International Journal of Pharmaceutics 434 (2012) 90–98

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Design and characterization of submicron formulation for a poorly soluble drug: The effect of Vitamin E TPGS and other solubilizers on skin permeability enhancement

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ARTICLE INFO

Article history:
Received 20 January 2012
Received in revised form 19 April 2012
Accepted 15 May 2012
Available online 23 May 2012

Keywords:
Transdermal drug delivery
Wet media milling
Permeation rate
Vitamin E TPGS
Nanoparticles
Crystal growth
Porcine skin

ABSTRACT

In transdermal drug delivery systems (TDDS), it is a challenge to achieve stable and prolonged high permeation rates across the skin since the concentrations of the drug dissolved in the matrix have to be high in order to maintain zero order release kinetics. Several attempts have been reported to improve the permeability of poorly soluble drug compounds using supersaturated systems, however, due to thermodynamic challenges, there was a high tendency for the drug to nucleate immediately after formulating or even during storage. The present study focuses on the efficiency of drug crystals at the submicron/nano range in presence of different solubilizers to improve the permeation rate. Effect of several solubilizers, e.g. Pluronic F-127, Vitamin E TPGS, propylene glycol were studied on the submicron suspension systems of ibuprofen as a model drug. Various stabilizers such as hydroxylpropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP) were examined to evaluate their crystal inhibitory effects on particle growth of the drug compound at submicron range. The overall permeation enhancement process through the skin seems to be influenced by the presence of solubilizers and also the presence of submicron drug crystal. The most promising stable formulation was developed with Vitamin E TPGS + HPMC submicron suspension, which produced higher permeation rate compared to other vehicles.

1. Introduction

Of all the non-invasive routes of administration, the transdermal route seems to be one of the most promising approaches for drug delivery. However, one of the challenges in transdermal drug delivery is the ability to overcome the barrier properties of the skin and to deliver effective amounts of drug for the desired therapeutic action. It is well understood that the stratum corneum (SC), the uppermost dead layer of cells in the epidermal layer, acts as the rate controlling barrier layer for percutaneous drug delivery. The challenge gets more pronounced in the case of poorly soluble drugs.

The basic parameters of the skin affecting the absorption of drug include (i) skin integrity and regional variation, (ii) dimensions of orifices, aqueous pores, and lipidic fluid paths, and (iii) density of appendages. Recently several approaches were used to overcome the skin barrier and allow drugs to reach the desired therapeutic site of action. New delivery systems such as microspheres, micro- and nanoparticles were evaluated with promising results (Toll et al., 2004). Different formulation approaches, such as, microparticles, solid lipid nanoparticles, and nano lipid carriers were also evaluated (Jana et al., 2009; Müller et al., 2002; Mehnert and Mäder, 2001; Mühlen et al., 1998), however, these carriers were not able to penetrate the SC at high concentrations. They were, however, able to deliver drugs to the skin surface and into the hair follicles. On the other hand, ethosomes, niosomes, and transferosomes have been shown to change their morphology and squeeze past the stratum corneum cells and achieve systemic delivery (Alvarez-Román et al., 2004; Rai et al., 2010). The crucial factors that need to be considered for formulation design include drug loading, skin permeability, stability, cost of manufacturing and mode of application. The properties of the drug molecule need to be considered for selecting the best approach.

The mechanism responsible for skin penetration of nano and micro-particles depends in part on the size of the carriers. Previously, it was reported that (Bolzinger et al., 2011) particles below 3 μm in diameter, can penetrate the SC through intracellular pathway and particles ranging from 3–10 μm penetrate through sebaceous follicles. In another study it was shown that when the particle size was higher than 5 μm, almost no penetration was observed through the stratum corneum, however particles with a diameter of about 750 nm demonstrated better permeation into the hair follicle of the human skin (Lademann et al., 2007).

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http://dx.doi.org/10.1016/j.ijpharm.2012.05.031

Studies were also reported for cosmetic formulations containing sunscreens and pigments for make-up products using inorganic particles (titanium dioxide, zinc oxide, etc.) in the nano range (Cross et al., 2007). Solid lipid nanoparticles with smaller diameters (about 208 nm) improved the penetration of diclofenac sodium through rat skin (Liu et al., 2010).

One of the simple approaches studied recently, was to reduce the size of the drug crystals using wet media milling approach. This kind of approach helped to improve the rate of release of drug substance by increasing the surface area of the crystals during the micronization process. Once the particle size decreased, probably to the submicron range, the saturation solubility increased. This increase probably promoted the enhancement of the permeation rate through the skin due to an increased concentration gradient. Drug crystals in nano or submicron range already gained lot of popularity in the pharmaceutical industry for the oral delivery of poorly soluble actives. Recently this formulation principle was applied to cosmetically used compounds such as rutin, hesperidin, resveratrol and ascorbyl palmitate, which are all poorly soluble entities (Mishra et al., 2009; Kobierski et al., 2009). In all these studies the effect of particle size was studied. However, in addition to particle size, skin absorption was also influenced strongly by the type of excipients used in the formulation. Whichever skin penetration pathway is ultimately used by the active moiety, the uptake of drug particles requires adequate wetting and thus the presence of solubilizers/surfactants play an important role in the formulation. In this study the effect of different solubilizers/co solvents such as Vitamin E TPGS, Pluronic F127 and propylene glycol were investigated on the permeability of a drug having a submicron particle size. Studies were reported in the past about the importance of using TPGS to improve the bioavailability of orally administered drugs (Rajebahadur et al., 2006; Varma and Panchagnula, 2005). However, not many reports were published to study its effect on the skin delivery. A systematic study was performed to evaluate the effect from individual components such as particle size of drug crystals and also the type of the vehicle used. Various characterization studies including the permeation rate were performed with these formulations.

