Cellular Permeation Pathways: Current focus of Permeation Enhancers for Effective Drug Delivery

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Abstract

Certain agents that enhance drug transport through the skin, including surfactants, bile salts, and fatty acids, have been shown to exert a similar effect on the oral mucosa. Oral drug delivery offers an attractive method of needle-free drug administration. Unfortunately, oral delivery is often hampered by the poor permeability of drugs across the intestinal epithelium. Although several single chemical permeation enhancers have been shown to alleviate permeability difficulties, these often occur at the expense of safety. The stratum corneum, oral mucosa and buccal mucosa pose a formidable challenge to formulators of drug delivery systems. Several approaches have been utilized to facilitate entry of drugs into the lower skin layers or mucosal layers. Traditionally, permeation enhancers are designed to deliver high drug concentrations across the barrier into the systemic circulation. The use of many of these agents results in unpleasant or toxic side effects. However, in recent years there has been a search for compounds that exhibit low toxicity, and maintain their enhancing activity. Research in the area of permeation enhancement or retardation is yielding valuable insights into the structure activity relationships of enhancers as well as retardants. The purpose of this review is to identify the major differences in the structural and chemical nature of the permeability barriers between the oral mucosa and skin, to clarify the mechanisms of action of penetration enhancers, and to identify the limitations of certain models that are used to assess the effect of penetration enhancers.

Keywords: Penetration Enhancers; Oral; Transdermal; Buccal; Technology; Nanocarriers.

Introduction

Permeation enhancers are incorporated in different types of formulations in order to improve drug flux through diverse membranes. Permeation enhancers are also known as penetration enhancers or absorption promoters or sorption promoters or accelerants. They decrease the barrier resistance and are widely used in oral, buccal, nasal, ocular and transdermal drug delivery systems. Oral mucosa and skin are remarkably efficient barriers causing difficulties for drug delivery of therapeutic agents. One long standing approach to increase the range of drugs that can be effectively delivered is by the use penetration enhancers which are chemicals that interact with mucosa and skin constituents to promote drug flux. To date though a vast array of chemicals has been evaluated as penetration enhancers, yet their inclusion has become limited since the underlying mechanisms of action of these agents are seldom clearly defined [1]. The purpose of the study is to give an overview on the role of permeation enhancers and their properties in various formulations for effective drug delivery.

Mechanism of action of chemical penetration enhancers

[2]

Structural Modification

Lipid modification: They (azone, terpenes, fatty acids, DMSO and alcohols) disrupt the stratum corneum lipid organization making it permeable or increasing its fluidity. The accelerant molecules jump into the bilayer, rotating, vibrating and translocating, forming microcavities and increasing the free volume available for drug diffusion.

Protein modification: Ionic surfactants, Decylmethylsulphoxide and DMSO interact well with keratin in corneocytes, opening up the dense protein structure, making it more permeable.

Partitioning promotion: Many solvents (Ethanol, Azone, Propylene glycol, DMSO) enter stratum corneum, change its solution properties by altering the chemical environment, and thus increase partitioning of a second molecule into the horny layer.

Mechanism of mucosal penetration

The mechanisms by which penetration enhancers are thought to improve mucosal transport include the following.

Increasing the fluidity of membrane lipid bilayers: The disruption of intercellular lipid packing by interaction with either lipid or protein components is thought to increase permeability. Biophysical techniques have demonstrated that, there is indeed,
a correlation between increased lipid fluidity and enhanced membrane permeability. Varying degrees of insult may occur in tissues that are in intimate contact with the enhancer. Therefore, a transient increase in the fluidity of the intercellular lipids may be thought of as a relatively nontoxic effect, whereas extraction of the intercellular lipids or denaturation of cellular proteins may be viewed as being somewhat more drastic.

**Affecting the components involved in the formation of intercellular junctions:** This could be particularly important in the case of intestinal membranes, where the barriers to paracellular diffusion of molecules and ions are the tight junctions or ‘zona occludens’.

**Overcoming the enzymatic barrier:** Protease inhibitors for endo and exo peptidases are potential penetration enhancers. Although various peptidases and proteases are present within the oral mucosa, and it is possible that metabolism may act as an enzymatic barrier, the intercellular pathway is thought to be deficient in proteolytic activity. However, changes in membrane fluidity induced by penetration enhancers may indirectly alter enzymatic activity.

**Chemical classification of enhancers**

The data suggest that enhancers may be placed into several groups depending on their activity. Those compounds that enhance drug concentrations across the skin (transdermally), into the skin (locally) and through the oral mucosa those that enhance the permeation of drugs, those that increase local skin-drug concentrations, but which do not produce significant transdermal enhancements and those that act as retardants, producing low local-drug concentrations and low transdermal fluxes. Table 1.0 lists various types of some of the important penetration enhancers [3].

**Oxazolidinones**

They have the ability to localize co-administered drug in skin layers, resulting in low systemic permeation. Oxazolidinones such as 4-decyloxazolidin-2-one has been reported to localize the delivery of many active ingredients such as retinoic acid and diclofenac sodium in skin layers [4].

**Urea**

Cyclic urea permeation enhancers are biodegradable and nontoxic molecules consisting of a polar parent moiety and a long chain alkyl ester group. As a result, enhancement mechanism may be a consequence of both hydrophilic activity and lipid disruption mechanism [5]. Urea is a hydrating agent (a hydrotrope) used in the treatment of scaling conditions such as psoriasis, ichthyosis and other hyper-keratotic skin conditions. In water in oil vehicle, urea alone or in combination with ammonium lactate produces significant hydration of the stratum corneum to improve barrier function when compared to the vehicle alone in human volunteers in vivo. Urea also has keratolytic properties, usually when used in combination with salicylic acid for keratolysis. The penetration enhancing activity of urea results from a combination of increasing

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**Table 1.0:** Types of chemical penetration enhancers classified by functional groups and chemical structure

| Category                      | Examples                                                                 |
|-------------------------------|--------------------------------------------------------------------------|
| Sulfoxides and similar       | dimethylsulfoxide, dimethylacetamide, dimethylformamide                   |
| compounds                    |                                                                          |
| Pyrrolidones                  | 2-pyrrolidone, N-methyl-2-pyrrolidone, 1-lauryl-2-pyrrolidone             |
| Alcohols                      | ethanol, 1-octanol, 1-hexanol, 1-decanol, lauryl alcohol,                 |
| Glycols                       | propylene glycol, butane-1, 2-diol, polyethylene glycol 400              |
| Urea and derivatives urea    | 1-dodecylurea, 1-dodecyl-3-methylurea,                                   |
| Azone and derivatives         | Azone (laurocapram; 1-dodecylazacycloheptan-2-one),                      |
| Enzymes                       | Acid phosphatase, calonase, papain                                       |
| Iminosulfur                   | 5, 5-dimethyl-N-(5-nitro-2-pyridy1) iminosulfurane,                      |
| Cyclodextrins                 | 2-hydroxypropyl-β-cyclodextrin,                                          |
| Fatty acid esters             | cetyl lactate, butylacetate, isopropyl myristate                         |
| Fatty acid                    | alkanoic acids, oleic acid, lauric acid, capric acid                     |
| Surfactant                    | sorbitan monopalmitate, sodium lauryl sulfate                            |
| Terpenes                      | limonene, nerolidol, farnesol, carvone, menthone                          |
| Polymers                      | β-D-glucopyranosyl-terminated oligodimethyl siloxanes, 1-alkyl-3-β-D-glucopyranosyl-1,1,3,3-tetra methyl disiloxanes |
| Monoolein                     | Monoolein                                                                |
| Oxazolidinones                | 4-decyloxazolidin-2-one, 3-acetyl-4-decyloxazolidin-2-one                 |
stratum corneum water content (water is a valuable penetration enhancer) and through the keratolytic activity [6].

