CHAPTER 1.0

INTRODUCTION, REVIEW OF LITERATURE AND AIM & OBJECTIVES
1.0 Introduction, Review of Literature and Aim & Objectives

The present aim of this introduction is to place the investigations of my Ph.D work in the perspective of recent pharmaceutical industry strategy and approach to develop and produce new pharmaceutical formulation, particularly, drug nanosuspension formulations.

1.1 Nanotechnology in Drug Delivery

An ideal drug therapy acquires effective concentration of the drug at the target for a particular period of time in order to reduce general and local side effects. An exciting challenge for developing appropriate drug delivery systems targeted for topical diseases is one of today’s most important focuses of pharmaceutical scientists. Development in the field of nanotechnology and its uses to the field of pharmaceuticals and medicines has revolutionized the twentieth century. Nanotechnology is the study of very small structures. The prefix “nano” is derived from greek word, which means, “dwarf”. The word “nano” means extremely small or miniature size. Nanotechnology is the treatment of individual atoms, molecules, or compounds into structures to manufacture materials and devices with unique properties. Nanotechnology engage work from top down i.e. decreasing the size of big structures to smallest structure e.g. photonics applications in nano engineering and nano electronics, top-down or the bottom up, which involves altering individual atoms and molecules into nanostructures and more closely resembles chemistry biology. Nanotechnology deals with materials in the size range of 0.1 - 100 nm; though, it is also inherent that these materials should exhibit diverse properties such as chemical reactivity, electrical conductance, magnetism, physical strength and optical effects, from bulk materials as a result of their small size. Nanotechnology applies on matter at dimensions in the nanometer scale length (1-100 nm), and therefore can be used for a broad range of applications and the formation of various types of nano devices and
nano materials. The short rationalization of pharmaceutical nano system is shown in the figure 1.1; pharmaceutical nanotechnology is classified into two basic types of nano tools viz. nano devices and nano-materials. These materials can be sub classified into nano crystalline and nano structured materials. Nano structure made up of nanoparticles (NPs), drug conjugates, dendrimers, micelles, metallic NPs etc. Carbon nano tubes are small macromolecules that are distinctive significance for biomedical application (Nikalje, 2015).

Figure 1.1: Schematic diagram of various types of pharmaceutical nano systems.

The application of nanotechnology in the health care segment, diagnostics, in imaging, drug delivery and therapeutics, also described as nanomedicine, has gained popularity over the last 5 years. This can be noticed from the increment in the USA budget for nanomedicine research, in addition to an enhancement in the number of nanopharmaceutical patents. Nanomedicine has been used since the 1960’s, with the first lipid vesicles recognized as liposomes. The recent development in this field is mostly due
to the advancement in nanoscience in better approaches of molecular assembly and the design of further controlled and well-organized nanomaterial.

The advantages of nanotechnology application are the opportunity of controlling the size of the resultant particles and devices (Sahoo et al., 2007; Sahoo et al., 2008). Therefore, while combined with advancement in imaging, bioinformatics, as well as systems biology, it offers the potential to put in innovative functionality in the areas of sensor technologies, medical diagnostics, cosmetics and pharmaceutical product delivery (McNeil, 2005). The drug delivery systems based on nanotechnology can lead to better half-life, controlled release over a short or long time periods, and highly specific site-targeted delivery of therapeutic agents.

1.1.1 Challenges of Nanotechnology in Drug Delivery

Although nanotechnology in drug delivery has been successful, as evidenced by some nano drug products in the market, not all approaches have met with the same success. New nanomaterials being developed come with the challenges which have to be surmounted. However some of the challenges encountered have been and are still being tackled by modification of the physicochemical characteristics of the nanomaterials to improve on properties such as long circulation in the blood, increased functional surface area, protection of incorporated drug from degradation, crossing of biological barriers and site-specific targeting. Another challenge of research and development (R&D) of nanomaterials for drug delivery is large scale production. There is always a need to scale up laboratory or pilot technologies for eventual commercialization. A number of nanotechnology based drug delivery systems may not be scalable due to the method and process of production and high cost of materials employed. The challenges of scaling up include low concentration of nanomaterials, agglomeration and the chemistry process – it
is easier to modify nanomaterials at laboratory scale for improved performance than at large scale. Maintaining the size and composition of nanomaterials at large scale is also a challenge. Despite the number of patents for nano drug delivery technologies, commercialization is still at its early stage. This is partially due to the fact that most of the research studies in nano drug delivery are carried out by researchers in academia. Therefore, for these technologies to get to the market there has to be increased partnership with the pharmaceutical companies. Unfortunately, a number of the major pharmaceutical industries are yet to consider nanotechnology as one of their priorities due to lack of regulatory guidelines and challenges of scaling up (Ochekpe et al., 2009). However, it is envisaged that with the expiration of more patents and market loss, more pharmaceutical industries will take up the production of nano drug products in order to compete favorably. Advances in nano drug delivery technology also provide new challenges for regulatory control. There is an increasing need to have regulations that would account for physicochemical and pharmacokinetic properties of nano drug products, which are different from conventional drug products. The United States’ Food and Drug Administration (FDA) and the European Medicines Evaluation Agency (EMEA) has taken the initiative to identify some possible scientific and regulatory challenges.

Furthermore, the International Organization for Standardization has set up a technical committee for the field of nanotechnologies to develop standards pertaining to terminology and nomenclature; measurement and characterization; and health, safety and environment amongst other standards. These standards are still under development.
1.2 Importance of Physicochemical Properties of NPs on the Efficiency of Drug Delivery

A drug delivery system is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and location of release of drugs in the body. When material is at a nanometer size range, it acquires unique physical and chemical properties. More specifically, the parameters that play a major role in drug delivery and have significant pharmacological effects are: particle size, surface area, hydrophobicity, surface charge and crystallinity.

1.2.1 Particle Size

The sub-micron size of NPs offers a number of distinct advantages over microparticles particularly in drug delivery due to the fact that these particles are in the size region of macromolecules. This physical characteristic, particularly for particles less than 100 nm in size allows these particles to reach virtually all tissues in the body (McNeil, 2005). NPs have in general, relatively higher intracellular uptake compared to microparticles. This was demonstrated by Desai et al., 1997, whereby 100 nm size NPs showed 2.5-fold greater up-take compared to 1µm and 6-fold higher uptake compared to 10 µm microparticles in Caco-2 cell line (Desai et al., 1997). This aspect of intracellular uptake is more so critical for intracellular pathogens such as infectious diseases, where the drug needs to act intracellularly. Thus by nanoencapsulating the drug, one can attain intracellular delivery of drugs. Furthermore these particles can cross barriers that in general make it difficult for conventional therapeutic compounds to reach the target. Reports on NPs crossing the blood brain barrier (BBB), the stomach epithelial and even the skin have been presented (Koziara et al., 2003). When orally taken, free therapeutic agents are absorbed into the systemic circulation via the portal blood and
undergo first pass metabolism, leading to poor bioavailability of drugs. However, when encapsulated, transport of the drugs will, in addition to entry via the portal blood, be through the Peyer’s patches, followed by uptake via the M cells, entry into the intestinal lymphatic transport and thus into the systemic circulation. This mode of transport, which includes, endocytosis and phagocytosis will thus minimize the first pass hepatic metabolism of the therapeutic agents, therefore improving their bioavailability (Desai et al., 1996; Desai et al., 1997). These nanocarriers can also be optimized to nanoencapsulate both hydrophilic and hydrophobic therapeutic compounds. In addition, nano sized particles have very high surface area per unit volume, and this has revolutionized the field of drug delivery through improved bioavailability of most therapeutic compounds that generally have poor bioavailability.

### 1.2.2 Surface Charge

The surface charge in NPs reflects the electrical potential of particles and is influenced by the chemical composition of the particle and the medium in which it is dispersed. NPs with a high positive or negative zeta potential have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles (Koziara et al., 2003; Müller and Keck, 2004). In the case of drug delivery, opsonisation, a process that involves the adsorption of proteins particularly of the complement system, to any foreign material, is also influenced by zeta potential. These proteins make the particle more susceptible to phagocytosis and thus leading to their clearance from the body. To circumvent this effect, various groups have coated the particles with hydrophilic polymers, such as Polyethylene Glycol (PEG), Pluronics etc, thus affecting both the surface charge and hydrophobicity of the particles, and therefore increasing the circulation time of the particles in the blood, and in turn prolonging the release of the drugs from the particles. Highly negative zeta potential values in serum are indicative of
particles that have become highly opsonised, and may also indicate poor formulations (Freiberg and Zhu, 2004; Mohanraj and Chen, 2006). Thus, minimising opsonisation via changing the surface charge is important for controlled release formulations. On the other hand a positive surface charge of the NPs, which can be attained by attaching positively charged polymers like chitosan on the surface of the particles, enhances its attachment to the negatively charged cellular membrane, thus improving intracellular uptake. Chitosan-based or coated particles have been reported to be efficient taken up by cells and also cross cellular barriers such as the BBB. This is as function of chitosan opening the tight junctions between cells, and thus facilitates transcellular particle transport (Park et al., 2010).

1.2.3 Hydrophobicity

The charge on the surface of the NPs is not the only factor influencing cellular uptake. A combination of both particle surface charge and increased hydrophobicity of the material has been reported to improve gastrointestinal uptake in case of oral delivery. Hydrophobicity also plays a role in the drug release profile by impacting the kinetics of the degradation of the polymeric shell. Mittal et al., 2007 reported that by changing the hydrophobicity of a nanocarrier, the structure/composition of the polymer/copolymer or the molecular weight, the polymer degradation and thus the drug release mechanism and/or duration is impacted (Mittal et al., 2007). NPs have the advantage of improving the solubility of drugs, particularly for the very hydrophilic or poorly soluble drugs which in most cases are not easy to formulate and have poor bioavailability. By encapsulating these drugs into polymeric particles, which are coated with hydrophilic polymers, the solubility of the drugs can be greatly enhanced, in turn improving the bioavailability of the drug. In addition, by coating the polymeric particles with hydrophilic polymers, the half-life of the drugs can be improved, and thus their efficacy. This approach can reduce
the dose and dose frequency of many effective but poorly soluble drugs, and thus in turn minimize the side effect since less doses will be administered.

