Contents

2.1 DEMENTIA

Dementia is a term used clinically to describe reduced intellectual functioning indications that affects normal life tasks such as memorizing, thinking, calculating, learning, reasoning and decision making. However, consciousness remains unaffected. The loss in cognitive function is usually accompanied, and seldom preceded, by decline in emotional control, social conduct, or motivation. Dementia is not a natural part of ageing. It is linked with diseases that disturb cerebral cortex, the portion of brain deals with thoughts, actions, memories and personality. Dementia is among the major reasons of disability and dependency in older people worldwide. Dementia has a physical, emotional, social, and financial impact, not only on the people who are

suffering from it, but also on their care-givers and families. Owing to the lack of awareness and understanding about dementia, there exist defamation and barriers to diagnosis and care.

2.1.1 Prevalence

Dementia is a syndrome where a decline in memory, thinking, behavior and the ability to perform everyday activities are evident. Though it mainly affects older people but it is not a usual part of ageing. Around 50 million people worldwide have dementia with addition of nearly 10 million new cases every year.

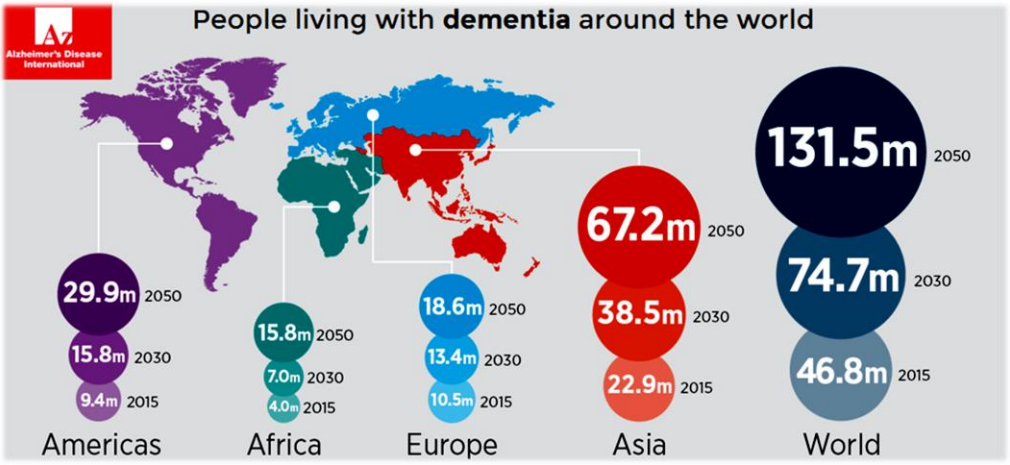


Fig. 2-1. Estimates of Dementia prevalence worldwide, adapted from 'World Alzhiemer Report-2015' [1]

The estimated percentage of the overall population aged 60 years and abover with dementia at any given time is between 5-8 %. Dementia is increasing rapidely in Asia placing an enormous burden on carers, who are primarily family members. In the Asia Pacific, only Australia, Japan and the Republic of Korea have framed public health policies to overcome the burden of dementia. In the rest of the Asia, caregivers bear the impact of the burden, medical and social backing are underdeveloped or non-existent and the enormity of the forthcoming dementia epidemic is still usually ignored. In terms of emerging dementia policies, majority of the action is still in Europe. Yet India is waking up to this [2].

2.1.2 Signs and symptoms

Based on the impact of the disease and the individual's personality before becoming ill, dementia affects different people in a different way. The signs and symptoms associated to dementia can be categorized in three stages. Early stage with gradual onset of symptoms (Table) that are widely overlooked. Middle stage with clearer and more restricting signs and symptoms. Late stage with serious memory disturbances where physical signs and symptoms become more obvious resulting in total dependence and inactivity. Common symptoms in each stage are given in Table 2.1.

Table 2.1 Common signs and symptoms associated with dementia

Early stage	Middle stage	Late stage
• Forgetfulness • Losing track of the time • Losing recognition of familiar places	• Forgetting recent events and people's names • Becoming lost at home • Increasing trouble with communication • Requiring help with personal care • Facing behavioral changes, like wandering, repeated questioning.	• Becoming unaware of the time and place • Facing difficulty in recognizing relatives and friends • Increasing necessity for assisted self-care • Facing difficulty in walking • Facing intense behavioural changes including aggression

2.1.3 Common types of dementia

There are many different forms of dementia. However, based on the underlying disease, dementia can be categorized in four main types amounting around 95 percent of people diagnosed with dementia. The most common type is Alzheimer disease that may contribute to 60–70% of cases. Other major types include vascular dementia, dementia with Lewy bodies (abnormal masses of protein that grow inside nerve cells), and a group of ailments that contribute to frontotemporal dementia (deterioration of the frontal lobe of the brain). The boundaries among different types of dementia are indistinct and mixed types frequently co-exist.

2.1.4 Treatment and care

There is no known cure to dementia or to alter its progressive course. However, to support and improve the lives of people having dementia, their caregivers and families, below approaches can be offered:

- Early diagnosis to promote optimal management
- Optimizing physical well-being, cognition and activity
- Identifying and treating associated disorders
- Treating psychological and behavioral symptoms
- Providing information and long-term support to caregivers.

There are medicines and other remedies that can relief some of the symptoms. A number of drugs are available today to improve brain functioning. The most effective anti-dementia agents among them are acetylcholinesterase inhibitors such as Tacrine, Rivastigmine, Donepezil, Galantamine, Dihydroergotamine, Piracetam and Noopept etc. Acetylcholine is one of the chemical substances that allow brain cells to communicate with one another, the so-called neurotransmitters. Research suggests that acetylcholine is reduced in the brain of Alzheimer's dementia patients. This kind of drugs prevents acetylcholine to be eliminated too quickly, prolonging its ability to conduct chemical messages between brain cells. It could be shown in clinical trials that, with this kind of drugs, the deterioration of the disease could be delayed by at least 12 months. Apart from preserving and partially improving mental capacities, and coping with daily activities, a delayed onset of behavioral disturbances and a reduction in caring time could also be demonstrated.

