CHAPTER 1
INTRODUCTION

1.1 SKIN

The skin constitutes one of the largest interfaces between the body and environment. The skin of an average adult body covers a surface area of approximately 2m² and receives about one-third of blood circulating through the body.

1.1.1 STRUCTURE OF SKIN

It has a complex structure with multiple layers, the uppermost layer being the stratum corneum, followed by the viable epidermis and dermis and it contains appendages, sweat glands, sebaceous glands and hair follicles as shown in Fig.1.1 (Figure adapted from www.healthy-skin-guide.com/skin-diagram.html). Even with all these, it has a thickness of only a few millimeters (2.97±0.28mm) and separates the underlying blood circulation network and viable organs from the outside environment. Generally the skin is described in terms of three tissue layers as follows.

Figure 1.1: Schematic representation of the structure of human skin (Figure adapted from www.healthy-skin-guide.com/skin-diagram.html).
**Introduction**

**a) Epidermis:** The epidermis can again be divided into two.

1. **Stratum corneum (SC):** The barrier property of the skin as part of its protective function is mainly due the stratum corneum, which is the uppermost desquamating horny layer. It comprises of 15-20 layers of flat, separated, nonviable, cornified, almost nonpermeable coenocytes embedded into a continuous lipid bilayer made of various classes of lipids. Structurally, this layer is best described by the brick and mortar model. The thickness of this layer ranges from 10-25 µm depending upon the region of the body and is the thickest in the palms of the hand and sole of the feet. This SC is remarkably more formidable barrier to drug transport in comparison to the epithelial barriers of gastrointestinal, nasal, buccal, vaginal or rectal drug delivery routes. These horny cells have lost their nuclei and physiologically rather inactive. They are formed and continuously replenished by slow upward migration of cells produced by the basal cell layer of the stratum germinativum, which is the regenerative layer of epidermis. Studies have shown that the stratum corneum is replenished about every two weeks in a mature adult [1, 2]. In normal SC, the cells have a water content of only 20% compared to the normal physiological level of 70% in the physiologically active stratum germinativum.

   The SC requires a minimum moisture content of 10% (w/w) to maintain flexibility and softness. In the thicker parts of the skin, the transition from the living cells of the germinativum to the dead cornified cells of the SC is made prominent by three layers, the stratum spinosum (prickly layer), stratum granulosum (granular layer) and stratum lucidum (clear layer) as shown in Fig.1.2 (Figure adapted from http://dynamicnaturesite.blogspot.in/2009/07/skin-care-study-of-skin-structure.html). Like SC, the stratum granulosum and stratum lucidum are also physiologically important [3].

2. **Viable epidermis:** The thickness of the viable epidermis is about 150 µm and comprises of many layers below the SC as mentioned above. Furthermore, the melanocytes, producing melanin for light protection and the langerhans cells responsible for the immune response of the skin are localized in the viable epidermis. There is no vascularization in this layer.
b) Dermis: The thickness of dermis ranges from 3-5 mm. This consists of a matrix of connective tissue composed of collagen, elastin and reticulin and is interdispersed by skin appendages. The network of connective tissue is actually a gel containing oriented tropocollagen (polypeptide) macromolecules. This network or gel structure is responsible for the elastic properties of the skin. Furthermore, the nerves, blood and lymphatic vessels are located in this layer.

c) Subcutaneous tissue: This is a sheet of fat-containing areolar tissue, known as superficial fascia, attaching the dermis to the underlying structures [3]. This mainly acts as a heat insulator and stores readily available high energy chemicals.

Figure 1.2: Schematic representation of the different layers of epidermis of human skin (Figure adapted from http://dynamicnaturesite.blogspot.in/2009/07/skin-care-study-of-skin-structure.html).
1.1.2 FUNCTIONS OF SKIN

The major function of human skin is to protect the body against physical, chemical and microbial injury, loss of water and other endogenous substances as well as thermoregulation of the body and serves as an excretory organ. The potential for these functions is highly dependent on the complex structure of skin, the stratum corneum playing the major barrier function. Imagine it is a few layers of dead cells with their highly organized structure are ultimately responsible for the protection of our body! Apart from this the skin is biologically active both as biosynthetically and metabolically. The skin lipids and vitamin D are synthesized in the skin while various drugs like testosterone [4] and benzoic acid [5] are metabolized. Even though all the major enzymes can be found in the skin, they seem to be less active in comparison to liver [6]. This is the reason why a reduced first pass effect is often associated with transdermal delivery.

1.2 SKIN CANCER

Skin cancer is one of the most common types of cancer with increasing number of cases worldwide. There are three main types of skin cancer as (a) basal cell carcinoma; (b) squamous cell carcinoma and (c) melanoma, affecting melanocytes. The first two are commonly termed 'nonmelanoma skin cancers' or 'epithelial skin cancers', and are among the most common cancers in white-skinned populations [7]. The incidence of all these types of skin cancers is increasing at an alarming rate in most western countries [8]. As per the reports of WHO, between 2 and 3 million non-melanoma skin cancers and 132,000 melanoma skin cancers occur globally each year. One in every three cancers diagnosed is a skin cancer and according to Skin Cancer Foundation Statistics, one in every five Americans will develop skin cancer in their lifetime. The most prevalent risk factors that contribute to the pathogenesis of epithelial skin cancers include excessive exposure to ultraviolet (UV) radiation, pigmentary traits and genetic predisposition. Exposure to UV radiation occurs when bare skin is exposed to solar UV rays or artificial UV rays from indoor tanning devices (sun beds or solariums). There are two main types of UV radiation: UVA and UVB. The difference lies in the length of their waves, which corresponds to their...
capacity to penetrate the skin. DNA damage caused by UVA and UVB exposure ages the skin and can also lead to malignant transformation of skin cells and cancer. To put it simply, ‘no tanning is safe’ for the skin [9]. The main types of skin cancers are as follows.

