishnan et al., 2004) when used topically. SLNs are preferred over liposomes owing to greater physical stability, cost effectiveness, convenience of scale-up and fabrication (Wissing and Müller, 2003).Furthermore, SLNs are comparable to liposomes in epidermal penetration (Liu et al., 2007b), transport through follicles (Münster et al., 2005) and controlled delivery of drugs (MuÈller et al., 2000). Thus, SLNs

possess several merits, and circumvent demerits of other colloidal systems for drug transportation such as liposomes, polymeric nanoparticles and microemulsions (Utreja and Jain, 2001).

SLNs technique has been successfully utilized to enhance the effective delivery of numerous antifungal drugs such as clotrimazole (Das et al., 2012), fluconazole (Gupta and Vyas, 2012), econazole (Sanna et al., 2007), miconazole (Bhalekar et al., 2009), terbinafine (Vaghasiya et al., 2013) and amphotericin B (Patel and Patravale, 2011) via both the oral and topical routes of administration.

SLNs are very easy to formulate. Several methods such as temperature modulated solidification technique (Patel et al., 2014), high-pressure homogenization (Gupta et al., 2017), high-speed stirring, emulsification-solvent diffusion (Emami et al., 2015), high shear homogenization (Domb, 2005), melt emulsification (Ahlin et al., 1998), hot homogenization (Lander et al., 2000, Jahnke, 2001), cold homogenization (Zur Mühlen, 1996),ultrasonication (Eldem et al., 1991), solvent emulsification (Sjöström and Bergenståhl, 1992), supercritical fluid method (Chen et al., 2006), double emulsion method (Cortesi et al., 2002) and phase inversion temperature method (Gao and McClements, 2016) have been useful in the fabrication of SLNs. In the temperature modulated solidification technique of SLNs formation, usually lipid matrix is first molten by heating to the melting point of the lipid. The drug is dissolved in this molten lipid. The solution is then dispersed in an aqueous solution of a surfactant maintained at the same temperature.After homogenization, the hot dispersion is gradually congealed. As the temperature drops down, the drug-loaded molten lipid solidifies. The drug may exist in the amorphous state in the lipid matrix or may recrystallize with less intensity. As SLNs are usually of < 1 µm particle-size, the loaded drug, if recrystallized, also exist in nanocrystalline state.

Lipids and emulsifiers are part and parcel of SLNs. Various lipids, such as triglycerides tricaprin (Domb, 1995), trilaurin (Westesen and Bunjes, 1995, Bunjes et al., 1996), trimyristin (Müller et al., 1996), tripalmitin (Siekmann and Westesen, 1996), tristearin (Ahlin et al., 1998) and hydrogenated coco glycerides (Almeida et al., 1997)] and hard fats witepsols (Almeida et al., 1997, Westesen et al., 1997), cetylpalmitate (Freitas and Müller, 1998), glycerylmonostearate (Cavalli et al., 1999), glycerylbehenate (Zur Mühlen,

1996), glycerylpalmitostearate (Müller et al., 1996), stearic acid (Bocca et al., 1998), palmitic acid (Gasco et al., 1992), decanoic acid (Gasco et al., 1992), behenic acid (Cavalli et al., 1997) and acidan N12 (Cavalli et al., 1997)] have been exploited in the development of SLNs. Likewise, emulsifiers such as soybean lecithin (Yang et al., 1999), egg lecithin (Gasco, 1993), phosphatidylcholine (Liu et al., 2007a), poloxamers (Almeida et al., 1997, Müller et al., 1997), poloxamine (Müller et al., 1996), tyloxapol (Siekmann and Westesen, 1994), polysorbates (Müller et al., 1997), sodium cholate (Liu et al., 2007a), sodium glycocholate (Bunjes et al., 2003), sodium taurocholate (Cavalli et al., 2002) and sodium docusate (Yuan et al., 2009) have been employed in the fabrication of SLNs.

In the present study, ketoconazole-loaded SLNs were formed with GMS and palmitic acid using temperature modulated solidification technique in conjunction with emulsification-solvent diffusion method and high speed stirring (Patel et al., 2014, Emami et al., 2015). The particle-size and release rates of the formulations were determined. Characterization of the optimized formulation was perused using X-ray diffraction (XRD), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Fourier-transform infrared (FTIR) spectroscopy.

2. Materials and Methods

2.1 Materials

Ketoconazole (assay ≥ 99%) and palmitic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Glyceryl monostearate was from BDH Laboratory Supplies (Poole Dorset, UK). Tween 80 was from Daejung Chemical Co. (Siheung, South Korea). All other chemicals were of reagent grade.

2.2 Preparation of Ketoconazole-Loaded SLNs

Ketoconazole-loaded SLNs were prepared with GMS and palmitic acid using temperature modulated solidification technique in conjunction with emulsification-solvent diffusion method, high speed stirring and hot homogenization (Patel et al., 2014, Emami et al., 2015). The complete composition of ketoconazole loaded SLNs is given in Table 1.

bath, centrifuged (5000xg)for 5 minutes

Table 1.Composition of SLNs.

Components (g)	Formulations						
	K1	K2	K3	K4	K5	K6	K7
Ketoconazole	0.2	0.2	0.2	0.2	0.2	0.2	0.2
GMS	0	0.333	0.666	1	1.333	1.667	2
Palmitic Acid	2	1.667	1.333	1	0.666	0.333	0

Solution of ketoconazole was formed in 15 ml ethanol in a 100 ml beaker at 70°C. Accurately weighed (Weighing balance TX323L, Shimadzu, Kyoto, Japan) quantity of GMS was added to the ethanolic solution of the drug maintained at 70°C followed bypalmitic acid. Both GMS and Palmitic acid were melted at 70°C. To the resultant homogeneous ethanoicmixture of dissolved constituents, 50 ml of 4% (w/v) aqueous solution of Tween 80, previously heated and maintained at 70°C, was added to it and mixed for a minute by using magnetic stirring to get a pre-emulsion. This pre-emulsion was then incorporated into 150 ml of 4% (w/v) aqueous solution of Tween 80 maintained at the same temperature in a 500 ml beaker and homogenized for 10 minutes employing a high-speed homogenizer (SilentCrusher M, Heidolph Instruments, Schwabach, Germany) at 70°C. The resultant dispersion was slowly cooled in an ice-and the supernatant was discarded. The residue, consisting of SLNs, was rinsed thoroughly with cold distilled water and used for further study.

2.3 Effect of Agitator Speed on Droplet-Size of the Scattered Phase

For assessing the influence of agitator speed of the homogenizer (SilentCrusher M, Heidolph Instruments, Schwabach, Germany) on the average droplet-size of the scattered phase, five samples of a formulation consisting of ketoconazole, GMS and palmitic acid (0.2/1.0/1.0, w/w/w) were prepared using the above-mentioned preparation method separately. Each of the five samples was homogenized at a fixed agitator speed of 1000 rpm, 5000 rpm, 10,000 rpm, 15,000 rpm or 26000 rpm. Twenty microliter of each hot dispersion was diluted with 5 ml of 4% (w/v) aqueous solution of Tween 80 maintained at 70°C. One milliter of this diluted dispersion was analyzed using Zetasizer (Malvern instruments, Malvern, UK) and

the average droplet-size of the scattered phase was measured.

