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Al-Quds University

Formulation of Topical Pregabalin for the Treatment of Neuropathic Pain

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M.Sc. Thesis

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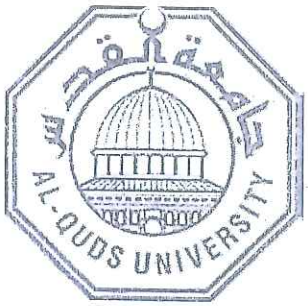

Thesis Approval

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Dedication

I lovingly dedicate this thesis to my beloved parents, who never stop giving of themselves in countless ways for their endless support and encouragement. To My beloved brothers and sister, who supported me each step of the way and have been my constant source of inspiration. They all have given me the drive and discipline to tackle any task with enthusiasm and determination. Without their unfailing support and help this work would not have been possible. To all my supportive friends during this journey.

Halima Thwaib

Declaration

I certify that the thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Signed: Halima .....

Halima Ahmad Ibrahim Thwaib

Date: 25.11.2018

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Halima Ahmad Thwaib

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Abstract

Pregabalin is an anticonvulsant drug used to treat epilepsy and neuropathic pain associated with diabetic peripheral neuropathy and post-herpetic neuralgia marketed by Pfizer under the brand name Lyrica®. It is available in capsules, oral solution, and extended-release tablets and is associated with a number of undesirable side-effects especially on the kidneys.

The aim of this study is to develop pregabalin microemulsion for topical treatment of neuropathic pain in order to overcome the troubles associated with its oral delivery and to investigate the effect of different PEs on drug permeation rate.

The experimental studies had been implemented in three stages: **the first stage**, the construction of pseudo-ternary phase diagram using aqueous titration method. The microemulsions were formulated using different oil phases such as oleic acid, IPM and R(+)-Limonene oil which were also used as PEs, 0.1 M pregabalin as the aqueous phase with PG in different ratios. Tween 80 as the surfactant and ethanol as the cosurfactant which were used in different ratios.

The second stage, the selection of microemulsion formulations: three systems (B,H and N) were selected to figure out the best PE among the three oils (Oleic acid, IPM and R(+)-Limonene) according to the highest permeability coefficient value (P) and three other systems (E,K and Q) were selected to choose the system having the highest permeability coefficient (P) in the presence of PG.

The third stage is the diffusion study of the selected systems. Diffusion parameters that were determined: Cumulative amount of drug released per unit area (Mt), the lag time (T_L), diffusion coefficient (D) ,the partition coefficient (K) and the permeability coefficient (P).

Among the three systems (B,N and H), oleic acid was the best PE among the three oils since it has the highest permeability coefficient (P). On the other hand, between the three other systems (E, K and Q) system K has the highest permeability coefficient value (P) in the presence of PG. Which indicates that PG works synergistically with IPM in system K in enhancing the drug permeability.

The results indicate that microemulsion formulation can be used as a feasible alternative to conventional formulations of pregabalin with advanced permeation characteristics for improved topical drug delivery. Short initial lag times were observed indicating that pseudo-steady state conditions were quickly achieved in all cases.

Keywords: Pregabalin, neuropathic pain, microemulsion, topical drug delivery, Franz diffusion cell, penetration enhancer.

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List of Abbreviations

%	Percentage
A	Surface area of diffusion
API	Active pharmaceutical ingredient
A_T	Monophasic
^oC	Celsius
C	Number of independent chemical constituents
C_1	Concentration in the membrane in the donor compartment
C_2	Concentration in the membrane in the donor compartment
C_0	Concentration of the drug in the donor compartment
cm	Centimeter
Cn	Concentration of penetrant (mg/ml) determined at nth sampling interval
Conc.	Concentration of penetran (mg/ml)
CNS	Central nervous system
C_r	Concentration of the drug in the donor compartment
D	Diffusion coefficient
DMSO	Dimethyl sulphoxide
DPN	Diabetic peripheral neuropathy
e.g	Exempli gratia (for example)
F	Number of possible independent changes of state or degrees of freedom
FDC	Franz diffusion cell
Fig	Figure
g	Gram
GABA	Gamma-Aminobutyric acid
GAD	Generalized anxiety disorder
GRAS	Generally regarded as safe
h	The thickness of the skin (stratum corneum) or the diffusion path length in the skin
HLB	Hydrophilic lipophilic balance
HPMC	Hydroxypropyl Methyl Cellulose

HPLC	High-performance liquid chromatography
hr	Hour
i.e	*id est* (that is)
IPM	Isopropyl myristate
J	Steady state flux
K	Skin/vehicle partition coefficient.
K_0	Rate constants of zero order
K_1	Rate constants of first order
K_H	Rate constant of Higuchi equation
K_p	Permeability coefficient
LPP	Lipid-protein partitioning
M	Molarity
m	Mass
ME	Microemulsion
mg	Milligram
min	Minutes
ml	Milliliter
mm	Millimetre
µm	Micrometer
M_t	Cumulative amount per unit area
MWCO	Molecular weight cut off
NaOH	Sodium Hydroxide
nm	Nanometer
NP	Neuropathic pain
NSAID	Nonsteroidal anti-inflammatory drug
o/w	Oil in water
P	Number of phases present in system
PBS	Phosphate buffer saline
PE	Penetration Enhancer
PEG	Polyethylene glycol
pH	Potential of Hydrogen

PHN	Postherpetic neuralgia
PG	Propylene glycol
ppm	Part per million
PVA	Polyvinyl alcohol
PVDF	Polyvinylidene Fluoride
PVP	Polyvinylpyrrolidone
Q_0	The initial amount of drug
Q_t	The amount of drug release in time
R^2	Correlation coefficient
rpm	Round per minute
RSD	Relative standard deviation
s	Second
S	Cross section (area) of the membrane
SAD	Social anxiety disorder
SC	Stratum corneum
SD	Standard deviation
SQRT	Square root of time
T	Time
TDDS	Transdermal drug delivery system
T_L	Lag time
UV	Ultraviolet
V	Volume
v/v	Volume per Volume
w/o	Water in oil
wt /wt	Weight per weight
x	The distance perpendicular to the surface of the barrier

Chapter One

Introduction

1.1. Skin

The skin is the largest human organ. Over the last 2-3 decades, the skin has become an important route for the delivery of drugs for topical, regional, or systemic action. The skin, however, has evolved as a physical and biochemical protective barrier, which prevents the loss of water from the body, and guards against entry into the body of external toxic chemicals and infectious agents, thereby maintaining homeostasis. This role of the skin as a barrier to the external environment renders the absorption and transdermal delivery of most drugs problematic. The stratum corneum (Fig. 1.1), which is the outermost layer of the skin and comprised of keratin-rich cells embedded in multiple lipid bilayers, has been considered the rate-limiting structure governing percutaneous absorption of many kinds of permeants [1].

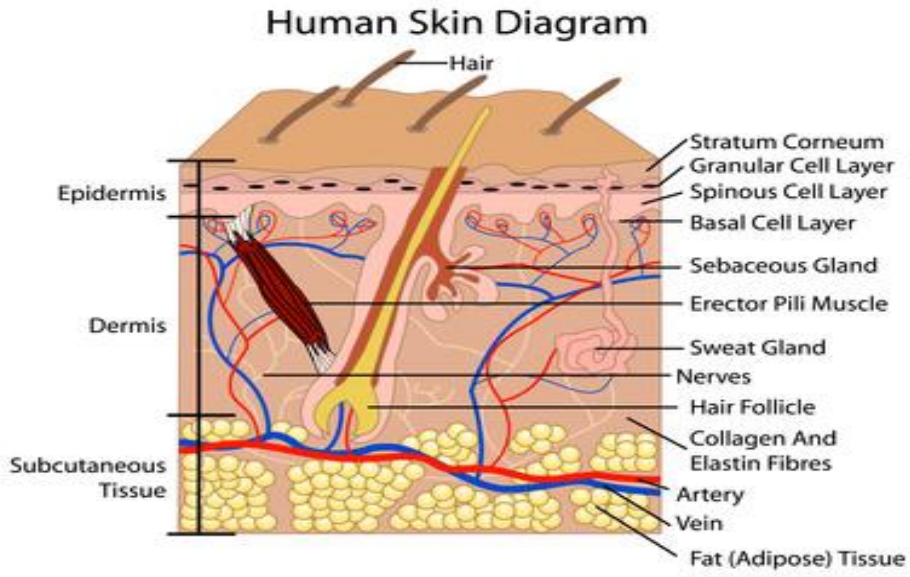

Fig1.1: Diagrammatic representation of the cross-section of human skin [2]

1.1.1. Skin Structure

The principal parts of the skin are the following :

a. Epidermis: It is the outermost layer of skin. It forms waterproof defensive wrap upon body's surface and formed up of stratified squamous epithelium underlying basal lamina. It contains no blood vessels and is nourished by diffusion from dermis and has role in regulation of body's temperature. The types of cells in the epidermis are Keratinocytes, Melanocytes, Langerhans cells, and Merkel cells. It is further divided into 5 sublayers: Stratum corneum, Stratum lucidum, Stratum granulosum, Stratum spinosum and Stratum germinativum. Stem cells in the stratum basale undergo continuous cell division, producing keratinocytes for the other layers [3,4].

b. Dermis: It is the layer of skin underneath the epidermis that consists of connective tissue and mitigates the body from stress and strain. It is rigidly connected to epidermis by basement membrane. It contains hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels and blood vessels. The dermis is structurally segregated into two areas; a superficial area called as papillary region and the reticular region [3].

c. Hypodermis: Its rationale is to attach the skin to underlying bones and muscles. It consists loose connective tissue [3].

1.1.2. Routes of Drug Penetration through the Skin

The outermost layer of the skin is SC. It attributes to the barrier function of the human skin. Properties of this barrier are based on specific content and composition. The bilayer lipids and surrounding corneocytes produces 'Brick-and-Mortar' model. The high density of the skin and

the low hydration of the skin considered as skin barriers [4]. There are two general options for drug substances to permeate the SC (Fig. 1.2):

1.The transepidermal route: It can be divided into:

a. The transcellular route : The more direct route is the transcellular. Here the drug has to cross the skin by directly passing through both the lipid structures of the SC and the cytoplasm of the dead keratinocytes. This is the shortest route for the drug substance, but the substances encounter significant resistance to permeation because they have to cross both lipophilic and hydrophilic structures [5].

b. The intercellular route : **The more common route** for drugs to permeate the skin is the intercellular route. Here the permeant overcomes the SC by passing between the corneocytes. The intercellular spaces consist of a mixture of lipids–ceramides, free fatty acids and their esters, and cholesterol and its sulphates that are structured in bilayers [5] .