1. **Melanoma**: The most aggressive cancer caused by UV exposure is malignant melanoma, which is related to both UVA and UVB exposure. They appear as black or brown skin lesion with irregularities in symmetry, color and border.

2. **Squamous cell carcinoma** (SCC): The second most aggressive cancer, which can metastasize, is squamous cell carcinoma, mainly related to UVB exposure. They appear as a scaly, flat lesion closely resembling eczema. Once it develops, it typically becomes a small lump on the skin.

3. **Basal cell carcinoma** (BCC): The most common skin tumor and is relatively benign. It does not usually metastasize, and can be related to either UVA or UVB exposure [10]. They appear as pink, waxy bumps and have a network of tiny blood vessels on the surface which bleed upon a minor injury.

The most serious among all three is melanoma. The incidence of melanoma has rapidly increased over the past several decades [11]. Approximately 10% of all patients who are diagnosed with melanoma eventually die from this cancer. This is clear from the American Cancer Society estimates for the year 2011, which shows that the total number of estimated new cases of skin cancer in United States in 2011 is 76330 out of which 70230 cases are of melanoma of skin and only the remaining are of other nonepithelial skin cancers. The estimated number of deaths from these is 3900 out of which 3040 can be due to melanoma of skin [12]. The risk for any of this skin cancer is higher for organ transplant patients since the immunosuppressant therapy make the cells incapable of repairing or destructing the UV damaged cells.

**Treatment**

As for any other type of cancer the treatment involves surgery, radiation and chemotherapy. The surgical removal is the most common method and may involve cryosurgery, Moh’s micrographic surgery or an excision. Chemothearapy either as single or combinational therapy with drugs like cisplatin, 5-Fluorouracil, doxorubicin and mitomycin are used for treatment SCC and BCC. Paclitaxil either alone or in
combination with cisplatin or carboplatin, a combination of cisplatin, vinblastine and dacarbazine or dacarbazine, carmustine, cisplatin and tamoxifen (Dart mouth regimen) are used in treatment of melanoma. The radiation therapy is usually used as an adjuvant therapy. The usual side effects of systemic chemotherapy as mentioned below are common here.

- hair loss
- mouth sores
- loss of appetite
- increased risk of infection due to low WBC
- easy bleeding (due to low platelets)
- fatigue (due to low RBC)

Apart from this there may be issues associated with the nonspecific distribution of the drug in the body resulting in low tumor concentrations and systemic toxicity when the treatment is with a conventional formulation. There are various toxicities like bone marrow depression, peripheral neuropathy, pulmonary fibrosis etc. reported due to this nonspecific distribution in the body. Such problems can be overcome to great extent by using novel formulations like polymeric nanoparticles for better targeting to the tumor site [13]. Nanoparticles can be used for site specific delivery either by passive or by active targeting. Passive targeting is possible because of the nanoscale size of these particles which permit them to pass through the leaky tumor capillary fenestrations into the tumor interstitium and cells by passive diffusion or convection. Active targeting involves site specific drug delivery based on molecular recognition [14].

1.3 TRANSDERMAL DELIVERY: A NOVEL APPROACH

There is considerable interest in the use of skin for the delivery of drugs since this route is one of the most important modes of drug administration and has made an important contribution to medical practice. Transdermal delivery represents an attractive alternative to oral delivery of drugs and hypodermic injections [15-18]. The first drug delivered through skin was dimethyl sulfoxide in 1900 and nitroglycerine ointment for the management of angina in 1950. But these were short lived because
of the lack of strong scientific foundation. The first transdermal product approved by US Food and Drug Administration (FDA) is in the year 1979. Since then, there is a rapidly growing interest in this mode of drug delivery and transdermal delivery systems of few drugs are approved by US FDA. Even with small number of drugs delivered via this route, the market revenue for the transdermal delivery system is huge and is based mainly between USA, Europe and Japan. However, the transdermal market still remains limited to a narrow range of drugs. Advances in transdermal delivery depend on the ability to overcome the challenges faced in permeation and methods to reduce skin irritation [19]. Anyway transdermal products for various diseases are at different stages of formulation and clinical development [20] and from this it is evident that there is a greater future for transdermal delivery of drugs. Today there are 19 transdermal delivery systems of all these types for delivery of drugs like estradiol, fentanyl, lidocaine and testosterone; combination patches containing more than one drug for contraception and hormone replacement etc [21].

The first generation transdermal systems were in clinical use to deliver small, lipophilic, low dose drugs and this is limited primarily by the barrier posed by the stratum corneum. These systems primarily rely on appropriate drug properties that permit skin penetration. The second generation systems recognize the need for enhancement in skin permeation to increase the scope of this delivery and use chemical enhancers, noncavitational ultrasound and iontophoresis. Third generation delivery systems target their effects to skin’s barrier layer of stratum corneum using microneedles, thermal ablation, electroporation, cavitational ultrasound etc. Despite these progresses, the delivery efficacies are still limited due to skin toxicity in case of chemical enhancers (e.g. surfactants and organic solvents) [16], inconvenience of using electrical/mechanical apparatuses [23, 24], or high production costs of sophisticated drug delivery systems [25, 26]. More importantly, these delivery systems can cause potential infection due to the prolonged disruption of stratum corneum, the first defense barrier of our body [24, 27]. Recently, novel approaches for delivering active ingredients through the skin without any deterioration of skin tissues have been investigated [28-34]. It is reported that the amine-functionalized
PLGA (poly (D,L-lactide-co-glycolide) nanocapsules showed better diffusion of encapsulated chemical drugs across the skin [29]. Protein transduction domains (PTDs) known as cell-penetrating peptides, ‘Tat’, known as a cell-penetrating peptide and ‘AT1002 peptide’ as well as Chitosan, a mucoadhesive (bioadhesive), positively charged polysaccharide also enhance skin permeability [28, 30].