2.4 Effect of Relative Quantity of Constituents on Droplet-Size of the Scattered Phase

In order to investigate the impact of relative quantity of GMS and palmiticacid on droplet-size of the scattered phase, several SLN formulations were prepared by the same method with varying quantities of GMS and palmitic acid while keeping ketoconazole quantity constant. The agitator speed was fixed at 26000 rpm. A detailed composition of all the formulations is displayed in Table 1.Twenty microliter of each hot-dispersion was diluted with 5 ml of 4% (w/v) aqueous solution of Tween 80 at 70°C.One milliter of this diluted dispersion was analyzed using a Zetasizer (Malvern instruments, Malvern, UK) and the average droplet-size of the scattered phase was measured.

2.5 Drug Content and Entrapment Efficiency

Ketoconazole, GMS and palmitic acid were completely dissolved to make an absolutely homogeneous system at 70°C in the molten state; therefore, it was considered that the entrapment efficiency was equivalent to the drug content (Luo et al., 2006, Patel et al., 2014). Each SLN formulation, equivalent to 10 mg ketoconazole, was dissolved in 100 ml ethanol in a 100 ml measuring flask and diluted appropriately to 25 µg/ml expected concentration of the drug. This dilution was analyzed by the UV-visible double beam spectrophotometer (Dynamica Scientific, Pagnell, UK) at 222 nm for determining actual concentration. The ketoconazole content in SLNs was calculated by the following formula:

$K_e = K_a/K_t * 100$. ----------Eq. 1

Where, K_e is ketoconazole content, K_a is actual concentration measured by the UV-visible spectrophotometer, and K_t is the theoretical concentration (25 µg/ml). Moreover, drug loading capacity (K_{lc}) was also determined by the following formula:

$K_{lc} = K_l/K_t * 100$. ----------Eq. 2

Where, K_l is actual quantity of ketoconazole entrapped in the inner phase, and K_t is the total quantity of the lipids (GMS and palmitic acid) against K_l plus amount of drug added (Luo et al., 2006, Ramasamy et al., 2012).

2.6 Drug Release Analysis

Each SLN formulation, containing 20 mg ketoconazole, was scattered in 1 ml of 4% (w/v) aqueous solution of Tween 80. This dispersion was transferred to a

dialysis bag (Spectra/Por® dialysis membrane, MWCO 12000, Spectrum Laboratories, Rancho Dominguez, CA, USA) and sealed appropriately. The filled dialysis bag was placed in a glass beaker carrying 100 ml of the phosphate buffer saline (PBS) adjusted at pH 7.4 and temperature maintained at 37±0.5°C by the outer water-bath. The magnetic stirring was adjusted at 100 rpm. At predetermined time intervals, 1 ml sample was withdrawn and the medium was compensated with 1 ml fresh PBS maintained at 37±0.5°C. After filtration (0.2 µm PTFE syringe filter) and appropriate dilution with PBS, 2 ml sample was analyzed by the UV-visible double beam spectrophotometer (Dynamica Scientific, Pagnell, UK) at 222 nm. Blank PBS was used as reference during analysis.

2.7 XRD Analysis

Crystalline aspects of ketoconazole-loaded SLNs, all individual constituents and their physical mixture were assessed using an X-ray diffractometer (D/MAX-2500 PC, Rigaku Corporation; Tokyo, Japan). A Cu-Kα_1 monochromatic radiation source was used by applying a voltage of 50 kV and a current of 100 mA. Each XRD pattern was recorded by scanning in the stretch of 8°-72° with 2θ scanning mode. The scan speed and step size were 5°/minute and 0.02°/second, respectively. The physical mixture was prepared by triturating solid ketoconazole, GMS and palmitic acid in the weight ratio of 0.2/1.0/1.0, respectively.

2.8 DSC Analysis

Thermal features of ketoconazole-loaded SLNs, all individual constituents and physical mixture were examined with a differential scanning calorimeter (DSC Q20, TA Instruments; New Castle, Delaware, USA). Each sample (about 5-10 mg) was properly sealed in an aluminium crucible. Then, the sealed crucible was heated in the stretch of 30-300°C with heating speed of 10°C/minute. A blank aluminum pan with lid was placed as a reference in the heating chamber.

2.9 SEM Analysis

Shape and surface morphology of ketoconazole-loaded SLNs was studied using scanning electron microscope (S-4800, Hitachi, Japan). The double adhesive tape was clung on an aluminum stub. Very minute quantity of SLNs was adhered to the exposed surface of the tape with the help of a common pin and observed after coating.

2.10 TEM Analysis

The morphology of ketoconazole-loaded SLNs was also inspected using a

transmission electron microscope (JEM-1230; JEOL, Tokyo, Japan). After appropriate dilution, sample was applied to a film-coated copper gird. Subsequently, a drop of 3% phosphotungstic acid was poured onto the film and left it to evaporate for 15 minutes. Then, the stained sample was examined using TEM (Ramasamy et al., 2012, Shi et al., 2012).

2.11 FTIR Analysis

Each of ketoconazole-loaded SLNs, individual constituents and physical mixture were analyzed by an FTIR spectrophotometer (Nicolet-6700, Pittsburgh, PA, USA) in the stretch of 400-4000 cm^{-1} with 2 cm^{-1} resolution.

3. Results and Discussion

Solid lipid nanoparticles (SLNs), prepared using GMS and Tween 80, have been very successful in improving release rate and oral bioavailability of various drugs such asefavirenz (Gaur et al., 2014), cryptotanshinone (Hu et al., 2010) and vinpocetine (Luo et al., 2006). In the present study, ketoconazole loaded SLNs were prepared using GMS, palmitic acid and Tween 80. The melting points of ketoconazole, GMS and palmitic acid are 148-152°C , 58-59°C and 62.9°C, respectively. The boiling point of ethanol is 78.37°C. In hot state, SLNs are (o/w) emulsions. The drug is loaded in the oil phase. The solubility of ketoconazole in warm ethanol is 20 mg/ml. The solubility of GMS in ethanol is 0.75 g/ml at 60°C (Organization, 2006). The solubility of palmitic acid in ethanol is 0.319 g/ml at 40°C (Seidell, 1919). In the present work, first of all, the drug was dissolved in a little quantity of ethanol to get a clear solution. This solution was heated at 70°C (i-e, above the melting points of GMS and palmitic acid but below the boiling point of ethanol). Then, GMS was added to it which melted in this hot ethanolic solution of the drug. Subsequently, palmitic acid was added after GMS completely dissolved. To this ternary solution, containing completely dissolved ketoconazole, GMS and palmitic acid, 50 ml of 4% (w/v) aqueous Tween 80 solution (which was previously heated and maintained at70°C) was transferred into it and stirred to get a pre-emulsion. This pre-emulsion was then incorporated into 150 ml of 4% (w/v) aqueous Tween 80 solution at 70°C and homogenized. The benefit of this method is that greater drug loading and entrapment efficiency can be ensured.

3.1 Effect of Agitator Speed on Droplet-Size

The five samples of the formulation

consisting of ketoconazole, GMS and palmitic acid (0.2/1.0/1.0, w/w/w), each sample run at one of the fixed agitator speed of 1000 rpm, 5000 rpm, 10000 rpm, 15000 rpm or 26000 rpm, resulted in the average droplet-size (*n = 3*) of 1273±49 nm, 914±44 nm, 734±40 nm, 478±37 nm and 326±30 nm, respectively (Fig. 1A). It is obvious that with increasing agitator speed, droplet-size was reduced (Yang et al., 2013); therefore, an agitator speed of 26000 rpm was fixed for further investigation.