Psychotropic drugs can be used as a supportive therapy in the treatment of behavioral problems in dementia. For instance, antipsychotic medications (typically used to treat disorders like schizophrenia) can be effective in reducing delusions and hallucinations. Anti-anxiety medications (typically used to treat anxiety disorders) can also be prescribed to help treating agitation and restlessness. Likewise,

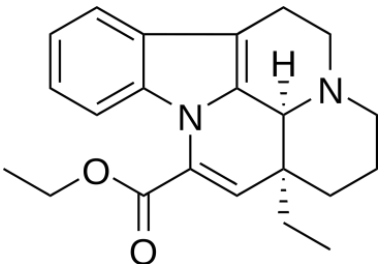
antidepressant medication can be prescribed to alleviate depressive symptoms. Treating depression symptoms is important as depression makes it harder for a person with dementia to remember things and enjoy life. It also adds to the difficulty of caring for someone with dementia. Significant improvements can be made by treating depression, as the patient's mood and their ability to participate in activities may be improved.

A growing number of herbal remedies, vitamins and other dietary supplements such as Fisetin, L-Theanine and Vinpocetine etc. are also promoted as treatments for Alzheimer's dementia and related diseases. They can be appealing to some people as they come from natural ingredients. Although many of these remedies may be valid candidates for treatment, using these drugs as an alternative or in addition to physician-prescribed therapy raise legitimate concerns.

2.2 VINPOCETINE (VPN)

Vinpocetine is a semisynthetic derivative alkaloid of vincamine (a synthetic ethyl ester of apovincamine) [3], an extract from the periwinkle plant. It is reported to have cerebral blood-flow enhancing [4] and neuroprotective effects [5], and is used as a drug for the treatment of cerebrovascular disorders and age-related memory impairment [6]. Vinpocetine is widely marketed as a supplement for vasodilation and as a nootropic for the improvement of memory. In other words, Vinpocetine may help support brain functions such as concentration and memory by activating cerebral metabolism. Vinpocetine has been identified as a potent anti-inflammatory agent that might have a potential role in the treatment of Parkinson's disease and Alzheimer's disease [7, 8]. The 15-30 mg/day of vinpocetine in three divided dose is generally recommended in treating dementia or cerebrovascular disorders.

2.2.1 Vinpocetine Profile



Synonyms: Cavinton,
Ethyl apovincaminate,
Ethyl apovincaminate, (+/-)-isomer,
Kavinton,
RGH-4405, TCV-3B

Mol formula: $C_{22}H_{26}N_2O_2$

Mol weight: 350.454 g/mol

Description

Vinpocetine (vinpocetine-ethyl apovincaminate) was synthesized in the late 1960s from the alkaloid vincamine, extracted from the leaf of the lesser periwinkle plant (Vinca minor). Vinpocetine was made available under the trade name Cavinton in 1978 and has since been used widely in Japan, Hungary, Germany, Poland, and Russia for the treatment of cerebrovascular-related pathologies. Several clinical studies have confirmed the neuroprotective effects of this compound.

Pharmacokinetics

Vinpocetine, when taken on an empty stomach, has an absorption rate of 6.7 percent. When taken with food, absorption increases 60-100 percent. Vinpocetine reaches the bloodstream approximately one hour after administration, whether taken with food or on an empty stomach. The elimination half-life of the oral form is one to two hours and the majority of vinpocetine is eliminated from the body within eight hours. Recent studies, either following i.v. infusion of vinpocetine in patients with cerebrovascular disorders or using positron emission tomography (PET) scans in animals, have shown that vinpocetine crosses the blood-brain barrier and is taken up by cerebral tissue. PET studies have also clearly shown in human subjects vinpocetine is preferentially absorbed in the central nervous system at twice the level that would be expected according to total body distribution. The highest uptake of vinpocetine was seen in the thalamus, putamen, and neocortical regions.

Mechanisms of Action

Vinpocetine appears to have several different mechanisms of action that allow for its antioxidant, vasodilating, and neuroprotective activities.

Voltage-dependent Sodium Channel Inhibition: It has been hypothesized that vinpocetine's application in ischemic stroke is secondary to its effect on voltage-dependant sodium channels in the brain. Inhibition of sodium channels in neural tissue is the primary mechanism of several different drugs reported to have neuroprotective effects in experimental ischemia. This action, effectively blocking accumulation of sodium in neurons, decreases the damage of reperfusion injury and may be beneficial in lessening the toxic effects of oxidative stress resulting from anoxia.

Phosphodiesterase-1 Inhibition: Vinpocetine inhibits Ca+2/calmodulin-dependent phosphodiesterase (PDE) type 1.11 This effect would theoretically lead to an increase of cyclic AMP over cyclic GMP and may be responsible for the benefits in cerebral circulation and decreased platelet aggregation observed after vinpocetine administration.

Antioxidant Effects: Like vitamin E, vinpocetine is an effective scavenger of hydroxyl radicals. It has also been shown to inhibit lipid peroxidation in synaptosomes of murine brain tissue and to protect against global anoxia and hypoxia in animals. Vinpocetine has decreased areas of neuronal necrosis in animal models up to 60 percent in experimentally-induced ischemia.

Other Neuroprotective Effects: Vinpocetine has been shown to protect neurons from the toxicity of glutamate and N-methyl-d-aspartate (NMDA).14 Vinpocetine lowers blood viscosity in patients with cerebrovascular disease, has significant vasodilating properties, decreases platelet aggregation, and increases and maintains erythrocyte flexibility under oxidative stress, all of which are potentially beneficial in

cerebrovascular disease. Vinpocetine causes a selective increase in cerebral blood flow and increases cerebral metabolic rate.

