Nanotechnology Approaches for Pain Therapy Through Transdermal Drug Delivery

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Abstract: The paper focuses on the advances in the field of pain treatment by transdermal delivery of specific drugs. Starting from a short description of the skin barrier, the pharmacodynamics and pharmacokinetics including absorption, distribution, action mechanism, metabolism and toxicity, aspects related to the use of pain therapy drugs are further discussed. Most recent results on topical anesthetic agents as well as the methods proved to overcome the skin barrier and to provide efficient delivery of the drug are also discussed. The present review is proposing to summarize the recent literature on the pharmacotherapeutic principles of local anesthetics and non-steroidal anti-inflammatory drugs, generally used to alleviate pain but also the drugs as nanoformulations with potential applications in transdermal delivery. A special attention is given to efficient formulations meant for transdermal penetration enhancement of anesthetics where the drug is encapsulated into macrocyclic molecules (cyclodextrins, cyclodextrin derivatives), liposomes or polymer nanoparticles and hydrogels.

Keywords: Local anesthetics, NSAID, transdermal, skin, cyclodextrin, liposome, polymer, hydrogel, nanoparticle.

INTRODUCTION

The pain removal is one of the most important problems related to our health and, since the beginning of humanity people strived to find a cure. Minimal invasiveness represents an important requirement and empirical formulations of curative mixtures were developed and perfected through generations as creams, ointments, pastes, powders, liquid extracts of animal or vegetal origins. Since the transdermal drug administration provides many advantages over oral treatments or injections, the approach of transdermal drug delivery systems has attracted the attention of many researchers. Among these advantages there can be mentioned several facts: the gastrointestinal environment is avoided and, therefore, all related inconvenient for drug formulations, the physiological contraindications of the oral route are obsolete, the patient compliance increases due to non-invasive treatment approach, first-pass effect is avoided, drugs with short biological half-live or narrow therapeutic window are more effective, the plasmatic concentrations of drugs with high biological activity or high toxicity can be accurately controlled. The goal of transdermal drug administration is to achieve local and surface effects, to target deeper tissues or even systemic circulation. The complexity of such drug delivery systems and their study should consider the skin characteristics, the type and biological activity of drug and drug-skin interactions. All these elements are specifically related to the biological availability of the drug in a certain time frame having finally as result the therapeutic effect. The modulation of this effect can be achieved taking into account the influence of the skin barrier and the overall diffusion characteristics of the drug molecules translated into their physical-chemical properties. Also, equally important is the drug delivery platform

and its interaction with the skin layer. Thus, in order to better address the needs of an improved pain therapy one should consider understanding the biological effects of the administered drug. The chemical and pharmacological properties of drugs determine their clinical features. Knowing the general mechanism of action of these compounds is useful in choosing the right therapy in clinical practice. This article summarizes and critically reviews the available literature on the pharmacotherapeutic principles of local anesthetics and non-steroidal anti-inflammatory drugs, which, in general, are used to alleviate pain and also, the drugs formulations with nanotechnological background, aimed for transdermal delivery, currently available in the scientific literature.

The transdermal permeation can be visualized in series of sequences, as follows: i. adsorption of a penetrant molecule onto the surface layers of stratum corneum; ii. diffusion through stratum corneum (SC) and through viable epidermis; iii. diffusion through the papillary dermis into the microcirculation. The permeation improvement through the stratum corneum is essential in the evaluation of the overall percutaneous absorption of therapeutic agents. When compared with the skin layers, the dermis and the hypodermis, the diffusion coefficients through stratum corneum for any given therapeutic agent are consistently lower. Usually, a drug/vehicle for a transdermal delivery cannot easily pass through the stratum corneum. Nanotechnology plays a promising role in transdermal drug delivery:

- smaller sized drug carriers can facilitate the transfer of therapeutic agents across the skin;

- drug permeation/penetration can be modified by controlling the release of active substances and increasing the period of permanence on the skin;

- assures a direct and tight contact with stratum corneum and skin appendices;

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- drug can be protected against chemical or physical interactions with skin environment.

The delivery of drugs across the skin membrane can be achieved by taking advantage of natural routes provided by the nature itself through passive diffusion or through active transportation routes. The skin has been naturally developed as the main barrier against the surrounding environment. Therefore, the main challenge which arises in topical drug administration is to find appropriate ways to overcome this barrier. Mechanisms of enhancing drug permeation may refer to increase in drug diffusivity in the vehicle and/or in the skin, increase in drug partitioning and diffusion, disturbance in the corneocytes barrier and increased local blood flow.

The main strategies employed in resolving these tasks are correlated with the skin modification in order to facilitate the drug diffusion or with drug molecule which can be modified or included in carriers capable to cross the intact skin. The first option, regarding the skin modification supposes two main approaches, a physical and a chemical one, respectively. The current paper will further focus on describing the advances in the field of drug enhanced permeation through skin and, especially, the nanotechnological pathways to achieve effective local pain therapy.

HISTOPHYSIOLOGY OF THE SKIN

The skin, the outer covering of the organism, accounts for about 15% of the total adult body weight [1]. The skin is composed of epidermis and dermis. The epidermis represents a stratified squamous epithelium and consists of cells named keratinocytes, arranged in 5 layers: basal layer or *stratum basale*, spinous layer or *stratum spinosum*, granular layer or *stratum granulosum*, *stratum lucidum* and cornified layer or *stratum corneum* [1-13]. Histologically, two varieties of epidermis are described: the thick epidermis

disposed at palmes and soles and the thin epidermis covering the rest of the body [1-3]. The stratum corneum or the superficial layer contains flattened cells with no nucleus and organelles, but filled with keratin. The cells are flat, with acidophilic appearance and permanently desquamate isolated or in very fine flaps [1, 10].

Thin epidermis is different from the thick epidermis and because of its reduced thickness, the spinous layer has fewer cell rows, the granular layer is discontinuous, the lucidum layer may be absent and the corneum layer is thinner [3, 10]. In addition to keratinocytes, other types of cells are also found: melanocytes, Langerhans cells and Merkel cells [1, 6]. Melanocytes derived from the neural crest represent dendritic cells scattered among the low columnar cells of the basal layer or in dermis.

The dermis, consisting of connective tissue, represents the support of the epidermis, also ensuring the nutrition of the epithelial cells. The dermis is well vascularized and innervated and it is divided into two areas: the superficial papillary dermis consisting of loose connective tissue rich in type III collagen fibers and the deep reticular dermis, a dense connective tissue which contains the skin appendages (sebaceous glands, sweat glands and hair follicles [1, 4].

The hair follicle presents two portions: a free portion or the hair shaft and another portion in the thickness of the skin or the root. The terminal portion of the root is called the hair bulb. The hair follicle has a central medulla with fewer cells slightly keratinized surrounded by the cortex, with highly keratinized cells. Outside the cortex, the cuticle of the hair follicle, the internal epithelial sheath, the external epithelial sheath and a fibrous connective tissue sheath can be identified [1, 4].

The skin presents sensory receptors for tactile sensations, which are divided into free or encapsulated nerve endings, depending on the absence or presence of the connective tissue capsule. From the
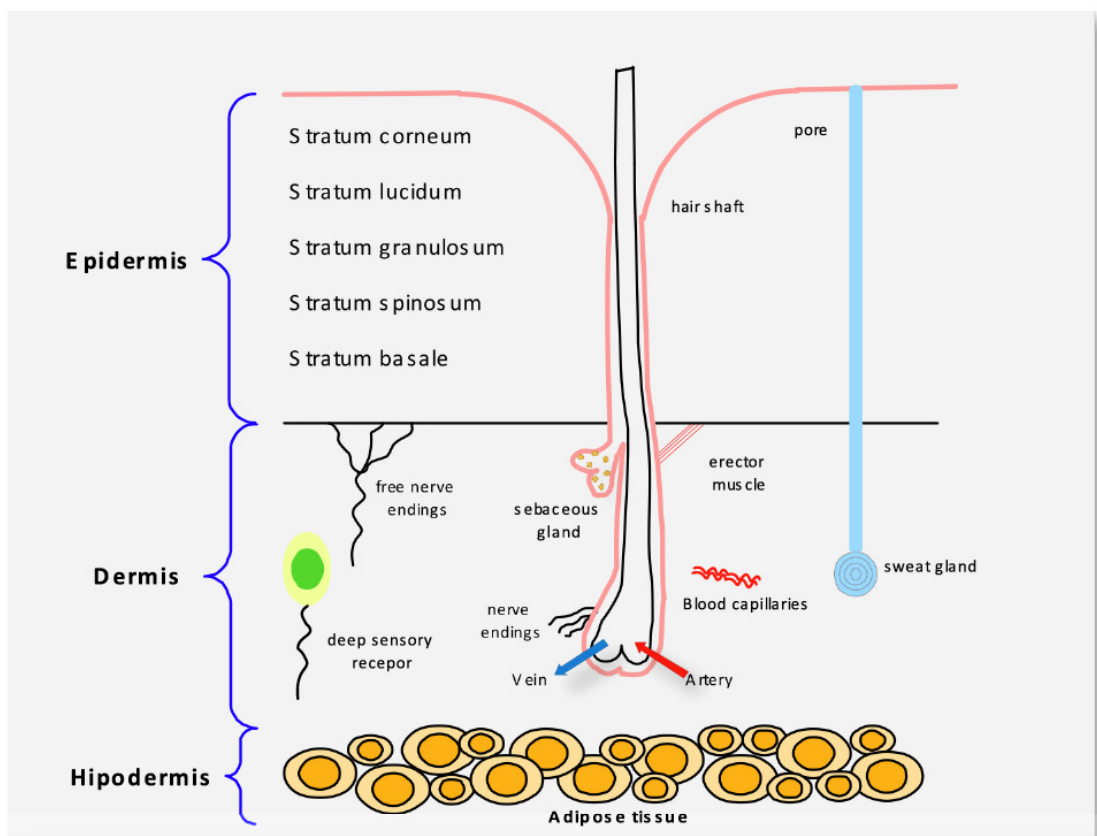