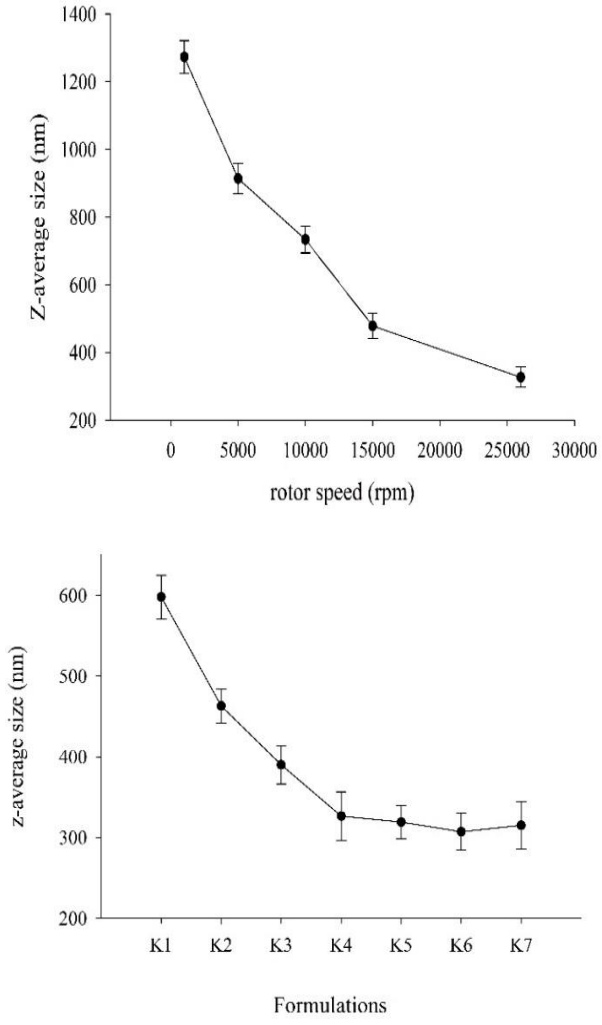

Fig. 1. Effect of rotor speed (A) and formulation composition (B) on the average droplet-size of the inner phase

3.2 Effect of Constituents on Droplet-Size

Effect of the compositions of SLNs on the average droplet-size (*n = 3*) of the inner phase is shown in (Fig. 1B). The full composition of SLNs is displayed in Table 1. With increasing concentration of GMS and decreasing concentration of palmitic acid in formulations K1-K4, the particle size was reduced significantly. This indicated that GMS is more efficiently dispersible than palmitic acid in the aqueous solution of Tween 80. However, further increase in GMS in formulations K4-K7 had no significant influence on droplet-size reduction. The surfactant is essential for reducing interfacial tension between lipid and water in hot state. Moreover, it prevents coalescence during cooling to get an SLN formulation (Zhang et al., 2009).

3.3 Drug Release Analysis

The release profiles of ketoconazole from SLNs formulations are shown in Fig. 2. In 10 minutes, the mean release of ketoconazole (*n = 6*) with plain drug powder, K1, K2, K3, K4, K5, K6 and K7 was 5.06±2.59%, 8.94±2.70%, 28.95±3.87%, 45.40±3.57%, 73.27±8.03%, 78.24±10.16%, 85.48±4.09% and 86.19±5.20%, respectively. With increasing concentration of GMS in formulations

(K1-K4), the release rate was increased, while the results of release rate with formulations K4-K7 were not significantly different from one another. This suggested that these results were in accordance with the droplet-sizes of the formulations. The acceleration in release rate with decreasing droplet-size might be because of the more exposed surface area to the outer medium (Purvis et al., 2006). The initial faster release might be owing to the drug existed in the vicinity of peripheral region of the nanoparticle and/or attached on its surface (Patel et al., 2014, Li et al., 2008).

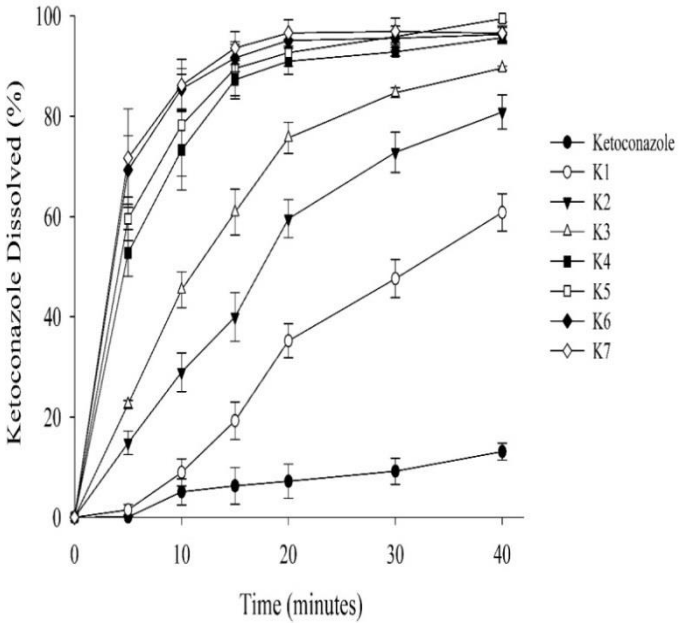

Fig. 2. Release profiles of ketoconazole from various formulations. Each value appears as mean±S.D. (n = 6)

Thus, formulation K4, containing ketoconazole, GMS and palmitic acid at the weight ratio of 0.2/1.0/1.0, was chosen for further study. The release rate of formulation K4 was 73.27±8.03%, which was not significantly different from formulations K5, K6 and K7. Likewise, average droplet-size (*n = 3*) with K4 was 326.50±30.00 nm and a PDI value of 0.259. The droplet-size distribution by intensity of SLNs that ranged from 150 nm to1000 nm as shown in (Fig. 3).

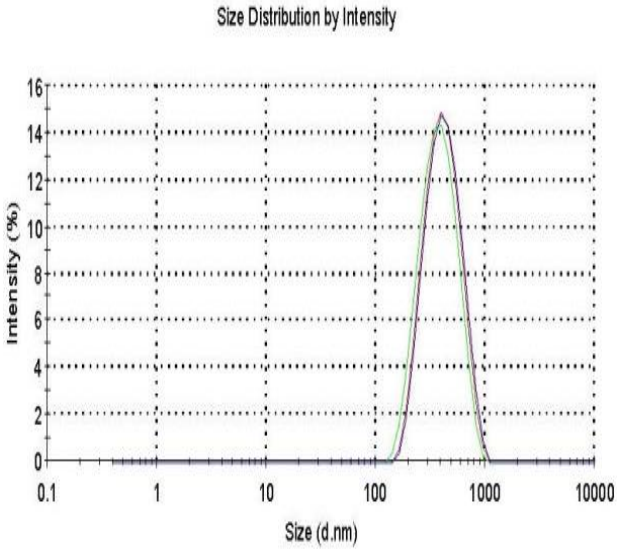

Fig. 3. Size distribution by intensity of SLNs of formulation K4

3.4 Drug Content and Entrapment Efficiency

The drug entrapment efficiency of SLNs preparations was determined. The drug content or entrapment efficiency of K4 formulation calculated was 99.67±0.29 % (*n = 3*). Thus, the entrapment efficiency of SLNs preparation was considered equivalent to the drug content in our studies (Luo et al., 2006, Patel et

al., 2014).