Clinical Indications

Chronic Cerebral Vascular Ischemia: Two PET studies in chronic stroke patients have shown that vinpocetine has a significant effect in increasing glucose uptake and metabolism in the healthy cortical and subcortical regions of the brain, particularly in the area surrounding the region of the stroke. A study in 15 chronic ischemic stroke patients found that a two-week vinpocetine trial significantly increased cerebral blood flow in the non-symptomatic hemisphere. Recent studies using Doppler sonography and near infrared spectroscopy have shown increased perfusion of the middle cerebral artery in patients with chronic cerebrovascular disease given a single infusion of vinpocetine.

Acute Ischemic Stroke: Although small studies have shown that vinpocetine has an immediate vasodilating effect in cerebrovascular circulation, a meta-analysis of the existing studies examining short- and long-term fatality rates with vinpocetine was unable to assess efficacy. In the analysis of eight studies in acute stroke patients (vinpocetine was administered within two weeks of event), only one study met the meta-analysis criteria. In the selected trial, three weeks after onset of intravenous vinpocetine therapy, 8 of 17 vinpocetine patients and 12 of 16 placebo patients were determined "dependent" (unable to live without assistance), and all were still alive. The meta-analysis authors were unable to determine a beneficial effect of vinpocetine, but did state that considering the in vitro studies and animal data, vinpocetine has potential to be effective in acute stroke. Properly designed studies have not yet been conducted.

Degenerative Senile Cerebral Dysfunction: A meta-analysis of six randomized, controlled trials involving 731 patients with degenerative senile cerebral dysfunction showed that vinpocetine was highly effective in the treatment of senile cerebral dysfunction. Using several psychometric testing scales in addition to physical symptoms (speech

and movement capacity, muscular coordination and strength, sensory-perceptual ability) the researchers were able to show a highly significant effect of vinpocetine on both cognitive and motor functions.

Alzheimer's Disease: Although evidence has been limited to one small study, the results suggest that vinpocetine supplementation may not be effective as a therapy for Alzheimer's disease. A double-blind, placebo-controlled study of vinpocetine in 15 Alzheimer patients, treated with increasing doses of vinpocetine (30, 45, and 60 mg per day) in an open label pilot trial during a one-year period, resulted in no improvement.

Tinnitus/ Meniere's Disease/Visual Impairment: Vinpocetine has been used in the treatment of acoustic trauma with subsequent hearing loss and tinnitus. Disappearance of tinnitus occurred in 50 percent of those who started vinpocetine within one week of the trauma. Regardless of the time since the incident, 79 percent of patients had improved hearing and 66 percent had a significant decrease in the severity of the tinnitus. Vinpocetine has also been found to be effective in treating Meniere's disease and in visual impairment secondary to arteriosclerosis.

Drug Interactions

Because Vinpocetine decreases platelet aggregation it should be avoided in patients on blood thinning medications.

Safety/Toxicity

Some studies have noted flushing, rashes, or minor gastrointestinal problems in some subjects; however, these side effects did not warrant discontinuation of the medication. In one study no significant side effects were reported, even in larger doses of 20 mg three times daily.

Dosage

All of the above studies used either 10 mg vinpocetine 3 times daily orally or intravenous vinpocetine. Patients with chronic cerebrovascular disorders that were included in the meta-analysis had been on an oral dosage of 10 mg three times daily.

2.2.2. Problems associated with VPN oral medication

Although it has been shown to increase the cerebral flow in the ischemic patients with cerebrovascular disease, to increase red cell deformability in stroke patients and to have neuroprotective abilities against brain ischemia [9], the clinical use of its marketed commonly used oral formulations is limited by its poor dissolution profile, and extensive first pass metabolism that results in very low bioavailability (~7%) [10]. This poor oral bioavailability together with the small $t_{1/2}$ (2.54 ± 0.48 hrs) implies the necessity of frequent drug dosing (three times daily), a situation that is inconvenient for patients of dementia and results in poor compliance [11, 12]. A review of work done on improvement of Vinpocetine delivery is summarized in Table 2.2.

Table 2.2. Review of work done on Vinpocetine

Author (Ref. no.)	Formulation (Route)	Technique	Polymer/ Excipient used	Conclusion
El-Laithy H.M. et al., 2011 [10]	Proniosomes (Transdermal)	By dissolving components in warm ethanol and phosphate buffer (pH 7.4) at 65±3 °C in stoppered tube	Sugar ester (sucrose laurate L-1695), Lecithin, Cholesterol	Achieved 91% permeation after 48 hours under occlusive condition with 206% relative bioavailability when compared to the commercial oral tablet
Ribeiro L et.al., 2005 [13]	Swellable HPMC tablet of multicomponent complexes (Oral)	Complexation using lyophilization followed by tablet using direct compression	βCD, SBEβCD, HPMC, PVP K30, Tartaric acid, Methocel, monohydrate lactose, magnesium stearate	Achieved better percent VPN release in 7 h (PD_{7h}) and dissolution efficiency parameter in 12 h (DE_{12h}) via VPN-CD-TA complexes
Xu H. et.al., 2009 [14]	Proliposomes (Oral)	Sorbitol addition to drug lipid solution in ethanol at 55 °C followed by ethanol evaporation	Soybean phosphatidylcholine, Cholesterol, Sorbitol, Ethanol	Utilized less toxic and more efficient method and achieved proliposomes with 350 % relative bioavailability as compared to VPN suspension