2. Via pores route: Since the skin appendages (glands and hair follicles) occupy only 0.1% of the total human skin surface, the contribution to the pore route was primarily considered to be small. Follicular penetration may also be an important pathway for the penetration of topically administered substances. The follicular apparatus of hair follicles, the sweat glands and microlesions in the interfollicular horny layer were introduced as theoretical vertical pathways for percutaneous penetration [5] .

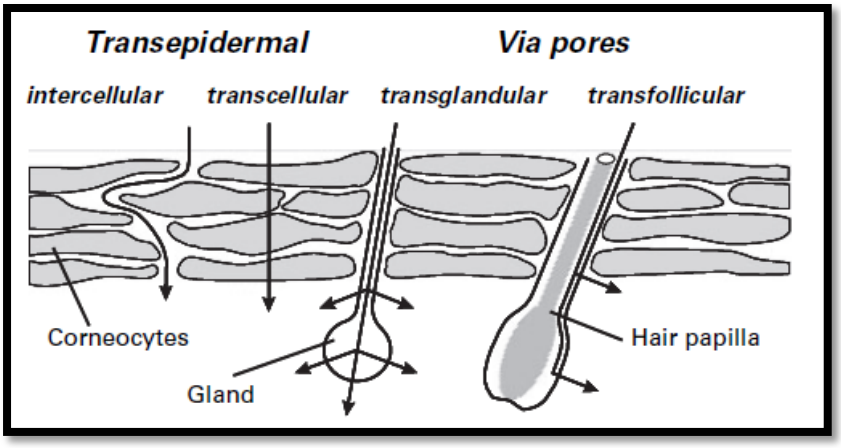

Fig 1.2: Options of drug penetration a crosss the staratum cornum schematically [5]

1.1.3. Physicochemical aspects of skin penetration

Even though the skin is such a heterogeneous membrane in most cases, the underlying transport process through the skin is controlled by simple passive diffusion. Fick's law of diffusion can be used to describe these processes:

$$J = \frac{DK\Delta C}{h}$$

where: J is the flux per unit area, D is diffusion coefficient of the drug in the skin, K is skin/vehicle partition coefficient, ΔC is the concentration difference across the skin, h is the thickness of the skin (SC) or the diffusion path length in the skin.

According to Fick's law, enhancement in the permeability of the drug can be achieved by altering any or all of the three parameters D, K, or h. The improvement of the drug permeation could be due to an increased diffusion within the skin, an increased partitioning or a decrease in

diffusion path length [1]. The mathematics of skin penetration will be fully illustrated in section (1.8).

1.2. Transdermal drug delivery

Advancements in drug delivery strategies are taking place at much prompt pace than the last decade. Currently 74% of drugs are taken orally but not found to be as effective as desired because of various obstacles coming in path of oral drug delivery. Today transdermal drug delivery system is one of the most capable modes of drug application. TDDS is defined as a dosage form, which when applied to the skin, deliver the drug through the skin at control rate to the systemic circulation. Currently transdermal delivery is one of the most promising methods for drug application. Increasing number of drugs are being added to the list of therapeutic agents that can be delivered to the systemic circulation via skin [6].

1.2.1. Advantages of Transdermal drug delivery

Transdermal drug delivery offers the following advantages over the oral route for controlled drug delivery [6,7,8]:

1. Avoidance of hepatic first pass metabolism and gastrointestinal incompatibility.
2. Ability to discontinue administration by removal of the system.
3. The ability to control drug delivery for a longer time than the usual gastrointestinal transit of oral dosage form.
4. The ability to modify the properties of the biological barrier to absorption .

5. Reduces side effects due to optimization of the blood concentration-time profile
6. Provides greater patient compliance due to the elimination of multiple dosing schedules
7. Enhances therapeutic efficacy.
8. Ensures ease of self administration.

1.2.2. Limitation with Transdermal Drug Delivery System

The outermost layer of the skin i.e. the SC provides the greatest resistance to penetration thereby limiting transdermal bioavailability of the drug and it is the rate limiting step in percutaneous absorption. Thus the transport of the drug across the skin membrane is a complex phenomenon. Transdermal drug delivery offers the following limitations [6]:

1. Local irritation may develop at the site of application.
2. A drug has large molecular size makes absorption difficulty. So drug molecule should ideally be below 800-1000 Daltons.
3. Many drugs with a hydrophilic structure having a low penetration through the skin and slowly to be of therapeutic benefit.
4. The drug molecule has to penetrate across the SC barrier in order to reach the deeper dermal region.
5. Drugs with high melting points (ideally less than 200 °C)

1.3. Penetration Enhancer

These are the substances that facilitate the absorption to penetrate through the skin by temporarily diminishing the impermeability of the skin. Penetration enhancers (PE) interact with structural components of SC i.e., proteins or lipids. They alter the protein and lipid packaging of the SC, thus chemically modifying the barrier functions of the skin leading to increased permeability of the drug through the skin. They are also known as **accelerants, absorption promoters** as they promote the penetration of topically applied drugs. PE can increase the drug diffusivity in the SC by dissolving the skin lipids or denaturing skin proteins. The type of enhancer employed has a significant influence on the design and development of the product. Permeability can be enhanced by altering the structure of the skin or by increasing the solubility of the drug in the skin [6].

1.3.1. Ideal characteristics of penetration enhancer

The ideal characteristics if any PE are the following. No single PE can possess all the required properties [2,5,6]:

1. It should be pharmacologically inert, non-toxic, non-irritating and non-allergenic.
2. It should be compatible with the drug and excipients.
3. It should not lead to the loss of electrolytes and body fluids, skin should immediately regain its barrier properties on its removal.
4.It should readily formulate into dermatological preparations, transdermal devices and skin adhesives.
5. It should be inexpensive and cosmetically acceptable.

1.3.2. Mode of action of penetration enhancers:

It is generally recognized that PEs enhance the permeation of drug across the skin via several mechanisms. However, to date the exact mechanism of action of PEs is only partially known. Skin PEs may exert their effects through one or a combination of the following mechanisms [1]:

1.The First suggested mechanism is solvent action. The PEs may plasticize or solubilize the skin-tissue components. The use of solvent that can extract the lipids in the SC and decrease its resistance to penetration.

2.The Second proposed mechanism is the interaction of enhancers with intercellular lipids leading to disruption of the highly ordered lamellar structure, thereby increasing the diffusivity of drugs through the lipid domain.

3.The Third proposed mechanism is the interaction of enhancers with intracellular protein to promote permeation of drugs through the corneocyte layer.

4.The Fourth proposed mechanism is an increase in the partitioning of drugs, co-enhancers or co-solvents into the SC.

The latter three mechanisms have been described as the lipid-protein partitioning (LPP) theory. In this theory, various possible locations of enhancer action within the intercellular and intracellular regions of SC have been proposed. In the intercellular region, three active sites where a PE may act in order to enhance the permeation of permeants have been suggested. These

three active areas are the area of polar head groups of the lipids, the aqueous region between the lipid head groups, and the lipid region of the hydrophobic tails within the bilayers [1] (Fig. 1.3):

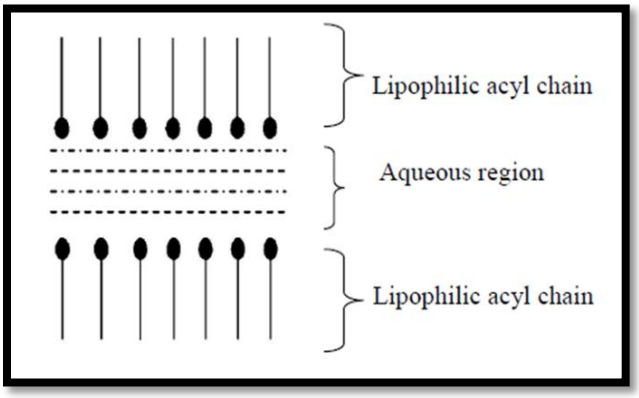

Fig 1.3: Diagrammatic representaion of he lipid bilayers of human SC [1].

1.The area of polar head groups of the lipids

The interaction of the enhancers with the polar head groups of the lipids. The lipid-lipid head group interactions and the packing order of the lipids are thus disturbed. The result is the facilitation of the diffusion of hydrophilic drugs. By the increased flow the content of free water molecules between the bilayers is increased, which leads to an augmentation of the cross-section for polar drug diffusion. Simple hydration can be used in structure modification which results in changes to drug penetration. By hydration of the SC, the penetration of most drugs can be increased. Normally in the SC the water content is 5–10%. The water content can be increased up to 50% under occlusive conditions. The head group disturbance of lipids by polar enhancer substances can also affect the hydrophobic parts of the lipids and leads to rearrangements in these bilayer areas. This also explains the penetration improvement for lipophilic drugs by use of lipid head group-influencing hydrophilic PEs [5].

2. The aqueous region between the lipid head groups

The effect of PE on the aqueous region by increasing its water content, there can be a direct action whereby the domain temporarily changes its bulk chemical constitution. With high concentration of solvents such as DMSO, PG or ethanol in a vehicle or device may penetrate into the aqueous region of the tissue that becomes a better solvent for molecules such as hydrocortisone. In other words, the operational partition-coefficient now favors an elevated drug concentration in the skin. The solvent then diffuses out into the dermis followed by the drug diffusion down its concentration gradient [5].

3.The lipid region of the hydrophobic tails within the bilayers

The interaction of lipophilic PEs with the hydrocarbon chains of the bilayer lipids. The penetration of lipophilic drugs is facilitated this way by packing order disturbance due to an increased fluidization of the hydrocarbon chains. These changes also influence the order of the polar head groups, which explains the penetration enhancement of hydrophilic drugs by use of a lipophilic enhancer substance [5].

(Fig. 1.4) Illustrates the influence of PEs on both the lipophilic pathway and the hydrophilic pathway of drug penetration.

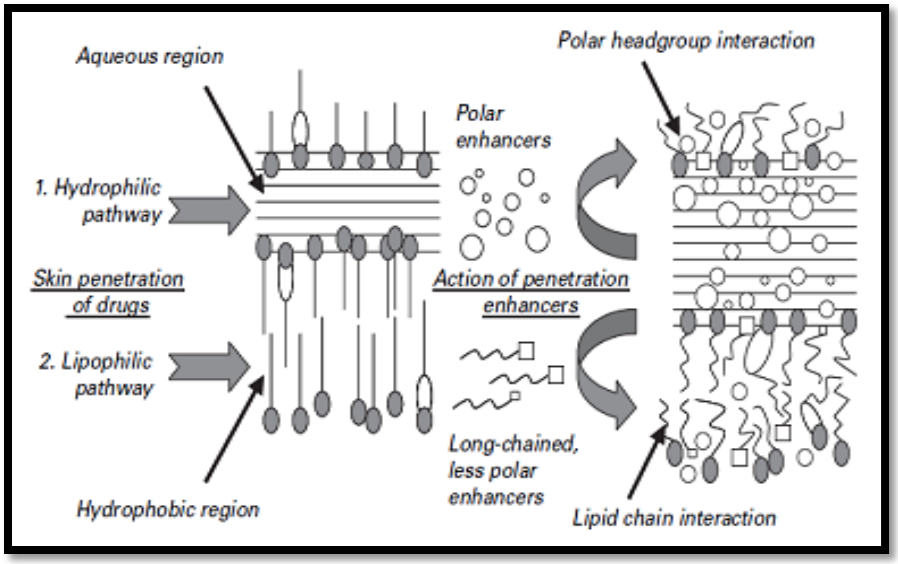

Fig1.4: Hydrophilic and lipophilic pathways of drug penetration and action mode of penetration enhancer [5].