Fig. (1). Schematic representation of skin structure.

structural point of view, these receptors are either dendritic nerve endings or specialized non-neuronal cells that are responsible for the perception of tactile sensations and pressure. They are generally known under the name of mechanoreceptors, such as: Pacini corpuscles, Meissner corpuscles, Ruffini endings, Merkel corpuscles, nerve fibers around the hair follicle, and free nerve endings [1, 4]. Free nerve endings are receptors that generally detect sensations of pain, heat and cold. These receptors are present in the dermis where they are intensely branched, cross the basal membrane and spread into the deeper layers of the epidermis [4, 13] Fig. **1**.

PHARMACOLOGY OF LOCAL ANESTHETICS

Pharmacodynamics

Local anesthetics drugs interrupt nerve conduction by interfering with neuronal cell membrane excitation and inhibiting the influx of sodium ions through channels and ionophores at this level.

Normally these channels are in resting state, in which sodium ions entry is denied. When the nerve is stimulated, the sodium ionophore's structure alters. The channels pass from the resting state in the activated or open state during which the sodium ions diffuse into the cell initiating depolarization. An insight on the sodium channel states helps to understand the preferential sensitivity of local anesthetics for various classes of neuronal fibers. Neural fibers with more rapid firing rates and smaller fibers are more susceptible to local anesthetic drugs given the fact that a certain volume of local anesthetic solution can easily block the required number of sodium channels for impulse transmission. Thus, the sensitivity of different fibres to firing is decreasing in the series: tiny autonomic fibers > sensory fibers > somatic motor fibers. In respect to nonmyelinated fibers versus myelinated fibers, the latter are blocked easier due to the fact that local anesthetic drugs pool in proximity to the axonal membrane. This explains why C-fibers, which are nonmyelinated and with a smaller diameter, are the most resistant to local anesthetics. During the sodium channel blockade, the local anesthetic binds in the "inner vestibule" of the Na+ channel [14] (Fig. **2**).

As the membrane potential increases (to around 20 mV) this triggers the closure of the inner h gate and the ionophore enters an inactive state. The deactivated state is formed as a result of closure of the outer m gate once the membrane potential reaches -60 mV. Whilst in an inactivated or deactivated state, the nerve is resistant to further stimulation. When the sodium channel is closed the local anesthetic can only gain access via the membrane as free base. However, when the sodium channel is open, as is the case in the activated, and to a lesser extent in the inactivated state, the ionized local anesthetic can also gain access to the nerve via the channel. In addition to this, the ionized form may enter from outside if the ionophore is repeatedly activated or opened. This gives rise to frequency-dependent block. The onset of block with agents that have more ionized molecules outside the membrane can therefore be accelerated by stimulation [15]. The effects dependent on frequency

and voltage of local anesthetics happen due to the repeated depolarization which increases the probability that a local anesthetics molecule will find a Na+ channel in the activated state. Thus, a resting nerve is less sensitive to local anesthetic blockade than a nerve that is repetitively stimulated. The pharmacodynamics effects of local anesthetics also depend on their spread away from the site of injection, their fixation at local binding sites such as surface proteins and lipids, especially myelin, and the inherent permeability of nerve fibers.

Pharmacokinetics

Most drugs are either weak acids or weak bases and exist as a combination of particles in ionized (charged) and un-ionized forms [14-41]. The degree of ionization is dependent upon the physiochemical properties of the drug and the environment in which it is located, thus the pKa and pH are particularly important. The pKa is the pH where 50% of the local anesthetic will remain in uncharged form and 50% will exist in charged form. For local anesthetics to become soluble, they are prepared as hydrochloride salts. After injection, the base is liberated by the relatively alkaline pH of tissue fluids. In tissue fluid the anesthetic will be present in both an ionized and un-ionized form. Movement of anesthetic molecules occurs by diffusion along a concentration gradient. The cationic form is primarily responsible for blocking the sodium channel and preventing the action potential from being propagated. Also the uncharged form may contribute by penetrating the lipid membrane. It diffuses through the nerve sheath, perineuronal tissues, and the neuronal membrane to reach the axoplasm and it inserts itself into the lipoprotein matrix, reducing the sodium channel diameter and consequently, sodium conductance. Ultimately, both forms of the local anesthetic are important for neural blockade.

Absorption

The absorption of local anesthetics into systemic circulation depends on total dose and concentration of drug administered, the route of administration, the vascularity of the administration site, and the presence of added vasoconstrictor. Intact skin is a near complete barrier to local anesthetic penetration. In this matter, special local anesthetic formulations or delivery methods are employed to facilitate transcutaneous transfer.

The permeation of drugs through the skin involves the diffusion through the intact epidermis through the skin appendages (hair follicles and sweat glands) [16]. The process is limited by the stratum corneum. The main known penetration routes are: the intercellular lipid route, the transcellular route and the follicular penetration. Other factors affecting absorption include the degree of ionization, lipophilicity and plasma protein binding. Absorption tends to be related to local tissue perfusion but the rate of dissociation of the respective local anesthetic from both proteins and adipose tissue at the site of injection is the agent specific determinant.

Local anesthetics exhibit an initial rapid phase followed by a later slow phase, whose contribution to overall uptake depends on
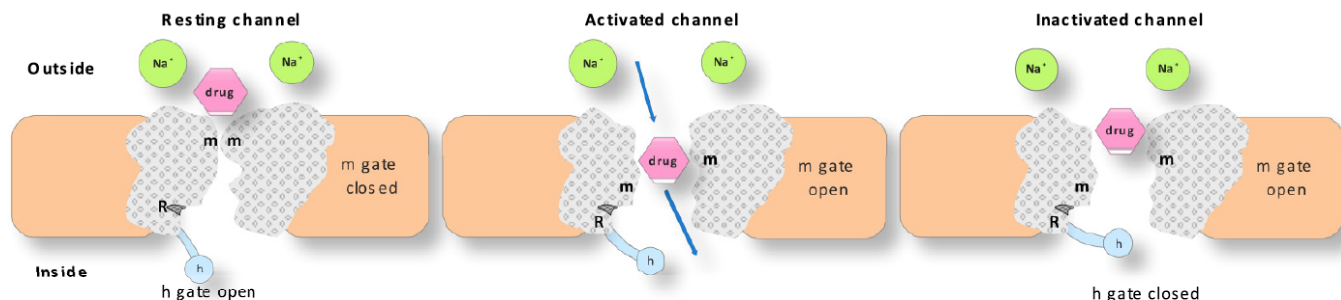

Fig. (2). Action mechanism of analgesic drugs.

the extent of local tissue binding of the individual agent. Plasma protein binding is believed to correlate closely with the degree of protein binding on the neuronal membrane.

Distribution

Generally, local anesthetics are distributed to and absorbed by organs with a high blood supply, such as the heart, brain, lungs and liver. Adipose tissue on the other hand has a high affinity for local anesthetics [16]. The distribution of the drug is influenced by the degree of tissue and plasma protein binding of the drug. The main plasma proteins involved in drug binding are albumin, 1-acid glycoprotein and the lipoproteins. 1-acid glycoprotein is capable of binding with high affinity the basic drugs, such as local anesthetics [18]. The potency of the local anesthetics and the duration of action depend on how highly the proteins bind on the drug [19].