**Pyrrolidones**

N-methyl-2-pyrrolidone (NMP) and 2-pyrrolidone (2P) are the most widely studied enhancers of this group. Pyrrolidones have been used as permeation promoters for numerous molecules including hydrophilic (e.g. mannitol, 5-fluouracil and sulphaguanidine) and lipophilic (betamethasone-17-benzoate, hydrocortisone and progesterone) permeants. As with many studies, higher flux enhancements have been reported for the hydrophilic molecules. NMP is employed with limited success as a penetration enhancer for captopril when formulated into a matrix type transdermal patch [7]. The pyrrolidones partition well into human corneum stratum and within the tissue, they may act by altering the solvent nature of the membrane and pyrrolidones have been used to generate ‘reservoirs’ within skin membranes. Such a reservoir effect offers potential for sustained release of a permeant from the stratum corneum over extended time periods. However, as with several other penetration enhancers, clinical use of pyrrolidones is precluded due to adverse reactions. An in-vivo vasoconstrictor bioavailability study demonstrated that pyrrolidones caused erythema in some volunteers, although this effect was relatively short lived. Also, a toxic hygroscopic contact reaction to N-methyl-2-pyrrolidone has recently been reported [8].

**Amines and Amides**

Some excipients might intercalate into the structure of lipids of the skin and disrupt the ordered packing making the structure more fluid and influencing positively the diffusion coefficient. Azone and its analogues have been widely studied in this respect, and it has been shown that the hydrogen bonding between the polar head group in Azone probably interacts with the skin ceramides. The greatest barrier disruption activity is recorded for compounds with long alkyl chains between C8-C16. The chemical has low irritancy, very low toxicity (oral LD50 in rat of 9 g/kg) and little pharmacological activity although some evidence exists for an antiviral effect [9].

**Fatty Acids and Esters**

An increase of 6.5-fold to 17.5-fold in the permeation rate of flurbiprofen through rat skin by unsaturated fatty acids, while no significant increase is observed with saturated fatty acids. Moreover, they have a greater enhancing effect on lipophilic drugs. Addition of oleic acid to an Ethanol: water (50:50) cosolvent system markedly improves the skin permeation of zalcitabine, didanosine, and zidovudine, whereas addition of the same to ethanol: TCP (50:50) produces no enhancement across hairless rat skin. The fatty acid extract of cod liver oil is found to be as good a permeation enhancer as oleic acid. The most effective transdermal penetration enhancer is palmitoleic acid, which results in a 640-fold increase in hydrocortisone flux through hairless mouse skin. Incorporation of pure cod liver oil in a PG vehicle did not improve the hydrocortisone permeability, suggesting that the unsaturated fatty acids have to be in the free form to be able to act as skin permeation enhancers. Oleic acid has been described to decrease the phase transition temperatures of the skin lipids with a resultant increase in rotational freedom or fluidity of these lipids. A typical example of an ester acting as a penetration enhancer is isopropyl myristate. Isopropyl myristate might show a double action by influencing on the partition between vehicles and skin by solubilisation and disruption of lipid packing, thus increasing the lipid fluidity [10].

**Sulphoxides and similar chemicals**

Dimethylsulphoxide (DMSO) is one of the earliest and most widely studied penetration enhancers. DMSO alone has also been applied topically to treat systemic inflammation, although currently it is used only to treat animals. Thus, it has been shown to promote the permeation of, for example, antiviral agents, steroids and antibiotics. The effects of the enhancer are concentration dependent and generally co-solvents containing >60% DMSO are needed for optimum enhancement efficacy. However, at these relatively high concentrations DMSO can cause erythema and wheals of the stratum corneum and may denature some proteins. As well as an effect on the proteins, DMSO has also been shown to interact with the intercellular lipid domains of human stratum corneum. Considering the small highly polar nature of this molecule it is feasible that DMSO interacts with the head groups of some bilayer lipids to distort to the packing geometry. In addition to the activities of penetration enhancers within the intercellular domain, high levels of potent solvents may have more drastic effects. They may damage desmosomes and protein-like bridges, leading to fissuring of the intercellular lipid and splitting of the stratum corneum squames. Solvent may enter the corneocyte, drastically disrupting the keratin and even forming vacuoles [11].

**Surface Active Agents**

Surfactants are found in many existing therapeutic, cosmetic and agro-chemical preparations. Usually, surfactants are added to formulations in order to solubilise lipophilic active ingredients, and so they have potential to solubilise lipids within the stratum corneum. Typically composed of a lipophilic alkyl or aryl fatty chain together with a hydrophilic head group, surfactants are often described in terms of the nature of the hydrophilic moiety. Anionic surfactants include sodium lauryl sulphate (SLS), cationic surfactants include cetyltrimethyl ammonium bromide, the nonoxynol surfactants are non-ionic surfactants and zwitterionic surfactants include dodecyl betaine. Both anionic and cationic surfactants swell the stratum corneum and interact with intercellular keratin. Non-ionic surfactants tend to be widely regarded as safe. Surfactants generally have low chronic toxicity and most have been shown to enhance the flux of materials permeating through biological membranes. Anionic materials themselves tend to permeate relatively poorly through human stratum corneum upon short time period exposure but permeation increases with application time. Non-ionic surfactants have only a minor enhancement effect in human skin whereas anionic surfactants can have a more pronounced effect. The effect of surface active agents on the skin barrier function depends on the agent's chemical
structure. In general, anionic surfactants tend to be more effective than cationic where as nonionic surfactants are considerably less effective. Nonionic surfactants might increase the membrane fluidity of the intercellular regions of the stratum corneum (e.g., Brij) and may extract lipid components and additionally, though of minor importance, they might interfere with keratin filaments and create a disorder within the corneocytes [11].

**Need for absorption enhancement**

Oral dosing is generally considered to be the most patient friendly and convenient route of drug administration. However, many pharmacologically active compounds cannot be administered orally because of inadequate oral bioavailability and this may limit the usefulness of these compounds. Poor oral bioavailability can be caused by poor aqueous solubility, degradation within the gastrointestinal contents, poor membrane permeability, or presystemic metabolism. Compounds can have poor membrane permeation due to large-molecular weight, as is the case with proteins and other macromolecules, or insufficient lipophilicity to partition into biological membranes, as with many hydrophilic, low-molecular weight compounds. There are numerous pharmacologically effective compounds currently used that must be administered by injection because of inadequate bioavailability by non-injection routes. Table 2.0 lists some of the compounds for which absorption enhancement technologies have been proposed and tested, clinically in many cases [13]. The compounds belong to proteins, polypeptides & peptides, non-peptide macromolecules and hydrophilic small molecules. Many proteins and peptides have demonstrated highly potent and selective pharmacologic activities toward various therapeutic targets. While some of these have been