The major advantages of transdermal delivery include [21]

- ability to bypass first pass metabolism in liver
- noninvasive and self administration is possible
- can provide drug release for long periods (up to 1 week)
- increased patient compliance
- inexpensive

The major challenge in this mode of delivery is to overcome the barrier functions of skin. For a better understanding of this, it is essential to know more about the anatomy of skin.

1.3.1 PERCUTANEOUS ABSORPTION

An understanding of skin absorption is of great importance for the assessment of safety aspects of chemicals, drugs and cosmetics, at the same time utilizing this noninvasive route for the delivery of drugs to skin and to systemic circulation. The former is of increased concern today because of the use of nanoparticles for various applications [35]. But as discussed earlier, the limitation of this route is that only limited number of substances can easily penetrate the skin.

1.3.2 SKIN ABSORPTION PATHWAYS

Skin absorption pathways can be divided mainly into two (a) across the intact stratum corneum and (b) via the skin appendages (Appendageal route or shunt pathway) as shown in Figure 1.3[19] where the route 1 and 3 are the appendageal routes. The relative importance of one versus other is being debated a lot by the scientific community [36-38] but the lack of proper experimental methods to distinguish the pathways of permeation again makes it more complicated. Finally a growing number of investigators are inclined to accept both the routes, with the relative importance depending upon the characteristics of the penetrating molecules.
In the initial transient diffusion stage, the drug molecules may penetrate the skin along the hair follicles or sweat ducts and are then absorbed through the follicular epithelium and the sebaceous glands. When a steady diffusion state has been reached, diffusion through the stratum corneum becomes the dominant pathway [39]. Moreover, it is generally accepted that as the appendages comprise a fractional area for permeation of approximately 0.1% only [40], their contribution to steady state flux of most drugs is minimal. This has further resulted in majority of researches focusing on enhancing the penetration across stratum corneum rather than appendages.

Figure 1.3: Simplified representation of skin showing routes of penetration: 1. through the sweat ducts; 2. directly across the stratum corneum; 3. via the hair follicles [Ref.41].

1) Transport across the intact stratum corneum

SC is the major rate limiting barrier in transdermal permeation. As mentioned earlier, it comprises of a multi-layered “brick and mortar” like structure of keratin-rich corneocytes (bricks) in an intercellular matrix (mortar) composed primarily of long chain ceramides, free fatty acids, triglycerides, cholesterol, cholesterol sulfate
and sterol/wax esters [42]. The lipid phase behavior of stratum corneum is different from other biological membranes because of its lipid composition.

**Figure 1.4:** Diagrammatic representation of the stratum corneum and the intercellular and transcellular routes of penetration [Ref.41].

The drugs that are transported across intact stratum corneum can either pass (a) through intercellular space- intercellular route or (b) through the interior of cells- the transcellular (intracellular) route as seen in Fig. 1.4 [20]. Traditionally it was thought that small hydrophilic molecules diffuse within the aqueous regions near the outer surface of intracellular keratin filaments (intracellular route) while lipophilic substances diffuse through the lipid matrix between the filaments (intercellular route) [43]. Based on available data, the intercellular route is considered as the major pathway of permeation even though it is a very tortuous and therefore much longer in distance than the entire thickness of stratum corneum. This is because this route is considered to yield much faster absorption due to the high diffusion coefficient of most of drugs within the lipid bilayer. The transport through the transcellular pathway
requires partitioning into and diffusing across multiple hydrophilic and hydrophobic domains [20].

2) Transport via the skin appendages (shunt pathway)

This consists of the glandular and the follicular pathways, the latter being the major one. This route is significant in the initial stages of skin absorption as well as in case of large hydrophilic compounds and ions.

1.3.3 KINETICS OF TRANSDERMAL PERMEATION

Drug permeation across the stratum corneum obeys Fick’s first law (Equation 1) where steady-state flux \( J \) is related to the diffusion coefficient \( D \) of the drug in the stratum corneum over a diffusional path length or membrane thickness \( h \), the partition coefficient \( P \) between the stratum corneum and the vehicle, and the applied drug concentration \( C_0 \) which is assumed to be constant:

\[
\frac{dm}{dt} = J = \frac{D C_0 P}{h}
\]  

Equation 1 aids in identifying the ideal parameters for drug diffusion across the skin. The influence of solubility and partition coefficient of a drug on diffusion across the stratum corneum has been extensively studied [44]. Molecules showing intermediate partition coefficients will have adequate solubility within the lipid domains of the stratum corneum to permit diffusion through this domain whilst still having sufficient hydrophilic nature to allow partitioning into the viable tissues of the epidermis.

Optimal permeability has been shown to be related to low molecular sizes [37, 45] as this affects diffusion coefficient and low melting point which is related to solubility. When a substance possess these ideal physicochemical properties (as shown in Table 1.1) passive transdermal delivery is possible [46] otherwise it is necessary to modify drug or vehicle or apply some methods to enhance the skin permeation.
1.3.4 PENETRATION ENHANCEMENT TECHNIQUES

The enhancement in skin permeation will definitely improve the systemic drug delivery whereas in case of diseases like skin cancers the enhanced permeation as well as retention in the skin layers is required. The efficacy of treatment for skin cancers can be greatly improved via the transdermal delivery due to EPR effect achieved by using nanocarriers with appropriate size and surface properties.