Addition of chemical radicals to the aromatic or the amine end of local anesthetics can increase its binding to proteins. However changes in protein binding of the local anesthetics do not change the plasma concentrations of free drugs.

Metabolism

Ester and amide compounds have different metabolization pathways. The aminoester-linked local anesthetics are hydrolyzed rapidly by plasma and tissue cholinesterases, consequently they have a short elimination half-life. Esters metabolites excretion is renal. Amides are metabolized in the liver by amidases, a much slower process [17]. The clearance of the drug will depend on both hepatic blood flow and the capacity of the enzyme systems. Metabolites may retain local anesthetic activity and toxicity potential, but usually at lower potency than the initial compound.

Toxicity

Toxicity results from a high plasma concentration of local anaesthetic agent. Acidosis, hypoxia and hypercarbia all potentiate the local anaesthetic toxicity [17]. Unforeseen local anesthetic toxicity could be a consequence of the drug metabolism alterations determined by co-morbidities like cardiac or hepatic insufficiency, modifications in plasma protein binding or interactions with other drugs.

Clinically, the toxicity is usually heralded by initial central nervous system features later to be followed by cardiac arrhythmia, myocardial depression as well as peripheral toxicity manifested as irreversible conduction blockade or other neurological symptoms. Complications such as hypersensitivity reactions, allergic reactions or methemoglobinemia can also occur with all local anesthetics administration.

An Update on Topical Anesthetics

There are important advantages of topical anesthetics, including superior safety profile and easy administration, but also disadvantages such as impaired absorption, local procedures, delayed onset of anesthesia, local side effects, and also high costs of the drug [25].

All anesthetic agents share a similar structure, consisting of: an aromatic part, usually a lipophilic benzene ring, an hydrophilic amine part, and intermediate chain. The lipophilic part ensures diffusion through the nerve membrane, being directly related to the anesthetic potency [32, 33]. Besides lipid solubility, other qualities of the anesthetic such as the pH, pKa level, protein binding, or vasodilator effect, also influence its efficacy. This is also dependent on the skin area on which the anesthetic is applied, especially its vascularity and surface, as well as on the duration of application [34]. There are many dosage forms available, such as patches, sprays, gels, lotions, creams, or ointments, ensuring various options, to be chosen by the clinician according to the case. Some of them also provide increased absorption of the anesthetic, such as the transdermal patch [35, 36].

Current available agents used for topical anesthesia include benzocaine, butamben, dibucaine, dyclonine lidocaine, oxybuprocaine, pramoxine, proparacaine, proxymetacaine, and tetracaine.

They are applied on the skin, or on mucosal surfaces, such as the mouth, conjunctiva, or genitalia [37, 38]. Due to the complex structure of the skin, penetration of topical anesthetics applied on it remains a puzzling issue. While solid agents are only superficially absorbed when applied on intact skin, eutectic mixtures as they melt at lower temperatures than their single components, ensure higher concentrations of the active principle, thus superior local anesthesia. Trying to overcome this, various liposomal preparations, nanovehicles, iontophoresis, skin needling, or occlusion procedures have been used [39].

A large number of topical anaesthetics are currently approved, the most commonly used ones being presented in the table below in alphabetic order (Table **1**). As these agents try to validate their efficacy, new agents are constantly being developed [40, 41]. A promising approach is that of using nano-formulation to increase skin penetration of topical anaesthetics. Recent publications present promising results using a lidocaine nano-formulation.

PHARMACOLOGY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID)

Ketoprofen (2-(3-benzoyl phenyl)-propionic acid), a well-known example of this class, is a NSAID derived from propionic acid (similar to ibuprofen, naproxen, flurbiprofen, dex-ketoprofen) which possesses analgesic and antipyretic properties [42, 43].

Pharmacokinetics

Ketoprofen absorption has been studied in a variety of forms including solid dispersions, tablets or oral capsules, tablets or controlled release capsules, topical gels, microspheres, microemulsions, nanocapsules and rectal suppositories.

Ketoprofen is extensively linked to plasmatic albumin (99%). The distribution volume is of 0.1 L/kg. The liver intensely metabolizes ketoprofen and the conjugation products are eliminated by renal excretion.

Action Mechanisms

Like all NSAIDs, the underlying process of ketoprofen pharmacodynamic activity is the inhibition of cyclooxygenase and through it the inhibition of the arachidonic acid metabolism [44, 45]. Activation of the arachidonic acid cascade leads to formation of eicosanoids, through three major pathways: cyclooxygenase (COX) pathway, lipoxygenase pathway and P450 cytochrome pathway. Cyclooxygenase was purified for the first time in 1976 and cloned in 1988. In 1991 a second enzyme was identified, consequently named COX-2. The two isoforms are almost identical in structure but different in substrate, inhibitory selectivity and intracellular position. COX-1 is a structural enzyme expressed in numerous cells, implicated in ensuring homeostasis: maintaining gastric mucous integrity, renal blood flow in injured kidney, platelet aggregation, pregnancy and childbirth. COX-2 is an inducible enzyme triggered by inflammatory and mitosis influencing factor, being involved in inflammatory response and cancer. It is located in the brain and spinal cord where it may be implicated in neural transmission, especially in pain and fever. Both COX-1 and COX-2 play a role in inflammation and pain modulation, being fairly important for cardiovascular, renal and reproductive processes [46, 47].

Ketoprofen is a nonselective COX-1 and COX-2 inhibitor, which explains the anti-inflammatory effect but also some side effects like those on the gastrointestinal tract. Unlike other NSAIDs, ketoprofen has a central analgesic action in addition to its peripheral one, which is expressed on a medullar and supermedullar level [48].

OVERCOMING THE SKIN BARRIER

As it was already mentioned, *stratum corneum* represents the main barrier against drug transport and despite the major advantages of the transdermal delivery of drugs; the latter is limited by

Table 1.   Marketed topical anaesthetics.

Generic name	Examples of brand name	Anesthetic Concentration	Dosage Forms	Duration of anesthesia (minutes)
Benzocaine	Americaine, Anacaine, Cepacol, Dermoplast, Lanacane, Solarcaine	0.5-20 (% mass)	cream, gels, lotion, ointment, solution	15-45
Dibucaine	Nupercainal, Rectacaine	1 (% mass)	cream, ointment	30-60
Dyclonine	Cepacol, Sucrets Complete	0.5-1 (% mass)	aerosol, lozenge	30-60
Lidocaine	ELA-Max, Lidoderm, Nervocain, Xylocaine	2-5 (% mass)	cream, liposomes, ointment, patch	15-45
Pramoxine	ProctoFoam,	1 (% mass)	aerosol foam, cream, ointment	30-60
Tetracaine	Pontocaine, Niphanoid	0.5-2 (% mass)	cream, gel, solution	30-60
Lidocaine/ Prilocaine	EMLA, Dolocaine	2.5/2.5 (% mass)	cream, patch	30-60
Lidocaine/ Tetracaine	Synera Patch, Tetralid	70 mg / 70 mg	cream, patch	30-60

stratum corneum intercellular lipids composed of ceramides, free fatty acids, cholesterol sulphate and minor lipids. These lipids are playing an essential role in barrier functionality and also limit the applications of transdermal drug delivery due to the fact that they minimize the permeability of *stratum corneum.* In the following section some of the drug delivery systems will be described in the context of transdermal drug delivery of the local anesthetics.

The physical methods of active trandermal drug delivery are invasive and generally ensure the skin penetration by creating spaces between the corneocytes. The simplest way is to peel locally the upper layer of skin cells prior drug administration. Another method is skin microscission, which comprises a jet of abrasive particles, such as Al_2O_3, through a mask to result in a certain number of pores with defined size(50-200 μm) on the skin surface [49]. Among the physical methods with lower degree of skin damage, generally used to increase skin permeability for drugs passage, there can be mentioned: iontophoresis, sonophoresis, electroporation, microneedles, needleless jet injectors, magnetophoresis, photomechanical waves, electron beam irradiation [50] or creating skin disturbances applying heat (chemical heating, thermoporation, thermal ablation, laser ablation) [51]. Also, these methods can be used in synergistic combinations with other penetration methods: chemical-iontophoresis, chemical-electroporation, chemical-ultrasound, iontophoresis-ultrasound, electroporation-iontophoresis, electroporation-ultrasound and pressure waves-chemicals.