Finally during the milling process, highly energized systems are formed due to the increase of surface area of drug compounds. Therefore it is very important to select a proper stabilizer in order to minimize any crystal growth of the submicron/nanoparticles. The most common approaches of stabilization are steric and/or electrostatic technique. Steric stabilization is achieved due to polymer adsorption on the surface of drug molecule. Two different polymeric stabilizers (hydroxypropylmethylcellulose (HPMC 3 cps) and polyvinylpyrrolidone (PVP K-30)) were used in this study to compare their efficiency on crystal growth inhibition and Ibuprofen was used as the model drug. This drug compound is poorly soluble in water and also has a high tendency of crystal growth during or after the size reduction process.

2. Materials and methods

2.1. Materials

Ibuprofen, an anti-inflammatory drug from Doctors Organic Chemical Limited (Tanaku, AP, India), has been used as a model drug in this study. The free base form of this drug is poorly water soluble with an equilibrium water solubility of 0.02 mg/ml and molecular weight of 206.28 g/mol. Among the different excipients used in this study, D-alpha-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS) was obtained from Eastman Chemical. Co. (Kingsport, TN, USA), Pluronic F-127 was obtained from BASF (Florham Park, NJ, USA), propylene glycol (PG) was obtained from Fisher's Scientific (Fair Lawn, NJ), HPMC 3 cps was obtained from Dow Chemical Company (Midland, MI, USA) and PVP K-30 was obtained from BASF (Florham Park, NJ, USA). Deionised water was used as dispersion media. All other materials used were of analytical grade.

2.2. Experimental set-up

2.2.1. Preparation of suspension and particle size reduction of drug crystals

During the manufacturing process, the drug substance and other inactive excipients were first dispersed in the water. Once an uniform suspension was formed, it was wet milled with the ceramic grinding media of 0.2 mm size, using a conventional planetary mill (Model PM400, Retsch GmbH, Germany, equipped with beaker having a chamber volume of 50 ml). The agitation rate of the mill was 400 rpm. High shear force generated during collision of the media with the solid drug particles provides the energy to fracture drug crystals into smaller particles and submicron suspension was formed. The drug loading (5%, w/v) and the ratio between the suspension and the grinding media (1:1, v/v) were kept constant at during this study. The samples were collected at different time points for characterization studies. The details of the formulation design are described in Table 1.

2.2.2. Short term stability study

The submicron formulations were kept on short term stability (2–8 °C; 25 °C and 40 °C) for studying crystal growth. Samples were collected at different time points between 0 and 6 weeks.

2.2.3. Microscopy study

The size of drug crystals in the suspension was studied by Olympus microscope (BX50, Tokyo, Japan) at a magnification of 100×. A drop of sample was placed on a glass slide and a cover slip was placed on the sample to spread the sample uniformly. The image of the sample was taken using an 11.2 Color Mosaic camera (Diagnostic Instruments, Inc.) attached to the microscope.

2.2.4. Particle size analysis

The growth of drug crystals was detected by Photon Correlation Spectroscopy. Photon Correlation Spectroscopy determines velocity distribution of particles movement by measuring dynamic fluctuations of intensity of scattered light. The solution was characterized by intensity-weighted particle size using PCS particle size analyzer (Beckman Coulter, Jersey City, NJ, USA). The cuvette was shaken for about 10 s by hand and placed immediately inside the sample holder of particle size analyzer. Once the required intensity was reached, analysis was performed to get the mean particle size and polydispersity index (PI). Analysis was done in triplicate using similar study protocol (angle – 90°, diluent – water, temp. – 25 °C, run time – 200 s).

2.2.5. Modulated DSC (MDSC)

Modulated differential scanning calorimetry (MDSC) was performed using a differential scanning calorimeter Q1000DSC (TA instruments, New Castle, Delaware, USA). The sample was placed into an aluminum DSC pan, and its weight was accurately recorded. The pan was covered with a lid with pin holes. The measurements were performed in dynamic nitrogen atmosphere with a flow rate of 50 ml/min. The sample was equilibrated at −25 °C and the modulation of ±1.00 °C at every 60 s was applied. Under these conditions, the sample was initially allowed to isothermally equilibrate for additional 8 min, before ramping the temperature until 250 °C (2 °C/min).

Table 1
Formulation design of ibuprofen submicron suspension using different combinations of solubilizer/polymer systems.

Code	TPGS % (w/v)	Pluronic % (w/v)	PG % (w/v)	Drug % (w/v)	HPMC % (w/v)	PVP % (w/v)
F1				5	2	
F2				5		2
F3	5			5	2	
F4	2.5			5	2	
F5	1			5	2	
F6	5			5		2
F7	2.5			5		2
F8	1			5		2
F9		5		5	2	
F10		2.5		5	2	
F11		1		5	2	
F12		5		5		2
F13		2.5		5		2
F14		1		5		2
F15			1	5	2	
F16			1	5		2
F17			25	5	2	
F18			25	5		2

2.2.6. Permeation study

Three different membranes were used for this screening study: (a) silicon membrane of 10 K MWCO (CoTran™ 9728, Membrane Ethylene Vinyl Acetate (EVA) Membrane from 3 M), (b) dialysis membrane of 10 K MWCO (Slide-A-Lyzer Dialysis cassettes from Thermo Scientific) and (c) Regenerated cellulose membrane of 10 K MWCO (Millipore). After washing and equilibration with PBS buffer, the synthetic membranes were mounted on static vertical Franz Diffusion cells – Permegear Inc., Bethlehem, PA (receptor volume 5.1 ml), donor area 0.64 cm^2 by clamping them between the donor and receptor compartments. The receptor compartment was filled with PBS (pH 7.4) which was maintained at $37 \pm 0.5\,^{\circ}C$ and constantly stirred at 600 RPM. Formulation was added (0.5 ml) to the donor compartment at an infinite dose to completely cover the membrane surface. Samples were collected from the receptor compartment at predetermined time points and replaced with equivalent amount of buffer. The drug content in the samples was analyzed by HPLC. In the second part of the study, permeation rates were determined using porcine (pig) skin. Dermatomed (~500 μm) pig skin was obtained from the abdominal regions of young Yorkshire pigs (26.5–28 kg, UMDNJ, Newark, NJ). The skin was stored at $-80\,^{\circ}C$. Prior to each experiment; the skins were allowed to thaw at room temperature, equilibrated and then used immediately for in vitro permeation studies.