The K4 formulation exhibiting 99.67±0.29% entrapment of the drug was equivalent to 10 mg ketoconazole theoretically; however, the actual drug present in the formulation equivalent to 10 mg ketoconazole was 9.967 mg. The weight of the whole formulation (0.2/1.0/1.0, w/w/w) equivalent to 10 mg drug was 110 mg. Thus, the drug loading capacity calculated was 9.061% (Luo et al., 2006, Ramasamy et al., 2012).

3.5 X-Ray Diffracttometry (XRD)

Crystalline property of the drug and other samples was assessed by the XRD technique. Figure 4, shows the XRD patterns of the samples. Ketoconazole plain drug powder exhibited typical crystalline peaks between about 15°-30° (Fig. 4a). The prominent peaks of GMS wasobserved between 8.5°-25.5°(Fig. 4b).Whereas, Palmitic acid showed very sharp peaks in between 16°-25.5° as shown in figure. 4c. This suggested that both the GMS and palmitic acid were of crystalline or semi-crystalline nature. The distinguishing peaks of ketoconazole in the pattern of physical mixture were located at 16.14°, 16.70°, 26.17° and 27.70° (Fig. 4d). All other peaks were overlapped by those of either GMS or palmitic acid. The appearance of peaks in the pattern of physical mixture was indicative of the existence of crystalline nature of the components. In the pattern of K4 formulation, distinguishing peaks of ketoconazole were absent; however, some peaks related to GMS and palmitic acid appeared in the pattern with reduced intensity (Fig. 4e). This suggested that the drug might be transmuted from crystalline state to the amorphous state in the SLNs. However, GMS and palmitic acid were recrystallized with low intensity might be because of entrapment of the drug.

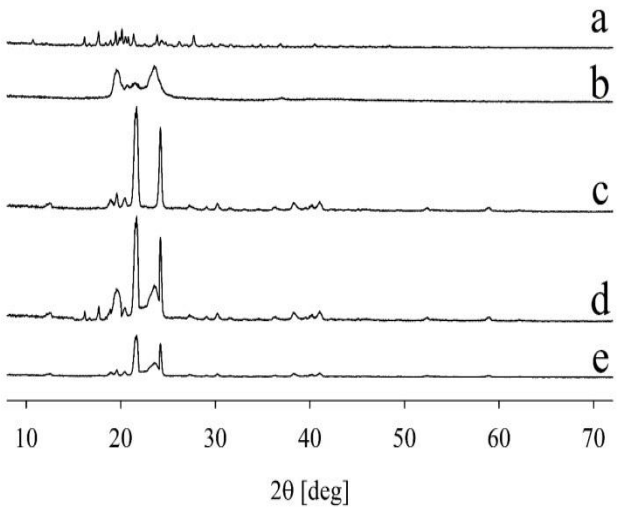

Fig. 4. XRD patterns: (a), ketoconazole; (b), GMS; (c), palmitic acid; (d) physical mixture; (e) SLNs of formulation K4

3.6 Differential Scanning Calorimetry (DSC)

Thermal properties of the samples are shown in (Fig. 5). Ketoconazole produced a deep endotherm at about 150°C commensurate to its melting point confirming its typical crystalline nature

(Fig. 5a). GMS also resulted in a sharp endotherm at about 60°C commensurate to its melting point (Fig. 5b). Palmitic acid gave a deep endotherm at about 65°C commensurate to its melting point (Fig. 5c). These results suggested that all these samples were crystalline in nature. Moreover, melting points of GMS and palmitic acid were very close to each other. Each of these endotherms appeared at the same location in the thermogram of physical mixture (Fig. 5d). Peaks related to GMS and palmitic acid appeared at their respective places in the thermogram of formulation K4 suggesting that they were recrystallized to some extent during SLNs formation (Fig. 5e). However, very interestingly, peak associated with ketoconazole was either vanished completely or diminished too much in the thermogram of SLNs. This confirmed the transmutation of crystalline form of the API into the amorphous form in the SLNs (Fig. 5e).

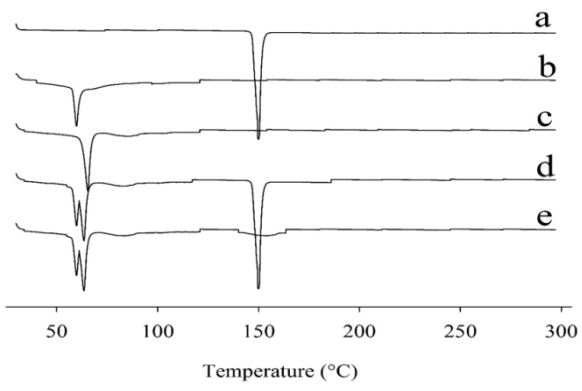

Fig. 5. DSC thermograms: (a), ketoconazole; (b), GMS; (c), palmitic acid; (d) physical mixture; (e) SLNs of formulation K4

3.7 Scanning Electron Microscopy (SEM)

The morphology and surface characteristics of ketoconazole plain powder and SLNs formulation K4 are shown in (Fig. 6A and 6B), respectively. Ketoconazole was consisting of cylindrical particles with irregular shapes and sizes (Fig. 6A). On the other hand, (Fig. 6B) is showing spherical SLNs of about 300 nm size having almost smooth surfaces.

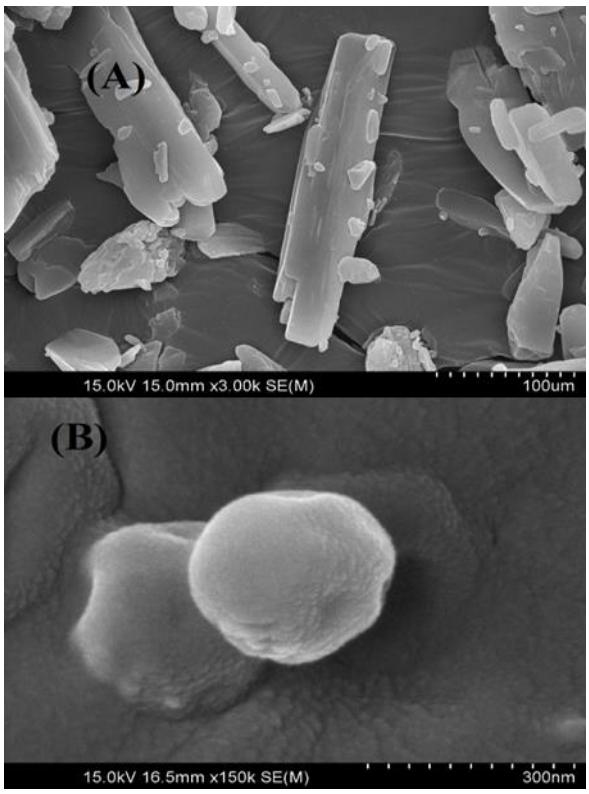

Fig. 6.SEM images: (A) ketoconazole plain powder (x 3000), (B) SLNs of formulation K4 (x 150,000)

3.8 Transmission Electron Microscopy (TEM)

TEM results revealed that the SLNs were spherical in shape and smooth surface

with a few aggregations (Fig. 7). This suggested that most of the particles contributed towards monodispersed behavior. Moreover, there were numerous particles with different sizes. However, each individual particle was of < 350 nm particle size. This seems in well covenant with the particle size determination with Zetasizer.