| Drug                   | Uses                           | Properties                                      | Comments                                      |
|------------------------|-------------------------------|------------------------------------------------|-----------------------------------------------|
| **Peptides, Proteins** |                               |                                                 |                                               |
| Calcitonin             | Postmenopausal osteoporosis   | 32 amino acid peptide, MW ~3,455               | Injection and nasal (F=3–5%) products are available |
| Desmopressin (DDAVP)   | Diabetes insipidus, nocturnal enuresis | 9 amino acid peptide, MW 1,183 | Oral (F=0.16%) and nasal (F=5–10%) products Insulin Diabetes |
| Insulin                | Diabetes                      | 51 amino acid peptide, MW ~5,800, hexamer form | Various injection products and one inhaled form available |
| Leuprolide             | Endometriosis, prostate cancer | 9 amino acid peptide analog, MW ~1,200         | Solution and depot injections and implant forms available |
| Octreotide             | Acromegaly, carcinoid tumors  | Cyclic octapeptide, MW ~1,000                  | IV and SC injection use only (50–500 mg tid dose) |
| **Non-peptide macromolecules** |                               |                                                 |                                               |
| Heparin                | Anticoagulant                 | Highly sulfated polymer, MW 12,000–15,000      | IV and SC use only                           |
| Low-molecular weight heparin (enoxaparin) | Prevention and treatment of thrombosis | MW ~4,500, sulfonate and Carboxylate groups | IV and SC use only, usually 30–40 mg/day |
| Fondaparinux           | Factor Xa inhibitor, anticoagulant | Pentasaccharide, MW ~1,727, sulfonate and carboxylate groups | SC injection only, usually 2.5–10 mg/day |
| Oligonucleotides       | Modulate various biological pathways | Hydrophilic, high MW | Emerging as potential parenteral Products |
| Vancomycin             | Antibiotic                    | Glycopeptide, MW 1,449                         | IV use, high doses, oral product for colitis only |
| **Hydrophilic small molecules** |                               |                                                 |                                               |
| Aminoglycosides        | Antibiotics                   | MW 500                                         | IV and IM use, high doses, some topical products |
| (e.g., amikacin, gentamycin) |                               |                                                 |                                               |
| Amphotericin B         | Antifungal                    | MW 924, low log P, high-polar surface area     | IV use                                        |
| Bisphosphonates        | Osteoporosis                  | Strongly acidic phosphate groups, MW approx. 250–325 | Oral bioavailability <1% for many in class |

Table 2.0 Drugs Potential for oral and transmucosal absorption enhancement technologies
developed into marketed injectable products, there is clearly a need for non-injection alternatives, especially for compounds that are used chronically and require frequent dose administration.

**Permeation enhancers used in various formulations**

**Topical and transdermal formulations**

It is generally accepted that the bioavailability of most topically applied drugs remains low.

Various methods are used to increase this bioavailability. One of the approaches is the use of permeation enhancers, and over the years, there has been a great interest in new chemical permeation enhancers. It is an important issue to predict the rate at which drugs or other xenobiotics penetrate the skin. Following two decades of commercialization, various transdermal membranes are reported in diverse therapeutic areas. Conventional forms of TDS are reservoir and matrix types where solid drugs are incorporated into polymeric vehicles [14]. Some of them include Solvents (Ethanol, acetone, polyethylene glycol, glycerol, propylene glycol), Surfactants (Brij30, brij72, Span 20), Azones (N-Acyl hexahydro-2-oxo-1H-azepines, N-Alkylmorpholine-2,3-diones), Terpenes (Limonene, Carvone), Fatty alcohols & Fatty acids (Lauryl alcohol, linolenyl alcohol, oleic acid and lauric acid) and Miscellaneous (Lecithin, sodium de-oxy cholate). Fig 1.0 illustrates the intercellular and intracellular penetration of drugs [15].

**Oral and buccal formulations**

Oral delivery is one of the most preferred routes of drug administration, particularly for proteins and peptides [16]. Although this type of drug delivery avoids the pitfalls associated with the use of needles, low bioavailability remains a problem due to the poor permeability of intestinal epithelia to therapeutic macromolecules [18]. One of the most widely studied means of addressing limited drug transport is the use of chemicals to promote drug uptake across the epithelium [19].

Chemical permeation enhancers increase the permeability of the intestinal epithelium through disruptions of the cellular membrane and/or changes in the structure of the tight junctions between epithelial cells [20]. These effects aid absorption through the transcellular and paracellular routes, respectively. There are clear differences between the oral mucosal membrane and other epithelial membranes of the intestine, nasal cavity and rectum. The oral mucosal membranes are less keratinized than the skin membranes and show a more loosely packed intercellular lipid domain. In terms of function of the absorption enhancement through the oral mucosal membrane, it can be said that it occurs principally through the lipid-filled intercellular spaces. There are only a limited number of studies comparing the systematic changes in the structure of enhancers and their influence on the oral mucosal membranes. A study related to the buccal bioavailability of testosterone indicated the absorption enhancing effect of hydroxypropyl-β-cyclodextrin with a relative bioavailability of 165% versus the administration without absorption enhancers. This effect was probably due to an increased solubility of testosterone, although cyclodextrins might also extract lipids from the intercellular matrix. The buccal mucosa, as a route for systemic drug delivery, offers distinct advantages over per-oral administration. These advantages include bypass of first-pass effect, avoiding presystemic elimination within the GI tract, and a better enzymatic flora for certain drugs. Though these benefits make the buccal route attractive, the low flux associated with it most often makes the attainment of therapeutic plasma levels difficult. One approach in overcoming this problem has been the use of permeation enhancers. Menthol is a monocyclic terpene with a pleasant taste and odor. It is widely consumed as a flavoring agent in oral dosage forms and as a fragrance in topical formulations. The effect of menthol on transdermal absorption of several drugs has been reported. The effect of ethanol on the permeability of propranolol in the presence of menthol has also been reported. A major benefit of using menthol as a permeation enhancer is its safety profile. This does not only result from the greater surface area provided by the small intestine, but also from the structural differences between each of the tissues, as demonstrated in fig. 4. Based on epithelial structure alone, it is not surprising that the simple columnar epithelium covering the small intestine provides less resistance to drug transfer than the stratified squamous epithelium covering the skin and buccal mucosa. The cellular organization of epithelia lining the buccal mucosa is typical of a stratified squamous epithelium, where the epithelial cells are surrounded by a hydrophilic intercellular matrix. Since drug delivery through the buccal mucosa is limited by the barrier nature of the epithelium and the area available for absorption, various enhancement strategies are required in order to deliver therapeutically relevant amounts of drug to the systemic circulation. Various methods, including the use of chemical penetration enhancers, prodrugs, and physical methods may be employed to overcome the barrier properties of the buccal mucosa [22]. The lipophilic cell membranes of the epithelial cells are thus surrounded by relatively polar intercellular lipids on the cell exterior and a hydrophilic aqueous cytoplasm on the cell interior. This is somewhat analogous to the situation in the intestine, where the epithelial cells are separated by a hydrophilic intercellular compartment, albeit, the intercellular spaces of the intestinal mucosa lack the polar lipids seen in the intercellular spaces of the buccal mucosa. Over the last few decades, researchers have investigated various approaches for the efficient oral delivery of peptides. Entrapment into particulate systems [23], mucoadhesive polymer formulations [24], and use of permeation enhancer [25] and protease inhibitor [26] adjuvants are among the most commonly utilized strategies. The use of
absorption enhancers, that improve the mucosal permeation of macromolecules without causing serious tissue damage, has been the focus of many research groups. Various permeation enhancers have been investigated for the improvement of peptide absorption through the intestinal membrane. Common examples of non-specific permeation enhancers are surfactants, chelating agents, bile salts, and fatty acids [27].