1.2.4 Surface Area

Nano-sized particles have a larger surface area due to the fact that a decrease in particle size results in an increase in surface-to-volume ratio and that size is inversely proportional to specific surface area. With NPs, during formulation the drug adsorbs onto the outer layer of the NPs, particularly in emulsion based techniques of preparation, resulting in the initial burst release due to the large surface area, thus affecting the drug release kinetics (Soppimath et al., 2001). Kondo et al., 1993 documented an increase in bioavailability as a result of a 10-fold reduction in particle size, which is as a result of an increase in surface area and consequently an increase in the dissolution rate. Furthermore, a larger surface area allows for a higher loading of the drug, thus leading to a reduction in the dose administered (Kondo et al., 1993).

1.2.5 Crystallinity

Crystallinity in NP formulation is an important consideration during the development process that greatly affects the solubility and dissolution characteristics of the drug. In general, amorphous (noncrystalline) forms of the particles typically may present faster dissolution rate compared to the crystalline forms. When considering the crystallinity of polymeric NPs it is suggested that degradation occurs first in the amorphous regions of the particle, followed by a slower degradation in the crystalline regions. This observation suggests that the crystallinity of the polymer affects the degradation rate and thus the drug release kinetics (Mahato, 2007). Izumikawa et al., 1991 observed that at low drug loading, the polymer dominated the crystalline properties of the particles and no crystallinity was observed for the drug. At high drug loading capacity, crystallinity was dependent on the organic solvent removal process, i.e. at slow
solvent removal rates particle crystallinity was observed for both the drug and the polymer, but faster removal, resulted in amorphous spheres (Izumikawa et al., 1991). Due to the slow degradation of the polymeric shell, the drugs can diffuse out of the particles in a slow and steady manner, thus increasing the active agent’s half-life. Thus, the agent’s effective therapeutic window in the target tissue is maintained. With this approach, it is postulated that the dosage and the dose frequency of most therapeutic compounds will be reduced. Their bioavailability will also be improved as a function of minimized first pass metabolism. Thus, most of the therapeutic agents being administered via the parenteral route due to poor absorption from the gastrointestinal tract (GIT) can safely be administered orally via the nanocarriers. Furthermore, since the carrier systems can be targeted to specific sites of disease, the dose level of therapeutic agent being administered is minimized. This greatly reduces the unwanted systemic side effects associated with conventional free therapeutic agents.

1.3 The Significance of Nanosuspensions as Drug Carrier Systems

1.3.1 Nanosuspensions: Formulation and Manufacturing

Nanosuspension (aqueous suspensions) refers to production of sub-micron-sized particles by subjecting the combination of drug and an appropriate stabilizer to the process of milling or high-pressure homogenization. Conventional milling and precipitation processes generally result in particles with sizes that are much greater than 1 mm. As such, a critical step in the nanosuspension preparation is the choice of the manufacturing procedure to ensure production of sub-micron particles. Nanosuspension formulations can be used to improve the solubility of poorly soluble drugs. A large number of new drug candidates emerging from drug discovery programs are water insoluble, and therefore poorly bioavailable, leading to abandoned development efforts. These can now be rescued by formulating them into crystalline nanosuspensions.
Stabilizers include excipients that enable nanogrinding of the drug particles, prevent crystal growth or NP aggregation during storage, pH-buffering substances, preservatives, and other components that may be needed for further processing (e.g., transforming into a solid form) or administration to patients (e.g., sweeteners, colorants) (Merisko-Liversidge et al., 2003). The term nanosizing, as used in this work, describes the reduction of suspended drug particles down to the submicron size range.

The main challenge in nanosuspension technology is prevention of particle agglomeration or aggregation and crystal growth (Merisko-Liversidge et al., 2003). At the nanometer scale, attractive van der Waals and dispersive forces between particles come into play. Such attractive forces increase dramatically as particles approach each other, which ultimately results in irreversible aggregation (Peukert et al., 2005; Kesisoglou et al., 2007). Key to drug NP technology is the successful compensation of the extra free energy of freshly exposed surfaces (Choi et al., 2005). The tendency of the smaller particles in a suspension to dissolve and re-crystallize on the larger particles represents a mode of instability, termed Ostwald ripening. Ostwald ripening becomes important with particles smaller than 0.5 µm. In general, the speed of Ostwald ripening is governed by molecular diffusion or surface reaction. Diffusion-controlled growth predominates if the particle size distribution in the suspension is large (i.e., in presence of an important fraction of smaller particles) and the solubility is high. In this situation, Ostwald ripening can be lowered by narrowing the particle size distribution. The alternative mechanism of surface reaction predominates under very low supersaturation, i.e., when the solubility of the smaller and larger particles is similar. Ostwald ripening via reaction-controlled mechanism can be prevented by the addition of polymeric stabilizers to the suspension. Irrespective of the mechanism, particle growth can be prevented or at least minimized by steric hindrance and/or electrostatic repulsion. Steric
hindrance is primarily achieved by adsorbing polymers onto particles, while for
electrostatic repulsion, ionic surfactants or polymers are used. Use of surfactants or
surface active polymers additionally promotes wetting and dispersion of the drug
particles, which are usually very hydrophobic. Commonly used polymeric stabilizers for
nanosuspensions include cellulose ethers, such as hydroxypropylcellulose (HPC) and
hydroxypropylmethylcellulose (HPMC), povidone, and poloxamers (types 188, 407 and
338). Commonly used surfactant stabilizers are either non-ionic, such as the polysorbate
types, or anionic, such as sodium dodecyl sulfate (SDS) and docusate sodium (SD). For
effective nanosuspension stabilization, the drug substance: stabilizer ratio may vary
between 20:1 to 2:1 (w/w). While insufficient amounts of stabilizers remain ineffective
for preventing particle agglomeration, excessive quantities may promote crystal growth
by Ostwald ripening (Merisko-Liversidge et al., 2003). Naturally, only excipients with
established safety profiles should be used for the stabilization of nanosuspensions
(Kesisoglou et al., 2007). Particle size reduction can be achieved mainly by bottom-up
and top-down processes:

(1) Particle formation through micro-precipitation, chemical synthesis, or complexation
(Verma et al., 2009; Kawabata et al., 2011).

(2) Particle comminution (nanosizing) through high energy homogenization (Liversidge
and Cundy, 1995; Müller and Keck, 2006).

Processes can also be combined to achieve synergistic effects as adopted in the
NANOEDGE® technology platform (Müller and Keck, 2006). A widely used process in
nanosuspension technology is wet media milling (NanoCrystals® technology) in high-
shear energy mills (Liversidge and Cundy, 1995). In this process, drug substance powder
is suspended in an appropriate medium (mostly an aqueous or aqueous-organic solution
containing appropriate stabilizers). To the drug dispersion, milling medium is added
under continuous stirring; typical milling media comprise beads of ceramics (cerium- or yttrium-stabilized zirconium dioxide), stainless steel, glass or highly cross-linked polystyrene resin, all of which can be obtained in different sizes (typically between 0.1 and 1 mm). The slurry of drug suspension and milling beads is then introduced into the milling chamber of an appropriate mill. Shear forces generated by the movement of the milling medium lead to particle size reduction. Required milling time mainly depends on the hardness of the drug particles, viscosity of the drug suspension, temperature, size and density of the milling medium, and total energy input during milling (Stenger et al., 2005). Milling time can last from about 30 min to several hours or even days (Shegokar and Müller, 2010). Nanomilling can be done at lab scale with as little as 100 mg in a few millilitres of medium by using the Nanomill® system (élan Drug Discovery, PA, USA). Production can be readily scaled up to several litres, e.g., by using Dynomill® (chamber volumes of 300 and 600 ml; Glen Mills, Cliffton, NJ, USA) in flow-through mode or Netzsch® mills (chamber volumes of 2, 10 and 60 L; Netzsch, Exton, PA, USA). To produce large-size batches, mills are preferably configured in circulation mode. The NanoCrystal® technology has successfully expanded the use of nanosuspensions for oral, inhalation, intravenous, subcutaneous, intramuscular and ocular administration (Merisko-Liversidge and Liversidge, 2008; Shegokar and Müller, 2010). The various methods of production of nanosuspension are illustrated in the figure 1.2.
Figure 1.2: Schematic diagram of preparation methods of nanosuspension.

Some other methods for nanosuspension formulation are also recommended (Kumar et al., 2013):

i. Laser fragmentation.

ii. Nanojet technology.

iii. Emulsion solvent diffusion method.

iv. Melt emulsification method.

v. Supercritical fluid method.