The penetration enhancement techniques work either by modifying the drug/vehicle or by modifying the stratum corneum as shown below. For an easy understanding and convenience, the methods can be grouped into three as

1. Physical methods
2. Chemical enhancers
3. Use of suitable carriers

1.3.4.1 PHYSICAL METHODS: This involve various techniques like electroporation (application of short micro to milli-second high voltage (100-1000V/cm) electrical pulses to create transient aqueous pores in lipid bilayers), iontophoresis (driving charged molecules into the skin by application of small direct current of approximately 0.5 mA/cm²), sonophoresis (application of low frequency (20kHz to 16 mHz)) ultrasound energy leading to gaseous cavities and increased fluidity of SC), laser radiation and photomechanical waves (direct and controlled exposure to laser radiation results in pressure waves causing ablation of SC) and also

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### Table 1.1: Ideal physicochemical parameter limits for passive transdermal delivery [Ref. 46].

<table>
<thead>
<tr>
<th>Critical properties for transdermal delivery</th>
<th>Requisite for passive skin penetration</th>
<th>Properties of curcumin</th>
<th>Properties of 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous solubility</td>
<td>≥1mg ml⁻¹</td>
<td>Insoluble</td>
<td>12mg ml⁻¹</td>
</tr>
<tr>
<td>Lipophilicity</td>
<td>10&lt; Ko/w &lt; 1000</td>
<td>1850</td>
<td>-9</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>&lt; 500 Daltons</td>
<td>368.39 Da</td>
<td>130.1 Da</td>
</tr>
<tr>
<td>Melting point</td>
<td>&lt; 200 °C</td>
<td>183 °C</td>
<td>283 °C</td>
</tr>
<tr>
<td>pH of saturated solution</td>
<td>5.9</td>
<td>~ 6</td>
<td>~ 4.75</td>
</tr>
</tbody>
</table>

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methods like magnetophoresis, thermophoresis and application of radiofrequency are used but to a less extent.

1.3.4.2 CHEMICAL ENHANCERS: A number of chemicals and solvents act as penetration enhancers by reversibly damaging or altering the physicochemical nature of the stratum corneum to reduce the diffusional resistance and increase permeability. Many enhancers such as azone, DMSO, alcohols, fatty acids and terpenes have shown to increase permeability by disordering or fluidising the lipid structure of the stratum corneum [19]. In addition to their effect on SC lipids, chemicals such as dimethyl sulphoxide (DMSO), decylmethyl sulphoxide, urea and surfactants also interact with keratin in the corneocytes [47], resulting in the disruption of order within the corneocyte. This causes an increase in diffusion coefficient ($D$ in Eqn.1). These molecules may also modify the peptide/protein material in the lipid bilayer domain to enhance permeability. A number of solvents such as ethanol, propylene glycol etc. increase the permeant partitioning into and solubility within the SC hence increasing $P$ in the Fickian equation (Eqn.1). A combination of such chemical enhancers with enhancement effect on diffusivity ($D$) and partitioning ($P$) will result in multiple effect and even synergistic effect have been shown by many combinations like azone and propylene glycol [48], terpenes and propylene glycol [49] etc.

But the major drawback with the use of these chemical enhancers is the resulting skin irritancy and toxicity. This has limited their usefulness in clinical application.

1.3.4.3 CARRIERS: Various micro and nanocarriers as well as vesicles have been studied in this context. Solid lipid nanoparticles have been studied for enhanced skin delivery of sunscreens, vitamin A and E, triptolide and glucocorticoids [50-57]. These solid lipid nanoparticles as well as micro and multiple emulsions forms an occlusive film on the skin surface because of their high oil phase content resulting in an increase in skin hydration. Liposomes and other modified vesicles like niosomes - vesicles composed of non-ionic surfactants [58-63] ethosomes - liposomes with high alcohol content [64-67] and transferosomes- vesicles composed of phospholipid as main ingredient with 10-25 % surfactant and 3-10% ethanol, are carriers of interest.
for many cosmetic products. The conventional liposomes are known to remain near the skin surface, dehydrate and fuse while the deformable vesicles like transfersomes penetrate via the pores in the SC and follow the hydration gradient [68-71].

1) Nanocarriers

As the present work focuses on polymeric nanocarriers, a little description of this group of carriers is in order. The origin of polymer nanocarriers for drug delivery dates from the late 70s and is attributed to Peter Speiser [72-74]. One of the applications of these initial nanoparticle drug delivery systems made of polymethacrylates was to enhance the immunogenicity of antigens, in other words, to have an adjuvant effect [72]. Unfortunately, this novel research was not developed further due to the safety concerns associated to these nanoparticles [75]. But now, several nanocarrier-based drug delivery systems are approved for clinical applications. Nanomedicine addresses a central problem in pharmacotherapy: the selective delivery of biologically active compounds from the site of administration directly to the therapeutic target. Thereby, the nanocarriers can serve as depot to deposit the drug at a defined region in the body, delivering it in a sustained/controlled release mode to selected tissues. More generally, pharmaceutical nanocarriers aim to reduce drug degradation and inactivation upon administration and aim to reduce adverse side effects. Nanocarriers are also intended to enhance drug absorption by facilitating diffusion through the epithelium, ultimately improving the pharmacokinetics and tissue distribution of the drug [76].