**Nasal formulations**

Only a few studies are available related to the effect of known absorption enhancers on the pulmonary absorption of poorly absorbable drugs, including peptides and proteins. Hydroxpropyl-β-cyclodextrin and especially dimethyl-β-cyclodextrin have been shown to enhance the pulmonary bioavailability of insulin in rats, and indicate relatively low acute mucotoxicity. Pulmonary insulin absorption is reported to be increased in the presence of glycocholate and Spans. The use and efficacy of absorption enhancers for nasal peptide and protein delivery is of utmost important. The enhancing effect of bile salt seems dependent on its lipophilicity. The bioavailability of gentamicin increases with increasing lipophilicity of trihydroxy bile salts. In the past years, much research has concentrated on the use of cyclodextrins to enhance bioavailability of peptides and proteins especially because of their mild and reversible effect on the nasal mucociliary clearance.

**Rectal formulations**

Due to a combination of poor membrane permeability and metabolism at the site of absorption, rectal bioavailability of peptide and proteins is low. Bile salts are also used for the enhancement of drug absorption, but several studies indicated severe damage due to their use in rectal drug delivery. Sodium tauro- 24, 25-dihydrofusidate (STDHF) has a positive effect on the availability of cefoxitin, vasopressine, and insulin in rats.

**Ocular formulations**

Enhancement of corneal penetration is advocated as one of the possible strategies for overcoming the poor topical bioavailability of ophthalmic drugs. Basically, corneal drug penetration can be improved by increasing the drug lipophilicity through ion-pair formation or produg derivatization or by transiently altering the corneal permeability with substances known as ‘penetration enhancers’ (PE). Surfactants produce ultrastructural changes in the cell membranes by partial solubilization and removal of phospholipids, thus improving the permeability characteristics of the corneal epithelium. The ocular use of PE, however, is potentially associated with eye irritation and cellular damage. Ocular irritants are currently identified/evaluated by the Draize test, which is a generalised gross method concentrating on the effects on rabbit cornea, iris and conjunctiva. This test is currently criticised on the basis of ethical considerations and unreliable prognosis of human response, and alternative methods have been recommended. The development of a simple and reproducible method for assessing the long- and short-term ocular toxicity of PE might lead to more efficient and safer topical medications and to benefits for many patients. The evaluation of cytotoxicity of pharmaceuticals on ocular cell cultures is proving a promising prognostic tool for oculotoxicity in vivo. The cytotoxicity of some potential ocular penetration enhancers (benzalkonium chloride, cetylpyridinium chloride, EDTA) is evaluated on immortalized corneal epithelial cell cultures using the cell proliferation reagent WST-1. Rabbit (RCE) and human (HCE) cells are used to assess possible species differences in the toxic response to the same agents [28].

**Nanocarriers as permeation enhancers**

The types of nanocarriers that are used today have significantly increased in the last decades. These systems are designed around the two characteristics that are sought in the modern pharmacy: temporal delivery and spatial location [29]. Some of the advantages include improvements in drug solubility, permeability, half-life, bioavailability, and stability [30] but sometimes with low load capacity in many cases and lack of stability of the system per se [31]. The physicochemical properties of nanocarriers determine the interaction with biological systems and nanocarrier cell internalization [32]. The main physicochemical properties that affect cellular uptake are size, shape, rigidity, and charge in the surface of nanocarriers. Nanocarriers can be administered by almost all routes [33] since they offer several advantages over other delivery systems, but with its own limitations. The most used and investigated nanocarriers for topical/transdermal drug delivery in the pharmaceutical field [35] as a function of the material used to prepare them.

**Liposomes**

Liposomes are lipid bilayer systems that can carry hydrophilic drugs inside the core and lipophilic drugs between the bilayer membrane barriers [38]. The lipid composition, and total lipid concentrations. Liposomes have been used successfully to transport drugs across the membrane barriers [38]. The lipidic lipid content makes the interaction of these entities with biological membranes easier.

**Transfersomes**

Some liposomes may have a deformable structure and pass through the SC or may accumulate in the channel-like regions in the SC, depending upon their composition. The driving force is nothing more than osmotic pressure. These liposomes are called transfersomes or transformable liposomes [39]. The need to reach the narrow tubes that make up the skin, to deliver drugs, led to the invention of transfersomes. The original idea to use liposomes as drug delivery systems was very smart, as they are made of lipids similar to biological membranes, but they have rigid structure. The
incorporation of elements in the lipid bilayer to make it flexible has made these carriers successful. Traditional transformable liposomes are made using surfactants in the lipid bilayer. In transdermal drug delivery, the paracellular and intercellular pathways are very important but appendage routes have been of increasing interest lately [40]. The use of flexible liposomes (transformable liposomes) is an invaluable strategy to reach the objective of drug delivery via the transdermal route. The use of these kinds of nanocarriers seems to be more effective than liposomes, and their flexibility allows the possibility of using them as transdermal vaccine vectors.

Ethosomes

The idea of making another kind of flexible liposome has been the goal of a lot of scientists. To that end, the ethosomes, which contain alcohol in the lipid bilayer to make them more flexible and able to be deformed when pressure is applied [41] were created. These carriers allow drugs to reach deeper skin layers and systemic circulation. Ethosomes are easy to prepare, and they are considered safe and efficient. For these reasons, they could have wide future applications [42]. Their main characteristics are softness and malleability, and they are considered good drug-delivery systems. Ethosomes are able to contain and deliver a lot of molecules because they can transport highly lipophilic drugs, e.g., testosterone, minoxidil, and cationic molecules such as propranolol and trihexyphenidil [43]. Ethosomes can carry and deliver a lot of drugs, and in the future these systems offer a huge opportunity to make better therapies, besides which they can transport molecules through the skin and biological membranes.

Niosomes

Niosomes are made of cholesterol and nonionic surfactants, which are biodegradable and minimally toxic. Niosomes were created with the same goal as transfersomes and ethosomes [44]. In addition, the incorporation of nonionic surfactants let the liposomes be more stable. The niosomes were originally used in the cosmetics industry, and the versatility of these systems has allowed their use to spread to other areas. For example, in pharmaceutical products, they are formulated for drug delivery. They are used for many routes of administration like oral, parenteral, ocular, vaginal and transdermal [45] The application of niosomes in transdermal drug delivery has been very important, because they can carry anti-aging agents and antifungal molecules, among other drugs.

Dendrimers

Dendrimers are nonpeptidic fractal 3-D structures made of numerous small molecules. The term “dendrimer” is Greek: “dendra” means tree and “meros” means part. This name was coined in the late 1970s by a research group formed by Vögtle, Denkewalter, Tomalia, and Newkome. The structure of these molecules results in relatively uniform shapes, sizes, and molecular weights. They are a very good alternative for drug delivery systems. Dendrimers can be used in antiviral and anticancer pharmaceutical therapies, including vaccines [46]. The first material used and still most commonly used for dendrimer fabrication is poly(amidoamine), which was initially synthesized by Dow Laboratories between 1979 and 1985. In the context of controlled chemical delivery, dendrimers have been explored for drug delivery, gene therapy, and delivery of contrast agents [47]. The use of dendrimers to encapsulate hydrophobic and labile molecules has been a successful road. The permeability of dendrimers through the skin depends on physicochemical characteristics like generation size, molecular weight, surface charge, composition, and concentration [48]. Dendrimers as transdermal drug delivery systems are relatively new, but there are numerous recent papers [49]. These have been used to transport photosensitizers for photochemical therapy and antifungal molecules.

