DESIGN AND EVALUATION OF SPECIAL DRUG DELIVERY TECHNIQUES OF POORLY SOLUBLE DRUG FOR ENHANCING SKIN PERMEABILITY.

By

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A Dissertation submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Graduate Program in Pharmaceutical Sciences

written under the direction of

Professor Bozena Michniak-Kohn, Ph.D.

and approved by

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New Brunswick, New Jersey

May 2012

ABSTRACT OF THE DISSERTATION

Design and Evaluation of Special Drug Delivery Techniques of Poorly Soluble Drug for Enhancing Skin Permeability.

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Dissertation Director: Professor Bozena Michniak, Ph.D.

In transdermal drug delivery systems (TDDS), it is a challenge to achieve stable and prolonged high permeation rates across skin since the concentration of the drug dissolved in the matrix has to be high in order to maintain zero order release kinetics of the drug. In case of poorly soluble drugs, due to thermodynamic challenges, there is a high tendency for the drug to nucleate immediately after formulating or even during storage. In this research we have designed two potential techniques – supersaturated solution and submicron suspension using Vitamin E TPGS / HPMC and other solubilizer / polymer systems in order to enhance the permeability of poorly soluble drug, like ibuprofen, through the skin.

A promising supersaturated formulation was developed with vitamin E TPGS, which produced better results as compared to propylene glycol (PG) or Pluronic F-127 formulations during *in vitro* permeation studies using synthetic membrane or porcine skin. In presence of polymeric stabilizer, the onset of crystallization was delayed. The optimization of the formulation with HPMC 3 cps resulted in inhibiting crystal growth during stability studies.

In the second technique, top down wet media milling process was used to micronize the drug crystal into the submicron range. The resulting high surface area demonstrated 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 significantly influenced the drug delivery from the formulations due to the formation of a supersaturated solution around the crystals and thus a high concentration gradient was maintained between the drug and skin surface. The most promising formulation was developed with Vitamin E TPGS, which produced higher permeation rates compared to other vehicles tested. Besides increasing the solubility of the drug, vitamin E TPGS also played an important role in promoting diffusion by altering the skin structure, by modifying partition phenomena and thus making the barrier more lipophilic.

Based on the above results, we designed gel formulations of supersaturated solution and submicron suspension with Vitamin E TPGS / HPMC. The submicron gel system produced higher permeation rate as compared to supersaturated gel formulations. Based on this observation, the submicron gel system was selected for a DOE study. From this study, a clear correlation was observed between the Vitamin E TPGS and particle size of submicron crystals with the permeation rate (flux) of ibuprofen through the porcine skin. In summary, a number of factors including the particle size of the drug crystals, surface properties of the carrier, interaction of drug molecule with the stabilizer needed to be considered while designing a suitable dermal formulation for poorly soluble compound. This study confirmed the potential benefit of the formulations for the enhancement of therapeutic action of ibuprofen as compared to other conventional drug delivery systems.

Acknowledgement

I would like to thank everyone who contributed significantly during my graduate school education, especially all of the wonderful professors who taught me, Novartis Pharmaceuticals who funded and supported me and all of my family and friends who encouraged me. I would like to thank the Department of Pharmaceutics at the Ernest Mario School of Pharmacy for giving me the opportunity to do my PhD program.

I would like to thank my advisor, Dr. Bozena Michniak-Kohn, who gave me the chance into the Pharmaceutical Sciences program, supported me intellectually and guided me extensively throughout my PhD program.

I would like to thank Dr. Tamara Minko, Dr. Guofeng You, and Dr. Maria Silvina Tomassone, members of my thesis committee, for taking the time out of their schedules to guide me through this process. Your guidance has truly been appreciated.

I would like to thank everyone at the New Jersey Center for Biomaterials who has played a role in my research. I would like to convey my special to Theresa Bello, Ami Shah and Hakim Robinson from Ernest Mario School of Pharmacy and Joshua Wang from Biomedical Engineering for their support with the permeation experiments and HPLC studies.

I like to thank Victoria Kai from Novartis for MVDA modeling support.

I would like to thank: current and past members of the Drug Delivery Lab from Center of Biomaterials including Priya Batheja, Vishwas Rai, Brian Kilfoyle, Sonali Bose, PeiChin Tsai, Golshid Keyvan for all of their assistance and friendship.

I would like to thank some of the special persons like Dr. Colleen Ruegger and Dr. Michael Motto from Novartis Pharmaceuticals for their whole hearted support. I would also like to thank Hui Pung from Ernest Mario School of Pharmacy and

Barbara Sirman from Graduate School of New Brunswick for their guidance through out the program.

Most importantly, I would like to convey my sincere thanks and gratitude to my family members; my thesis would not have completed without their patience, encouragement and support. My best friend and wife Malini and my son Shounak gave me strength and support during the last six years of my course. Finally I like to thank my parents, Ms. Nivedia Ghosh and Late Shiba Prosad Ghosh and my in-laws, Dr. Anjali Majumdar and Mr. Kanoj Majumdar for their moral support and confidence on me. All your confidence gave me the strength to have this wonderful achievement.

I appreciate all of you for being so kind on me. Thank you!

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Chapter 1. Introduction

1.1. Background

Among the different non-invasive routes available to the patient, the transdermal route has become increasingly popular with and accounting for about 12% of the global drug delivery market. Due to the ability of ease of application and delivery, the field is experiencing a high growth rate (1). The market value for transdermal delivery products was $12.7 billion in 2005 and it is expected to increase to $21.5 billion in 2010 and $32 billion by 2015 (2-3).

1.2. Drug delivery systems using passive diffusion

The most conventional route of transdermal delivery utilizes passive delivery in the form of ointments, creams, gels and patches. Some examples of transdermal patch design are as follows (4-5).

a) Membrane moderated transdermal drug delivery systems (TDDS): This device consists of four layers; an impermeable backing membrane, adjacent polymer layer that acts as a drug reservoir, a microporous membrane and an adhesive film. This technique was used by Transderm- Scop ® (scopolamine) for motion sickness (Ciba); Transdermal-Nitro® (nitroglycerin) for angina (Ciba); Catapres TTS® (clonidine) for hypertension (Boehringer Ingelheim) and Estraderm® (Estradiol) (Novartis) for hormone therapy.

b) Adhesive dispersion type TDDS: This drug reservoir is formulated by directly dispersing the drug in adhesive polymer. This technique was successfully

used by Deponit® (nitroglycerin) (Pharma Schwartz) and Frandol® (nitroglycerin) (Toaeiyo) for the treatment of angina.

c) Matrix diffusion controlled TDDS: This drug reservoir consists of a hydrophilic or lipophilic polymer containing the dispersed drug. Nitro-dur® (nitroglycerin) (Key) used this technique for the treatment of angina.

d) Micro reservoir TDDS: Drug is carried in micro-reservoir dispersed in a polymeric matrix. Example of this delivery system is Androderm® (testosterone) (Watson) that is used for the treatment of hormone therapy.

1.3. Barrier property of the skin

One of the challenges of transdermal drug delivery system is to deliver effective amounts of the drug through the skin layers. Skin consists of three main layers - the epidermis, dermis, and subcutaneous tissue. The outermost layer of the epidermis is the *stratum corneum*, which is composed of dead, anucleated keratinocytes. The other layers, in descending order, are viable epidermis, stratum lucidum, stratum granulosum, stratum spinosum, and the stratum basale (6-7). Being the outermost layer of the skin, the *stratum corneum* presents itself as the foremost defence of the body against pathogenic invaders (Figure 1). It is composed of "quasi-continuous lipidic matrix, largely in crystalline state". Water concentration gradient drops from 75% in the viable epidermis to only 10 to 30% on the surface. The hydrophilic pathways assist in transporting drugs. Thus permeation through the skin becomes a rate limiting step for poorly soluble drugs.

1.4. The use of chemical enhancers and its limitations

The penetration of drug through the skin is influenced by three different factors – a) physicochemical properties of the applied molecule, b) the type of formulation and c) delivery method. Different novel approaches have been used for delivering the drug through the skin (8).

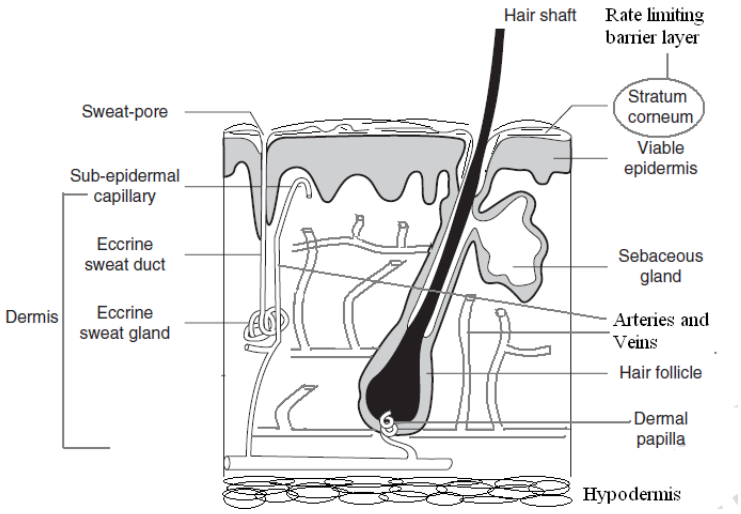

Figure 1 Diagrammatic representation of structure of skin (1)

There are many published articles that describe the use of penetration enhancers for enhancing the rate and extent of drug delivery through the skin. Characteristics of an ideal vehicle/permeation enhancer have been listed by Sloan et al. (9) and Pfister et al. (10) and others. Important issues related to permeation modifiers are their safety profiles. At the molecular level, several biochemical changes have been observed in the skin due to disruption of the barrier properties following the application of chemicals.

Some chemical penetration enhancers, in spite of their excellent permeation enhancement capabilities, have been associated with a number of adverse effects in animal/human skin models. Some of the adverse effects associated with commonly used chemical penetration enhancers are described below:

a) Greater transepidermal water loss (TEWL), erythema, observed with saturated fatty alcohols (11);

b) Erythema, scaling, contact urticaria, stinging, burning and systemic symptoms caused by sulfoxides (e.g. DMSO) (12);

c) Higher skin irritation and increased TEWL values observed with unsaturated fatty acids compared (13);

d) TEWL and skin morphological changes caused by fatty acids, azones, terpenes, lecithin (14);

e) Strong barrier disruption, erythema, dryness and skin irritation observed with surfactants (sodium lauryl sulfate alone and in combination with other surfactants and enhancers) (15-16);

f) Erythema and other irritant cutaneous reactions on human skin caused by prrrolidones (17-18).

1.5. Energy related devices for transdermal drug delivery

Alternate techniques have been explored for improving the permeability parameters through the skin. These include iontophoresis, electroporation, mechanical skin ablation and other energy-related techniques, as described in the following sections.

a) Iontophoresis: A small electric current is applied to increase permeation of charged and neutral compounds across the skin (Figure 2). Iontophor® (Life Tech., Inc.) and Phoresor-Iontocaine® (Iomed Inc.) utilize this technique in the device in order to deliver lidocaine (19).

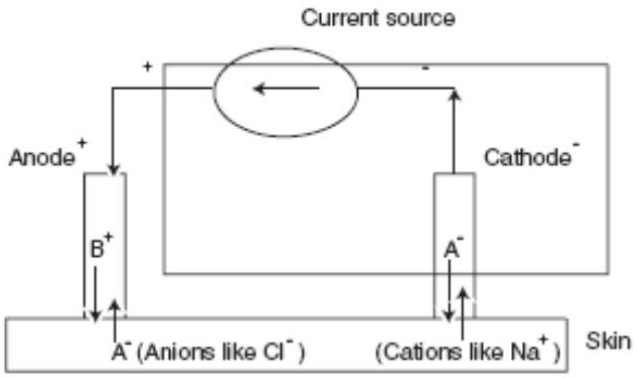

Figure 2 An anodal iontophoretic system (19).

b) Electroporation: In this technique, high voltages (≥100 V) and short treatment durations (milliseconds) are employed. SynConTM (Inovio Biomedical Co.) used this technique for PennVax(TM)-B HIV DNA Vaccine (under clinical trials) (20-21).

c) Microneedle based devices: Microneedles of length 50–110 mm can penetrate the SC and epidermis to deliver the drug from the reservoir (Figure 3). Macroflux® microprojection array (Zosano- formely Alza) & others used this technique for vaccine delivery (22-23).

d) Skin Puncture and Perforation: Passive or active delivery of drug takes place after disruption of the skin barrier and creation of holes with needle-like structure or blades similar to the microneedle device. Imprinter TMR (Imprint

Pharmaceuticals) used this approach for delivering low to high viscosity formulations and solid particulates to different depths of the skin (21).

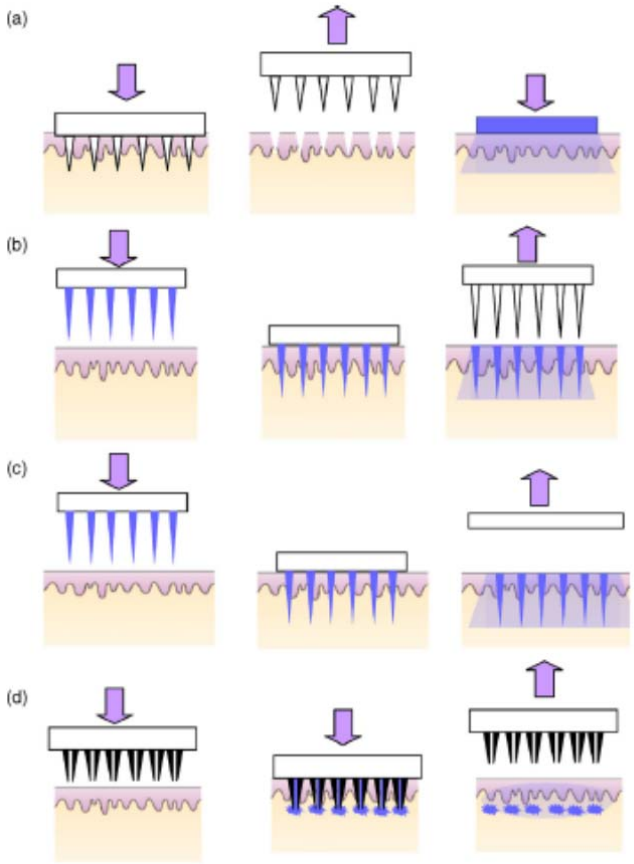

Figure 3 Schematic of drug delivery using different designs of microneedles: (a) solid microneedles for permeabilizing skin via formation of micron-sized holes across stratum corneum, (b) solid microneedles coated with dry drugs or vaccine for rapid dissolution in the skin, (c) polymeric microneedles with encapsulated drug or vaccine, (d) hollow microneedles for injection of drug solution (24).

e) Ultrasound (sonophoresis or phonophoresis): This approach uses oscillating pressure low-frequency ultrasound wave (55 kHz) for an average duration of 15 s to enhance skin permeability. SonoPrep® device (Sontra Medical Co.) used this technique for delivering local anesthetics and insulin (25).

f) Temperature: Heat application to skin increases drug diffusivity due to increased SC lipid fluidity. The flux of drug through skin is controlled by skin surface temperature (Figure 4). Controlled heat-aided drug delivery (CHADD) patch (Zars Inc., Salt Lake City, UT) used this approach for the delivery of local anesthetic system (26).

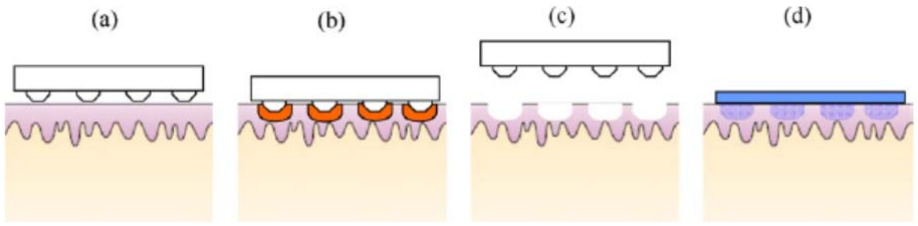

Figure 4 Schematic of drug delivery using thermal ablation: (a) micro-electrodes are pressed against the skin, (b) skin is ablated via heating due to RF energy or resistive heating in the electrodes, (c) after removing the ablation device, (d) micropores formed are covered with drug patch for delivery (24).

Reports were also published of using other sophisticated techniques such as laser radiation, high frequency altering current, magnetophoresis, and powder injection (Figure 5). However, all these above mentioned devices are limited by their size, cost, complexity and their potential to cause pain and irritation.

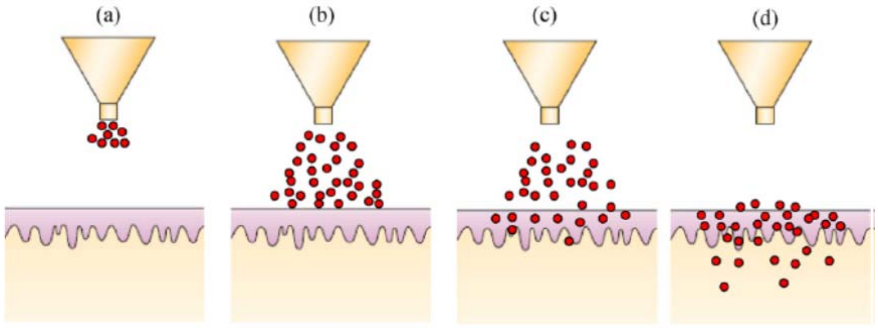

Figure 5 Schematic of drug delivery using powder injector: (a) ejection of particles from nozzle, (b) impact of particles on skin surface, (c) penetration of particles across stratum corneum, (d) completion of delivery. Particles which penetrate into the skin are mostly distributed in stratum corneum and viable epidermis (24).

1.6. Use of supersaturated systems for topical application

Another technique used extensively in the topical area is the supersaturation approach (Figure 6). Supersaturated systems are defined as formulations in which the concentration of drug in the solution exceeds its saturation solubility. The application of supersaturated systems to topical and transdermal drug delivery has been researched popular for many years (27-29). Pullett et. al. studied the synergistic effects on the enhancement of flurbiprofen permeation across the human skin using supersaturated solution and oleic acid. Also Megrab et. al. demonstrated enhancement of permeation of estradiol due to synergistic effect between supersaturation and increased amount of propylene glycol in the formulation. However, one of the drawbacks for these systems is the production of stable formulations due to the formation of crystals (Figure 7) at their metastable state (30-31).

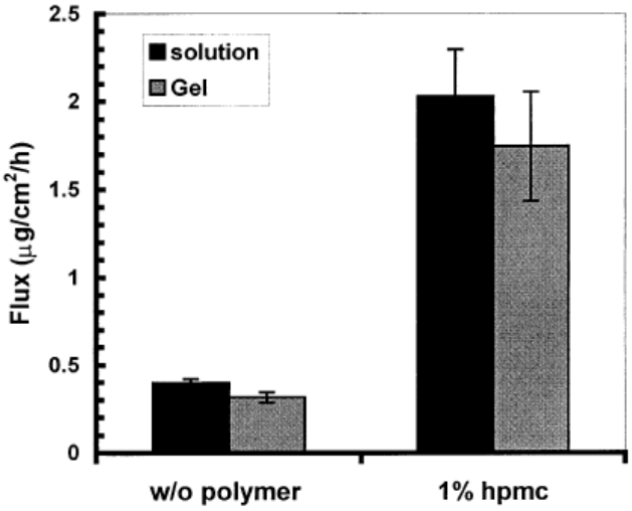

Figure 6 Comparison of the fluxes of HA (hydrocortisone acetate) from a solution and a gel (4.8x saturation) (30).

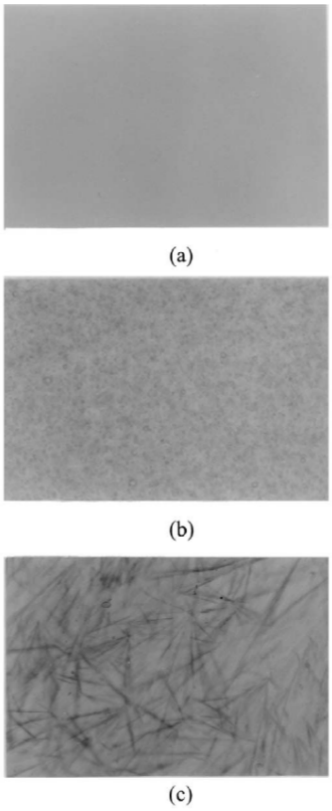

Figure 7 Microphotographs of different PIB (polyisobutylene as adhesive matrix) ketoprofen patches: (a) patch without ketoprofen; (b) patch before crystallization containing dispersed ketoprofen; (c) patch after crystallization containing ketoprofen crystals (32).

1.7. Application of nanotechnology for dermal application

Nanotechnology has an incredible potential for revolutionizing the therapeutics and diagnostics by using various formulation approaches and techniques. Different systems that are extensively studied over the last few years for delivering the compounds for topical and transdermal administrations are summarized in the following sections:

a) Liposomes: Liposomes are spherical vesicles with lipid bilayers made from either phospholipids or cholesterol enclosing an aqueous centre. The deformability of

liposomes decreases with increasing cholesterol content (33). The size of liposomes can range from between 50 to several hundred nanometers and are classified based on size or number of lipid bilayers. If classified by size, there are two categories: small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV). Based on the number of lipid bilayers, there are two categories as well: multilamellar vesicles (MLV) and unilamellar vesicles (ULV). Techniques for producing liposomes include dry lipid film hydration, emulsification, reverse phase evaporation, freeze-thaw processes, and solvent injection. All of these processes are performed with homogenization methods such as sonication and removal of the unloaded drug is done by centrifuge, filtration, or dialysis. The disadvantages in the production of liposomes lie in the need for special equipment, the solvents used, which are sometimes pharmaceutically unacceptable, and complex entrapment procedures. Liposomes can undergo lipid oxidation, hydrolysis, aggregation, and fusion, all of which are harmful to their stability and undesired due to the presence of an aqueous phase (33). Over twenty years ago, Mezei introduced the first demonstration of liposomes as a delivery agent for active materials (34). They were discovered to be an excellent carrier of steroids and retinoids, delivering concentrations up to five times higher than simple lotions, and were thus extensively studied for topical delivery agents (33-34). However, skin penetration of these vesicles has received much debate due to multiple factors such as size, composition, lamellarity, and charge (34-35). Possible mechanisms, which allow the liposomes to penetrate through the skin, are shown in Figure 8.

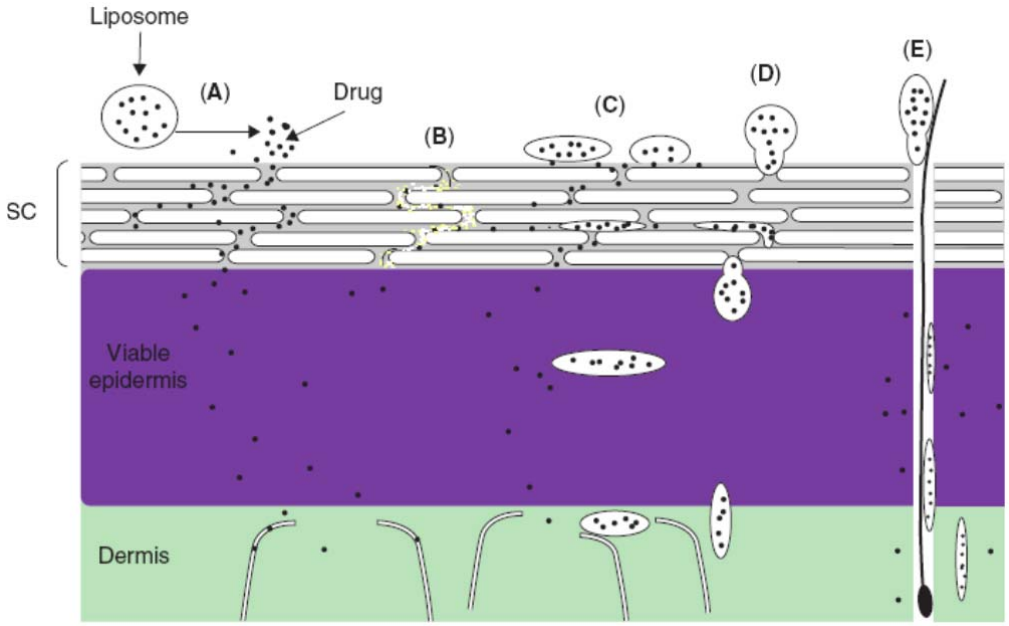

Figure 8 Possible mechanisms of action of liposomes as skin drug delivery systems. A. The free drug mechanism. B. The penetration-enhancing process of liposome components. C. Vesicle adsorption to and/or fusion with the stratum corneum (SC). D. Intact vesicle penetration into or into and through the intact skin. E. Delivery through an appendage (36).

b) Particle based nanosystems—SLN and NLC: The first two generations of nanoparticles begin with solid lipid nanoparticles (SLN) and the second with nano structured lipid carriers (NLC). The production of SLN is performed by removing the liquid lipid from an oil/water emulsion (Figure 9) and substituting it with a lipid that is solid at body temperature and has a perfect crystalline lattice. These contain 0.1% w/w to 30% w/w solid lipids and can be stabilized with a surfactant. The size of SLN ranges from 40 to 1000 nm (37). NLC, on the other hand, are produced with a blend of solid and liquid lipids in a ratio from 70:30 up to 99.99:0.1 (37). As with SLN, this blend is required to be solid at body temperature. As a result, the lattice can be one of three types: imperfect type, amorphous type, or multiple types (38). Both SLN and NLC can be positively or negatively charged. The advantages that NLC holds are a

higher loading capacity for drugs, a lower water content, and lower expulsion of drugs during storage (39). High-pressure homogenization (hot and cold) and microemulsion formation are two ways to produce SLN. High-pressure homogenization is the most popular and widely used method as it offers many advantages: easy scale up, absence of organic solvents and finally smaller production time. The composition and structure of SLN and NLC make them very rigid and impervious to structural change (40-41). As a result, these particles are unable to completely pass through the *stratum corneum*, consequently lingering at the skin surface. The prevention of absorption allowed these formulations to be applied as a carrier for cosmetics, repellents, and sunscreens.

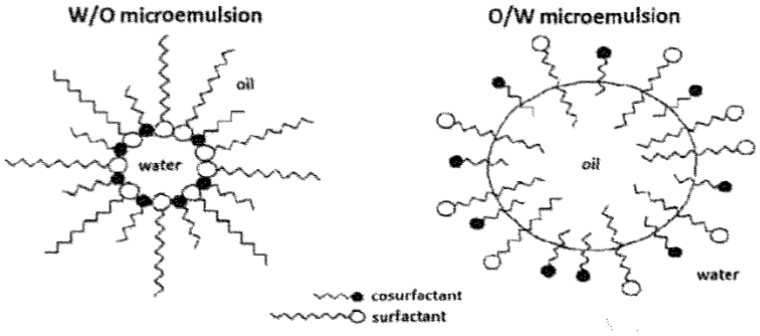

Figure 9 Schematic representation of w/o microemulsion and o/w microemulsion structure (42).

c) Polymeric Microparticles and Nanoparticles: Polymeric particles are spheres or capsules, and are mostly oily particles covered with polymers. The size of these particles is on the micro and nano scale, and the particles may be positively or negatively charged. Particle size, surface charge, surface modification, and hydrophobicity of the particles influence the targeting capability of nanoparticles. Penetrations of these particles have been shown to stay at the skin surface and were unable to pass through the *stratum corneum*. In a study done by Alvarez-Román *et al*.,

fluorescent polystyrene particles were applied to porcine skin (diameters 20 and 200 nm). The study revealed that the particles accumulated in the hair follicular openings. However, in a more recent study performed by Zhang *et al.*, the permeation ability of poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles was investigated in conjunction with microneedles, in order to enhance the skin's permeability towards the drug (43). The results not only showed that all the nanoparticles were deposited into the skin, but also that the amount deposited significantly increased with the use of microneedles.

Two types of nanoparticles where studied using human abdominal skin at full thickness with vertical diffusion cells, and PBS as the receptor fluid. The first formulation was made of iron oxide nanoparticles that were coated with TMAOH (tetramethyl ammonium hydroxide) and mixed in aqueous TMAOH. The second was made with iron nanoparticles coated with AOT (sodium sulfo succinate), and mixed in aqueous AOT. After they were applied to the skin for twenty-four hours, they were found to be present in all layers of the *stratum corneum*, in the area between the *stratum corneum* and the stratum granulosum, and in the hair follicles[44]. It should be noted that no particles were able to cross the length of the skin.

d) Vesicle based particles: The methods described above allow the delivery of a drug into the superficial layers of the skin. There are other lipid vesicular systems (Figure 10) that are capable of penetrating into the deep skin strata and allowing transdermal absorption. The mechanism responsible for skin penetration of nano and micro particles depends in part on the size of the carriers (Figure 11).

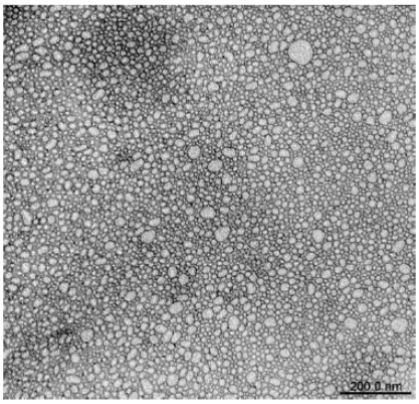

Figure 10 Transmission electron microscope (TEM) picture of nanoparticles (Vehicles) (45).

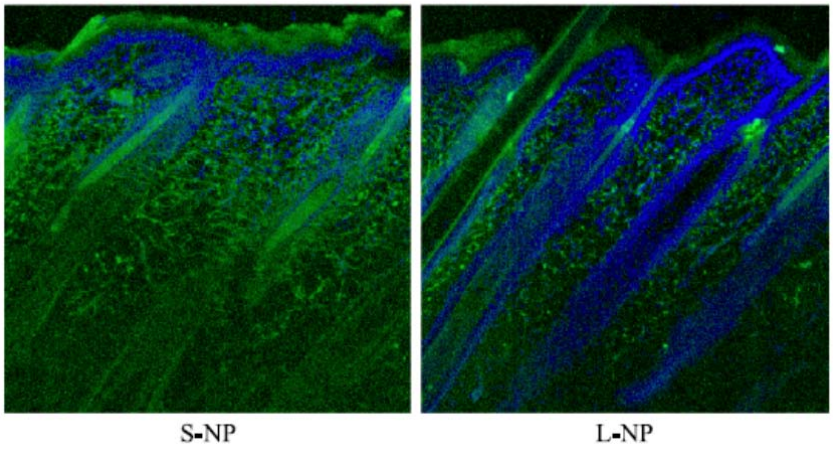

Figure 11 CLSM image of a cross-section of Albino Hartley guinea pig skin fluorescent-labelled nanoparticles applied for 12 h. S-NP: 40-nm size of nanoparticles, L-NP: 130-nm size of nanoparticles (10 pieces of z-direction optical cross-sectioned tissue images were merged) (45).

i) Ethosomes: Ethosomes are comprised of phospholipids and high concentrations of ethanol and water, containing up to 20% to 45% ethanol which allows for a high flexibility of the vesicles (46). The particles are negatively charged with fluid lipid bilayers. The composition of ethosomes affects their size, with their average diameter between tens of nanometers to one micron. An increase in ethanol concentration from 20% to 45% caused a size reduction from 193 nm to 103 nm; an

increase in phospholipid content from 0.5% to 4% caused the vesicle to double from 118 nm to 249 nm. Due to the ethanol composition and high vesicle lamellarity, ethosomes are able to efficiently trap molecules that are hydrophilic, lipophilic, and amphiphilic. Studies were performed to show that ethosomes were able to encapsulate testosterone and minoxidil up to 90% and 83%, respectively. Ethosomes are manufactured by dissolving the lipid and drug in ethanol, and then they are mixed with a constant stream of aqueous solution in a sealed container. There are a variety of mechanisms by which ethosomes deliver the drug into the skin. Ethanol can be released to increase permeation of the skin, ethosomes release lipids to interaction with the skin's own lipids in order to enhance permeation and finally, ethosomes may squeeze through the skin layer, fuse with skin lipids in the deeper layers, allowing systemic drug absorption.

ii) Surfactant based particles: Niosomes and Transfersomes are some of the examples of this category. Niosomes are made up of a nonionic surfactant and cholesterol, with small amounts of phospholipids (46). They are prepared in a similar manner to liposomes by film hydration of a surfactant and lipid followed by homogenization and size reduction. The surfactants that can be used include, polyoxyethylene alkyl ethers, sorbitan esters, polysorbate-cholesterol mixtures, crown ethers, perfluoroalkyl surfactants, and alkyl glycerol ethers. The size of niosomes range from 100 to 200 nm after sonication, and from 50 to 100 nm after a microfluidizer or high-pressure homogenizer. The skin permeability of niosomes has been shown to alter by varying their cholesterol content, their ability to modify the *stratum corneum* intercellular lipid structure, and their adsorption and fusion with the skin surface. Thus follicular transport was proved to be a potential pathway for dermatotherapy and cosmetics.

Finally the particle size of these nanoparticles / nanocarriers has an important role on the skin permeability. Previously, it was reported that particles below 3 um in diameter, can penetrate the SC through intracellular pathway and particles ranging from 3 um – 10 um penetrate through sebaceous follicles. However, several recent studies using smaller sized particles provided new correlations between particle size and penetration routes. In one study the in vitro permeation profile of nanoparticles (40 nm – 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 larger sized particles due to the tight network of epidermal Langerhan's cells (47) (Figure 12). 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. Further the enhancement was promoted when the size of the particles was decreased (45). Thus follicular transport was proved to be a potential pathway for dermatotherapy and cosmetics. In one study it was shown that when the particle size was higher than 5 um, 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 (44).

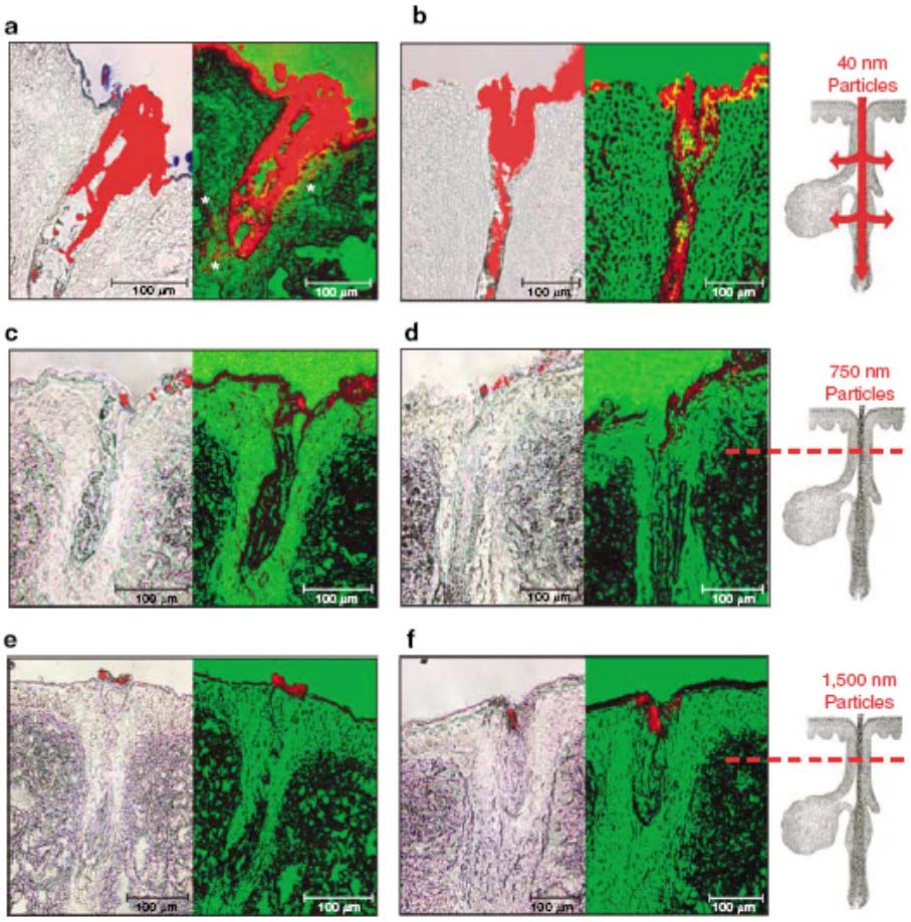

Figure 12 40 nm, but not 750 or 1,500 nm, nanoparticles penetrate via the vellus hair follicle into the surrounding tissue. Laser scan microscopy were performed on cryosections of skin samples treated with (a) 40 nm (0.1% solids, $2.84x10^{13}$ particles/ml, n=6), (b) 750nm (0.1% solids, $1.08x10^{10}$ particles/ml), or (c) 1,500nm (0.1% solids, $1.35x10^{9}$/ml) nanoparticles. Digital image overlay was used to localize the fluorescent signal on the tissue sections. (a, b) The authors found that 40nm nanoparticles, in contrast to the larger particles, penetrated deep into vellus hair follicles. Transcutaneously applied (c, d) 750nm and (e, f) 1,500 nm fluorescent nanoparticles, in contrast, aggregated in the infundibulum of human vellus hair follicles. No penetration to deeper parts of the hair follicles and no penetration into viable epidermis was observed in any of the samples. (a–f) Bar=100 mm (47).

e) Application of nanocrystal technology: The nanocrystal technology is one of the most popular technologies to improve the bioavailability of poorly soluble drugs. In this process the particle size of drugs was reduced down to the sub-micron range (Figure 13). As the dissolution rate of the poorly soluble drug is proportional to

the surface area, therefore nanocrystal approach is a potential technique to improve drug release (Figure 14). Further, the saturation solubility of the drug also increases with reduction of particle size of the drug substance. The technology was applied to several compounds and five oral products are already in the market (48).