2.2.7. HPLC analysis

The assay was determined by using a gradient HPLC (Waters 2695 HPLC system) equipped with UV–vis detector (Waters 2487, Dual I Absorbance Detector) and a C18 column detection (X Terra column, Waters, Ireland, analytical C18 column, 5 μm particle size, 4.6 mm × 150 mm). The mobile phase was a mixture of acetonitrile and phosphate buffer (pH 3.5) with a ratio of 60/40 (v/v). The detection wavelength was 230 nm, the flow rate was 1.2 ml/min and run time was 6 min (Iervolino et al., 2000). The method was validated and the linearity of the calibration curve was recorded.

3. Results and discussion

3.1. Formulation design

As shown in Table 1, several formulations were evaluated using different solubilizers and polymeric stabilizers. Among the solubilizers, Vitamin E TPGS and Pluronic F127 were used as non-ionic surfactants and propylene glycol was used as solubilizer and permeation enhancer. The drug concentration was fixed at 5% (w/v). HPMC 3 cps and PVP K-30 were used as polymeric stabilizers during this study. Both these polymers were used at 2% (w/v) concentration. After about 4 h of wet media milling process, significant particle size reduction was observed for the drug crystals (Fig. 1) and submicron suspension (nanosuspension) was formed.

3.2. Particle size analysis

One of the most important characterization studies of a suspension was the particle size of the drug crystals. The particle size was determined using fixed-angle routine photon correlation spectrometer, PCS. The mean values and also the polydisperisity index (PI) were collected from photon correlation spectroscopic (PCS) analysis. PCS is a very powerful method to detect the size of small particles even at the nano range. During this study samples were analyzed to measure d10, d50 and d90 values at regular intervals during the process. Significant reduction of particle size of the drug crystals was observed with the increase of milling time. After 1–2 h of micronization process, although the d50 of the particles was observed to be in the sub-micron range, however few large crystals were observed and d90 was close to or above 1 μm. However, after 4 h, no large crystals were observed and d90 was close to 500 nm. The steady decrease of polydispersity index (PI) also indicated the gradual elimination of larger drug crystals in the suspension. During the micronization process, the crystals fracturing process continually produces fresh surfaces. The breakage rate was high until 2 h due to the presence of larger crystals. After certain time the

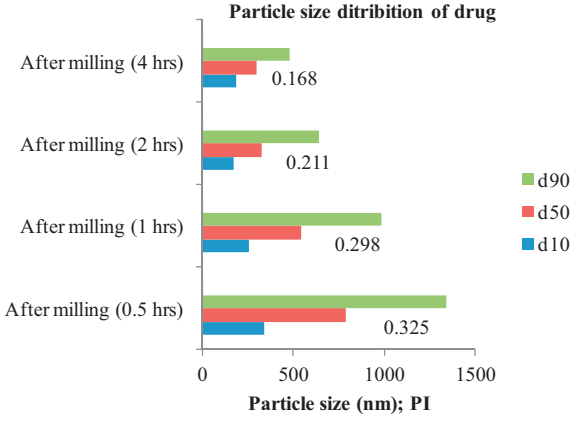

Fig. 1. Particle size distribution of ibuprofen drug crystals in F5 formulation during the micronization process.

number of larger crystals reduced in the suspension and thus he rate of reduction of particle size decreased and became almost constant.

For most of the formulations, a significant reduction of particle size of drug was observed within first few hours of micronization process and submicron size drug crystals were produced. However, a significant effect was observed from the different components used in the formulations. The most effective particle size reduction was observed with the formulation containing HPMC 3 cps or PVP K-30 without any solubilizers. A trend in the increase of particle size was observed when the solubilizer was incorporated into the system, probably due to Ostwald ripening. Also, HPMC 3 cps was shown to stabilize the smaller particles more effectively as compared to PVP K-30. While studying the effect of different solubilizer concentrations (Fig. 2) no significant difference between the particle size was observed with Vitamin E TPGS or Pluronic F127 using HPMC 3 cps as stabilizer. In all cases, the d50 of the drug particles was below 500 nm. However, at the higher concentration of propylene glycol, the size of the drug crystals was significantly larger.

The process of the size reduction of the drug crystals seems to be a complex phenomenon, where multiple effects have to be considered at the same time. Due to the high attrition force, the larger crystals break into small particles and due to the formation of a high surface energy, the smaller particles attempt to agglomerate at the same time. It is therefore very important to understand the properties of the drug for example, the solubility of the drug in the vehicle, the drug interactions with these vehicles and also the nature of the adsorption process. It was observed that the instability of the suspension was directly proportional to the solubility of the drug in that particular system. The increase of solubility of the drug in Vitamin E TPGS or Pluronic F127, in the range of 1–5% (w/v), was not very significant. However, a significant increase in drug solubility was observed in propylene glycol from 1% to 25% (w/v). This explained the reason why using 25% propylene glycol the particle sizes below 500 nm was not observed. Propylene glycol was selected at a 25% (v/v) based on the previous studies (Davis and Hadgraft, 1991), which reported the effective concentration required to obtain sufficient enhancement levels. It was decided to use the solubilizers (Pluronic F127 and Vitamin E TPGS) above their CMC value (critical micelle concentration). Also we selected to use lower concentrations of TPGS and Pluronic F-127 in the formulation because these surfactants could potentially cause skin irritation due to exposure at higher concentration based on the MSDS (Material Safety Data Sheet). However, no systematic study was conducted to identify the threshold concentration to trigger skin irritation.