Author (Ref. no.)	Formulation (Route)	Technique	Polymer/ Excipient used	Conclusion
Zhuang C.Y. et.al., 2010 [15]	Nanostructured lipid carriers (Oral)	High pressure Homogenization at 800 bar pressure and about 70 °C temperature	Compritol 888 ATO, Lecithin, Solutol HS-15, Poloxamer 188, Miglyol 812N	Achieved 322% relative bioavailability via VPN-NLC as compared to VPN suspension after oral administration in Wistar rats
Luo Y. et.al., 2006 [16]	Solid Lipid Nanoparticles (Oral)	Ultrasonic-solvent emulsification technique at 0 °C	Glyceryl monostearate, Soya lecithin, Polyoxyethylene hydrogenated castor oil, Tween 80, dichloromethane	Achieved around 400 % relative bioavailability from VPN-SLN as compared to VPN solution.
Hua L. et.al., 2004 [12]	Microemulsion (Transdermal)	Pseudo-ternary phase diagram	Oleic acid, Labrasol, Transcutol P	Achieved 3160-fold higher VPN solubility than that in water and a flux of around 36 mg/cm2/h.
Chen Y. et.al., 2008 [17]	Self-Micro emulsifying Drug Delivery System (Oral)	Pseudo-ternary phase diagram	Cremophor EL, Labrafac, Transcutol P, Oleic acid, Oleylamine	Achieved 200 % relative bioavailability from VPN-SMEDDS as compared to VPN powder
Mao Y.T. et.al., 2013 [18]	Ethosomes (Transdermal)	Coarse Dispersion - Ultrasonication	Lecithin, Ethanol, Cholesterol, Vitamin E	Achieved 3.56 $\mu g/cm^2/h$ transdermal flux and 220% relative bioavailability as compared to oral tablet
Moghaddam A.A. et.al., 2014 [19]	Nanoethosomes (Transdermal)	Solvent evaporation technique	Phospholipid 90 G, Tween-80, Ethanol, Chloroform, Di-ethyl ether	Achieved 50.57 nm mean particle size, 97.51% entrapment efficiency and 925.60 $\mu g/cm^2/h$ mean transdermal flux

Author (Ref. no.)	Formulation (Route)	Technique	Polymer/ Excipient used	Conclusion
Ning M. et.al., 2011 [20]	Elementary Osmotic Pump System (Oral)	Core tablet pan coated and drilled by laser	Citric acid, Lactose, Mannitol, HPMC K4M, Magnesium Steric, Cellulose acetate 398-3, PEG-6000, Diethyl-o-phthalate, Acetone	Achieved bioequivalent but prolonged drug release (2.5-fold increase in MRT as compared to IR tablet.
El-Zahaby S.A. et.al., 2017 [21]	self-nanoemulsifying osmotic pump tablet (Oral)	S-SNEDDS core tablet dip-coated with Opadry CA and drilled by micro-drill	Cremophor EL, PVP K30, Transcutol HP, Maisine 35-1, Aeroperl® 300 Pharma, Opadry CA, HPMC-K4M, Avicel PH101, Lubripharm, Sodium chloride, Acetone, Glycerol,	Achieved 2-fold increase in bioavailability as compared to oral tablet
Wang R. et.al., 2016 [22]	Nanoparticles (injection)	Emulsion solvent evaporation method	mPEG 2000, PLA, Sodium cholate and Tween-80	Achieved 2.87-fold increase in bioavalability as compared to VPN injection

2.3 NOOPEPT (NPT)

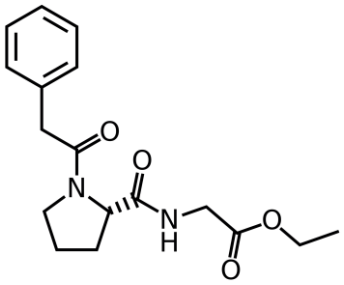
Noopept or N-phenylacetyl-L-prolylglycine ethyl ester is a synthetic dipeptide molecule derived from the endogenous dipeptide cycloprolylglycine which is a combination of the amino acids glycine and proline in a cyclic configuration [23]. It is commonly prescribed as cognitive enhancer in Russia while readily available and legal in USA. It is well-known for its neuroprotective properties which include an anti-oxidant and anti-inflammatory effect as well as the ability to prevent neurotoxicity caused by excessive calcium and glutamate. It is also found to exert a positive impact on memory formation, consolidation and retrieval [24] and to possess an anxiolytic effect as well as mild psychostimulant-like effects [25]. The mechanism of action is not fully understood but, like the Racetams, Noopept appears to positively modulate acetylcholine neurotransmission. It is observed to increase expression of both neurotropic factors NGF and BDNF in the hippocampus [26]. This may explain why improvements in memory are

more apparent following long-term dosage administrations. Noopept is further reported to reduce depression and anxiety in both human and animal studies [24].

2.3.1 Noopept Profile

Synonyms: Ethyl 1-(phenylacetyl)-L-prolylglycinate, GVS-111

Mol formula: $C_{17}H_{22}N_2O_4$



Mol weight: 318.373 g/mol

Log P: 1.23

Flash point: 284.8° C

Occurrence and Usage

Noopept (N-phenylacetyl-L-prolylglycine ethyl ester, GVS-111, Nupept, Zynapse) is a piracetam analogue and cycloprolylglycine prodrug that has been used clinically in Russia since 1995 as a nootropic agent. It is supplied as the nearly neutral substance in 10–30 mg tablets or capsules and in pure powder form for oral administration. Adult doses are normally 10–30 mg 1–3 times daily. Noopept is available in the U.S. as a dietary supplement.

Blood Concentrations

A single oral 20 mg dose given to 3 healthy men resulted in peak plasma noopept concentrations of approximately 13–33 µg/L at 30–60 minutes, declining with an elimination half-life of approximately 1 hour.

Metabolism and Excretion

Noopept administered to experimental animals was subjected to ester hydrolysis, amide hydrolysis, N-deacylation and aromatic hydroxylation. N-Phenylacetyl-L-prolylglycine, N-phenylacetyl-L-proline and L-prolylglycine were identified as metabolites in plasma.

Toxicity

The adverse effects of noopept may include restlessness, tremor, agitation and insomnia.

Analysis

Noopept has been determined in biological specimens by liquid chromatography with ultraviolet or mass spectrometric detection. Gas

chromatography with flame-ionization or mass spectrometric detection has also been employed.

2.3.2 Problems associated with NPT oral medication

Preliminary findings based on serum concentrations and excretion kinetics suggested that oral doses of 50 mg/kg are roughly equivalent to injected dosages of 5 mg/kg [27]. This implies that oral bioavailability is only 10% compared to injections. A review of work done on improvement of Vinpocetine delivery is summarized in Table 2.3.