In the nanocarriers, the drug is dissolved, entrapped, encapsulated or attached to the matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. In recent years polymeric nanoparticles have attracted considerable attention as potential drug delivery devices in view of their applications in the controlled release of drugs, their ability to target particular organs / tissues, as carriers of DNA in gene therapy, and in their ability to deliver proteins, peptides and genes through a peroral route of administration [77-79]. The biodegradability of the matrix helps to avoid accumulation of the polymer matrix on repeated dosing which is a major advantage with biodegradable polymeric systems.
Polymeric nanoparticles can be prepared by various methods such as emulsification, precipitation, co-acervation, ionic gelation etc [80]. Most of the nanovehicles are found to show Enhanced Permeability and Retention (EPR) effect at solid tumors contributing to passive targeting. This is mainly due to the size and surface properties of the nanovehicles as well as the microenvironment at the tumor sites. According to the EPR concept, biocompatible macromolecules accumulate at much higher concentrations in tumor tissues than in normal tissues or organs, even higher than those in plasma [81-83]. The microenvironment at the tumor site which includes active angiogenesis and high vascular density, extensive production of vascular mediators that facilitate extravasation, defective vascular architecture and impaired lymphatic clearance of macromolecules and lipids from interstitial tissue is responsible for this [84]. The first three factors contribute to enhanced permeability while the last one is responsible for retention effect as seen in Fig.1.5 [85]

![EPR effect at solid tumors](image)

**Figure 1.5: Image showing EPR effect at solid tumors [Ref. 85]**

Many of the nanosystems as mentioned above, because of their nanosize and surface properties have proven to be effective in enhancing the transdermal penetration [86, 87]. It is also demonstrated by different authors [88-91] that the use of nanoparticles as transdermal drug delivery systems (TDDS) enhances the rate and
extent of transport across skin, without compromising the skin barrier function. Although such systems were undoubtedly able to enhance skin penetration and distribution, the mechanism by which this enhancement was achieved is still unclear [88, 92].

This work deals with the use of a unique class of polymeric nanocarriers called nanogels. Nanogels are modified versions of hydrogels which find various applications in the medical field due to their special features.

a) Hydrogels

Hydrogels in general have got more attention in the drug delivery industry a long time back because of their tunable chemical and three-dimensional (3D) physical structure and good mechanical properties. Their most attractive features are their high water content, biocompatibility, environment responsiveness and desirable mechanical properties [93, 95]. These unique properties offer great potential for the utilization of hydrogels in tissue engineering, biomedical implants, and more importantly drug delivery [95-102]. The hydrophilic gel was put to biological use for the first time in the early 1960’s by Wichterle and Lim [103]. By definition, hydrogels are three-dimensional polymeric networks capable of imbibing high amounts of water or biological fluids [104]. Polymers with hydrophilic pendant groups like as –OH, –CONH–, –CONH2–, and –SO3H have greater tendency to swell and absorb water, thus becoming candidates for hydrogels [105]. Their ability to absorb water, swell and yet not get dissolved is because of the cross linked networks present in the structure. These can be either physical or chemical in nature and can be covalent bonds, hydrogen binding, Vander Waals interactions, or physical entanglements [104]. One of the most interesting features of hydrogels is their ability to resemble living tissue thus finding application in tissue engineering as scaffolds. This unique property has been attributed to their high water content, their soft and rubbery consistency, and low interfacial tension with water or biological fluids [106, 107]. Drug release from hydrogels can be categorized as:

- Diffusion-controlled
- Swelling-controlled, and
Chemical-controlled

It is the diffusion-controlled release that is most often associated with the hydrogels [108] and this is in turn dependent on the mesh size within gel matrix [109]. Mesh size of the gel matrix is a very important factor for hydrogels as it decides physical properties of the network like mechanical strength, diffusivity, and biodegradability [108, 109]. The mesh size is dependent on the monomers, degree of crosslinking and the intensity of external stimuli like pH, temperature etc.

Hydrogels have become a very popular candidate in the race for designing the ideal drug delivery system. The reason for this growing interest is their exceptional physicochemical and biological characteristics [110]. One of the earliest works dealing with the use of hydrogels in drug delivery [111] focused on using poly (vinyl alcohol) hydrogel for rectal administration of indomethacin. Another research was conducted [112] on an implantable collagen- poly (HEMA) hydrogel loaded with 5-fluorouracil with an aim to treat solid tumors. Later, freeze-dried chitosan-poly (ethylene oxide) hydrogels were used for site-specific antibiotic delivery in the stomach [113]. Inulin hydrogels were studied [114] as carriers for colonic drug targeting. A study dealing with thermosensitive hydrogel based on quaternized chitosan and PEG for nasal drug delivery applications is reported [115]. There has been a lot of work of this kind on the utilization of hydrogels in the field of drug delivery.

All these attractive properties and characteristics resulted in research being conducted into the nanoscale variations of hydrogels. Although very popular, these hydrogels also have drawbacks. One of the biggest drawbacks when hydrogels are used in stimuli–responsive applications is that the transduction of signals will be limited by the rate of diffusion. This can be removed by introducing interconnected pores in the polymer structure to form capillary networks in the matrix and by decreasing diffusion paths by downscaling the size of hydrogels and thus reducing the lag time in the induction of responses [116]. In recent years, much effort has been directed to the micro- and nano-scaled hydrogels (micro/nanogels) due to their unique
features such as size, colloidal stability, high loading capacity and large surface area [117].

b) Nanogels

Nanogels are nanosized versions of hydrogels. They are composed of an ionic or non-ionic network of amphiphilic or hydrophilic polymer chains. Their usefulness in the drug delivery arena became evident with the fact that they can be designed to spontaneously absorb biologically active molecules through formation of salt bonds, hydrogen bonds, or hydrophobic interactions [118]. The various advantages that nanogels provide are as listed below:

- High biocompatibility: This is by virtue of the high water content and low surface tension [119]
- High loading capacity [120]
- Controlled release of payload [119]
- Flexibility in design [104]
- Versatility in drug loading and release [104]
- High water absorbtivity: Nanogels in the unloaded swollen state contain a considerable amount of water [104]
- The rapid response to external stimuli [119]
- Increased and prolonged circulation time [119]: They hence have a better chance of targeting the site of interest
- High stability in aqueous solution [120]: Nanogels consist of polycore and hydrophilic shell to make zero Gibb’s energy and are able to enclose a water-insoluble drug in the polycore and thus these drugs are protected from interactions with the surrounding biological fluid.