1.4. Approaches to increase skin permeation

1.4.1. Chemical Penetration enhancer :

Various chemical PE have been investigated for overcoming the SC barrier and enhancing the transdermal delivery of drugs. Chemical PEs are relatively inexpensive and easy to formulate, they offer flexibility in their design, are simple in application and allow the freedom of self administration to the patient [6].

1.4.1.1. Surfactants

Surfactants can be classified into four main categories according to the presence of formally charged groups in the head, It is generally recognized that nonionic surfactants possess the least

toxicity and skin irritation potential, and therefore they have been widely investigated as skin penetration enhancers [1,2,9].

- ✓ **Anionic surfactants:**

In general, anionic surfactants are more effective than cationic and nonionic surfactants in enhancing skin penetration of target molecules. Some anionic surfactants interact strongly with both keratin and lipids and alter the permeability of the skin by acting on the helical filaments of the SC, thereby resulting in the uncoiling and extension of keratin filaments to produce keratin. Then they cause an expansion of the membrane, which increases permeability [9].

- ✓ **Cationic surfactants:**

The cationic surfactants interact with the keratin fibrils of the cornified cells and result in a disrupted cell-lipid matrix. The cationic surfactants may interact with anionic components of the stratum corneum, change the electronic property there, and stimulate the transfer of the anionic drug into the skin [9].

- ✓ **Nonionic surfactants:**

The nonionic surfactants such as Tween 80 (Fig. 1.5) enhance absorption by inducing fluidization of the SC lipids. They are two possible mechanisms by which the rate of transport is enhanced using nonionic surfactants. Initially, the surfactants may penetrate into the intercellular regions of SC, increase fluidity and eventually solubilize and extract lipid components. Secondly, penetration of the surfactant into the intercellular matrix followed by interaction and binding with keratin filaments may results in a disruption within the corneocyte [9].

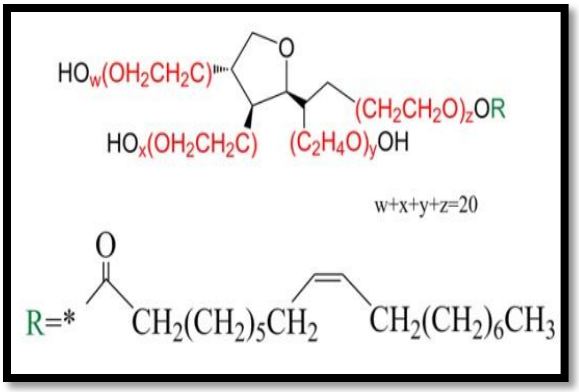

Fig 1.5: Polysorbate 80 (Tween 80) [10].

✓ **Zwitter ion:**

Zwitterionic surfactants have both cationic and anionic centers attached to the same molecule. The cationic part is based on primary, secondary, or tertiary amines or quaternary ammonium cations. The anionic part can be more variable and include sulfonates [9].

✓ **Biosurfactants:**

Biosurfactants are surface-active substances synthesized by living cells. Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions, and potential applications in environmental protection [9].

1.4.1.2. Water

Water is the most natural PE. Hydration state of the SC is important in determining penetration enhancement of a given drug. Increased hydration of the SC enhances transdermal flux of a variety of drugs [7].

1.4.1.3. Hydrocarbons

Several hydrocarbons including alkanes, alkenes, halogenated alkanes, squalene and mineral oil have been used as vehicles or PEs to increase permeation of a variety of drugs across the skin. These PEs work by partitioning into the SC and disrupting the ordered lipid bilayer structure [6].

1.4.1.4. Alcohols, fatty alcohols and Glycols

Alcohols such as ethanol which is commonly used in many transdermal formulations. Higher glycols e.g. PEG do not penetrate SC significantly and may form hydrogen bonds with penetrants, thus reducing the thermodynamic activity of the compounds and penetration rates (Fig. 1.6) [6].

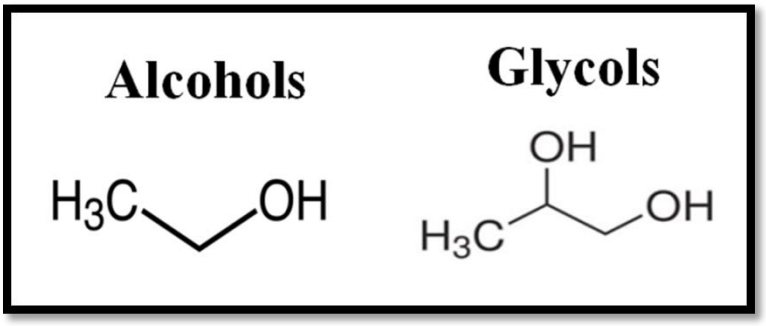

Fig: 1.6 Structer of Ethanol and Propylene Glycol [5]

Some compounds (example includes PEG, PG) used as cosolvent to saturate the solution of the active ingredients and thereby they maximize the thermodynamic activity of the penetrant and thus increase their absorption through the skin. PG has been used as a ‘stand-alone’ PE, but is more widely used as a vehicle for the application of accelerants and its efficacy is mixed as PE reported in literature. PG also works synergistically with many enhancers, including Azone, oleic

acid, fatty alcohols and terpenes. Its mechanisms of action, are probably same as alcohol (Ethanol). Permeation of the solvent through the tissue could alter the thermodynamic activity of the vehicle which would modify the driving force for diffusion [6,9].

1.4.1.5. Fatty Acids

Numerous fatty acids have been employed as PEs. It was appeared that saturated alkyl chain lengths of around C10 to C12 attached to a polar head group yields a potent enhancer, whereas with unsaturation in the alkyl chain then C18 chain lengths appear near optimum. The most potent straight-chain fatty acid enhancer appears to be lauric acid (C12), whereas the cis-unsaturated oleic acid (C18) (Fig. 1.7) is generally one of the prime enhancers selected for permeation investigations [6,9].

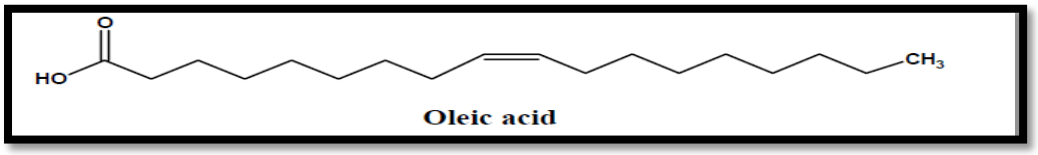

Fig 1.7: Chemical structure of oleic acid [1]

Mode of action; Considerable effort has been directed at investigating the mechanisms of action of oleic acid as a PE in human skin. From thermal analysis, it is apparent that the enhancer with the lipid domains within the SC as would be expected for long-chain fatty acid. A new structure was shown in the intercellular lipids in the presence of oleic acid, suggesting that a novel lipid domain is induced in the barrier lipids on exposure to this enhancer. The formation of such pools would provide permeability defects within the bilayer lipids, thus facilitating permeation of hydrophilic permeants via these defects. Lipophilic molecules are less hindered by the lipid

domains within the SC and hence their permeation is not enhanced to as great an extent as for the hydrophilic molecules. Irrespective of the mode of action, oleic acid has the considerable advantage over some other PEs of low irritancy [6,9].

1.4.1.6. Amides

Cyclic and acyclic amides form another large class of chemicals studied as PEs. Cyclic and amides such as Azone (Fig.1.8) and its analogues along with pyrrolidones are the most extensively used amides [6].

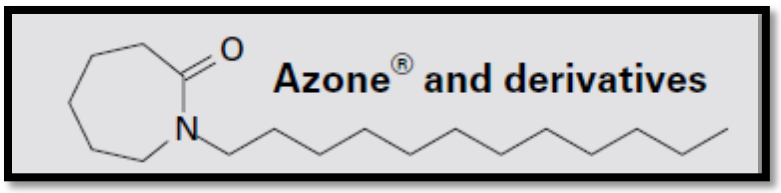

Fig 1.8: Chemical structure of Azone [5]

1.4.1.7. Esters

Esters of fatty acids have been used in several studies and show skin permeation enhancement of a wide variety of drugs. IPM (Fig. 1.9) is the most widely studied ester along with several other esters of fatty acids. These chemicals generally work by partitioning themselves in the ordered lipid domains of the SC [6].

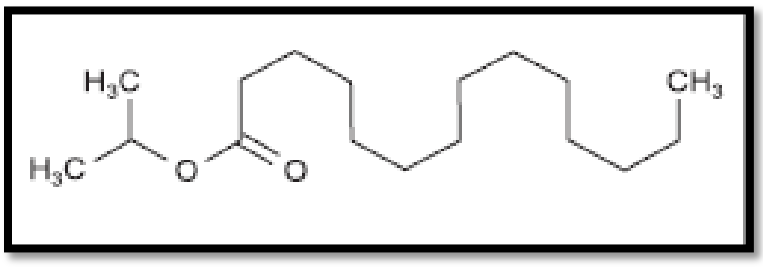

Fig1.9: Chemical structure of IPM [10]

1.4.1.8. Sulphoxide

Dimethyl sulphoxide (DMSO) (Fig. 1.10) is reported as skin PE and cosolvent. Properties exhibited by DMSO as, displacement of bound water from keratin, extraction of skin lipids, change in keratin conformation and interaction with lipid alkyl chains in SC. The problem with DMSO, is the high amounts which appears to be needed for penetration enhancement and associated issues of irritation. Due to its potential toxicity and adverse reactions, it could not be a better choice [7].