The use of ultrasounds leads to local ablation in stratum corneum but having as secondary effects the tissue disruption and may affect the nearby organs down to a certain depth [52]. Methods with disruption effects more localized to the skin upper layer are infrared laser light [53] or high frequency radio-field [54]

The topical administration of lidocaine was accelerated by applying a short (~15 s) ultrasonic pretreatment (Sonoprep®, Echo Therapeutics Inc.) of the skin exposed to a strong ionic surfactant (1% SDS) [55]. Also, the active administration of ketoprofen through sonophoresis [56] and sodium nonivamide acetate II through iontophoresis were reported [57].

The chemical treatment of the skin aimed for improving the drug diffusion uses the so called chemical enhancers. They directly affect the skin structure by acting on corneocytes or on intercellular lipids. Thus, the chemical enhancers may promote the extraction of lipids resulting in formation of diffusion pathways or they can induce, by partitioning, the destructuration of highly ordered lipid lamellae, hence, causing their fluidization. Also, the transport of the drug can be stimulated by chemical enhancers through increased thermodynamic activity of active principles in the topical formulation. Among the chemical enhancers there can be mentioned several categories: water, acids (fatty acids mostly), alcohols, amines, hydrocarbons, amides (azones are among the first chemical enhancers to be used), esters, surfactants, sulfoxides, *etc.* [58]

The use of single chemical enhancers give reasonable performances in skin penetration, however, increasing the concentration at desired levels of drug diffusion can raise the enhancer concentration at toxic levels. Therefore, combinations of different enhancers are used provided that their association has synergistic effects. For example, the combination of certain solid drugs with chemical enhancers can lead to the formation of hydrophobic eutectic mixtures, at room temperature. Thus, the drug in fluid form can easily penetrate through skin layers either by increased partitioning, either by disrupting the lipid lamellae. Some examples of pain therapy drugs tested for topical administration in liquid form are based on eutectic mixtures of ibuprofen with terpenes [59] or lidocaine with menthol [60]. However, the best known example is the EMLA cream containing eutectic mixture of lidocaine and prilocaine.

Inclusion Complexes

The most well known molecules used for the preparation of drug inclusion complexes are cyclodextrins and their derivatives. Cyclodextrin molecules with pharmaceutical relevance, α-, β-, γ-cyclodextrin, are composed of 6, 7 or 8 glucopyranose rings bound in a 1, 4-configuration and forming rings with a lipohilic cavity and hydrophilic exterior. The inner cavity plays an important role concerning the pharmaceutical applications of these molecules allowing the formation of inclusion complexes of lipophilic drugs resulting in increased solubility in hydrophilic environment.

The formation of the inclusion complex with a certain CD molecule is dependent on the guest properties such as polarity and geometry which are dictating the type of the molecule to be included into CD cavity [61]. In some cases the complexation may be influenced by the electrical nature of the guest. For example, the formation of host-guest complexes between cucurbituril and local anesthetic is greatly influenced by the protonation state of the guest molecule [62].

The complexation of guest molecules is a reversible process allowing the release of the entrapped organic compounds. According to the structural particularities of either the host or guest this

complexes are characterized by a stability constant, characteristic to any given guest/host combinations. The physical-chemical properties of cyclodextrins can be adjusted by chemical modification at the level of hydroxyl groups, thus the resulting derivatives have different complexation abilities and solubility. Derivatives of β-cyclodextrin with increased water solubility (e.g. hydroxypropyl-β-cyclodextrin, HP-β-CD, sulfobutylether-β-CD, SBE-β-CD, randomly methylated-β-CD, RAMEB) are most commonly used in pharmaceutical formulations. The use of drug inclusion complexes in CD is related to several effects on the pharmaceutical product [63, 64]:

- Effect on drug solubility and dissolution by increasing the solubility and dispersability of hydrophobic molecules making them available in higher concentrations than normal conditions.

- Effect on drug bioavailability by acting as permeation enhancers through different biological membranes e.g., skin, mucosa, or the eye cornea.

- Effect on drug safety by ameliorating the irritation caused by a certain dose of drugs. The reduction of the dose required for optimum therapeutic activity due to increased drug solubility.

- Effect on drug stability by improving the stability of labile drugs against degradation by dehydration, hydrolysis, oxidation, and photodecomposition and thus increase the shelf life of drugs.

Complexation with cyclodextrins has been reported to both increase and decrease skin penetration. In their review, Loftsson *et al.* concluded that the effect on skin penetration is influenced by cyclodextrin concentration [65]. Other authors [66] are attributing the skin penetration enhancement by cyclodextrins to extraction of stratum corneum lipids, thus opening penetration channels for the drug molecules. Thorough studies stated that only methylated CDs applied in high concentrations (10-20%) in aqueous solutions can have influence on the stratum corneum [67-69]. The effect of skin lipids extraction was not observed for β-, HP-β- and γ-CDs [70]. Evaluation of the permeation enhancement activity of β-CD and 2-HP-β-CDs alone and complexed with other penetration enhancers for the model lipidic drugs, using human cadaver skin, demonstrated that the penetration enhancement didn't occurred and complexation of lipophilic terpene with the CDs reduced enhancer efficacy [71].

In general, most of the studies seem to agree that drug diffusion through the skin may be improved by the addition of cyclodextrins into the drug formulations, but in certain amounts depending on the drug and other particular conditions. The improvement effect is not due to the fact that CD/drug complexes play the role of carrier, as there weren't found clear evidences for skin penetration of the complexed drug. The CD alone has low performances in skin penetration [72]. Nevertheless, finding traces of these complexes would be a difficult task since decomplexation is possible. Thus, the mechanism skin permeation of drugs in presence of CD and their derivatives is related to the increased availability of the drug molecules at the skin surface as CDs would contribute to a better homogenization of the hydrophobic drug formulations. Increasing the cyclodextrin concentration decreases the formulation ability to penetrate the skin and may reduce the bioavailability of the system [73].

Dollo *et al.* [74, 75] studied the complexation behavior of local anesthetics in cyclodextrins. The inclusion complex-forming abilities of five local anaesthetics of the amide-type, bupivacaine, etidocaine, lidocaine, mepivacaine and prilocaine were proved. Moreover, 2-hydroxypropyl-β-cyclodextrin and heptakis (2, 6-diomethyl)-β -cyclodextrin showed the best complexing abilities, forming the strongest complex with bupivacaine. Furthermore, the formation of inclusion complexes can affect the *in vitro* transfer rate of drug.

The effect of sulfobutylether-β-cyclodextrin (SBE-β-CD) on the biopharmaceutics of local anesthetic bupivacaine (BVC) in a rabbit sciatic mode was also studied [76], showing that injectable aqueous formulation of complexed drug allowed an improvement of the therapeutic index of BVC. The effectiveness of supercritical carbon dioxide technique for preparing solid complexes between β-cyclodextrin and three local anesthetic agents (benzocaine, bupivacaine, and mepivacaine) has been studied by Al-Marzouqi *et al.* [77]. Also, the effects of β-cyclodextrin on the solubility properties of lidocaine base were shown to appreciably influence the physico-chemical and pharmaceutical properties of drug in its products, possibly via weak inclusion complexation [78]. De Lima *et al.* [79] reported the improvement of tetracaine antinociceptive effect by inclusion in cyclodextrins. The local anaesthetics procaine, tetracaine, prilocaine and dibucaine, along with procainamide were found to form stable guest-host complexes with cucurbituril in aqueous solution, as the result of ion-dipole interactions between the protonated amine groups on the guests and the carbonyl-rimmed portals of the host molecules [62]. The steric physical connectivity with the host of the guest molecules included in the host cavity depend on the solution pH and the relative sites of protonation.

In spite of the fact that the complexation ability of a wide range of local anesthetics has been studied, there is a rather low number of products derived from these studies which are clinically applied for transdermal drug delivery. In fact, there are several patents, already reviewed [80], which refer to various combinations of cyclodextrin (derivatives)/local anesthetics compositions [81-84]

The complexation of NSAIDs (nonsteroidal antiinflamatory drugs) with β-cyclodextrin has also been studied by several groups [85-88]. However, there is only one study mentioning the transdermal delivery of ketoprofen from its β-CD and HP-β-CD inclusion complexes [89]. In fact the authors showed that diffusion rate of ketoprofen from its inclusion complexes was depending on the drug enviroment in the order of carbopol gel - oil/water emulsion - fatty ointment.