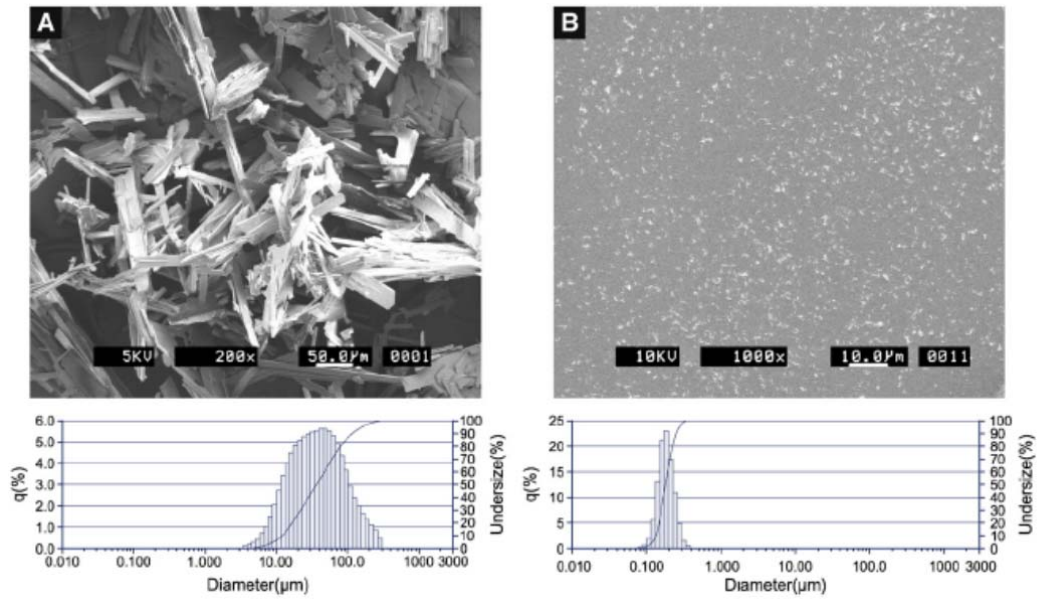

Figure 13 Nanosized drug crystals. The scanning electron micrographs of posaconazole, a poorly-water soluble antifungal agent, are shown before (A) and after nanosizing using wet media milling technology (B). Also shown are the particle size distribution curves performed using laser light diffraction and deionized distilled water as the diluent. The mean particle size of the unprocessed crystals~53 μm with a broad distribution profile while the nanosized dispersion has a distribution profile with a mean size ~0.185 μm (185 nm) (49).

The principle of nanocrystals is that, they improve the transport of drugs across a barrier/membrane. So far pharmaceutical attention focused only on oral and i.v. administration. Other areas such as dermal administration were completely neglected. Since poorly soluble molecule has more lipophilic character, and should therefore penetrate even better when the solubility problem and the low-dissolution velocity can be overcome by nanocrystal production. Basically, there is no difference

in the procedure for producing nanocrystals for oral, intravenous or dermal administration/application. Also incorporation of nanocrystals into dermal products is very simple. A concentrated nanosuspension can be added to the water phase to form creams or lotions.

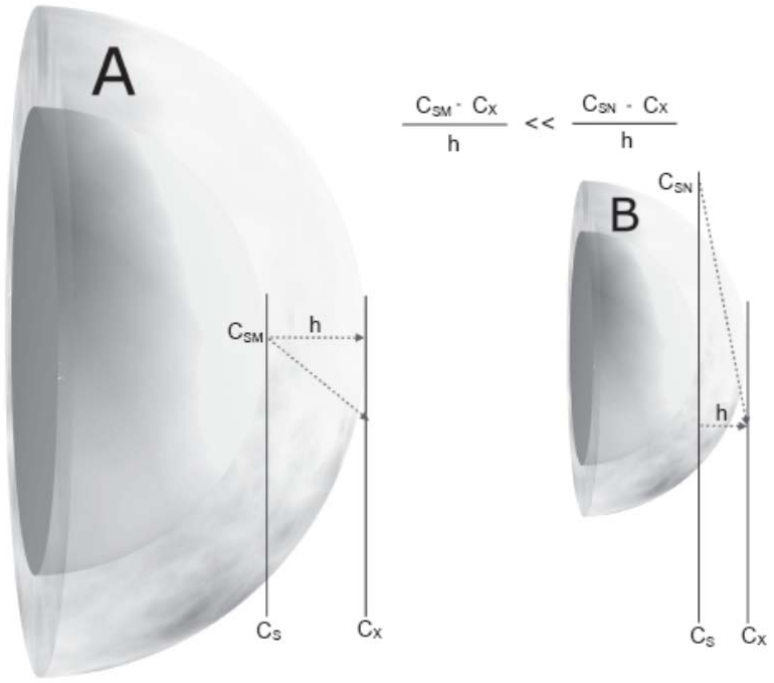

Figure 14 Comparison of a microcrystal (A) and a nanocrystal (B) and their surface curvature and concentration gradient over the diffusional distance (h). Abbreviations: cs, drug-saturated water at surface (M, microcrystal; N, nanocrystal); Cx , bulk concentration at diffusional distance; h, diffusional distance. dc / dt ~ (cs – cx) / h (50).

During the formation of nanocrystals, due to the change of Gibbs free energy, thermodynamically unstable system is formed, which results in agglomeration or crystal growth due to Ostwald ripening. This in turn may impact drug release due to formation of larger particles with decreased surface area. Therefore proper selection of stabilizers is required during the preparation of nanocrystals to stabilize the

nanoparticles by preventing them from aggregating due to the attractive force between the particles. In many cases a combination of stabilizers are more beneficial (51).

Nanocrystals can be produced by two basic techniques. In the bottom up approach, nanoparticles are produced by precipitation method. However, some of the drawbacks for this kind of approach are low drug loading and process scale-up. The alternate technology used for the production of nanoparticles is the top down approach, in which nanocrystals are produced by media milling approach or by high pressure homogenization approach or combination of both. Lack of complexity in the process, easy scale-up and elimination of solvent are some of the benefits of media milling technology (48).

In case of nanocrystals, nanoparticles are dispersed in a liquid and the system is termed a "nanosuspension". In contrast to many nanocarriers, nanocrystals can be prepared from regulatorily approved excipients, which provide an advantage for the formulations to use in clinical studies and also to enter the regulatory market. Basically these formulations are simple systems consisting of drug and stabilizer with no organic solvent, which also make this system eco friendly.

Only recently has the thought of using Nano crystals for dermal application become popular. The mechanism of dermal delivery of nanocrystals has been explained using the example of the delivery of rutin and hesperidine (51-52). These compounds are poorly soluble antioxidants. After conversion into nanocrystals, the formulation increased the sun protection factor (SPF) by 59%. Similar data were reported for hesperidine with an increase in SPF of 36% (Figure 15).

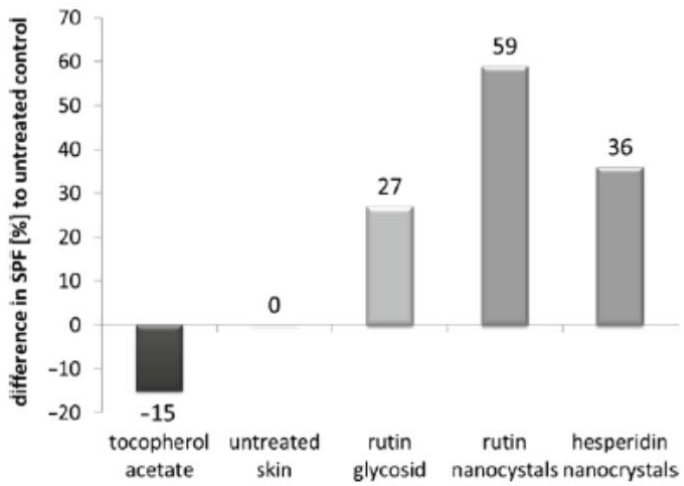

Figure 15 Change in % of SPF of human skin after treatment with formulations containing alpha tocopherol acetate, water soluble rutin glucoside, rutin nanocrystals and hesperidin nanocrystals (48).

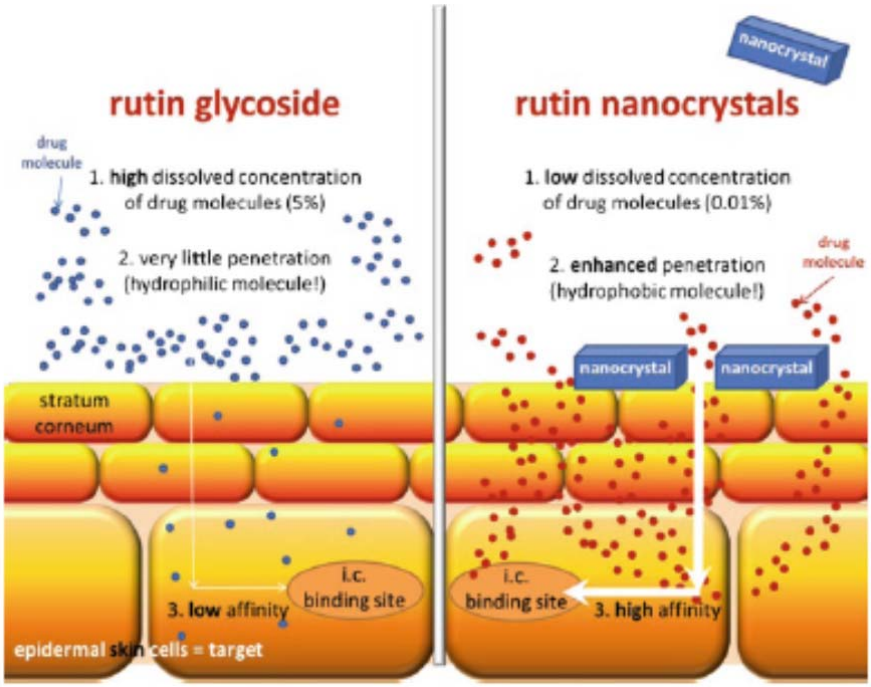

Figure 16 Mechanism of improved dermal action of rutin nanocrystals (right) and water soluble rutin glucoside (48).

f) Toxicological aspects of nano particles: According to the nanotoxicological classification system (NCS) four categories were defined based on the drug particle

sizes and the degree of biodegradability of the inactive ingredient used in the formulation. When the particle size of the nanoparticles remain in the range of about 100–1000 nm, it can be only taken up by macrophages and thus resulted to lower toxicological risk due to the minimum access into the body cells. However, if the particle sizes of nanoparticles remain below 100 nm, they can access any cell of the body by endocytosis/pinocytosis, and therefore pose a higher potential risk. Also if the inactive ingredients used in the formulation are biodegradable in the body, they will be excreted and , thus potential undesired effects will be low.

However, non-biodegradable materials can cause dermal irritation. . Based on these considerations, the nanocrystals are classified as Class I – Class IV, depending on the size (above or below 100 nm) and also whether the inactive ingredients are biodegradable or not (Figure 17).

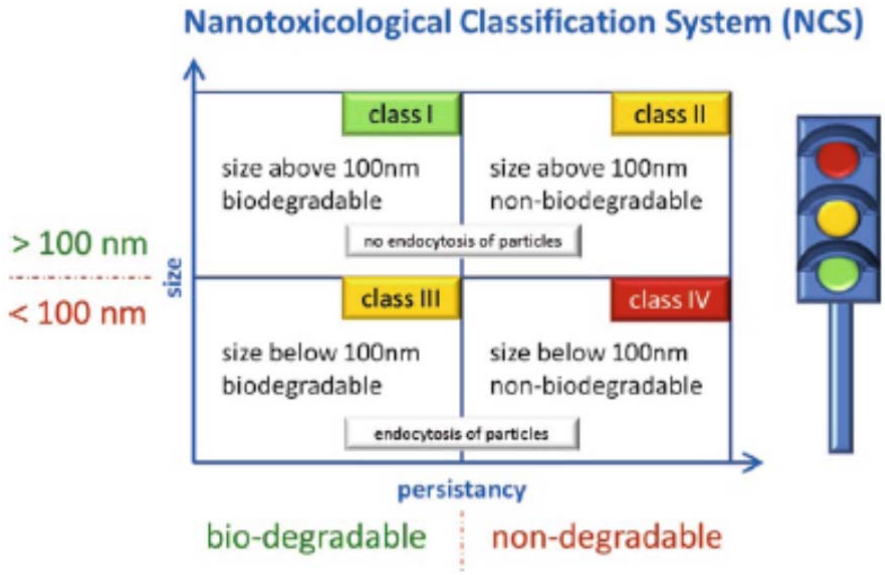

Figure 17 The nanoparticles are differentiated as Class I–IV with increasing toxicological risk, based on size (<100 nm, 100–1000 nm) and biodegradability/non-biodegradability (i.e. persistence in the body) (48).

g) Tocopheryl polyethylene glycol succinate (TPGS) is a water-soluble derivative of a natural source of vitamin E and functions as a surfactant with an HLB value of 13.2. Several studies have demonstrated that TPGS improves the oral bioavailability of poorly soluble drugs. The enhancement of bioavailability is due to enhanced solubility, improved permeability, and reduced intestinal metabolism (54). In one of the study, TPGS was able to enhance the solubility and hence the permeability of estradiol.

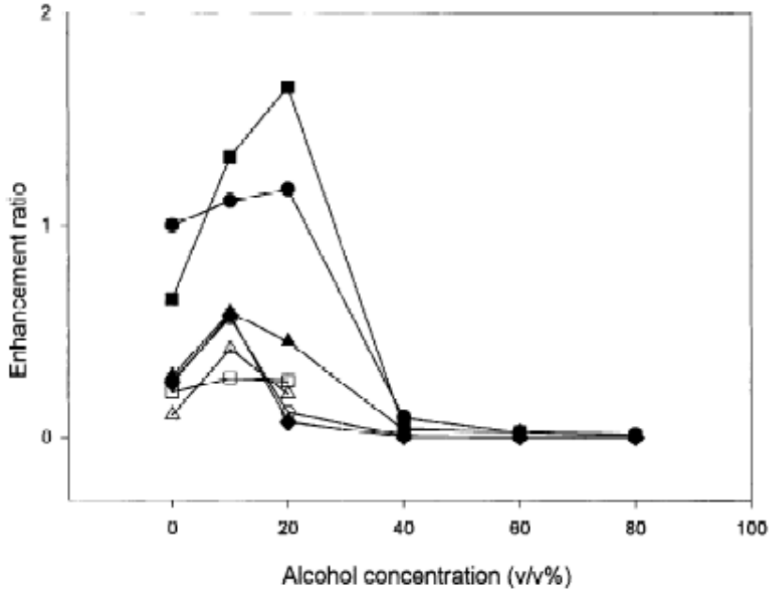

Figure 18 Enhancement ratio of estradiol in different EtOH/TPGS cosolvent systems (54).

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Chapter 2. Background and significance

Of all the non-invasive routes of administration, the transdermal route seems to be one of the most promising approaches for drug delivery. Transdermal drug delivery systems (TDDS) have offered multiple advantages over the oral drug delivery since last few decades. The transdermal drug has already grown into a $2 billion market share in USA. These drugs target various therapeutic areas such as local pain control, hormonal abnormalities, contraception, motion sickness, pain control, depression etc. In this delivery technique, the drug is targeted for localized effect at the site of application or delivered to the skin layers by virtue of drug permeation. Among several challenges of this delivery system, one of the limitations is the permeation of effective concentration of drug molecule through the skin barrier for desired therapeutic action. The stratum corneum, which is the uppermost layer of skin, acts as the barrier layer for percutaneous drug delivery. Therefore the permeation challenge gets more pronounced in case of poorly soluble drug molecules. Although these molecules should principally enhance the permeation rate due to higher lipophilicity, however, the dissolution velocity and rate of drug release becomes rate limiting for those kinds of compounds. Unfortunately, significant numbers of new molecule entities (NCE) generated during the drug discovery are poorly soluble or practically insoluble in water. Thus for the improvement of permeability rate through the skin, there is an imminent need in designing novel formulation strategies.

Different enhancement techniques including physical and chemical approaches have been attempted. Physical systems, such as iontophoresis and phonophoresis require complex and expensive delivery devices. Chemical methods involve

penetration enhancers. In general, penetration enhancers alter the barrier properties of the stratum corneum and trigger skin irritation in many cases. Therefore, supersaturated formulation approach provides an attractive alternative.

Several studies were reported in the literature about using supersaturated systems for the permeability enhancement of drug compounds through the skin. Supersaturation is defined as a state at which the amount of drug solubilized in a vehicle is greater than its equilibrium solubility. Linear relationships had been shown to exist between drug content in the transdermal matrix and drug release, resulting in an increased drug flux due to higher thermodynamic activity. When these systems were applied to the skin, the superficial layer of the SC also became supersaturated with the drug and thus enhanced the permeation rate. This technique offers the advantage of being inexpensive and does not alter the integrity of the stratum corneum. However, such kinds of systems were thermodynamically unstable because of crystallization of drug molecules immediately after formulation or even during storage.

Propylene glycol was used widely as a solubility and permeability enhancer in combination with PVP K-30 as a crystal inhibitor in such kind of supersaturated systems. However, not much work has been done to study the effect of non-ionic surfactants such as vitamin E TPGS, Pluronic F-127 etc. on the permeability of poorly soluble drug through the skin. These excipients were widely used for oral delivery and promising results were observed in terms of improving the bioavailability of several molecules. Thus we like to investigate the effect of these non-ionic surfactants on improving the dissolution velocity of the drug and the permeability rate.

Also in case of supersaturated system, there is a tendency of drug crystallization or precipitation on the skin surface which ultimately lower the permeation rate. In a separate study, we like to perform a comparative evaluation between HPMC 3cps and PVP K-30 for their crystal inhibition effects and their implication on the permeation enhancement of the drug.

Additionally, 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 the nanotechnology approach has been extensively explored for transdermal drug delivery. The mechanism responsible for skin penetration of nano and micro particles depends on the size of drug compound. Recent studies conducted using smaller size particles gave new ideas about the correlation between particle size and penetration route. Ethosomes, niosomes, and transferosomes have been shown to change their morphology and squeeze past the *stratum corneum* cells and achieve systemic delivery. 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.

The crucial factors need to be considered for formulation design include drug loading, stability of drug compound, variability between batches and most importantly, the permeability factor. Although the nanocarrier approaches mentioned above had shown promising results in virtue of skin delivery, however, these are still confined within the academic research and not achieved popularity in the industry because of several complexities like cost, batch-batch reproducibility and scale up factors.

One of the popular techniques used in industrial application in order to improve the bioavailability of poorly soluble compounds is to reduce the size of the drug crystals using wet media milling. This kind of approach helped to improve the dissolution velocity of drug substance by increasing the surface area of the crystals during the micronization process. This technology can be applied to micronize the drug crystals probably to the submicron or nano range, for improving the permeation rate of the molecules through the skin.

Also during the milling process thermodynamically unstable system is formed due to the change of Gibbs free energy. This results in agglomeration or crystal growth of smaller particles due to Ostwald ripening. Flocculation or crystal growth formation during the process or during shelf life may impact its performance due to formation of larger particles with reduced surface area. Therefore proper selection of stabilizers is required during the preparation of submicron formulation. Stabilizers are needed to stabilize the submicron by preventing them from aggregating due to the attractive force between the particles. The two different techniques used for stabilizing such kinds of systems are steric and ionic stabilization. Among these, steric stabilization is recommended in some cases because of possible skin irritation of ionic stabilizers (like sodium lauryl sulphate / SLS).

In addition to particle size, skin absorption is also influenced strongly by the type of vehicles 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. Therefore it might be beneficial to perform a systemic study of the submicron crystals in presence of different solubilizers / co solvents such as Vitamin

E TPGS, Pluronic F127 and propylene glycol to evaluate its effect on the permeability of a drug through the skin.

Finally gels are one of the commonly used techniques for topical formulations and are widely used in the pharmaceutical and cosmetic field. One of the principle advantages of gel system is to provide an extended time of contact with the skin surface. The final formulations optimized from supersaturated solution and submicron suspension can be converted into the gel for delivering the drug at the desired site of action. It is also required to investigate various gel forming polymers like hydroxypropylmethylcellulose (HPMC K100), sodium carboxymethylcellulose (Na-CMC) and polaxamar in order to study their individual effect on the rheological properties, stability and drug release kinetics.

Considering the fact that, most inflammatory diseases occur locally and near the surface of the body, topical application of non-steroidal anti inflammatory drugs (NSAIDs) to the inflamed site can offer the advantage of delivering the drug directly to the diseased site and producing high local concentrations. This bypasses gastric irritation and also reduces adverse systemic effects. Ibuprofen is a potential NSAID used in painful, inflammatory, and certain non-rheumatic conditions. This drug also exhibits antipyretic and analgesic activity.

We planned to do a study with the above formulation approaches (supersaturated and sub-micron system) in order to investigate its impact on improving the permeability of ibuprofen. Comparison study will be performed to identify the system responsible for better permeability. Finally the release rate,

steady-state flux, and permeability coefficient of the drug from the gel formulations will be compared to conventional gel formulations of ibuprofen.

Chapter 3. Specific Aims

The objective of this study is to design an effective and stable formulation for a poorly soluble compound to enhance the permeability through the skin. In transdermal drug delivery systems (TDDS), it is a challenge to achieve stable and prolonged high permeation rates across skin since the concentration of the drug dissolved in the matrix has to be high in order to maintain zero order release kinetics of the drug. In case of poorly soluble drugs, due to thermodynamic challenges, there is a high tendency for the drug to nucleate immediately after formulating or even during storage. The goal of our research is to perform a comparative evaluation study between the two different systems - (supersaturated solution and submicron suspension) in order to enhance the permeability of the poorly soluble drug.

Ibuprofen will be used as the model drug for this study. This drug possesses very poor aqueous solubility and also has high tendency to form crystals from saturated or supersaturated solutions. The free base form of this drug is poorly water soluble with an equilibrium water solubility of 0.1 mg/ml. Ibuprofen is one of the most potent non-steroidal anti inflammatory agents that also have antipyretic activity. Ibuprofen is well absorbed following oral administration; however, its use has been limited by a number of side effects, including bleeding and ulceration. Transdermal administration can overcome these side effects.

Specific Aim 1: Evaluation of supersaturated solution system using Vitamin E TPGS and other solubilizers – formulation development and crystal inhibition studies.

This study will focus on the efficiency of Vitamin E TPGS / HPMC supersaturated solution and other solubilizer / polymer systems to improve the

solubility of the drug and inhibit crystal growth in the transdermal formulation. Effect of several solubilizers e.g. Pluronic F-127, vitamin E TPGS and propylene glycol (PG) will be studied on the supersaturated systems of ibuprofen as model drug. Various stabilizers such as hydroxylpropylmethylcellulose (HPMC 3 cps) and polyvinylpyrrolidone (PVP K-30) will be examined to evaluate their crystal inhibitory effects. Different analytical tools will be used in this study as a screening tool to optimize the final formulation with minimum crystal growth.

Specific Aim 2: Permeability study of supersaturated systems using synthetic membranes and porcine skin.

One of the critical parameters to be considered while enhancing the drug permeability is to produce a high concentration gradient between the drug crystals and the skin surface. This should result in a higher drug release from the topical system due to the formation of a supersaturated solution around the crystals and fast replacement of diffused molecules. In this research work, we will investigate the effect of non-ionic surfactants such as vitamin E TPGS, Pluronic F-127 and compare them with propylene glycol (PG) on the permeation rate of drug molecules through the membrane.

While testing the effect of different solubilizers / polymer combinations on the permeability rate, initially synthetic membranes will be used for screening the supersaturated systems. Finally, porcine skin membranes will be used to determine the flux and permeability coefficient to compare with the data obtained from synthetic membranes.

Specific Aim 3: Evaluation of submicron suspension system– formulation development and process optimization.

The purpose of this study is to develop a submicron suspension using wet media milling technique. During the screening study, different ratios of drug and solubilizers will be used to evaluate its effect on stabilization of smaller crystals during the process and also during the storage. Studies will be performed to investigate any change of polymorphic properties and morphology of the crystals during the wet milling. Based on this preliminary study, a model formulation and processing condition will be selected to be considered in the next part of the research which will focus on the permeation study to identify the influence of micronization of drug crystals on drug release and skin penetration.

Specific Aim 4: Permeability enhancement study of submicron systems using synthetic membranes and porcine skin.

In this study the effect of different solubilizers / co solvents such as Vitamin E TPGS, Pluronic F127 and propylene glycol will be investigated on the permeability of a drug having submicron particle size. A systematic study will be performed to evaluate the permeability enhancement profile influenced from each individual component such as particle size of drug crystals and also the type of the vehicle used in the formulation.

Specific Aim 5: Design and characterization of gel formulations using high viscosity polymers.

The optimized formulations from the previous studies will be converted into a gel as the final dosage form for topical application. Various gel forming polymers like hydroxypropylmethylcellulose (HPMC K100), sodium carboxymethylcellulose (Na-CMC) and polaxamar (Pluronic F127) will be studied to evaluate their effect on formulation properties and stability profile.

Specific Aim 6: Comparing the gel formulations (supersaturated and submicron systems) and investigating the permeability enhancement of the drug through the skin.

A statistical analysis will be performed to evaluate the effect of individual components and the interaction between these parameters. A 2^3 factorial design with three critical parameters at two different levels (High and Low) will be executed. 3 replicates will be used for each formulation during the permeation study. Based on the above study, we should be able to predict –

- the gel system (supersaturated solution vs. Submicron suspension) having better permeability.
- any significant effect from the amount of solubilizers used.
- effect from the viscosity of the gel on the permeation rate.

Chapter 4. A comparative study of Vitamin E TPGS / HPMC supersaturated system and other solubilizer / polymer combinations to enhance the permeability of a poorly soluble drug through the skin.

4.1 Introduction

For poorly soluble drugs, attaining adequate bioavailability after oral admistration is always a challenge. Several formulation approaches such as solid dipersion, microencapsulation, complex formation, salt formation, prodrug design, etc. have been attempted to improve the solubility of drug. However, if the drug also has a tendency to cause gastrointestinal disturbances the dermal route is an alternative option to minimize these side effects. On the other hand, drug delivery through the skin also has its own challenges due to the low permeability of the stratum corneum (SC), the outermost layer of the skin. Due to the chemical nature of the skin, the molecules should have a good balance between lipophilicity and hydrophilicity for enhanced permeation through the skin (1). Various approaches with chemical enhancers have been attemped in the past for improving the drug permeability, especially for relatively insoluble compounds (2).

Several studies reported in the literature used supersaturated systems for increasing the permeability of drug through the skin (3-5). Linear relationships have been shown to exist between drug content in the transdermal matrix and drug release, resulting in an increased drug flux with increasing thermodynamic activity. When these supersaturated systems were applied to the skin, the superficial layer of the SC also became supersaturated with the drug and thus enhanced the permeation rate (6-8). However, such transdermal patches are generally thermodynamically unstable because the drug shows the tendency to nucleate immediately after formulating or

even during storage. If the drug precipitates, its flux becomes independent of the administered concentration and the release becomes no longer a zero order (8). In such a case it is possible to use polymers or other additives to stabilize the supersaturated matrix (9). The success of the prevention of the nucleation process of the dissolved drug by the addition of excipients depends on the ability of these stabilizers to inhibit nucleation. Therefore, careful selections of polymer and solubilizer systems are very important to formulate a stable transdermal film that will release the drug in zero order fashion (10-11).

In this paper, the authors attempt to summarize a systemic investigation of different solubilizers / co-solvent for producing supersaturated systems by rapid change of solubility of poorly soluble drug and thus increasing the permeability rate. Polymeric stabilizers were also used for inhibiting crystal growth.

One of the critical parameters to be considered when enhancing the drug permeability is to produce a high concentration gradient between the drug crystals and the skin surface. This should result in a higher drug release from the topical system due to the formation of a supersaturated solution around the crystals and fast replacement of diffused molecules. In the previously published papers, propylene glycol was used as a solubility and permeability enhancer with PVP K-30 as a crystal inhibitor (8-9). In this research work, the authors investigated the effect of non-ionic surfactants such as vitamin E TPGS, Pluronic F-127 and compared them with propylene glycol (PG) by enhancing the solubility of the drug and hence the permeation rate, by converting it to the supersaturated state. Vitamin E TPGS was used previously as a plasticizer (at a concentration of 1-5%w/v) to produce films by hot melt extrusion process (12). In another study, vitamin E TPGS was evaluated to

enhance the solubility and permeability of estradiol. It was reported that, although vitamin E TPGS was able to improve the solubility of the drug by micellar solubilization, however, it was not responsible for the enhancement of drug penetration (13).

Also in case of supersaturated system, there might be a tendency of drug crystallization or precipitation on the skin surface which ultimately lower the permeation rate. Therefore, in a separate study, a comparative evaluation was performed with HPMC 3cps and PVP K-30 on their crystal inhibition effects and their implication on the permeation enhancement of the drug.

Ibuprofen was used as the model drug for this study that possesses very poor aqueous solubility and also has high tendency to form crystals from saturated or supersaturated solutions. The free base form of this drug is poorly water soluble with an equilibrium water solubility of 0.1 mg/ml. While testing the effect of different solubilizers / polymer combinations on the permeability rate, initially synthetic membranes were used for screening the supersaturated systems in order to evaluate the effect from individual components of the formulation. Finally, porcine skin membranes were used to determine the flux and permeability coefficient to compare with the data obtained from synthetic membranes.

4.2 Materials and Methods

4.2.1 Materials

Ibuprofen was obtained from Doctors Organic Chemical Limited (Tanaku, AP, India). The 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 (poloxamer) 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.

4.2.2 Preparation of saturated and supersaturated solutions

Supersaturated solutions were produced by dissolving the drug in water which contain the cosolvent (propylene glycol) or the solubilizer (vitamin E TPGS / Pluronic F-127). In this method, initially the vehicle (cosolvent / solubilizer) was dissolved in water. Excess drug was then added into this system and the suspension was stirred for 48 hrs. at 37°C using an insulated shaker (Innova 4000, New Brunswick Scientific, Edison, NJ, USA). The suspension was then centrifuged using a centrifuge (CT422, Jouan Inc., Winchester, VA, USA) at 3000 rpm and the supernatant clear solution was collected and divided into two portions. The first portion was mixed with the polymeric stabilizer (HPMC 3 cps / PVP K-30) and the second portion was used as such without any stabilizer. The vehicles added into the system, increased the solubility of the drug above its saturation level. The polymeric stabilizer was used to inhibit crystallization. The compositions of the different formulations are outlined in Table 1 (A-C).

Figure 1 Centrifuge apparatus

Table 1 Screening of different variants to study crystal growth (A: without any stabilizer; B: with HPMC; C: with PVP)

A.

Ingredients	**V-1**	**V-2**	**V-3**	**V-4**
Drug	Excess	Excess	Excess	Excess
Propylene Glycol		25%		
Vitamin E TPGS			5%	
Pluronic F-127				5%

B.

Ingredients	**V-1A**	**V-2A**	**V-3A**	**V-4A**
Drug	Excess	Excess	Excess	Excess
Propylene Glycol		25%		
Vitamin E TPGS			5%	
Pluronic F-127				5%
HPMC 3 cps	2%	2%	2%	2%

C.

Ingredients	**V-1B**	**V-2B**	**V-3B**	**V-4B**
Drug	Excess	Excess	Excess	Excess
Propylene Glycol		25%		
Vitamin E TPGS			5%	
Pluronic F-127				5%
PVP K-30	2%	2%	2%	2%

The saturated solution was produced by dissolving excess drug into the water which did not contain any vehicle and the suspension was stirred for 48 hrs. at 37°C using the similar insulated shaker. The saturated solution was used as a control in this study. The supersaturation factor was estimated of these formulations, by dividing the

concentration of the drug in the above solutions by its saturated solubility in the water media.

4.2.3 Short Term Stability study

The clear solution was kept on short term stability for studying crystal growth. The samples were kept at 5°C and also at ambient conditions (25°C). Samples were collected at different time points from 0 to 1 week to study the crystal growth and subjected to microscopy and particle size analysis as described in the following sections.

4.2.4 Light Microscopy study

The presence of crystals in the solutions was observed using an Olympus microscope, (BX51 & BX50, Tokyo, Japan) at a magnification of 100 X. 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.) and Digital camera (QImaging Retiga, BC, Canada) attached to the microscope.

Figure 2 Light microscope

4.2.5 Particle size analysis

The growths of crystals were detected by Photon Correlation Spectroscopy using Beckman Coulter particle size analyzer (N4 plus, Jersey City, NJ, USA) and Laser Diffraction particle size analyzer from Malvern Instruments (Mastersizer 2000, Worcestershire, United Kingdom). Photon Correlation Spectroscopy determined the velocity distribution of particles movement by measuring dynamic fluctuations of intensity of scattered light. While doing this analysis, the cuvette was shaken for about 10 sec. by hand and placed immediately inside the sample holder of particle size analyzer. Once the required intensity was reached, analysis was performed to obtain the mean particle size and polydispersity index (PI). Analysis was performed in triplicate (angle - 90 deg.; diluent – water; temp. - 25°C; run time – 200 sec.). Laser Diffraction method was used for detecting the larger particles that were in the micron

range. During analysis low agitation was used without any sonication to prevent any change of crystal size. This test was also performed in triplicate.

Figure 3 Laser Diffraction Particle size analyzer

Figure 4 PCS Particle size analyzer

4.2.6 Solubility study

Solubility studies were performed by mixing excess amount of drug into water containing various concentrations of surfactant (vitamin E TPGS, Pluronic F-127) /

co-solvent (propylene glycol) and kept at 37°C with constant shaking until 72 hours. After that the solution was centrifuged using a centrifuge (CT422, Jouan Inc., Winchester, VA, USA) at 3000 rpm, the supernatant liquid was collected and the concentration of drug dissolved was analysed using HPLC (as described below).

4.2.7 Permeation study

The permeation study was performed using similar method described previously[14]. Two different membranes were used for this screening study: a) silicon membrane of 10 K MWCO (CoTran™ 9728, Membrane Ethylene Vinyl Acetate (EVA) Membrane from 3M) and b) dialysis membrane of 10K MWCO (Slide-A-Lyzer Dialysis cassettes from Thermo Scientific).

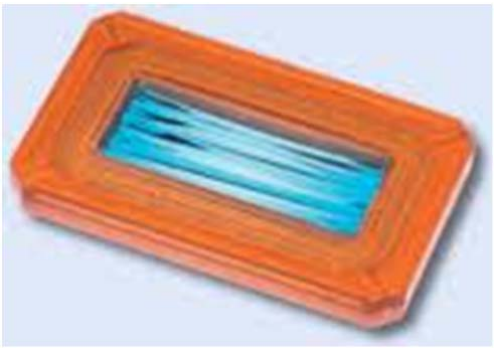


Figure 5 Dialysis membrane

After washing and equilibration with PBS, the synthetic membranes were mounted on static vertical Franz Diffusion cells –Permegear Inc., Bethlehem, PA (receptor volume 5.1 ml, donor area 0.64 sq. cm.) by clamping them between the donor and receptor compartments. The receptor compartment was filled with PBS (pH 7.4) which was maintained at 37°C $\pm$ 0.5°C and constantly stirred at 600 RPM (Table 2). Formulation was added (0.5 ml) to the donor compartment as an infinite dose to

completely cover the membrane surface. Receptor samples were collected 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 skin. Dermatomed (~500 um) pig skin obtained from the abdominal regions of young Yorkshire pigs (26.5–28 kg, UMDNJ, Newark, NJ). The skin was stored at −80°C. Prior to each experiment; the skins were allowed to thaw to room temperature, equilibrated and then used immediately for *in vitro* permeation studies.

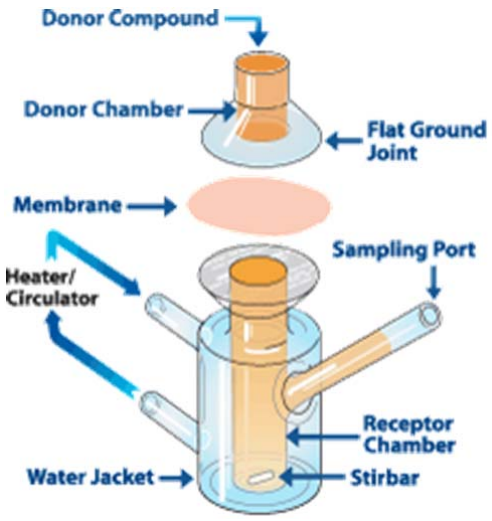


Figure 6 Vertical Franz cells (receptor volume 5.1)

4.2.8 HPLC analysis

The assay and degradation products were 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 um particle size, 4.6 x 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 minutes (4). The method was validated and the linearity of the calibration curve was recorded.