Loading of biological agent(s) is usually a spontaneous process in the case of these nanogels. The loaded drug would interact with the polymer matrix through electrostatic, Van der Waals and/or hydrophobic interactions. When the drug gets entrapped within the polymer matrix, these nanogels collapse forming stable nanoparticles. However to enhance the stability and prevent aggregation of nanogels, hydrophilic polymers, such as poly (ethylene glycol) (PEG) are introduced into their
Introduction

structure. These polymers form a protective hydrophilic layer when they become exposed during the collapse of the nanogel. This hydrophilic layer can prevent uptake of the nanogels by the mononuclear phagocytic system and thus helps to increase the circulation time in the blood stream [119]. Targeting of these nanogels is made possible by functional modification of the many functional groups exposed on its surface, with targeting moieties like antibodies. Research does show that various nanogels deliver their payload inside cells and across biological barriers. Such nanogels exhibit high stability and protect biological agents from degradation by cell’s metabolic systems. On the whole, nanogels demonstrate excellent potential for enhancing oral and brain bioavailability and systemic drug delivery of low molecular weight drugs [118].

Currently there are many approaches for the preparation of nanogels. They can be categorized as below [118].

- Physical self-assembly of interactive polymers
- Polymerization of monomers in homogeneous phase or micro- or nano-heterogeneous environment
- Chemical cross-linking of preformed polymers and
- Template-assisted nanofabrication of nanogels
- Nanogel synthesis by RAFT polymerization is an emerging technique for the control of architecture and potential bioapplications [121]

High drug loading capacities can be expected for hydrophilic nanogels and this loading would be much greater than those normally observed for other nanosized pharmaceutical carriers such as polymeric micelles, liposomes and biodegradable nanoparticles. This is because swollen nanogels are mainly comprised of water and therefore provide for a larger cargo space for incorporation of low molecular mass drugs and, especially, biomacromolecules. Also, the loading in nanogels is done under relatively mild conditions when compared to other carriers, which is very important for preservation of biological activity of labile drugs and biomacromolecules, such as proteins and polypeptides. The release of biological agents from these nanogels is as described for hydrogels in Figure 1.5 [118].
Intelligent nanogels that exhibit drastic response to various stimuli have been hot topics in the rapidly growing fields of smart materials and nanomedicine [122, 123]. A family of multi-responsive nanogels with different compositions and crosslinking degrees has been prepared by the miniemulsion copolymerization and their thermoresponsive behaviors, acid-triggered hydrolysis, and reduction-induced degradation were studied [117]. Thermo-responsive nanogels from poly(l-lactide)-g-pullulan (PLP1 and 2) copolymers with different lactide contents were investigated as an anticancer drug delivery carrier and the results suggest that self-assembled PLP nanogels, by means of a triggering temperature, can be used as a long-term drug delivery system in cancer treatments [124]. A novel technique to prepare cyclodextrin (CD) nanogels, in which the cross-linking takes place simultaneously with an emulsification/solvent evaporation process, has been implemented. The ability of the nanogels to host a molecule that can form inclusion complexes and to sustain its release was tested using 3-methylbenzoic acid (3-MBA) as a probe with a high affinity for both β-cyclodextrin (βCD) and γCD. Permeability tests confirmed that 3-MBA was indeed taken up by the nanogels and then slowly released [125]. A class of core shell structured hybrid nanogels to demonstrate the conception of integrating the
Introduction

functional building blocks into a single nanoparticle system for simultaneously optical temperature sensing, cancer cell targeting, fluorescence imaging, and combined chemo-photothermal treatment were reported. The hybrid nanogels were constructed by coating the Ag-Au bimetallic NP core with a thermo-responsive nonlinear poly (ethylene glycol) (PEG)-based hydrogel as shell, and semi-interpenetrating the targeting ligands of hyaluronic acid chains into the surface networks of gel shell. This study reported that the ability of the hybrid nanogels to combine the local specific chemotherapy with external NIR photothermal treatment significantly improved the therapeutic efficacy due to a synergistic effect [126].

Another class of water-dispersible hybrid nanogels for intracellular delivery of hydrophobic curcumin was reported. The core-shell structured hybrid nanogels were synthesized by coating the Ag/Au bimetallic nanoparticles (NPs) with a hydrophobic polystyrene (PS) gel layer as inner shell, and a subsequent thin hydrophilic nonlinear poly (ethylene glycol) (PEG)-based gel layer as outer shell. The uniqueness of these hybrid nanogels lies in the integration of the functional building blocks for combined curcumin and photothermal therapy to significantly improve the therapeutic efficacy [127].