Liposomes

There are already more than 50 years since Bangham discovered that in aqueous environment phospholipids, as almost all amiphilic molecules, formed closed vesicles having an aqueous core limited by a phospholipid bilayer [90]. Liposomes become in time a very reliable carrier for drugs due to theirs very important advantages such as [91]:

- Biocompatibility and biodegradability due to the lack of both toxicity and immunogenicity

- Similarity of liposomes with cells and other cellular organites from size and composition points of view [92-94]

- Simplicity of preparation and the fact that size of liposomes can be adjusted function of application needs

- The possibility of including both hydrophilic and hydrophobic active principles as liposomes improve the solubility of lipophilic and amphiphilic drugs

- pH and temperature sensitivity

- Minimal loss of entrapped drug storage

The size of liposomes depends on both composition and preparation method and usualy vary from around 25 nm to more than 1 μm in diameter. Liposomes are generally classified in MLVs - multilamellar vesicles range in size from 500 to 5000 nm which consist in several concentric bilayers; LUVs - large unilamellar vesicles range in size from 200 to 800 nm; and SUVs - small unilamellar vesicles are around 100 nm in size and formed by a single bilayer [95].

Liposomes are made from natural (sphingolipids, glycosphingolipids, sterol lipids) or synthetic phospholipids as saturated or unsaturated lipid, each class of materials presenting advantages

which become essential for a specific application. For example, in the natural phospholipids case, lecithins (phosphatidylcholine type lipids), these represent the major component of the cellular membrane so the resulted liposomes will not present any problem of biocompatibility and they will not be attacked by the immune system. On the other hand, synthetic phospholipids present a predictable and well defined composition and they can be designed to have certain properties, by varying acyl chain length and saturation degree [96].

Up to present many different methods were developed to prepare liposomes of different sizes, structure and size distribution. The methods can be rationalized as i. mechanical methods including lipid *thin film hydration and ultrasonication*; ii. methods which are based on the replacement of the solvent which include *reverse phase evaporation, ether or ethanol vaporization (ether or ethanol injection)*; iii. methods which are based on size transformation or fusion of preformed vesicles including *freeze-thaw extrusion, freeze-thaw sonication and dehydration-rehydration methods*. Anyway, the most frequently used methods are ultrasonication, reverse phase evaporation and detergent removal.

Regarding the use of liposomes for transdermal delivery of drugs, it was already demonstrated that they played several key roles in transdermal delivery such as retention effect into stratum corneum, penetration enhancers altering the intercellular lipid lamellae within this skin layer, depot for sustained release of drugs and drug carrier to deliver entrapped drug molecules into or across the skin [97].

The mechanism of interactions between liposomes and stratum corneum have been intensively investigated by many techniques such as: differential scanning calorimetry (DSC), X-ray diffraction, confocal laser scanning microscopy and most of the researchers concluded that the main mechanism of enhancement of drug delivery through the skin is the direct transfer of the drug between the phospholipid bilayers of liposomes and the lipid contents of the skin [98]. Recently, Brewer and his colleagues used Raster image correlation spectroscopy (RICS) in correlation with multiphoton excitation fluorescence microscopy in order to give a diffusion map at different levels of the stratum corneum using fluorescent loaded liposomes (with both hydrophilic and hydrophobic dyes) giving in the same time information about liposomes integrity. The authors consider that stratum corneum, which presents a low water content, is responsible for liposomes loss of integrity and content [99].

Nevertheless, there are a lot of controversions concerning the mechanism by which can cross the layers of the skin: one of the route is through hair follicules when liposomes can penetrate the deeper skin layers, via the lipid-rich channel which covers the follicular duct, with subsequent entry into the follicular cells [100]. Moreover, it seems that also the charge of liposomes surface presents an effect on transdermal penetration. For example, comparing positively charged with neutral liposomes, Qin and his coworkers demonstrated that the cationic liposomes enhanced the transdermal delivery [101].

Many types of liposomes have been used for local anaestetic encapsulation including conventional liposomes but also ultraflexible or ultradeformable liposomes, transfersomes, flexozomes, ethosemes, *etc*. [102, 103]. In the last years, conventional liposomes are replaced with "highly deformable" liposomes known for their skin penetration ability [104]. These liposomes contain an activator (chain of surfactant) which destabilizes the lipid bilayer from liposomes and making it elastic and flexible [105]. On the other hand, it was observed by Zhang *et al.*, that ethosomes present enhanced penetration ability due probably to the ethanol first of all by reducing the density of the epidermal membrane and second of all by making them more deformable and facilitating the skin penetration into deeper structures [106].

Currently, on the market there are only two commercially available formulations based on a local anaestetic: ELA-MAX, produced by Ferndale Laboratories a topical anesthetic cream with 4% lidocaine, and LMX-4, which is a topical liposomal formulation of 4% lidocaine and previously known as ELA-Max [107]. ELA-MAX is a drug used for pain relief resulting from minor cuts, abrasions, minor burns and skin irritations and is applied to intact skin for 15 to 40 minutes. LMX has a high absorption rate through the skin and within 20 to 30 minutes produces an anesthetic effect which lasts for approximately 1 hour after application. LMX-4 is approved in the USA as an analgesic drug in cases of pruritus associated with minor burns, cuts, and insect bites [108].

Clinical studies have been performed for comparing EMLA (commercial eutectic mixture of the local anesthetics lidocaine and prilocaine) with lidocaine liposomal formulations and the conclusion was that the latter presents a longer effect in comparison with non vesicular carriers [109]. Other studies, compared EMLA with liposomal formulation for dermal anesthesia which contains 5% tetracaine, showing that the liposomes based system were much more effective than EMLA formulation [110, 111].

In early '90 Foldvari and his coworkers compared the encapsulation of various local anesthetic agents into liposomes and formulate a topical tetracaine preparation which can provide deep anesthesia [112]. They have obtained a physically stable topical liposomal tetracaine (2%) product can be prepared with good application properties but with a relatively short shelf-life.

Few years later, Yu *et al.* prepared bupivacaine loaded liposomes for prolonging the effect of local anaestesia and demonstrated that, if the local anesthetic agent (bupivacaine) is encapsulated in MLV liposomes, a longer duration and higher intensity of local anesthesia in comparison with the free drug solution will be provided [113]. Currently, bupivacaine is clinically used but not for transdermal delivery but for postsurgical anesthesia [114].

Mura *et al.* prepared multilamellar (MLV) and small unilamellar (SUV) vesicles entrapping benzocaine using phosphatidylcholine-cholesterol as lipophilic phase and ethanol-water as hydrophilic phase and they have observed that all liposomal-benzocaine formulations showed sustained release properties and a more intense anaesthetic effect than plain drug [115].

Benzocaine loaded multilamellar liposomes based on phosphatidylcholine and cholesterol were prepared by the group of Singh and the *in vivo* tests revealed a longer duration of action in the case of liposomal formulations in comparison with the free drug [116].

The major advantage of local anesthetic drugs encapsulation into liposomes is the slow release rate prolonging the anaesthetic effect and in the same time minimizing the toxicity at cardiovascular respectively central nervous level [117].

Recent studies indicate that bupivacain represent the most cited local anaestetic used in liposomal formulations, whereas liposomes loaded with benzocain or tetracain are already used in commercial topic formulations such as OptisomeTM [80].

Few but promising studies are dedicated to other lipid systems such as **lipids microemulsions**. Microemulsions like liposomes have penetration enhancing properties. Local anaesthetics encapsulated in microemulsions load to fast transdermal penetration and effect [118]. A group of researchers analyzed *in vivo* transdermal permeation of tetracaine hydrochloride encapsulated in lecithin water-in-oil and oil-in-water microemulsion and they have observed that the analgesic response of tetracaine hydrochloride is dependent on the lecithin microemulsion composition and on drug concentration [119].

Encapsulation of low solubility drugs into liposomes implies the use of organic solvents and it is limited by the possible issues related to the destabilization of the lipid double layer. In addition,

the drugs immobilized in the lipid bilayer are much faster released after their administration than when are solubilized in the aqueous core of the liposomes, leading to the incomplete valorification of the "carrier" quality through biological membranes. The first way to overcome this drawback is to solubilized the drug into a solvent miscible with water like ethanol and to further continue with liposomes formation using this mixture as aqueous phase [120]. The second way is represented by the preparation of soluble inclusion complexes using cyclodextrins without use of organic solvents and obtaining in the end drug in *cyclodextrins in liposomes complex systems*. These are useful for improving the drug solubility and stability but also for an adequate *in vivo* behavior of the drug with low water solubility overcoming the rapid release, as it was observed in the case of conventional liposomes. In addition, complexation with cyclodextrins improves drug solubility and skin permeability favouring drug bioavailability after a topical administration.