Table 2 Parameters for permeation studies

Permeation study parameters	
Membrane	0.64 sq. cm. Equilibrated in PBS solution at RT for 30min
Receptor solution	5.1 ml of Phosphate buffer (Phosphate Buffer Saline tablet to be dissolved in 100 ml HPLC water and sonicated for 10 min)
Stirring speed	600 RPM
Temperature	37°C
Formulation volume	500 ul
Receptor sample volume	300 ul

4.3 Results and Discussion

4.3.1 Solubility study

The flux of a given drug is limited by its solubility. The permeation of drug through the skin depends on the chemical potential, which is controlled by extent of supersaturation of the drug in the solubilizer-polymer system. A solubility study was performed with three different solubilizers / co-solvent to determine the concentration

of the solubilizer needed for producing supersaturated systems. The solubility study showed that ibuprofen possessed the highest solubility in propylene glycol. The solubility was low at lower concentrations of propylene glycol, however it increased exponentially with increases in the concentration of this solubilizer. For vitamin E TPGS, a linear increase of solubility was observed with concentration. Pluronic F-127 showed less effect on the solubility of the drug (Figure 7).

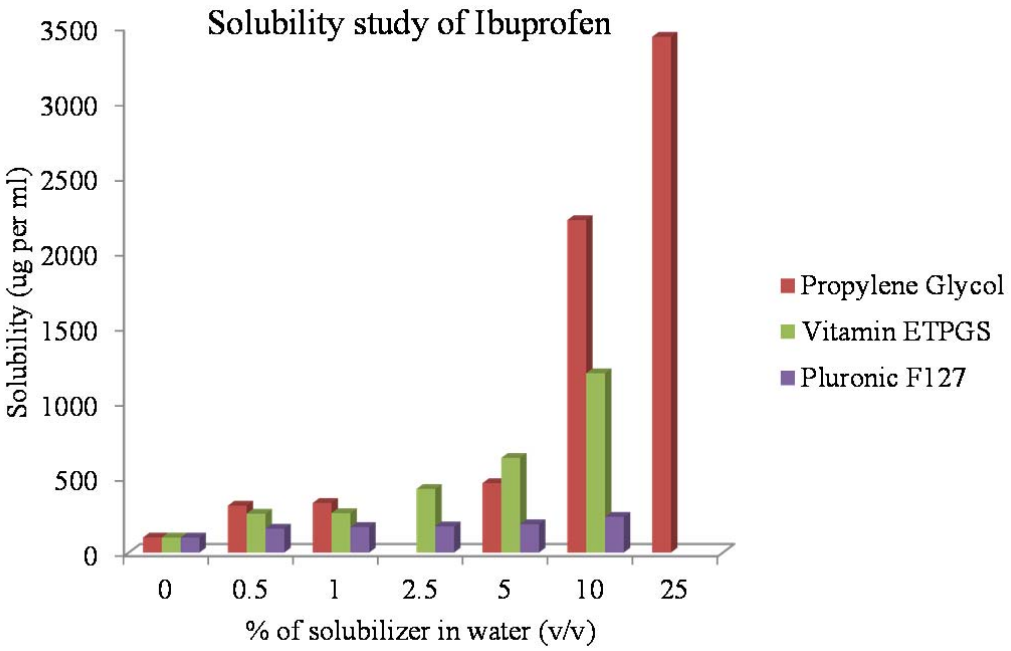


Figure 7 Solubility study of drug in water using different solubilizers / co-solvent.

Propylene glycol was used as a co-solvent to increase the solubility of the drug, whereas vitamin TPGS and Pluronic F-127 were used as non-ionic solubilizers to improve the solubility of the compound. Based on this study, the concentration of the formulation ingredients were selected for further investigation. Propylene glycol was selected at a 25% (v/v) based on the previous studies (7), which showed that this concentration is effective to improve the permeability of drug through the skin. It was

decided to use the solubilizers (Pluronic F127 and Vitamin E TPGS) above their CMC value (critical micelle concentration). In one of the previous studies, vitamin E TPGS was used at 5% w/v (12) in order to produce a transdermal film. Based on the data obtained from the solubility study and from published information, it was therefore decided to use 5% w/v of vitamin E TPGS. For comparison purposes, a similar concentration was also selected for Pluronic F127. Finally, these surfactants could also potentially cause skin irritation due to exposure at higher concentrations based on the MSDS (Material Safety Data Sheet). However, no systematic study was conducted to identify the threshold concentration to trigger skin irritation. The supersaturation factor was estimated from the solubility study as indicated in Table 3.

4.3.2 Stability study

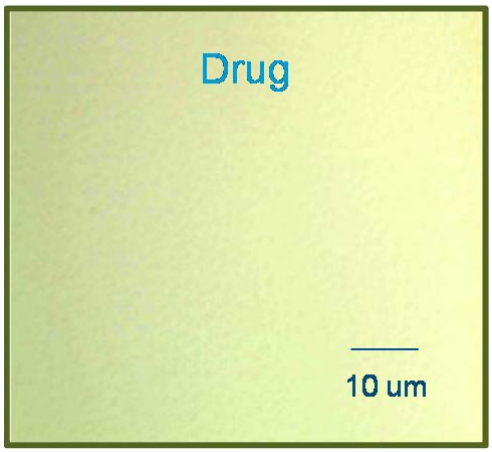
The stability study was conducted to identify the appropriate conditions for inhibiting crystal growth in the formulation. While using light microscopy, no crystal growth was observed with the drug dissolved in water, since the drug in the water remained in the saturated state. However, the rate of nucleation and crystal growth increased with the supersaturation level of the drug. Therefore, when propylene glycol was added, significant crystal growth was observed within 6 hours after sample preparation. In presence of vitamin E TPGS and Pluronic F-127 crystal growth was also observed. However, the sizes of crystals were comparatively smaller.

In presence of HPMC 3 cps, crystal growth was inhibited for Drug- propylene glycol system to a certain extent. For vitamin TPGS & Pluronic F-127, crystal growth was inhibited significantly and also the size of the crystals were smaller compared to

those observed with propylene glycol. Inhibition of crystal growth was not significant for PVP K-30 (Figure 8).

Table 3 Estimation of supersaturation factor of drug from solubility study

% of vehicle in water(w/v)	Supersaturation factor		
	25% Propylene Glycol	5% Vitamin ETPGS	5% Pluronic F127
0	-	-	-
0.5	3.12	2.59	1.59
1.0	3.30	2.62	1.70
2.5	-	4.23	1.74
5.0	4.62	6.30	1.88
10.0	22.14	11.94	2.38
25.0	34.35	-	-
50.0	110.19	-	-



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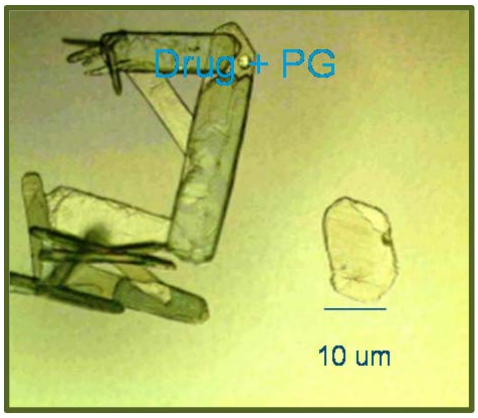

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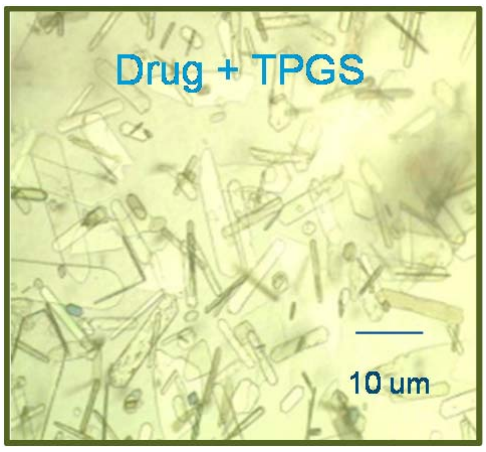

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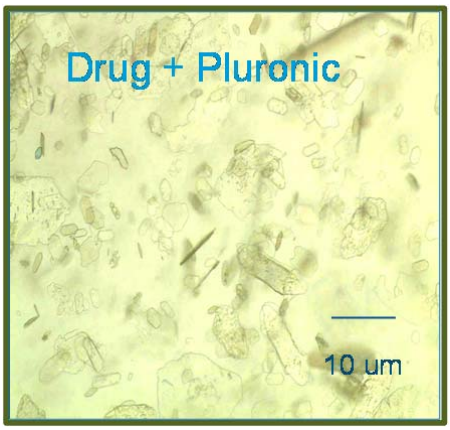

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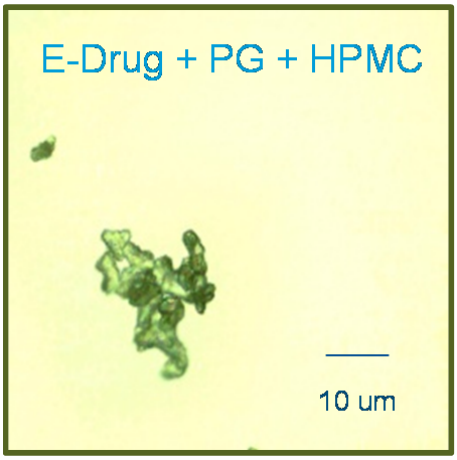

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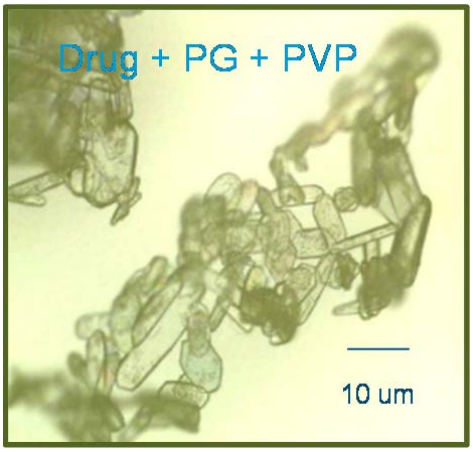

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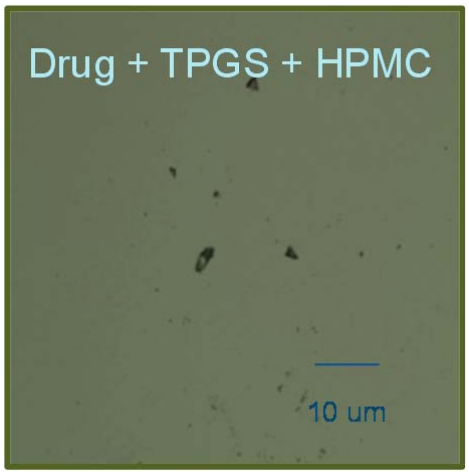

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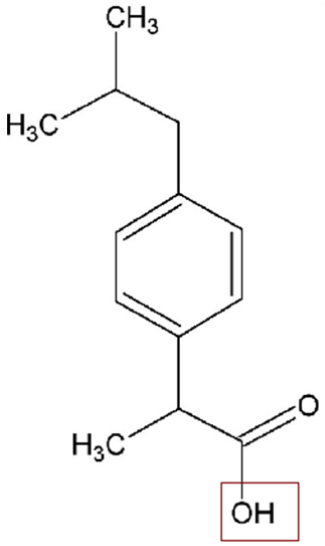
H

Figure 8 Microscopic study of crystal growth in supersaturated solutions after 1 week (A-Drug, B-Drug + PG, C-Drug + TPGS, D-Drug + Pluronic, E-Drug + PG + HPMC, F-Drug + PG + PVP, G-Drug + TPGS + HPMC, H-Drug + TPGS + PVP.

Therefore it was concluded that crystal growth inhibition caused by HPMC 3 cps was higher than that for PVP K-30. This can be explained by the fact that HPMC 3 cps interacts more strongly through hydrogen bonding with the drug as compared to PVP K-30 and probably provided improved surface coverage. The high affinity of HPMC 3 cps to the drug molecule can be explained by its open chain like structure whereas PVP K-30 has a more compact or coil shaped structure (Figure 9). In Table

4, authors tried to explain the functional groups responsible for forming hydrogen bond between the drug and different ingredients used in the formulations. The morphology of crystals formed from various additives seemed to be quite different since the habit of the crystals formed depended on the growth rate of crystallographic faces.



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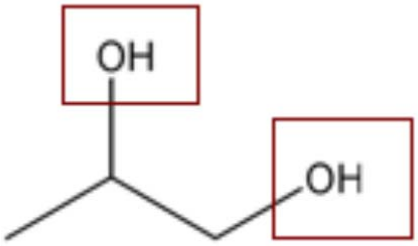

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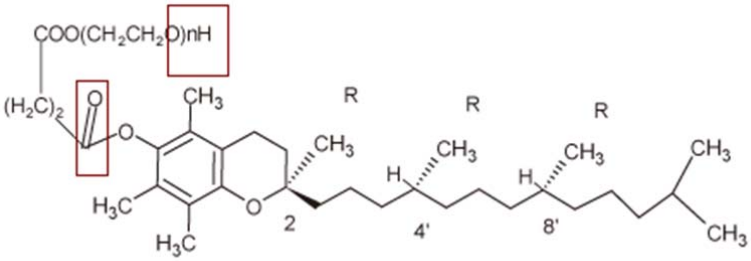

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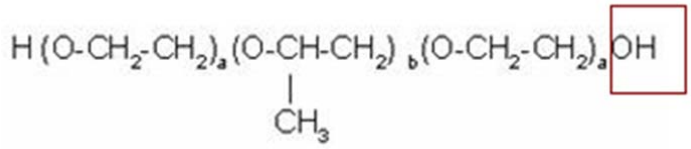

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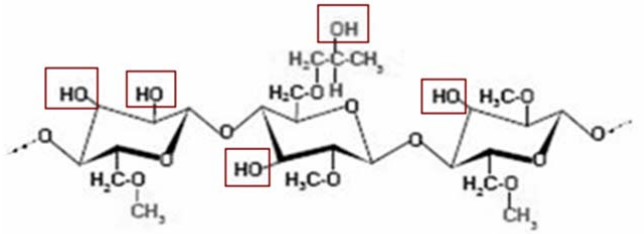

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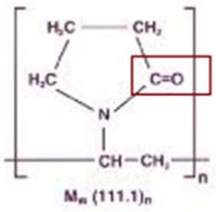

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Figure 9 Chemical structures, A. Ibuoprofen, B. Propylene Glycol (PG), C. Vitamin E TPGS, D. Pluronic, E. Hydroxypropylmethylcellulose (HPMC), F. Polyvinyl pyrrolidone (PVP). All the functional groups, responsible of producing hydrogen bond, were highlighted within the box.

Table 4 Functional groups in different compounds, responsible for forming the hydrogen bond

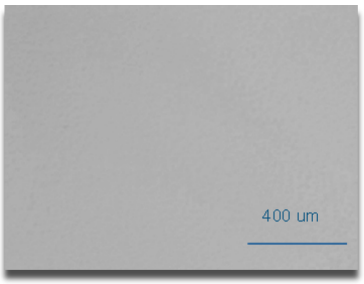
Compounds	**Functional groups responsible for hydrogen bonding**
Ibuprofen	acid group (-C00H)
Propylene Glycol	hydroxyl groups (-OH)
Vitamin E TPGS	alcohol group (-CH_2OH) and ester group (=C=O)
Pluronic	hydroxyl group (-OH)
Hydroxypropylmethylcellulose	multiple hydroxyl groups (-OH)
Polyvinyl pyrrolidone	ester group (=C=O)

In order to optimize the level of polymer needed to inhibit the crystallization, a study was performed to measure the crystallization time (time at which the first crystal was observed under the microscope). The crystallization time was increased by increasing the amount of HPMC in the supersaturated system (Table 5).

In the supersaturated state, instantaneous nucleation took place due to the collision of molecules that triggered the crystal growth. However, when the polymer was added the nucleation process became diffusion controlled and the onset of nucleation was delayed due to the increase in the amount of polymer. The strong hydrophobic interaction between the drug and the polymer had to be eliminated for the drug molecules to form the nuclei for crystal growth. The strength of interaction controlled the onset of the crystallization process that in turn depended on the storage time (Figure 10).

Table 5 Estimation of the onset of crystallization time of Vitamin E TPGS supersaturated solutions with different amounts of HPMC 3 cps

HPMC (%)	Onset of crystallization (hrs)
0	3
0.5	3
1.0	4
1.5	6
2.0	10
2.5	10
3.0	12



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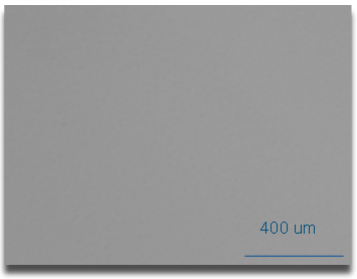

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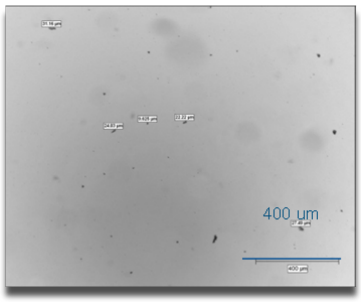

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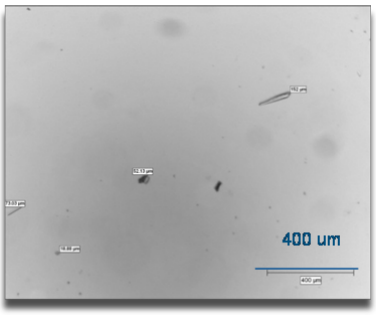

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Figure 10 Microscopic study of onset of crystallization with storage time for TPGS + HPMC supersaturated systems (A- 0 hr, B- 6 hr, C-12 hr, D-24 hr).

In a separate study, the detection of crystal growth was performed more quantitatively by using a combination of photon correlation spectroscopy (PCS) and the laser diffraction (LD) method. By using these analytical tools, the size of the crystals present in the solution could be measured. The PCS method was utilized to detect any presence of crystals in the sub-micron size range and the LD method was used to detect large crystals in the micron range. The results were very similar to visual observation using microscopy (Figure 11). Crystal growth was significantly inhibited using HPMC 3 cps and this effect was higher than that of PVP K-30. Here the polymer probably occupied the adsorption sites on the drug crystals by hydrogen bonding with the –COOH group of the drug and thus created a mechanical barrier. Crystal growth was also significantly less for Drug-Vitamin E TPGS-HPMC 3 cps

system as compared to Pluronic F-127 or propylene glycol. This finding can be explained by the strong hydrophobic interaction with the drug crystals due to the presence of hydrogen bonding between functional groups in vitamin E TPGS (Figure 3) that retained the drug in a solubilized state.

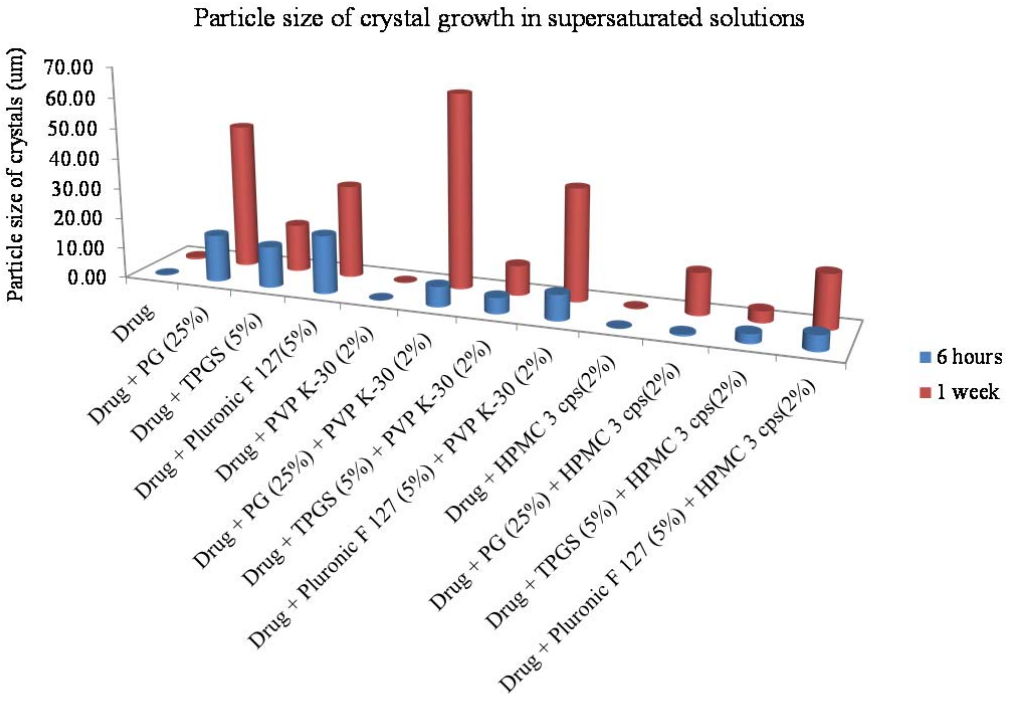

Figure 11 Particle size analysis of crystal growth in supersaturated solutions during storage.

In another study, the clear solution obtained after centrifugation was filtered through a 0.22 um filter. By using this additional step, crystal growth was significantly minimized for the vitamin E TPGS-HPMC 3 cps formulation. The filtration step most probably eliminated any crystal "seeds" present in the solution that would have triggered crystallization. However, we tested non-filtered samples in our screening studies to represent the worse case scenario.

4.3.3 Membrane selection study

Although synthetic membranes are not indicative of actual permeation in biological tissue, we used synthetic membranes to initially screen the relative permeability of drug in presence of different formulation vehicles. Also synthetic membranes are less complicated as compared to the biological skin. Thus in order to eliminate the possible variability of permeation parameters, the initial formulation feasibility studies was conducted by using the synthetic membranes. The permeation rate from these membranes was then compared with that obtained using pig skin.

All the membranes and skins were hydrated in PBS for thirty minutes prior to use. Permeability rates were highest for dialysis membranes followed by silicone membranes. As expected, the permeability through the skin was the lowest. The skin rates were closest to those obtained for the silicone membranes (Figure 12). Based on this study, silicone membranes were selected for further screening studies. The permeability parameters were estimated using the following equations.

a. Flux, Jss (ug/cm^2/h) was calculated from the slope of the cumulative drug amount permeated through the membrane (0.64 sq. cm.) versus time plot. The results were multiplied by a factor (1.56) in order to represent the data as ug/cm^2/h.

b. Enhancement ratio (ER) was estimated by using the equation; ER = Jss of test sample / Jss of control sample (saturated solution of drug in absence of any polymer and solubilizer).

4.3.4 In vitro permeation study using silicone membrane

The permeation study of the supersaturated solutions was conducted using silicone membranes. From this study the highest permeation rate was observed for the vitamin TPGS system, followed by Pluronic F-127 and finally the propylene glycol. This was in spite of using 25% (w/v) propylene glycol as compared to 5% (w/v) of vitamin E TPGS or Pluronic F-127 (Figure 13).

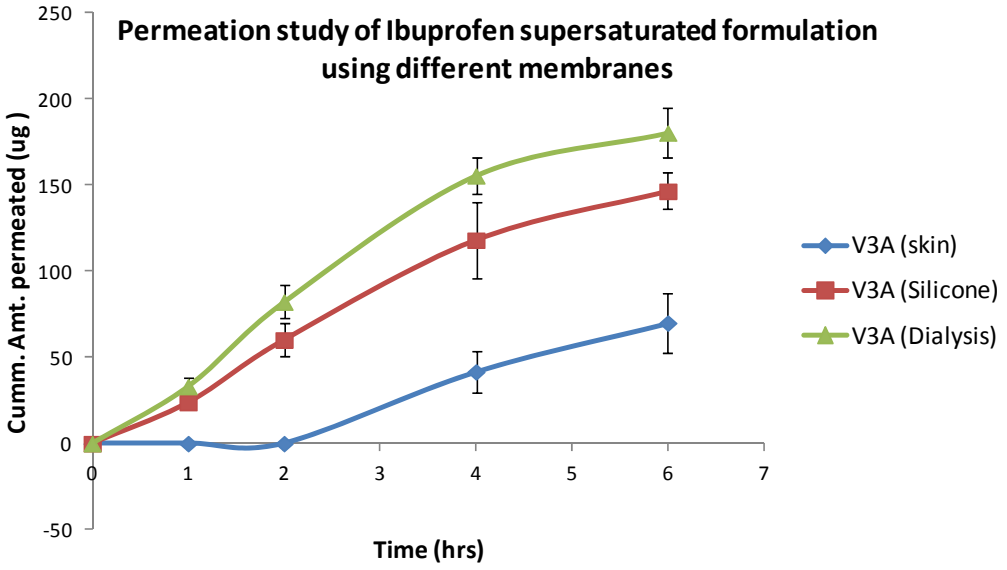

Figure 12 Permeation study of supersaturated solution of ibuprofen using various membranes.

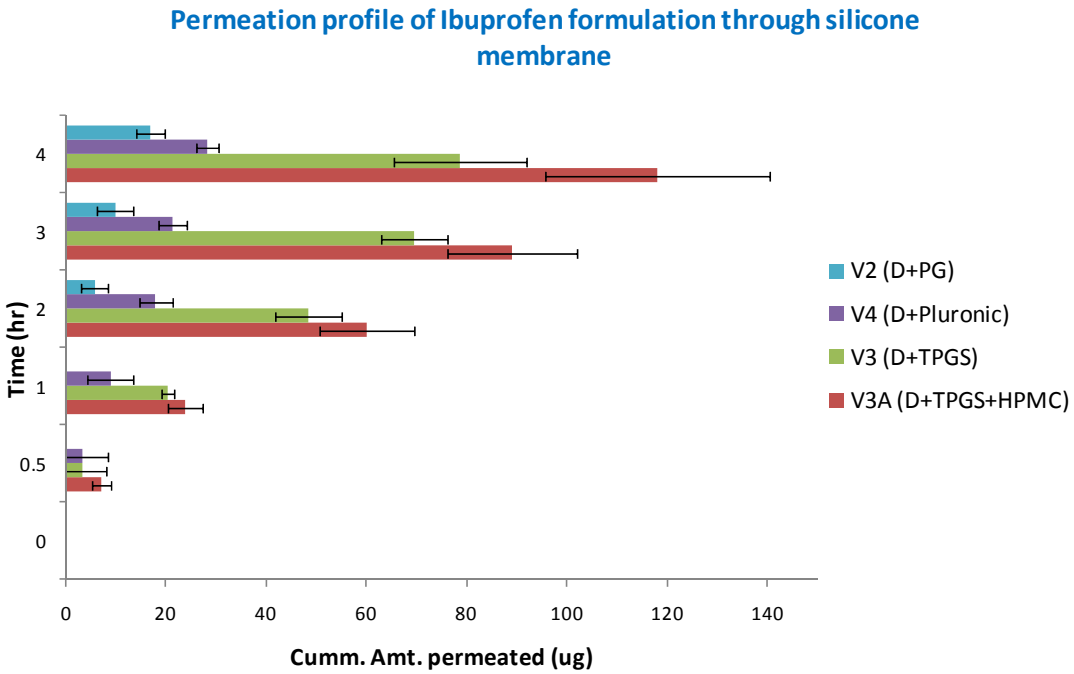

Figure 13 Effect of solubilizers-polymer combinations on permeability of ibuprofen through silicone membranes.

The permeation rate and enhancement ratio was determined for these formulations using Fick's law. Fick's law ($J_S = DKCs/h$) describes the flux (J) across a rate-limiting barrier (thickness h) at sink conditions including solubility (Cs), lipophilicity (partition coefficient K), and the molecular weight or size (diffusion coefficient D). The enhancement ratio (ER) is defined as the ratio between the mean flux of supersaturated system and the mean flux of the saturated drug solution without using any co-solvents.

The highest permeation rate was observed for vitamin E TPGS system (27.7 ug/cm^2/hr; SD-3.4; n=3) through the synthetic membrane, followed by Pluronic F-127 (8.3 ug/cm^2/hr; SD-1.3; n=3) and finally propylene glycol (5.5 ug/cm^2/hr; SD-1.3; n=3) (Table-6). In a separate study, while evaluating the effect of hydroxypropyl methylcellulose (HPMC 3 cps), the permeation rate of the drug through the membrane

was higher when HPMC 3 cps was used in the formulation due to crystal growth inhibition, which was in agreement with the stability study performed earlier. Therefore, HPMC 3 cps was identified as a potential stabilizer to inhibit crystallization and also improve the permeability rate of drug (39.1 ug/cm^2/hr; SD-6.7; n=3).

Table 6 Estimation of permeation parameters from supersaturated systems using silicone membrane (n=3)

Formulation	Flux, Jss (ug/cm^2/h)	Enhancement ratio, ER
V1 (Drug)	3.1 (SD-0.3)	-
V2 (Propylene Glycol)	5.5 (SD-1.3)	1.8
V3 (Vitamin E TPGS)	27.7 (SD-3.4)	8.9
V4 (Pluronic F-127)	8.3 (SD-1.3)	2.7
V5 (Vitamin E TPGS + HPMC 3 cps)	39.1 (SD-6.7)	12.6

Vitamin E TPGS (TPGS, D-a-tocopheryl polyethylene glycol 1000 succinate) has been utilized for numerous applications in pharmaceutical dosage forms. Due to the presence of both a lipophilic and a hydrophilic moiety, it is considered as an ideal surfactant with an HLB value of 13.2. The solubility of the drug improved in the presence of TPGS through micellar solubilization. The CMC value of vitamin E TPGS in water is around 0.2 mg / ml (0.02%), which is much lower than the amount of TPGS used in the formulation (5%).

The degrees of supersaturation involved in these systems are high and they tend to crystallize by spontaneous nucleation resulting to the decrease of driving force for permeation (15). While evaluating the effect of crystal inhibitor, HPMC had been shown to increase the permeability rate of drug probably by inhibiting the growth of crystals on the surface of the membrane and therefore helped the drug to remain in the supersaturated state. Once the crystal growth took place in the supersaturated system, the chemical potential was reduced which was responsible for lowering the drug permeation rate through the membrane. This interaction between the drug and the polymer resulted in inhibition of nucleation of the drug molecule and hence this helped the drug to remaining supersaturated state for a longer time. The above study was further extended to porcine skin in the following section, to investigate how the formulation components modulated the permeation enhancement properties

4.3.5 In vitro permeation study using porcine skin

When the silicone membrane was replaced by porcine skin, the permeation rate showed similar trends, with highest permeability observed with vitamin E TPGS (Figure 14).

While conducting the porcine skin permeation study, the permeation rate showed similar results in a 24 hours study, with highest permeability was observed with vitamin E TPGS and HPMC system with a permeation rate of 6.3 ug/cm^2/hr (SD-0.44; n=4) compared to 4.3 ug/cm^2/hr (SD-0.33; n=4) for the TPGS system without HPMC (Figure 15). The systems containing propylene glycol and Pluronic F-127 showed lower flux values (Table 7). The values reported in the PhD proposal defence were corrected due to an error from the calculation factor.

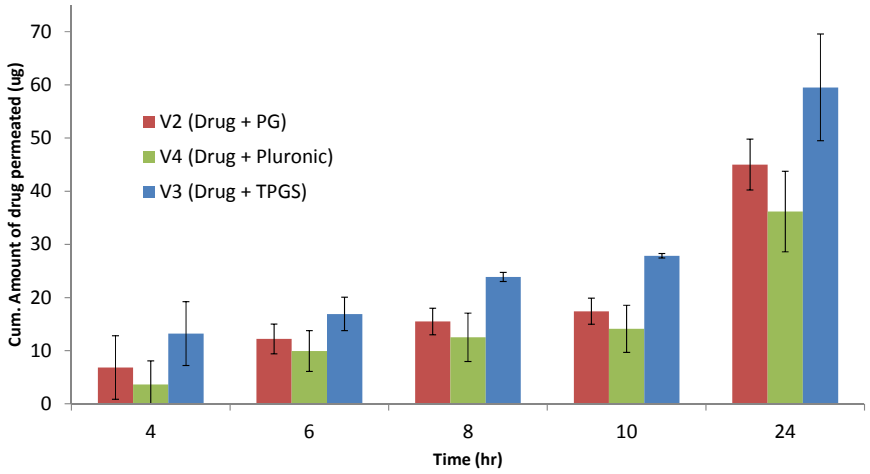

Figure 14 Permeation studies of different supersaturated systems containing solubilizers using porcine skin.

Table 7 Estimation of permeation parameters from supersaturated systems using porcine skin (n=4)

Formulation	Flux, Jss ($ug/cm^2/h$)	Enhancement ratio, ER
V1 (Drug)	0.7 (SD-0.4)	-
V2 (Propylene Glycol)	3.0 (SD-0.32)	4.3
V3 (Vitamin E TPGS)	4.3 (SD-0.33)	6.1
V4 (Pluronic F-127)	2.5 (SD-0.15)	3.6
V3A (Vitamin E TPGS + HPMC 3 cps)	6.3 (SD-0.44)	9.0

Theoretically, the formulation containing propylene glycol should have shown highest flux based on the thermodynamic activity resulting from higher solubility

compared to vitamin E TPGS. Our data did not show this to be the case. This unexpected result may be explained by the following information.

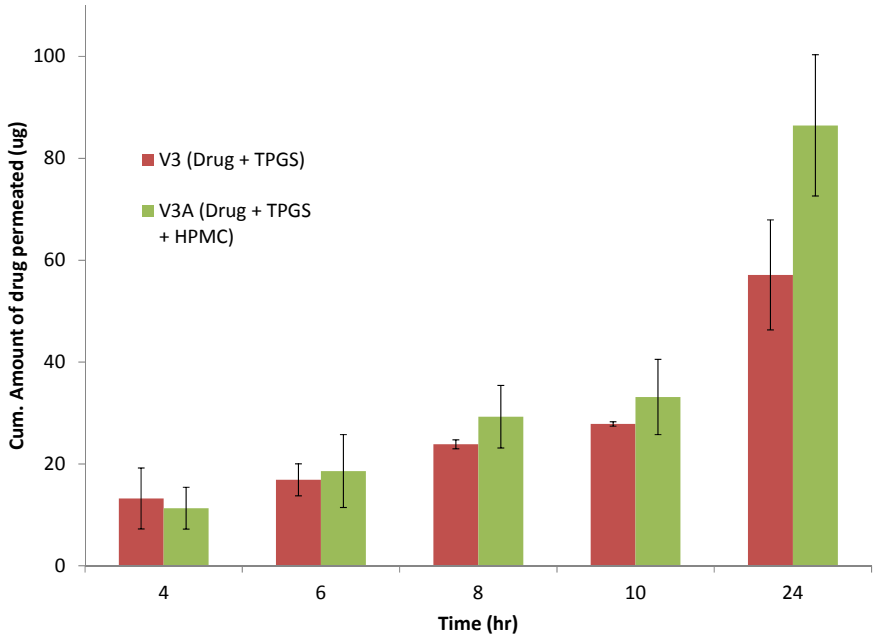

Figure 15 Effect of crystal inhibitor on the permeation of drug from supersaturated solution.

Besides increasing the solubility of the drug, vitamin E TPGS also plays an important role in promoting diffusion by altering the skin structure (*D*), by modifying partition phenomena by making the barrier more lipophilic (*K*) and thus reducing the interfacial tension. This makes the SC more permeable to poorly water soluble compounds such as ibuprofen. Hence this can explain the enhanced flux through the skin which is a novel finding. Previous studies reported the importance of vitamin E TPGS in improving the absorption of orally administered drugs e.g. paclitaxel (14) and nifedipine(15). In addition, other studies reported its unique role as an absorption enhancer(16).

Propylene glycol although reported (17) to have similar effects in skin permeation studies, its effects appeared to be less than those of vitamin E TPGS. Although Pluronic F-127 is a non-ionic surfactant like Vitamin E TPGS, however its role on the permeability enhancement was not significant. Probably it results in an increase in the driving force for drug transport, however, had no effect on the barrier function of the skin. Also polymers such as HPMC 3 cps were used to inhibit nucleation on the surface of the skin which would forfeit the advantage of the supersaturated systems (Figure 16). The permeation rate of the drug through the skin was lower as compared to that for the synthetic membranes (Table 8). This may be due to the rougher surface of the SC that may induce crystal growth (18).

Table 8 Effect of supersaturation factor on the flux of ibuprofen using synthetic membranes and porcine skin.

Study parameters	PG	Vitamin ETPGS	Pluronic F127
Supersaturation factor	34.5	6.3	1.9
Flux through silicone membrane (ug/cm^2/h)	5.5	27.7	8.3
Flux through porcine skin (ug/cm^2/h)	4.3	6.1	3.6

Finally, due to higher thermodynamic activities in the supersaturation system, the drug always has a tendency to crash out, and this result in the crystallization or precipitation phenomena. Previous studies had used conventional parameters such as the use of co-solvents, increase of temperature, change of pH, etc. in order to produce supersaturated systems. In this research study, the authors were able to optimize a

simple system by using a solubilizer (Vitamin E TPGS) and crystal inhibitor (HPMC 3 cps). This system has shown enhanced permeability and improved stability.

Figure 16 HPMC helped to prevent crystal growth in supersaturated solution

Besides HPMC 3 cps, Vitamin E TPGS also probably assisted the drug to remain in the supersaturated state by hydrogen bonding with the drug molecule.