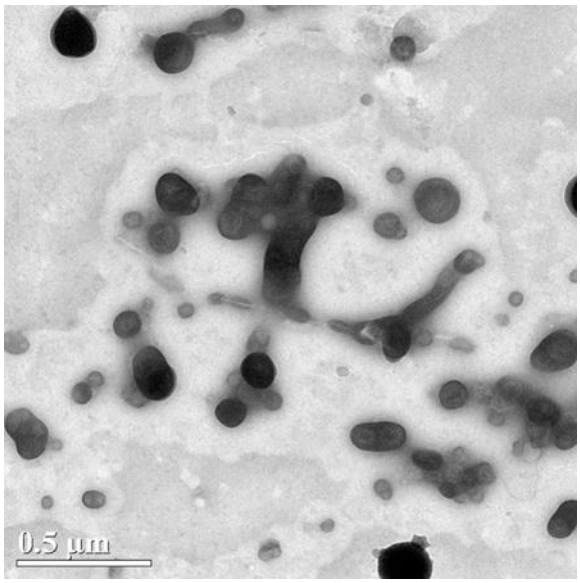

Fig. 7. TEM image of SLNs of formulation K4

3.9 Fourier Transform Infrared Spectroscopy (FTIR)

The drug excipients compatibility studies were carried out using FTIR spectroscopy to detect any possible interaction between pureketoconazole with excipients used in the formulations. FTIR results of ketoconazole, GMS, palmitic acid, physical mixture and SLNs formulation K4 are given in (Figs. 8a, 8b, 8c, 8d and 8e), respectively. The recorded infrared spectra of pure ketoconazole, GMS, palmitic acid, physical mixture and SLNs formulation K4, indicated that no drug-excipients interaction occurred. The chief distinctive peaks of ketoconazole which appeared in the spectrum of physical mixture were located at 585 cm^{-1}, 664 cm^{-1}, 811 cm^{-1}, 901 cm^{-1} and 1645 cm^{-1}. These spikes also witnessed in the spectrum of SLNs; there was no shift of these peaks. Thus, it indicated that ketoconazole had no covalent interaction with GMS or palmitic acid in the SLNs.

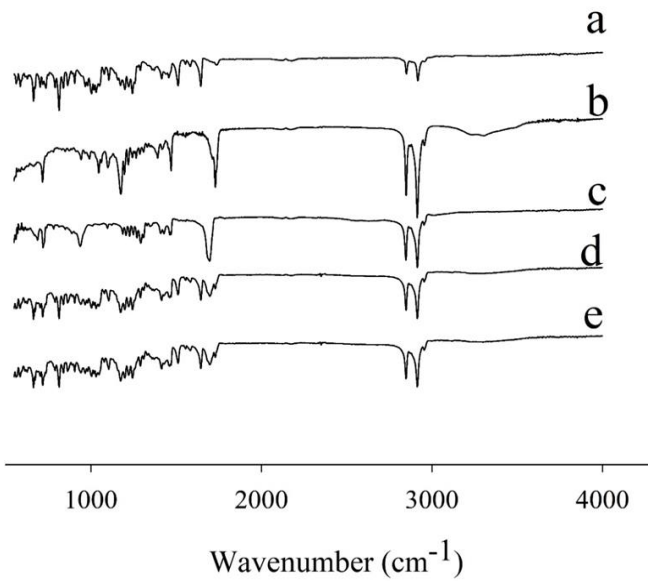

Fig. 8. FTIR spectra: (a), ketoconazole; (b), GMS; (c), palmitic acid; (d) physical mixture; (e) SLNs of formulation K4

4. Conclusions

SLNs formulation K4, consisting of ketoconazole, GMS and palmitic acid (0.2/1.0/1.0, w/w/w), furnished average droplet-size of 326.50±30.00 nm and a

PDI of 0.259, and a release rate of 73.27±10.16% (15-fold quicker than that of the plain drug powder) in 10 minutes. The API existed in the amorphous form in the smooth-surface round-shaped SLNs. The drug had no vigorous bonding with the excipients. The accelerated release rate of ketoconazole in SLNs can be ascribed to: (i) decreased particle-size, (ii) increased surface area exposed to the outer medium, (iii) transmutation of the crystalline drug into its amorphous counterpart or decreased crystallinity, and (iv) presence of the drug in the vicinity of peripheral region of the nanoparticle and/or attached on its surface. Thus, this SLNs formulation can be incorporated into hydrogel, cream, lotion or some other suitable vehicle for topical use or can be utilized in a suitable dosage form to be used orally for more effective delivery of ketoconazole.

References

Ahlin P, Kristl J & Smid-Korbar J (1998). Optimization of Procedure Parameters and Physical Stability of Solid Lipid Nanoparticles in Dispersions. *Acta Pharm,* 48**:** 259-267.

Almeida AJ, Runge S & Müller RH (1997). Peptide-Loaded Solid Lipid Nanoparticles (Sln): Influence of Production Parameters. *Int J Pharm,* 149**:** 255-265.

Aziz HA, Peh KK & Tan YTF (2007). Solubility of Core Materials in Aqueous Polymeric Solution Effect on Microencapsulation of Curcumin. *Drug Dev Ind Pharm,* 33**:** 1263-1272.

Badawi A, Sakran W, Ramadan M & El-Mancy S (2012). Improvement of the Microbiological Activity of Topical Ketoconazole using Microemulsion Systems. *J Drug Deliv Sci Technol,* 22**:** 473-478.

Balata G, Mahdi M & Bakera RA (2010). Improvement of Solubility and Dissolution Properties of Ketoconazole by Solid Dispersions and Inclusion Complexes. *Asian J Pharm Sci,* 5**,** 1-12.

Becker KL (2001). *Principles And Practice Of Endocrinology And Metabolism,* Lippincott Williams & Wilkins.

Bhalekar MR, Pokharkar V, Madgulkar A, Patil N & Patil N (2009). Preparation and Evaluation of Miconazole Nitrate-Loaded Solid Lipid Nanoparticles for Topical Delivery. *Aaps Pharmscitech,* 10**:**289-296.

Bhattacharyya A & Bajpai M (2013). Development and Oral Bioavailability of Self Emulsifying Formulation of Ketoconazole. *Int J Pharm Sci Nanotechnol,* 5**:** 1858-1865.

Bocca C, Caputo O, Cavalli R, Gabriel L, Miglietta A & Gasco MR (1998). Phagocytic Uptake of Fluorescent Stealth and Non-Stealth Solid Lipid Nanoparticles. *Int J Pharm,* 175**:** 185-193.

Borelli D, Bran J, Fuentes J, Legendre R, Leiderman E, Levine H, Restrepo A & Stevens D (1979). Ketoconazole, an Oral Antifungal: Laboratory and Clinical Assessment of Imidazole Drugs. *Postgrad Med J,* 55**:** 657-661.