The use of cyclodextrins in topic formulations present important advantages [121] such as elimination of irritative/toxic effects at the skin and mucosa level, elimination of the use of excipients like pH regulators, solubilisation agents or organic solvents, improvment the drug absorption by enhancing its bioavailability and reducing drug metabolism at the administration site. The use of cyclodextrins has also limitations such as low complexation efficiency, high mass differences between cyclodextrins and "guest" which can lead to a limited loading even when the complexation efficiency is very good (this is very important for the drugs that need high doses or concentration) and, finally, the modification of the rheological characteristics of the drug formulation.

Solid Lipid Nanoparticles

Among the carriers used for transdermal drug delivery there should be mentioned the solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugates [122]. SLNs and NLCs differ mainly by lipid composition; SLNs are composed only by solid lipids, whereas NLCs are made from a combination of liquid and solid lipids [123]. The encapsulation of local anesthetics in SLN has been performed in the case of tetracaine. The release of drug was fast with a high burst initial release, as generally observed for these type of drug carriers [124]. However, reinforcing the SLN leaded to a better carrier physical stability and better tetracaine release profiles [125]. Another example consists in lidocaine loaded SLN and NLC included in hydrogels formulations [126]. The topical applications of these formulations were proved to be superior to a commercial (Xylocaine gel). NLC dispersions containing benzocaine and lidocaine exhibited slow *in vitro* release and high drug encapsulation efficiency. *In vivo* experiments demonstrated that the lidocaine and benzocaine loaded NLCs are capable of improved local anesthesia in rats [127].

Hydrogels

Hydrogels are tridimensional polymer networks capable to accommodate water or biological fluids in the polymeric matrix. Among the most interesting hydrogel systems which are currently attracting attention of researchers, the so called "smart" polymers are capable of reversibly change according to external stimuli like pH, temperature or electric field. The transdermal delivery is particularly concerned by the thermosensitive hydrogels which are able to increase their viscosity at body temperature, being liquid at lower temperatures [128]. The topical administration of lipidic local anesthetics via hydrogels allows the simultaneous use of various penetration enhancers. For example, Jin *et al.* studied an hydrogel system based on hydroxypropyl methylcellulose and poloxamer 407 containing procaine and glycols, bile salts and non-ionic stabilizers [129]. They showed that the drug diffusion from such formulation is temperature dependent. In fact, poloxamer 407 was largely used for its thermogelling properties to prepare various drugs formula-

tions: indomethacin/isopropyl myristate or (+)-limonene [130], capsaicin and nonivamide [131], ketoprofen/ limonene nerolidol, fenchone and thymol [132] ketoprofen/ethyl alcohol, isopropyl alcohol, propylene glycol, polyethylene glycol and glycerin/ lauric acid, oleic acid, capric acid, myristic acid, lauryl alcohol [132], nonivamide/ chitosan and carboxymethylcellulose [133], sodium naproxen/ Azone® and Transcutol® [134], *etc*.

Loughlin *et al.* [135] proposed an poly(vinylalcohol) - tetrahydroxyborate anion hydrogel containing water soluble lidocaine and D-mannitol as mixture stabilizer. The local anesthetic drug was proved to be an effective delivery system, its release mechanism being dependent on temperature, thus possessing the properties of a reservoir system.

Al-Suwayeh *et al.* [136] developed a topical gel formulation for improved skin penetration of lornoxicam for enhancement of its analgesic activity. The gel composed of hydroxypropyl cellulose and carbopol were loaded with permeation enhancers as hydroxypropyl β-CD and exhibited significant improvement of lornoxicam permeation and analgesic activity comparable to marketed drug injection (Xefo).

Another representative example is given by the study of Sallam *et al.* [137]. The authors compared two different diflunisal loaded formulations, lipogel (Lecithin organogels) and hydrogel microemulsions (Carbomer microemulsion-based hydrogel), from the point of view of their efficacy in releasing the drug across the human skin. One of the main conclusion of their study was that lipogels is able to release higher amount of drugs in comparison with the hydrogels, probably due to the presence of lecithin which they assumed that enhance the skin permeability.

The pilot clinical studies of the tetracaine/Carbomer-940 gel with the combination of 5% menthol and 70% ethanol as penetration enhancers indicated its anesthetic effectiveness was appropriated for most medical applications [138].

Nanosized Polymer Systems

An important breakthrough in drug supporting, carrying, and targeting systems was attained in the last two decades through the advent and implementation of the remarkable innovations that popped-out at a fast pace from the field of nanotechnologies and nanoscaled products. A major contribution to these achievements was given by the advances registered in the synthesis, formulation, processing, and conditioning of new and effective polymer-based nano formulations in which drugs are entrapped, encapsulated, physical mixed, or chemically attached. Such systems were also attempted for the transdermal delivery route of analgesics, under the form of polymeric nanoparticles like nanocapsules or nanospheres, dendritic nanocarriers, nanogels, nanostructured patches and dressings [139].

It is well known that the main issue affecting the efficiency of transdermal drug delivery consists in the very low permeability of the compact, external protective skin barrier represented by stratum corneum. Moreover, this permeability varies from a subject to another, from a body part to another, is affected by the circadian rhythm, diseases and external factors like prolonged exposure to sunlight, water, and chemicals (i.e. cosmetics, soaps). The successful transdermal delivery is furthermore complicated by the lipophilic gradient between the lipid lamellae of stratum corneum and comparative more hydrated layers of dermis and viable epidermis. This means that analgesics and their nanocarriers should have an appropriate hydrophobic- hydrophilic balance to achieve the desired effect on the free nerve endings from dermis [140].

Drug transportation across wealthy intact skin commonly takes place through passive diffusionalong transepidermal(intercellular andtranscellular)or transappendageal(through hair follicles, sweat and sebaceous glands pores) pathways. Permeation via the intercellular lipid matrix, which is abundant in ceramides, fatty acids and

cholesterol, dominates the route along the keratin fibrils of corneocytes, while the transappendageal pathway is frequently considered of minor importance since it is active on only about 0.1% from total skin surface [141]. On the other hand, the pores associated with hair follicles and sweat glands are large enough to afford the transport of less hydrophobic and higher molecular weights molecules, and may serve as nanoparticle depots, playing animportant role in the permeation of nanoencapsulated drugs [142].

The permeation improvement through the stratum corneum is essential in the evaluation of the overall percutaneous absorption of analgesics since the diffusion coefficients are consistently lower for any given therapeuticagent as compared with the viable skin layers. In this context, the polymeric nanocarriersadd multiple contributions to the successful drug delivery. Their small sizes facilitate the transfer of therapeutic agents across the skin and may assure the penetration through natural or artificial pores. The flexible hydrophobic polymer surfaces permit the intercalation between the corneocytes and may inducedisruption of the stratum corneum lipids, thus facilitating the drug passage. Flexibility, elasticity and deformability enhance the transport across stratum corneum by taken advantage from the existing hydration gradient, allowing the use of higher dimensional nanocarriers and/or supplementary drug loading [143]. The polymer chains may also self-organize into the skin lipid matrix, indirectly contributing to the skin permeation enhancement, and could assure a direct and tight contact with stratum corneum and skin appendices. Furthermore, a given drug may be protected by the external polymer layer against unwanted chemical or physical interactions within the skin environment.

The nanoparticle penetration through the healthy skin is still controversial [144]. It was generally considered that nanoparticles of 20 nm and above cannot penetrate into the viable tissue underneath stratum corneum [145]. However, skin permeation into viable dermal tissues was reported for drug carrier nanoparticles of up to about 50 nm [146], while penetration into the hair follicles was achieved for particles of hundreds of nanometers [147]. Moreover, the fact that nanoencapsulation greatly extends the duration and reduces the systemic toxicity of local anesthetics is known from long time ago [80]. The additional advent of tailored, precisely designed nanostructured materials like polymer nanocarriers has allowed the preparation of more sophisticated drug carrier systems for better control of the permeation and release kinetics of the drug [49, 148]. Such nanocarriers containing physically encapsulated, entrapped, dissolved, and/or chemical linked drugs are becoming more and more important as reservoirs of drugs since their use modifies, delays and increase the permanence of the effect of active principle, and allowsnot onlythe control of drug release, but also the skin permeation [149].