4.4 Conclusion

A promising supersaturated formulation was developed with vitamin E TPGS (V3-A), which produced better results compared to propylene glycol (PG) or Pluronic F-127 formulations during *in vitro* permeation studies using synthetic membrane or porcine skin. In presence of polymer, the onset of crystallization was delayed. The optimization of the formulation with HPMC 3 cps resulted in inhibiting crystal growth during stability studies as compared to PVP K-30, which also increased the permeation rate of drug through the skin. In this study, the amount of polymer used was relatively low (2%), which probably did not play any significant role on the diffusional resistance on the drug molecules to prevent nucleation. The hydrophobic interaction between the drug and the polymer was probably responsible for the inhibition of nucleation. Future studies will be conducted to optimize a gel formulation with increased amounts of polymer to increase the viscosity of the supersaturated systems and provide additional crystal growth inhibition by diffusional resistance.

4.5 References

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Chapter 5. Design and characterization of submicron suspension for a poorly soluble drug: The effect of Vitamin E TPGS and other solubilizers on skin permeability enhancement.

5.1 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. In addition, the prediction of adequate skin delivery of drugs from formulations has always been difficult. 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. Although these molecules should principally possess enhanced permeation rates due to their higher lipophilicity, it is usually reported that the rate of dissolution of the drug in the delivery system becomes rate limiting for these types of molecules. As a result zero order permeation is not achieved.

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 (1). Different formulation approaches, such as, microparticles, solid lipid nanoparticles, and nano lipid carriers were also evaluated

(2-4), 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 (5-6). The crucial factors that need to be considered for formulation design include drug loading, skin permeability, stability, and 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 (7) particles below 3 um in diameter, can penetrate the SC through intracellular pathway and particles ranging from 3 um – 10 um penetrate through sebaceous follicles. However, several recent studies using smaller sized particles provided new correlations between particle size and penetration routes. In one study the in vitro permeation profile of nanoparticles (40 nm – 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 larger sized particles due to the tight network of epidermal Langerhan's cells (8). 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. Further, the enhancement was promoted when the size of the particles was decreased (9). Thus, follicular transport was proved to be a potential pathway for dermatotherapy and cosmetics. In one study it was shown that when the particle size was higher than 5 um, 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 (10).

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 (11). Besides inorganic materials, a wide variety of applications were reported for organic nano and microparticles using different formulation approaches such as polymer particles used for encapsulation of drug, solid lipid nanoparticles, etc. Solid lipid nanoparticles with smaller diameters (about 208 nm) improved the penetration of diclofenac sodium through rat skin (12).

One of the simple approaches studied recently, was to reduce the size of the crystals for poorly soluble drug compounds. 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 (13-14). 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 (15-16). 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 micronization 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.

5.2 Materials and methods

5.2.1 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.

5.2.2 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.

5.2.3 Short Term Stability study

The submicron formulations were kept on short term stability (2-8^0 C) for studying crystal growth. Samples were collected at different time points between 0 to 6 weeks.

5.2.4 Microscopy study

The size of drug crystals in the suspension was studied by Olympus microscope, (BX50, Tokyo, Japan) at a magnification of 100 X. 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.

5.2.5 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 sec. 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 deg., Diluent – Water, Temp. - 25^0 deg. C, Run time – 200 sec.).

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

Code	TPGS % (w/v)	Pluronic % (w/v)	PG % (w/v)	Drug % (w/v)	HPMC % (w/v)	PVP % (w/v)
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

5.2.6 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 aluminium 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 ^{0}C and the modulation of ± 1.00 ^{0}C at every 60 seconds was applied. Under these conditions, the sample was initially allowed to isothermally equilibrate for additional 8 minutes, before ramping the temperature until 250 ^{0}C (2 deg. C per min).

5.2.7 Permeation study

Two different membranes were used for this screening study: a) silicon membrane of 10 K MWCO (CoTran™ 9728, Membrane Ethylene Vinyl Acetate (EVA) Membrane from 3M) and b) dialysis membrane of 10K 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 sq. cm. by clamping them between the donor and receptor compartments. The receptor compartment was filled with PBS (pH 7.4) which was maintained at $37^0 \pm 0.5$ 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 um) 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°C. Prior to each experiment; the skins were allowed to thaw at room temperature, equilibrated and then used immediately for *in vitro* permeation studies.

5.2.8 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 um particle size, 4.6 x 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 minutes (17). The method was validated and the linearity of the calibration curve was recorded.

5.3 Results and discussion

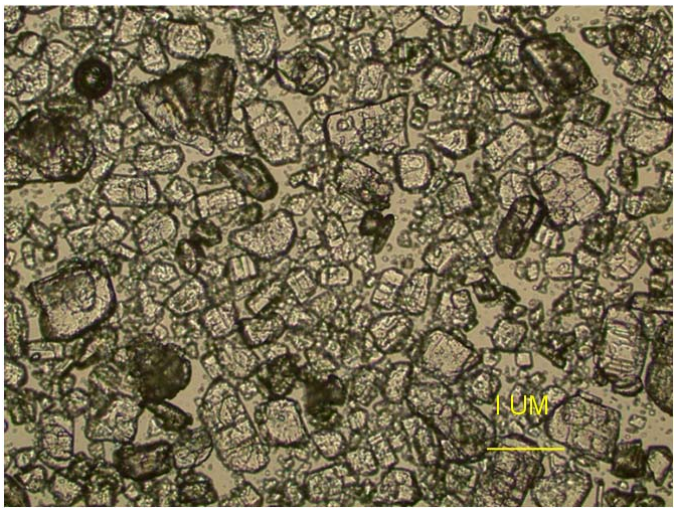
5.3.1 Formulation design

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 hours of micronization process, significant particle size reduction was observed for the drug crystals (Figure 1) and submicron suspension (nanosuspension) was formed.

5.3.2 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 analysed 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 hrs. 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 um. However, after 4 hours, no large crystals were observed and d90 was close to 500 nm (Figure 2). 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 hours due to the presence of larger crystals. After certain time the number of larger crystals reduced in the suspension and thus he rate of reduction of particle size decreased and became almost constant.



A

B

Figure 1 Light microscpe picture of ibuprofen drug crystals (A-before micronization; B-after micronization)

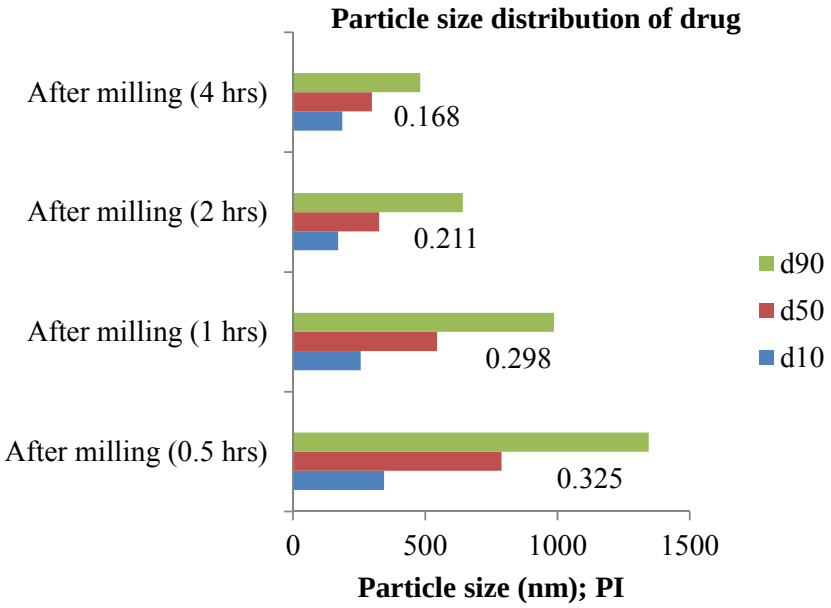


Figure 2 Particle size distribution of ibuprofen drug crystals during the micronization process.

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.

The particle size distributions of submicron suspensions containing Vitamin E TPGS or Pluronic F127 at 1% level are shown in Table 2. When no solubilizers were used, for both the polymeric stabilizers (HPMC 3 cps and PVP K-30), the d90 of drug was below 500 nm which was very promising. However, when the surfactants were incorporated into the system, a slight increase in particle size was observed due to the solubilization effect. While studying the effect of different solubilizer concentrations, (Figure 3) no significant difference between the particle sizes 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 (Figure 4).

Table 2 Particle size distribution of ibuprofen sub micron suspension for different formulations.

Sample	D10 (nm)	D50 (nm)	D90 (nm)	PI (Polydispersity index)
HPMC stabilizer (2%)				
5% Drug with 1% TPGS	251.3	390.3	613.0	0.188
5% Drug with 1% Pluronic F127	186.9	298.5	481.4	0.179
5% Drug	153.4	256.2	435.4	0.165
PVP stabilizer (2%)				
5% Drug with 1% TPGS	442.1	759.2	1320.2	0.365
5% Drug with 1% Pluronic F127	426.1	720.4	1446.5	0.355
5% Drug	138.4	255.1	472.5	0.201

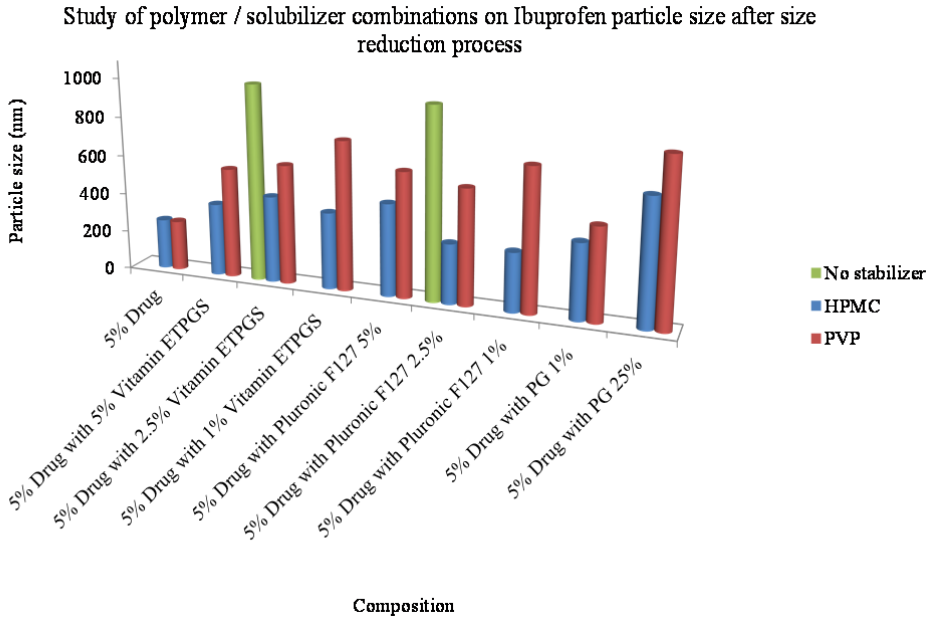


Figure 3 Effect of different solubilizers / polymers on the efficiency of particle size reduction of drug crystals.

The increase of solubility of the drug in Vitamin E TPGS or Pluronic F127, in the range of 1% to 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 (18), 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.

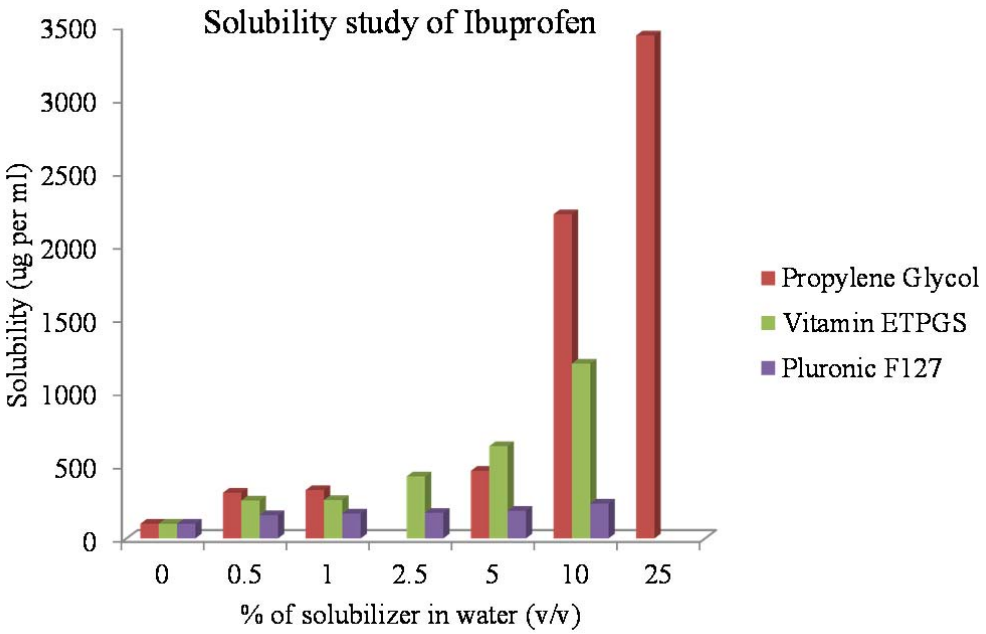


Figure 4 Solubility study of ibuprofen in water using different vehicles.

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.

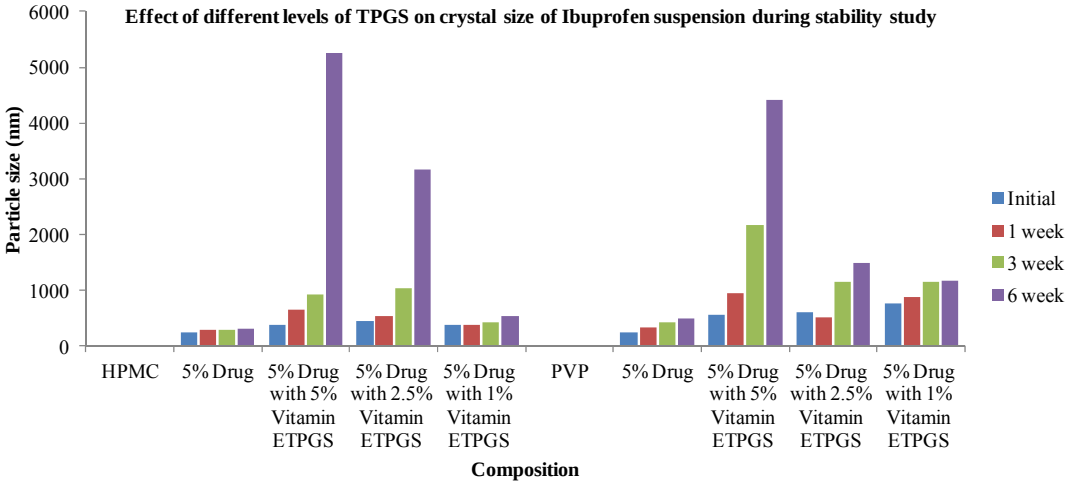
5.3.3 Stability study

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 2-8^0 C. and the particle size of the samples was tested at initial, 1 week, 3 weeks and 6 weeks time points.

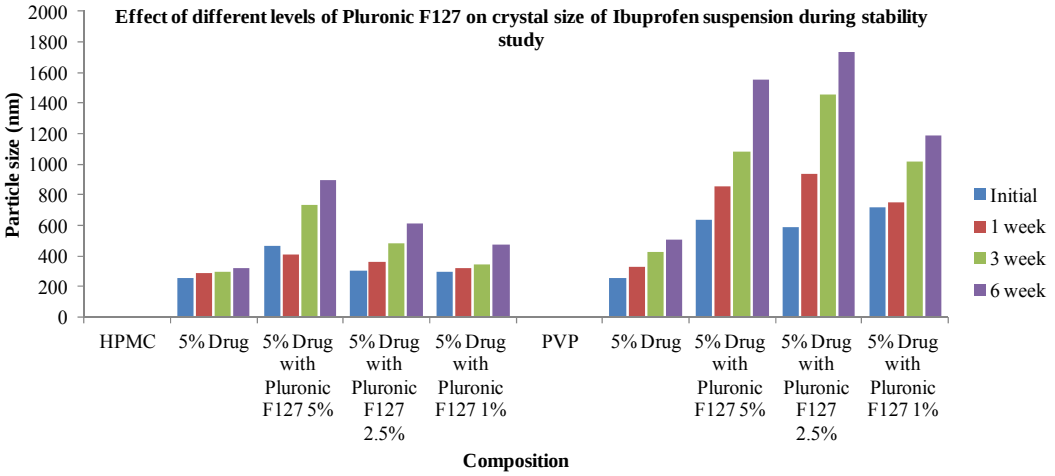
During the stability study of the formulation containing Vitamin E TPGS, significant growth of particle size was observed with increasing concentrations of

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 (Figure 5A). 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 (Figure 5B).

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.

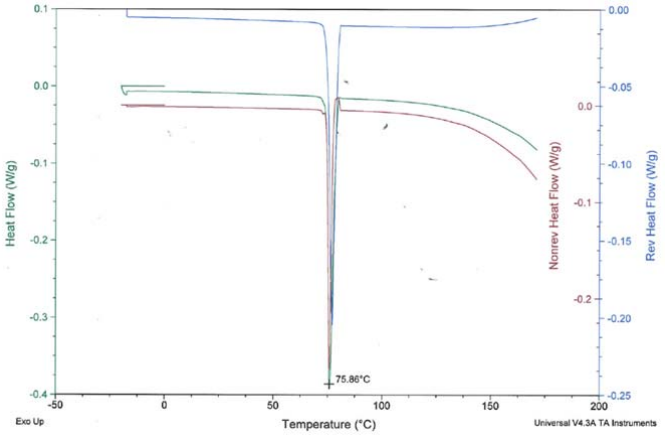


A.

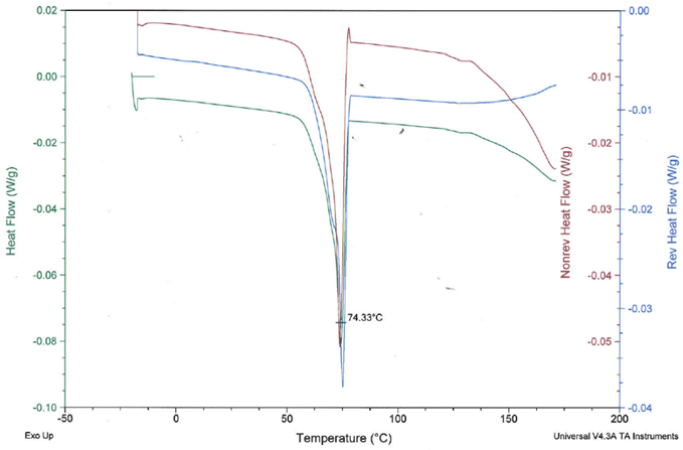


B.

Figure 5 Study of different concentration of solubilizers on the growth of drug crystals during stability study (A-effect of TPGS; B-effect of Pluronic F127).


Heat Flow (W/g)
Nonrev Heat Flow (W/g)
Rev Heat Flow (W/g)
75.86°C
Exo Up
Temperature (°C)
Universal V4.3A TA Instruments

A.


Heat Flow (W/g)
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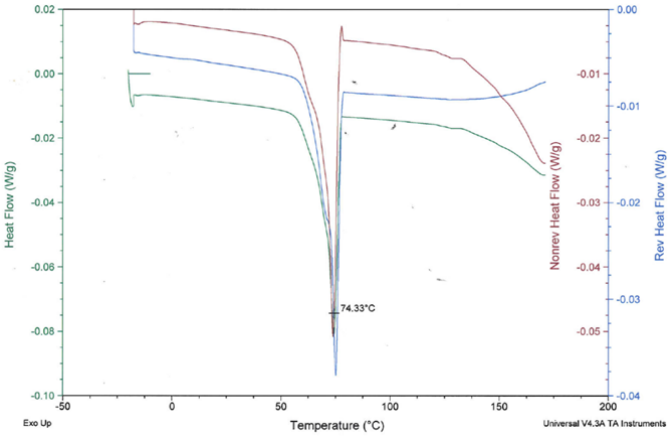
B.


Heat Flow (W/g)
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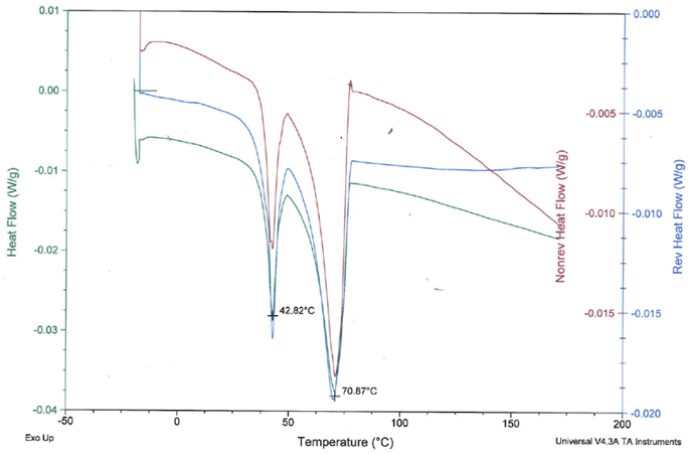
C.



D.

Figure 6 DSC study showing the crystallinity of drug retained after micronization process in presence of different vehicles (A-ibuprofen drug substance; B-submicron suspension with Pluronic F127; C-submicron suspension with PG; D-submicron suspension with TPGS).

5.3.4 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. The modulated differential scanning calorimetry (MDSC) study was performed with suspension formulated with the different vehicles. The results showed no change of crystallinity of the drug substance (Figure 6). Also no change of melting pointing was observed after milling. An additional peak was observed for TPGS system, close to its melting point (41^0 C).

5.3.5 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 minutes prior to use. Permeability rate was highest for dialysis membrane followed by regenerated cellulose membrane and finally for the silicone membrane (Figure 7). Based on this study, the silicone membrane was selected for further screening experiments.

5.3.6 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 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).

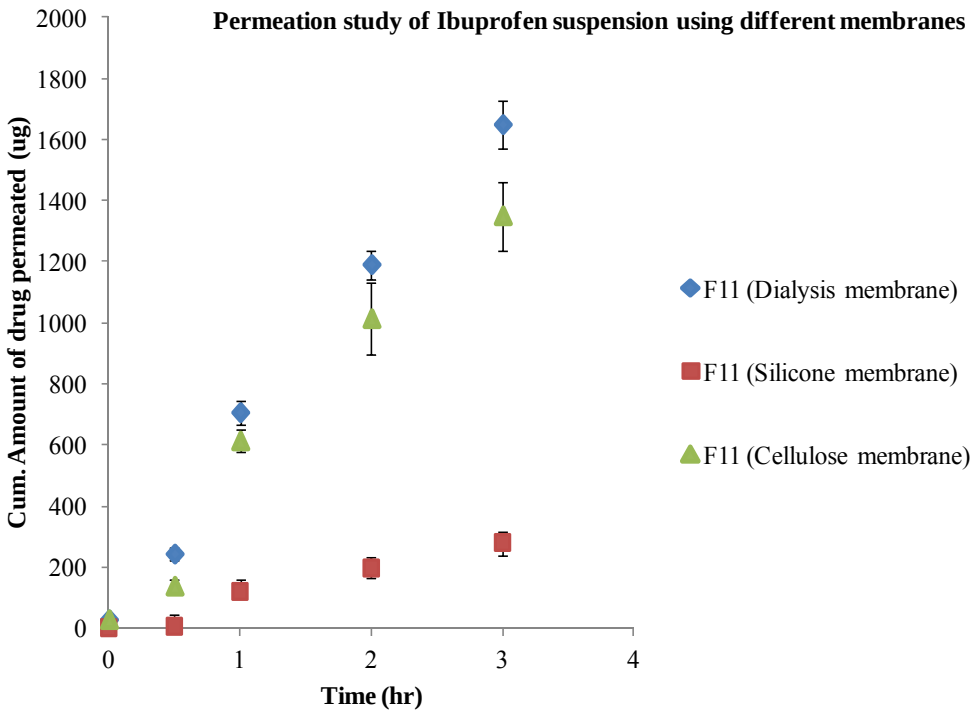


Figure 7 Permeation study of ibuprofen submicron suspension through the synthetic membranes.

These permeability parameters were estimated using the following equations:

a. Flux, Jss (ug/cm^2/h) was calculated from the slope of the cumulative drug amount permeated through the membrane (0.64 sq. cm.) versus time plot. The results were multiplied by a factor (1.56) in order to represent the data as ug/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 (Figure 8) 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 crystallization and also improve the permeability rate of the drug through the skin.

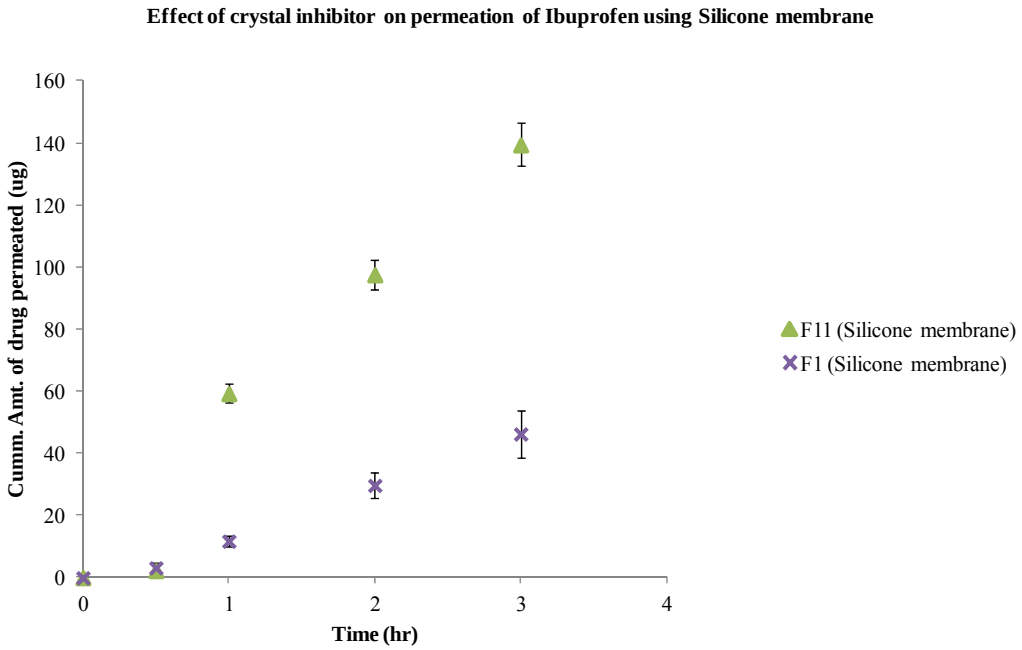


Figure 8 Effect of crystal inhibitor on the permeation rate of ibuprofen submicron suspension through the synthetic membrane.

5.3.7 In vitro permeation study using porcine skin

5.3.7.1 Effect of particle size

A study was performed to evaluate the effect from the drug crystal particle size on the drug permeability through 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 hour of milling. With the increase of milling time, large residual

particles in the suspension were actually reduced into smaller particles. From Figure 9, it was observed that samples collected at 15 min, 30 min and 180 min produced particle size having d50 values close to 891 nm (SD-220 nm; PI-0.33), 655 nm (SD-112 nm; PI-0.21) and 365 nm (SD-78 nm; PI-0.17) respectively.

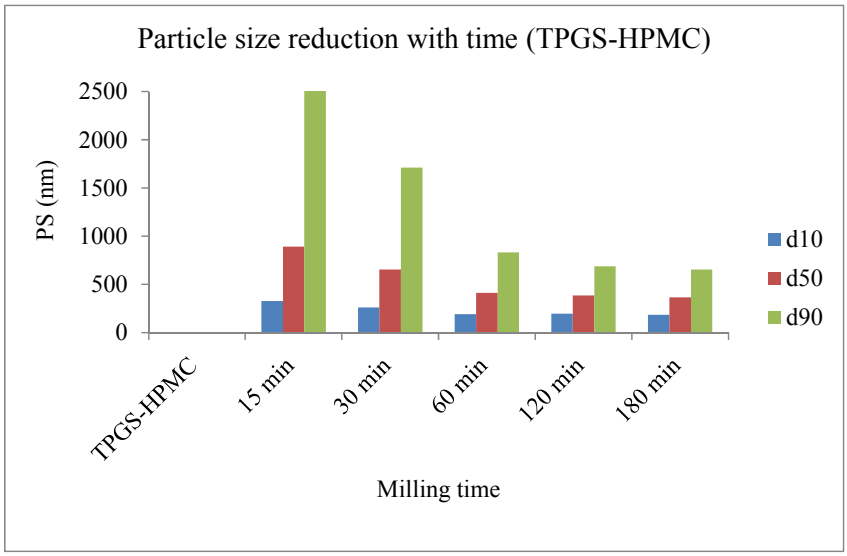


Figure 9 Production of ibuprofen submicron (nano) suspension using TPGS-HPMC 3 cps system

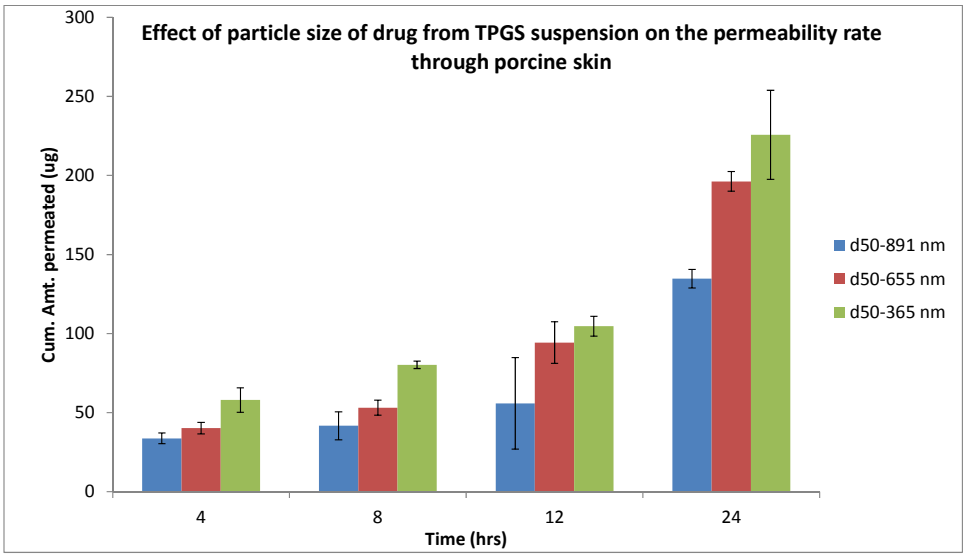


Figure 10 Effect of particle size of drug crystals (using TPGS – HPMC suspension) on the permeability of ibuprofen through the pig skin.

The flux values are shown in Table 3. In the past, particles were defined as "nanoparticles" if their size (d90) was below 1 um. Recently, an additional class has been introduced which was named "sub micron particles". As per this current classification system, we divided the particles into three groups – nanoparticles (less than 100 nm), submicron particles (100 nm – 1 um) and microparticles (1 um – 1 mm) (7). Our permeation study was performed using the drug crystals in the submicron range (250-750 nm).

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

Vitamin E TPGS suspension	**Flux, Jss (ug/cm^2/h)**
365 nm	15.1 (SD-0.2)
655 nm	13.3 (SD-0.2)
895 nm	8.9 (SD-0.04)

3.7.2 Effect of solubilizers

While studying the effect of different solubilizers, the highest permeability was observed with Vitamin E TPGS (Figure 11A), followed by Pluronic F127 and finally with propylene glycol. The flux observed for Vitamin E TPGS was 17.0 ug/cm^2/hr (SD-1.72; n=3) compared to 10.1 ug/cm^2/hr (SD-0.2; n=3) for Pluronic F127 and 5.5 ug/cm^2/hr (SD-0.5; n=3) for propylene glycol (Table 4). The system without any solubilizer showed lower flux values of 6.2 ug/cm^2/hr (SD-0.8; n=3). The

reported flux values in the PhD proposal were modified, while correcting the error with the calculation factor.

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 5), however, without any micronization process. The non-micronized suspension demonstrated similar trends, with the highest permeability observed with Vitamin E TPGS, followed by Pluronic and PG (Figure 11B).

The flux observed for Vitamin E TPGS was 9.1ug/cm^2/hr (SD-0.41; n=3) compared to 4.92 ug/cm^2/hr (SD-0.97; n=3) for Pluronic F127 and 2.31 ug/cm^2/hr (SD-0.24; n=3) for propylene glycol (Table 5). Significantly low flux of 0.7 ug/cm^2/hr (SD-0.4; n=3) was observed for the system which did not contain any solubilizer.

From the estimated enhancement factor (ER), it was observed that:

- Without any solubilizer, the permeability rate increased by 9 fold.
- With all 3 solubilizers (TPGS, PG and Pluronic), permeability rate increased by about 2 fold (Table 4).

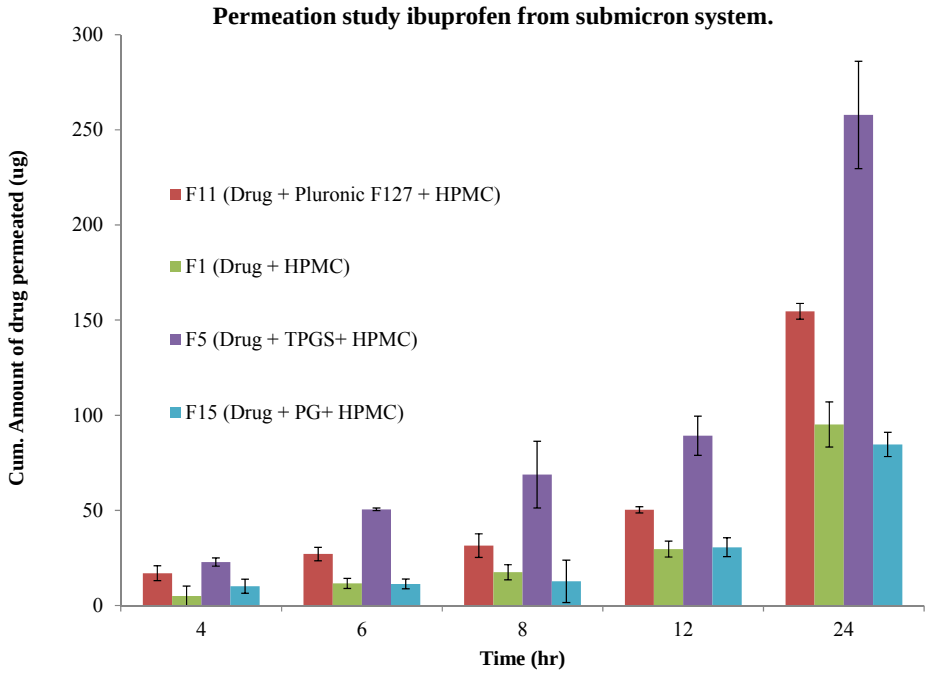
Table 4 Estimation of permeation parameters from micronized and non-micronized suspension in presence of various solubilizer / stabilizer.

Formulation	Flux, Jss ($ug/cm^2/h$)		Enhancement ratio due to micronization; ER
	Before micronization	After micronization	
Suspension (Drug + HPMC)	0.7 (SD-0.4)	6.2 (SD-0.8)	8.9
Suspension (Propylene Glycol + HPMC)	2.31 (SD-0.24)	5.5 (SD-0.5)	2.4
Suspension (Vitamin E TPGS + HPMC)	9.1 (SD-0.41)	17.0 (SD-1.72)	1.9
Suspension (Pluronic F-127 + HPMC)	4.92 (SD-0.97)	10.1 (SD-0.2)	2.1

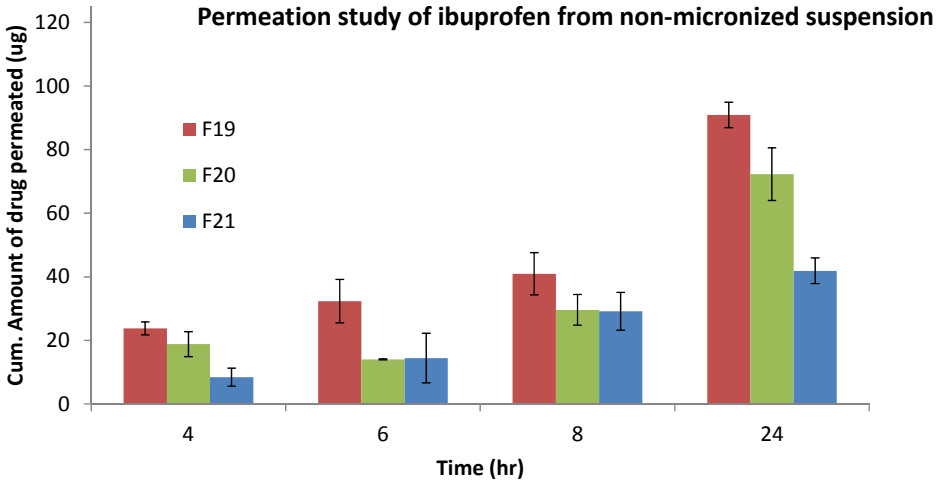
Table 5 Formulation design of ibuprofen non-micronized suspensions.

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%)

It can therefore be seen that the effect of solubilizers on the permeability enhancement appeared to be more critical as compared to the micronization process (Figure 12) and Vitamin E TPGS was observed to be the most effective solubilizer.



A.



B.