1.3.2 Nanosuspension Stabilization Concept

During both, bottom-up and top-down nanosuspension preparation processes, the surface area of the particles will be enlarged, resulting in a change in Gibbs free energy (Lee and Cheng, 2006). Consequently, the particulate system tends to minimize the total surface energy by surface area reduction, leading to agglomeration of the particles. The interaction between two particles is illustrated by the DLVO theory, which plots the total potential energy versus the interparticle distance (Eerdenbrugh et al., 2008). Within small
distances between particles the attractive forces prevail, leading to agglomeration. Therefore, a stabilization of drug nanosuspension particles is required. Generally, two concepts of nanosuspension particle stabilization are pursued. Electrostatic stabilization is provided by the physical adsorption of charged molecules onto the nanosuspension particles’ surface. Approaching particles in the formulation suffer from repulsive forces due to the electrostatic barriers, as shown in figure 1.3(b). SDS (Dolenc et al., 2009), dioctyl sulfosuccinate sodium salt (Langguth et al., 2005) and sodium deoxycholate (Chaubal and Popescu, 2008) are mainly used as electrostatic stabilizers for the nanosuspension preparation. The second concept of steric stabilization includes the formation of a sterical barrier around the particles by adsorption of polymers onto the surface. Within this kind of stabilization, an elastic and an osmotic contribution is discussed. The elastic mechanism includes the volume restriction for each polymer chain by approaching of two particles. Additionally, osmotically driven water influx into the space between two particles occurs due to a local increase of polymer concentration by approach of two particles (Moghimi and Szebeni, 2003). Both mechanisms lead to a repulsion of the NPs and therefore to the stabilization of the system, which is schematically shown in figure 1.3(c). Typical steric stabilizers used in the manufacturing procedure are the polymers HPC, (Fakes et al., 2009; Kim et al., 2011; Ain-Ai and Gupta, 2008) HPMC (Hill et al., 2012; Eerdenbrugh et al., 2009) and polyvinylpyrrolidone (Bhakay et al., 2011) with varying chain lengths and typical molecular weights between 15,000 to 50,000 Da (Choi et al., 2008). Other polymers which decrease the interfacial tension between particle and dispersion medium comprised e.g. of polysorbates, (Li et al., 2009; Kayaert et al., 2010) PEO-PPO-PEO block copolymers (Jacobs et al., 2000), α-tocopheryl polyethylene glycol 1000 succinate (Gao et al., 2010) and fatty acid esters of PEG (Zhao et al., 2010). Often a combination of both stabilization approaches shows
advantageous properties in case of nanosuspension particle size stability (Ghosh et al., 2011).

(a) Enlarged outlook on NPs

(b) Ionic/electrostatic stabilization

(c) Steric stabilization

Figure 1.3: Scheme of submicron sized particles in nanosuspension.

1.3.3 Physicochemical Aspects of Nanosuspensions

Due to their material properties and their small size, a nanosuspension shows various distinctive physicochemical properties. At first, the dissolution velocity of nanosuspensions is increased in contrast to microparticulate systems (Chaubal and
Popescu, 2008). The Noyes-Whitney-equation describes the correlation of dissolution velocity \(\frac{dm}{dt}\) with solute diffusion coefficient in the dissolution media \(D\), particle surface \(A\), thickness of the diffusion layer around the particles \(h\) and concentration gradient of saturation solubility \(C_s\) minus concentration of substance at time \(t\) \(C_t\), as depicted in Eq. (1.1).

\[
\frac{dm}{dt} = \frac{D \cdot A}{h} (C_s - C_t)
\]

By decreasing particle sizes to the nanometer range, the surface area of particles will be enhanced, which in turn leads to higher dissolution rates. Besides the modification of dissolution rates, nanosuspensions can also be able to increase the saturation solubility of drug substances according the Ostwald-Freundlich relationship, as shown in the Eq. (1.2):

\[
\log \frac{C_s}{C_{co}} = \frac{2 \sigma V}{2.303 RT \rho c} (C_s - C_t)
\]

Whereas \(C_s\) and \(C_{co}\) display the drug solubility and the solubility of the solid consisting of large particles, respectively, \(\sigma\) the interfacial tension, \(V\) the molar volume of the particle material, \(R\) the gas constant, \(T\) the absolute temperature, \(\rho\) the density of the solid and \(r\) the radius of the particles. The increase in a substance’s saturation solubility is described for particles below 1 \(\mu\)m in diameter (Müller and Peters, 1998). However, it should be mentioned that a particle size decrease down to approximately 220 nm, a typical size for nanosuspensions produced with e.g. the wet milling method, does only lead to a 15% increase in saturation solubility (Eerdenbrugh et al., 2010). Therefore, the impact of enlarged saturation solubility is relatively small in comparison to an increase in dissolution velocity. A third correlation, which describes an increase in drug solubility, is the Laplace Eq. (1.3):
\[ \Delta p = \frac{2\sigma}{\tau} \]

Where, \( \Delta p \) illustrates the dissolution pressure. As the particle radius decreases with an increasing surface curvature, the dissolution pressure value will be enlarged, (Rasenack and Müller, 2004) resulting in enhancement of the drug solubility.

1.3.4 Potential Role of Nanosuspension Technology for Drugs with Poor Solubility

There are following potential benefits of nanosuspension technology (Kumar et al., 2013):

1. Reduced particle size, increased drug dissolution rate, increased rate and extent of absorption, increased bioavailability of drug, area under plasma versus time curve, onset time, peak drug level, reduced variability, and reduced fed/fasted effects. Due to the particle size reduction, the penetration capability of topical nanosuspension preparations increases significantly (Figure 1.4).

(a) Large, traditional topical preparation
2. Nanosuspensions can be used for compounds that are water insoluble but which are soluble in oil. On the other hand, nanosuspensions can be used in contrast with lipidic systems, and successfully formulate compounds that are insoluble in both water and oils.

3. NPs can adhere to the gastrointestinal mucosa, prolonging the contact time of the drug and thereby enhancing its absorption.

4. A pronounced advantage of nanosuspension is that there are many administration routes for nanosuspensions, such as oral, parenteral, pulmonary, dermal and ocular.

5. Nanosuspension of NPs offers various advantages over conventional ocular dosage forms, including reduction in the amount of dose, maintenance of drug release over a prolonged period of time, reduction in systemic toxicity of drug, enhanced drug absorption due to longer residence time of NPs on the corneal surface, higher drug concentrations in the infected tissue, and suitability for poorly water-soluble drugs, and smaller particles are better tolerated by patients than larger particles; therefore, NPs may represent auspicious drug carriers for ophthalmic applications.

6. Nanosuspension has low incidence of side effects by the excipients.
7. Nanosuspensions overcome delivery issues for the compounds by obviating the need to
dissolve them and by maintaining the drug in a preferred crystalline state of size
sufficiently small for pharmaceutical acceptability.

8. Increased resistance to hydrolysis and oxidation and increased physical stability to
settling.

9. Reduced administration volumes, essential for intramuscular, subcutaneous, and
ophthalmic use.

10. Finally, nanosuspensions can provide the passive targeting.

1.4 Applications of Nanosuspension in Drug Delivery

Nanosuspensions can be applied to all phases of pharmaceutical development
from pre-clinical, clinical phase 1 and 2 to phase 3 and commercial, depending on the
drug substance characteristics and application. Nanosuspensions can be formulated as a
liquid, semi-solid or a solid formulation depending on its application and the development
phase. Their major advantage remains on the NPs size and the consequent increase in
surface area and low toxicity. Particle size engineering can be used to achieve the
intended dissolution rate in case the drug substance adsorption is dependent on the
solubility or both solubility and permeability. The NPs nanosize and increase in the
specific surface area constitute both their major advantage and manufacturability and
stability challenges. Therefore, one of their obvious critical to quality attributes is the
particle size distribution as well as re-dispersion both in the gastric intestinal fluids or
plasma. Nanosized drug particles in liquid or dried nanosuspension formulations offer
several therapeutic benefits. Firstly, drug NPs exhibit enhanced dissolution rate, which is
due to the greatly increased specific surface area, as described by the Nernst–Brunner and
Noyes–Whitney equations.
Secondly, drug NPs also show increased solubility, which is due to the vapour and dissolution pressure of solid particles that increases when particles sizes decrease below 1000 nm, and more particularly below 100 nm), as described by the Freundlich–Ostwald equation (Kesisoglou et al., 2007). From an industrial perspective, nanosuspensions are advantageous, because they can be applied by many different routes, most importantly the oral and parenteral routes, including intravenous administration. Upon parenteral administration, drug NPs offer the potential for passive or active targeting. When administered intravenously, drug NPs can be taken by the mononuclear phagocyte system (MPS) providing targeting for macrophage related disorders and accumulation in liver and spleen (Shegokar and Müller, 2010). To lower and retard uptake by the MPS and maintain the drug NPs longer in circulation, particles can be decorated with hydrophilic polymers, such as PEG. Such PEGylated drug NPs would then passively accumulate in tumour tissue by the so-called enhanced permeation and retention effect (Merisko-Liversidge et al., 2003; Chaubal, 2004). For active targeting, drug NPs can be coated with specific functional groups. For example, thiamine coated NPs demonstrated preferential uptake in the brain via the blood–brain–barrier thiamine transporter (Chaubal, 2004). In oral administration, drug NPs show enhanced bioavailability and more balanced drug absorption between fed and fasted states. Taken together, all the beneficial properties of drug NPs may offer means for accelerating lead selection and drug development processes (Chaubal, 2004).