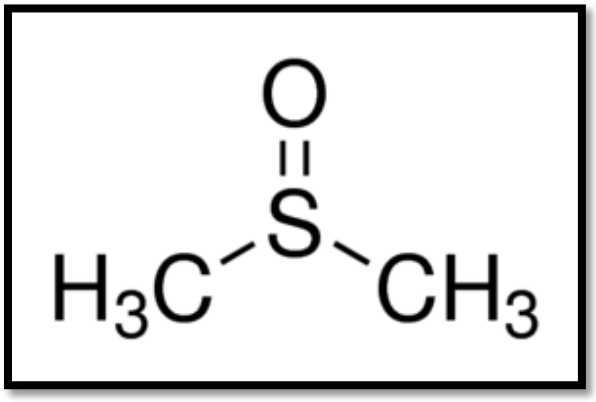

Fig 1.10: Chemical structure DMSO [5]

1.4.1.9. Terpenes, terpenoids and essential oils

Chemical structure of terpenes and terpenoids consist of number of repeated isoprene (C_5H_8) units which is used to classify terpenes. Terpenes are found in essential oils, and are compounds comprising of only carbon, hydrogen and oxygen atoms, but which are not aromatic. Numerous terpenes have long been used as medicines as well as flavoring and fragrance agents. They are in general clinically acceptable and relatively safe skin PE for both lipophilic and hydrophilic drugs. In general, they have low systemic toxicity and skin irritancy in addition to having good

penetration enhancing abilities. Many terpenes permeate human skin well and large amounts of terpenes have been found in the epidermis after application from a matrix-type patch. Terpenes may also modify drug diffusivity through the membrane. An example of terpenes is R(+)-Limonene (Fig. 1.11) which is natural cyclic monoterpen. It is extracted from rinds of citrus fruits [7,11,12].

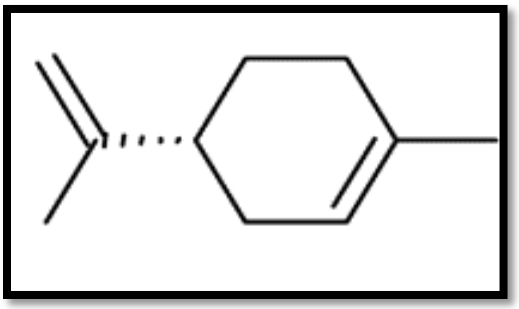

Fig 1.11: Chemical structure of R(+)-limonene [5]

1.4.2. Drug Vehicle Based

Drug vehicle based method of penetration enhancement does not change SC function like chemical and physical penetration enhancement method. This method is based on drug selection, vesicles, prodrug, chemical potential of drug and eutectic system. The interaction of enhancers with SC and the development of structure activity relationships for enhancer will aid in the development of enhancers with optimal characteristics and minimal toxicity [11]. Some of the carriers and vesicles:

a. Liposomes

Liposomes are colloidal particles formed as concentric bimolecular layers that are capable of encapsulating drugs. Phospholipids in liposomal systems can disrupt the bilayer fluidity in the SC. Liposomes used for high molecular weight and low solubility drug [2,11].

b. Ethosomes ("soft vesicles")

Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water. Ethosomes improving the drug's efficacy, enhancing patient compliance and comfort and reducing the total cost of treatment [11].

c. Microemulsion

Microemulsions are isotropic, thermodynamically stable solutions in which substantial amounts of two immiscible liquids are brought into a single phase by means of an appropriate surfactant or surfactant mixture. It provides large concentration gradient and interact with the rigid lipid bilayer structure and acts as a chemical enhancer [3,11].

1.4.3. Physical techniques

1.4.3.1. Iontophoresis

It is a process which involves the transport of ionic or charged molecules into a tissue by the passage of direct or alternating electric current through an electrolyte solution containing the ionic molecules to be delivered using an appropriate electrode polarity. The process involves the

transfer of ions into the body by an electromotive force. Ions with positive charge are driven into the skin at the anode and those with negative charge at the cathode. The current intensity should be increased slowly, maintained for the length of the treatment and decreased slowly at the end of the treatment. The current must be within comfortable tolerance of the patient. The drawbacks associated with the iontophoresis technology include the possibility of electric shock, skin irritation, burns and cost of treatment [2,6].

1.4.3.2. Electroporation

This process involves the application of transient high voltage electrical pulse to cause rapid dissociation of the SC through which large and small peptides and other drugs can pass. The degree of enhancement achieved in-vitro is related to the applied voltage, number and duration of the pulses offering the possibility of a controllable phenomenon [2,6].

1.4.3.3. Sonophoresis

This process involves the usage of high frequency ultrasound waves. The application of low frequency ultrasound can increase the permeability of human skin to many drugs including high molecular weight proteins by several orders of magnitude, thus making transdermal administration of these molecules potentially feasible [2,6].

1.4.3.4. Microfabricated Microneedles

It is a novel technology which employs micron-sized needles made from silicon. Microneedles penetrate the skin about 10-15 mm deep inside the skin but do not reach the nerves found in

deeper tissue, so are painless. These microneedle arrays, after insertion into the skin create conduits for transport of drug across the skin. The drug after crossing the SC diffuses rapidly through deeper tissue and taken up by capillaries for systemic administration. A microprocessor is attached to a tiny pump for delivering tiny amounts of the drug [6].

1.5. Microemulsion

Microemulsions are isotropic, thermodynamically stable transparent systems of oil, water and surfactant. They are frequently in combination with a cosurfactant with a droplet size usually in the range of 20 to 200 nm. The use of microemulsions is advantageous not only due to the facile and low cost preparation, but also because of the improved bioavailability. The increased absorption of drugs in topical applications is attributed to enhancement of penetration through the skin by the carrier [11].

It has been estimated that approximately half of the approved drugs are lipophilic and have poor absorption characteristics when administered from oral route. To meet with such challenge, drug discovery programs searched new alternative route for drug administration with new drug delivery platforms. Microemulsions based formulations intended to skin application brings out very promising results as solubility enhancement drug; modify drug permeability in topical microemulsion vehicle and quick penetration of drug in skin. Topically administered microemulsion systems have been shown to provide improved drug stabilization in comparison to topical formulation developed conventionally [11,12].

1.5.1. Components of Microemulsion

The components of microemulsion formulation as oils and surfactants are largely present but due to their toxicity, irritancy and unclear mechanism of action retards their use. For mild microemulsions to form, biocompatible, clinically acceptable, non toxic components should be screened. Therefore greater emphasis is laid upon use of GRAS excipients [3,13].

- **Oil phase**

The selection of oil is based on the nature of the drug as well as the route of administration. The oil influences the curvature and has the capability to swell the tail group of surfactant. Saturated and unsaturated fatty acids have penetration enhancing activity of their own. The fatty acids increase the permeability by disrupting densely packed lipids and filled up in extracellular spaces of SC. Amongst unsaturated fatty acids, oleic acid is an effective skin PE. Also penetrating effect of fatty acids is selective of individual drug [3].

- **Surfactant**

The actual purpose of surfactant is to lower the interfacial tension to negligible value facilitating the process of dispersion during preparation of microemulsion. It presents the microemulsion with pertinent lipophilic character to furnish accurate curvature. This adsorption behavior can be attributed to solvent nature and to the chemical nature of surfactant that combines both polar and non polar group in a single molecule. Due to their dual nature these amphiphiles “sit” at interfaces so that their hydrophobic moiety is repelled from strong solvent interactions. Surfactant screening can be done with help of HLB (Hydrophilic lipophilic balance) value. The

HLB provides a numerical value that suggests whether o/w or w/o emulsion will form. The accepted fact is that generally low HLB surfactants are favorable for w/o microemulsion and high HLB surfactants are suited best for o/w microemulsions [3,14].

Generally, surfactants derived from natural sources are preferred over synthetic surfactants. Non ionic surfactants are good replacement for naturally occurring surfactants because of their good cutaneous tolerance, lower irritation potential and toxicity. Tweens have been investigated for their minimal toxicity. In general, the surfactant concentration should be kept to a minimum [3,14].

- **Cosurfactants**

Most of the time, surfactant alone cannot lower the oil/water interfacial tension sufficiently to yield a microemulsion. Liquid crystalline phases are often formed when the surfactant film is too rigid. It is necessary to add cosolvents (amphiphilic short chain molecules) or cosurfactants to bring the interfacial tension close to zero. The amphiphilic nature of these additives (with the length of the carbon chain ranging from (C2 to C9) enables them to interact with surfactant molecules at the interface, thereby affecting their packing. Cosurfactants penetrate the surfactant monolayer providing additional fluidity to the interfacial film by disrupting liquid crystalline phases. Commonly used cosurfactants include alcohols, derivatives of glycols or PG [14].

- **Aqueous phase**

Most commonly, water is used as aqueous phase. As in case of microemulsions used for parenteral administration aqueous phase should be isosmotic to blood which is adjusted by sodium chloride, glycerol and sorbitol [3].

1.5.2. Phase behavior

The relationship between the phase behavior of mixture and its composition can be confined with the support of phase diagram. The phase behavior of simple microemulsion systems comprising oil, surfactant and cosurfactant can be studied with the aid of ternary phase diagrams (Fig. 1.12) in which each corner of the diagram represents 100% of the meticulous component. However, almost always in case of microemulsions, they contain an additional component as cosurfactant and/or drug. In the case where four or more components are involved, pseudo-ternary diagrams are constructed where a corner represents binary mixture of two components as surfactant/cosurfactant, water/drug or oil/drug [3,15].

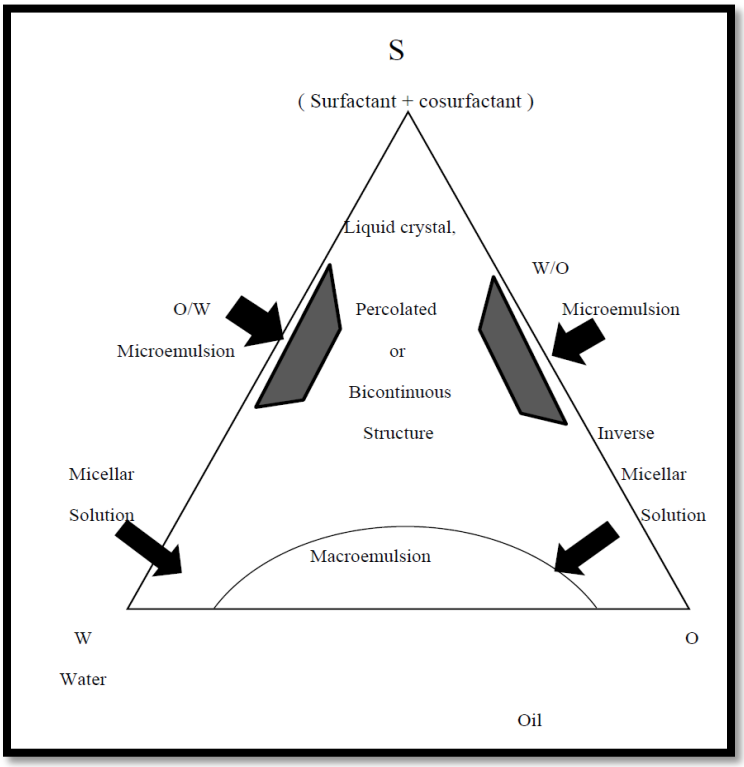

Fig 1.12: Pseudo-ternary phase diagram of oil, water and surfactant showing microemulsion region [3].