Figure 11 Permeation study of ibuprofen suspensions through the porcine skin (A-submicron suspensions; B-non-micronized suspensions)

Vitamin E TPGS (TPGS, D-a-tocopheryl polyethylene glycol 1000 succinate) has been utilized for numerous applications in pharmaceutical dosage forms. In addition to stabilizing drug crystals, TPGS also 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. Previous studies have reported the importance of Vitamin E TPGS for improving the absorption of drugs when these were administered orally (19).

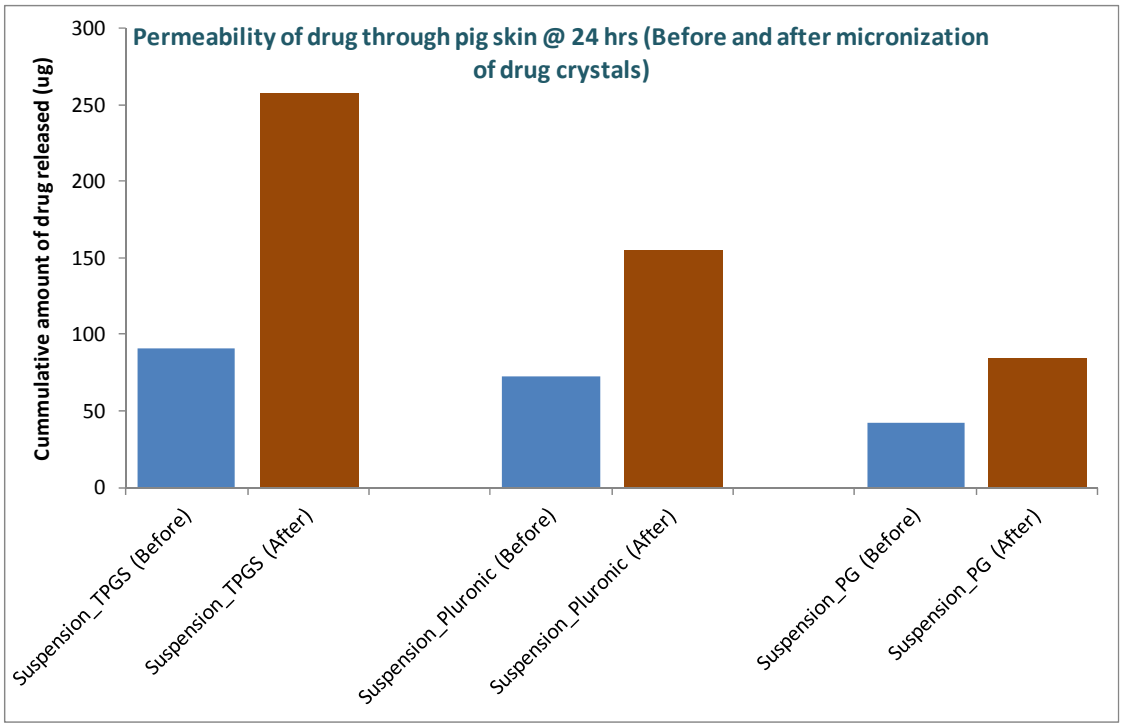


Figure 12 Overall comparison of permeation profile of ibuprofen drug crystals through the porcine skin (before and after micronization process).

Propylene glycol was reported by Herkenne et al., 2008 (20) 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 crystal particle size. 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 (21). 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 Figure 13.

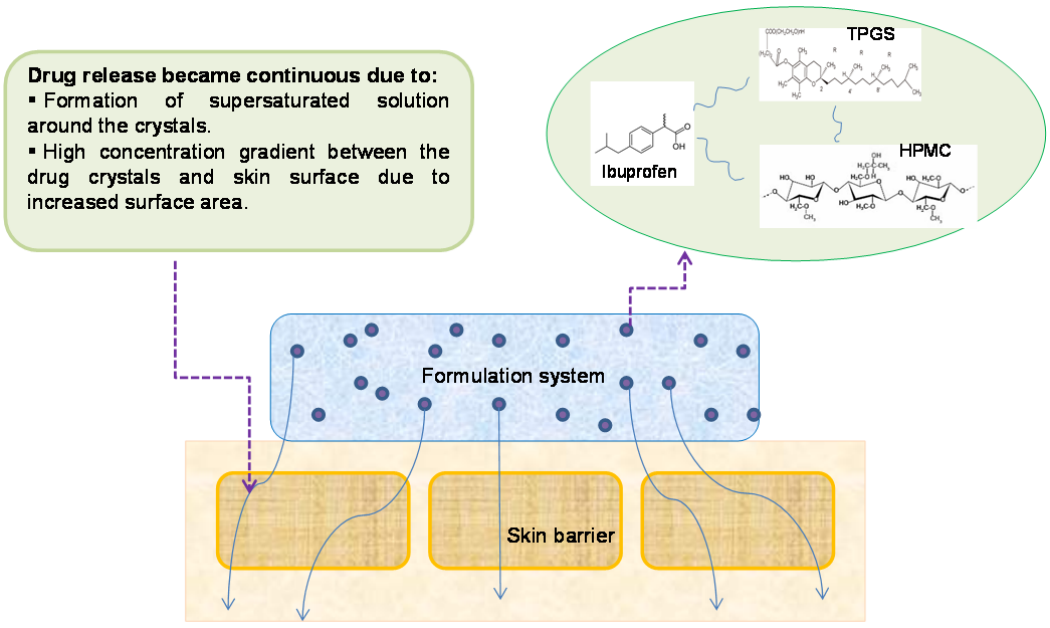


Figure 13 Mechanism of permeation profile of ibuprofen drug crystals from the submicron suspension system.

5.4 Conclusion

During the micronization 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 due to hydrogen bonding. 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.

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Chapter 6. Comparing supersaturated and submicron gel formulations of a poorly soluble drug compound for enhancing skin permeability - A case study.

6.1 Introduction

Administration of drugs to the skin provides two important goals: topical and transdermal absorption. Topical formulations are designed to administer the drug into deeper regions of the skin. Transdermal formulations aim to deliver the drug into systemic circulation. The formulations target various therapeutic areas such as acne, skin infection, melanoma, pain control, motion sickness, angina, and many others. Oral drug delivery has many disadvantages and this becomes even more apparent when this route is used to treat localized disease in the skin. For example, the anti-inflammatory (NSAID) drugs used for the treatment of acute and chronic arthritic conditions can cause gastric mucosal damage which may result in ulceration and/or bleeding. Therefore topical delivery of these classes of drugs overcomes many side effects such as gastric complications (1). There is great interest to develop such topically applied dosage forms to improve patient compliance and to provide relatively consistent drug levels at the application site for prolonged periods.

Despite extensive research and development efforts, only a limited number of drugs can be administered by the topical/transdermal route due to various limitations. One of the reasons is the limitation in permeation of effective concentrations of drugs through the skin barrier for desired therapeutic action. It is well understood that the stratum corneum (SC), the uppermost dead layer of cells of the epidermis, acts as the rate controlling barrier layer for percutaneous drug delivery. Therefore the permeation challenge becomes more pronounced in case of poorly soluble drug molecules (2). Even though these molecules should possess enhanced permeation rates due to their

higher lipophilicity, the rate of release of the drug becomes rate limiting for these compounds.

Several studies have been reported in the literature using topical gels for enhanced drug delivery through the skin (3-5). In order to improve the permeability of the drug through the skin, penetration enhancers were incorporated into the gel. This approach has succeeded in many cases in overcoming the skin barrier, however, is restricted by the skin irritation that may be caused by some of these compounds.

Another approach to achieve drug enhancement involves the use of supersaturated systems with co-solvents and solubilizers (6-9). Linear relationships have been shown to exist between drug content in the transdermal matrix and drug release, resulting in an increased drug flux due to higher thermodynamic activity. However, these systems are often thermodynamically unstable due to the crystallization of drug molecules immediately after formulation or even during storage. Due to the depletion of drug concentration, the flux becomes no longer zero order release. In such a case it is essential to use polymers or other additives to stabilize the supersaturated matrix (10). The success of the prevention of the nucleation process of the dissolved drug by the addition of suitable excipients depends on the ability of these stabilizers to interact with the drug molecules.

Recently nanotechnology has been extensively explored for transdermal drug delivery enhancement. The crucial factors that need to be considered for formulation design include drug loading, stability of drug compound, scale-up ability and most importantly, the permeability factor. The mechanism responsible for skin penetration of nano- and micro-particles depends on the particle size. Recent studies conducted

using smaller sized particles provided new insights concerning the correlation between particle size and skin penetration route. It was shown that 40 nm nanoparticles penetrated the skin via the follicular route; however, limited penetration was observed for larger sized particles due to the tight network of epidermal Langerhan's cells (11). In another study it was also shown that when the particle size was higher than 5 um, 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 (12).

One of the approaches studied recently, was to reduce the size of crystals by the wet media milling approach for poorly soluble drug compounds (13-14). This helped to improve the rate of release of drug substance by increasing the surface area of the crystals during the milling process. Once the particle size was decreased, probably to the submicron or nano range, the saturation solubility increased. This increase promoted the enhancement of the permeation rate through the skin due to an increased concentration gradient.

The novelty of this current study is the comparative evaluation between two different systems - (a supersaturated solution and a submicron suspension / nanosuspension) used to enhance the permeability of a poorly soluble drug through the skin. Vitamin E TPGS and HPMC 3 cps was used as the basic components for the two systems used in this investigation. In a separate study, the authors evaluated the significance of these compounds in the topical formulation (15). Vitamin E TPGS was used in the supersaturated solution to enhance the solubility of the poorly soluble drug. Also it was used to stabilize the system by hydrophobic interactions. Additionally, it was also evaluated as an enhancer during the permeation of the drug

though the skin. HPMC K4 was used as a steric stabilizer to inhibit crystal growth of the drug.

This manuscript is divided into three main sections. The first deals with the design and assessment of gel formulation (using gel forming polymers such as HPMC K100, Na- CMC, Pluronic F127) in order to optimize the final variant, which was tested for permeability using porcine skin. Based on the results from the comparative study between the supersaturated and submicron systems, the submicron formulation was selected as a more preferable formulation.

In the second section of the research, a factorial design study was conducted to evaluate the individual effects from the three critical components (particle size of drug nano crystals, concentration of Vitamin E TPGS and concentration of gel forming polymer) on the permeation rate (flux) of drug through porcine skin. In the third or final part of the study, the optimal formulation selected from the factorial design experiment was tested on human skin to confirm the permeability assessment.

Ibuprofen was used as the model drug for this study. It is a potent non-steroidal anti-inflammatory (NSAID) drug often used for the treatment of acute and chronic arthritic conditions. Although topical delivery is the preferred approach to overcome the challenges of gastric complications occurring with oral delivery of this drug, the drug molecule exhibits poor aqueous solubility and also has a high tendency of crystal growth in the high energized system. We hope to overcome these challenges but designing an optimal topical formulation for ibuprofen.

6.2 Materials and methods

6.2.1 Materials

Ibuprofen, an anti-inflammatory drug from Doctors Organic Chemical Limited (Tanaku, AP, India), was 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. The excipients used in this study include, D-alpha tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS) from Eastman Chemical. Co. (Kingsport, TN, USA), Pluronic F-127 from BASF (Florham Park, NJ, USA), propylene glycol (PG) from Fisher's Scientific (Fair Lawn, NJ), HPMC K4 and HPMC K100 from Dow Chemical Company (Midland, MI, USA). Deionised water was used as dispersion media.

6.2.2 Preparation of gel formulations prepared from supersaturated solutions.

Initially vitamin E TPGS was dissolved in water at 70-80° C to produce a final concentration of 5% (w/v). Excess drug was added into this system and the suspension was stirred for 48 hrs at 37°C using an insulated shaker (Innova 4000, New Brunswick Scientific, Edison, NJ, USA). The suspension was then centrifuged using a centrifuge (CT422, Jouan Inc., Winchester, VA, USA) at 3000 rpm and the supernatant clear solution was collected. This aliquot was mixed with 2 % w/v of HPMC K4 as steric stabilizer. After forming the supersaturated solution, gel forming polymers were dispersed into the solution using high speed homogenizer and the formulation was kept overnight in order to achieve complete hydration. Three different polymers were used at different concentrations and the compositions of these formulations are outlined in Table 1.

6.2.3 Preparation of gel formulations prepared from submicron suspension

During this process, vitamin E TPGS was dissolved in water at 70-80° C to produce a 1% w/v solution. The stabilizer (HPMC K4) was dissolved in the solution (2% w/v). The drug substance (5% w/v) was dispersed into the system and the

Table 1 Formulation design of ibuprofen supersaturated solution using different polymer systems.

Code	TPGS % (w/v)	HPMC K4 % (w/v)	HPMC K100 % (w/v)	Na-CMC % (w/v)	Pluronic F127 % (w/v)
S1	5	2	1		
S2	5	2	2.5		
S3	5	2	5		
S4	5	2		1	
S5	5	2		2.5	
S6	5	2		5	
S7	5	2			10
S8	5	2			20
S9	5	2			25

resulting suspension was wet milled with the grinding media (0.2 mm diameter) using a conventional planetary mill, Model PM400, Retsch GmbH, Germany, equipped with beaker with a chamber volume of 50 ml. The agitation rate was maintained at 400

rpm. High shear force generated during collision of the milling media with the solid drug provided the energy to fracture drug crystals into smaller particles. Due to the collision of the drug crystals with the beads and with the wall of the grinding chamber, small crystals at sub micron or nano size range were produced.

Once the desired submicron suspension was formed, gel forming polymers were dispersed into the solution using high speed homogenizer. Similar to the supersaturated formulations, three different polymers were used at varying concentrations. The compositions of the different formulations used are outlined in Table 2.

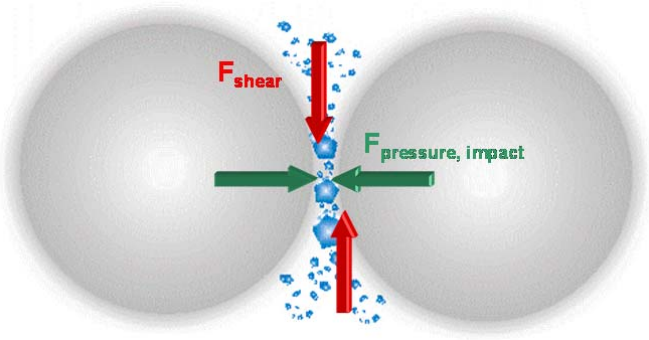


Figure 1 Formation of nanocrystals by media milling approach

6.2.4 Evaluation of gel formulations

The gels were evaluated for drug content and viscosity. The drug content of the gels was determined by dissolving about 200 mg of gel in Acetonitrile-Water mixture (1:1), which was diluted with PBS solution (pH 7.4). The drug content was estimated by HPLC method that was discussed in section 2.3.8. Viscosity of gels was determined using a Brookfield DV-E viscometer at 37 ◦ C temperature (Middleboro, MA, USA).

6.2.5 Short Term Stability study

The stability study was conducted to identify the appropriate conditions for inhibiting the crystal growth in the formulations. The gel formulations were kept on short term stability and the crystal growth was monitored. The formulations were stored at 2-8° C and samples were collected at different time points from 0 to 6 weeks for analytical characterization, using microscopy and particle size analysis.

Table 2 Formulation design of ibuprofen submicron suspension

Code	TPGS % (w/v)	HPMC K4 % (w/v)	Drug % (w/v)	HPMC K100 % (w/v)	Na-CMC % (w/v)	Pluronic F127 % (w/v)
N1	1	2	5	1		
N2	1	2	5	2.5		
N3	1	2	5	5		
N4	1	2	5		1	
N5	1	2	5		2.5	
N6	1	2	5		5	
N7	1	2	5			10
N8	1	2	5			20
N9	1	2	5			25

6.2.6 Microscopy study

The presence of drug crystals in the gel produced from supersaturated solution was studied using a polarized microscope, (Olympus BX50, Tokyo, Japan) at a

magnification of 100 X. A drop of sample was placed on a glass slide with a coverslip, to spread the sample uniformly. The image of the samples was captured using an 11.2 Color Mosaic Camera, (Diagnostic Instruments Inc.) attached to the microscope.

6.2.7 Particle size analysis

The growth of crystals in the submicron gel system was detected by Photon Correlation Spectroscopy. Photon Correlation Spectroscopy determines velocity distribution of particle movement, by measuring dynamic fluctuations of intensity of scattered light. The suspensions were characterized by intensity-weighted particle size using PCS particle size analyzer (Beckman Coulter, Jersey City, NJ, USA). Once the required intensity reached, analysis was performed to obtain the mean particle size and polydispersity index (PI). Analysis was performed in triplicate (Angle - 90 deg., Diluent – Water, Temp. - 25 ° C, Run time – 200 sec.).

6.2.8 Permeation study

Permeation rates were determined using porcine skin. Dermatomed (~500 um) 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°C. Prior to each permeation experiment; the skins were allowed to thaw at room temperature. After washing and equilibration with PBS, the skin was mounted on static vertical Franz Diffusion cells –Permegear Inc., Bethlehem, PA (receptor volume 5.1 ml, donor area 0.64 sq. cm.) by clamping them between the donor and receptor compartments. The receptor compartment was filled with PBS (pH 7.4) and maintained at 37 $\pm$ 0.5° C with constant stirring at 600 RPM. Formulations were added to the donor compartment as

an infinite dose to completely cover the membrane surface. Receptor samples were collected at predetermined time points and then replaced with equivalent amount of buffer. The drug content in the samples was analyzed by HPLC.

6.2.9 Statistical analysis

A statistical analysis was performed with submicron gel formulations in order to evaluate the effect of individual components and the interaction between these parameters. A 2^3 factorial design with three critical parameters at two different levels (High and Low) was executed. Three replicates were used for each formulation during permeation study. The concentration of HPMC K4 and drug substance was kept constant during this study. The overall study design is shown in Table 3.

The Pareto chart was used as a statistical tool to analyze the effect and magnitude of the above parameters. The objective for this statistical analysis was to investigate the change of permeability profile from each of the following components:

- Size of drug nano crystals.
- Vitamin E TPGS concentration.
- Concentration of gel forming polymer

6.2.10 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 um particle size, 4.6 x 150 mm). The mobile phase consists of a mixture of acetonitrile and phosphate buffer (pH 3.5) with a ratio of 60/40 (v/v). The detection

wavelength used was 230 nm with a flow rate of 1.2 ml/min and run time of 6 minutes**.**

6.3 Results and discussions

6.3.1 Formulation development of gel system prepared from supersaturated solution

6.3.1.1 Solubility study

The flux of a given drug through a membrane such as skin is limited by its solubility. The permeation through the skin depends on the chemical potential, which is controlled by extent of supersaturation of the drug in presence of a solubilizer. Initially a solubility study was performed with different concentrations of TPGS to determine the concentration required for producing supersaturated systems. A linear increase in solubility was observed with concentration. TPGS basically acted as non-ionic solubilizer and improved the solubility of the compound. Based on this study, 5% w/v TPGS was selected for further investigation. Also a low concentration of TPGS was selected due to the potential skin irritation that could occur at higher concentrations as discussed in our previous publication (15). The supersaturation factor was estimated from the solubility study data (Table 3).

6.3.1.2 *Optimization of polymer concentration*

After forming the supersaturated solution, gel forming polymers were dispersed into the solution using high speed homogenizer and kept overnight for complete hydration. Three different polymers (HPMC K100, Na-CMC and Pluronic F127) were used at three different concentrations to produce optimal formulations

with regards to physical properties. After 12 hours a transparent gel was formed in most cases; however a significant difference in viscosity was observed in the different formulations. The viscosity was measured using a Brookfield viscometer. The results

Table 3 Estimation of supersaturation factor of ibuprofen in TPGS solution

% of vehicle in water(w/v)	Solubility (ug ml ml)	Supersaturation factor
0	100	-
0.5	258.66	2.59
1.0	262.26	2.62
2.5	423.3	4.23
5.0	630.06	6.30
10.0	1193.88	11.94

are shown in Figure 2. It was observed that viscosity increased linearly with the concentration of the polymer in the gel. Also among the three polymers, the viscosity of Na-CMC was higher than for HPMC K 100 at all three concentrations tested (1%, 2.5% and 5% levels). Among all polymers, the viscosity of Pluronic F127 gel was the lowest even at 25%.

6.3.1.3 Stability study

The stability study was conducted to identify the appropriate formulation for inhibiting crystal growth. From the light microscopy study (Figure 3), it was observed that Pluronic F127 was less effective in terms of crystal inhibition. For Pluronic F127, crystal growth was observed even at the higher concentration of 25%. For other gel systems (Na-CMC and HPMC K100), few crystals were observed at lower concentrations (1%). However, no or minimum nucleation occurred at 2.5% and 5% until the 6 week time point. This observation may be explained by the following mechanisms.

In the supersaturated state, instantaneous nucleation occurred due to the collision of molecules that triggered the crystal growth. The diffusion resistance produced by increasing the viscosity of the gel system reduced the nucleation process, which actually prevented crystal growth during storage. Therefore, crystals growth was significantly eliminated for HPMC K100 and Na-CMC systems at 2.5% and above. For Pluronic F127 significant crystal growth was observed probably due to the lower viscosity.

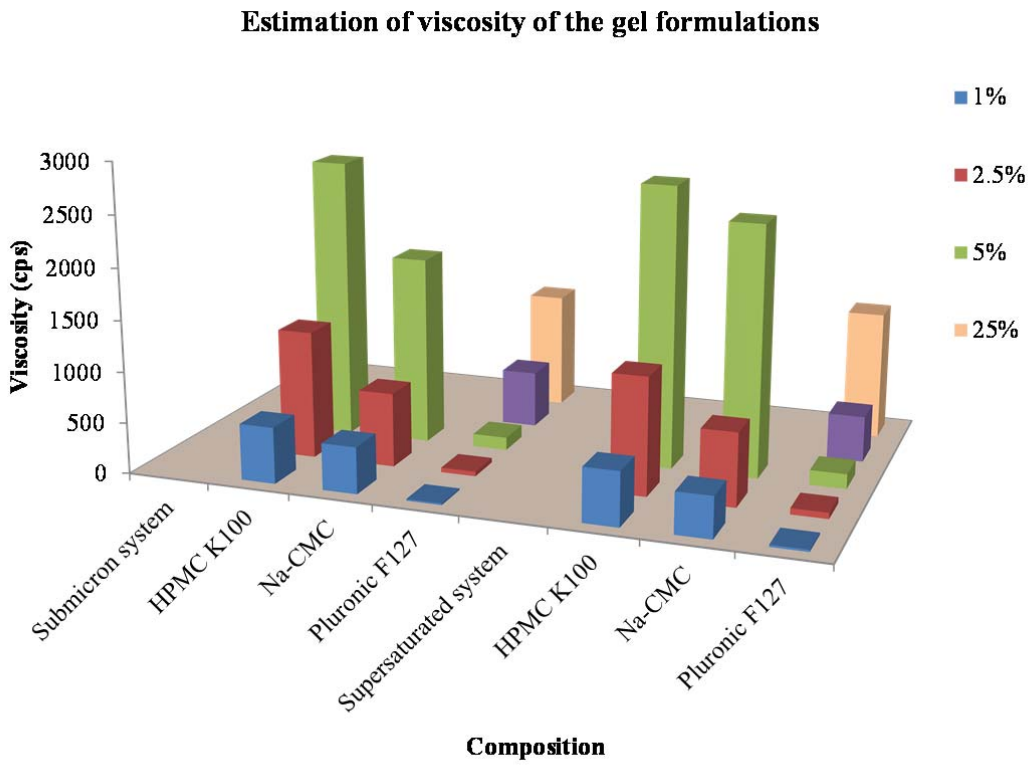
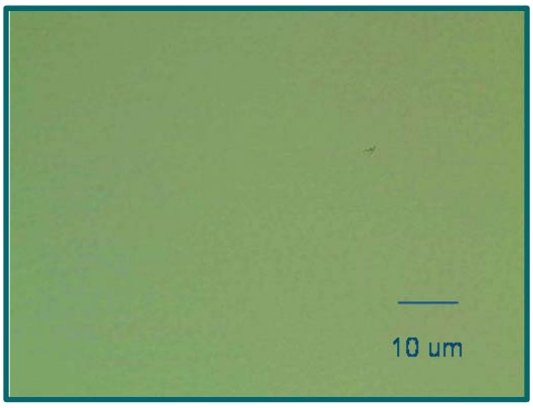


Figure 2 Effect of polymers on the viscosity of ibuprofen gel system

During the early screening study, when the HPMC K4 was added, the nucleation process became diffusion controlled and the onset of nucleation was delayed due to the strong hydrophobic interaction between the drug and the polymer



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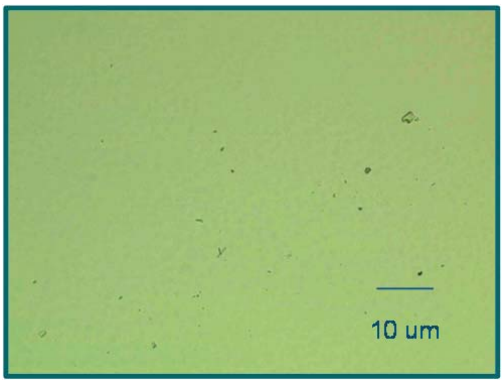

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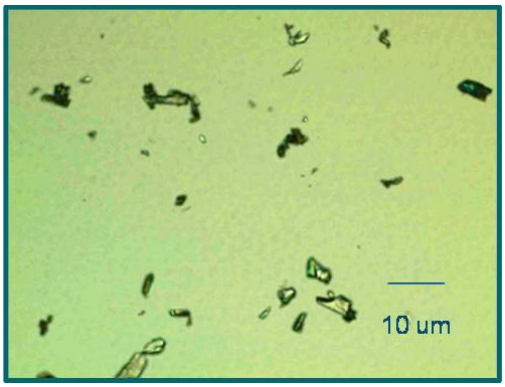

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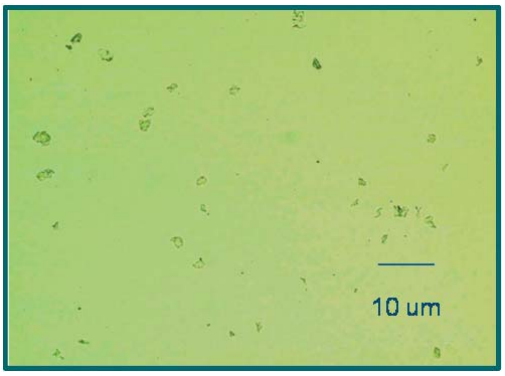

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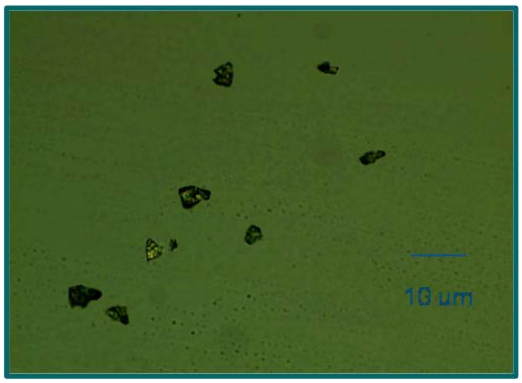

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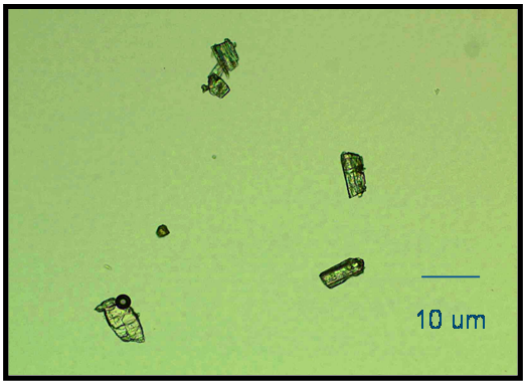


F

Figure 3 Light microscpe picture of drug crystals in supersaturated solution after 6 weeks storage. (A- 2.5% HPMC K100 gel; B-2.5% Na-CMC gel; C-1% HPMC K100 gel; D-1% Na-CMC gel; E- 10% Pluronic F127 gel and F-25% Pluronic F127 gel).

(15). However, the strength of interaction tended to be weaker on long term storage. The presence of high viscosity polymers at higher concentrations actually produced an additional effect of producing diffusion resistance that resulted in better stabilization of the system. Based on the above studies the supersaturated gel systems (S2 and S5) were selected for skin permeation study.

6.3.2 Formulation design of gel system produced from submicron suspension

As shown in Table 2, several formulations were evaluated using submicron suspensions. The submicron suspensions were prepared by the wet milling process using 5% drug, 1% TPGS and 2% HPMC K4 polymer. The advantages of using the top down media milling approach for the formation of submicron suspensions included high drug loading capacity, elimination of organic solvent and easy scale-up. In a separate study a detailed evaluation was conducted in order to optimize the TPGS and HPMC K4 polymer concentrations based upon the success of producing submicron drug crystals during the milling process. The hydrophilic hydroxyl group of the HPMC polymer formed hydrogen bonds with the submicron drug particles thus providing a steric barrier and inhibiting the crystal growth.

Similar to the supersaturated systems, in the preliminary experiments we used different types of polymers at three different concentrations (Table 2). Viscosity and particle size were recorded for the different formulations. As shown in Figure 1, the viscosity of these formulations was similar to that of the supersaturated gel formulations.

6.3.3 Particle size analysis of submicron gel system

One of the most important characterization studies for the submicron suspensions was the particle size analysis 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 PCS analysis. We have defined our system as a "submicron suspension" for the following reasons. In the past, particles were defined as nanoparticles if the size (d90) was below 1 um. At

present, an additional class has been introduced named “sub micron particles”. As per this current category, particles were divided into 3 groups – nanoparticles (less than 100 nm), submicron particles (100 nm – 1 um) and microparticles (1 um – 1 mm). The reason for this kind of classification was due to the unique properties of smaller particles (less than 100 nm) as compared to relatively larger particles (16).

The two factors that need to be considered during the milling process were the breaking of the larger drug crystals and agglomeration of the smaller sized particles. As the particle size of the drug crystals changed to submicron range, the total surface area of the particles increased significantly. In such a case, it is very important to cover the surfaces of submicron particles effectively. This can be accomplished either by increasing the polymer level or by using optimal polymer composition, which is responsible for strong interactions with the drug molecule. In some cases higher amounts of polymer may produce a negative impact on the drug release due to the increase of formulation viscosity.

The particle size distribution of gel formulations using different polymers are presented in Figure 4. As shown, the size of drug crystals increased significantly in presence of Pluronic F127, most probably due to the high concentration of this surfactant used in the gel system. However, the particle size increase was comparatively lower for HPMC K100 and Na-CMC at 2.5%. For HPMC K100, almost no particle size increase was observed. The reason for the low particle size increase for HPMC K100 can be explained by the fact that HPMC possessed a hydrophobic interaction with the drug crystals, resulted to inhibition of crystal growth. A short term stability study was performed for evaluating the efficiency of different stabilizers used in the formulations. The stability study was performed at 2-8

deg. C. and the particle size of the samples was tested at initial, 1 week, 3 weeks and 6 weeks time points.

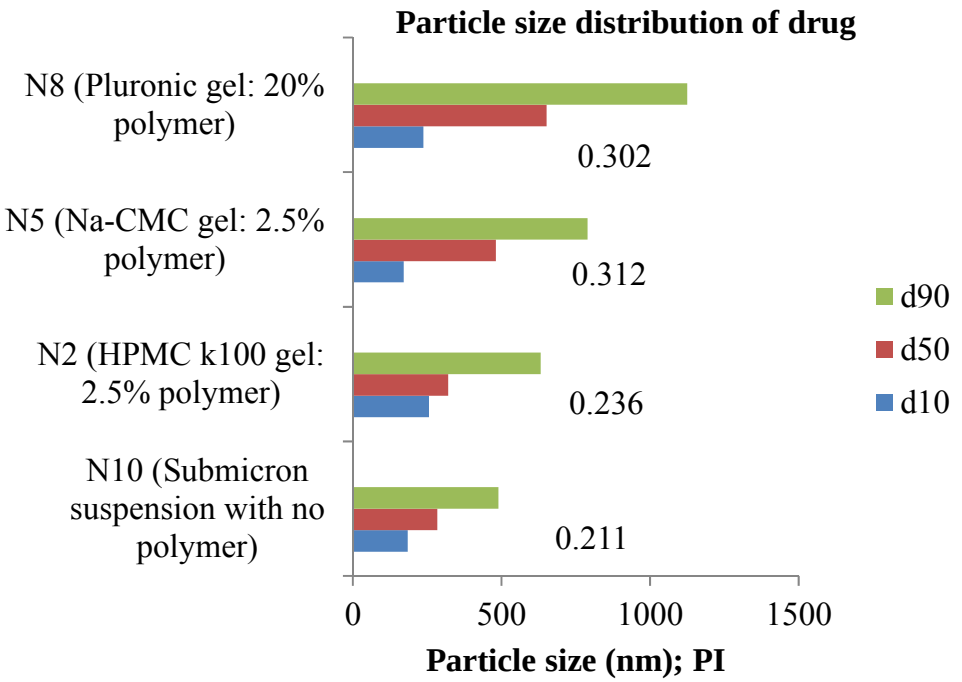


Figure 4 Mean Particle size of drug crystals in submicron gel system using different polymer system.

Once the size of the drug crystal is reduced into the submicron or nano range, the particles exhibit Brownian motion, which leads to aggregation. Ostwald ripening of smaller particles also occurs at the same time due to the higher solubility of the drug in presence of surface active agents such as vitamin E TPGS. During the stability study of the formulation containing HPMC K100, the lowest level of particle size growth was observed. However, significant particle size growth was observed for the other two gel systems formulated with Na-CMC and Pluronic F127 (Figure 5). The stabilizer should have a sufficient affinity for the particle surface of the drug in order to stabilize the submicron system. Also it should be adsorbed onto the particle surface

in order to provide sufficient repulsion between particles to decrease or eliminate aggregation. HPMC K100 probably was adsorbed onto drug crystals due to the interaction of the hydrophobic (methoxyl) and hydrophilic (hydroxypropyl) groups with the drug and provided steric stabilization as described earlier. Similar results have been reported in the published literature (10). Based on the particle size distribution of the gel formulations, N2 and N5 variants were selected for further permeation studies.

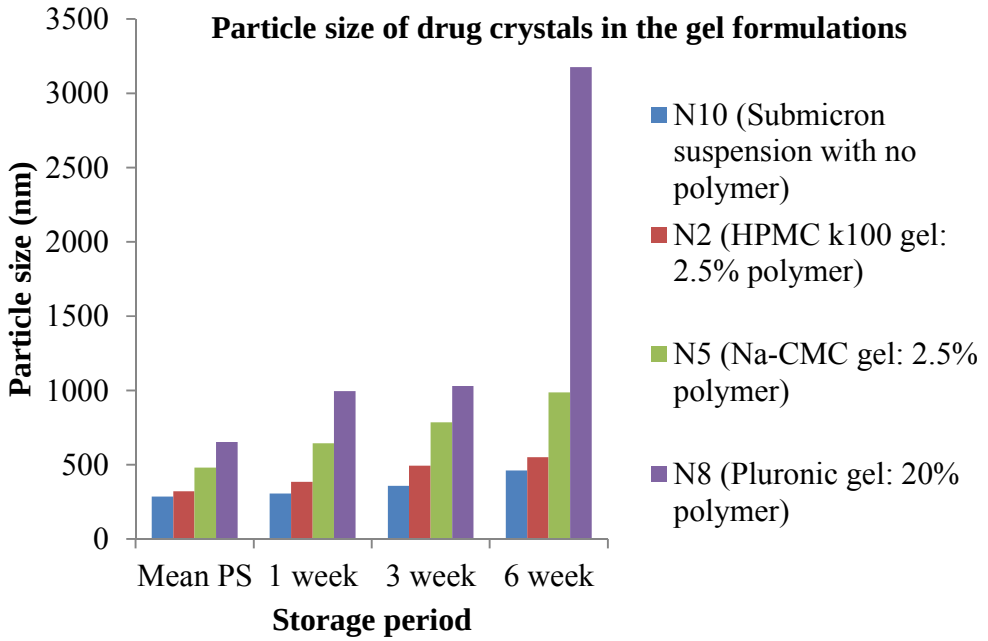


Figure 5 Effect of different polymers on the growth of drug crystals during stability study.

6.3.4 In vitro permeation study using porcine skin

The permeation rate and enhancement ratio for the gel formulations was determined using Fick's law. Fick's law (*JsDKCs*/*h*) describes the flux (*Js*) across a rate-limiting barrier (of thickness, *h*) at sink conditions including solubility (*Cs*),

lipophilicity (partition coefficient, *K*), and the molecular weight or size (diffusion coefficient, *D*). The enhancement ratio (ER) is defined as the ratio between the mean flux of test sample and the mean flux of the control sample. The permeability parameters were estimated using the following equations.

a. Flux, Js (ug/cm^2/h) from the slop of the cumulative portion permeated per unit area versus time plot.

b. Enhancement ratio, ER using the equation; ER = Js of test sample (submicron gel system)/ Js of control sample (supersaturated gel system).

6.3.4.1 Effect of polymers on the permeation of drug from submicron gel system.

While evaluating the effect of polymer type on the permeation rate, the highest permeation was observed for HPMC K100 followed by Na-CMC (Figure 5). The flux observed for HPMC K100 was 15.2 ug/cm^2/hr (SD-0.8; n=3) compared to 12.0 ug/cm^2/hr (SD-0.8; n=3) for Na-CMC polymer (Table 4).