Two types of novel nanogels were prepared using shell cross-linking of Pluronic F127 micelles with polyethylenimine (PEI) (F127/PEI nanogel), and penetrating network of poly(butylcyanoacrylate) (PBCA) in Pluronic F127 micelles (F127/PBCA nanogel). Poorly soluble anticancer drug, paclitaxel (PTX) and 10-hydroxycamptothecin (HCPT) were used as model drugs and incorporated into nanogels. The data demonstrated that these novel nanogels improved stability towards dilution, increased solubility and showed better cellular uptake by cells compared with free drug [128]. In another study, a nanogel system was developed as an effective drug delivery system for the simultaneous topical delivery of two anti-inflammatory drugs, spantide II (SP) and ketoprofen (KP). For this, a skin permeating nanogel system (SPN) containing surface modified polymeric bilayered nanoparticles along with a gelling agent was developed. Poly-(lactide-co-glycolic acid) and chitosan were used to prepare bilayered nanoparticles (NPs) and the surface was
modified with oleic acid (NPSO). Hydroxypropyl methyl cellulose (HPMC) and Carbopol with the desired viscosity were utilized to prepare the nanogels. The nanogel system was further investigated for in vitro skin permeation, drug release and stability studies [129].

Nanogels have been recently used for gene as well as protein delivery. In a recent study reported a mutant of FILIP 1 L gene was delivered to human ovarian cancer cells by novel biodegradable cationic heparin-polyethyleneimine nanogels [130]. The preparation of redox-responsive single-protein nanocapsules for intracellular protein delivery is being reported. In this study, the target protein was noncovalently encapsulated, through an \textit{in situ} interfacial polymerization, into a positively charged polymeric shell interconnected by disulfide-containing crosslinkers. The dissociation of the polymeric shell under reducing conditions and the subsequent release of protein were confirmed using cell free assays in the presence of glutathione [131]. A near- infra red light responsive core shell nanogels based on Au-Ag nanorods coated with DNA cross linked polymeric shells was constructed for targeted drug delivery. In this the DNA complimentarity has been applied to develop a polyacrylamide based sol-gel transition system to encapsulate anticancer drugs to the gel scaffold. The study reported that on exposure to NIR irradiation, the photothermal effect of AU-Ag nanorods lead to a rapid rise in temperature of the surrounding gel, leading to fast release of the encapsulated drug with good controllability [132]. Lipid coated nanogels with crosslinked human serum albumin in the core for carrying the cargo, has been studied for integrin v3 targeting of various anticancer drugs. This showed promising results in animal models [133].

Compared to the conventional macroscopic hydrogels, nanogels show much faster responsiveness, thereby enabling a better control of drug delivery and bioimaging [126], temporally and/or spatially. These are capable of responding to external stimuli by changing their physicochemical properties such as volume, water content, refractive index, permeability and hydrophilicity-hydrophobicity [134]. Among various intelligent nanogels, the most extensively studied are those responsive to changes in pH [135-139], temperature [140-142], reduction potential
Introduction

[143-145] or their combination [146, 147]. It is well known that there are numerous pH gradients in the physiological and pathological processes, and a significant difference of redox potential exists between the extracellular environments and the intracellular compartments [148-151]. In addition, temperature can be conveniently used to tune the drug release [152,153] or to control the drug loading of polymeric nanoparticles [154-156].

Thus, nanogels form a very distinct class of drug vehicles offering many benefits over existing drug carriers. They provide very good encapsulation of small biologically active agents and biomacromolecules. The advantages of these systems, as mentioned above include simplicity of formulation with the drugs, high loading capacity and stability of the resulting formulation in dispersion. Engineering optimal drug loading and release of drugs is possible of the unique swelling and collapsing of nanogels, a property solely exhibited by this class of nanocarriers. In response to external environmental factors, nanogels can undergo rapid volume changes, and allow for stimuli-controlled release of encapsulated biologically active compounds including charged or hydrophobic drugs and biopolymers [118, 157].

1.4 CHITIN

Among the novel families of biological macromolecules, whose relevance is becoming increasingly evident, are chitin and its main derivative, chitosan [158]. Chitin which is the most widely found renewable nitrogen-bearing compound, is an insoluble linear polymer of β-1, 4-linked N-acetyl glucosamine residues and was first described by Henri Braconnot in 1811. The structure of chitin is as shown in [Fig.1.6] [158].
Chitin exists in three polymorphic forms that differ in the arrangement of the molecular chains in the crystal cell. \( \alpha \)-chitin is the most abundant crystalline variant and is tightly compact. In \( \alpha \)-chitin the chains are arranged in an anti-parallel fashion. In \( \beta \)-chitin, where the chains are parallel, is a crystalline hydrate and water can penetrate between the chains of the \( \beta \)-chitin lattice (Figure 1.7) [159]. \( \gamma \)-chitin is a mixture of \( \alpha \) and \( \beta \)-chitin with two parallel chains in one direction and the third one in the opposite direction. The most common source of crystalline \( \alpha \)-chitin is the exoskeleton of large crustaceans [160].
Since a large amount of crab and shrimp shells are produced every year as food waste, further utilization of α-chitins as processed materials or a commodity substance is highly desired [161]. Chitin’s high crystallinity, high strength, and biocompatibility makes this material very attractive for use in the fabrication of implant devices, wound dressing materials, drug delivery systems, and regenerative medical components for bones and other medical materials [162]. Chitin flakes are produced from crustacean shells by a three step process. The first step is used to remove protein by using weak sodium hydroxide. The second step uses hydrochloric acid to remove minerals and finally the flakes so obtained are washed with water. Chitin has several attractive features. It is biodegradable, bio-absorbable, non-toxic, anti-microbial and as mentioned above biocompatible [163]. The polymeric nanocarrier used in this study is chitin nanogel. The functional groups of chitin that make it viable for the formation of nanogel particles are –OH and –NHCOCH$_3$. The chitin used here is 72.4% acetylated and so the remaining 28% monomers contain free amine groups, giving an overall cationic charge which may be helpful for the selective binding to anionic lipids in the skin structure. The study on chitin nanogel in this work has been aimed at finding out how well this nanogel will act as a drug delivery carrier via the transdermal route. In order to investigate this, 2 anticancer drugs were selected. The drugs used were the hydrophobic curcumin and hydrophilic 5-Fluorouracil.