NPs have found wide use in recent years for various oral, injectable, inhalational and even intradermal applications (Chaubal, 2004). The first nanosuspension-based product launched on the market using the NanoCrystal® technology was the immune suppressant sirolimus (Rapamune®, Wyeth) in 2000, followed by aprepitant (Emend®, MERCK) in 2003, fenofibrate (TriCor®, ABBOTT) in 2003, and megestrol acetate
(Megace, ES PAR PHARM) in 2005. All these products are administered orally. One of the first demonstrations of the biopharmaceutical benefits of nanosuspensions in oral administration was with danazol, a poorly soluble gonadotropin inhibitor for treatment of, e.g., menorrhagia. The absolute bioavailability of conventional danazol microsuspensions in beagle dogs was only 5.2%, but 82.3% when administered as an aqueous nanosuspension (at same dose of 200 mg); at the same time, \( T_{\text{max}} \) was reduced and \( C_{\text{max}} \) increased approx. 15-fold (Liversidge and Cundy, 1995). Another example is aprepitant, a low solubility drug substance used on the drug product Emend® which has been approved for the prevention of chemotherapy-induced nausea and vomiting. Aprepitant nanosuspensions are prepared by nanogrinding using hydroxypropyl cellulose and sodium dodecyl sulfate as stabilizers (Wu et al., 2004). The NanoCrystal® dispersion provided 3.5-fold increase in exposure in humans at a dose of 100 mg compared with a tablet formulation made of micronized particles and also eliminated food effects on absorption in a dog model (Wu et al., 2004). In various instances, intravenous drug injection is required to meet the clinical needs. However, when a formulation is administered intravenously, particle size must be smaller than 5 \( \mu \)m to avoid capillary blockade and embolism. Nanosuspensions offer a good alternative as they can be injected intravenously using relatively high doses in low volumes and achieve 100% bioavailability (Gao et al., 2012). Merisko-Liversidge, 2011 compared the performance of marketed paclitaxel solution (Taxol®), which is formulated as microemulsion with Cremophor® EL and ethanol to solubilise the drug substance, with paclitaxel nanosuspension. The results demonstrated that the nanosuspension was better tolerated and more efficacious. More recently, nanosuspensions have also been introduced as long-acting injectable formulations. Tailoring the particle size distribution at the nanosize level may afford control of dissolution rates, while offering the advantages of high drug dose and stability.
Injectable long-acting formulations are currently available in several therapeutic areas requiring long-term treatment or prophylaxis, such as for psychiatric disorders or contraception (Van’t Klooster et al., 2010). A successful long-acting nanosuspension is Invega® Sustenna® (Jansen), a once-a-month extended release nanosuspension of paliperidone palmitate for intramuscular (deltoid or gluteal muscle) injection. Paliperidone palmitate nanosuspension is obtained by nanogrinding in presence of a surfactant and marketed as a single-use prefilled syringe (Samtani et al., 2009; Merisko-Liversidge and Liversidge, 2011). Another example of a long-acting (once-a-month) injectable nanosuspension is with the very poorly water soluble antiretroviral anti-HIV rilpivirine. Rilpivirine is currently in development as a long acting nanosuspension formulation, for treatment and prophylaxis against HIV (Baert et al., 2009). Rilpivirine nanosuspension with 200 nm sized particles was injected intramuscularly (i.m.) or subcutaneously (s.c.) in dogs as a single-dose and achieved stable plasma concentration profiles detectable up to 3 months. The 200 nm sized particles provided improved early release (higher $C_{\text{max}}$) as compared with 400 or 800nm sized particles. Pharmacokinetic simulations demonstrated that once-a-month s.c. or i.m. administrations of rilpivirine in humans will maintain plasma concentrations above the minimal therapeutic concentrations of 73 to 95 ng/ml as observed after daily oral dosing with 25 mg rilpivirine (Baert et al., 2009; van’t Klooster et al., 2010). The following, are further examples of applications of nanosuspensions. Docetaxel (Duopafei®) is a practically water-insoluble drug substance with low bioavailability and high toxicity; it is used in the treatment of different types of cancer (ovarian, breast and other type of tumors). The presently marketed product contains high concentration of the non-ionic surfactant Tween 80. Docetaxel-loaded nanosized lipid carrier was prepared by high pressure homogenization using soya lecithin as surfactant followed by freeze-drying in presence of mannitol.
The mean particle size of the freshly prepared and the freeze-dried drug-loaded lipid particles were 200.0 ± 3.4 nm and 223.3 ± 4.3 nm, respectively. At two weeks after i.v. injection into B16 melanoma-bearing mice, the group treated with the nanosuspension showed smaller tumor volumes ($P<0.05$) and lower tumour weights ($P<0.01$) than the Duopafei® group; this suggests that the nanosuspension inhibited effectively tumour growth, while no antitumor effect was observed in the blank group. Besides the higher antitumor efficacy, the nanosuspension –treated group showed also increased survival rate and strongly reduced drug toxicity (Wang et al., 2011). In Sporanox® i.v. for intravenous administration, itraconazole is solubilized by hydroxypropyl-β-cyclodextrin and propyleneglycol. Because of the cyclodextrin, Sporanox® i.v. must not be administered to renally impaired patients. To overcome this limitation and to accommodate maximal dose, an itraconazole nanosuspension with mean particle size of 581 ± 18 nm (measured after 24 months storage at 5°C) was developed. When administered i.v. to rats, the nanosuspension was better tolerated than itraconazole solution, especially at higher doses. The higher tolerated drug levels achieved with the nanosuspension in the target organs reduced Candida albicans colony counts and increased survival rates (Rabinow, 2004).

The investigational anticancer compound SN 30191 is practically water-insoluble inhibitor of phosphatidylinositol-3-kinase (PI3-K). The activation and/or over-expression of PI3-K has been associated with a number of human cancers; PI3-K has been shown to disturb the fine equilibrium between cell division, growth and apoptosis, which leads to abnormal cell growth and possibly resistance to therapy. SN 30191 was formulated by high-pressure homogenization as a nanosuspension with a mean particle size of less than 150 nm. A toxicity study in mice (i.v. administration of 2.5 – 20 mg/kg body weight) revealed that the tolerated dose with the nanosuspension was at least four times higher (10
mg/kg versus 2.5 mg/kg) than with the solution formulation (consisting of 10% (v/v) DMSO, 40% (v/v) PEG 300, 50% (v/v) PBS and 0.5% (w/v) Cremophor® ELP) (Sharma et al., 2011). Another case where nanosuspension technology has proven to be highly beneficial is with camptothecin, a natural alkaloid and topoisomerase I inhibitor with strong antitumor activity. The compound had, however, to be abandoned in early clinical trials, because of its poor solubility and toxicity. A camptothecin nanosuspension was developed using a solvent precipitation method, which yielded particle sizes in the range of 200 nm to 700 nm. When tested in MCF-7 xenografted BALB/c mice, the nanosuspension suppressed significantly tumor growth with the drug concentration in the tumor being five times higher at 24 h as compared to a drug solution in propylenglycol-water mixture (Zhang et al., 2011). A final example concerns the topoisomerase II inhibitor asulacrine with activity against breast and lung cancers (Ganta et al., 2009). Asulacrin nanosuspension was prepared by high pressure homogenization using poloxamer 188 as stabilizer for the drug NPs (d50 0.133 ± 0.02 µm; d90 0.702 ± 0.02 µm). In vitro dissolution testing revealed a sustained profile reaching 42% of the dose (5 mg in 500 ml medium) within 5 h (as compared to 6% when a micronized powder was used). A pharmacokinetic study in mice showed that the nanosuspension produced reduced C_{max} (12.2 ± 1.3 µg ml⁻¹ versus 18.3 ± 1.0 µg ml⁻¹) and AUC-values (18.7 ± 0.5 µg ml⁻¹ h versus 46.4 ± 2.6 µg ml⁻¹ h) as compared to the drug solution in a 1:1 mixture of dimethylacetamide and propylenglycol. The plasma profiles indicated that the nanosuspension formulation maintained low plasma drug for a longer period of time, which can potentially help to overcome the dose-dependent toxicity of asulacrin. The authors of the study that asulacrine nanosuspensions may give added value by allowing a reduction in either the dose or its frequency of administration (Ganta et al., 2009).
As reported by Majuru and Oyewumi, 2009 the feasibility of applying NPs in topical/cosmetic preparations has been a subject of several commentaries. In any case, this dosage form utilizes the advantages of NPs such as: (i) protection of labile compounds; (ii) controlled release of incorporated drugs; (iii) ability of solid lipid NPs to act as occlusives to increase the water content of the skin; and (iv) ability of NPs to serve as physical barriers on the skin for blocking UV light and, as such, for use in sunscreen formulations (Majuru and Oyewumi, 2009).

1.5 Aloe vera as a Burn Wound Healing Promoter

According to the World Health Organisation (WHO): A burn injury of the skin occurs when some or all the different layers of cells in the skin are destroyed by a hot liquid (scalds), a hot solid (contact burns), or a flame (flame burns). Injuries of the skin and other tissues due to ultraviolet/infrared radiation, radioactivity, electricity, or chemicals are also considered to be burns (WHO, 2010). The skin is the body’s largest organ and is made up of three layers; the epidermis, the dermis and the subcutis. These layers work together to: act as a waterproof, insulating shield, guarding the body against extremes of temperature, damaging sunlight, and harmful chemicals. It also exudes antibacterial substances that prevent infection and manufactures vitamin D for converting calcium into healthy bones. Skin additionally is a huge sensor packed with nerves for keeping the brain in touch with the outside world. At the same time, skin allows us free movement, proving itself an amazingly versatile organ (National Geographic, 2010). A burn to the skin can be relatively minor or result in life threatening complications depending on the severity of the burn. Burns are categorized according to the extent of damage to the skin. There are three levels of burns: Superficial – (also referred as first degree burns) cause damage to the first or top layer of skin. The burn site will be red and painful. Partial thickness – (also referred as second degree burns) includes damage to the
first or second skin layers. The burn site will be red, peeling, blistering and swelling with clear or yellow-colored fluid leaking from the skin, and is very painful. Full thickness – (also referred as third degree burns) involves damage to both the first and second layers, plus the underlying tissues, muscle, bone and organs. The burn site generally appears black or charred with white exposed fatty tissue or bone. The nerve endings are generally destroyed so there is little or no pain experienced at the site of the full thickness burn but surrounding partial thickness burns will be very painful (Better Health Channel, 2010). Another important classification for a burn injury is the total body surface area (TBSA) which is affected. Generally, a burn to greater than 10 per cent TBSA is classified as a major burn.