Brass C, Galgiani J, Blaschke T, Defelice R, O'reilly R & Stevens D (1982). Disposition of Ketoconazole, An Oral Antifungal, in Humans. *Antimicrob Agents Chemother,* 21**:**151-158.

Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG & White TC (2012). Hidden Killers: Human Fungal Infections. *Sci Transl Med,* 4**:** 165rv13-

165rv13.
Bunjes H, Koch MH & Westesen K (2003). Influence of Emulsifiers on the Crystallization of Solid Lipid Nanoparticles. *J Pharm Sci Exp Pharmacol,* 92**:** 1509-1520.
Bunjes H, Westesen K & Koch MH (1996). Crystallization Tendency and Polymorphic Transitions in Triglyceride Nanoparticles. *Int J Pharm,* 129**:** 159-173.
Cavalli R, Caputo O, Carlotti ME, Trotta M, Scarnecchia C & Gasco MR (1997). Sterilization and Freeze-Drying of Drug-Free and Drug-Loaded Solid Lipid Nanoparticles. *Int J Pharm,* 148**:** 47-54.
Cavalli R, Gasco MR, Chetoni P, Burgalassi S & Saettone MF (2002). Solid Lipid Nanoparticles (Sln) as Ocular Delivery System for Tobramycin. *Int J Pharm,* 238**:** 241-245.
Cavalli R, Peira E, Caputo O & Gasco MR (1999). Solid Lipid Nanoparticles as Carriers of Hydrocortisone and Progesterone Complexes with B-Cyclodextrins. *Int J Pharm,* 182**:** 59-69.
Chen Y, Jin R, Zhou Y, Zeng J, Zhang H & Feng Q (2006). Preparation Of Solid Lipid Nanoparticles Loaded With Xionggui Powder-Supercritical Carbon Dioxide Fluid Extraction And Their Evaluation In Vitro Release. *Zhongguo Zhong Yao Za Zhi= Zhongguo Zhongyao Zazhi= China Journal Of Chinese Materia Medica,* 31**:** 376-379.
Cortesi R, Esposito E, Luca G & Nastruzzi C (2002). Production of Lipospheres as Carriers for Bioactive Compounds. *Biomaterials,* 23**:** 2283-2294.
Daneshmend TK & Warnock DW (1988). Clinical Pharmacokinetics of Ketoconazole. *Clin Pharmacokinet,* 14**:** 13-34.
Das S, Ng WK & Tan RB (2012). Are Nanostructured Lipid Carriers (Nlcs) Better than Solid Lipid Nanoparticles (Slns): Development, Characterizations and Comparative Evaluations of Clotrimazole-Loaded Slns and Nlcs? *Eur J Pharm Sci,* 47**:**139-151.
Dismukes WE, Stamm AM, Graybill JR, Craven PC, Stevens DA, Stiller RL, Sarosi GA, Medoff G, Gregg CR & Gallis HA (1983). Treatment Of Systemic Mycoses With Ketoconazole: Emphasis On Toxicity And Clinical Response In 52 Patients. *Ann Intern Med,* 98**:**13-20.
Domb AJ (1995). Long Acting Injectable Oxytetracycline-Liposphere Formulations. *Int J Pharm,* 124**:** 271-278.
Domb AJ (2005). Lipospheres For Controlled Delivery of Substances. *Microencapsulation: Methods and Industrial Applications,* 297.
Eldem T, Speiser P & Hincal A (1991). Optimization of Spray-Dried and-Congealed Lipid Micropellets and Characterization of their Surface Morphology by Scanning Electron Microscopy. *J Pharm Res,* 8**:** 47-54.
Emami J, Mohiti H, Hamishehkar H & Varshosaz J (2015). Formulation and Optimization of Solid Lipid Nanoparticle Formulation for Pulmonary Delivery of Budesonide Using Taguchi and Box-Behnken Design. *Res Pharm Sci,* 10**:** 17.
Faergemann J, Borgers M & Degreef H (2007). A New Ketoconazole Topical Gel Formulation in Seborrhoeic Dermatitis: An Updated Review of the Mechanism. *Expert Opin Pharmacother,* 8**:**1365-1371.
Finkel R, Clark MA & Cubeddu LX (2009). *Pharmacology,* Lippincott Williams & Wilkins.
Freitas C & Müller RH (1998). Spray-Drying of Solid Lipid Nanoparticles (Sln Tm). *Eur J Pharm Biopharm,* 46**:** 145-151.
Fromtling RA (1988). Overview of Medically Important Antifungal Azole Derivatives. *Clin Microbiol Rev,* 1**:** 187-217.
Gao S & Mcclements DJ (2016). Formation and Stability of Solid Lipid

Nanoparticles Fabricated using Phase Inversion Temperature Method. *Colloids Surf A Physicochem Eng Asp,* 499**:** 79-87.

Gasco M, Cavalli R & Carlotti M (1992). Timolol in Lipospheres. *Die Pharmazie,* 47**:** 119-121.

Gasco MR (1993). Method for producing Solid Lipid Microspheres having a Narrow Size Distribution. Google Patents.

Gaur P K, Mishra S, Bajpai M & Mishra A (2014). Enhanced Oral Bioavailability of Efavirenz by Solid Lipid Nanoparticles: In Vitro Drug Release and Pharmacokinetics Studies. *Biomed Res Int,* 2014.

Gupta M & Vyas SP (2012). Development, Characterization and In Vivo Assessment of Effective Lipidic Nanoparticles for Dermal Delivery of Fluconazole against Cutaneous Candidiasis. *Chem Phys Lipids,* 165**:** 454-461.

Gupta S, Kesarla R, Chotai N, Misra A & Omri A (2017). Systematic Approach for the Formulation and Optimization of Solid Lipid Nanoparticles of Efavirenz by High Pressure Homogenization using Design of Experiments for Brain Targeting and Enhanced Bioavailability. *Biomed Research International,* 2017.

Harde H, Das M & Jain S (2011). Solid Lipid Nanoparticles: An Oral Bioavailability Enhancer Vehicle. *Expert Opin Drug Deliv,* 8**:** 1407-1424.

Heeres J, Backx L, Mostmans J & Van Cutsem J (1979). Antimycotic Imidazoles. Part 4. Synthesis and Antifungal Activity of Ketoconazole, A New Potent Orally Active Broad-Spectrum Antifungal Agent. *Open J Med Chem,* 22**:** 1003-1005.

Hu L, Xing Q, Meng J & Shang C (2010). Preparation and Enhanced Oral Bioavailability of Cryptotanshinone-Loaded Solid Lipid Nanoparticles. *Aaps Pharmscitech,* 11**:** 582-587.

Huang X, Tanojo H, Lenn J, Deng CH & Krochmal L (2005). A Novel Foam Vehicle for Delivery of Topical Corticosteroids. *J Am Acad Dermatol,* 53**:**S26-S38.

Huang Y, Colaizzi J, Bierman R, Woestenborghs R & Heykants J (1986). Pharmacokinetics and Dose Proportionality of Ketoconazole in Normal Volunteers. *Antimicrobial Agents And Chemotherapy,* 30**:** 206-210.

Jahnke S (2001). The Theory of High-Pressure Homogenization. *Paperback Apv,* 42**:** 7-30.