Table 2.3. Review of work done on Noopept

Author (Reference no.)	Formulation (Route)	Technique	Polymer/ Excipient used	Conclusion
Boiko S.S. et.al., 2005 [28]	Lyophilized injection	Lyophilization	Mannitol	Achieved similar pharmacokinetic profile as compared to standard parent substance
Boiko S.S. et.al., 2004 [29]	Tablet (Oral)	-	-	Observed only 7.09% absolute bioaccessibility of noopept in rats and 9.33% in rabbits.
Borisovich S.S. et.al., 2004	Tablets / Capsules (Oral)	Wet granulation followed by compression or encapsulation in hard gelatin capsules.	Lactose, Microcrystalline cellulose, Polyvinylpyrrolidone, Magnesium Stearate	Achieved disintegration in 4-7 minutes and the dissolution of around 90% drug in 45 minutes in water
Borisovich S.S. et.al., 2005	Amorphous Powder or friable tablet for injection	Aseptic sterile filtration followed by lyophilization	Lactose, Olive oil, Mannitol	Showed antiamneasic effect of developed injection in hemorrhagic stroke models

2.4 TRANSDERMAL ROUTE FOR DRUG DELIVERY

Limitations suffered by oral administration of the Vinpocetine and Noopept necessitate the utilization of some other delivery route for better sustained plasma level of the drug throughout the duration of therapy. In this context, the consideration has been given to non-invasive, user-friendly transdermal route which is reported to have the potential of avoiding first-pass metabolism, achieving infusion like zero

order drug delivery profile, avoiding the trauma associated with parenteral therapy, improving patient compliance by reducing the frequency of administration for short half-life medications [30]. The transdermal route also provides an opportunity to cease absorption in the event of an overdose or other problems.

2.4.1 Transdermal route

The skin (cutis) is the largest human organ contributing around 4% to total body weight. It consists of two main layers viz, epidermis and dermis. The epidermis is composed mostly of stratified keratinocytes layers, where the corneocytes are covered in a protein-rich envelope followed by an outer lipid envelope and further surrounded by an extracellular lipid matrix. In humans, the epidermis is generally 0.02–0.2 mm (typically 0.05-0.15 mm) thin. The underlying dermis layer contains a diversity of cell types, blood vessels, lymphatics and nerves entrenched in a dense network of connective tissues.

Viable epidermis is found above the basement membrane that separates it from dermis. The viable epidermis comprised of a variety of cells with specialized functions like melanocytes for melanin production, Merkel cells for sensory perception and Langerhans cells for immunological function. Keratinocytes are also found interspersed amongst the viable epidermis. These cells undergo keratinization where the cell differentiates and moves upward towards the outermost stratum corneum (SC) layer. On reaching the SC, cells become anucleated and flattened and are eventually disposed off. In addition to the organized cellular components, there exist skin appendages such as apocrine and eccrine sweat glands and the pilosebaceous units comprising hair follicles and associated sebaceous glands.

2.4.2 Breaching the Stratum corneum barrier

The outermost horny skin layer (stratum corneum), composed of dead keratinized tissue, is about 5–20 μm thick and is the major obstacle to diffusion. Winsor and Burch [31] were first shown that barrier function of skin reside in the stratum corneum (SC) or horny layer in

1944. Blank [32] further confirmed the barrier property of SC using more sophisticated tape-stripping experiments in 1953. The brilliance of this lipid-rich barrier to transport is greatly demonstrated by the steep water concentration decline from almost 75% in the viable epidermis to just 10–30% at an air-exposed skin surface. However, its thickness varies appreciably between body sites, gender, race and species. A variety of physical and chemical methods have been explored to breach SC including use of penetration enhancers, lasers, electrical energy, ultrasound, radio frequency, thermal energy and microneedles. A review of literatures on application of these techniques for transdermal penetration enhancement are presented in **Table 2.4**.

Table 2.4. Review of work done on transdermal permeation enhancement by chemical and physical means

References	Permeant	Permeation enhancer	Observations
Southwell D. et.al., 1983 [33]	Caffeine	Dimethyl formamide	DMF increased flux (12-fold) across human skin through irreversible membrane damage
Hoogstraate A.J. et.al., 1991 [34]	Desglycinamide arginine vasopressin (DGAVP)	Azacycloheptan-2-ones (Azones)	Dodecyl-Azone showed 3.5-fold increase in permeability by perturbing the lipid arrangement via insertion in to the lipid bilayers
Park E.S. et.al., 2001 [35]	Captopril	N-methyl-2-pyrolidone	Limited success as a penetration enhancer when formulated in a matrix-type transdermal patch
Shin S.C. et.al., 2002 [36]	Triprolidone	long-chain fatty acids	Enhanced permeation via mechanism similar to Azones
Morimoto Y. et.al., 2002 [37]	Morphine HCl	L-menthol	Enhanced permeation through hairless rat skin
Cornwell P.A. et.al., 1994 [38]	5-flurouracil.	Sesquiterpene oil	Increased absorption of 5-flurouracil
Asbill C.S. et.al., 2000 [39]	Retinoic acid	4-decyloxazolidin-2-one	Localize the delivery of retinoic acid in skin layers resulting in low systemic permeation
Pershing L.K. et.al., 1990 [40]	Estradiol	Ethanol	Enhanced the flux through human skin *in vivo*

References	Permeant	Permeation enhancer	Observations
Banga A.K. 1998 [41]	-	Iontophoresis	Enhanced permeation by applying low-level electric current for electro-repulsion of charged electro-osmosis of uncharged and electro-perturbation of both charged and uncharged permeant
Liu H. et.al., 2006 [42]	Cyclosporine A	Sonophoresis	Enhanced permeation via creation of gaseous voids in the intercellular lipids on exposure to ultrasound
Murthy S.N. 1999 [43]	Benzoic acid (Diamagnetic solute)	Magnetophoresis	Enhanced flux by applying magnetic field as an external force and to induce structural alterations
Shomaker T.S. et.al., 2001 [44]	Testosterone	Thermophoresis	Enhanced flux via increased lipid fluidity at elevated temperatures
Sintov A.C. et.al., 2003 [45]	Granisetron HCl	Radio-frequency	Enhanced flux via heat-induced microchannels to achieve 30 times higher blood plasma levels in rats
Svedman P. 2000 [46]	Morphine	Suction ablation or skin erosion	Enhanced permeation by applying vacuum to remove epidermis and induce hyperaemia in underlying dermis
Lee W.R. et.al., 2003 [47]	Vitamin C	Laser radiation	Enhanced permeation by exposure to laser radiation for controlled SC disruption