Nanoparticles

The main goal of delivery systems is to reach the organ of interest and often to go through it. Recently, scientists have developed lots of nanocarriers for helping to improve drug transport into the skin and through biological membranes. The skin is an important route to go into the body, and with its larger contact area it can be very useful to administer drugs locally and systemically [50]. Nanotechnology in the pharmaceutical sciences opens a new avenue of therapies for the treatment of many diseases and represents hope that people may be helped to have a better life. Nowadays, it is possible to encapsulate a variety of molecules into nanoparticles like drugs, proteins, peptides and DNA. Moreover, gold nanoparticles, has been used for transdermal delivery to encapsulate proteins to enable percutaneous delivery. The interaction between the gold nanoparticles and the skin barrier leads to an increase of skin permeability and effectively prompts percutaneous absorption of the coadministered proteins [51]. The main advantage of codelivery is that it does not require the loading of drugs into the nanoparticulate system. Therefore, compromise in activity can be minimized for both protein drugs and nanoparticles because of the exclusion of complicated drug loading processes. This highlights a new strategy for percutaneous protein delivery, with obvious advantages in terms of simplicity and cost-effectiveness. Also, a combined multiphoton-pixel analysis method was developed for semiquantitation of gold nanoparticle penetration into different skin layers [52]. Gold nanoparticles are also commonly used in cosmetic products such as facial gold masks. Protein nanofiber gold nanoparticle creams and gold nanoparticle masks have been claimed to enhance the firmness of skin and to have a rejuvenating action [53]. Silver nanoparticles are similar to solid-drug nanoparticles in that the active agent appears to be the breakdown product of the particle. Silver nanoparticles exhibit minimal penetration into skin and are consequently considered safe. Studies of long-term occupational exposure to silver ions and silver nanoparticles have concluded that they are relatively nontoxic [54]. They can also be classified depending on the material from which they are made. Nanoparticle-preparation techniques are based on their physicochemical properties. They are made by emulsification–diffusion by solvent displacement, emulsification–polymerization, in situ polymerization, gelation, nanoprecipitation, solvent evaporation/extraction, inverse salting
out, dispersion polymerization, and other techniques derived from these. [49, 55]. Two of the main options for transdermal delivery are the solid-lipid nanoparticles and nanostructure lipid carriers. Aside from polysaccharide nanoparticles, polymeric nanoparticles are very good options for transdermal delivery because they can be tailor-made in different sizes and it is possible to modify their surface polarity in order to improve skin penetration [56]. From the upper skin, lipid nanoparticles can reach deeper skin regions because they exhibit mechanical flexion. Nano carriers can even travel from the skin to lymph nodes, representing a promising tool for immunomodulation [57].

**Nanoemulsions**

Nanoemulsions are isotropic dispersed systems of two nonmiscible liquids, normally consisting of an oily system dispersed in an aqueous system, or an aqueous system dispersed in an oily system but forming droplets or other oily phases of nanometric sizes. Despite drug loading issues, they can be stable for long periods due to their extremely small size and the use of adequate surfactants. Hydrophobic and hydrophilic drugs can be formulated in nanoemulsions because it is possible to make water/oil or oil/water nanoemulsions [49]. They are nontoxic and nonirritant systems, and they can be used for skin or mucous membranes and parenteral and nonparenteral administration in general, and they have been utilized in the cosmetic field. Transdermal delivery using nanoemulsions has decreased due to the stability problems inherent to this dosage form. Some examples of drugs using nanoemulsions for transdermal drug delivery are gamma tocopherol, caffeine, plasmid DNA, aspirin, methyl salicylate, insulin and nimesulide [49]. In general, the advantages and limitations of using nanocarriers for transdermal drug delivery are their tiny size, their high surface energy, their composition, their architecture, and their attached molecules [58].