1.5 CURCUMIN

Curcumin, a natural polyphenol, found in the rhizomes of curcuma longa (turmeric), a member of the ginger family Zingiberaceae. Chemically curcumin is a $\alpha, \beta$-unsaturated β-diketone with IUPAC name (1E,6E)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione and commonly called as diferuloylmethane, which exhibits keto-enol tautomerism having a predominant keto form in acidic and natural solutions and stable enol form in alkaline medium. It is a hydrophobic molecule and it is practically insoluble in aqueous solutions [164,165]. Its chemical structure has been shown in Fig.1.8. Curcumin is known to possess multiple therapeutic effects with remarkable effect on multiple cancers [166]. The
other interesting fact about this phytodrug is that it acts by multiple molecular mechanisms [167]. In case of skin cancers also curcumin act on more than one molecular target [168]. This drug has a poor bioavailability by virtue of its hydrophobicity and research is being conducted in order to find a carrier that can increase its bioavailability. Curcumin has very low water solubility, which makes it difficult to achieve the optimum plasma concentration at the target site. Another issue with this drug is that the soluble portion is rapidly degraded at physiological pH making it very unstable in the human body and less active. Various methods have been tried to improve its water solubility as well as stability by complexation and similar techniques. In one such study, the stabilization is done by complexation with divalent cations in glycerol/water system [169]. Thus to increase its efficiency researchers over the world have come out with newer and better ways to carry the drug into the body and make sure the required concentration is obtained at the effect site.

\[ \text{Figure 1.8: Chemical structure of curcumin} \]

1.6 5-FLUOROURACIL (5-FU)

5-Fluorouracil (5-FU) belongs to a family of antitumor drugs. 5-Fluorouracil, a pyrimidine analogue, displays a broad spectrum of activity against several solid tumors by interfering with thymidylate synthesis [170]. It is one of the oldest antitumor drugs, commonly used in clinical oncology practice. It is widely used in clinical treatment of several solid cancers such as gastrointestinal, pancreas, breast, colorectal, liver and brain cancer [171]. It finds use in some skin cancers as a topical cream. The target sites of 5-FU are all the organs of the human body, specially the gastrointestinal tract. 5-FU is quickly metabolized in the body; therefore, the
maintenance of high serum concentrations of this drug is needed to improve its therapeutic activity. The maintenance of these serum concentrations requires continuous administration but 5-FU shows severe toxic effects; and of course reaching and/or exceeding the toxic concentrations must be avoided [172]. Hence the aim of the nanocarrier in this thesis is to reduce associated side effects and improve the therapeutic index of this drug. The structure of 5-FU has been shown in Fig 1.9. The drug is slightly soluble in water and can thus be aptly described as partially hydrophilic. As of now the methods of administration mainly include intravenous route and topical application in the form of cream and solution is recommended for various superficial skin conditions like multiple actinic keratoses, psoriasis etc. These formulations are also approved by US FDA for the basal cell carcinoma (BCC). But in these cases the efficacy is limited due to inadequate penetration through SC because of the hydrophilic nature of 5 FU. Attempts have been made to increase the penetration by use of iontophoresis and penetration enhancers [173,174]. But the use of a nanogel formulation is not being reported so far.

![Chemical structure of 5-Fluorouracil](image)

Figure 1.9: Chemical structure of 5-Fluorouracil

Keeping all the above facts in mind, the study was aimed to deliver the above two drugs using chitin nanogels via the transdermal route. Chitin based nanogel can be an ideal candidate for skin penetration for many reasons. As the permeability of a substance through skin is inversely proportional to its size under certain conditions [175], the nanoscale size of curcumin/5-FU loaded chitin nanogels is one major factor
facilitating penetration. The cationic charge of chitin can also facilitate the penetration through intact skin. As a good transdermal system, beyond allowing desirable amount of drug to cross the skin barrier, the system has to be biocompatible, preferentially biodegradable and non-irritant to skin [176]. Chitin is a very good candidate in these aspects as well. Because of these advantages, a nanocarrier based on chitin is thought to have EPR effect on the skin layers enabling passive targeting to the melanocytes for better treatment of melanoma.

**Research questions**

- Will it be possible to develop a nanogel from chitin for transdermal drug delivery applications?
- Will the chitin nanogel be effective in transdermal delivery of curcumin/5-fluorouracil?
- What will be the anticancer effects *in vitro* of these formulations in melanoma?

**Hypotheses**

- Chitin nanogel can be prepared from biodegradable chitin with desirable properties for transdermal drug delivery.
- Chitin nanogel will be an effective carrier for curcumin/5-fluorouracil to cross the skin barrier (stratum corneum).
- Chitin nanogel loaded with Curcumin/5-Fluorouracil will have potential for the treatment of skin cancer (melanoma).
1.7 OBJECTIVES OF THE STUDY

The specific objectives of this study include:

- Formulation and characterization of the chitin nanogel carrier.
- Formulation and characterization of hydrophilic 5-FU/hydrophobic curcumin loaded chitin nanogels.
- *In vitro* swelling, drug release, blood compatibility, cytotoxicity and cell uptake studies of the drug loaded chitin nanogels.
- *Ex vivo* skin permeation studies of curcumin/5-FU loaded chitin nanogels using porcine skin model.
- Comparison of curcumin/5-FU loaded chitin nanogels for their efficacy in melanoma cancer as well as skin penetration.