Table 4 Estimation of permeation parameters from supersaturated solution and submicron suspension using porcine skin (n=3).

Formulation (Constant level - TPGS + HPMC K4)	**Flux, Jss (ug/cm^2/h)**		**Enhancement ratio due to micronization; ER**
	SS solution	**Submicron system**	
HPMC K100	8.7 (SD-0.6)	15.2 (SD-0.8)	1.8
Na-CMC	8.8 (SD-1.4)	12.0 (SD-0.8)	1.4

6.3.4.2 Effect of polymers on the permeation of drug from supersaturated gel system.

For the supersaturated system, no difference of permeability was observed between the HPMC K100 and Na-CMC system (Figure 6). The flux observed for HPMC K100 was 8.7 ug/cm^2/hr (SD-0.6; n=3) compared to 8.8 ug/cm^2/hr (SD-1.4; n=3) for Na-CMC (Table 4).

Based on the above results, enhancement ratios were calculated in order to evaluate the influence of submicron gel over supersaturated gel system. From the estimated enhancement factors, it was observed that the–

- Submicron system produced higher permeation rates as compared to the supersaturated system (ER = 1.4-1.8) and
- HPMC K100 produced a higher permeation rate as compared to Na-CMC gel system.

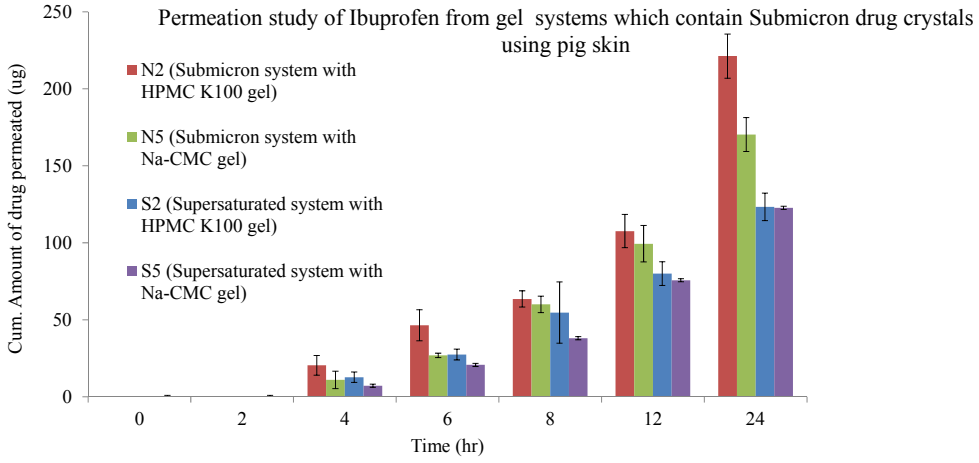


Figure 6 Effect of polymers on the permeate rate of ibuprofen through pig skin

6.3.5 Statistical design of gel system.

On the basis of the preliminary results obtained from the permeation study discussed above, formulations were designed using the submicron gel system in order to investigate the effects of critical formulation parameters. The influence of the three critical components –size of drug particles in submicron range, level of Vitamin E TPGS in the suspension and the concentration of gel forming polymer (HPMC K100) were studied on the skin permeability of ibuprofen. These parameters were adjusted in a factorial design analysis in order to evaluate their significance in determining the flux. Permeation studies were performed using high and low levels of each of these parameters. All three parameters were varied with two different 'levels', which resulted in a 2^3 factorial design study. For each formulation, three replicates were tested during the permeation study.

The permeation study was conducted using dermatomed porcine skin. The Franz cell receptor samples were collected at predetermined time points (4, 8, 12, 24, 36, 48, 72 hrs.) and analysed using HPLC (Table 5). The flux was determined for each formulation in order to identify the significance of the variables. Significant increase of permeation rates was observed for the 72 hrs. The following presents the results of these studies and the significance of the findings.

As shown in the plot (Figure 7), the slope of the permeation profile for each of the formulations was different. The effects from individual components are evaluated. The influence from the particle size (Figure 8), concentration of vitamin E TPGS (Figure 9) and concentration of HPMC K100 polymer (Figure 10) are summarized, keeping the other parameters constant.

Flux values were determined for each permeation study (Table 6) and these values were used as response factors in the factorial design. The results from the permeation studies demonstrated a rank order in correlation between the formulation parameters and drug permeability through the skin.

Table 5 Critical formulation parameters used for factorial design analysis.

Run	X1 (Drug particle size, nm)	X2 (TPGS level, %)	X3 (concentration of gel forming polymer, %)
F1	+1 (300)	+1 (2.0)	+1 (1.0)
F2	+1 (300)	+1 (2.0)	-1 (3.0)
F3	-1 (900)	+1 (2.0)	-1 (3.0)
F4	+1 (300)	-1 (0.1)	-1 (3.0)
F5	-1 (900)	-1 (0.1)	-1 (3.0)
F6	-1 (900)	+1 (2.0)	+1 (1.0)
F7	-1 (900)	-1 (0.1)	+1 (1.0)
F8	+1 (300)	-1 (0.1)	+1 (1.0)

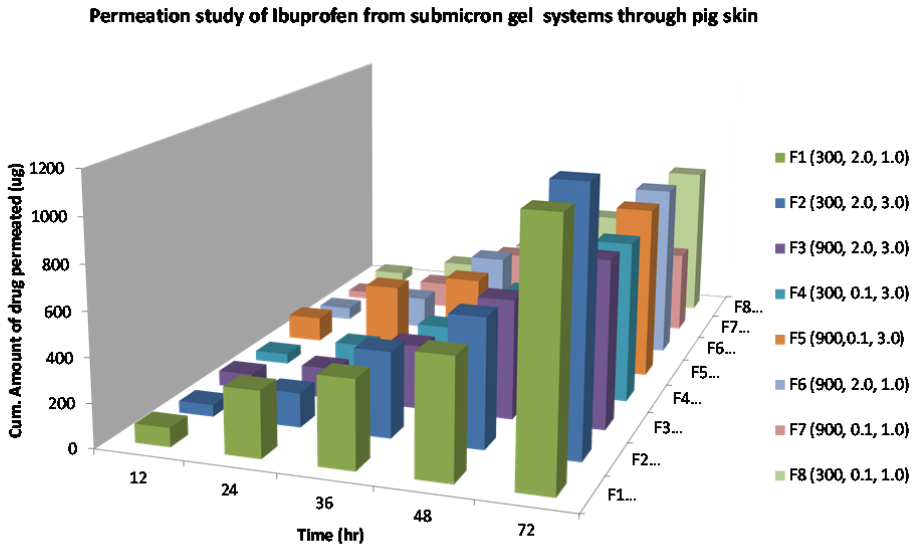
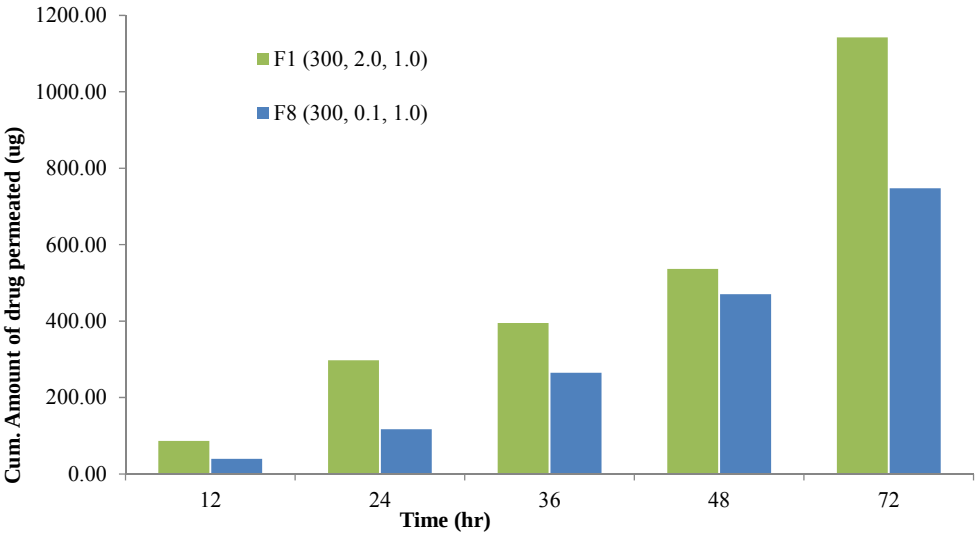


Figure 7 Permeation profile of ibuprofen from submicron gel formulations, through pig skin

Table 6 Estimation of flux of ibuprofen gel formulations during statistical analysis

RUN#	X1 PS	X2 TPGS level	X3 K100 level	Flux (ug/sq.cm/hr)	SD
F1	+1 (300)	+1 (2.0)	+1 (1.0)	26.0	3.6
F2	+1 (300)	+1 (2.0)	-1 (3.0)	29.7	4.1
F3	-1 (900)	+1 (2.0)	-1 (3.0)	19.3	2.4
F4	+1 (300)	-1 (0.1)	-1 (3.0)	19.3	3.3
F5	-1 (900)	-1 (0.1)	-1 (3.0)	14.1	2.2
F6	-1 (900)	+1 (2.0)	+1 (1.0)	19.8	0.9
F7	-1 (900)	-1 (0.1)	+1 (1.0)	12.5	2.2
F8	+1 (300)	-1 (0.1)	+1 (1.0)	17.7	2.4



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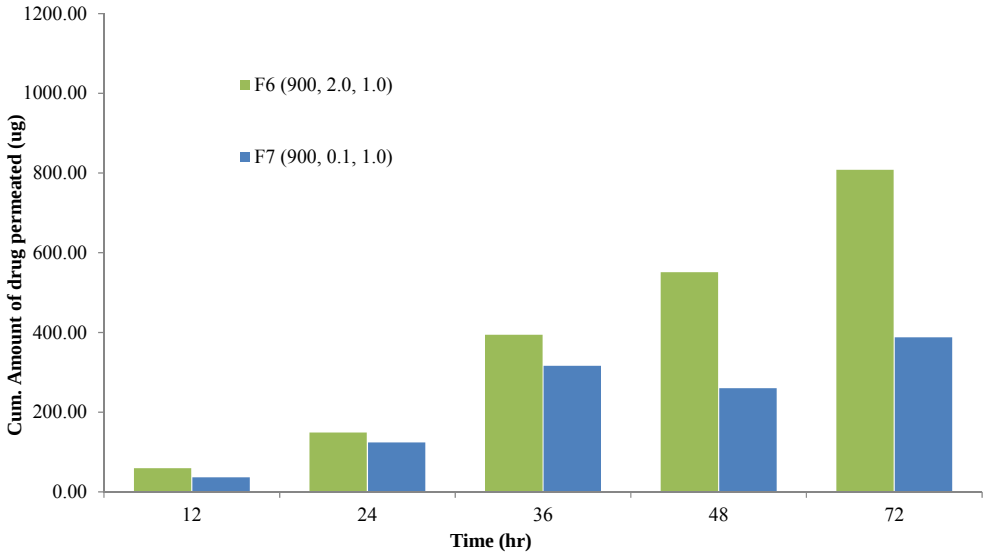

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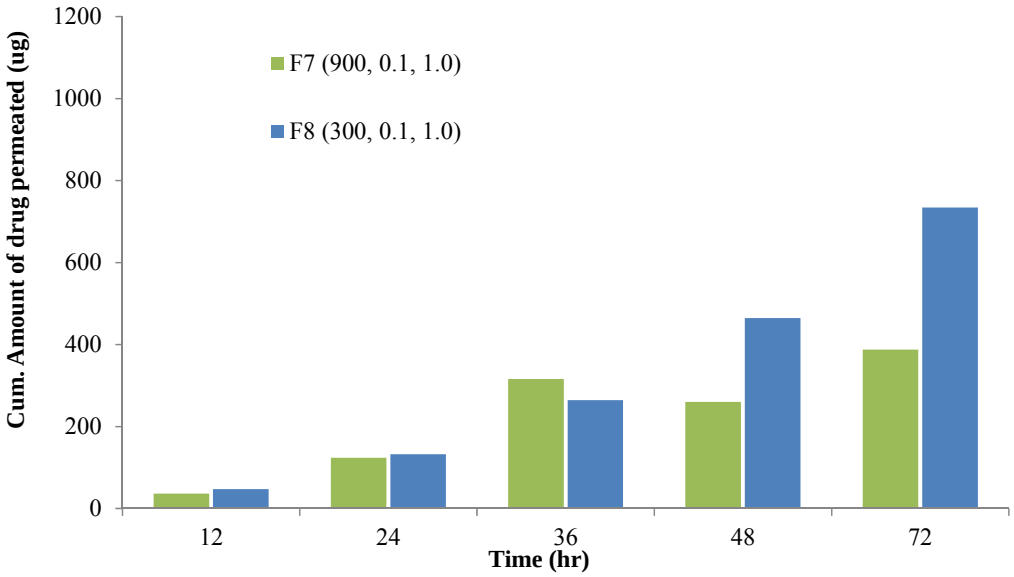
Figure 8 Influence of Vitamin E TPGS on the permeability of drug through the pig skin (A- 300 nm particle size; B – 900 nm particle size).



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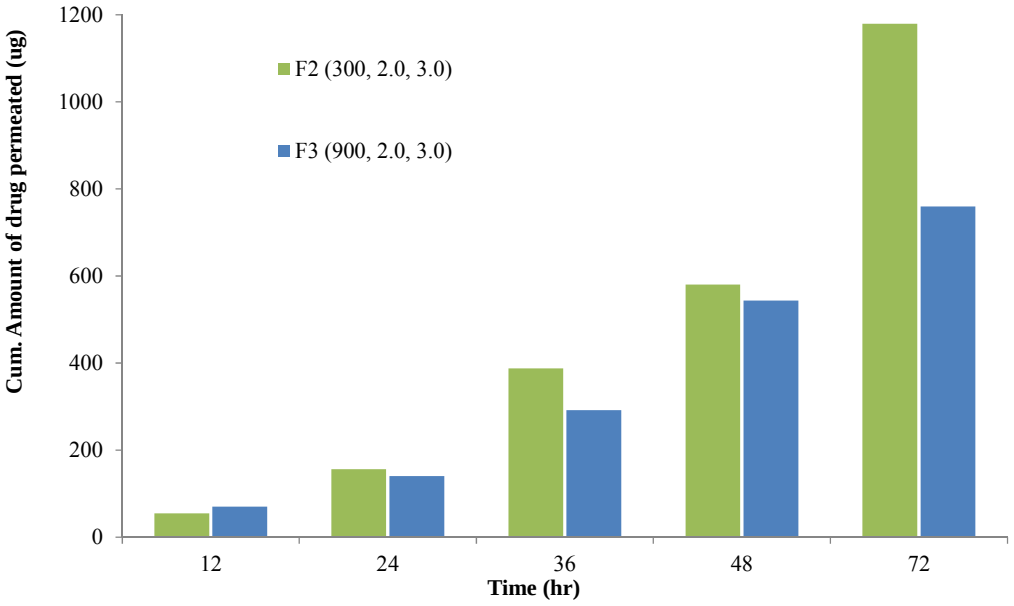

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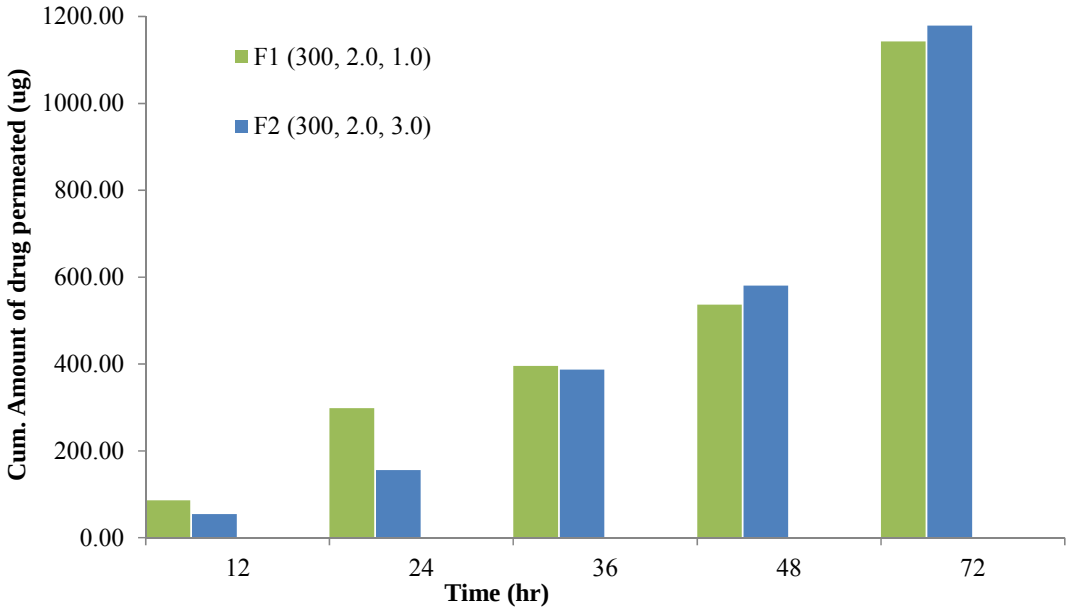
Figure 9 Influence of particle size of submicron drug crystals on its permeability through the pig skin (A- 0.1% TPGS; B – 2.0% TPGS).



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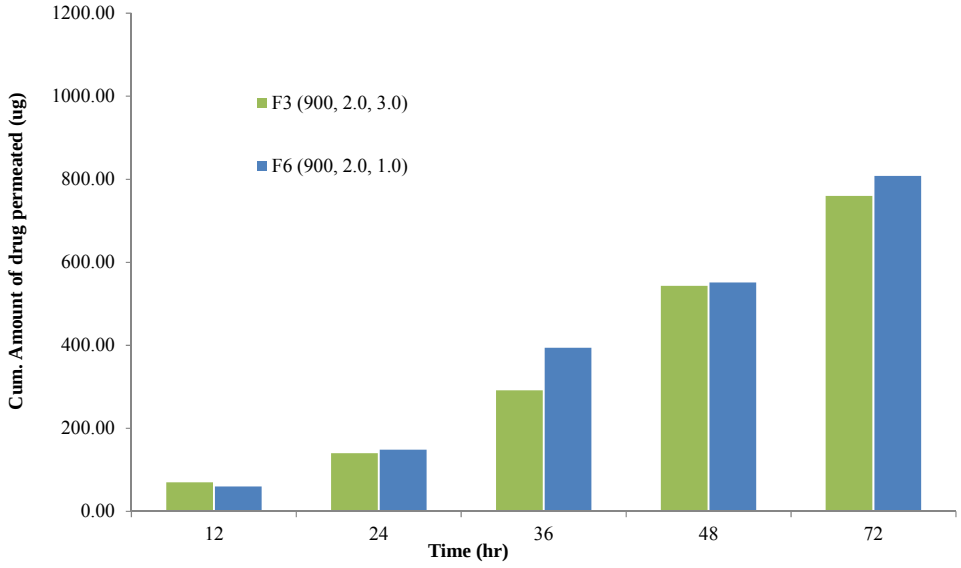

B

Figure 10 Influence of HPMC K100 concentration on the permeability of drug through the pig skin (A- 300 nm particle size; B – 900 nm particle size).

From the Pereto chart (Figure 11), concentration of TPGS seemed to be the most significant parameter (p value < 0.005). The magnitude of effect was positive (+). The increase in TPGS concentration influenced the drug solubility and also the permeability of the drug through the skin.

The particle size of drug crystals was the second most significant parameter (p value < 0.005). The magnitude of effect was negative (-). The decrease of crystal size influenced the drug solubility rate and hence the drug fluxes through the skin. The influence of particle size on the permeability can be explained by the larger surface area and potentially higher solubility of the submicron system.

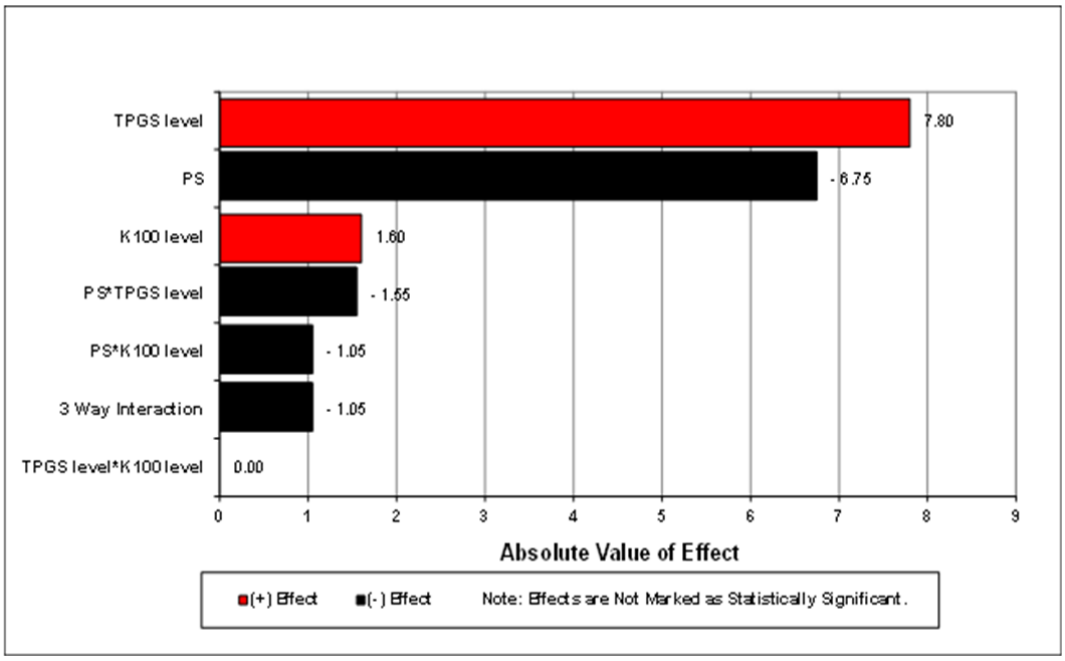

Figure 11 Pareto chart as a statistical tool to analyze the effect and magnitude of the critical formulation parameters from ibuprofen submicron gel formulations

The phenomenon can be explained by the following Ostwald–Freundlich's equation:

$$\ln S/S_0 = 2M\gamma/\rho rRT \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2),$$

where S is the solubility, S_0 is the solubility of a flat sheet ($r = \infty$), M is the molecular weight of the solid, γ is the interfacial tension, R is the gas constant, T is the absolute temperature, r is the radius, and ρ is the density of the solid. As can be seen from this equation, the solubility of a certain material is inversely correlated to the particle size. Based on the principle, as the particle size decreases, the solubility increases. Therefore, the gel system that contained larger drug particles (~ 900 nm) resulted in a concentration gradient between the differently sized particles due to the higher solubility of small particles and the lower solubility of large particles. At the vicinity of the skin surface, the smaller particles easily diffused from the high concentration to the low concentration and precipitated on the surface of the large particles. This resulted in a decrease in the permeability rate of the drug.

The concentration of HPMC K100 binder seemed to be the non-significant parameter (p value > 0.005). However, the analysis of HPMC K100 parameter revealed interesting insights. The magnitude of effect was positive (+), that contradicted the prediction, which assumed that the level of HPMC K100 concentration should have a negative (-) impact on the permeability of drug through the skin. The increase in concentration of HPMC K100 should have reduced permeability rate by increasing the viscosity of the gel system. However, an additional effect needed to be considered. The increase of HPMC K100 level inhibited the formation of crystal growth of the drug and thus improved the permeation rate to

certain extent. At the same time, by increasing the amount of HPMC K100, the viscosity of the suspension also increased. This provided additional resistance to the Ostwald ripening process. Thus the performance of these stabilizers can be explained by the combination of steric hindrance of polymer and diffusion resistance due to higher viscosity of the system. Finally the use of HPMC K100 along with Vitamin E TPGS could have a synergistic effect in stabilizing the highly energized crystals.

The above result suggests that factorial design is a useful tool for identifying the impact of individual formulation parameters on the drug permeability profiles through the skin. It becomes apparent that presence of surfactant (TPGS) and size of submicron crystals have a significant impact on the permeability profile. The submicron gel system with optimized formulation was prepared and used for the subsequent permeation study through human skin.

The major contribution to the enhanced supersaturation of a poorly soluble drug like ibuprofen can be explained by Noyes-Whitney equation (1).

$$dC/dt = DA(Cs-C)/h \text{……..(1)}$$

where dC/dt is the rate of dissolution of the drug particles, D is the diffusion coefficient of the drug in the formulation matrix, h is the thickness of the diffusion layer around each drug particle, Cs is the saturation solubility of the drug in solution in the diffusion layer and C is the concentration of the drug in the gel.

Several hypotheses may be generated to explain the experimental findings during the research.

- increase of total area (A) of the particles due to size reduction;
- decrease in the thickness (h) of the diffusion layer surrounding the particles;
- increase of saturation solubility (Cs) by decreasing the particle size to submicron range (< 500 nm), which can be explained due to the increase of particle curvature as interpreted by the Ostwald Freundlich equation (2). In addition, the increase in particle curvature increases the dissolution pressure, which consequently increases the saturation solubility. However, Grant and Brittain suggested that an increase in solubility due to increased particle curvature might only become significant for particles having a radius of less than about 10 nm (17).

In the present study the increase of drug solubility occurred due to the addition of surface active agent (Vitamin E TPGS in this case). In a separate study, the unique property of Vitamin E TPGS (TPGS, D-a-tocopheryl polyethylene glycol 1000 succinate) has been evaluated on the permeability enhancement of the drug through the skin. This property of TPGS can be explained by it possibly altering the skin structure (*D*), by transforming the skin barrier to be more lipophilic (*K*) and thus reducing the interfacial tension to make the SC more favourable for the poorly water soluble drug such as ibuprofen to pass through. More detailed investigations on the mechanism of skin permeation of ibuprofen at the submicron state and the influence of Vitamin E TPGS will possibly shed some light on the observed effects.

The overall permeation enhancement process through the skin seems to be influenced by the presence of solubilizers as well the particle size of the drug crystals.

These factors resulted in higher drug release due to the formation of supersaturated solution around the crystals and thus a high concentration gradient was produced between the drug crystals and skin surface. Therefore, fast replacement of diffused molecules occurred due to fast and continuous dissolution from the new crystal surface and thus drug release became continuous or zero order as shown in Figure 12. In the case of supersaturated systems, there might be a tendency for the drug to crystallize on the skin surface and this ultimately lowered the permeation rate.

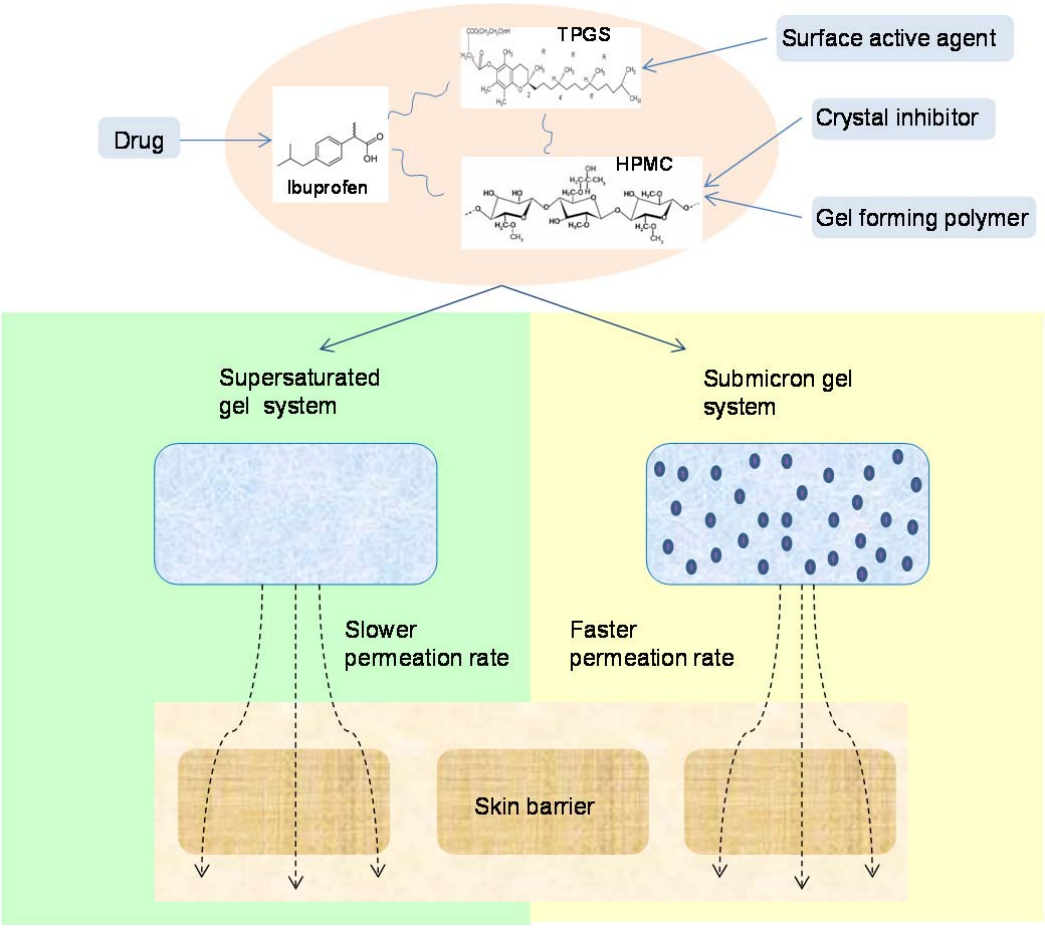

Figure 12 Mechanism of skin permeation of ibuprofen drug from supersaturated solution and submicron suspension using a gel system

6.4 Conclusion

In summary this study demonstrated a clear correlation between the Vitamin E TPGS and particle size of submicron crystals with the permeation rate (flux) of ibuprofen through the porcine skin. The explanation for the high permeation rate through the skin was mainly because of high surface area created in the formulation system that resulted in a high and continual drug release from the formulation to the external phase as a result of a constant driving force. In addition, the components used in the system also influenced the drug delivery potential from the formulation that improved the wettability of the poorly soluble drug and thus affected the mobility parameters through the skin. The formulation developed with Vitamin E TPGS and HPMC 3cps provided hydrophobic interactions that resulted in submicron particle stabilization. In conclusion a number of factors including the particle size of the drug crystals, surface properties of the carrier, interaction of drug molecule with the stabilizer needed to be considered while designing a suitable dermal formulation for the poorly soluble compound. In summary, for BCS II compounds like ibuprofen, submicron / nanosuspension gel formulations seem to be an attractive approach for improving the drug permeability through the skin.

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Chapter 7. Additional studies.

7.1 Zeta potential of submicron suspension

7.1.1 Introduction: Zeta potential is the potential at the hydrodynamic shear plane and can be determined from the particle mobility under an applied electric field. The mobility of the particle is dependent on the effective surface charge.

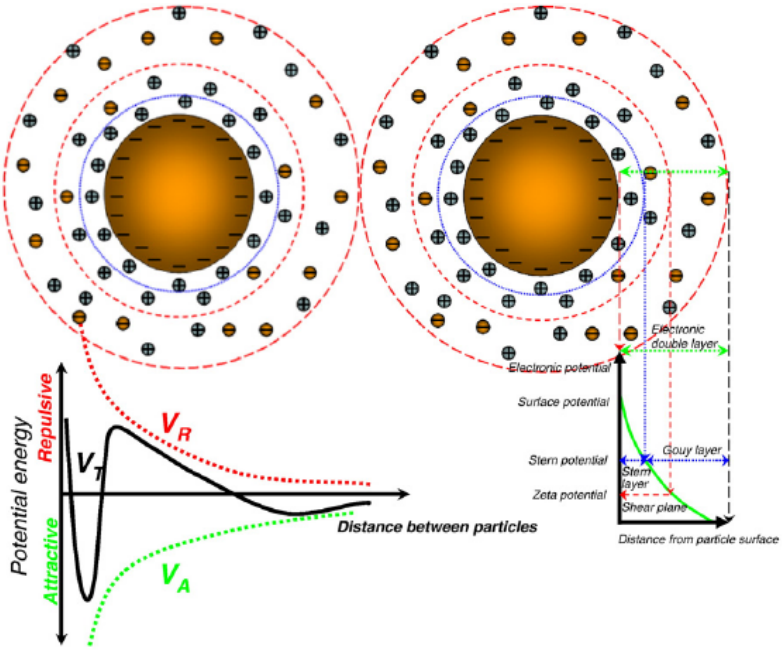


Figure 1 Illustration of classical DLVO theory. Attractive forces are dominant at very small and large distances, leading to primary and secondary minimum, while repulsive forces are prevailing at intermediate distances and create net repulsion between the dispersed particles, thus preventing particle agglomeration (1).

7.1.2 Measurement of zeta potential: Electrophoretic mobility of the wet milled particles in the submicron formulations were measured by forward scattering through transparent electrode method using Delsa Nano C (Beckmann Coulter, CA) at room temperature.

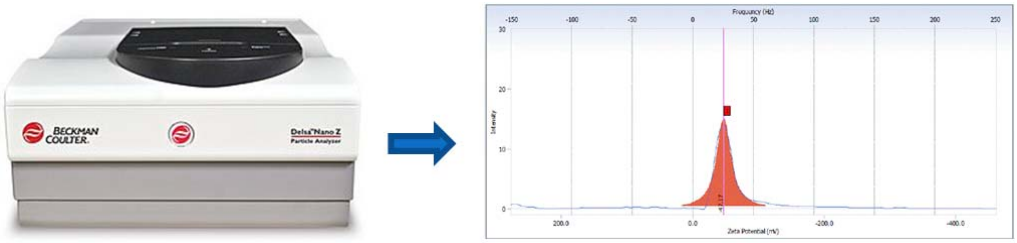


Figure 2 Measurement of zeta potential using Beckman Coulter Delsa Nano analyzer

7.1.3 Results and Discussion:

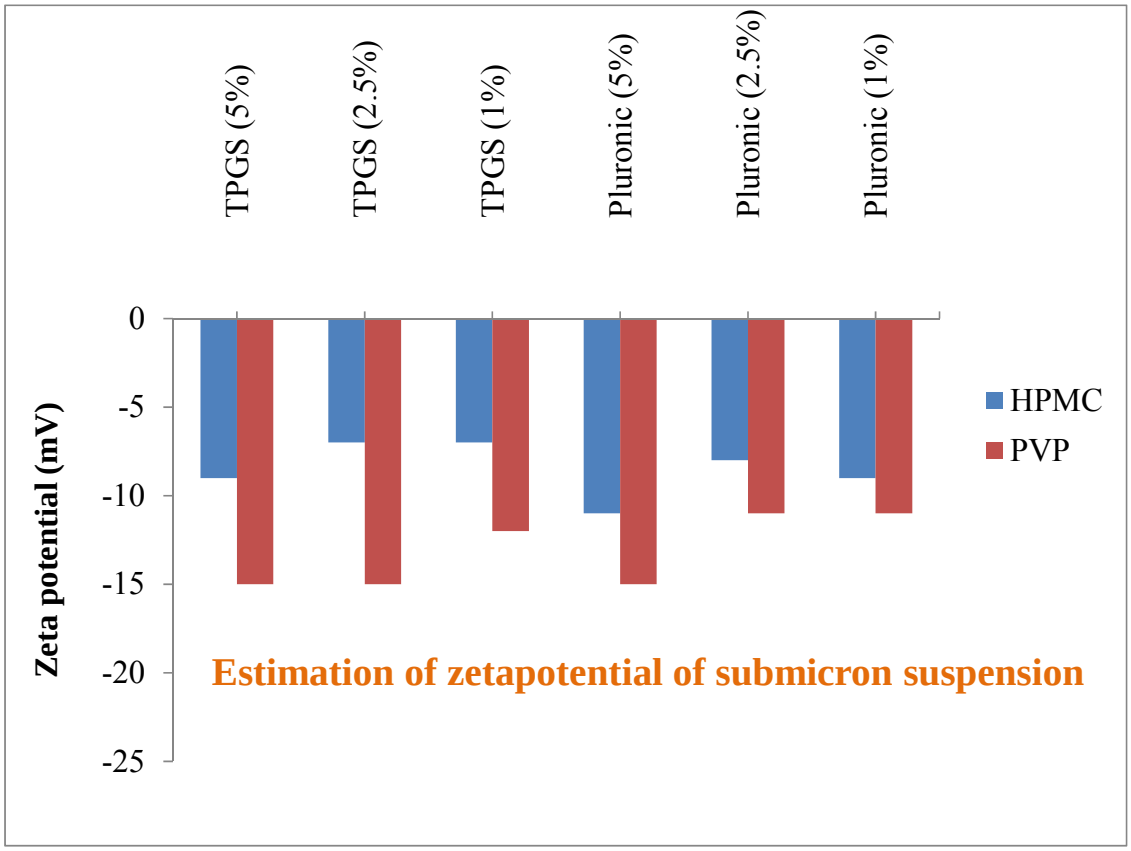


Figure 3 Estimation of zeta potential of submicron suspension formulations

In general, nanosuspension stabilization can occur by electrostatic or steric stabilization or combination of both. Given the fact that TPGS is a nonionic stabilizer, nonzero zeta potentials are caused by charges associated with the compound surfaces.