In addition to the solubility of drug in these vehicles, their adsorption affinity with the drug also needed to be considered for inhibiting crystal growth during the process and also during the storage. Detailed explanations of this interaction mechanism are presented in the following section of stability study.

3.3. Stability study

A short term stability study was performed in order to evaluate the comparative stabilization efficiency of different polymers used in the formulations. The stability study was performed at three different conditions (2–8 °C; 25 °C and 40 °C). The particle size of the samples was tested at initial, 1 week, 3 weeks and 6 weeks time points. During the particle size measurement at different time points, reduction of particle size was observed at higher temperature. This can be explained due to the fact that, higher temperature increased the solubility of active compound and therefore the particle size was slightly reduced due to dissolution effect. When the formulations were stored at low temperature (2–8 °C), the solubility of the active was reduced, leading to crystal growth. Since the purpose of our study was to investigate the influence of different components on crystal growth, therefore lower storage temperature was selected for formulation screening. Similar observation was reported by Mishra et al., 2009.

During the stability study of the formulation containing Vitamin E TPGS, significant growth of particle size was observed with increasing concentrations of TPGS. The instability of the submicron suspension may have been caused by nucleation and particle growth of drug crystals at higher concentration of Vitamin E TPGS. However, at a lower concentration of 1%, no significant growth of particle size was observed (Fig. 3A). HPMC 3 cps was used in suspension as polymeric stabilizer (2%, w/v). HPMC 3 cps polymer may have been adsorbed onto the drug crystals due to the interaction of its hydrophobic (methoxyl) and hydrophilic (hydroxypropyl) groups with the drug molecules. The formation of this hydrogen bonding between the drug and the stabilizer is most probably responsible for stabilizing the highly energized crystals. Similar effects were observed for Pluronic F-127. However, the growth of particle size was comparatively faster when higher concentrations of Pluronic were used (Fig. 3B).

During the storage of these formulations, two important factors needed to be considered. The micronized particles have a tendency to grow in size due to Ostwald ripening. At the same time, solubilizers were adsorbed on the surface of drug by steric interaction. However, as the storage time increased, steric stabilization became weaker and thus crystal growth occurred. In this study lower amount of polymer was used (2%), which probably did not play a significant role on the diffusional resistance on the drug molecules. Transferring the suspension to a suitable gel formulation containing high viscosity polymers may improve the stability of the formulation by inducing diffusional resistance.

3.4. MDSC study

One of the critical factors that need to be considered during the particle size reduction process for compounds such as ibuprofen, which exhibits a low melting point, is the conversion of the drug substance into the amorphous state due to crystal lattice structure breakdown. Since the mobility of the drug is higher in amorphous phase as compared to crystalline phase, therefore crystalline drug is more preferable in the final product to avoid stability issues. Previous reports had shown the influence of nano sizing on the polymorphic properties of drug substance, e.g. the effect of milling on the changes of solid state of indomethacin (Sharma et al., 2009).

The modulated differential scanning calorimetry (MDSC) study was performed with submicron suspensions formulated with the different vehicles. The results showed no change of crystallinity of the drug substance. Also no change of melting pointing was observed after milling. An additional peak was observed for Pluronic F127 system, close to its melting point (55 °C).

3.5. Membrane selection study

The goal of this study was to identify a synthetic membrane that would allow the permeation of small compounds, such as ibuprofen. Although synthetic membranes are not identical to biological tissues, they can still be used as an initial screen to differentiate formulations and the relative permeability of drugs.

All membranes used were hydrated in PBS buffer for 30 min prior to use. Permeability rate was highest for dialysis membrane followed by regenerated cellulose membrane and finally for the silicone membrane (Fig. 4). Based on this study, the silicone membrane was selected for further screening experiments.

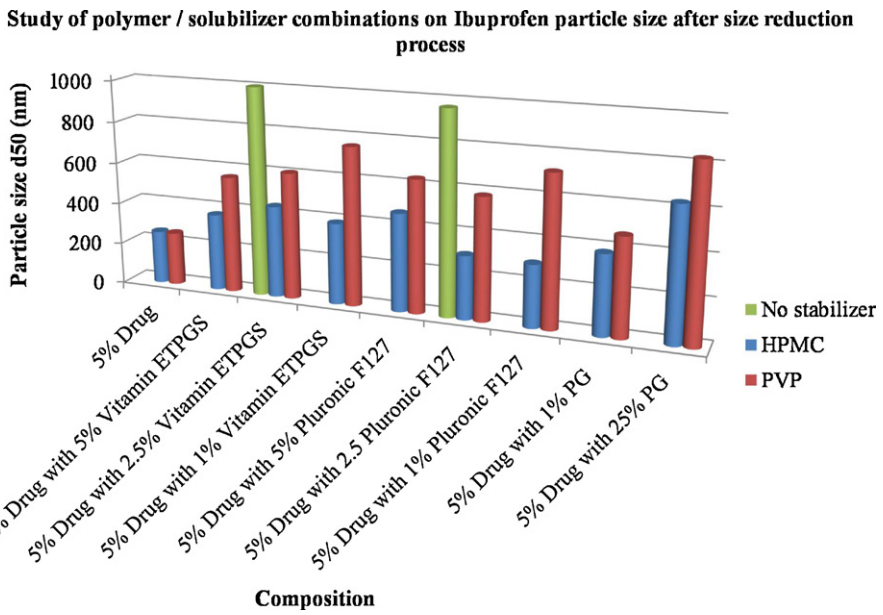

Fig. 2. Effect of different solubilizers/polymers on the efficiency of particle size reduction of ibuprofen drug crystals.