**Permeation Enhancement Technologies of some products**

One of the few absorption enhancers to have advanced to a marketed product is cyclopentadecalactone, also referred to as pentadecalactone. This agent is proprietary to Bentley Pharmaceuticals, Inc. and is now being promoted as CPE-215 by CPEX Pharmaceuticals, a spin-off of Bentley. This absorption enhancer is currently used in a transdermal testosterone product Testim, marketed by Auxillium. The formulation contains up to 8% pentadecalactone in a gel formulation primarily comprised of ethanol. CPEX Pharmaceuticals is also currently pursuing a nasal insulin delivery product utilizing CPE-215 as an absorption promoter, which is in early clinical trials. Nasal bioavailability of insulin, relative to subcutaneous injection, is reported to be 10–20% and the formulation is well tolerated [59]. Emisphere Technologies, Inc. is developing products utilizing its proprietary Eligen technology, a library of absorption-enhancing compounds of which sodium N-[8-(2-hydroxybenzyl)amino]caprylate (also referred to as SNAC and salcaproate sodium) is the lead. Emisphere contends that SNAC enhances absorption by forming a noncovalent complex with the active compound that enables transcellular absorption, without altering tight junctions [60]. For proteins, the mechanism may involve a reversible change in protein conformation and protection against degradation prior to absorption. SNAC is found to increase the absorption of cromolyn approximately eightfold, and the mechanism appears to be related to an increase in membrane fluidity, since SNAC has no effect on cromolyn lipophilicity [61]. A subchronic toxicity study in rats indicates a no observable adverse effect level of 1,000 mg/kg/day or greater [62]. It is interesting to also note that Caco-2 cells exposed to SNAC shows evidence of cell damage using various cytotoxicity assays, including lactate dehydrogenase, mitochondrial dehydrogenase activity, trypan blue exclusion and neutral red binding [61]. The current lead products in development utilizing SNAC are calcitonin, which is in phase 3 trials in partnership with Novartis. Earlier in development are products intended to deliver glucagon-like peptide-1 and peptide Y via the oral route. A structurally related absorption enhancer also originating from Emisphere is 8-(N-2-hydroxy-5-chloro-benzyl)-aminocaprylic acid, or 5-CNAC, which is in the clinical trial phase of development in an oral calcitonin formulation being developed by Nordic Biosciences in partnership with Novartis. A tablet containing 200 mg 5-CNAC and 0.8 mg calcitonin provides greater calcitonin absorption and greater effects on a biomarker of bone resorption than nasal calcitonin but absorption is influenced by fed state and the volume of water taken with the tablet [63]. A 14-day clinical trial of twice daily oral calcitonin with 5-CNAC suggests potentially useful reductions in biomarkers of bone resorption and cartilage degradation [64]. Another drug delivery specialty company focused on improving the oral delivery of existing drugs with poor absorption is Merrion Pharmaceuticals. Their proprietary formulations, collectively referred to as gastrointestinal permeation enhancement technology (GIPET), are based on the use of medium chain fatty acids and salts and derivatives of medium chain fatty acids. The products in development include two bisphosphonates, alendronate and zoledronic acid, a gonadotropin-releasing hormone antagonist and fondaparinux, a pentasaccharide factor Xa antagonist. The Merrion absorption-enhancing excipients and active drug are preferably delivered using an enteric-coated dosage form. The excipients, the main enhancer being sodium caprate, are claimed to have generally recognized as safe (GRAS) status based on prior use as food additives. Using the GIPET formulation approach, it is possible to achieve 5–9% oral bioavailability of low-molecular weight heparin and to increase alendronate oral bioavailability 12-fold relative to the existing marketed product, to approximately 7% [65]. In clinical phase 1 and 2 studies conducted so far, GIPET formulations appear to have been well tolerated. Sodium caprate has also been utilized as an excipient to improve the oral absorption of an antisense oligonucleotide of molecular weight 7701 (ISIS 104838) in preclinical and clinical studies conducted by Isis Pharmaceuticals. In the absence of an absorption enhancer, ISIS 104838 had undetectable oral bioavailability in rats, dogs and pigs. But in dogs, administration of enteric coated tablet containing...
sodium caprate and ISIS 104838, systemic oral bioavailability averaged 1.4% compared to IV administration [66]. Tissue histology of the small intestine and large intestine indicates no changes after once daily dosing of tablets containing approximately 1 g of sodium caprate for seven consecutive days. Oral ISIS 104838 is also evaluated in humans using solid formulations designed to combine immediate release and delayed release sodium caprate (660 mg total) in an enteric-coated capsule [67]. The formulation providing the greatest average oral bioavailability results in 12.0% average bioavailability relative to subcutaneous injection and ranging from approximately 2% to 27.5% in ten fasted subjects. Average bioavailability and inter-subject variability are similar in the fed state. Modifying the release of sodium caprate is thought to have prolonged the duration of exposure of the intestinal membrane to the enhancer, as well as expanding the surface area exposed. Oral dosing of this antisense oligonucleotide in specifically designed formulations with sodium caprate could feasibly result in systemic exposure at levels required for therapeutic efficacy. Formulation technology is also apparently key to the effectiveness of an absorption enhancing approach being pursued by Chiasma, who refers to their proprietary technology as a transient permeability enhancer (TPE) system. While the Chiasma TPE technology is not disclosed, intellectual property covering absorption-enhancing formulations is described by scientists affiliated with Chiasma [68]. These formulations consist of a suspension of a medium chain fatty acid salt, exemplified by sodium caprylate, and a matrix-forming polymer in a hydrophobic medium, such as glyceryl triglyceride, and their utility in improving the oral bioavailability of octreotide, exenatide, and other macromolecules is demonstrated. Using the TPE system, Chiasma is in early clinical studies with an oral form of octreotide acetate. Scientists have long sought for an oral dosage form for insulin delivery. In addition to offering an alternative to daily injections for the millions of diabetic patients requiring insulin therapy, the oral route of insulin delivery could have a physiological advantage of mimicking insulin secretion from the pancreas via the portal circulation to the liver [69]. Oral insulin delivery requires protection from degradation in the stomach and intestinal lumen, as well as enhancement of its permeation across the intestinal membrane. One of the companies developing an oral insulin product is Oramed Pharmaceuticals. In a formulation comparison study in healthy subjects, one orally administered Oramed insulin formulation exhibits pharmacologic response (glucose and c-peptide lowering) and is well tolerated [70]. The formulation composition is not known, but the patent literature suggests that the formulation may include one or more protease inhibitors (such as aprotinin and soybean trypsin inhibitor), EDTA or a bile acid or bile salt as a permeation enhancer, and an omega-3 fatty acid in an enteric-coated formulation [71]. The extent of insulin oral bioavailability afforded with this approach is not known. Diabetology Ltd. also performed clinical trials with an oral insulin formulation referred to as Capsulin, which employs unknown GRAS excipients for absorption enhancement. Oral 150 and 300 U insulin doses produced hypoglycemic effects with modest increase of plasma insulin concentration [72]. An alternative approach to achieving systemic insulin exposure and effects, which is under continuing clinical investigation, is the buccal delivery technology of Generex Biotechnology. This employs a combination of several proprietary excipients, which may include sodium lauryl sulfate, fatty acids, bile acids, and other excipients in a liquid mixed-micellar spray [73]. The permeation-enhancing excipients are claimed to be GRAS, and the system was said to provide 10% absorption [74]. This has already been marketed in some countries outside USA and is being studied in USA under a treatment IND. A formulation strategy has been described by Unigene scientists combining a permeation-enhancing excipient with an acid to lower the local pH of the intestinal fluids to a pH where protease activity is reduced [75]. This is preferably formulated as an enteric-coated tablet, and for the oral delivery of salmon calcitonin, the preferred permeation enhancer is lauroyl L-carnitine. Unigene has licensed the oral calcitonin delivery technology to Tarsa, and a product is in late-stage clinical trials for the treatment and prevention of postmenopausal osteoporosis in collaboration with Novartis. Another strategy that has been utilized for protecting a peptide drug from degradation in the stomach and small intestinal lumen is encapsulation of the drug within the inner aqueous phase of a reverse micelle stabilized by polymers. The components of the reverse micelle may also increase intestinal permeability. This is the formulation approach Solgenix (formerly DorBiopharma) expects to use to deliver leuprolide in clinical trials. Solgenix claims that with this lipid polymer micelle formulation, oral bioavailability in rats and dogs is improved from 2.2% to 20–40%. This technology is early in development. While many of the absorption enhancing technologies discussed so far have centered on oral drug delivery, there have also been advances made in transmucosal absorption enhancement. One of the companies focusing on nasal drug absorption enhancement is Aegis Therapeutics, with their group of proprietary enhancers referred to as Intravail. These agents, which are initially developed at the University of Alabama at Birmingham, are a group of medium chain alkylglycosides including dodecylmaltoside and tetradeckylmaltoside. Enhanced nasal bioavailabilities of calcitonin, insulin, and human growth hormone are demonstrated in rats [76], and the enabling excipients are said to be well tolerated [77]. In a study in healthy human subjects, nasal bioavailability of calcitonin is improved from 6.6% with a control formulation to 35.9% with dodecylmaltoside [78]. Aegis seems to be positioning its technology more for outlicensing rather than developing products internally. Another company with proprietary technology being applied toward nasal drug delivery enhancement is Archimedes Pharma, which is using chitosan to develop nasal formulations with increased bioavailability. Archimedes technology is being used in clinical development candidates for the nasal delivery of morphine, granisetron, and vaccines. While chitosan has both mucoadhesive and permeation enhancing properties, some chitosan derivatives such as N-trimethyl chitosan, have been shown to have greater permeation enhancement, especially at neutral pH [79]. Thiolated polycarbophil is another structurally modified pharmaceutical excipient designed to
maximize its effects as an absorption promoter [80]. Finally, the permeation enhancer dodecyl-2-N,N-dimethylamino propionate (DDAIP) has been used in topical alprostadil products which are approved in some countries and is in late-stage clinical studies for US registration. This technology referred to as NexACT is developed by Apricus Bio (formerly NexMed) and is claimed to enhance the absorption of various types of compounds through skin, buccal, or intestinal absorption sites. Other potential products are at earlier stages of development. Table III provides a list of some of the companies utilizing absorption enhancers and their technologies and development candidates. Most commonly these companies control some form of intellectual property around a specific technology, and the technology is being applied to non-proprietary compounds, in addition to the possibility of licensing the technology or the products in development to partners. There may also be companies that recognized a need or potential application of an absorption enhancing technology for their proprietary compounds or for a therapeutic area of particular interest and have initiated product development with non-proprietary absorption enhancing excipients. Table 3.0 shows a list of pharmaceutical companies and their technologies along with the corresponding drugs for permeation enhancement for drugs under study [1].