Jain A, Gautam SP, Gupta Y, Khambete H & Jain S (2010). Development and Characterization of Ketoconazole Emulgel for Topical Drug Delivery. *Der Pharmacia Sinica,* 1**:**221-231.

Jain SH, Sadow PM, Nosé V & Dluhy R G (2008). A Patient with Ectopic Cortisol Production derived from Malignant Testicular Masses. *Nature Reviews. Endocrinology,* 4**:** 695.

Jenning V, Gysler A, Schäfer-Korting M & Gohla SH (2000a). Vitamin A Loaded Solid Lipid Nanoparticles for Topical Use: Occlusive Properties and Drug Targeting to the Upper Skin. *Eur J Pharm Biopharm,* 49**:** 211-218.

Jenning V, Schäfer-Korting M & Gohla S (2000b). Vitamin A-Loaded Solid Lipid Nanoparticles for Topical Use: Drug Release Properties. *J Control Release,* 66**:** 115-126.

Kakkar V, Singh S, Singla D & Kaur IP (2011). Exploring Solid Lipid Nanoparticles to enhance the Oral Bioavailability of Curcumin. *Mol Nutr Food Res,* 55**:** 495-503.

Kanaujia P, Lau G, Ng WK, Widjaja E, Hanefeld A, Fischbach M, Maio M & Tan RB (2011). Nanoparticle Formation and Growth During In Vitro Dissolution of Ketoconazole Solid Dispersion. *J Pharm Sci,* 100**:** 2876-2885.

Khandpur S, Suman M & Reddy BS (2002). Comparative Efficacy of Various Treatment Regimens for Androgenetic Alopecia in Men. *J Dermatol,* 29**:** 489-

498.
Lander R, Manger W, Scouloudis M, Ku A, Davis C & Lee A (2000). Gaulin Homogenization: A Mechanistic Study. *Biotechnol Prog,* 16**:** 80-85.
Li X, Xu Y, Chen G, Wei P & Ping Q (2008). Plga Nanoparticles for the Oral Delivery of 5-Fluorouracil using High Pressure Homogenization-Emulsification as the Preparation Method and In Vitro/In Vivo Studies. *Drug Dev Ind Pharm,* 34**:** 107-115.
Liu J, Gong T, Wang C, Zhong Z & Zhang Z (2007a). Solid Lipid Nanoparticles Loaded with Insulin by Sodium Cholate-Phosphatidylcholine-Based Mixed Micelles: Preparation and Characterization. *Int J Pharm,* 340**:** 153-162.
Liu J, Hu W, Chen H, Ni Q, Xu H & Yang X (2007b). Isotretinoin-Loaded Solid Lipid Nanoparticles with Skin Targeting for Topical Delivery. *Int J Pharm,* 328**:**191-195.
Loli P, Berselli ME & Tagliaferri M (1986). Use of Ketoconazole in the Treatment of Cushing's Syndrome. *The J Clin Endocrinol Metab,* 63**:**1365-1371.
Luo Y, Chen D, Ren L, Zhao X & Qin J (2006). Solid Lipid Nanoparticles for Enhancing Vinpocetine's Oral Bioavailability. *J Control Release,* 114**:** 53-59.
Mahtab A, Anwar M, Mallick N, Naz Z, Jain GK & Ahmad FJ (2016). Transungual Delivery of Ketoconazole Nanoemulgel for the Effective Management of Onychomycosis. *Aaps Pharmscitech,* 17**:** 1477-1490.
Maia CS, Mehnert W & Schäfer-Korting M (2000). Solid Lipid Nanoparticles as Drug Carriers for Topical Glucocorticoids. *Int J Pharm,* 196**:** 165-167.
Mcelwee KJ & Shapiro J (2012). Promising Therapies for Treating and/or Preventing Androgenic Alopecia. *Skin Therapy Lett,* 17**:** 1-4.
Mehnert W & Mäder K (2001). Solid Lipid Nanoparticles: Production, Characterization and Applications. *Adv Drug Deliv Rev,* 47**:**165-196.
Muèller RH, Maèder K & Gohla S (2000). Solid Lipid Nanoparticles (Sln) for Controlled Drug Delivery–A Review of the State of the Art. *Eur J Pharm Biopharm,* 50**:**161-177.
Muller, R. & Lucks, J. (1997). Arzneistofftrager Aus Festen Lipid-Teilchen, Feste Lipidnanospharen (Sln),(1996) European Patent No. *Ep0605497 (Issued 1996). W. Mehnert, A. Zur Muhlen, A. Dingler, H. Weyhers, Rh Muller, Solid Lipid Nanoparticles (Sln)—Ein Neuartiger Wirkstoff-Carrier Fur Kosmetika Und Pharmazeutika: Ii. Wirkstoff-Inkorporation, Freisetzung Und Sterilisierbarkeit, Pharm. Ind,* 59**,** 511-514.
Müller R, Maaben S, Weyhers H & Mehnert W (1996). Phagocytic Uptake and Cytotoxicity of Solid Lipid Nanoparticles (Sln) Sterically Stabilized with Poloxamine 908 and Poloxamer 407. *J Drug Target,* 4**:**161-170.
Müller RH, Radtke M & Wissing S A (2002). Solid Lipid Nanoparticles (Sln) and Nanostructured Lipid Carriers (Nlc) in Cosmetic and Dermatological Preparations. *Adv Drug Deliv Rev,* 54**:** S131-S155.
Müller RH, Rühl D, Runge S, Schulze-Forster K & Mehnert W (1997). Cytotoxicity of Solid Lipid Nanoparticles as a Function of the Lipid Matrix and the Surfactant. *J Pharm Res,* 14**:** 458-462.
Münster U, Nakamuranachname C, Haberland A, Jores K, Mehnert W, Rummel S, Schaller M, Korting H, Zouboulis CC & Blume-Peytavi U (2005). Ru 58841-Myristate–Prodrug Development for Topical Treatment of Acne and Androgenetic Alopecia. *Pharmazie,* 60**:**8-12.
Organization, WH (2006). *The International Pharmacopoeia,* World Health Organization.

Patel HC, Parmar G, Seth A, Patel J & Patel S (2013). Formulation and Evaluation of O/W Nanoemulsion of Ketoconazole. *Int J Pharm Sci,* 4**:** 123-129.

Patel MN, Lakkadwala S, Majrad MS, Injeti ER, Gollmer SM, Shah ZA, Boddu S HS & Nesamony J (2014). Characterization and Evaluation of 5-Fluorouracil-Loaded Solid Lipid Nanoparticles Prepared via a Temperature-Modulated Solidification Technique. *Aaps Pharmscitech,* 15**:**1498-1508.

Patel PA & Patravale VB (2011). Ambionp: Solid Lipid Nanoparticles of Amphotericin B for Oral Administration. *J Biomed Nanotechnol,* 7**:** 632-639.

Phillips R & Rosen T (2001). Topical Antifungal Agents. *Comprehensive Dermatologic Drug Therapy. 1st Ed. Philadelphia: Wb Saunders***:**497-523.

Piérard-Franchimont C, Goffin V, Henry F, Uhoda I, Braham C & Piérard G (2002). Nudging Hair Shedding by Antidandruff Shampoos. A Comparison of 1% Ketoconazole, 1% Piroctone Olamine and 1% Zinc Pyrithione Formulations. *Int J Cosmet Sci,* 24**:**249-256.