3.6. *In vitro permeation study*

The permeation rate and enhancement ratio were determined for the different formulations tested. Fick's law ($Js = DKCs/h$) describes the flux (J) across a rate-limiting barrier (of thickness, h) in sink conditions and solubility (Cs), lipophilicity (partition coefficient, K), and the molecular weight or size (diffusion coefficient, D). Another important parameter calculated was the enhancement

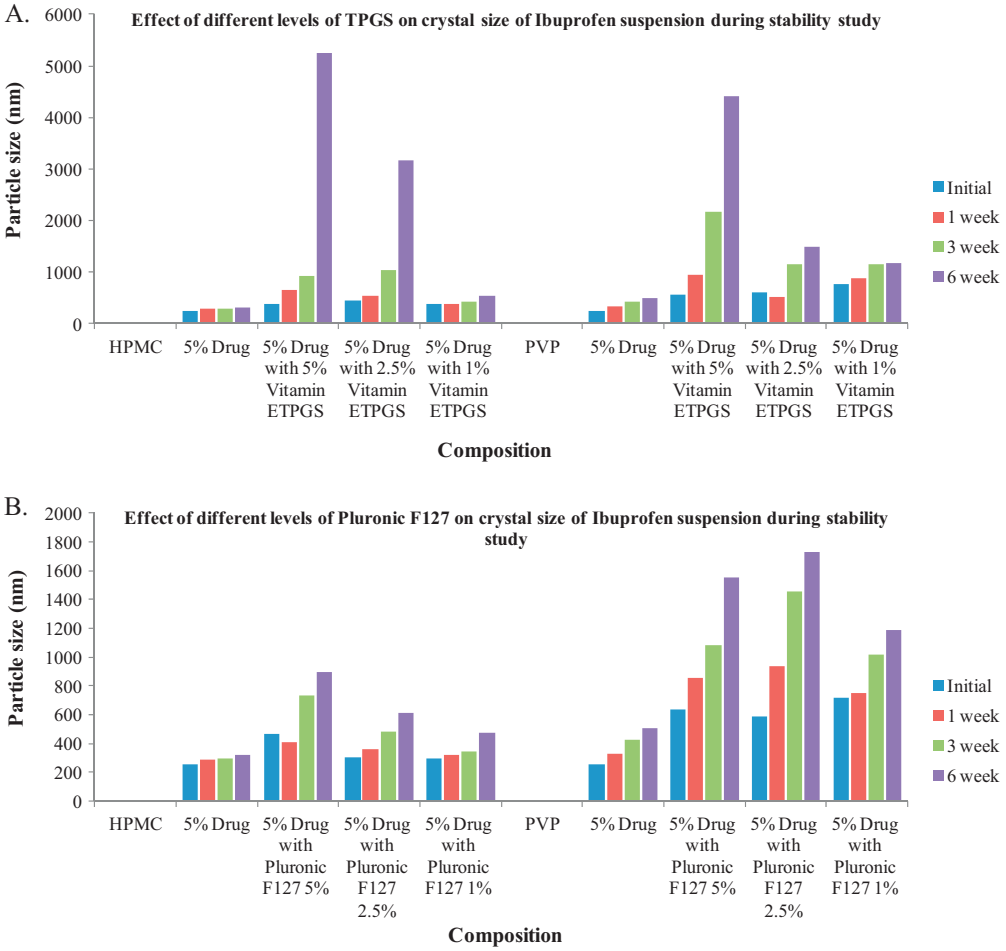

Fig. 3. Study of different concentration of solubilizers on the growth of ibuprofen drug crystals during stability study (A: effect of TPGS; B: effect of Pluronic F127).

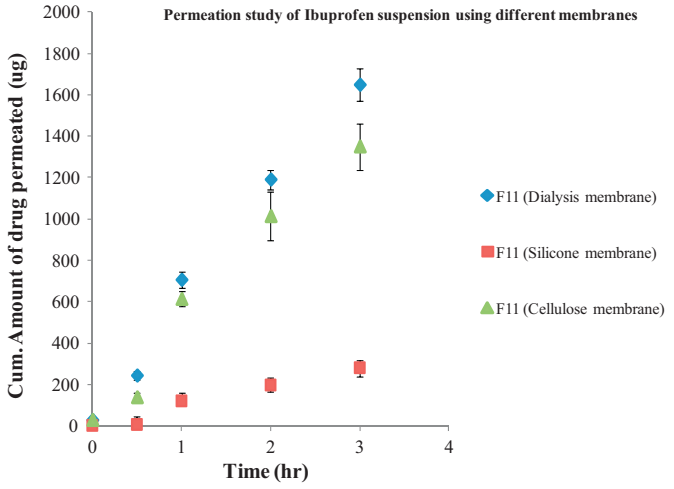

Fig. 4. Permeation study of ibuprofen submicron suspension through the synthetic membranes.

ratio (ER), which is defined as the ratio between the mean flux of the submicron system and the mean flux of the control (un-micronized suspension with or without any solubilizer). These permeability parameters were estimated using the following equations:

(a) Flux, *Jss* (μg/cm^2/h) was calculated from the slope of the cumulative drug amount permeated through the membrane (0.64 cm^2) versus time plot. The results were multiplied by a factor (1.56) in order to represent the data as μg/cm^2/h.
(b) Enhancement ratio, ER using the equation; ER = *Jss* of test sample/*Jss* of control sample (un-micronized suspension with or without the corresponding vehicle).