| Company                     | Enhancer/technology                                      | Drugs                                      |
|-----------------------------|----------------------------------------------------------|--------------------------------------------|
| CPEX Pharmaceuticals        | Cyclopentadecalactone                                     | Transdermal testosterone (Market); nasal insulin (phase 2) |
| Emisphere Technologies      | Sodium N-[8-(2 hydroxylbenzoyl)amino] caprylate (SNAC)    | Calcitonin (phase 3), vitamin B12, GLP-1, peptide Y |
| Nordic Biosciences          | 8-(N-2-hydroxy-5-chloro benzoyl)-amino-caprylic acid (5-CNAC) | Calcitonin (phase 3)                       |
| Merrion Pharmaceuticals     | Medium chain fatty acids, salts, and derivatives          | Alendronate (phase 2), zoledronic acid (phase 3), gonadotropin-releasing hormone antagonist (phase 1/2), fondaparinux (phase 1) |
| Isis Pharmaceuticals        | Sodium caprate, modified release formulation              | Antisense oligonucleotide (phase 1)        |
| Chiasma                     | Sodium caprylate suspension in hydrophobic medium with matrix forming polymer | Octreotide (phase 2)                      |
| Oramed Pharmaceuticals      | Protease inhibitor and omega 3 fatty acid                 | Insulin (phase 2), glucagon-like peptide 1 analog |
| Diabetology Ltd.            | Unknown GRAS excipients                                  | Insulin (phase 2)                          |
| Generex                     | Liquid mixed-micelle spray                               | Insulin (approved in some countries, treatment IND in USA) |
| Unigene/Tarsa               | Combo of protease inhibitor, permeation enhancer, pH modifier, enteric coating | Calcitonin (phase 3)                       |
| Soligenix (Dor)             | Lipid polymer micelle                                    | Leuprolide (preclinical)                   |
| Aegis Therapeutics          | Alkylglycosides                                           | Feasibility claimed for various intranasal peptides |
| Archimedes Pharma           | Chitosan                                                  | Intranasal morphine (phase 3), intranasal granisetron (phase 1) |
| NexMed/Apricus Bio          | Dodecyl-2-N,N-dimethylamino propionate (DDAIP)            | Topical alprostadil (NDA) and possibly other topical agents |

Table 3.0 Pharmaceutical Companies and Permeation Enhancement Technologies

**Conclusions**

Several technologies for enhancing absorption of poorly bioavailable compounds have progressed from the early studies demonstrating permeation enhancement in an isolated membrane model, and a number of absorption-enhancing technologies are now in clinical trials. Some of these utilize specific excipients in a new way or in different concentrations or combinations than have been used in existing products. Others utilize new excipients for their permeation-enhancing function. These new excipients and formulations increase systemic exposure after oral, transmucosal, or transdermal dosing, as indicated by improved bioavailability or bioactivity, and appear to represent feasible alternatives to existing products, which afford non-optimal bioavailability or must be administered by injection. It seems likely that gradually absorption-enhancing formulations will be more broadly accepted in the international market in the near future. One of the barriers to regulatory approval may be the requirement for demonstrating safety of a new excipient, which itself has biological activity. Understanding the mechanism of absorption enhancement may be very useful toward registration. However, it seems reasonable that once a delivery technology is proven to be successful for one particular drug, that technology might be readily adapted to improving the delivery of other poorly absorbed drugs. New
absorption enhancers that are designed to function through specific mechanisms and are more potent and specific than those currently in clinical trials may follow. These technologies may afford alternatives for proteins and peptides currently only administered by injection. In addition, and as important, these technologies may enable the development of new chemical entities with good pharmacologic activity, but poor biopharmaceutical properties, that otherwise would not be developed into drugs.

References
1. Bruce Aungst J 2012 Absorption Enhancers: Applications and Advances. AAPS J, 14(1): 10-18.
2. Mbah CJ, Uzor PF, Omeje EO 2011 Perspectives on Transdermal Drug Delivery. J. Chem. Pharm. Res, 3(3): 680-700.
3. Songkro S 2009 Songklanakarin J. Sci. Technol: 31(3), 299-321.
4. Asbill CS, Michniak BB 2000 Percutaneous penetration enhancers: Local versus transdermal activity. Pharm Sci Technol Today: 3(1), 36-41.
5. Singh PB, Chaudhry PK 2007 Penetration enhancers for transdermal drug delivery of systemic agents. J Pharm Res: 6.
6. Gloor M, Fluhr J, Wasik B, Gehring W 2001 Clinical effect of salicylic acid and high dose urea applied in standardized NRF formulations, Pharmazie, 56(10): 810-814.
7. Park ES, Chang SJ, Rhee YS, Chi SC 2001 Effects of adhesives and permeation enhancers on the skin permeation of captopril, Drug Dev Ind Pharm, 27(29): 975-980.
8. Jungbauer FH, Coenraads PJ, Kardaun SH 2001 Toxic hygroscopic contact reaction to N-methyl-2-pyrrolidone, Contact Dermatitis, 45(5): 303-304.
9. Adrian Williams C, Brian Barry W 2004 Penetration enhancers, Adv Drug Deliv Rev, 56(5): 603-618.
10. Touitou E, Godin B, Karl Y, Bujanover S, Becker Y 2002 J Control Release, 80(1-3): 1-7.
11. Mbah CJ, Uzor PF, Omeje EO 2011 Perspectives on Transdermal Drug Delivery. J. Chem. Pharm. Res, 3(3): 680-700.
12. Cazares Delgadillo J, Naik A, Kalia YN, Quintanar Guerrero D, Ganem Quintanar A. 2005 Int. J. Pharm, 297: 204-212.
13. Aungst BJ. 2012 Absorption Enhancers: Applications and Advances The AAPS Journal, 14(1): 10-18.
14. Padula C, Nicoli S, Aversa V, Colombo P, Falcon F et al. 2007 Bioadhesive film for dermal and transdermal drug delivery, Eur J Dermatol, 17(4): 309-312.
15. Mohamed MI 2004 Optimization of chlorphenesin emulgel formulation. AAPS J, 6(3): 81-87.
16. Sinha V, Singh A, Kumar RV, Singh S, Kumria R et al. 2007 Oral colon-specific drug delivery of protein and peptide drugs, Crit Rev Ther Drug Carrier Syst, 24(1): 63-92.
17. des Rieux A, Fievez V, Garinot M, Schneider YJ, Preat V. 2006 Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J Control Release, 116(1): 1-27.
18. Goldberg M, Gomez Orellana I. 2003 Challenges for the oral delivery of macromolecules. Nat Nat Rev Drug Discov, 2(4): 289-295.
19. Thanou M, Henderson S, Kydonieus A, Elson C 2007 N-sulfonato-N, O-carboxymethylchitosan: a novel polymeric absorption enhancer for the oral delivery of macromolecules. J Control Release, 117(2): 171-178.
20. Jevprasesphant R, Penny J, Attwood D, D’Emanuele A 2004 Transport of dendrimer nanocarriers through epithelial cells via the transcellular route. J Control Release, 97(2): 259-267.
21. Emily F, Craig D, Edward W 2007 Disruption of epithelial tight junctions by yeast enhances the paracellular delivery of a model protein. Pharm Res, 24(1): 37-47.
22. Joseph Nicolazzo A, Barry Reed L, Barrie Finnin C 2005 Department, Buccal penetration enhancers—How do they really work? J Control Release, 105(1-2): 1-15.
23. Damge C, Maincent P, Ubirch N 2007 Oral delivery of insulin associated to polymeric nanoparticles in diabetic rats. J Control Release, 117(2): 163-170.
24. Werle M, Makhlof A, Takeuchi H 2010 Carbopol-lectin conjugate coated liposomes for oral peptide delivery. Chem Pharm Bull (Tokyo), 58(3): 432-434.
25. Fetih G, Habib F, Okada N, Fujita T, Attia M, Yamamoto A 2005 Nitric oxide donors can enhance the intestinal transport and absorption of insulin and [Asu1, 7]-eel calcitonin in rats. J Control Release, 106(3): 287-297.
26. WerleM, Takeuchi H. 2009 Chitosan–aprotinin coated liposomes for oral peptide Delivery: Development, characterisation and in vivo evaluation, Int J Pharm: 370(1-2), 26-32.
27. Khafagy ELS, Morishita M, Onuki Y, Takayama K 2007 Current challenges in Non invasive insulin delivery systems: a comparative review. Adv Drug Deliv Rev, 59(15): 1521-1546.
28. Susi Burgalassi , Patrizia Chetoni, Daniela Monti, Fabrizio Saettone M 2001 Cytotoxicity of potential ocular permeation enhancers evaluated on rabbit and human corneal epithelial cell lines. Toxicol Lett, 122(1): 1-8.
29. Papakostas D, Rancan F, Sterry W, Blume-Peytavi U, Vogt A 2011 Nanoparticles in dermatology. Arch Dermatol Res, 303(8): 533–550.
30. Cho K, Wang X, Nie S, Chen ZG, Shin DM 2008 Therapeutic nanoparticles for drug delivery in cancer. Clin Cancer Res, 14(5): 1310–1316.
31. Blynskaya EV, Alekseev KV, Alyautilin RN 2012 Perspectives of the development of pharmaceutical nanotechnology. Russ J Gen Chem, 82: 519–526.
32. Panariti A, Miserocchi G, Rivolta I 2012 The effect of nanoparticle uptake on cellular behavior: disrupting or enabling functions? Nanotechnol Sci Appl: 5: 87–100.
35. Pailler-Mattei C, Bec S, Zahouani H 2008 In vivo measurements of the elastic mechanical properties of human skin by indentation tests. Med Eng Phys, 30(5): 599–606.