2.4.3 Microneedle based transdermal delivery

Microneedles or other microporation techniques provide a minimally invasive, painless way of creating microchannels in skin which can then allow the transport of drug delivery vehicle across the previously impervious barrier i.e. SC, deeper into the skin and systemic circulation. The structures are small enough that they do not penetrate into the dermis; they do not reach the nerve endings and thereby avoid sensation of pain [48]. The dimensions of the pore created in skin by microneedles are typically around 50-200μm through which even nanosized particulate drug carriers can easily be delivered transepidermally [48]. These microconduits, in general, designed to deliver hydrophilic molecules but can also be utilized to create a

reservoir of lipophilic drugs for infusion like delivery [30]. A variety of microneedles like metal, silicon, titanium, glass or maltose have been developed [49]. Among them, silicon or metal microneedles suffers the problems of requiring dedicated and costly clean room facilities and chances of accidental needles break off in the skin which may arise complications. In contrast, microneedles made up of hydrophilic biodegradable polymers avoid such drawbacks and can be made by a simple and cheap micromoulding technique, dissolve in the skin to create microchannels [50].

2.5 NANOCARRIERS AS DRUG DELIVERY VEHICLES

Incorporation of drugs in to suitable nanocarriers that can easily get deposited and continuously release the drug in the vicinity of papillary area having rich capillary network may ensure better systemic availability of the drugs. To serve the aforementioned purpose, biodegradable nanoparticles and ultradeformable liposomes displayed a great potential.

2.5.1 Polymeric nanoparticles (PNP)

Nanoparticles have been extensively studied for oral and parenteral administration owing to their sustained drug release. This property of nanoparticles could also be utilized for topical drug administration to support the skin with drug over a prolonged period and to maintain a desired drug concentration in the skin. Many researchers had attempted to use nanoparticles for topical drug delivery, and they found that the drug permeation was enhanced by gradual drug release from the nanoparticles on the skin surface, but did not find the nanoparticle carriers inside the skin [51]. Some other researchers attempted to verify the penetration of nanoparticles across the skin, but found that only few of nanoparticles were able to permeate into the skin passively through the hair follicles while most nanoparticles were primarily restricted to the uppermost SC layer and unable to penetrate the skin [52]. Here, skin microporation with microneedles could be of

great use to assist nanoparticles to overcome the SC barrier and create a mini depot beneath the epidermis for prolonged drug release.

2.5.2 Ultradeformable liposomes (UDL)

In contrast to nanoparticles, ultradeformable liposomes have the ability to penetrate the skin intact owing to their vesicle deformability. and act as a drug reservoir to continuously transport drug through the skin [53]. Additionally, solubility of poorly water-soluble drugs can be significantly improved in elastic liposomes than in aqueous solution, which leads to a greater concentration gradient across the skin and subsequently improves permeation [54]. The combination of elastic liposomes and microneedles may provide higher and more stable transdermal delivery rates of drugs without the constraints of traditional diffusion-based transdermal devices, such as molecular size and solubility. Incorporation of these nanocarriers in to hydrogels may also improve the stability and handling of the formulations. A review of literatures on application of nanocarriers for transdermal penetration enhancement are summarized in Table 2.5.

Table 2.5. Liposomal and nanoparticulate formulations for transdermal delivery

References	Formulation & Drug	Problem addressed	Observations
Dubey V. et.al., 2007 [55]	Ethanolic liposomes of Melatonin	Poor skin permeation and long lag time	Observed transdermal flux of 59.2 µg/cm^2/h through human cadaver skin
Fang J.Y. 2005 [56]	Tea catechins loaded anionic Liposomes with ethanol	Poor skin permeation	Five to seven fold increase in catechin permeation
Mishra D. et.al., 2007 [57]	Elastic liposomes of Propranolol HCl	Low permeability	Observed transdermal flux of 16.19 µg/cm^2/h with enhancement ratio of 8.8 through human cadaver skin
Jain S.K. et.al., 2008 [58]	Elastic liposomes of Acyclovir sodium	Poor and highly variable oral bioavailability ranging from 10 to 30%	Observed 6.3 times higher transdermal flux (6.21 µg/cm^2/hr) and decreased lag time (0.6 hr) than conventional liposomes
Bendas E.R. et.al., 2007 [59]	Ethosomes of Salbutamol sulphate	First-pass metabolism in the liver and gut wall	Reported higher transdermal flux as well as cumulative drug permeated through mice skin over 24 hours (~22%).

References	Formulation & Drug	Problem addressed	Observations
Patel K.K. et.al., 2012 [60]	Niosomal gel of Lopinavir	Poor bioavailability (~20%) and extensive first pass metabolism	cumulative drug permeated through rat skin over 24 hours (~18.32%).
Shim J. et.al., 2004 [61]	Block-copolymer Nanoparticles of Minoxidil	Skin permeation pathway of nanoparticles	Nanoparticles penetration mainly via shunt routes like hair follicles, resulting in skin absorption of solutes
Ortega M.J.S. et.al., 2009 [62]	Model drugs loaded starch-derivative Nanoparticles	Potential for transdermal drug delivery	Observed a clear enhancer effect for flufenamic acid.
Bhaskar K. et.al., 2009 [63]	Lipid Nanoparticles of Flurbiprofen	Bioavailability improvement	Relative bioavailability with reference to oral route was found to increase by 4.4 times when gel formulations were applied
Jana S. et.al., 2014 [64]	Chitosan-egg albumin Nanoparticles of Aceclofenac	Transdermal delivery of Aceclofenac	Observed sustained ex vivo permeation of aceclofenac over 8 h through mouse skin
Liu D. et.al., 2010 [65]	Solid lipid nanoparticles of Diclofenac sodium	Transdermal delivery of Diclofenac	SLNs showed improve dermal localization of Diclofenac sodium
Abdellatif A.A.H. et.al., 2015 [66]	Transfersomal Nanoparticles of Clindamycin	Transdermal delivery of Clindamycin	Transfersomal gel showed a significantly higher drug permeation and flux than its transfersomal suspension.