1.5.2.1. Phase rule

The phase rule enables identification of the number of variables depending on system compositions and conditions. It is depicted as:

$$F = C - P + 2$$

Where, F is the number of possible independent changes of state or degrees of freedom, C the number of independent chemical constituents and P the number of phases present in system. The F value determines the system to be invariant, monovariant, bivariant , and so on, depending on its value whether zero, 1, 2 or so on. At low surfactant concentration, there is series of equilibria between phases, referred as winsor phases [3,15] (Fig. 1.13):

Winsor I: The microemulsion phase (o/w) is in equilibrium with the upper excess oil. The surfactant rich water phase coexists with oil phase where surfactant is only present as monomers at small concentration.

Winsor II: The upper microemulsion phase (w/o) is in equilibrium with excess of water. The surfactant rich oil phase coexists with surfactant poor aqueous phase.

Winsor III: The middle microemulsion phase (o/w plus w/o called bicontinuous) is in equilibrium with excess oil and lower excess water. Surfactant rich middle phase coexists with both excess water and oil surfactant poor phase.

Winsor IV: Here oil, water and surfactant are homogenously mixed to form isotropic single phase micellar solution.

Inter-conversion between these phases can be produced by adjusting the proportions of components [3,15].

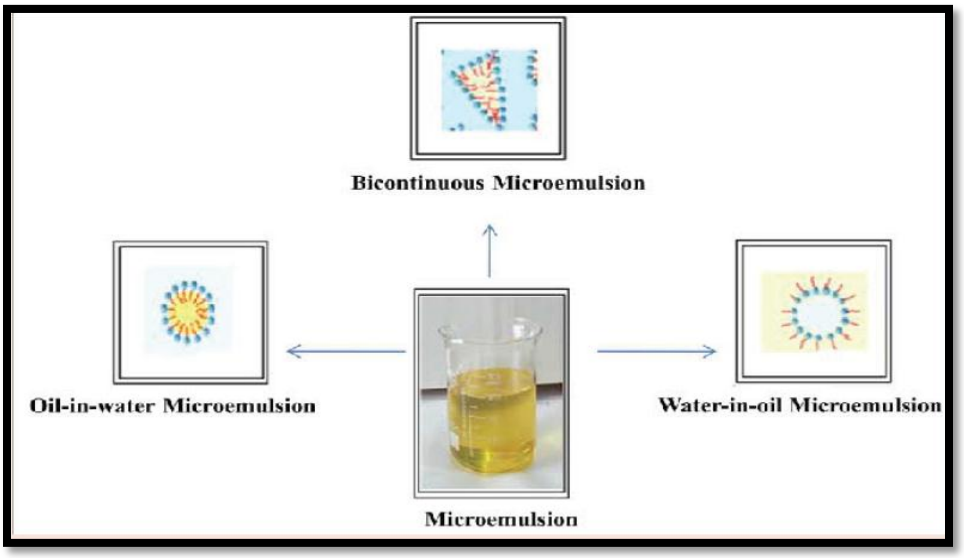

Fig 1.13: Different types of microemulsions depending on the composition [3].

1.5.2.2. Method of Preparation

A. Phase titration method:

Microemulsions are prepared by spontaneous emulsification method, which is illustrated with help of phase diagrams. Phase diagram construction is practical approach to study complex series of interaction which occurs when different components are mixed. The aspect of the phase diagram is phase equilibrium and demarcation of phase boundaries. Most often pseudo-ternary phase diagrams are constructed to figure out microemulsion zone as quaternary phase diagram is time consuming and difficult to interpret [3,15].

B. Phase inversion method:

Phase inversion of microemulsion happens upon addition of excess of dispersed phase. Phase inversion leads to radical physical changes as change in particle size that alter drug release. During cooling, this system crosses the point of zero spontaneous curvature and minimal surface tension, prompting the formation of finely dispersed oil droplets. Microemulsions can be prepared by controlled addition of lower alkanols to milky emulsions to produce transparent solutions comprising dispersions of either o/w or w/o or colloidal dispersions. The lower alkanols are called co-surfactants. They lower the interfacial tension between oil and water sufficiently low for almost spontaneous formation [3,15,16].

1.5.3. Advantages Microemulsions as Topical/Transdermal Delivery Systems

The interest in microemulsions as topical/transdermal delivery systems results from the multiple advantages that these systems present, as described below. Some of the features described here are not exclusive of microemulsions and are displayed by other dermatological formulations and delivery systems. However, few of them combine all the features described as microemulsions do, which provides a reasonable explanation for the popularity of microemulsion use for topical and transdermal delivery. The advantages of microemulsions include the following [7,13]:

1.Thermodynamic stability.

2. The ease of preparation, as only low energy input is required.

3. The cost of preparation being generally low.

4. The possibility of incorporating both hydrophilic and lipophilic drugs (at the same time, if desirable), due to the presence of hydrophilic and lipophilic domains.

5. The increased drug loading, since the amphiphilic interface can be viewed as an additional region for drug solubilization if compared to non-structured oily or aqueous vehicles.

6. The penetration-enhancing ability.

From all of the above mentioned properties, the last is probably one of the most relevant when it comes to the use of microemulsions as a delivery system to the skin. Due to the small droplet size and large amount of inner phase in microemulsions, the density of droplets and their surface area are assumed to be high. Therefore, droplets settle down to close contact with the skin providing high concentration gradient and improved drug permeation. Moreover, low surface tension ensures good contact to the skin. Also, the dispersed phase can act as a reservoir making it possible to maintain an almost constant concentration gradient over the skin for a long time [7,13,17] .

1.6. Pregabalin

1.6.1. Neuropathic pain

Neuropathic pain (NP) is defined as “pain initiated or caused by a primary lesion or dysfunction in the nervous system”. Patients experience pain described most frequently as burning, tingling or electric shock. It is generally persistent and/or chronic in nature and can be further categorized as either peripheral or central depending on the origin of the lesion or dysfunction [18,19].

NP is caused by nerve injury, which may have several different origins, including trauma, diabetes, herpes infection, and cancer. Due to the heterogeneity of etiologies and the complexity of the underlying mechanisms, the treatment of painful neuropathies can be challenging because of the large number of medications available to treat this condition and potential differences between medications in effectiveness or harms [18].

NP can impair the quality of life and affect activities of daily living. It can have physiologic implications, affect mood, reduce occupational performance, and generate a major health care costs. Pharmacologic therapy is the first line of treatment for NP. Current treatment for neuropathic pain includes antidepressants such as tricyclic agents and selective serotonin reuptake inhibitors, anticonvulsant agents (e.g. gabapentin and pregabalin), topical lidocaine, and oral opioids (e.g. morphine). The efficacy of these medications varies from patient to patient depending on factors such as location of the pain, age of the patient, and/or any comorbid systemic disease. Pregabalin has been extensively characterized in terms of its efficacy and safety and is currently one of the recommended first-line treatments for neuropathic pain [20,21].

Systemic pharmacotherapy is often accompanied by unpleasant side effects, including sedation, dizziness, and weight gain. Medications used for neuropathic pain may also be contraindicated for the medically compromised or elderly patient. Topical application of medications constitutes a unique method of local drug delivery that is less likely to induce systemic side effects or interact with other medications. Local application should require simpler dose titration processes compared with the complicated titration commonly necessary for systemic therapies [21].

1.6.2. Pregabalin

Pregabalin (Fig. 1.14) is the international non-proprietary name for (3*S*)-3-(aminomethyl)-5-methylhexanoic acid. It belongs to the group of 3-substituted analogues of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), such as gabapentin and baclofen. Discovered by Silverman et al in 1991, Pregabalin is an anticonvulsant drug used to treat epilepsy and neuropathic pain marketed by Pfizer under the brand name Lyrica®, which is available in more than 60 countries. It has been studied in the treatment of patients with DPN, PHN, GAD, and SAD and as an adjunctive therapy in adults with partial-onset seizures. It is considered as one of the drugs of the future due to its high and broad therapeutic activity and reaches blockbuster status (1 billion $ annual sales or more); thus, the development of simple efficient synthetic processes has become an important research goal for medicinal and organic chemists [22,23].

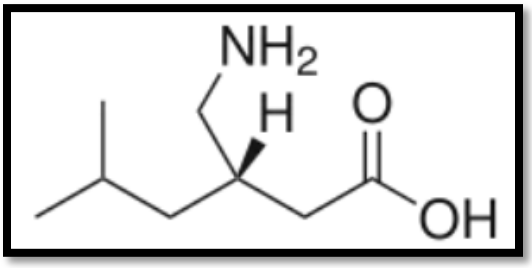

Fig 1.14: Chemical structure of Pregabalin [22].

1.6.2.1. Chemistry

Pregabalin ($C_8H_{17}NO_2$) is a white to off-white, highly crystalline solid powder . Pregabalin has a molecular weight of 159.23 and a melting point about 196.67^oC. It has two pKa values, 4.2 and 10.6, corresponding to the carboxylic acid and the amine groups, respectively. It is sparingly soluble in water [22].

1.6.2.2. Mechanism of action

Pregabalin binds to the α2-δ-1 subunit of voltage gated calcium channels of presynaptic neurons and modifies channel functional properties. Pregabalin modulates hyperexcited neurons via its potent binding at the α2-δ-1 subunit that is associated with decreased calcium influx at nerve terminals and reduced release of several excitatory neurotransmitters (e.g., glutamate, noradrenaline, serotonin, dopamine and substance P) [23]. The mechanism of action is shown in (Fig.1.15).