Wound healing is a complex biological process where the main goal of clinical intervention is the promotion of tissue restoration (Mendonça et al., 2009). Wounds can result from many conditions including burns, arterial disease, surgery and trauma, and can be classified as acute or chronic. Acute wounds are wounds that follow a predictable and timely repair process which results in the restoration of sufficient anatomical and functional integrity if healing proceeds normally (Lazarus et al., 1994). This predictable sequence of events can be broken down to initial inflammation, collagen and fibroblast deposition (scar tissue formation), angiogenesis (new blood vessel formation), wound contraction and scar remodeling. Chronic wounds, however, are wounds where the repair process has been disrupted (e.g. infection, immunosuppression) and healing has been subsequently delayed.

*Aloe vera* (also known as *Aloe barbadensis* Mill., *Aloe indica* Royle, *Aloe perfoliata* L. var. *vera* and *A. vulgaris* Lam) is a plant belonging to the Liliaceae family, of which there are over 360 known species (Vogler and Ernst, 1999). They are cactus-like perennial succulents and are characterized by stem less, large, thick, fleshy leaves that are
lance shaped and have a sharp apex and a spiny margin (Steenkamp and Stewart, 2007). The plant provides two distinct products: the yellow latex, which is referred to as aloe juice, and the leaf pulp which is the innermost portion of the leaf and is composed of the parenchyma cells whose baseline function is for storage of food and nutrients that contain the *Aloe vera* gel (Figure 1.5).

![Figure 1.5: Aloe vera leaf containing gel like substance (Aloe vera gel).](image)

The raw pulp contains about 98.5% water with the remaining 1.5% containing a range of compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids (Hamman, 2008). The leaf pulp is commonly delivered as a topical ointment on wounds in a gel, cream or mucilage form (the mucilage being the thick, glue-like gel substance that is derived from the leaf pulp of the *Aloe vera* plant). *Aloe vera* has been used in wound healing since ancient times, with evidence suggesting it was well-known to the ancient Egyptian, Greek and Indian cultures. The use of *Aloe vera* was mentioned in the Ebers papyrus, widely considered as an important medical document of ancient Egypt and dating back 1550 BC. In 330 BC, the famous Greek king Alexander the Great was said to be persuaded by his mentor Aristotle to capture the island of Socotra (now part of modern day Yemen) in the Indian
Ocean, famed for its supply of aloe which he needed to heal his wounded soldiers (Atherton, 1998).

Evidence from animal studies has highlighted the possible effects of *Aloe vera* in wound healing (Chithra et al., 1998; Mendonça et al., 2009; Takzare et al., 2009). Prostaglandin and bradykinin hydrolysing enzymes in *Aloe vera*, including carboxypeptidase and bradykinase, are hypothesized to reduce pain and inflammation (Steenkamp and Stewart, 2007; Chithra et al., 1998). Aloe-derived polysaccharides such as mannose-6-phosphate have been postulated to be active growth substances, especially in epithelialization (Davis et al., 1994; Boudreau and Beland, 2006; Steenkamp and Stewart, 2007). Davis hypothesized that the binding of mannose-6-phosphate to fibroblast receptors induces fibroblastic proliferation, which ultimately helps promote collagen deposition and tissue reorganisation. Acemannan, another polysaccharide, has been shown to up-regulate white blood cell activity in the wound healing process (Boudreau and Beland, 2006; Tamura et al., 2009; Kuzuya et al., 2001). Similarly, suggested that the antibacterial properties of anthraquinones, an organic compound responsible for the natural pigment of *Aloe vera*, is beneficial in minimizing infection (Tamura et al., 2009).

### 1.6 Clinical Evidence of SSD

Silver and silver compounds have been routinely used as general antimicrobial agents for over a century (Klasen, 2000; Lansdown, 2002; White, 2002). Silver, as the common ionic (active) form (Ag⁺), is generally recognized as a safe, broad-spectrum antimicrobial agent. It is only the ionic form that has the antimicrobial activity (Russell and Hugo, 1994). Silver ions are made bioavailable through the interaction of aqueous fluid, typically wound exudates, with metallic (elemental) silver, or directly from silver salts (compound). This latter mechanism is the basis for the antimicrobial activity of silver nitrate, chloride and sulphadiazine, and may avoid the need for chemical oxidation
in order to provide the ‘active’ species as is the case with metallic silver (Gibbins, 2003). Thus, the availability of silver ions is dependent upon dissociation of silver salts, or, on their solubility in wound fluid; this is pH dependent. However, wound fluid is a complex mixture which can vary considerably in composition; it is broadly similar in composition to serum as it contains proteins, lactate and electrolytes (e.g. Na⁺, K⁺, Cl⁻), urea and glucose. Ionic silver Ag⁺ is a highly reactive chemical species, which interacts with functional organic groups such as thiols. As integral protein side-groups, thiols and chemically similar species are key components of most proteins—including enzymes, and prokaryotic (bacterial) cell wall structures, and also nucleic acids. It is this binding that forms the basis of antimicrobial activity. Silver was formulated as the salt of the sulphonamide antibiotic, sulphadiazine, in the 1960s by Fox (Fox, 1968). Silver sulphadiazine (SSD) is a non-ionised, water insoluble, fluffy white powder that is formed when sulphadiazine, a weak acid, reacts with silver nitrate to form the complex silver salt. A polymeric structure for SSD has been proposed, where six Ag⁺ ions bind to six sulphadiazines via the nitrogen atoms of the sulphadiazine pyrimidine rings (Fox, 1983). Since the introduction of SSD into clinical use, it has been used extensively in the topical treatment of infected burns. More recently, it has been utilized in chronic wounds and the latest application is its incorporation into medical devices, such as catheters and dressings, for the prevention and treatment of infections. SSD has largely been marketed as a 1% SSD cream as Flamazine™ (Smith and Nephew, Hull), and as a cream with 1% SSD in combination with 0.2% chlorhexidine digluconate as Silvadene™ (Monarch Pharmaceuticals, Tennessee, USA). This review will examine the laboratory evidence of its antimicrobial activity and cytotoxicity, and the clinical evidence of its efficacy. The advantages and limitations of SSD will also be explored.
Sulphadiazine is one member of four sulphonamide groups (others include sulfacetamide, mafenide, sulfasalazine and sulfisoxazole); it is characteristically rapidly absorbed and excreted from the gastrointestinal tract (Goodman and Gilman, 1990). From the topical route, Ag110 (radiolabelled silver) clearance has been measured in rats, and in ex-vivo, burned human skin (Harrison, 1979). Peak attachment of radiolabelled silver was observed to be 1% of the administered dose in 24 hours, leading to the deduction that SSD functions via the slow release of silver. Whereas low concentrations of silver may be distributed into the tissues, the plasma concentration of sulphadiazine may approach therapeutic concentrations, depending on the surface area treated. Hence, silver is largely confined to cutaneous tissues and the sulphadiazine penetrates into the systemic circulation (Lockhart et al., 1983). This differential distribution pattern is reflected in varying therapeutic- and side-effects, which will be discussed later. In 1985, Fox studied the detail human pharmacology of SSD in depth (Fox, 1985).

1.6.1 Antimicrobial potentials of SSD

The mode of action of SSD is not clearly elucidated, and whether the broad-spectrum antimicrobial activity is attributable to either the silver or the sulphadiazine moieties, or a synergistic combination of both, have been debated (McDonnell and Russell, 1999). In general, sulphonamides are broad-spectrum antibiotics that are predominantly bacteriostatic by inhibiting the formation of dihydropteroic acid, an intermediate of the folate pathway. SSD has been seen to cause surface and membrane blebs in susceptible (but not SSD-resistant) bacteria (Coward et al., 1973). Fox and Modak, 1974 showed that silver, rather than sulphadiazine, binds to bacteria, and suggested that only a relatively small amount of available sulphadiazine appears to be active in this context (Fox and Modak, 1974). There is evidence of antifungal and
antiviral activity, attributable to the silver moiety (Wright et al., 1999; Thurman and Gerba, 1989).

In wound treatment, it is probable that SSD functions by delivering sustained, low concentrations of silver (approx 1–2 ppm) into the wound environment, and that this interferes with, or modulates, multiple cellular processes. There are multiple deleterious effects in micro-organisms rather than a single, specific inhibitory mechanism (Fox and Modak, 1974). Given the anionic content of wound fluid, it is interesting to consider how silver solubility and available ions might exert a bactericidal effect. The chloride anion (Cl\(^{-}\)) is found in serum, and wound exudate. It will influence the availability of Ag\(^{+}\) in solution. At low chloride concentrations (around a 100 mM) as present in wound fluid, soluble silver will bind to the bacterial cell surface, inhibiting respiration (Bragg and Rainnie, 1974). Moderate concentrations of chloride remove the silver as insoluble silver chloride precipitate, and, at higher chloride concentrations (such as might be found in exudate) silver is brought back into solution as the bioavailable anion AgCl\(^{2-}\) (Gupta et al., 1998). The ability of highly diluted heavy metals (including silver in water) to inhibit micro-organisms was termed ‘oligodynamic’ by von Nägeli in 1893; this term has often been applied to silver. Silver products are thought to interact with microbial thiol groups, carboxylates, phosphates, hydroxyls, amines, imidazoles, and indoles, either singly, or, in various combinations (Grier, 1983). Trevors et al., 1987 reviewed the possible cellular effects of silver toxicity (Trevors et al., 1987). These include:

- Binding of silver to base pairs in DNA, and thereby blocking transcription.
- Binding to cell surface components, interfering with bacterial respiration and uncoupling ATP synthesis, and.
- Preventing uptake of phosphate, and causing the release of components (such as glutamine, proline, succinate and phosphate from *Escherichia coli*).
Certain effects (such as phosphate release) may be reversed by the presence of thiol groups; such thiolated compounds as dithiothreitol and mercaptoethanol can combine with silver to form a stable, inactive complex that reduces toxicity to bacteria. Fox, 1968 suggested that prolonged contact of SSD with biological fluids containing chloride and sulphydryl groups, leads to the solubilisation of sulphadiazine, and thus enhances the oligodynamic action of silver with the additional antibacterial activity of the released sulphonamide (Fox, 1968). Gupta et al., 1998 studied the effect of halides, e.g. chloride on susceptibility to silver in two strains of E. coli (Gupta et al., 1998). High concentrations of halides increased sensitivity of both a silver sensitive strain and a silver resistant strain to silver in In vitro tests, whereas low concentrations of chloride emphasized the differences between the sensitivities of the strains.