2.5.3 Nanocarriers loaded Microneedle patches

An attempt was also made to incorporate these nanocarriers in dissolving microneedle patch (MNP) to combine skin microporation and drug administration in single step. This may also be beneficial for delivering a constant and calculated fraction of drug each time, providing occlusive condition to prolong the time for which the pore remains opened, better handling and storage of the formulation, providing environmental protection to the ingredients and avoiding any microbial invasion through such pores. A review of literatures on application of microneedles for transdermal penetration enhancement of various formulations including nanocarriers are given in **Table 2.6**.

Table 2.6. Transdermal delivery through microneedle treated skin

References	Formulation & Drug	Problem addressed	Observations
Qiu Y. et al., 2008 [67]	Elastic liposomes of Docetaxel	High molecular weight and poorly water soluble drug	Enhanced transdermal flux (1.3–1.4 μg/cm^2/h) and 70 % decrease in lag time through microneedle-treated skin
Patel D. et.al., 2015 [68]	Lopinavir spray	Poor bioavailability (~20%) and extensive first pass metabolism	Improved permeation (PER, 1.77) and transdermal flux (52.5 μg/cm^2/h) through microporated pig ear skin
Thakkar H.P. et.al., 2015 [69]	Ethosomal hydrogel of Raloxifene HCl	Poor aqueous solubility and extensive first pass metabolism	Enhanced transdermal flux (6.194 μg/cm^2/h) through the microporated skin
Chen G. et.al., 2015 [70]	Triptolide loaded liposome hydrogel patch	Severe digestive, liver, cardiac, hematopoietic system and urogenital toxicities	Combining patch with microneedle technology could provide a safe and efficient administration method of triptolide for treating Rheumatoid Arthritis
Coulman S.A. et.al., 2009 [71]	Antigen loaded nanoparticles	Poor skin penetrability of macromolecules	Microneedle array devices significantly enhanced the intra/transdermal delivery of nanoparticle formulations
Martanto W. et.al., 2004 [72]	Insulin solution	Poor skin penetrability of macromolecules	Higher insulin concentration in donor solution, shorter microneedle insertion time, and fewer repeated insertions showed larger drops in blood glucose level and higher plasma insulin concentrations
Zhang W. et.al., 2010 [73]	PLGA nanoparticles	Poor skin penetrability of nanoparticles	Permeation of nanoparticles into the skin was enhanced by microneedles
Vucen S.R. et.al., 2013 [74]	Ketoprofen-loaded nanoparticles	Poor flux with sustained release system	Enhanced flux of ketoprofen in microneedles treated skin over a prolonged period of time

References	Formulation & Drug	Problem addressed	Observations
Ramadan E. et.al., 2016 [75]	Lamivudine loaded nanoparticles	Short half-life of Lamivudine	Enhanced steady state flux by > 2 folds using microneedle-mediated transport
Andar A.U. et.al., 2017 [76]	Gas vesicle Nanoparticles	Permeation enhancement	Permeation enhanced after microneedle-treatment of pig skin

2.6 QUALITY BY DESIGN

Quality by design (QbD) is a systematic, holistic, scientific, risk-based and proactive approach that deals with identification of product characteristics that are critical to product quality, relate them with the material attributes or process parameters and establish how these critical attributes can be varied to consistently produce quality product. Thus, pharmaceutical QbD may include following elements:

2.6.1 Defining quality target product profile (QTPP)

QTPP is the quality characteristics that the drug product must possess so as to reproducibly deliver the therapeutic benefit claimed in the label. It is generally acknowledged as a tool for setting the tactical foundation for drug product development. QTPP plays a vital role in the entire drug product development process like, effective optimization of a drug product, decision-making within an organization and constructive communication with regulatory bodies.

2.6.2 Product designing

In order to design and develop a robust product with desirable QTPP, a serious consideration must be given to the physical, chemical, and biological properties of all form of drug substance for which there is an interest in development and which can potentially be created during processing or *in vivo*. Consideration must also be given to another critical formulation challenge, drug-excipient compatibility to reduce undesirable stability issues and enhance understanding of drug-excipient interactions. Such studies are termed as preformulation studies that need to be conducted routinely to appropriately align dosage form

components and processing with drug substance and performance criteria.

2.6.3 Process designing

The process selection depends upon the materials' properties and product design. Factors like facility, equipment, manufacturing variables, material movement and QTPP should be consider for designing a process. It is often necessary to perform preliminary feasibility trials based on the existing knowledge about the product to be developed, and type of process. Utilization of computer-aided process design (CAPD) and process simulation in drug product and process design is also gaining importance to develop more robust processes capable of producing high quality products at a faster rate and lower cost.

2.6.4 Establishing CQA, CMA and CPP

Process development involves investigation of quality attributes raw materials and process parameters that results s large number of variables that are impossible to investigate in single shot. Hence, prior understanding and the risk assessment is frequently utilized to reduce variables to be examined. Critical quality attributes (CQA) can be defined as physical, chemical, biological, or microbiological characteristic direct or indirect control of which is essential to ensure the product quality. Critical process parameters (CPP) are process inputs and Critical material attributes (CMA) are drug substance and excipients' input that significantly influence CQA when altered within their operation range (control space). Ideally, a thorough understanding of the interactions between CMA and CPP is desirable so that any change in CMA can be compensated via fine tuning of CPP. Design of experiment (DOE) helps in identifying optimal conditions, critical factors that most influence CQAs and also about the interactions and synergies between CMA and CPP. Based on the acceptable lower and upper limits of CQA, the design space of CMA and CPP can be established. A review of literature on application of DOE in development of nanocarriers as well as microneedles are summarized in **Table 2.7**.