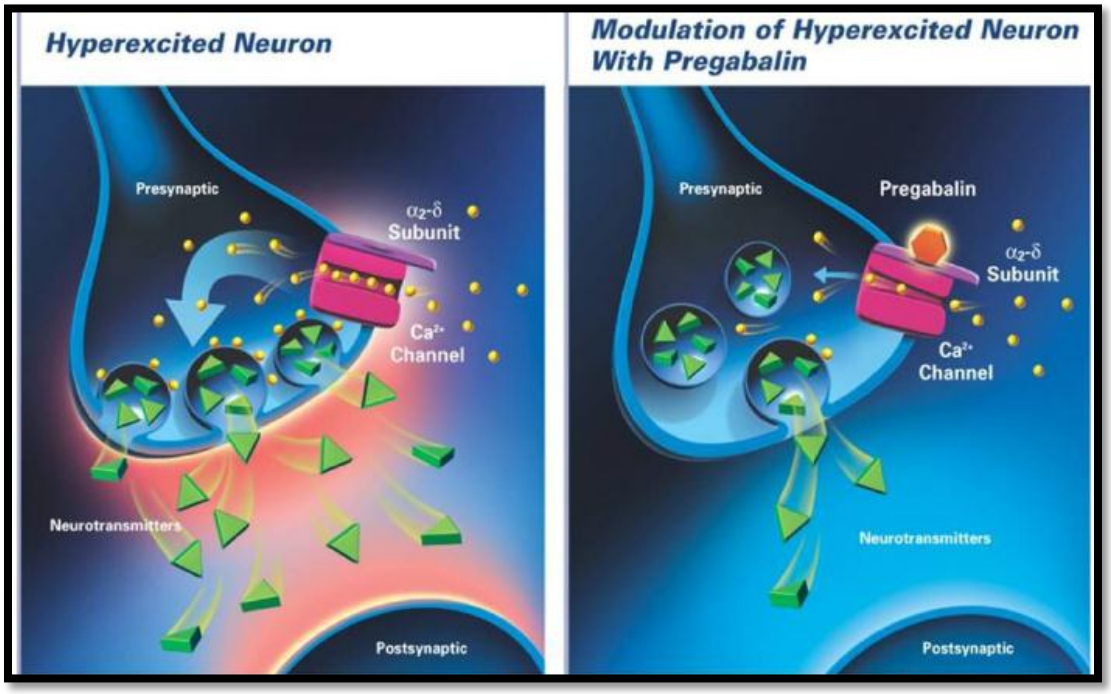

Fig 1.15: Mechanism of action of pregabalin [24].

1.6.2.3. Pharmacokinetics

Pregabalin is readily absorbed orally, and its oral bioavailability is equal to or greater than 90%. It has a half-life of about 6 hours. Pregabalin reaches a peak plasma concentration (means range from 0.04 to 9.5 µg/mL) within 1.5 hours. Following single-dose (25-300 mg) and multiple-dose (75-900 mg/day) administration, maximum plasma concentrations increase linearly, with steady state levels reached within 24 to 48 hours. Patients taking the recommended dosages have shown steady state concentrations up to 10 µg/mL. Pregabalin has a volume of distribution of 0.5 L/kg. Approximately 90% of the dose is excreted unchanged in the urine. The N-methylated derivative of pregabalin is the major urinary metabolite, accounting for only 0.9% of the dose. Pregabalin is removed by haemodialysis [22,23].

1.7. Franz diffusion cell

Topical and TDDS are advantageous in comparison to other administration routes. Within this context, the use of *in vitro* static diffusion cells to assess skin permeability has evolved into a major research methodology, providing key insights into the relationships between skin, drug and formulation. Such testing is highly useful not only for the design and development of novel formulations but also for toxicity screening and quality control purposes [25].

Crucially, Franz-type diffusion studies frequently involve the use of synthetic membranes to model real skin. Although the artificial membranes will not model the lipid perturbation effects undergone by biological samples, inferences regarding partitioning and diffusion phenomena can be made. Synthetic membranes may be preferred to skin tissue as they are more easily resourced, less expensive and structurally simpler. Furthermore, synthetic membranes exhibit superior

permeation data reproducibility as *in vivo* variables such as skin age, race, sex and anatomical site are eliminated [25,26].

1.7.1. Diffusion Cell Equipment

The Franz diffusion cell is the most popular technique for conducting in vitro release study. In such studies a receptor solution is placed into the receptor compartment, which is maintained at 32˚C. The cells are stirred with a magnetic stir bar, usually at a speed of 600 rpm. Samples are then withdrawn from the receptor solution at regular time intervals. Typical diagram of FDCs is depicted in (Fig. 1.16). It is made up of donor chamber and receptor chamber. Membrane is placed between donor chamber and receptor chamber. Sample is applied in donor chamber and over the membrane. Receptor chamber contains receptor medium, which is maintained at required temperature by heat circulation by water jacket [25].

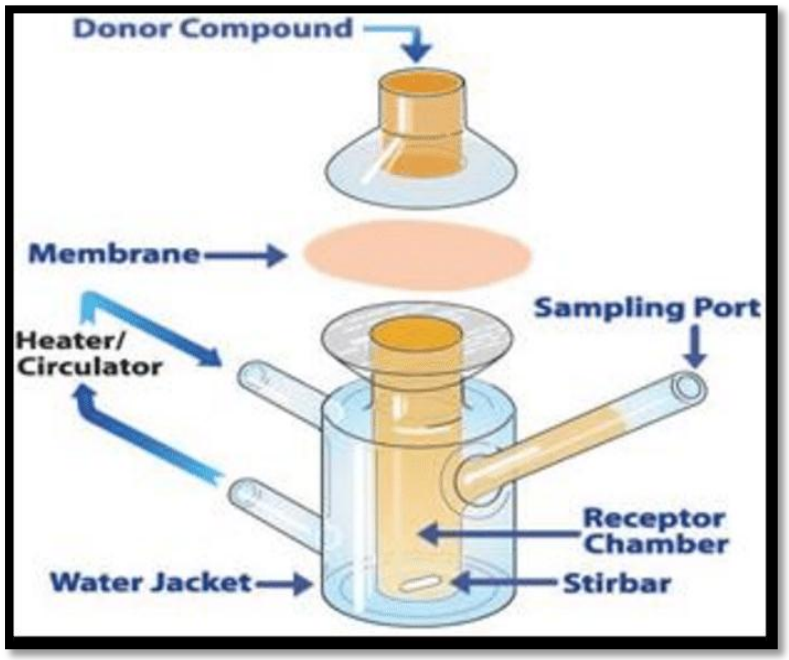

Fig 1.16: Typical diagram of Franz diffusion cell [25].

1.8. Principles of Diffusion through membrane

Diffusion is a process of mass transfer of individual molecules of substance due to concentration gradient by random motion. This diffusion through the SC is a passive one. Passive diffusion leads to change in concentration in a region over a period of time and space. Fick's laws of diffusion quantitate the amount of solute diffusing per unit time and area as a function of concentration gradient of solute in the direction of diffusion, and relate the changes in solute concentration in a given region over time to the change in concentration gradient of the solute over in that region [27].

1.8.1. Fick's First Law

Fick's law of diffusion postulates that the diffusing molecules go from regions of high concentration to regions of low concentration. The rate of diffusion, the amount of material (M) flowing through a unit cross section (S) of a barrier in unit time (t), is defined as the flux (J). Flux is related to the concentration gradient ($dC = C_1 - C_2$) between the donor region at higher concentration (C_1) and the receiving region at lower concentration (C_2) per unit distance (x) by the following expression [27].

$$J = \frac{dM}{dt} \times \frac{1}{S} \qquad \text{---------- (1.1)}$$

Where, J is the flux, in g/(cm^2 s), S is the cross section of the barrier, in cm^2 dM/dt is the rate of diffusion, in g/s (M = mass, in g; and t = time, in s)

The flux is proportional to the concentration gradient, dC/dx:

$$J = -D \times \frac{dC}{dx}$$ ---------- (1.2)

Where, D is the diffusion coefficient of a penetrant (also called diffusant), in cm^2/s , C is the concentration, in g/ml , and x is the distance perpendicular to the surface of the barrier, in cm.

Thus,

$$\frac{dM}{dt} = -D \times S \times \frac{dC}{dx}$$ ---------- (1.3)

The negative sign in this equation signifies that diffusion occurs in a direction of decreasing concentration. Thus, the flux is always a positive quantity. Although the diffusion coefficient, D, or diffusivity, as it is often called, does not remain constant. It is affected by changes in concentration, temperature, pressure, solvent properties, molecular weight and chemical nature of the diffusant. Therefore, D is more correctly referred as a diffusion coefficient rather than a diffusion constant. In the initial stage of diffusion, the drug molecules may penetrate the skin appendages (hair follicle and sweat glands) then the diffusion through SC becomes the dominant when a steady state has been reached [27].

1.8.2. Fick's Second Law

Fick's second law predicts changes in solute concentration over time caused by diffusion. It states that the change in concentration with time in a particular region is proportional to the change in the concentration gradient at that region in the system. Concentration of solute or diffusant, C, in the volume of the region, x, changes as a result of net flow of diffusing molecules

in or out of the region [27]. This change in concentration with time, t (i.e., dC/dt), is proportional to the change in the flux of diffusing molecules, J, per unit distance, x (i.e., d*J*/dx):

$$\frac{dC}{dt} = -\frac{dJ}{dx} \qquad \text{---------- (1.4)}$$

Differentiating the equation of flux, J, as per Fick's first law of diffusion ($J = -D \times dC/dx$), with respect to x, we obtain

$$-\frac{dJ}{dx} = D\frac{d^2C}{dx^2} \qquad \text{---------- (1.5)}$$

Therefore, concentration and flux are often written as C(x,t) and J(x,t), respectively, to emphasize that these parameters are functions of both distance x and time t. Substituting dC/dt for −dJ/dx, Fick's second law of diffusion can be expressed as

$$\frac{dC}{dt} = D\frac{d^2C}{dx^2} \qquad \text{---------- (1.6)}$$

1.8.3. Diffusion rate

Fick's first law of diffusion describes the diffusion process under steady state when the concentration gradient (dC/dx) does not change with time. The second law refers to a change in concentration of diffusant with time at any distance (i.e., a nonsteady state). Diffusive transport from a dosage form is usually slow, leading to most of the drug transport happening under steady state conditions. Therefore, it is important to understand the diffusive conditions under steady state [27,28].