No clear trend can be observed between the measured zeta potential values and the success rate. This type of observation can be explained by the fact that, agglomeration of nanoparticles is governed by hydrophobic interactions due to the presence of TPGS. A similar observation was reported in the past (2).

Even though the measured zeta potential was low, it was speculated that thick layer of stabilizers had an overwhelming effect on stabilization of drug in the TPGS system. Although, the zeta potential values were lower than 20 mV, sufficient stabilization was still achieved for this type of system. The result was consistent with previous reports for nanosuspension systems including rilpivirine, itraconazole and fenofibrate with TPGS as stabilizer (3, 4, 5).

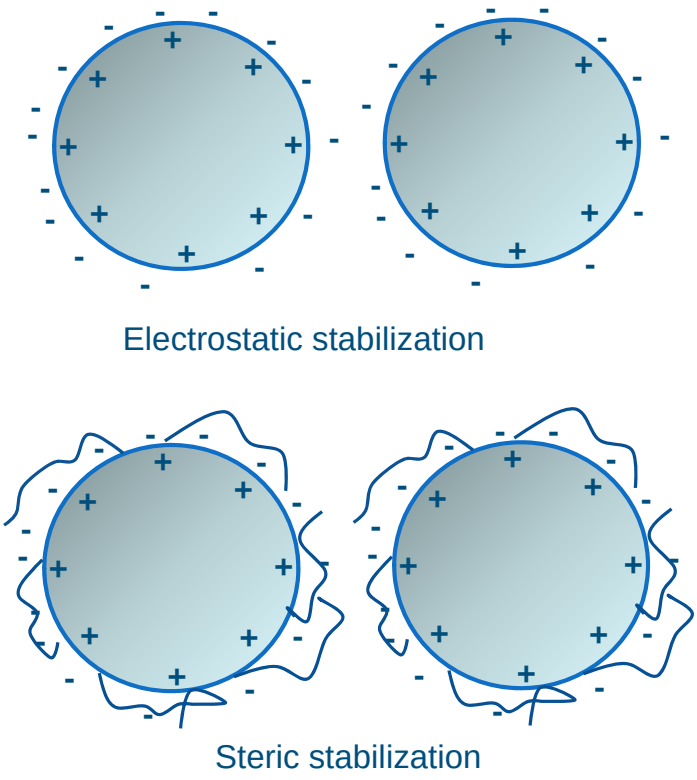


Figure 4 Mechanism of Steric and electrostatic stabilization

7.2 Assay of submicron gel formulations

7.2.1 Estimation of assay of gel formulations: The gels were evaluated for the drug content. The drug content of the gels was determined by dissolving about 100 mg of gel in Acetonitrile-Water mixture (1:1), which was diluted with PBS solution (pH 7.4) to make a volume of 100 ml. The drug content was estimated using HPLC.

Table 1 Assay of submicron gel formulations used in the DOE study

RUN#	X1 PS	X2 TPGS level	X3 K100 level	Assay (%) of gel
F1	900	0.1	1	96.38
F2	300	2.0	3.0	96.04
F3	300	0.1	3.0	98.50
F4	900	0.1	3.0	96.26
F5	300	2.0	1	100.07
F6	900	2.0	1	98.34
F7	300	0.1	1	96.15
F8	900	2.0	3.0	96.26

The assay for submicron formulation formulations were 95% (Table 1). Some drug loss takes place while separating the beads from the drug suspension. No significant change was observed after gel formation.

7.3 Particle size analysis of submicron particles in presence of different surfactants and polymeric stabilizers

7.3.1 Preparation of submicron suspension: 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 during this study. The samples were collected at different time points for characterization studies.

7.3.2 Particle size analysis: Photon Correlation Spectroscopy method was used for particle size analysis of submicron particles in gel the formulation. This equipment determines velocity distribution of particles movement by measuring dynamic fluctuations of intensity of scattered light. The Brownian motion of the particles results to the fluctuations of the local concentration of the particles. The details of the method are shown below:

Analysis mode – Size, Angle - 90 deg., Diluent – Water, Temperature - 25 deg. C, Run time – 200 sec.

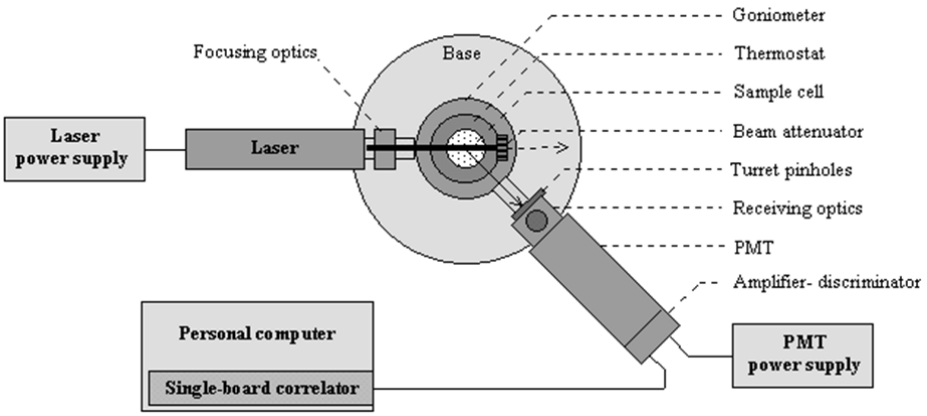


Figure 5 PCS-N4 (Beckman Coulter) Plus Particle size measuring unit was used for this study

7.3.3 Results and Discussions:

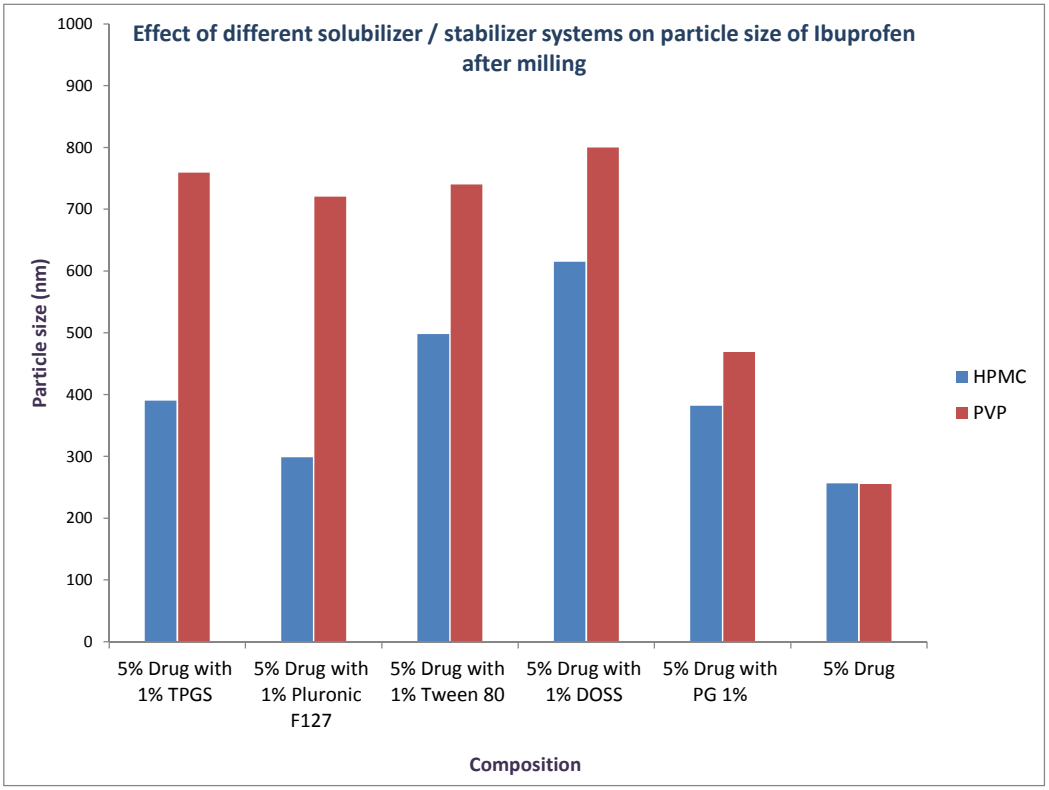


Figure 6 Mean particle size of submicron suspension formulations

Figure 6 highlighted the mean particle size of submicron drug crystals in presence of five different surfactants / solubilizers. Additionally HPMC or PVP was used as a steric stabilizer along with the surfactants / solubilizers. From the particle size distribution analysis, HPMC was indicated as a better stabilizer as compared to PVP. Also the particle size of submicron drug crystals in presence of TPGS, Pluronic and PG was smaller as compared to Tween and DOSS suspensions.

7.4 Permeation profile study of marketed product as compared to submicron gel formulations

7.4.1 Marketed product: One of the marketed products of ibuprofen (Deep Relief Gel, mfg. by Mentholatum Co. Ltd.) was selected as the reference product for this research. Deep Relief Gel gives relief of rheumatic pain, muscular aches, pains and swellings. Deep Relief Gel contains the active substances: Ibuprofen 5.0%w/w, Levomenthol 3.0%w/w along with other in active ingredients, such as, propylene glycol, carbomer, diisopropanolamine, ethanol and water.

7.4.2 Permeation study and method of analysis: Permeation rates were determined initially using porcine skin (pig skin) and then human skin. Dermatomed (~500 um) 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°C. Prior to each permeation experiment; the skins were allowed to thaw at room temperature. After washing and equilibration with PBS, the skin was mounted on static vertical Franz diffusion cells – Permegear Inc., Bethlehem, PA (receptor volume 5.1 ml, donor area 0.64 sq. cm.) by clamping them between the donor and receptor compartments. The receptor compartment was filled with PBS (pH 7.4) and maintained at 37 $\pm$ 0.5° C with constant stirring at 600 RPM. Formulations were added to the donor compartment as an infinite dose to completely cover the membrane surface. Samples from receptor compartment were collected at predetermined time points and then replaced with equivalent amount of buffer. The drug content in the samples was analyzed by HPLC.

The permeation rate and enhancement ratio of different optimized formulations was determined by Fick's law. Fick's law ($J_S = DKCs/h$) describes the flux (J) across a rate-limiting barrier (of thickness, h) at sink conditions including

solubility (*Cs*), lipophilicity (partition coefficient, *K*), and the molecular weight or size (diffusion coefficient, *D*). The enhancement ratio (ER) is defined as the ratio between the mean flux of submicron gel system and the mean flux of the control. The permeability parameters were estimated using the following equations.

a. Flux, Js (ug/cm^2/h) from the slop of the cumulative portion permeated per unit area versus time plot.

b. Enhancement ratio, ER using the equation; ER = Js of test sample / Js of control sample.

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 um particle size, 4.6 x 150 mm). The mobile phase consists of a mixture of acetonitrile and phosphate buffer (pH 3.5) with a ratio of 60/40 (v/v). The detection wavelength used was 230 nm with a flow rate of 1.2 ml/min and run time of 6 minutes (6)**.**

7.4.3 Results and Discussions: The permeation study was performed to compare the different submicron formulations from DOE study (Table 2) with ibuprofen Deep Relief Gel. The permeation study was conducted using dermatomed porcine skin. The samples from Franz cell receptor were collected at predetermined time points (4, 8, 12, 24, 36, 48, 72 hrs.) and analyzed by HPLC. As shown in the plot (Figure 7), the permeation profile of the marketed formulation (F9) was significant slower as compared to few of the variants from the DOE study (F2, F3, F5, F6 and F8). The flux was determined for each formulation for more quantitative estimation Table 3).

The enhancement factor of the above 5 submicron gel formulations was increased by 2-3 folds as compared to the marketed ibuprofen gel formulation.

Table 2 Composition of gel formulations used for permeation studies.

Run	X1 (Drug particle size, nm)	X2 (TPGS level, %)	X3 (concentration of gel forming polymer, %)
F1	-1 (900)	-1 (0.1)	1 (1.0)
F2	1 (300)	1 (2.0)	-1 (3.0)
F3	1 (300)	-1 (0.1)	-1 (3.0)
F4	-1 (900)	-1 (0.1)	-1 (3.0)
F5	1 (300)	1 (2.0)	1 (1.0)
F6	-1 (900)	1 (2.0)	1 (1.0)
F7	1 (300)	-1 (0.1)	1 (1.0)
F8	-1 (900)	1 (2.0)	-1 (3.0)
F9		Control	

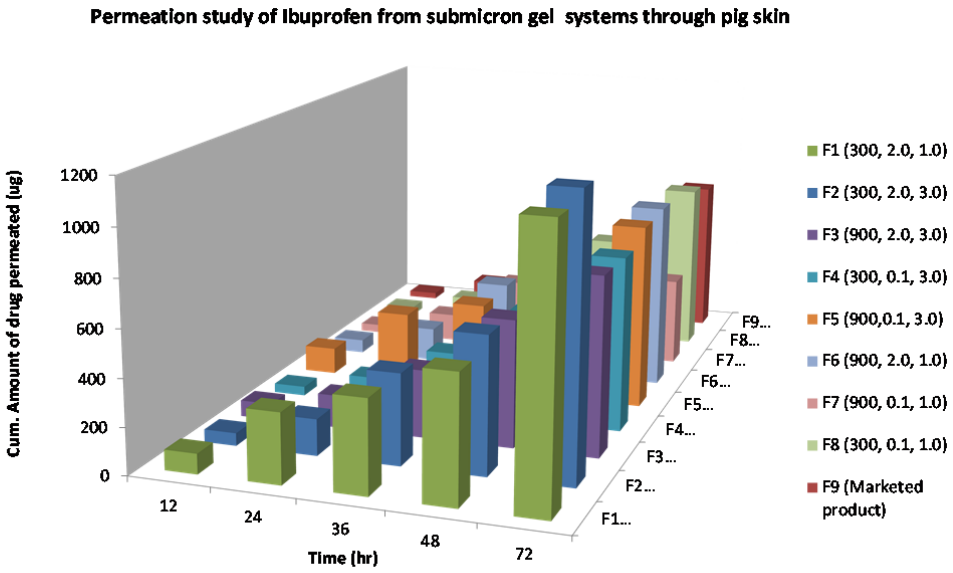


Figure 7 Permeation profiles of submicron gel formulations as compared to the marketed formulation.

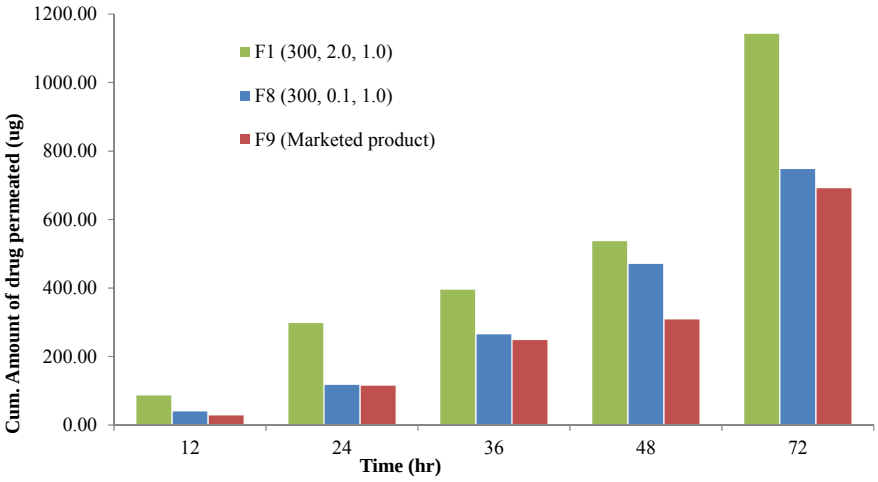


Figure 8 Permeation profiles of submicron gel formulations with different TPGS concentrations as compared to the marketed formulation.

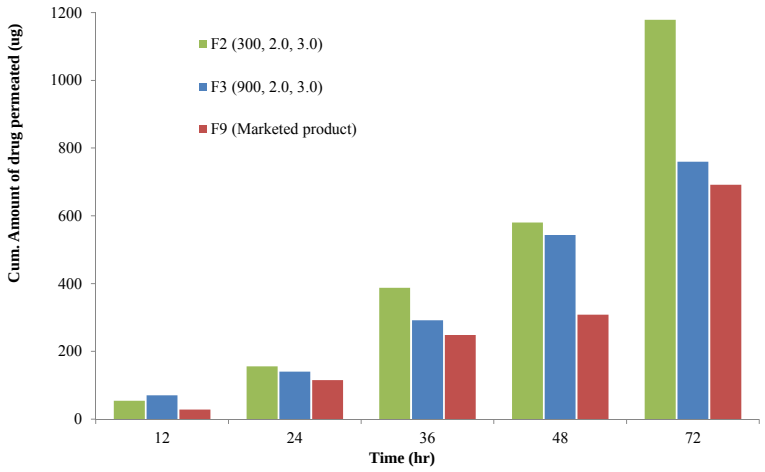


Figure 9 Permeation profiles of submicron gel formulations with different particle sizes as compared to the marketed formulation.

Table 3 Estimation of flux of ibuprofen gel formulations as compared to the control sample (marketed formulation).

RUN#	X1 PS	X2 TPGS level	X3 K100 level	Flux (ug/sq.cm/hr)	SD
F1	900	0.1	1	10.2	1.2
F2	300	2.0	3.0	29.7	0.7
F3	300	0.1	3.0	20.9	0.8
F4	900	0.1	3.0	10.2	2.3
F5	300	2.0	1	27.4	1.3
F6	900	2.0	1	21.9	1.5
F7	300	0.1	1	16.8	1.1
F8	900	2.0	3.0	21.9	0.7
F9		Control		11.5	0.8

7.4.4 Conclusion: By optimizing the critical parameters of the submicron gel formulations (such as drug particle size, TPGS and HPMC K100 concentration), we are able to enhance its permeation rate as compared to one of the marketed product of ibuprofen gel.

7.5 Permeation profile study of final gel formulation through human skin

7.5.1 Selection of optimized formulation: Based on the results from the DOE study the following formulation (Table 4) was selected in order to test the permeability profile through the human skin. Additionally the permeation profile was compared with the supersaturated gel formulation and marketed formulation (Tables 5 and 6).

Table 4 Composition of submicron gel formulation

Ingredients	% (w/v)
Ibuprofen	5
Vitamin E TPGS	2
HPMC 3 cps	2
HPMC K100	3
Purified water	qs

Table 5 Composition of supersaturated gel formulation

Ingredients	% (w/v)
Ibuprofen	5
Vitamin E TPGS	5
HPMC 3 cps	2
HPMC K100	3
Purified water	qs

Table 6 Composition of marketed gel formulation (Deep Relief gel, Mfg. by Mentholatum, UK) (7)

Ingredients	% (w/v)
Ibuprofen EP	5
Menthol	3
Carbomer	-
Propylene glycol	-
Di-isopropanolamine	-
Ethanol	-
Purified water	qs

7.5.2 Experimental set-up

7.5.2.1 Preparation of submicron suspension and gel system: During this process, vitamin E TPGS was dissolved in water at 70-80° C to produce a 2% w/v solution. The stabilizer (HPMC K4) was dissolved in the solution (3% w/v). The drug substance (5% w/v) was dispersed into the system and the resulting suspension was wet milled with the grinding media (0.2 mm diameter) using a conventional planetary mill, Model PM400, Retsch GmbH, Germany, equipped with beaker with a chamber volume of 50 ml. The agitation rate was maintained at 400 rpm. High shear force generated during collision of the milling media with the solid drug provided the energy to fracture drug crystals into smaller particles. Due to the collision of the drug crystals with the beads and with the wall of the grinding chamber, small crystals at

sub micron or nano size range were produced. Once the desired submicron suspension was formed, gel forming polymers were dispersed into the solution using high speed homogenizer.

7.5.2.1 Preparation of submicron suspension and gel system: During this process, vitamin E TPGS was dissolved in water at 70-80° C to produce a 2% w/v solution. The stabilizer (HPMC K4) was dissolved in the solution (3% w/v). The drug substance (5% w/v) was dispersed into the system and the resulting suspension was wet milled with the grinding media (0.2 mm diameter) using a conventional planetary mill, Model PM400, Retsch GmbH, Germany, equipped with beaker with a chamber volume of 50 ml. The agitation rate was maintained at 400 rpm. High shear force generated during collision of the milling media with the solid drug provided the energy to fracture drug crystals into smaller particles. Due to the collision of the drug crystals with the beads and with the wall of the grinding chamber, small crystals at sub micron or nano size range were produced. Once the desired submicron suspension was formed, gel forming polymers were dispersed into the solution using high speed homogenizer.

7.5.2.2 Preparation of supersaturated solution and gel system: Initially vitamin E TPGS was dissolved in water at 70-80° C to produce a final concentration of 5% (w/v). Excess drug was added into this system and the suspension was stirred for 48 hrs at 37°C using an insulated shaker (Innova 4000, New Brunswick Scientific, Edison, NJ, USA). The suspension was then centrifuged using a centrifuge (CT422, Jouan Inc., Winchester, VA, USA) at 3000 rpm and the supernatant clear solution was collected. This aliquot was mixed with 2 % w/v of HPMC K4 as steric stabilizer. After forming the supersaturated solution, gel forming polymers were dispersed into

the solution using high speed homogenizer and the formulation was kept overnight in order to achieve complete hydration.

7.5.2.3 Evaluation of gel formulations: The gels were evaluated for the drug content. The drug content of the gels was determined by dissolving about 100 mg of gel in Acetonitrile-Water mixture (1:1), which was diluted with PBS solution (pH 7.4) to make a volume of 100 ml. The drug content was estimated using HPLC.

7.5.2.4 Particle size analysis of submicron gel formulation: The growth of crystals in the submicron gel system was detected by Photon Correlation Spectroscopy. Photon Correlation Spectroscopy determines velocity distribution of particle movement, by measuring dynamic fluctuations of intensity of scattered light. The suspensions were characterized by intensity-weighted particle size using PCS particle size analyzer (Beckman Coulter, Jersey City, NJ, USA). Once the required intensity reached, analysis was performed to get the mean particle size and polydispersity index (PI). Analysis was done in triplicate (Angle - 90 deg., Diluent – Water, Temp. - 25 ° C, Run time – 200 sec.).

7.5.2.5 Permeation study: Permeation rates were determined using human skin from a 40 year old female Hispanic donor collected from right posterior leg. The skin was obtained from NY Firefighters (New York NY) and was dermatomed to 500 um.

The skin was stored at −80°C. Prior to each permeation experiment; the skins were allowed to thaw at room temperature. After washing and equilibration with PBS, the skin was mounted on static vertical Franz Diffusion cells – Permegear Inc., Bethlehem, PA (receptor volume 5.1 ml, donor area 0.64 sq. cm.) by clamping them between the donor and receptor compartments. The receptor compartment was filled

with PBS (pH 7.4) and maintained at 37 $\pm$ 0.5° C with constant stirring at 600 RPM. Formulations were added to the donor compartment as an infinite dose to completely cover the membrane surface. Receptor samples were collected at predetermined time points and then replaced with equivalent amount of buffer. The drug content in the samples was analyzed by HPLC.

The permeation rate and enhancement ratio of different optimized formulations was determined by Fick's law. Fick's law ($J_S = DKCs/h$) describes the flux (J) across a rate-limiting barrier (of thickness, h) at sink conditions including solubility (Cs), lipophilicity (partition coefficient, K), and the molecular weight or size (diffusion coefficient, D). The enhancement ratio (ER) is defined as the ratio between the mean flux of submicron gel system and the mean flux of the control. The permeability parameters were estimated using the following equations.

a. Flux, Js ($ug/cm^2/h$) from the slop of the cumulative portion permeated per unit area versus time plot.

b. Enhancement ratio, ER using the equation; ER = Js of test sample / Js of control sample.

7.5.2.6 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 um particle size, 4.6 x 150 mm). The mobile phase consisted of a mixture of acetonitrile and phosphate buffer (pH 3.5) with a ratio of 60/40 (v/v). The detection

wavelength used was 230 nm with a flow rate of 1.2 ml/min and run time of 6 minutes**.**

7.5.3 Results and discussions

7.5.3.1 Particle size analysis: The particle size distribution of submicron gel formulations is presented in Table 6. The mean value of the drug crystals was below 300 nm.

Table 6 Particle size distribution of optimal submicron formulation

Mean (nm)	PI	d90	d50	d10
267	0.18	553	288	114

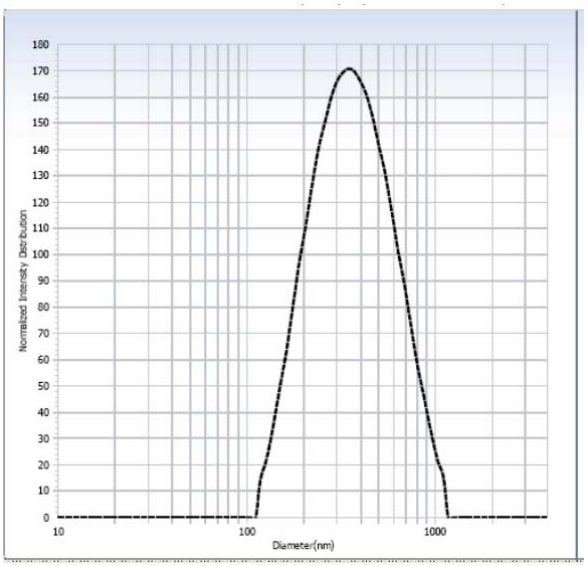


Figure 10 Particle size distribution plot obtained from Delsa nano particle size analyzer

7.5.3.2 Evaluation of gel formulations:

Table 7 Evaluation of submicron gel formulation (test) and marketed formulation (control)

Product	Assay
Marketed product (Deep Relief gel, Mfg. by Mentholatum, UK)	99.4
Submicron gel formulation	96.04

7.5.3.3 In vitro permeation study through human skin: The in vitro permeation study for was conducted with three different formulations (submicron gel formulation, supersaturated gel formulation and marketed product). From this study, highest permeation rate was observed for the submicron gel formulation as compared to supersaturated gel formulation and marketed product. The flux observed for submicron gel formulation was 23.1 ug/cm^2/hr (SD-0.7; n=3) compared to 9.9 ug/cm^2/hr (SD-0.9; n=3) for marketed formulation and 14.1 ug/cm^2/hr (SD-0.6; n=3) for supersaturated gel formulation.

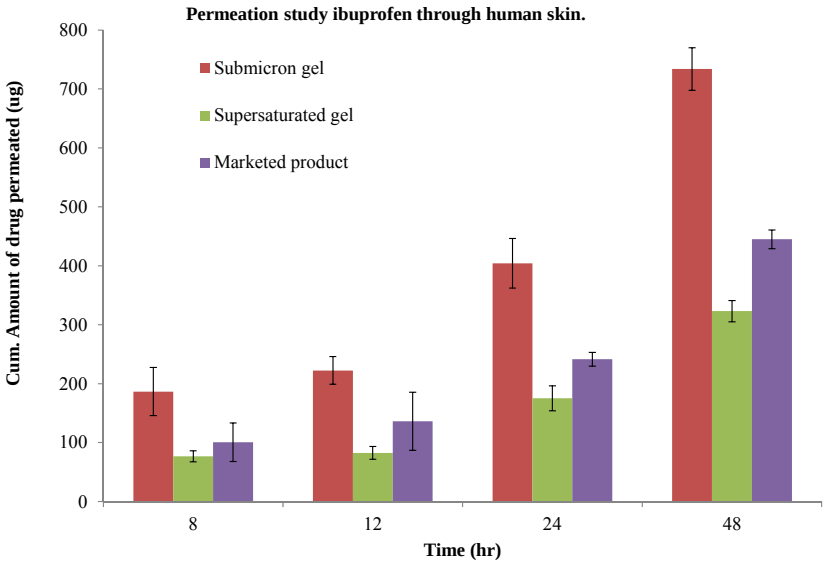


Figure 11 Permeation study profile of submicron gel formulation and supersaturated solution as compared to the marketed formulation (control) through the human skin

Table 8 Estimation of permeation parameters from submicron gel formulation (test) and marketed formulation (control) (n=3).

Formulation	Flux, Jss ($ug/cm^2/h$)	Enhancement ratio; ER
Marketed product (Control)	14.1 (0.6)	-
Submicron gel	23.1 (0.7)	1.64

7.6 MVDA (Multi Variant Data Analysis) modeling of submicron gel formulations

7.6.1 Objective: Besides the Factorial design analysis using Pareto chart approach, MVDA modeling was performed to study the influence of different components of submicron gel formulations on the permeation rate of the drug through pig skin. SIMCA P + (version 11.5) software was used for this analysis.

7.6.2 Introduction: MVDA (Multi Variant Data Analysis) is an important modeling tool for very large data set. It distinguishes between different classes and draw correlations between different process parameters and quality attributes. It can structure a method for connecting process with quality. Finally it finds relationships between variables measured on the process (X) (at N time points) and corresponding values of "result variables" (Y). In research, this model helps process understanding by identification of influential process parameters and establishing the correlation pattern among the process parameters. The basic functions of MVDA includes –

- Data overview
- Classification and discrimination
- Regression modeling (Relationship between X vs. Y).
- correlation pattern among the process parameters.

This model is established based on the equation: $y = f(x) + e$, where, $f(x)$ = the part explained by the model and e = noise (the remaining unexplained part of the data).

7.6.3 Results and discussions: By using the MVDA model, similar results were obtained as compared to the Pareto chart approach. Figure 13 and Figure 14 had shown the importance and magnitude of four different variables (particle size of drug crystals, TPGS concentration, concentration of gel forming polymer and viscosity of gel formulations) on the two response factors (cumulative amount permeated after 72 hrs and flux). The important observations are summarized below-

- TPGS level produced the highest influence on the permeation rate with a positive magnitude of factor.

- The next highest influence was obtained from the particle size of drug crystals, which had a negative magnitude of impact on the permeation rate of the drug.

- The lowest influencing factor seems to be the viscosity of the formulation (produced from the gel forming polymer). This factor also produced positive magnitude of impact on the permeation rate.

- Finally the model distributed the formulations into two groups. These groups (F1, F6, F7, F8 and F2, F3, F4, F5) were formed due to the difference with the concentration of gel forming polymer. The 2nd group (purple) had higher viscosity values as compared to the 1st group (blue) (Figure 15 and Figure 16).

- A linear relationship was observed between the concentration of gel forming polymers and the viscosity of formulations (Figure 17).

- The overall MVDA modeling study produced R^2 value of about 0.8 between observed vs. predicted plot. It demonstrated a strong agreement between the actual results of these runs vs. the calculated/predicted values.

Run	X1 (Drug particle size, nm)	X2 (TPGS level, %)	X3 (concentration of gel forming polymer, %)	X4 Viscosity of formulation (cps)	R1 Cumm. Amount permeated at 72 hrs (ug)	R2 Flux (ug/sq.cm/hr)
F1	300	2	1	625	1142.37521	26
F2	300	2	3	1800	1178.939989	29.7
F3	900	2	3	1750	759.6148813	19.3
F4	300	0.1	3	1550	747.5042064	19.3
F5	900	0.1	3	1575	799.4671901	14.1
F6	900	2	1	700	808.0482333	19.8
F7	900	0.1	1	550	387.863152	12.5
F8	300	0.1	1	525	747.5042064	17.7

Figure 12 Details of data used for MVDA modeling

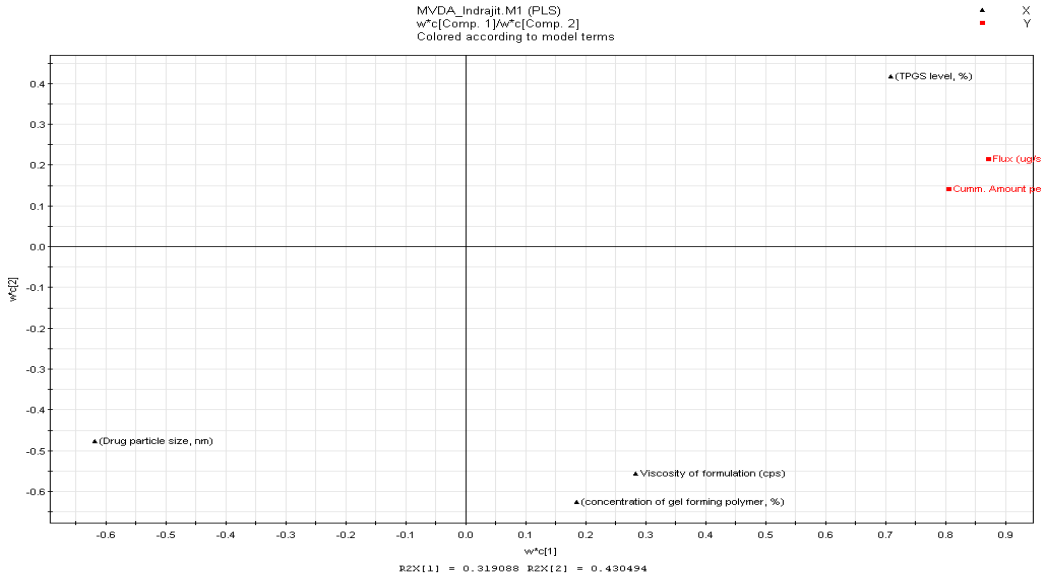


Figure 13 Loadings Plot - Summary of influence of variables (response variables are highlighted in red)

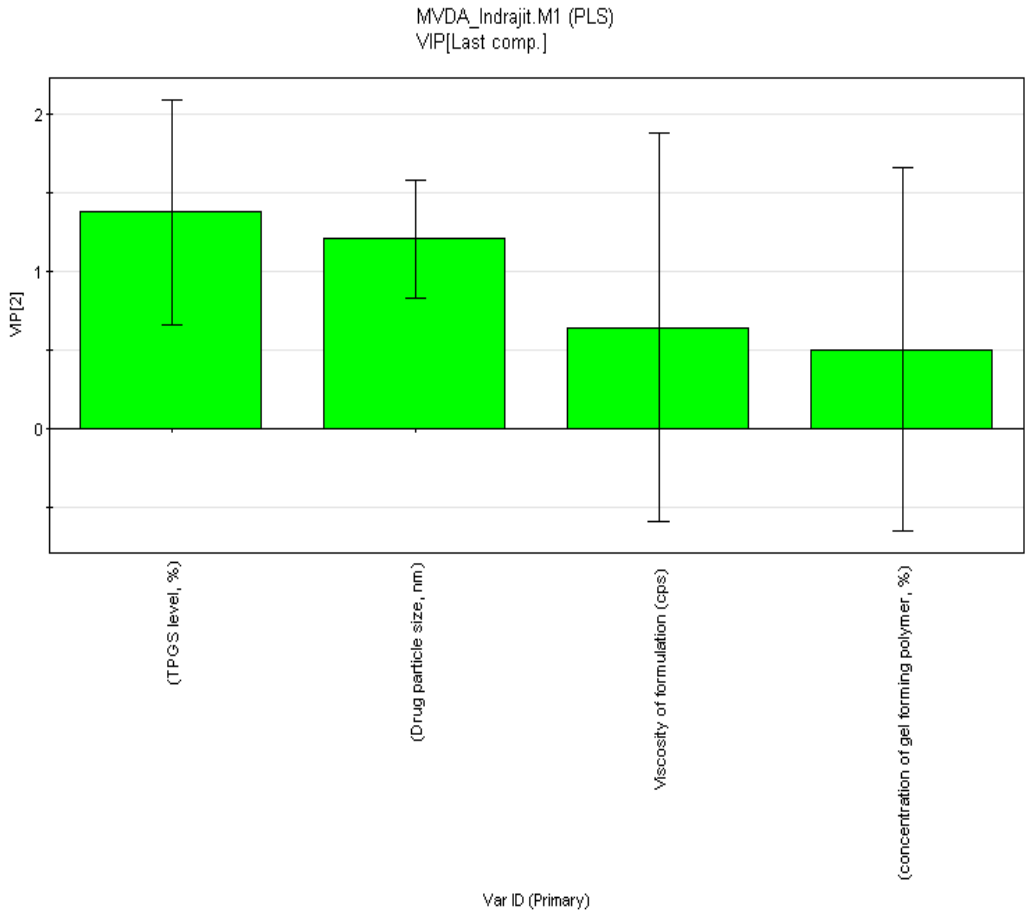


Figure 14 Variable importance plot

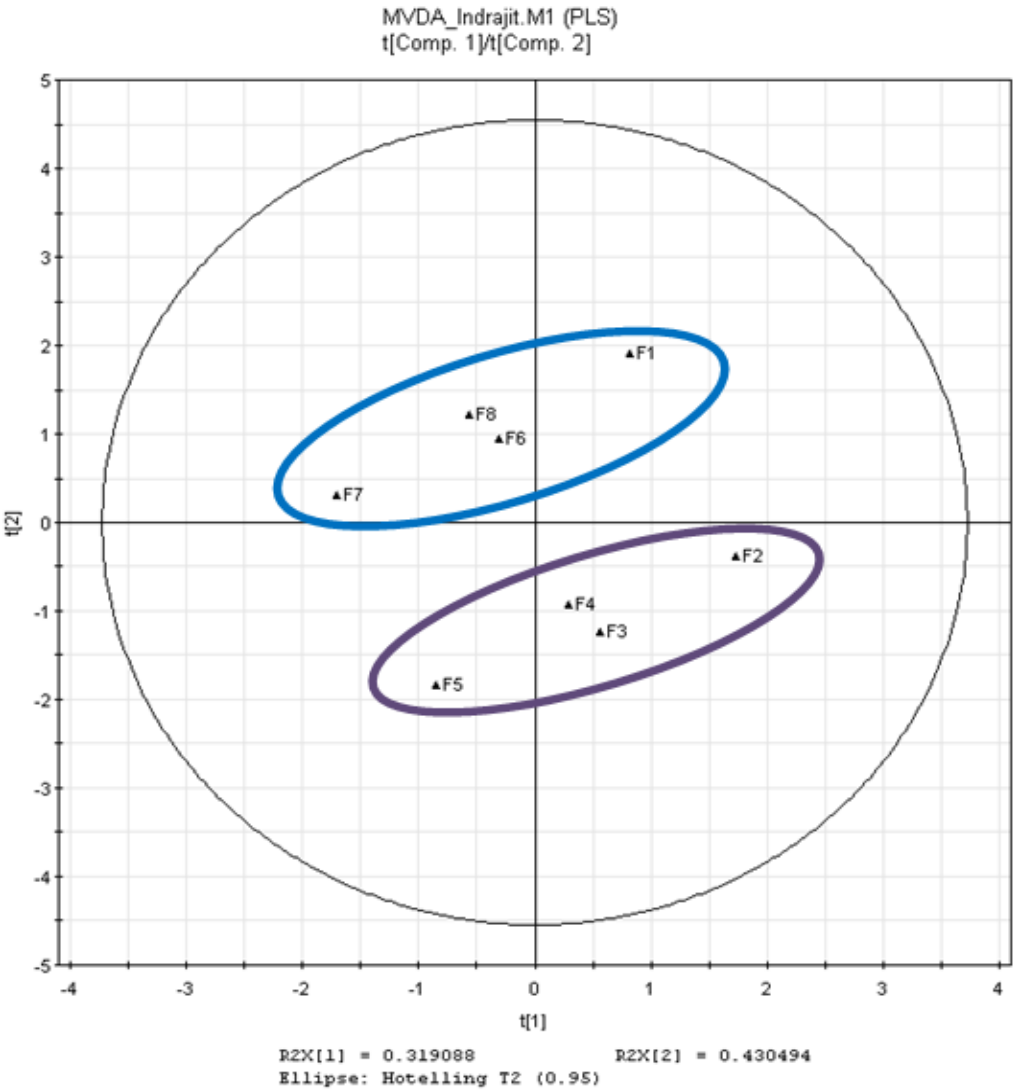


Figure 15 T1-T2 Scores Plot : Summary of observations (Runs)