While evaluating the effect of the polymeric stabilizer, the permeation rate of the drug through the membrane was found to be higher when HPMC 3 cps (Fig. 5) was used in the formulation, and this was probably due to the crystal growth inhibition. This observation was in agreement with the stability study performed earlier. Therefore, HPMC 3 cps was identified as a potential stabilizer to inhibit the crystal growth and also improve the permeability rate of the drug through the skin.

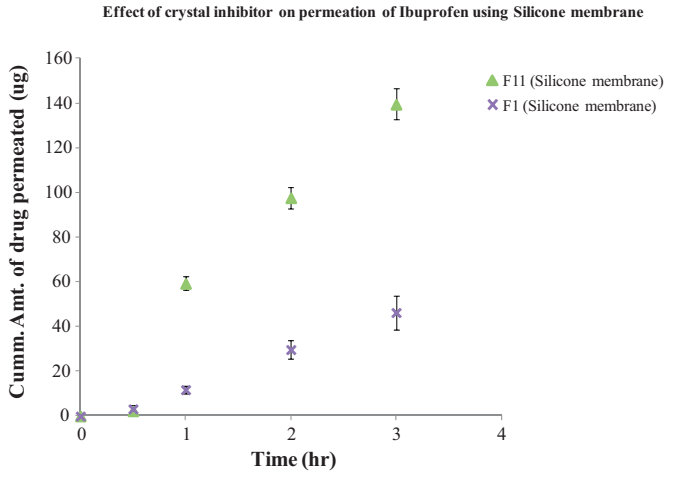

Fig. 5. Effect of crystal inhibitor on the permeation rate of ibuprofen submicron suspension through the synthetic membrane:.

Table 2
Effect of particle size of drug crystals on permeation parameters using porcine skin ($n = 3$).

Variant	Flux, *Jss* (μg/cm^2/h)
Vitamin E TPGS suspension	
365 nm	64.8 (SD-5.5)
655 nm	56.7 (SD-0.9)
895 nm	38.0 (SD-2.3)

3.7. *In vitro permeation study using porcine skin*

3.7.1. *Effect of particle size*

A study was performed to evaluate the effect from the drug crystal particle size on the drug permeability through the skin. Samples were collected at regular intervals (0, 15, 30, 45, 60, 120 and 180 min) during the micronization process. In the earlier part of this study, significant particle size reduction of the drug was observed during the first 1–2 h of milling. With the increase of milling time, large residual particles in the suspension were actually reduced into smaller particles. The samples were collected at 15 min, 30 min and 180 min, produced particle size having d50 values close to 891 nm, 655 nm and 365 nm respectively. These samples were evaluated for permeation study using porcine skin. As shown in Fig. 6 significant influence of drug crystal size was observed on the permeation profile of drug though the skin. The flux values are shown in Table 2. While comparing the flux between the submicron formulations (365 nm and 895 nm) the difference observed was statistically significant from *t* test ($p < 0.005$). In the past, studies were reported to explain correlations between particle size and penetration routes. In one study the in vitro permeation profile of nanoparticles (40–1500 nm) was investigated using human skin samples. It was shown that 40 nm nanoparticles penetrated the skin via the follicular route; however limited penetration was observed for 750 nm particles due to the tight network of epidermal Langerhan's cells (Vogt et al., 2006). Similarly in another study, it was shown that hair follicles and sweat ducts provided the main route for minoxidil-loaded nanoparticles to penetrate through the skin. The enhancement was promoted when the size of the particles was decreased from 130 nm to 40 nm (Shim et al., 2004). Thus follicular transport was proved to be a potential pathway for nanoparticles having less than 100 nm size.

However, in this research effective drug penetration through the skin was observed even with larger particle size (>100 nm). In this study we actually followed an alternative approach. By reducing the size of the particles to nano range, we tried to increase the dissolution velocity and hence the concentration gradient of the drug between the drug crystal and the skin. Thus we are able to enhance the penetration rate of the drug by using the whole stratum corneum as penetration route and not just depending on the limited number of follicles. Additionally Vitamin ETPGS also helped to promote the diffusion of the drug through the skin, as explained in the following section.

In the past, particles were defined as "nanoparticles" if their size (d90) was below 1 μm. Recently, an additional class has been introduced which was named "sub-micron particles". As per this current classification system, the particles are divided into three groups – nanoparticles (less than 100 nm), submicron particles (100 nm–1 μm) and microparticles (1 μm–1 mm) (Bolzinger Marie-Alexandrine, et al.). Our permeation study was performed using the drug crystals in the submicron range (250–750 nm).

3.7.2. *Effect of solubilizers*

While studying the effect of different solubilizers, the highest permeability was observed with Vitamin E TPGS (Fig. 7A), followed by Pluronic F127 and finally with propylene glycol. The

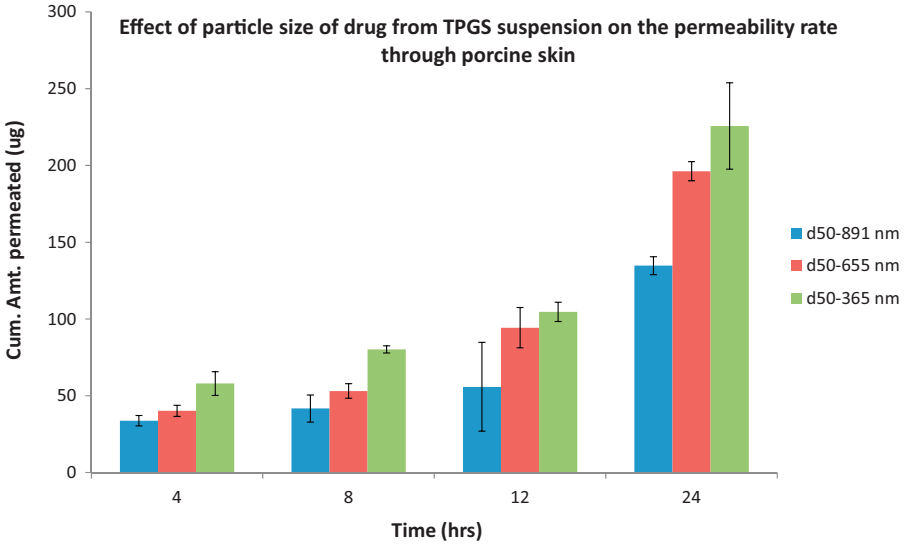

Fig. 6. Effect of particle size of drug crystals (using TPGS–HPMC suspension) on the permeability of ibuprofen through the pig skin.