If the concentration of permeant in the donor and in the receiver compartments are C_1 & C_2, respectively, the Fick's first law can be written as follows:

$$J = \frac{dM}{dt} \times \frac{1}{S} = D \times \frac{(C_1 - C_2)}{h} \quad \text{---------- (1.7)}$$

in which $(C_1 - C_2)/h$ approximates dC/dx. The Concentrations C1 and C2 within the membrane are determined by the partition coefficient of the solute ($K_{membrane/solvent}$) multiplied by the concentration C_{donor} in the donor compartment or $C_{receptor}$ in the receptor compartment. Thus,

$$C_1 = C_{donor} \times K_{membrane/solvent} \quad \text{---------- (1.8)}$$

and

$$C_2 = C_{receptor} \times K_{membrane/solvent} \quad \text{---------- (1.9)}$$

Therefore, the partition coefficient

$$K_{membrane/solvent} = \frac{C_1}{C_{donor}} = \frac{C_2}{C_{receptor}} \quad \text{---------- (1.10)}$$

Hence,

$$\frac{dM}{dt} = \frac{D \times S \times K_{membrane/solvent} \times (C_{donor} - C_{receptor})}{h} \quad \text{---------- (1.11)}$$

Under sink conditions, the drug concentration in the receptor compartment is maintained much lower than the drug concentration in the donor compartment, such that $C_{receptor} \rightarrow 0$. Therefore, Equation 3.11 can be simplified as

$$\frac{dM}{dt} = \frac{DSK_{membrane/solvent}C_{donor}}{h} \quad \text{---------- (1.12)}$$

This equation can also be expressed in terms of the permeability coefficient, P, in cm/s, defined as

$$P = \frac{D \times K_{membrane/solvent}}{h} \quad \text{---------- (1.13)}$$

as

$$\frac{dM}{dt} = P \times S \times C_{donor} \quad \text{---------- (1.14)}$$

If we measure the cumulative amount of diffusant, M , which passes per unit area through the membrane as a function of time, then a typical permeation profile can be drawn, as illustrated in (Fig. 1.17):

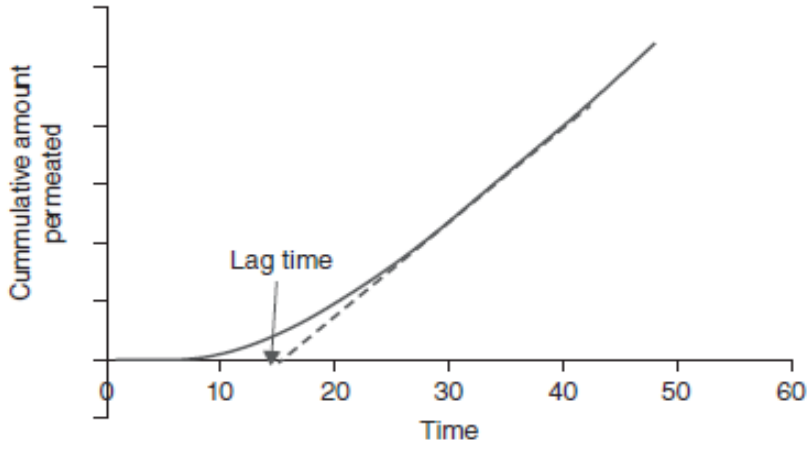

Fig 1.17: Typical permeation profile for an infinite dose application to human skin, obtained by plotting the cumulative amount per unit area of diffusant passing into the receptor compartment with time [28].

As can be seen, after sufficient time the plot approaches a straight line and from the slope we can obtain the steady state flux,

$dM/dt = DC_oK / h$ ---------- (1.15)

where dM/dt is the flux, usually termed J which is the cumulative mass of permeant that passes per unit area of the membrane in time t, C_0 is the constant concentration of the drug in the donor compartment in mg/ ml, K is the partition coefficient of the solute between the membrane and the bathing solution, h is the thickness of the membrane in (cm).

Steady state is achieved when the plot becomes linear: extrapolation of the linear portion to the time axis yields the lag time T_L. (the intercept on the time axis at M = 0) Lag time :

$T_L = h^2/6D$ ---------- (1.16)

1.9. Problem

Pregabalin, which was identified as an antiepileptic in the early 1990's, is now widely used for treatment of diabetic peripheral neuropathy. Unfortunately, though, it has serious problems when it administrated orally:

1.Central nervous system (CNS)-mediated side effects such as dizziness, somnolence (sleepiness), blurry vision, weight gain, sleepiness, trouble concentrating, swelling of hands and feet and dry mouth are common in pregabalin treated patients and have a significant impact on their quality of life.

2. Interaction with other medications may contradict the use of these medications or prevent their use in the medically compromised with elderly patients. The elderly patients are more vulnerable than younger persons since they often suffer from multiple medical and nutritional problems. This can limit treatment options with analgesic agents due to an increased risk of adverse effects and problems with complex drug interactions.

3.Renal Impairment: Pregabalin clearance is directly proportional to creatinine clearance. In addition, pregabalin is effectively removed from plasma by haemodialysis (following a four hour haemodialysis treatment plasma pregabalin concentrations are reduced by approximately 50%). Since renal elimination is the major elimination pathway, dosage reduction in patients with renal impairment and dosage supplementation following haemodialysis is necessary .

1.20. Aims and Objectives

The aim of this study is to prepare pregabalin microemulsion for topical treatment of neuropathic pain in order to provide patient with dosage form that:

1. Minimize or avoid dose-limiting CNS-mediated side effects of orally administered pregabalin and to increase the patients compliance specially for the elderly patients.
2. Have low cost.
3. Easy to prepare.
4. Have a thermodynamic stability.
5. Have a penetration enhancing ability.
6. Have the possibility of immediate withdrawal of the treatment if necessary.

The specific objectives are:

(1) To prepare different formulations of pregabalin microemulsions with various oils and different cosurfactant ratios

(2) To study the influence of different oils (Oleic acid, IPM and R(+)-limonene), cosurfactant ratio and PG ratio on the phase behavior and to select the optimal system that gives the best microemulsion region.

(3) To test the chosen formulation on the Franz diffusion cell to evaluate its uptake into a membrane and its ability to penetrate the skin.

(4) To select the best penetration enhancer from the three oils.

(5) To study the effect of PG in penetration enhancement.

Chapter Two

Literature review

According to the aim and objectives of my research work I have reviewed the following literatures as support for the microemulsion as TDDS and the attempts to formulate topical pregabalin to carry out the project to its logical conclusion.

2.1. Literature review for Microemulsions as TDDS

In recent years, microemulsions have attracted considerable interest as promising colloidal drug delivery vehicles. There are many advantages for microemulsions in pharmaceutical applications over other systems, including high solubilization capacity for both water-soluble and oil-soluble drugs, thermodynamic stability, transparency, and ease of preparation.

Callender et al., (2017) pointed out that for the period 1 January 2011–30 April 2016, 431 publications related to microemulsion drug delivery (Fig. 2.1) were identified and screened according to microemulsion, drug classification, and surfactant types. Results indicate the use of microemulsions predominantly in lipophilic drug delivery (79.4%) via oil/water microemulsions and nonionic surfactants (90%) for oral or topical administration. Cancer is the disease state most targeted followed by inflammatory diseases, microbial infections and cardiovascular disease. Key generalizations from this analysis include: 1) microemulsion formulation is largely based on trial-and-error despite over 1200 publications related to microemulsion drug delivery since their discovery in 1943; 2) characterization using methods including interfacial tension, droplet size,

electrical conductivity, turbidity and viscosity may provide additional information for greater predictability; 3) microemulsion drug delivery publications arise primarily from China (27%) and India (21%) suggesting additional research opportunities elsewhere. There has been a steady increase in these numbers leading to the conclusion that microemulsion systems are slowly being recognized as efficient drug delivery vehicles [29].

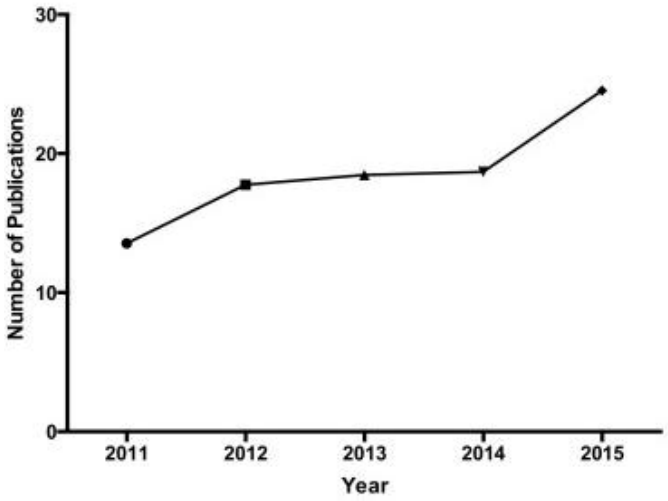

Fig 2.1: Number of microemulsion formulation publications Avaliable on PubMed 2011-2016 [29].

Jadupati *et al*., (2011) developed the Insulin-loaded microemulsions for transdermal delivery using IPM or oleic acid as the oil phase, Tween 80 as the surfactant, and isopropyl alcohol as the cosurfactant. The insulin permeation flux of microemulsions containing oleic acid through excised mouse skin and goat skin was comparatively greater than that of microemulsions containing IPM. The insulin-loaded microemulsion containing 10% oleic acid, 38% aqueous phase, and 50% surfactant phase with 2% DMSO as permeation enhancer showed maximum permeation flux through goat skin [30].

Xiaohui *et al.,* (2011) studied the microstructure characterization of microemulsion consisting of oleic acid, cremophor RH40, ethanol and water and investigate the influence of microstructure on the solubilization potential of the microemulsion to meloxicam. They concluded that the solubilization capacity of microemulsion is closely related with its microstructure. The solubilization of W/O microemulsion is the best, compared with other two (O/W, Bi continuous), where as the O/W is the weakest [31].

Ying *et al*., (2011) investigated a microemulsion system for transdermal delivery of ligustrazine phosphate. Microemulsions containing IPM, labrasol, plurol oleique® and water were investigated in pseudo-ternary phase diagrams. The optimized microemulsion with permeation flux of 41.01μg/cm^2/h across rat skin *in vitro*, showed no obvious irritation on back skin of rabbits. The results indicated that the studied microemulsion system might be a promising vehicle for transdermal delivery of ligustrazine phosphate [32].

Manish *et al.,* (2010) formulated Glipizide microemulsion by water titration method using oleic acid as oil phase, Tween 80 as surfactant and PG as cosurfactant. Microemulsions were characterized for pH, in vitro release profile, ex-vivo diffusion study, irritancy tests, stability and in vivo evaluation. Five microemulsion formulations were prepared. Oleic acid is used as oil phase in 2, 4, 6, 8, 10% concentration of formulation content and then 6% (ME-3) obtained in clear form and have higher cumulative percent release than others. Nonionic surfactant Tween 80 was selected because they are generally less toxic, produce less skin irritation. *In vivo* studies were carried out on Wistar rats. The optimized microemulsion formulation was found to be o/w

type emulsion. The results indicated that the developed microemulsion systems, especially ME-3, may be promising vehicles for the transdermal delivery of glipizide [33].

Arun *et al.,* (2009) formulated the two novel formulation of O/W microemulsion of ketoprofen for improving transdermal absorption. Formulations were prepared by constructing the pseudo-ternary phase diagrams using oleic acid, Tween 80, PG and water in different ratios and were gelled by incorporating fumed silica. The result of solubility study shown ketoprofen was highest in oleic acid, followed by ethyl oleate, IPM, and isopropyl palmitate. Therefore, oleic acid was chose as oil phase for microemulsion. The oily mixtures of oleic acid, Tween 80 and PG led to increased in drug solubility. After extensive screening for physical characteristics and appearance, final ratios of surfactants/cosurfactants were decided. *In-vitro* diffusion study was carried out using artificial semipermeable membrane [34].