Most published studies on the mode of action of SSD concern bacterial cells, but irreversible inactivation of phosphomannose isomerase by SSD was demonstrated in Candida albicans. Phosphomannose isomerase is a zinc metalloenzyme essential for the biosynthesis of cell walls in fungi; and a cysteine residue, at position 150, has been shown to be the site of action of SSD. The same enzyme in E. coli was unaffected by SSD (Wells et al., 1995). In addition to the antimicrobial effects of silver, positive benefits to the wound healing process have been reported (Lansdown et al. 1997; Demling and DeSanti, 2001; Kirsner et al., 2002). These focus on theoretical mechanisms involving:

- The displacement of zinc from metallothionines
- Changes in metalloprotein levels within the wound
- Effects on inflammatory cytokines.

Further research is needed to confirm and to clarify these mechanisms.
1.6.2 Cytotoxicity and Limitation of SSD

The systemic toxicity of the sulphonamide antibiotics is dependent upon their individual chemistry and metabolism (Shear et al., 1986). In general, the sulphonamides are known to induce agranulocytosis (Willoughby, 1977). Certainly, neutropaenia has been reported in burns treated with topical SSD, but this cannot be directly attributed to the sulphadiazine. It would appear to be the case that systemic or local toxicity from sulphadiazine, as delivered from 1% topical emulsion formulations dosed according to manufacturer’s instructions, is most unlikely. However, in a study on severely burned patients, where a large body surface area was treated with a topical SSD emulsion, measurable toxicity, including allergic contact reactions to the formulation excipients, e.g. Propylene glycol and cetyl alcohol has been reported (Degreef and Dooms-Goossens, 1985). Similar responses to the sulfadiazine were reported by Kulick et al., 1985, although this is contradicted by Degreef and Dooms-Goossens, 1985 who maintain that, ‘there is no evidence that SSD is contraindicated for patients with a history of sulfonamide hypersensitivity’ (Kulick et al., 1985; Degreef and Dooms-Goossens, 1985).

Toxic effects of silver include skin irritation and discolouration (argyria) which is widely reported for silver nitrate solutions and colloidal silver but rarely associated with topical SSD (Gettler et al., 1927; Buckley, 1963; Marshall and Schneider, 1977; Wright et al., 1998). Allergic contact dermatitis to silver as SSD and as the nitrate has been reported, although with SSD most such reactions are to the excipients (Fisher et al., 2003; Agarwal et al., 2002; Iliev and Elsner, 1998).

Cytotoxicity in vitro has been described and postulated as a cause of delayed wound healing as reported anecdotally (Poon and Burd, 2004). In wound treatment, any potential for cytotoxicity arising from silver-releasing wound dressings or SSD creams, can be avoided through the prudent use of such products – particularly avoidance of
unnecessary or prolonged use. Serum silver levels have been found to rise after topical SSD treatment (Maitre et al., 2002). Upon dissociation, the sulphonamide clears from the body more rapidly than does silver (Boosalis et al., 1987). Elevated serum silver (over 20 mg L\(^{-1}\)) is reported to cause renal dysfunction, liver and nerve toxicity: this occurs after prolonged exposure of leg ulcers and acute burns to 1% SSD (Maitre et al., 2002; Tsipouras et al., 1997), and, agranulocytosis in neonates (Viala et al., 1997).

Delayed wound healing is claimed to be the clinical manifestation of mild toxicity, as evidenced from in vitro studies involving various skin cells lines (Hidalgo and Dominguez, 1998; Poon and Burd, 2004). Undesirable characteristics associated with the clinical use of SSD are the emergence of resistant strains of microbial species, the claimed retardation of wound healing and the development of systemic side-effects, indicating the need for a better burn dressing (Lee and Moon, 2003; Stern, 1989; Cho Lee et al., 2005; Hussain and Ferguson, 2006). Disadvantages of SSD include the need for frequent dressing changes at least once daily, pain during dressing change, local maceration, cytotoxic to keratinocyte as well as fibroblast and bacterial resistance (Muangman et al., 2006). Recent advances in local wound care products have led to the development of moisture retentive dressings that have the advantages of reducing pain symptoms, reducing the frequency of dressing changes, and improving exudates management (Twomey, 2005). SSD cream, however, is relatively short-acting, requires reapplication at least daily, and is time-consuming and messy to apply and remove. Dressings containing SSD should not be used in patients with sensitivity to sulphonamide antibiotics or hepatic/renal impairment, or in pregnancy, during lactation or in newborns.

In 1970s, as an antibacterial agent for topical treatment of burns and wounds; silver is complexed to propyleneglycol, stearyl alcohol, and isopropyl myristate and mixed with the antibiotic Sulfadiazine producing a combined formulation made from
silver nitrate and sodium sulphadiazine by substituting a silver atom for a hydrogen atom in the sulphadiazine molecule and combining the inhibitory action of the silver with the antibacterial effect of sulphadiazine (Klasen, 2000; Stanford et al., 1969). Bacterial resistance to these products does develop. Impaired re-epithelialization has been described. Observed bone marrow toxicity with SSD is primarily due to the propylene glycol component (Atiyeh et al., 2007).

A number of adverse reactions and side effects have also been reported together with increasing resistance to SSD (Trop et al., 2006). In addition to adverse effects of sulphonamides, prolonged topical application of SSD cream can induce argyria (Chaby et al., 2005), even though it has never been reported yet as a result of topical application except locally (Dunn and Edwards-Jones, 2004). Direct silver-induced renal toxicity has also been reported and confirmed by high concentration of silver in blood and urine. Kidney function improved on withdrawal of the topical cream (Chaby et al., 2005). Leukopenia has been documented as well following prolonged SSD application and could be secondary to medullar toxicity (Chaby et al., 2005). In vitro studies showed that SSD is cytotoxic (McCauley et al., 1989) but that cytotoxicity can be reduced by controlling the delivery of the active agent (Kuroyanagi et al., 1991). Even though other in vivo studies have found no evidence for cytotoxicity (Geronemus et al., 1979) and despite the fact that after decades of use, the evidence for cytotoxicity is not clear and SSD remains the main topical product used in burn units (Dunn and Edwards-Jones, 2004; Fakhry et al., 1995). Various observed toxic effects confirm that this topical cream should not be used for long periods on extensive wounds (Chaby et al., 2005). Modern silver-containing dressings are antimicrobial, yet cellular toxicity is a serious side-effect (Ziegler et al., 2006). Though it has been reported traditionally that silver has a low mammalian cell toxicity (Kirsner et al., 2001; Wright et al., 2002; Paddock et al., 2002; Shi et al., 2006).
Rare but serious side effects associated with the use of SSD include sensitivity, hemolytic anemia, hyperbilirubinemia, methemoglobinemia, anaphylaxis, toxic epidermal necrolysis, Stevens-Johnson syndrome, agranulocytosis, leukopenia, and bacterial resistance. However, such side effects are uncommon and the actual incidence is unclear (Fuller, 2009). Several adverse reactions and side effects have been reported, such as resistance to SSD, renal toxicity, and leukopenia, thus confirming that this topical cream should not be used for long periods on extensive wounds (Atiyeh et al., 2007). Prolonged application on partial thickness burn wounds results in high patient care cost and complicates wound healing because inpatient follow up is needed (Hosseinimehr et al., 2010). Hepatic or renal toxicity and leukopenia may be caused by the topical application of SSD. In fact, these side effects have been observed in the treatment of large wounds (Chaby et al., 2005; Fraser et al., 2004).

Even after more than 40 years of use, SSD still is frequently referred to as ‘the gold standard’ in the treatment of partial thickness burns because of its excellent antibacterial properties and its wide availability, especially in developed countries. However, in recent years, several reports have shown that this standard therapy also has a number of substantial disadvantages. Application of SSD always results in the formation of a pseudoeschar layer on the burn wound which impairs evaluation of burn depth and healing status. Daily dressing changes are labour intensive, expensive and induce fear and pain, especially in children. A cytotoxic effect of SSD has also been demonstrated on epidermal cells with hair follicle death resulting in a slowing down of the healing rate and increased skin problems after healing. Because of these disadvantages of SSD, the quest for the ideal burn dressing is still ongoing. Improvements in technology and the expansion of our knowledge regarding wound healing, bacterial burden and drug delivery have led to the development of a wide range of new dressing options. Factors such as
fluid absorbing and releasing capacity (cfr ‘moist’ wound healing), number of dressing changes, ease of application and removal, pain and comfort for the patient, anti-bacterial properties, drug delivery as well as cost effectiveness have all become increasingly important in the search of the ideal wound dressing (Heyneman et al., 2016).
1.7 Review of Literature

Several studies described in literature as well as reviews of the recent developments in SSD topical drug delivery by nanotechnology, with different possibilities explored so far have been summarized here.

Yousefpoor et al., investigated the synergistic effects of AV-gel and silver NPs on the healing rate of the cutting wounds. In order to find out the dose of silver NPs in AV-gel, the MBC techniques were used for the majority of wound infection carrying bacteria such as, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The wounds (full-thickness) were created dorsal part of rat skin; then the rats were separated into 4 groups. The treatments rats group administered mixture of AV-gel and silver NPs, AV-gel alone and silver NPs alone with respect to control groups. The treatment was carried out for the period of 2 weeks and no significant difference (p<0.05) in healing rate between the mixture and control group. The AV-gel improved the rate of wound healing while the silver NPs had wound delayed effect; and when they were mixed, it was comparable to the average effect of both AV-gel and silver NPs (Yousefpoor et al., 2016).