Požnjak J (2011). *Solid Lipid Nanoparticles and Nanostructured Lipid Carriers in Cosmetic and Pharmaceutical Dermal Products.* Farmaceutsko-Biokemijski Fakultet, Sveučilište U Zagrebu.

Purvis T, Vaughn JM, Rogers TL, Chen X, Overhoff KA, Sinswat P, Hu J, Mcconville JT, Johnston KP & Williams RO (2006). Cryogenic Liquids, Nanoparticles, and Microencapsulation. *Int J Pharm,* 324**:**43-50.

Ramasamy T, Khandasami US, Ruttala H & Shanmugam S (2012). Development of Solid Lipid Nanoparticles Enriched Hydrogels for Topical Delivery of Anti-Fungal Agent. *Macromol Res,* 20**:** 682-692.

Reider N & Fritsch P (2012). Other Eczematous Eruptions. *Dermatology,* 1**:**220.

Rossi S (2013). Australian Medicines Handbook (2013 Ed.). Adelaide: The Australian Medicines Handbook Unit Trust. Isbn 978-0-9805790-9-3.

Saboji J, Manvi F, Gadad A & Patel B (2011). Formulation and Evaluation of Ketoconazole Microsponge Gel by Quassi Emulsion Solvent Diffusion. *Cell Tissue Res,* 11**:** 2691.

Sanna V, Gavini E, Cossu M, Rassu G & Giunchedi P (2007). Solid Lipid Nanoparticles (Sln) as Carriers for the Topical Delivery of Econazole Nitrate: In-Vitro Characterization, Ex-Vivo and In-Vivo Studies. *J Pharm Pharmacol,* 59**:** 1057-1064.

Seidell A (1919). *Solubilities Of Inorganic and Organic Compounds C. 2,* D. Van Nostrand Company.

Severino P, Andreani T, Macedo AS, Fangueiro JF, Santana MHA, Silva AM & Souto EB (2011). Current State-Of-Art and New Trends on Lipid Nanoparticles (Sln And Nlc) For Oral Drug Delivery. *J Drug Deliv,* 2012.

Shah KA, Date AA, Joshi MD & Patravale VB (2007). Solid Lipid Nanoparticles (Sln) of Tretinoin: Potential in Topical Delivery. *Int J Pharm,* 345**:** 163-171.

Shi F, Zhao JH, Liu Y, Wang Z, Zhang YT & Feng NP (2012). Preparation and Characterization of Solid Lipid Nanoparticles Loaded with Frankincense and Myrrh Oil. *Int J Nanomedicine,* 7**:**2033-2043.

Siekmann B & Westesen K (1994). Melt-Homogenized Solid Lipid Nanoparticles Stabilized by the Nonionic Surfactant Tyloxapol. I. Preparation and Particle Size Determination. *Pharm Pharmacol Lett,* 3**:** 194-197.

Siekmann B & Westesen K (1996). Investigations on Solid Lipid Nanoparticles prepared by Precipitation in O/W Emulsions. *Eur J Pharm Biopharm,* 42**:**104-109.

Sivaramakrishnan R, Nakamura C, Mehnert W, Korting H, Kramer K & Schäfer-Korting M (2004). Glucocorticoid Entrapment into Lipid Carriers—Characterisation by Parelectric Spectroscopy and Influence on Dermal Uptake. *J Control Release,* 97**:** 493-502.

Sjöström B & Bergenståhl B (1992). Preparation of Submicron Drug Particles in Lecithin-Stabilized O/W Emulsions I. Model Studies of the Precipitation of Cholesteryl Acetate. *Int J Pharm,* 88**:** 53-62.

Souto E & Müller R (2005). Sln and Nlc for Topical Delivery of Ketoconazole. *J Microencapsul,* 22**:** 501-510.

Souto EB & Müller RH (2010). Lipid Nanoparticles: Effect on Bioavailability and Pharmacokinetic Changes. *Drug Delivery.* Springer.

Tsume Y, Amidon G & Takeuchi S (2013). Dissolution Effect of Gastric and Intestinal Ph Fora Bcs Class Ii Drug, Pioglitazone: New In Vitro Dissolution System to Predict In Vivo Dissolution. *J Bioequiv Availab,* 5**:** 224-7.

Utreja S & Jain N (2001). Solid Lipid Nanoparticles. *Advances in Controlled and Novel Drug Delivery. New Delhi, India: Cbs Publishers***,** 408-425.

Vaghasiya H, Kumar A & Sawant K (2013). Development of Solid Lipid Nanoparticles Based Controlled Release System for Topical Delivery of Terbinafine Hydrochloride. *Eur J Pharm Biopharm,* 49**:** 311-322.

Van Der Pas H, Peeters F, Janssens D, Snauwaert E & Van Cutsem J (1983). Treatment of Vaginal Candidosis with Oral Ketoconazole. *Eur J Obstet Gynecol Reprod,* 14**:** 399-404.

Vyas S & Khar R (2002). Nanoparticles. *Targeted and Controlled Drug Delivery—Novel Carrier Systems, Cbs, New Delhi***,** 331-338.

Westesen K & Bunjes H (1995). Do Nanoparticles Prepared from Lipids Solid at Room Temperature always Possess a Solid Lipid Matrix? *Int J Pharm,* 115**:**129-131.

Westesen K, Bunjes H & Koch M (1997). Physicochemical Characterization of Lipid Nanoparticles and Evaluation of their Drug Loading Capacity and Sustained Release Potential. *J Control Release,* 48**:** 223-236.

Wissing SA & Müller RH (2003). The Influence of Solid Lipid Nanoparticles on Skin Hydration and Viscoelasticity–In Vivo Study. *Eur J Pharm Biopharm,* 56**:** 67-72.

Yang KY, Du Hyeong Hwang A MY, Kim DW, Shin YJ, Bae ON, Kim YI, Kim JO, Yong CS & Choi HG (2013). Silymarin-Loaded Solid Nanoparticles Provide Excellent Hepatic Protection: Physicochemical Characterization and In Vivo Evaluation. *Int J Nanomedicine,* 8**:**3333.

Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW & Yang CZ (1999). Body Distribution in Mice of Intravenously Injected Camptothecin Solid Lipid Nanoparticles and Targeting Effect on Brain. *J Control Release,* 59**:** 299-307.

Yuan H, Jiang SP, Du YZ, Miao J, Zhang XG & Hu FQ (2009). Strategic Approaches for Improving Entrapment of Hydrophilic Peptide Drugs by Lipid Nanoparticles. *Colloids And Surfaces B: Biointerfaces,* 70**:** 248-253.

Zelefsky M, Eastham J, Sartor O & Kantoff P (2011). Cancer: Principles & Practice of Oncology. Philadelphia: Lippincott Williams & Wilkins.

Zhang J, Fan Y & Smith E (2009). Experimental Design for the Optimization of Lipid Nanoparticles. *J Pharm Sci,* 98**:** 1813-1819.

Zur Mühlen A (1996). *Feste Lipid Nanopartikel Mit Prolongierter Wirkstoffliberation: Herstellung, Langzeitstabilität, Charakterisierung, Freisetzungsverhalten Und-Mechanismen.*