Table 3
Estimation of permeation parameters from micronized and non-micronized suspension in presence of various solubilizer/stabilizer.

Formulation	Flux, *Jss* (μg/cm²/h)		Enhancement ratio due to micronization; ER
	Before micronization	After micronization	
Suspension (drug + HPMC)	1.6 (SD-0.56)	16.3 (SD-0.9)	10.2
Suspension (propylene glycol + HPMC)	11.1 (SD-1.07)	14.5 (SD-1.4)	1.3
Suspension (Vitamin E TPGS + HPMC)	29.7 (SD-1.06)	46.3 (SD-4.4)	1.6
Suspension (Pluronic F-127 + HPMC)	17.0 (SD-0.99)	26.7 (SD-0.6)	1.6

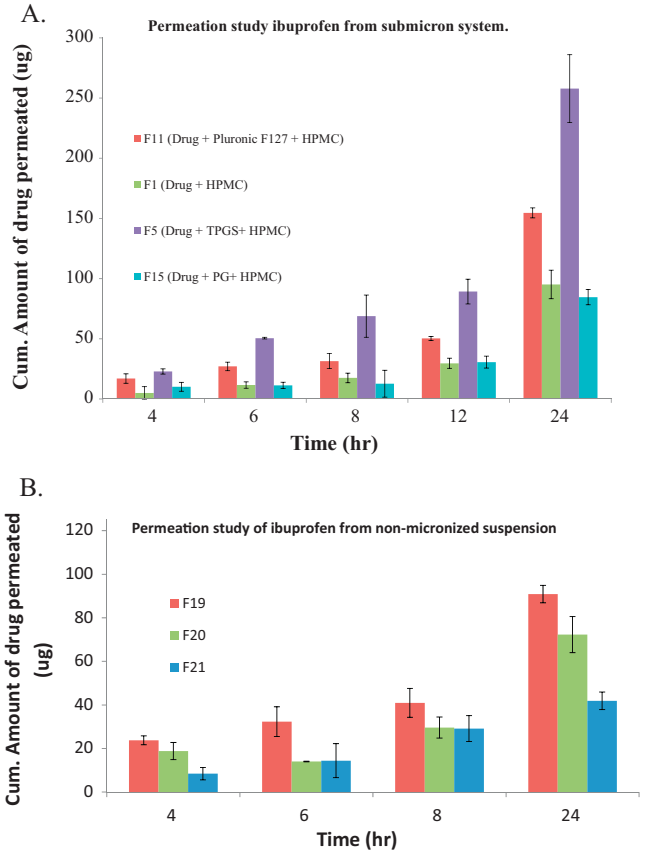

Fig. 7. Permeation study of ibuprofen suspensions through the porcine skin (A: submicron suspensions; B: non-micronized suspensions).

flux observed for Vitamin E TPGS was 46.3 μg/cm²/h (SD-4.4; $n = 3$) compared to 26.7 μg/cm²/h (SD-0.6; $n = 3$) for Pluronic F127 and 14.5 μg/cm²/h (SD-1.4; $n = 3$) for propylene glycol (Table 3). The system without any solubilizer showed lower flux values of 16.3 μg/cm²/h (SD-0.9; $n = 3$).

Based on the above results, an additional study was conducted in order to identify the critical factor between submicron drug particle and effect of solubilizer, responsible for the permeability enhancement of the drug. In this study the permeation experiment was carried out using the three solubilizers at similar concentrations used earlier (Table 4), however, without any nanosizing process. The non-micronized suspension demonstrated similar trends, with the highest permeability observed with Vitamin E TPGS, followed by Pluronic and PG (Fig. 7B).

The flux observed for Vitamin E TPGS was 29.7 μg/cm²/h (SD-1.1; $n = 3$) compared to 17.0 μg/cm²/h (SD-1.0; $n = 3$) for Pluronic F127 and 11.1 μg/cm²/h (SD-1.1; $n = 3$) for propylene glycol (Table 3). Significantly low flux of 1.6 μg/cm²/h (SD-0.6; $n = 3$) was observed for the system that did not contain any solubilizer.

From the estimated enhancement factor, ER (Table 3), it was observed that the effect of solubilizers on the permeability enhancement appeared to be equally critical as compared to the nanosizing process and Vitamin E TPGS was observed to be the most effective permeation enhancer.

Table 4
Formulation design of ibuprofen non-micronized suspensions.