36. Grice JE, Ciotti S, Weiner N, Lockwood P, Cross SE, Roberts MS 2010 Relative uptake of minoxidil into appendages and stratum corneum and permeation through human skin in vitro. J Pharm Sci, 99(2): 712–718.

37. Bakowsky H, Richter T, Kneuer C, Hoekstra D, Rothe U, et al. 2008 Adhesion characteristics and stability assessment of lectin-modified liposomes for site-specific drug delivery. Biochim Biophys Acta, 1778(1): 242–249.

38. Zaborova O, Sybachin A, Ballauff M, Varoslavov A 2011 Structure and properties of complexes of polyacationic brushes with anionic liposomes. Polymer Science Series A, 53: 1019–1025.

39. Gandhi AA, Chaskar S, Jadhav SP, Salunkhe KS. Transfersomes 2011. In: Ali Demir Sezer, editor. Recent Advances in Novel Drug Carrier Systems. Rijeka: InTech; In press.

40. Patel R, Singh S, Singh S, Sheth N, Gendle R. 2009 Development and characterization of curcumin loaded transfersomes for transdermal delivery. J Pharm Sci Res: 1(4), 71–80.

41. Madsen JT, Vogel S, Karlberg AT, Simonsson C, Johansen JD, et al. 2010 In vivo measurements of surfactant mixtures on skin structure and barrier properties. Ann Pharmacother, 44(17): 279–282.

42. Torres DR 2010 Transdermal nanocarriers. In: Escobar-Chávez JJ, Merino V, editors. Current Technologies to Increase the Transdermal Delivery of Drugs. Bussum: Bentham Science, 120–140.

43. Cappel MJ, Kreuter J 1991 Effect of nanoparticles on transdermal drug delivery. J Microencapsul, 8(3): 369–374.

44. Leary AC, Dowling M, Cussen K, O'Brein J, Stote RM 2008 Pharmaceutokinetics and pharmacodynamics of intranasal insulin spray (Nasulin) administered to healthy male volunteers: Influence of the nasal cycle. J Diabet Sci Tech, 2(6): 1054–1060.

45. Ding X, Rath P, Angelo R, Stringfellow T, Flanders E, et al. 2004 Oral absorption enhancement of cromolyn through noncovalent complexation. Pharm Res, 21(12): 2196–2206.

46. Riley MGI, Castelli MC, Paehler EA 2009 Subchronic oral toxicity of salcaprozate sodium (SNAC) in Sprague–Dawley and Wistar rats. Int J Toxicol, 28(4): 278–293.

47. Karsdal MA, Byrjalsen I, Christiansen C 2008 Optimizing bioavailability of oral administration of small peptides through pharmacokinetic and pharmacodynamic parameters: The effect of water and timing of meal intake on oral delivery of salmon calcitonin. BMC Pharmacol, 8(5): doi:10.1186/1472-6904-8-5.

48. Karsdal MA, Byrjalsen I, Henriksen K, Riis BJ, Christiansen C 2008 The clinical effect of oral salmon calcitonin delivered with 5-CNAC on bone and cartilage degradation in osteoarthritic patients: a 14 day randomized study. Osteoarthr Cartil, 18:150–159.

49. Mahler S, Leonard TW, Jacobsen J, Brayden DJ 2009 Safety and efficacy of sodium caprate in promoting oral drug absorption: from in vitro to the clinic. Adv Drug Del Rev, 61(15): 1427–1449.

50. Raafoo AA, Chiu P, Ramtoula Z, Cumming IK, Teng C, et al. 2004 Oral bioavailability and multiple dose tolerability of an antisense oligonucleotide tablet formulated with sodium caprate. J Pharm Sci, 93(6):1431–1439.
67. Tillman LG, Geary RS, Hardee GE 2008 Oral delivery of antisense oligonucleotides in man. J Pharm Sci, 97(1): 225–236.

68. Salama P, Mamluk R, Marom K, Weinstein I, Tzabari M 2010 Pharmaceutical compositions and related methods of delivery. US Patent Appl. 0105627 A1.

69. Arbit E, Kidron M 2009 Oral insulin: The rationale for this approach and current developments. J Diabet Sci Technol, 3(3): 562–567.

70. Eldor R, Kidron M, Arbit E 2010 Open-label study to assess the safety and pharmacodynamics of five oral insulin formulations in healthy subjects. Diabet Obes Metab, 12(3): 219–223.

71. Kidron M 2011 Methods and compositions for oral administration of proteins. US Patent Appl. 0014247 A1.

72. Luzio SD, Dunseath G, Lockett A, Broke-Smith TP, New RR, Owens DR 2010 The glucose lowering effect of an oral insulin (Capsulin) during an isoglycaemic clamp study in persons with type 2 diabetes. Diabet Obes Metab, 12(1): 82–87.

73. Bernstein G 2008 Delivery of insulin to the buccal mucosa utilizing the RapidMist system. Expert Opin Drug Deliv, 5(9): 1047–1055.

74. Modi P 2008 Methods of administering and enhancing absorption of pharmaceutical agents. U.S. Patent No. 7,087,215 B2

75. Crotts G, Ghebre Sellassie I, Sheth A 2002 Oral peptide pharmaceutical dosage form and method of production. US Patent 7,316819.

76. Arnold JJ, Ahsan F, Meezan E, Pillion DJ 2004 Correlation of tetradecylmaltoside induced increases in nasal peptide drug delivery with morphological changes in nasal epithelial cells. J Pharm Sci, 93(9): 2205–2213.

77. Arnold JJ, Fyrberg MD, Meezan E, Pillion DJ 2010 Reestablishment of the nasal permeability barrier to several peptides following exposure to the absorption enhancer tetradecyl-β-D-maltoside. J Pharm Sci, 99(4): 1912–1920.

78. Maggio ET, Meezan E, Ghambeer DKS, Pillion DJ 2010 Highly bioavailable nasal calcitonin—potential for expanded use in analgesia. Drug Del Tech, 10: 58–63.

79. Thanou M, Verhoef JC, Marbach P, Junginger HE 2000 Intestinal absorption of octreotide: N-trimethyl chitosan chloride (TMC) ameliorates the permeability and absorption properties of the somatostatin analogue in vitro and in vivo. J Pharm Sci, 89(7): 951–957.

80. Vetter A, Martien R, Bernkop-Schnurch A 2010 Thiolated polycarbophil as an adjuvant for permeation enhancement in nasal delivery of antisense oligonucleotides. J Pharm Sci, 99(3): 1427–1439.