Ali et al., identified the probable reasons of the color change in SSD cream manufactured by Julphar Company packed in plastic container 500 gm, in addition is to find suitable measures to overcome this difficulty. The prepared cream was filled into white plastic container with white cap, black plastic container and black cap and white container with white cap plus aluminum foil liner from inside. Primarily, samples were studied visually and were chemically analyzed. Three samples in different containers were kept in normal circumstance and under exposure to light for three months. The effect of these containers as well as environmental conditions later observed at weekly interval. At the end of study they found that upon exposure of the SSD to light the color of the cream in the white container was changed to dark, the color of the cream in the
white container with white cap plus aluminum foils was not changed remarkably as well as the color of the cream in the black container remain unchanged and this appear the significance of the packaging components on dosage form stability which shows that defective packaging of pharmaceutical dosage form can invalidate the most stable formulation. Accordingly, it is necessary that the choice of container materials for any particular formulation be made only after a thorough assessment has been made of the persuade of these materials on the stability of the formulation as well as of the effectiveness of the container in protecting the formulation throughout extended storage under changeable environmental condition of temperature, humidity and light. In spite of the wide range of new burn treatment agents, SSD cream still can be considered as the best typical antibacterial agent of major importance in treatment of the burn patient. The evolving issue of color alteration is caused due to the oxidization of silver which results from exposure to light. This problem can be short out by the use of light resistance container. Although, it is observed by this research that the discoloration issue will not change the therapeutic effect (Ali et al., 2016).

Jodar et al., developed an optimized hydrogel with impregnated SSD was pursued, topically used for the antimicrobial. The selected hydrogel shows a homogeneous appearance, with whitish coloration as well as free from any fractures or cracks. The impregnated SSD shows antimicrobial action, as predicted, signifying a extended drug release. The FTIR spectra of the hydrogel with impregnated SSD demonstrated that the drug did not engage in any bonds with the polymeric matrix, which otherwise might have lessen its antimicrobial action (Jodar et al., 2015).

Patel et al., developed an emulgel formulation involving SSD for topical use. The bio adhesive particulate system involving SSD in order to overcome the difficulty associated with conventional systems. The prepared as well as selected formulation has
better qualitative gelling characteristic, bio adhesive strength and wound healing action on rats. Although, standard market conventional powder (Silverex®) has synergistic antimicrobial action, and the prepared formulation indicate equivalent wound healing action on rats in burn wound model, that might be due to continuous drug supply to the wound and protective action towards microbes (Patel et al., 2015).

Adhya et al., conducted a research in the burn unit of IPGME&R & SSKM Hospital Calcutta, from January 2011 to August 2012. Patients with second-degree burn injury were randomly divided into SSD and Silver NPs treated group. Clinical evaluation of second-degree burn injury was done on every week till 4th week as well as on end of treatment. Silver NPs can be an efficient and better substitute to SSD for burn wounds; especially second-degree deep-dermal burns (Adhya et al., 2015).

Hamid and Soliman investigated the possible effect of Aloe vera on the angiogenesis procedure during healing of a full-thickness (FT) burn wound. Study conducted on 70 rats was separated into 3 groups- A (control), B and C. Group B was bare to FT skin burn. Group C was bare to FT skin burn with use of local AV-gel. Each of group B and C was grouped into 3 sub-groups from which skin specimens were taken at 4th, 8th, 12th days. Skin specimens were ready for histological as well as immunohistochemical study through hematoxlyin and eosin, Masson’s trichrome and alpha smooth muscle actin (α-SMA). All data were determined morphometrically and statistically examined. Subsequent to 4 days from creating FT burn, the early necrosis as well as inflammation progressively changed due to enhanced granulation tissue (GT) on 8th and 12th day skin samples. The collagen deposition of GT enhanced with time to make a coarse dense bundles, for the moment the newly formed capillaries of GT were enclosed by pericytes which shows considerable expression of α-SMA early on 4th and till 8th day specimens and reduced on 12th day specimens. AV-treated groups indicated relative
reduction of α-SMA detection particularly in 8th and 12th day specimens along with a considerable reduction in the inflammatory infiltrate in all phases and deposition of further mature as well as finer collagen fibers comparatively analyzed along with burn per se specimens. The FT skin burn, Aloe vera demonstrates a beneficial outcome by decreasing the inflammation extensively and giving a more mature GT that could speed up healing and might form a sound well-remodeled scar (Hamid and Soliman, 2015).

Dellera et al., developed chitosan oleate ionic micelles loaded with SSD to be allied with platelet lysate (PL) for the management of wound. The SSD loaded micelles were considered to be linked in wound healing with PL (a hemoderivative rich in growth factors). Unloaded micelles show good compatibility with both fibroblasts as well as PL. A significant rise (up to 100 times with respect to saturated solution) of SSD contents in micelle dispersion was attained. In addition, the encapsulation diminishes the cellular toxicity of the drug against fibroblasts and the drug incompatibility with PDGF-AB (platelet derived growth factor), chosen as representative of platelet growth factors (Dellera et al., 2014).

Sandri et al., developed a montmorillonite-chitosan based SSD nanocomposites for topical application for the management of chronic skin lesions. The loading of SSD in MT/CS nanocomposites focused at inhibiting the wound healing delayed effects, by reducing the cellular toxicity of SSD and keeping their antimicrobial potentials stable. Nanocomposites were formulated by employing different concentrations of MT (100-2000 mg) and 40 ml of a 1% (w/w) chitosan glutamate aqueous solution. Developed nanocomposite was characterised for in vitro biocompatibility and proliferation as well as for wound healing by the use of typical human dermal fibroblasts. Antimicrobial potentials were assessed towards four reference bacterial strains: Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. SSD loaded in
the 100 MT/CS nanocomposite shows good *in vitro* biocompatibility as well as gap
closure properties (fibroblasts) and retained SSD antimicrobial potentials, particularly
towards *P. aeruginosa*, that frequently complicates skin lesions (Sandri et al., 2014).

Dharashivkar et al., developed an advanced niosomally encapsulated SSD gel for
the management of burn by using thin film hydration technique. The *in vitro*
antimicrobial potential of niosomally encapsulated SSD gel was as good as that of
marketed cream towards *Staphylococcus aureus* even when employed in 1/2th the
concentration (0.5%) of commercial formulation (1%). Moreover SSD (0.5% w/w)
niosomal gel was formulated by carbopol 934 (1.6%). It was apparent by *in vitro*
permeation study that SSD release was greatly retarded from both niosomes as well as
niosomal gel as compared to marketed formulation thus lessening the dosing frequency.
In-vivo study indicated that a niosomal gel involving 0.5% w/w SSD was more efficient
in burn wound healing in comparison to 1% w/w marketed formulation even when used
once a day (Dharashivkar et al., 2014).

Morsi et al., developed a novel nano system based cubosomes, loaded with SSD
for management of burns topically. Dispersions of cubosomes were prepared by an
emulsification method employing different amounts of a lipid phase monoolein and
Poloxamer 407, the nonionic surfactant, with or without polyvinyl alcohol. Developed
cubosomal dispersions were analyzed with respect to morphology of particle, dimensional
distribution, size of the particle, and *in vitro* drug release. The optimized formulation was
further transferred into a chitosan, carbopol 940 or chitosan/carbopol mixture depend
hydrogels, to form cubosomal hydrogels (cubogels). *In vivo* and histopathological studies
were also carried out on rats to forecast the efficiency of the newly developed cubogels in
contrast with the marketed cream (Dermazin®) (Morsi et al., 2013).
Sandri et al., developed an advanced wound dressings based on SSD solid lipid NPs (SLN) for tissue repairing, to be utilize in alliance with PL for the management of skin lesions. The encapsulation of SSD in SLN permitted to inhibit the cellular toxicity of the drug on normal human dermal fibroblasts, in vitro, as well as the involvement of the drug with PL. Dressings depend on chitosan glutamate indicate antimicrobial action with and without PL representing to be a acceptable prototype for the management for skin lesions (Sandri et al., 2013).

Shahzad and Ahmed conducted a study and determined the possible role of AV-gel in comparison with 1% SSD cream for the management of superficial as well as partial thickness burns. In this study a total of 50 patients with superficial and partial thickness burns were separated into two equal groups arbitrarily by successive sampling process, one group were treated with AV-gel while the other was with 1% SSD cream, and the outcomes concerning duration of wound epithelialization, pain relief and cost of management were differentiated. Patients treated with AV-gel, healing of burn wounds were outstandingly early than those patients managed with 1% SSD. Patients treated with Aloe vera group were comforted of pain earlier in comparison with the patients who were treated with SSD (Shahzad and Ahmed, 2013).

Venkataraman and Nagarsenker prepared SSD nanosuspension by the use of microprecipitation–high-pressure homogenization method. An optimized formulation of SSD (0.5%) was developed by the use of Cremophor EL (6%) and Lauroglycol 90 (4%), subjected to 30 cycles of 1,000-bar pressure to generate a nanosuspension. It was apparent through XRD analysis that SSD was exists in amorphous state in both the cases (Microprecipitate and nanosuspension). SSD (0.5%) nanogel was formulated by the use of Carbopol 974 P (1%) for topical delivery of nanosized SSD. In vitro release studies indicated that SSD release was more rapidly from solutions and nanosuspensions in
comparison to gel formulation due to the influence of the gel matrix on SSD release. *In vivo* studies disclosed that a nanogel involving 0.5% SSD was more effective in wound healing in contrast to 0.5% and 1% marketed cream (Venkataraman and Nagarsenker, 2013).