The polymer species involved in transdermal delivery of analgesics must be certified for medical use, should not produce extensive skin irritations, allergic reactions, long term or irreversible alterations of the functional and protective layers of the skin. They are usually biodegradable and may be natural, bio-derived, or fully synthetic polymers. Poly(ε-caprolactone), poly (lactic acid), poly (glycolic acid) and poly (lactic-co-glycolic) copolymers, chitosan and cellulose derivatives are some of the most common polymers used in skin-penetrating nanocarriers and nanoparticles formulations [150, 151]. From a general point of view, there are two main methods for producing polymeric nanoparticles: the precipitation of pre-formed polymers and *in situ* polymerization [152, 153]. The polymerization in emulsion leads to matricial nanoparticles called nanospheres, while the addition of an organic solvent and oil in this medium gives by interfacial polymerization vesicular nanostructures or nanocapsules. Nanospheres and nanocapsules can be also prepared using pre-formed polymers by emulsification-diffusion, nanoprecipitation (without oil) or interfacial deposition of polymer (containing the oil). Another method is based on solvent extraction,

in which the oil in water emulsion is highly homogenized before addition of water and solvent evaporation [154].

The complexity of such drug delivery systems determines the consideration of several interconnected elements: drug structure and physical properties, potential drug-skin and drug-polymer interactions, skin characteristics, nanocarrier structure and properties (i.e. size, shape, specific surface area, electrical charge, stability, spreadability, occlusivity). All these elements are specifically related to the biological availability of the drug in a certain time frame, having as finally result the envisaged therapeutic effect. The modulation of this effect can be supplementary achieved by tuning the overall diffusion characteristics of the drug molecules through specific actions targeted to overcome the skin barrier [155].

Besides the various degree of permeability improvement given by the use of polymer nanoparticles, which enhance the stratum corneum penetration with or without generating a drug reservoir, the skin barrier could be compromised by local ablation, mechanical flexing, abrasion, or perforation with sonic or high voltage pulses, ballistic particles and cutaneous microneedles [49, 142]. These methods and especially the use of less invasive mechanical flexing and cutaneous microneedles procedures could be synergistically employed together with polymeric nanocarriers to highly enhance the delivery of drugs. Unlike other strategies that facilitates the transdermal drug delivery, such as electroporation, ultrasound, thermal ablation, iontophoresis and skin bombardment, the use of microneedles is more simple and do not require complex and expensive equipment for obtaining a significant clinical impact [156].

Progresses registered in the fabrication, design, and miniaturization of these minimally invasive devices has determined a wide applicability in the delivery of medications into and through the skin [157, 158]. It also attracted extensive research on new polymer nanocarriers suitable for use in microneedles-assisted drug delivery. Microneedles are specifically designed to penetrate only the outermost skin barrier layer, the stratum corneum, without stimulating the pain receptors oraffecting the blood capillaries that reside in the inner and basal derma. The transient pathways so formed in the stratum corneum afford a much better percutaneous drug permeation than chemical/lipid enhancers, increasing the drug delivery rates by orders of magnitude and supplementary allowing the transit of larger macromolecules and nanoparticles [159, 160].

Different technical approaches, fabrication procedures, geometries and materials from metals to biodegradable polymers are combined in a continuous effort to optimize and expand the medical applications of microneedles [161, 162]. Controlled and reliable drug delivery across the microneedles-breached skin barrier can be achieved with enough deformable and stable nano-sized carriers, which are typically composite colloids [49]. If properly designed and applied, these hetero-aggregates can open spontaneously and carry drugs through the cutaneous pores, enabling its deep deposition and prolonged action. Alternatively, drug could be dissolved and entrapped into a microneedle-integrated transdermal patch. For example, such a patch based on poly(ethylene glycol) diacrylate was reported to be able to load a high amount of lidocaine and release it for prolonged periods [163]. Lidocaine diffuse out of the reservoir patches integrated into microneedles through the porous channels left by dissolving drug particles.

Ones of the most studied polymers for transdermal delivery of analgesics are poly (lactic acid), poly (glycolic acid), and their poly (lactic-co-glycolic) copolymers. Thus, nanospheres and nanocapsules in the range of 160-300 nm obtained from poly (lactic-co-glycolic) copolymers (PLGA) with and without oily cores were successfully tested for the delivery of ropivacaine [164], benzocaine [165] and bupivacaine [166]. The encapsulation efficiency was low for ropivacaine (3.8%), and significantly much higher for benzocaine (60%) and uncharged bupivacaine (75%) mostly due to the charging and water solubility of ropivacaine. However, was noted

that encapsulation markedly decreased the systemic toxicity of ropivacaine. The composition of the oily core is also an important factor in boosting the analgesia efficiency [167]. It was furthermore reported that poly (lactic acid) encapsulation doubles the analgesia intensity and duration of free benzocaine, and could be more effective than PLGA and poly(ε-caprolactone) [168]. Alginate based nanoparticle have also provided high bupivacaine encapsulation efficiencies of 76-88% [169]. PLGA nanoparticles of 328 nm were also used to encapsulate the flufenamic acid, a lipophilic anti-inflammatory drug, resulting in a 1.8 fold increase of its concentration at dermal layer as compared with the free form [170]. Analogous results were attained by encapsulation in nanoparticles of 180 nm synthesized from starch derivatives [171]. The efficiency of encapsulation was very high (95%) and 14.8% of flufenamic acid was released within the first 12 hours. Comparable encapsulation efficiency (92.2%) was also reported for an anti-inflammatory glucocorticoid, clobetasol-17-propionate, loaded on lecithin/chitosan nanoparticles with a medium size of about 247 nm [172]. The skin accumulation of clobetasol-17-propionate was similar with that obtained by using a ten-fold more concentrated commercial cream.

The most common vesicular colloidal polymeric drug nanocarriers with an oily, lipophilic core surrounded by a thin wall of polymeric material are synthesized by the anionic polymerization of alkylcyanoacrylate monomers [173, 174]. This type of vesicles is also known under the name of polymersomes. Their main advantages are related with the ultrafine particle size, hydrophilic/hydrophobic surface characteristics, and oily vesicular nature. It should be mentioned here that these vesicles becomes spherical micelle when the hydrophilic part represent more than three quarters from the entire amphiphilic mixture. Such micelles are smaller than vesicles and could easily accommodate with skin pores, while the ability to encapsulate hydrophobic drugs remains very high [155, 175].

Another nanotechnological formulation that has made a breakthrough in transdermal drug delivery systems involves the multiphase nanoemulsions represented by micellar nanoparticles [176]. These nanoparticles are typically composed from five basic components (one or more active principles, oil, stabilizer, solvent and water) that are mixed together and subjected to a milling process. As result, the active principle may be contained in one or more different fractions, either solubilized in solvent or aqueous media, either associated with lipids, micelle or solid nanoparticles, in a more readily bioavailable form. Micellar nanoparticles allow the incorporation of active principles with very different aqueous solubility and a drug loading of up to 20% (w/w).

The aqueous media may be buffered to optimize the drug stability and maintain a specific pH, whereas the used oils (*i.e.* medium-chain triglycerides, squalane, mineral and vegetable oils) form the internal phase of the emulsion. The solvent (*i.e.* ethanol, low molecular weight glycols, N-methylpyrrolidinone) is a key factor for transdermal permeation since it exerts a major contribution to the control of drug solubilized fraction. Stabilizers aid the micellar phase formation and sterically maintain the oil droplets and newly formed supramolecular assemblies. They are usually non-ionic surfactants (*i.e.* glycerol and ethoxylated fatty acid esters, polyethylene glycol esters, block copolymers). The micellar nanoparticle technique allows drug penetration and deposition in the stratum corneum and epidermis, with comparable advantages as the patch technology [177].

The poly alkylcyanoacrylate nanocapsules prepared by interfacial polymerization, loaded with indomethacin and incorporated into a Pluronic-type gel formulation, was able to penetrate through the stratum corneum by disturbing its internal lipid organization, and deeper, reaching the blood vessels [178].

The major benefits of using these types of nanocapsules and micelles are that the shell properties (like surface charges and re-

leasing behavior reported to the envisaged active ingredients) could be controlled and tailored in accord with specific needs, transdermal drug delivery could be accomplished without any deterioration of the skin tissues, and open the gate to the transcutaneous transport of less hydrophobic or even hydrophilic macromolecules. In addition to the dramatically increase in skin penetration after encapsulation as a consequence of compromised barrier function of stratum corneum, it is important to note that the polymer matrix remains entrapped in this skin layer, while the active component deeply permeates into the skin.