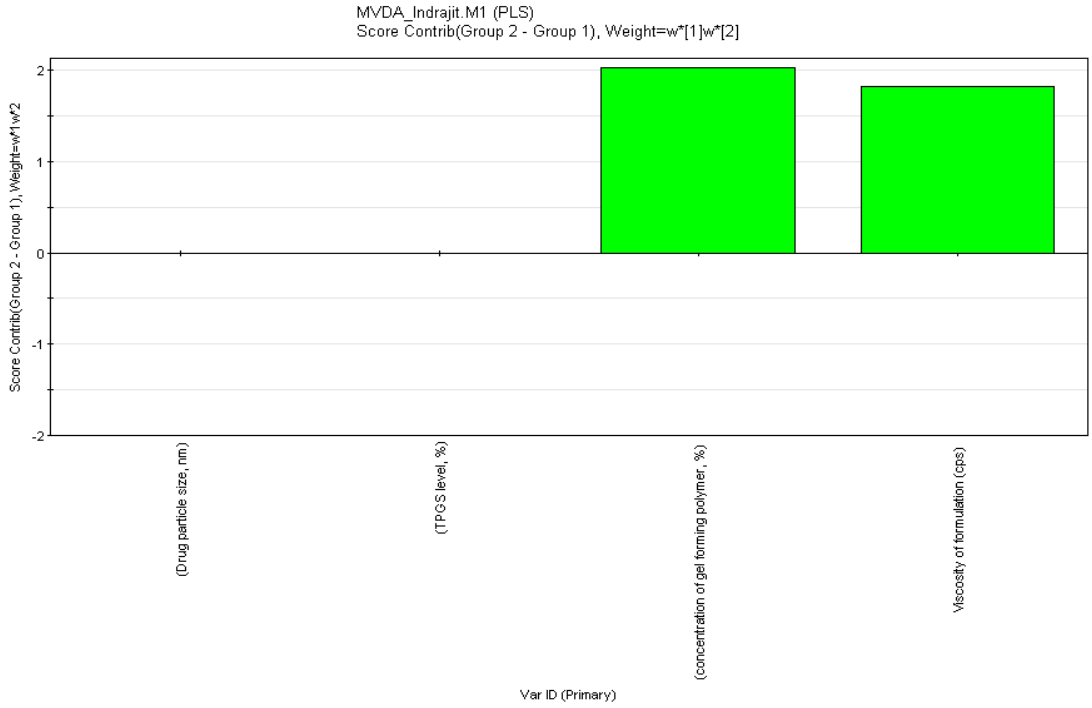
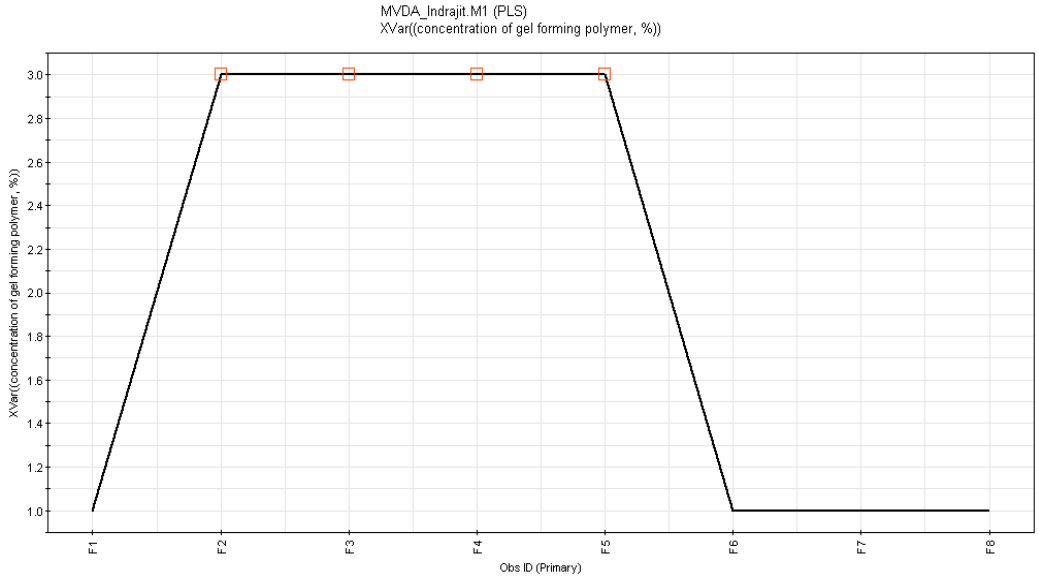
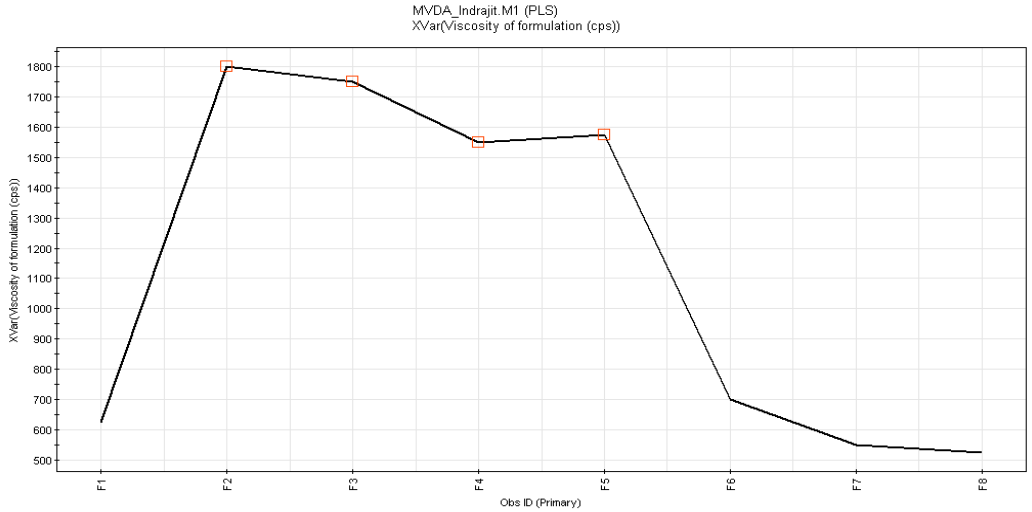


Figure 16 Contribution plot for the distribution of formulations into different sub groups



A



B

Figure 17 Influence of the concentration of gel forming polymer on the viscosity of formulations (A – Conc. of gel forming polymers; B – Viscosity of the gel formulations).

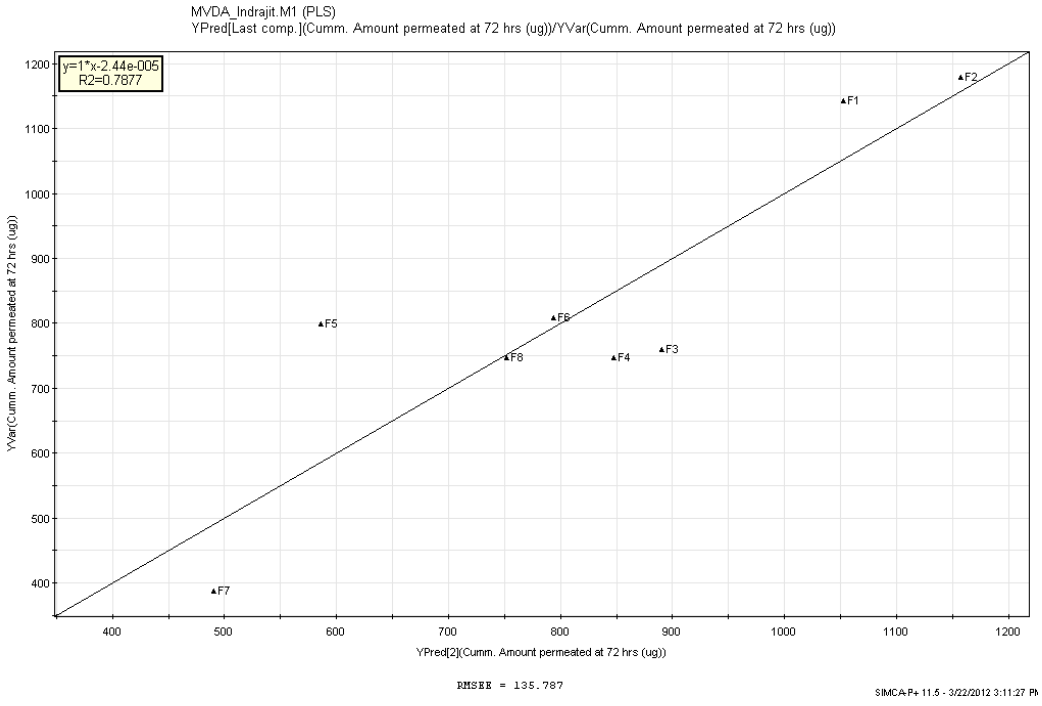


Figure 18 Observed vs. Predicted Plot: shows the agreement between the actual results of our runs vs. the calculated/predicted values by the PLS model.

7.7 References

1. Wu, L., Zhang, J. and Watanabe, W. Physical and chemical stability of drug nanoparticles. Advanced Drug Delivery Reviews 63 456–469, 2011.

2. Van Eerdenbrugh B., Vermant J., Martens J.A., Froyen L., Van Humbeeck J., Augustijns P., Van den Mooter G., A screening study of surface stabilization during the production of drug nanocrystals, J. Pharm. Sci. 98, 6, 2091-2103, 2009.

3. Van Eerdenbrugh B, Van den Mooter G, Augustijns P. Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products. Int J Pharm, 364:64–75, 2008.

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Chapter 8. Final Conclusion

In this research we successfully developed promising formulations using the supersaturation and nanomilling approaches. The supersaturated formulation was developed with vitamin E TPGS, which produced more favorable results as compared to propylene glycol (PG) or Pluronic F-127 formulations during in vitro permeation studies using synthetic membranes or porcine skin. In presence of polymer such as HPMC 3 cps, the onset of crystallization was delayed due to crystal growth inhibition. In this study, the amount of polymer used was relatively low, which probably did not play any significant role on the diffusional resistance on the drug molecules to prevent nucleation. The hydrophobic interaction between the drug and the polymer was probably responsible for the inhibition of nucleation. Therefore, the optimized formulation was converted into a gel for the improvement of stability of the system. Among the different gel formulations, HPMC K100 gel improved the stability of the system significantly as compared to Pluronic F127 and Na-CMC gel systems. Also higher permeability profiles were observed for HPMC K100 gel.

In the second approach, the coarse drug crystals were micronized using a top down media milling approach. During the micronization process the drug crystal size was reduced into the submicron (nano) range. The drastic increase of 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 due to hydrogen bonding. 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 need to be considered while designing a suitable submicron dermal formulation for poorly soluble compounds.

Similar to the supersaturated system, the Vitamin E TPGS – HPMC submicron suspension was also converted to gel formulations. A full scale factorial design study was performed to study the influence of different formulation parameters like drug particle size, concentration of TPGS and concentration of HPMC K100 on the permeation rate. This Pareto chart approach demonstrated a clear correlation between the Vitamin E TPGS and particle size of submicron crystals with the permeation rate (flux) of ibuprofen through the porcine skin. Also the permeation rate from the submicron gel formulations was significantly higher as compared to the supersaturated systems. The results from the MVDA (Multi Variant Data Analysis) correlates well with the analysis using Pareto chart approach.

Finally a human skin permeation study was performed, which had shown higher permeability rate for the optimized submicron gel formulation as compared to the marketed product of ibuprofen. In summary, for BCS II compounds like ibuprofen, submicron / nanosuspension gel formulations seem to be an attractive approach for improving the drug permeability through the skin and improving the therapeutic efficacy of the compound.

Appendices – I

Additional data

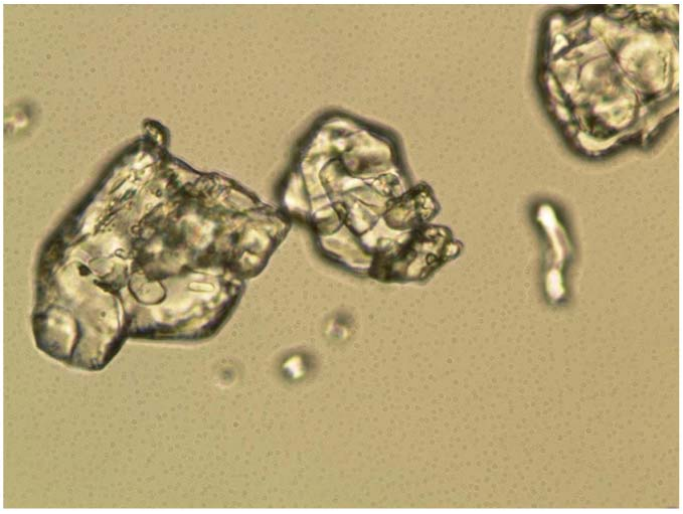
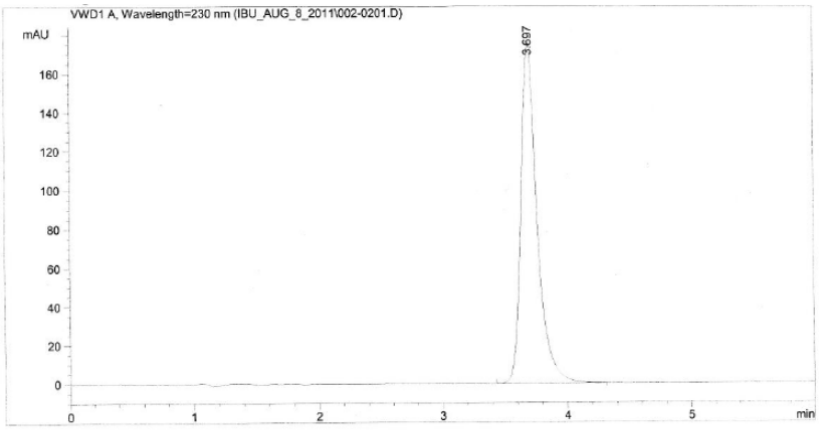


Figure 1 Light microscopic picture of ibuprofen crystals

Table 1 Parameters for HPLC analysis

Parameters	Specifications
Mobile phase	ACN:Phosphate buffer (pH 3.5) = 6:4 v/v
Column	C18, 5 um particle size, 4.6 X150 mm column
Injection volume	20 ul
Flow rate	1.2 ml per min
Temp.	Ambient
UV detection wavelength	230 nm
Run time	5-6 min
Retention time	~ 3.5 min



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

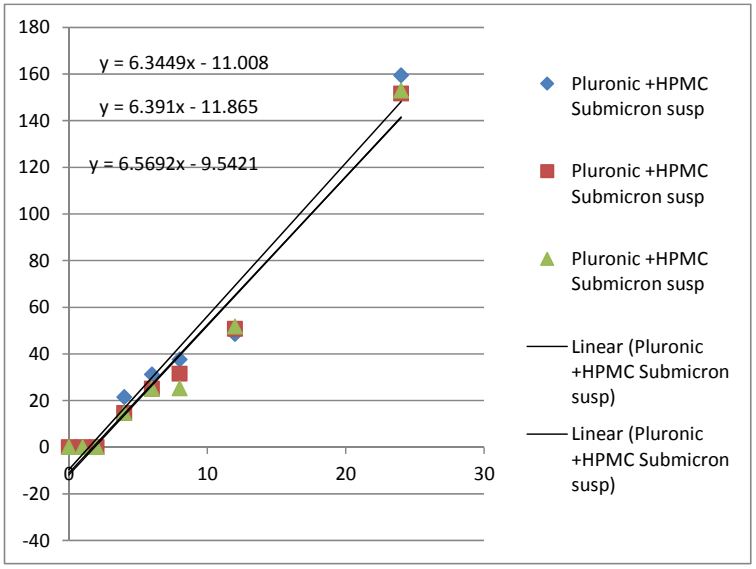
Signal 1: VWD1 A, Wavelength=230 nm

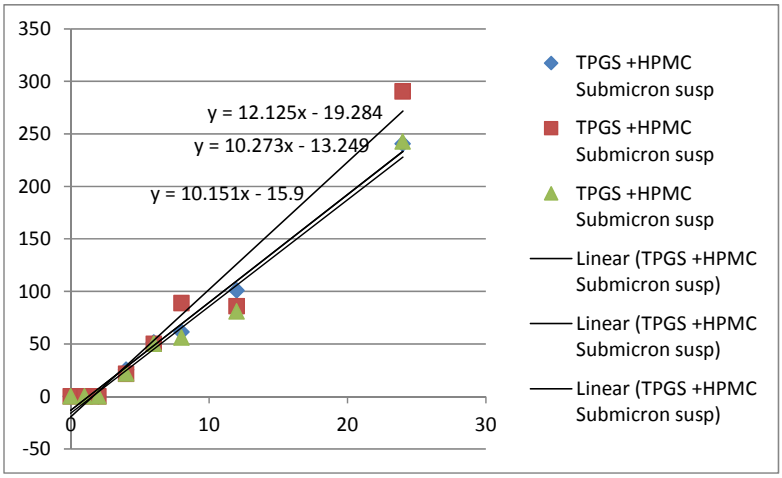
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	3.697	BB	0.1338	1576.83264	175.75612	100.0000
Totals :				1576.83264	175.75612	

Figure 2 HPLC chromatogram of ibuprofen standard solution.

Table 2 Absorbance values of ibuprofen from different concentrations of standard

Conc. (ug / ml)	Area
5	79.53
10	153.36
25	405.09
50	791.36
100	1646.27
500	7735.96
$R^2 = 0.9998$	
100-Inj. 1	1577
100- Inj. 2	1595
100- Inj. 3	1580
100- Inj. 4	1592
100- Inj. 5	1588
RSD = 0.49%	


y = 6.3449x - 11.008
y = 6.391x - 11.865
y = 6.5692x - 9.5421
Pluronic +HPMC Submicron susp
Pluronic +HPMC Submicron susp
Pluronic +HPMC Submicron susp
Linear (Pluronic +HPMC Submicron susp)
Linear (Pluronic +HPMC Submicron susp)


y = 12.125x - 19.284
y = 10.273x - 13.249
y = 10.151x - 15.9
TPGS +HPMC Submicron susp
TPGS +HPMC Submicron susp
TPGS +HPMC Submicron susp
Linear (TPGS +HPMC Submicron susp)
Linear (TPGS +HPMC Submicron susp)
Linear (TPGS +HPMC Submicron susp)

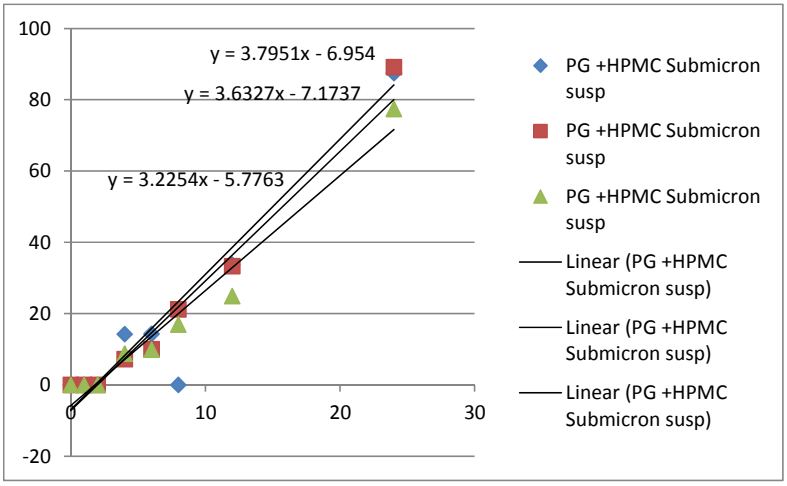
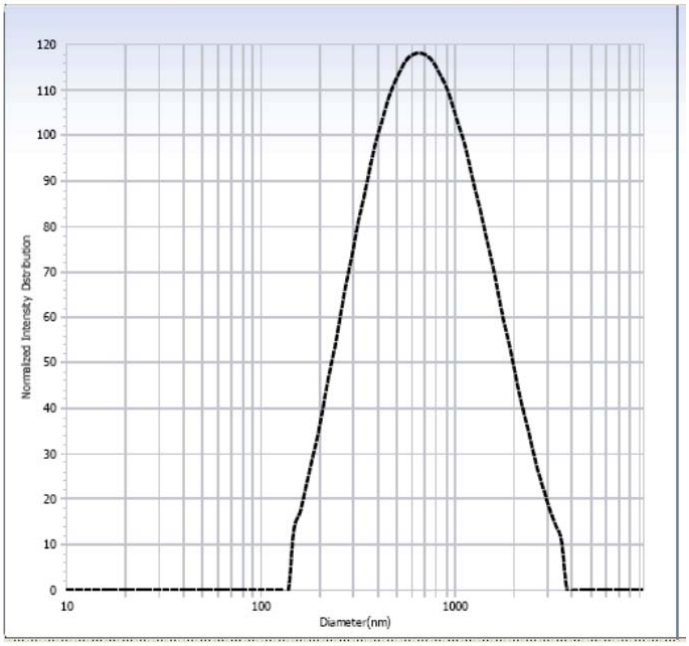
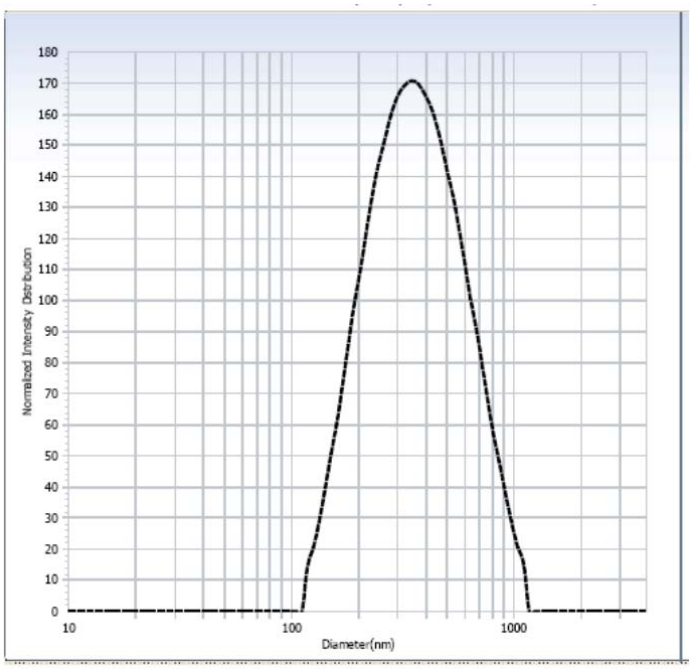


Figure 3 Estimation of flux of un-micronized suspensions from permeation study through the porcine skin.



A: Drug crystal size = +1



B: Drug crystal size = -1

Figure 4 Particle size distribution of drug crystals in submicron suspension (Beckman Coulter - Delsa Nano particle size analyzer).

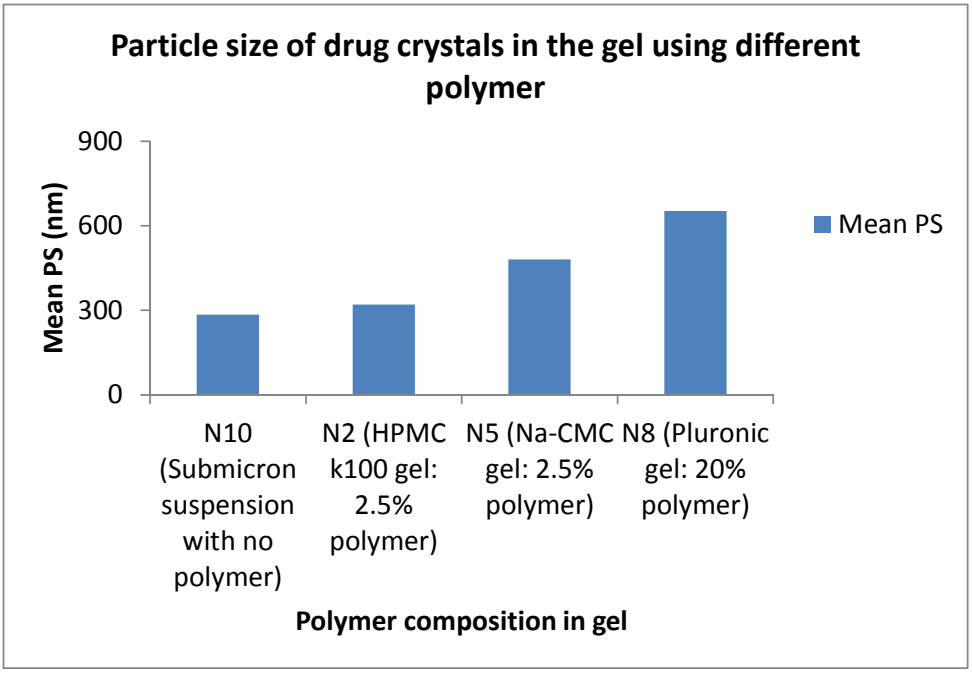


Figure 5 Particle sizes of drug crystals in gel formulations

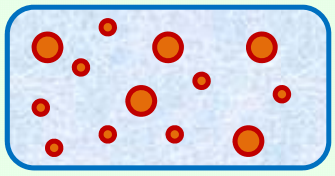
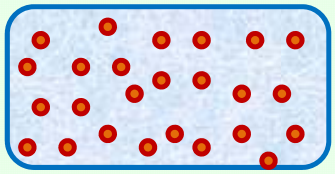
Submicron formulation - Drug crystals size = +1		d-50 < 600 nm d-90 < 1 um
Submicron formulation - Drug crystals size = -1		d-50 < 300 nm d-90 < 750 nm

Figure 6 Distribution of drug crystals in formulations having different particle size

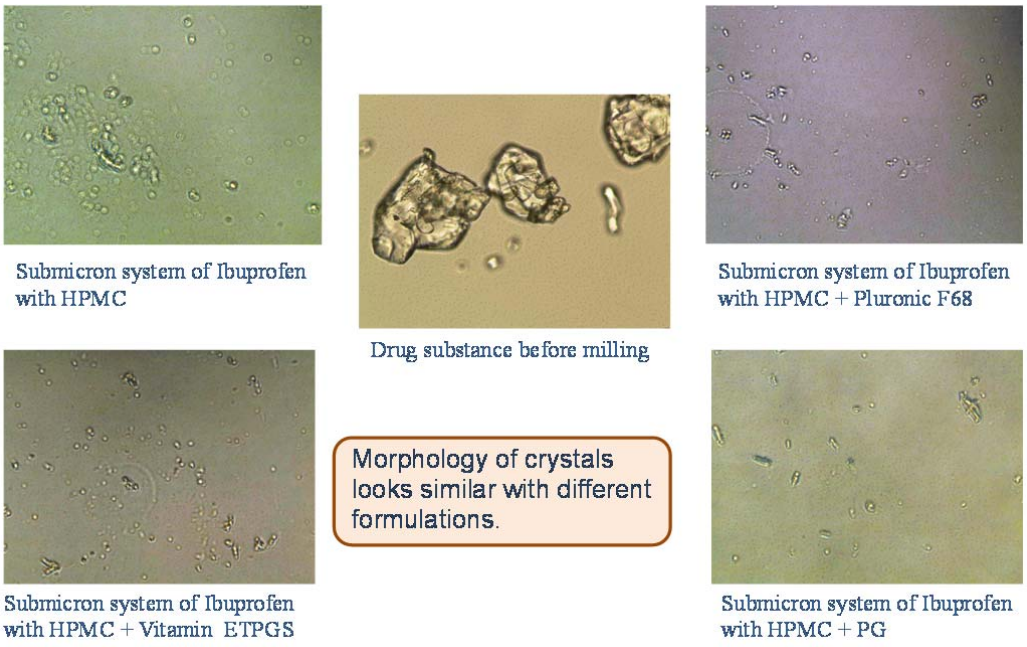


Figure 7 Microscopic pictures of submicron formulations

Appendices – II

Study protocols

A. Permeation study

1) Equilibrate the membrane and the skin in PBS solution for 15-30 min.

2) Clean the Franz diffusion cell with alcohol (by stirring for about 15 min).

3) Discard the alcohol.

4) Fill chamber with PBS until it reaches the top layer of the cell.

5) Place the skin on top of the cell.

6) Place the donor chamber on top of the skin and clamp it tightly.

7) Place the 1-2 (about 500 ul) drops of the sample into the donor chamber.

8) Sample at predetermined time interval. Cap the vials and label them properly.

9) Replace the PBS with the exact amount of solution.

10) Analyse the sample by using a gradient HPLC equipped with UV-vis detector and a C18 column detection (X Terra column, Waters, Ireland, analytical C18 column, 5 um particle size, 4.6 x 150 mm). The detection wavelength was 230 nm, the flow rate was 1.2 ml/min and run time was 6 minutes. The mobile phase was prepared using the following method.

11) a) Measure 600mL of acetonitrile.

b) Dissolve 2.72g of Potassium Phosphate in 100mL of HPLC water in a 400mL flask.

c) Test the pH of this solution. It should be 3.5-4.

d) To adjust the pH use a solution of 0.8g NaOH dissolved in 100mL of water. Make the volume with HPLC water to 400 ml.

e) Add the acetonitrile to this buffer solution and degas.

Appendices – III

Abbreviations

DOE – Design of Experiment

ER – Enhancement Ratio

HPLC – High Performance Liquid Chromatography

HPMC – Hydroxy Propyl Methyl Cellulose

LD – Laser Diffraction

MDSC – Modulated Differential Scanning Calorimetry

Na-CMC – Sodium Carboxy Methyl Cellulose

NS – Nano Suspension

PBS – Phosphate Buffered Saline

PCS – Photon Correlation Spectroscopy

PG – Propylene Glycol

PI - Polydispersity Index

PVP – Polyvinyl Pyrrolidone

SS – Supersaturated Solution

ST – Stratum Corneum

TDDS – Transdermal Drug Delivery System

MVDA - Multi Variant Data Analysis.

Appendix - IV

Resume

PROFESSIONAL EXPERIENCE

Novartis Pharmaceutical Corporation, East Hanover, NJ

Pharmaceutical Development Unit

Principal Scientist / Project Leader – June 2010 - Present

Senior Research Scientist – Feb. 2008 – June 2010

Research Scientist - February 2005 - Feb. 2008

Ranbaxy Pharmaceutical Inc. , New Brunswick, NJ

Formulation Development Scientist

October 2002- February 2005

Other generic companies:

Orchid Research Center, Zydus Cadila Healthcare & Dr.Reddy's Laboratories, India

Research Executive - April 1999 – October 2002.

EDUCATION

PhD, Pharmaceutical Science – Ph.D. May, 2012

Ernest Mario School of Pharmacy,

Rutgers State University of New Jersey, USA.

M. S. Pharmaceutical Sciences – 1997-1999

Jadavpur University, India

RESEARCH PROJECT

a) Design and Development of Transdermal Drug Delivery System

B. Pharm– 1993-1997

Jadavpur University, India

SCIENTIFIC PUBLICATIONS

1. Patents

1. **GHOSH INDRAJIT**, KOWALSKI JAMES, SNYDER JENNIFER, TONG WEI-QIN, VIPPAGUNTA SUDHA, (WO2010036686) GALENICAL FORMULATION COMPRISING ALISKIREN AND PROCESS FOR ITS PREPARATION BY MELT EXTRUSION GRANULATION, Novartis AG (2009).

2. **GHOSH INDRAJIT**, LI SHOUFENG, TONG WEI-QIN, VIPPAGUNTA SUDHA, HONG WEN (WO2010107971) GALENICAL FORMULATIONS OF A FIXED DOSE COMBINATION OF VALSARTAN AND ALISKIREN, Novartis AG (2010).

3. **GHOSH INDRAJIT**, SNYDER JENNIFER, TONG WEI-QIN, VIPPAGUNTA SUDHA, (KR1020090042961) METHOD FOR MAKING SOLID DISPERSIONS OF HIGHLY CRYSTALLINE THERAPEUTIC COMPOUNDS, Novartis AG (2009).

4. ALTENBURGER RALF, ..**GHOSH INDRAJIT**.., (EP2205233) GALENICAL FORMULATIONS OF ALISKIREN AND VALSARTAN, Novartis AG (2008).

5. NAGAMALLA Rajendra, KUMARAPERUMAL Natrajan, **GHOSH Indrajit**, MUDRI, Irena PODUVAL, Vidya RANE, Supriya, DESAI Ganpat, (WO2008056200) ORAL PHARMACEUTICAL COMPOSITIONS OF SIMETHICONE, RANBAXY LABORATORIES LIMITED (2006).

2. Publications

From Industry

1. **Indrajit Ghosh***, Sonali Bose, Radha Vippagunta, Ferris Harmon, Nanosuspension for improving the bioavailability of a poorly soluble drug and screening of stabilizing agents to inhibit crystal growth, International Journal of Pharmaceutics, 409 (2011) 260–268.

2. **Indrajit Ghosh**, Jennifer Snyder, Radha Vippagunta, Marilyn Alvine, Ronak Vakil, Wei-Qin (Tony) Tong1, Sudha Vippagunta*, Comparison of HPMC based polymers performance as carriers for manufacture of solid dispersions using the melt extruder, International Journal of Pharmaceutics, Volume 419, Issues 1-2, 31 October 2011, Pages 12-19.

3. **Indrajit Ghosh***, Radha Vippagunta, Shoufeng Li, and Sudha Vippagunta, Key considerations for optimization of formulation and melt extrusion process parameters for developing thermosensitive compound, Pharmaceutical Development and Technology, 2011, Early Online.

4. **Indrajit Ghosh***, Shoufeng Li, Importance of performing Bioequivalency study for a highly soluble and poorly permeable drug (BCS Class 3) – A Case study, International Journal of Pharmaceutics, Submitted.

From Academy

1. **Indrajit Ghosh**, Bozena Michniak-Kohn*, A comparative study of Vitamin E TPGS / HPMC supersaturated system and other solubilizer / polymer combinations to enhance the permeability of a poorly soluble drug through the skin, Drug Development and Industrial Pharmacy, Early online.

2. **Indrajit Ghosh**, Bozena Michniak-Kohn*, Design and characterization of submicron suspension for a poorly soluble drug: The effect of Vitamin E TPGS and other solubilizers on skin permeability enhancement, International Journal of Pharmaceutics, Submitted.

3. V. Rai, **I. Ghosh**, S. Bose, S.M.C. Silva, P. Chandra, B. Michniak-Kohn*, Transdermal review on permeation of drug formulations, modifier compounds and delivery methods, J. DRUG DEL. SCI. TECH., 20 (2) 75-87 2010.

4. A. Manna, **I. Ghosh**, N. Sen, R.S. Thakur, L.K. Ghosh, B.K.Gupta, Statistical Optimization of Transdermal Drug Delivery System of Terbutaline Sulphate, Journal of Bollettino Chemico Farmaceutico, Feb. 139, page 26, 2000.