Ghodekar et al., developed an emulgel to enhance the solubility and bioavailability of SSD by preparing solid dispersions, secondly in comparison between polaxomer188 and 407 for improving the solubility by using different processes of solid dispersion preparation and thirdly combine the advantages of emulsions and gels using formulating emulgel of SSD. The outcomes indicted that poloxamer 407 and 188, solid dispersions of poloxamer 407 were more amorphous than that of Poloxamer 188 and via employing both type of poloxamer. The formulation of emulgel with distinct gel former, Sepineo P600 lecithin gel as a gel forming agent was shows to have improved release as well as better gel characteristics in contrast to pluronic lecithin gel and Carbopol lecithin gel containing preparations (Ghodekar et al., 2012).

Strydom et al., developed an advanced Poly(amidoamine) (PAMAM) dendrimer mediated synthesis and stabilization of SSD NPs with improved antibacterial action. In this study, it was shown that PAMAM dendrimer complexes with sulfadiazine and silver could be utilized for a bottom up strategy to formulate highly soluble SSD NPs. These NPs were stabilized towards crystal growth via electrostatic layer by layer coating with different PAMAM dendrimers. In addition, Silver NPs can be enclosed in the dendrimer shells that improved SSD release. NP formulation in a cream base provided a topical drug delivery platform with improved antibacterial potential towards burn wound infections, comprising three nanostructures for example, nanoSSD, silver NPs as well as PAMAM dendrimers, in one proficient, elegant nanosystem (Strydom et al., 2012).
Kleinbeck et al., developed an advanced \textit{In situ} photopolymerized semi-interpenetrating networks (sIPNs) consisted of PEG. sIPNs work as drug delivery matrices for drugs burdened as either soluble or covalently associated components. Concurrent release of SSD and bupivacaine from the sIPN would offer multiple-hit treatment of dermal wounds which reducing infection, and minimizes pain as well as sIPN absorption of exudates and facilitate epidermal regrowth. Efficiency of released SSD was established \textit{in vitro} on methicillin resistant \textit{S. aureus}, \textit{Pseudomonas aeruginosa} and \textit{Staphylococcus aureus}. Bupivacaine loaded exclusive of SSD indicated deficient release; in contrast concurrent loading along-with SSD facilitated 100% bupivacaine release. SSD released at 98% exclusive of bupivacaine as well as 96% with bupivacaine. sIPNs efficiently release bupivacaine and SSD as maintaining the antimicrobial potential of SSD. Drug loaded sIPNs have capacity to enhance wound healing by given that multi-drug delivery with an efficient wound management (Kleinbeck et al., 2009).

Nascimento et al., developed a novel topical gel formulation comprises of chitosan containing 1\% SSD, as an option for the management of burn wounds. \textit{In vivo} experiment was completed with 21 Wistar rats separated into 3 groups. Group I was treated by chitosan gel exclusive of the antimicrobial, group II was treated by chitosan gel along-with 1\% SSD and group III was treated by marketed formulation 1\% SSD cream. Owing to its pseudoplastic attribute as well as excellent bioadhesiveness, the chitosan based gels indicated an agreeable retention time on the wound surfaces. Chitosan gel with SSD treated wounds indicated a high fibroblast production and a improved angiogenesis with respect to other groups that are imperative parameters for the assessment of the process of wound healing (Nascimento et al., 2009).

Maenthaisong et al., detailed the possible effect of \textit{Aloe vera} use in the management of burn wound and as a wound healing promoter. \textit{Aloe vera} has been
conventionally employed for the management of burn wound healing but clinical confirmation still uncertain. They conducted a systematic investigation to conclude the possible effect of topical used *Aloe vera* for the management of burn wounds. They electronically searched related research in South-East Asia Database, CINAHL, MEDLINE, Health STAR, DARE, Cochrane Library, Chinese Databases and several Thai local Databases (1918 - 2004). Only controlled clinical trials for burn wound were involved. Two reviewers autonomously collected data on study feature, patient characteristics, and intervention and result measure. From total of 371 patients study only four studies were included in this review. Cumulative confirmation tends to prop up that *Aloe vera* may be an efficient interventions applied in the management of burn wound healing (*1°, 2°* burns) (Maenthaisong et al., 2007).

Nesamony and Kolling developed IPM/DOSS/Water microemulsions as reactors for SSD nanocrystal synthesis, usually considered as safe for human application. Stable preparations devoid of a co-surfactant were developed by the use of isopropyl myristate (IPM), DOSS, and water. A ternary phase diagram was created for the IPM/DOSS/water system. The developed formulation was characterized by conductivity as well as dynamic laser light scattering (DLS). The outcomes of DLS exhibited that the emulsified water droplets had mean diameter in the range of 9.2 to 19.7nm, depends on composition. It was hypothesized that two individual microemulsion formulation having dispersed aqueous droplets of either sodium sulfadiazine or AgNO₃ would react when combined (Nesamony and Kolling, 2005).

Muller et al., described the inhibition of wound contraction (retardation of wound healing) by the use of topical antimicrobials and further explore that phenomenon and to investigate the action that other drugs for example *Aloe vera* may show on this process. Excised wounds (full-thickness) were formed on the dorsal part of Sprague-Dawley rats.
under anaesthetic condition. Developed wounds were managed by the application of topical agents 3 times a day for 14 days, and then observation made until healed. Animals were divided into seven groups as per the treatment option: saline control, placebo (aqueous cream) control, 1% SSD cream, 0.5% SSD, 1% SSD with the combination of Aloe vera, 1% SSD with the combination of nystatin, and nystatin alone. Surface areas of wound were calculated each 3 days. Time to 50% and 90% healing comparison was made employing ANOVA. The wound healing times and half-life were shortest in the case of SSD-Aloe vera and nystatin treated groups ($P < 0.05$) and highest in the 1% SSD and saline control treated groups. Contraction of wound was delayed by saline and SSD. Addition of Aloe vera and Nystatin to SSD reversed the wound contraction delayed effect (Muller et al., 2003).
1.8 Aim and Objectives

The present methods of developing new pharmaceutical formulations are insufficiently efficient. For every 5,000–10,000 molecules that come into the research and development pipeline, approx 250 molecules go through the preclinical program, five are tested in clinical trials, and only one receives approval for market beginning. From discovery to marketing and post-marketing phases, the development of a medicinal product/preparation should fulfill the current necessities of health regulatory authorities. To make certain the safety, efficacy and quality, the formulation and manufacturing process should be stable and robust, and offer medicinal products with consistent critical quality attributes such as reliable dose, drug dissolution and storage stability. Two key factors in the development of medicinal products/preparations are drug solubility and permeability, as they determine to a large extent the bioavailability of a drug substance (Shah and Agnihotri, 2011). In fact, the number of comparatively large-sized and/or less soluble molecules showing permeability-limited and/or solubility-limited absorption has greatly improved over the last years (Lipinski, 2001; Lipinski, 2002; Merisko-Liversidge et al., 2003; Di et al., 2012; Shah and Agnihotri, 2011). It has been reported that 75% of current and recent drug development candidates demonstrate low solubility in water, (Di et al., 2012; Kawabata et al., 2011) and hence belong to the classes II (high permeability) and IV (low permeability) of the Biopharmaceutical Classification System (BCS) (Amidon et al., 1995).

Numerous measures are at present applied to enhance the aqueous solubility of very slightly water-soluble or practically insoluble drug candidates (hydrophobic drugs), including production of an optimal salt form or a pro-drug, pH adjustment of the aqueous medium in case of weakly acidic or basic substances, addition or use of organic solvents, incorporated into cyclodextrins, micelles, microemulsions or liposomes, and, last but not
least, micronization as well as nanonization. Although such measures can appreciably improve aqueous solubility, they may also affect (positively or negatively) the permeability and stability of the candidate drug substances. Further, some of the measures concerning the utilization of organic solvents or surfactants may not give sufficient solubilisation effect at safe concentrations for low potency drugs requiring high doses (Merisko-Liversidge et al., 2003); indeed, for solubilizing imperative amounts of drug substance, high concentrations of excipients may be required, which may lead safety issues, particularly during long-term use in humans (Chaubal, 2004). In this respect, the best possible salt form, optimal pH of the aqueous medium and micro- and nanosuspension formulations are preferable. Early formulation development of very slightly water-soluble or practically insoluble (hydrophobic) compounds is critical to facilitate pre-clinical and clinical pharmacokinetic, efficacy and toxicity studies. Importantly, methods and dosage forms formulated for pre-clinical trials must be transferable to clinical formulations and their proposed administration route (Chaubal, 2004). Therefore, developability of an early formulation is critical. The permeation through topical route is always a difficult problem, so it is imperative to design a formulation which can enhance the permeation of the drug through skin to treat the burn wound effectively. This thesis deals with nanosuspensions as drug carrier system, which is able to overcome low aqueous solubility issues of drug substances, a major hurdle in drug research and development. The aim of this work was the development of novel nanostructured drug delivery system of SSD containing Aloe vera gel (AV-gel). To achieve the goal, the focus was laid on the following pillars:

- Selection of suitable excipients such as surfactants and co-surfactant.
- To enhance the solubility of the poorly soluble drug, SSD, by using nanonization approach as a formulation technique.
To evaluate its stability according to stability guidelines.

Physicochemical characterization of formulated nanostructured formulation.

To optimize the formulation on the basis of particle size distribution, shape, appearance, TEM or SEM, XRD analysis, *in vitro* release study.

Stability study (as per ICH guidelines).

Skin irritation study, and skin permeation study using Franz diffusion cell technique.

Screening of second degree burn wound healing potential of novel nanostructured SSD containing *Aloe vera* gel formulation on rat.

Histopathological study.