Topical nanostructurated gels could be used either to enhance drug permeation or as drug scavangers. Thus, theacid-functionalized nanogels based onpoly(N-isopropylacrylamide) could be used as effective cationic drugsscavangers, i.e. for bupivacaine, with excellent results in the treatment of local anesthetic overdoses [179], while other acrylate gels could facilitate the skin permeation by lidocaine [180]. Chitosan based polyelectrolytes complexes (with alginate, carrageenan, pectin and carboxymethylcellulose) have been developed as nanogels for hydrosoluble tramadol formulation by Kamel and Abbas [181]. From all analyzed formulations, chitosan - carrageenan nanogels indicated an optimization of release and permeation profile at skin level.

A particular case of polymer nanoparticles is represented by dendrimers, which have the advantage of presenting a numerous, but controlled number of surface functional groupsavailable for covalently drug binding, and act as permeability enhancers for lipophilic drugs [142]. The skin permeability depends on composition, concentration, surface charge, generation size, molecular weight. Dendrimers increase the stability and bioavailability of drugs, are easy to prepare and functionalize, but show a lower biodegradability and higher toxicity than common polymeric nanocarriers, and are effective only for hydrophilicdrugs. For example, polyamidoaminedendrimers were used for the transdermal delivery of non-steroidal anti-inflammatorydrugs like ketoprofen, diflunisal and indomethacin increasing their bioavailability by up to 2.73, 2.48 and respective 1.6 times after skin penetration [182, 183].

Hybrid Systems

Almost all the studies concerning drug delivery via the transdermal route uses combinations of different penetration enhancing strategies. In this respect, the skin breeching via nanosized transporters benefited from synergistic approaches combining elements that are convergent towards an improved drug delivery. A first example is the use of topical polymer gels in almost all the situations when a certain drug should be homogenized with other components of the drug delivery system. As it was mentioned earlier, cyclodextrin drug complexes were topically applied using various hydrogels.

Also, the encapsulation of low solubility drugs into liposomes implies the use of organic solvents and it is limited by the possible issues related to the destabilization of the lipid double layer. In addition, the drugs immobilized in the lipid bilayer are much faster released after their administration than when are solubilized in the aqueous core of the liposomes, leading to the incomplete valorification of the "carrier" quality through biological membranes. The first way to overcome this drawback is to solubilized the drug into a solvent miscible with water like ethanol and to further continue with liposomes formation using this mixture as aqueous phase [120]. The second way is represented by the preparation of soluble inclusion complexes using cyclodextrins without use of organic solvents and obtaining in the end drug in *cyclodextrins in liposomes complex systems*. These are useful for improving the drug solubility and stability but also for an adequate *in vivo* behavior of the drug with low water solubility overcoming the rapid release as it was observed in the case of conventional liposomes. In addition, complexation with cyclodextrins improves drug solubility and skin permeability favouring drug bioavailability after a topical administration.

The use of cyclodextrins in topic formulations present important advantages [184] such as elimination of irritative/toxic effects at the skin and mucosa level, elimination of the use of excipients like pH regulators, solubilisation agents or organic solvents, improvment the drug absorption by enhancing its bioavailability and reducing drug metabolism at the administration site. The use of cyclodextrins has also limitations such as low complexation efficiency, high mass differences between cyclodextrins and "guest" which can lead to a limited loading even when the complexation efficiency is very good (this is very important for the drugs that need high doses or concentration) and, finally, the modification of the rheological characteristics of the drug formulation.

The efficacy of such a combination which combine the solubilisation capacity of cyclodextrins with carrier function of liposomes through skin structures has been intensively studied by both conventional and ultradeformable liposomes, all these studies underlining the importance of chosing the cyclodextrins and of the complexation method for obtaining the maximum effect.

Anyway, since these complex systems are relatively recent, there is a limited number of references regarding the preparation of liposome-cyclodextrin complex loaded with an anesthetic drug. In the following the most representative examples will be shortly described:

a. Bragagni *et al.* [185] studied the effect of prilocaine complexation with hydroxypropyl-ß-cyclodextrin and inclusion into liposomes on the efficacy of anesthetic effect. The authors considered four different systems: liposomes loaded with prilocaine base complexes in hydroxypropyl β-CD dispersed in aqueous phase, liposomes loaded with prilocaine chlorohydrate in aqueous phase, liposomes loaded with prilocaine base in lipidic phase and liposomes loaded in both aqueous and lipidic phase. The experimental results showed the formulations containing complexed drug had a better drug loading and, moreover, increased the duration of the drug-anesthetic effect and reduced the initial lag time, in comparison with the corresponding liposomal formulations.

b. Also, the preparation and characterization of ketoprofen inclusion complexes in cyclodextrins encapsulated in liposomal formulations for transdermal pain therapy has been reported [186]. The prepared complexes, based on β-CD or hydroxypropyl β-CD showed an improved drug solubility up to 11 times. The complexed drug was encapsulated in multilamellar liposomes (MLV) which were affected in size but not in their structure. Experimental data suggested that hydroxypropyl β-CD leads to formation of more stable liposomal formulations as compared to the systems containing β-CD complexes. Also, an interesting observation was the fact that drug penetration into the skin tissues was deeper in the case of free drug formulations as compared with liposomal ones.

c. The same group, studied the liposomes with ultradeformable shape as drug delivery systems for benzocaine and butamben inclusion complexes in β-CD or hydroxypropyl β-CD [187]. The ultradeformable liposomes were obtained through addition of stearylamine in the initial lipid mixture. The liposomes, loaded with drug complexes in both lipidic and aqueous phases showed better anesthetic effect as compared with liposomes containing drug only in one phase.

Another hybrid delivery system based on drug cyclodextrin-epichlorohydrin polymer complexation and loading into nanostructured lipid carriers (NLC) has been produced in order to optimize ketoprofen therapeutic efficacy [188]. The drug would possess both the solubilizing and stabilizing properties of cyclodextrins and the prolonged release, high tolerability and percutaneous absorption enhancer properties of NLC. Two different polymeric systems β-CD-epichlorohydrin polymer and carboxymethylathed-β-CD-epichlorohydrin polymer were evaluated and two different methods to produce solid ketoprofen-polymeric Cd complexes were brought forward. The delivery system strengthened the benefits of both the utilized carriers, with regard to their potential for improving the drug therapeutic efficacy and safety, allowing an improvement in both the dissolution and the skin permeation properties of drug. β-CD based system exhibited higher solubilizing properties and co-grinding under dry conditions was the best complex preparation technique, giving rise to homogeneous amorphous particles of nanometric range, which enabled a greater entrapment efficiency into NLC.

The improvement of drug permeation through the skin has been achieved also through the combination of complexation abilities of CD with chemical penetration enhancement of amines. For example, a cationic polymer, based on hydroxypropyl-β-CD and poly-ethilenemine (PEI) was proposed as a transdermal penetration enhancer of diclofenac sodium [189]. The system was evaluated in terms of its preparation, cytotoxicity assay, irritation study, *in vitro* and *in vivo* permeation investigation. It was implied that the proposed system could change the secondary structure of keratin in stratum corneum by the means of improving the content of β-folded structure and cutting down the α-helical structure from keratin, and then result in more random accumulative structure of keratin and freer space for carbon movement, which could enhance the transdermal penetration and absorption of drugs. The irritation and toxicity of the polymers showed a that CD-PEI has better skin biocompatibility properties than PEI alone. Compared to the control group, the amount of diclofenac sodium penetrated into dermis and epidermis from drug solution increased.

CONCLUSION

Pain therapy via various drugs can benefit from drug delivery devices with possibility to control in a timely manner the drug amounts delivered directly to the affected area. A desirable transdermal delivery formulation must be able to prolong the drug effect, to lower applied drug concentration, low toxicity at local and systemic levels. The topical pain therapy using nanotechnologies as penetration enhancement strategies, especially the nanocarriers, macromolecular and cyclodextrin based enhancers has proved to be an alternative to active penetration methods using physical methods to create a transdermal passage for drug diffusion. It is safe to conclude that there is no such concept of simple or unique penetration enhancer as most of the strategies in the field are aiming for synergistic combinations of different origins (vesicular carriers, nanoparticles, polymers, cyclodextrins, etc.). Among the universally combined elements, hydrogels and cyclodextrins seem to have complementarities with other enhancers or with the drug molecules and to provide enough advantages in the drug delivery design. It is expected that polymer architectures based on cyclodextrin cores, thus retaining the complexation abilities, to be further employed in producing transdermal drug delivery systems for pain therapy.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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