Formulation code	Solubilizer	Stabilizer
F19	Vitamin E TPGS (1%)	HPMC 3 cps (2%)
F20	Pluronic F127 (1%)	HPMC 3 cps (2%)
F21	Propylene glycol (25%)	HPMC 3 cps (2%)

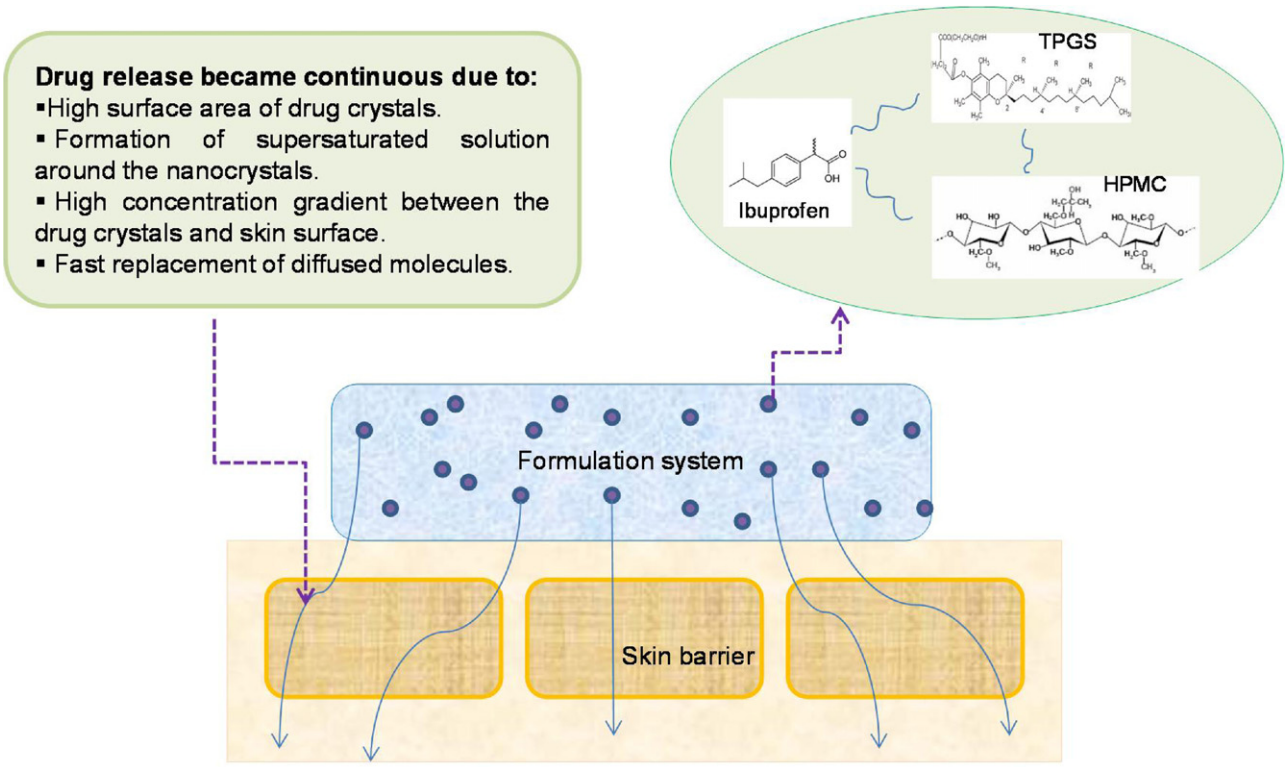

Fig. 8. Mechanism of permeation profile of ibuprofen drug crystals from the submicron suspension system.

Vitamin E TPGS (TPGS, D-alpha-tocopheryl polyethylene glycol 1000 succinate) has been utilized for numerous applications in pharmaceutical dosage forms. Previous studies have reported the importance of Vitamin E TPGS for improving the absorption of drugs when it was administered orally (Yu et al., 1999). However, very few studies were reported to demonstrate the influence of TPGS on skin permeability enhancement. In one study (Sheu et al., 2003), it was reported that although TPGS was able to improve the solubility of estradiol, however it was not responsible for the penetration enhancement when compared to effect from alcohol. In our study, TPGS plays an important role in promoting diffusion by altering the skin structure (D), by modifying partition phenomena (making the barrier more lipophilic (K)) and thereby reducing the interfacial tension and decreasing the SC barrier allowing poorly water soluble drugs such as ibuprofen to pass through the skin. Thus, the flux was enhanced significantly by simultaneous combination of the above mechanisms. More detailed study need be done in the future to identify the actual mechanism of interaction of TPGS with the skin.

Propylene glycol was reported by Herkenne et al. (2008) to have a similar effect during the permeation of the drug through the skin; however its effect appeared to be less compared to that of Vitamin E TPGS. Pluronic F127 on the other hand had little or no effect on the alteration of skin structure. Also polymers such as HPMC 3 cps were used to inhibit nucleation on the surface of the skin.

Therefore, the overall permeation enhancement process through the skin seems to be influenced by the presence of solubilizers and also the presence of submicron drug crystals. Both factors resulted in higher drug release due to the formation of a supersaturated solution around the crystals and thus a high concentration gradient between the drug and skin surface. Similar mechanism was explained by Müller et al. (2011) while discussing the penetration mechanism of nanoparticles through the biological membranes. Fast replacement of diffused molecules occurred due to rapid and continuing dissolution from the new crystal surface generated and thus drug release became continuous as shown in Fig. 8.

4. Conclusion

During the wet media milling process the drug crystal size was reduced into the submicron range. The resulting high surface area resulted in a higher and continuous drug release from the formulation into the external phase due to the constant driving force. In addition, the components used in the system also significantly influenced the drug delivery from the formulations. The improvement of the wettability of the poorly soluble drug probably affected the mobility parameters through the skin. The most promising formulation was developed with Vitamin E TPGS, which produced higher permeation rates compared to other vehicles tested. Along with TPGS, HPMC 3 cps also stabilized the submicron particles. In conclusion, a number of factors including the particle size of the drug crystals, nature and surface properties of the carrier, interaction with the stabilizer have to be considered while designing a suitable submicron dermal formulation for poorly soluble compounds.

Acknowledgements

The authors would like to thank undergraduates Theresa Bello and Ami Shah from Ernest Mario School of Pharmacy and Joshua Wang from Biomedical Engineering for their support with the in vitro permeation experiments and HPLC studies.

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