Table 2.7. Literature on formulation optimization by Response surface design

References	Formulation (Drug)	Response surface Design selected	Remarks
Varshosaz J. et.al., 2010 [77]	Solid Lipid Nanoparticles (SLN) of Amikacin	Central composite design	Ratio of drug to lipid, amount of lipid phase and volume of aqueous phase were optimized to get 149 nm particle size and 88 % drug-loading efficiency
Molpeceres J. et.al., 1996 [78]	Polycaprolactone Nanoparticles of Cyclosporin A	Central composite design	Temperature of the aqueous phase, needle gauge, volume of the organic phase, and the amounts of polymer and surfactant were optimized
Kollipara S. et.al., 2010 [79]	PLGA Nanoparticles of Paclitaxel	Central composite design	Amount of polymer, amount of miglyol and duration of ultrasonication were optimized
Thakkar H.P. et.al., 2012 [69]	Ethosomal Hydrogel of Raloxifene	Central composite design	Influence of lipid and ethanol concentration on vesicle size and entrapment efficiency was extensively investigated
Zeng C. et.al., 2016 [80]	Composite Phospholipid Liposomes of Tilianin	Central composite design	Amount of phospholipids, amount of cholesterol and weight ratio of phospholipid to drug were optimized to get 101.4 nm particle size and 90.28 % encapsulation efficiency
Tefas L.R. et.al., 2015 [81]	Quercetin-loaded Liposomes	D-optimal design	Dipalmitoylphosphatidylcholine (DPPC) concentration, DPPC: Cholesterol molar ratio and quercetin concentration were optimized
Mura P. et.al., 2008 [82]	Benzocaine Liposomes	D-optimal design	Qualitative factors (Potassium glycyrrhizinate, cholesterol, stearylamine, dicethylphosphate) and Quantitative factors (percent of ethanol and total volume of hydration phase) were optimized
Varshosaz J. et.al., 2012 [83]	Solid Lipid Nanoparticles of Amikacin	D-optimal design	Influence of type and concentration of cryoprotectants and Pre-freezing temperature on Freeze-drying process was investigated
Choisnard L. et.al., 2005 [84]	Cyclodextrin Nanoparticles	D-optimal design	Water fraction, acetone fraction and ethanol fraction were optimized
Amodwala S. et.al., 2017 [85]	Microneedle patch of Meloxicam	D-optimal design	Ratio of polyvinyl alcohol to polyvinyl pyrrolidone and solid content of matrix solution were optimized to maximize needle strength

References	Formulation (Drug)	Response surface Design selected	Remarks
Shi J. et.al., 2012 [86]	Liposomes gels of Paeonol	Box-Behnken design	DC-Chol concentration, molar ratio of lipid/drug and polymer concentration were optimized
Solanki A.B. et.al., 2007 [87]	Piroxicam Proniosomes	Box-Behnken design	Molar ratio of Span 60:cholesterol, surfactant loading, and amount of drug were optimized
Moghddam S.M.M. et.al., 2016 [88]	Nanostructured lipid carriers of Nimesulide	Box-Behnken design	Ratio of stearic acid: oleic acid, poloxamer 188 concentration and lecithin concentration were optimized
Sharma D. et.al., 2014 [89]	Polymeric Nanoparticles of Lorazepam	Box-Behnken design	Polymer, surfactant, drug, and aqueous/organic ratio were optimized
Wang F. et.al., 2013 [90]	Methazolamide-loaded Solid Lipid Nanoparticles	Box-Behnken design	Amount of GMS, amount of phospholipid, concentration of surfactant were optimized
Rahbar N. et.al., 2014 [91]	Chitosan-Coated Magnetite Nanoparticles	Box-Behnken design	Influence of pH, initial metal concentration and the amount of damped adsorbent on adsorption process was investigated
Bhavsar M.D. et.al., 2005 [92]	Nanoparticles-in-Microsphere	3^3 Full factorial design	Polymer concentration in organic phase, amount of nanoparticles added as internal phase, and the speed of homogenization were optimized
Lalani J. et.al., 2013 [93]	Protein functionalized PLGA nanoparticle of Tramadol	3^3 Full factorial design	Effect of polymer concentration, stabilizer concentration and organic:aqueous phase ratio were evaluated on particle size and entrapment efficiency
Chourasiya V. et.al., 2016 [94]	PLGA Nanoparticle of Atenolol	3^3 Full factorial design	Amount of polymer, surfactant concentration in aqueous phase and aqueous phase volume were optimized
Gambhire M.S. et.al., 2011 [95]	Dithranol-loaded Solid Lipid Nanoparticles	3^2 Full factorial design	Lipid ratio and Sonication time were optimized to get desired particle size and entrapment efficiency
Murthy R.S.R. et.al., 2004 [96]	Flutamide Liposomes	3^3 Full factorial design	Volume of organic phase, volume of aqueous phase, and drug: phosphotidylcholine:cholesterol molar ratio were optimized
Kiafar F. et.al., 2016 [97]	Filgrastim loaded Liposomes	3^2 Full factorial design	Molar ratio of Lipid/cholesterol and hydration time were optimized

2.6.5 Controlling the manufacturing process

The multidimensional combination and interaction of CMA and CPP that were revealed to provide quality assurance is known as Design space which is subjected to regulatory assessment and approval. Variation within the approved design space is not regarded as a change and hence, do not trigger a regulatory post-approval change process. A significantly smaller control space (or normal operating ranges of CMA and CPP to assure reproducibility) than the design space represents a robust process.

The FDA introduced Process Analytical Technology (PAT) to further advance identification, simulation, and control of pharmaceutical process. PAT deals with designing, analyzing and controlling manufacturing via timely measurements of CQA of raw and in-process materials and processes to ensure final product quality.

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