M Bajpai *et al.,* (2009) developed dexamethasone microemulsion systems for topical drug delivery and these system composed of water, oleic acid; Tween 80 and Isopropyl alcohol were investigated as potential drug delivery vehicles. Pseudo-ternary phase diagram was constructed at room temperature by titration, and the oil-to-surfactant/cosurfactant mass ratios that exhibit the maximum in the solubilization of water were found. The superior transdermal flux of dexamethasone was due to 1000 fold improvement in solubilization of dexamethasone by microemulsions using lecithin. It can be concluded from the study that the dexamethasone microemulsions can be potentially used for improved topical drug delivery [35].

2.2. Literature review for Pregabalin

Fukasawa *et al.*, (2014) in their research studied the transdermal administration of aqueous pregabalin solution as a potential treatment option for patients with NP to avoid CNS-mediated side effects. Since Pregabalin is a widely used as adjuvant therapy for patients with NP. It was speculated in this study that transdermal delivery would minimize centrally mediated side effects. To test this idea, it was evaluated the analgesic effects of pregabalin delivered through the transdermal route in animal models of NP. To test the effects of transdermally administered pregabalin, 1 mg of pregabalin was dissolved in 0.1% Tween 80 at 10 mg/mL and applied to both hind paws after the surgery and allowed to dry. In normal rats, 1 mg of pregabalin was dissolved in methanol at 10 mg/mL and applied in the same way. The right lower legs of sham mice were dipped into a solution of 2.5 or 7.5 mg/mL pregabalin in pure water for 5 min and allowed to dry. The results showed that transdermally administered pregabalin increased the pain thresholds in response to mechanical stimuli in a partial sciatic nerve ligation model in rats and a spinal nerve ligation model in mice, and surprisingly also in normal animals. It is noteworthy that simple transdermal application of an aqueous solution of pregabalin is effective. This could be a useful treatment option to avoid or minimize the CNS-mediated side effects of orally administered pregabalin [36].

Pregabalin and gabapentin (Fig. 2.2) are often considered first-line treatments for various neuropathic pain syndromes, generally irrespective of cause. Both pregabalin and gabapentin are antiepileptic medications that bare structural resemblance to GABA. The drugs efficacy in NP is linked to their ability to bind to voltage-gated calcium channels in the CNS, specifically to the alpha-2-delta protein. This binding decreases neurotransmitter release in the CNS as a result of

reduced calcium influx through the gated channels but pregabalin is more effective in treatment of the NP so it was very interested when Mbah *et al.,* (2014) studied the influence of cosolvent system and microemulsion formulation on in-vitro skin permeation of gabapentin, furthermore, to characterize the physicochemical properties of drug-loaded oil-in-water (o/w) and water-in-oil (w/o) cremophor 40-based microemulsions in comparison to the blank counterparts. In this research the cosolvent system prepared by homogenous mixing is composed of ethanol-water and propylene glycol-water mixture (90:10, 80:20, 70:30 v/v) respectively. The microemulsion consisted of coconut oil, water and mixture of cremophor 40 (surfactant) and ethanol (cosurfactant) and was prepared by aqueous phase titration method. Transdermal flux for gabapentin was studied in-vitro using modified FDCs. The ethanol-water system (70:30 v/v) gave higher flux for gabapentin when compared to PG-water system (70:30 v/v). The in vitro permeation data obtained from experimental work suggested that the cosolvent system (ethanol-water 70:30 v/v) and w/o microemulsion formulations respectively, can be successfully used as potential vehicles in developing transdermal therapeutic systems for gabapentin [37].

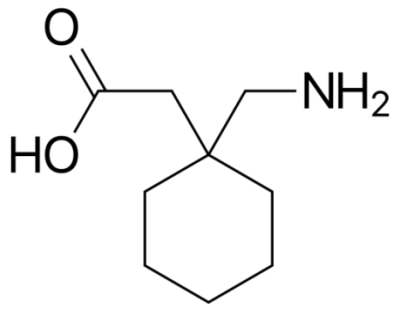

Fig 2.2: Chemical structure Gabapentin [38].

Nnadi *et al.*, (2012) studied the possibility of delivering gabapentin and glipizide transdermally using some cosolvent systems, and pharmaceutically acceptable o/w and w/o microemulsions with good rheological properties formulated with polyethoxylated castor oil (as surfactant), ethanol (as cosurfactant), locally sourced coconut oil (as oil phase) and distilled water (as aqueous phase) as vehicles. So different strengths (10, 20 and 30 % v/v) of ethanol and PG cosolvent systems were prepared by homogenous mixing. Various oil/water and water/oil microemulsions were prepared by aqueous titration method. The microemulsion areas were identified by constructing pseudo-ternary phase diagrams. Transdermal permeation of gabapentin and glipizide (in both cosolvent systems and microemulsions) through rat abdominal skin was determined by modified FDC. The result of this study showed that the optimized microemulsions for delivery of gabapentin and glipizide were w/o and o/w respectively. Cosolvent of ethanol-water (3:7) is the best cosolvent for transdermal delivery of both drugs from transdermal patches of acceptable sizes. Based on these findings, it could be suggested that cosolvent systems (ethanol-water 3:7) and microemulsions (surfactant: cosurfactant of 1:1 and 1:2) are possible vehicles for the development of transdermal products of both drugs investigated [38].

Bhatia *et al.,* (2012) studied the permeation of pregabalin from transdermal patch into microcirculation of skin. Matrix type TDDS of Pregabalin was prepared by the solvent evaporation technique. Several batches were prepared by using combination of HPMC and PVP; PVA and PVP; Eudragit RL-100 and Eudragit RS-100; HPMC and EC in different ratios. PG was used as plasticizer and DMSO was incorporated as a permeation enhancer. Patches were evaluated for their in-vitro drug release profile and *ex-vivo* skin permeation studies. Patches were also subjected to stability studies and skin irritation studies to determine their compatibility with

skin. Formulation containing HPMC and PVP in the ratio of 3:1 and PG, 5% w/v and DMSO, 6% w/v was found to be the most optimum formulation and was also found to exhibit maximum *in -vitro* % drug release of about 81.70 %. Result of evaluation studies revealed that pregabalin can be administered as a controlled drug delivery system to reduce frequency of drug administration [39].

Plaza-Villegas *et al.*, (2012*)* studied the effect of topical treatment with pregabalin and diclofenac on neuropathic orofacial pain induced by infraorbital nerve injury in the rat. This study was done by using sixty-four Sprague-Dawley rats underwent infraorbital nerve injury. Seven days after surgery, pain was verified and the rats randomly assigned to topical or systemic treatment with pregabalin or diclofenac, or to no treatment. Pain intensity and motor coordination were assessed at baseline, after surgery, and daily after treatment for 4 consecutive days. Medication plasma levels were assessed at the end of the study. Topical treatment with 10% pregabalin or 5% diclofenac reduced the pain significantly. A significant decrease in motor coordination was found in the systemic pregabalin. The medications plasma levels were significantly higher in the systemic treatment compared with the topical. Topical treatment with pregabalin or diclofenac can reduce neuropathic orofacial pain induced by nerve injury, but among the topical medications used in this study, the most effective in pain relief was topical pregabalin at 10%. This medication also demonstrated a minimal side effect profile [40].

Chapter Three

Materials, Methods and Experiments

3.1. Materials and reagents

Items	Source
Disodium Hydrogen Phosphate	Merk
Distilled water	Al-Quds University
R(+)-Limonene	Sigma-Adrich
Ethanol 99%	Biotech For Medical Supplies
IPM	Sigma-Adrich
n-octanol	Sigma-Adrich
Oleic acid	Sigma-Adrich
PG	Biotech For Medical Supplies
Potassium Dihydrogen Phosphate Monohydrate	Merk
Pregabalin	Jerusalem Pharmaceuticals
Potassium Cholride	Merk
Sodium Chloride	Merk
Tween 80	KOLB

Table 3.1: List of Materials and reagents used in this study

3.2. Tools and Equipments

The tools and equipment that were used in this study:

- Dialysis membrane Cellu Sep : Its nomial MWCO is 6000-8000 Daltons, the flat width is 23 mm and the wall thickness is 28 µm.
- Franz diffusion cell
- Glass test tubes with screw caps
- HPLC
- HPLC vials
- Magnetic Stirrer
- pH meter
- Precision Balance
- Sonicator
- Synthetic membrane filter PVDF : Its diameter is 47mm, the pore size is 0.22 µm and the thickness is 0.0125 cm.
- Thermometer
- Vortex

FDC, HPLC, HPLC vials and Sonicator were provided from Samih Darwazeh Institute of Industrial Pharmacy and the rest of the tools and equipments were provided from Al-Quds University.

3.3. Methodology

3.3.1. Preparation of solutions

The following solution will be required in this study:

a. Preparation of 0.1 M pregabalin:

1.5919 g of pregabalin was transferred to a clean, dry 100ml volumetric flask. Then slowly distilled water was added to the volumetric flask and gently swirled until all the pregabalin was dissolved. Adding water was continued until reached the 100ml mark on the neck of the flask.

b. Preparation of 0.1 M pregabalin and PG mixture in (1:1) ratio**:**

12.0278 g of 0.1 M of pregabalin was mixed with 12.0288 g of PG on vortex mixer.

c. Preparation of Tween 80 and ethanol mixture in (1:1) ratio:

8.0423 g of Tween 80 was mixed with 8.0419 g of ethanol on vortex mixer.

d. Preparation of Tween 80 and ethanol mixture in (2:1) ratio:

16.0468 g of Tween 80 was mixed with 8.0423 g of ethanol on vortex mixer.

e. Preparation of 0.9 % (w/v) Phosphate Buffered Saline:

PBS with pH 7.4 was prepared by dissolving 0.8 of sodium chloride, 0.2 g of potassium chloride, 1.44 g of disodium phosphate and 0.24 of potassium dihydrogen phosphate in 800 ml distilled water, and then the pH adjusted to 7.4 by sodium hydroxide solution. Then water added

to total volume of 1 liter. The solutions was sterilized by autoclaving (20 min, 121°C, liquid cycle). Then stored at room temperature.

3.3.2. Construction of pseudo-ternary phase diagram

In this study eighteenth phase diagrams (Table 3.2) were established they were divided into three groups depending on the oil phase that was used in each system as the following :

Group 1 : six phase diagrams with different PG and ethanol ratios using oleic acid as the oily phase.

Group 2 : six phase diagrams with different PG and ethanol ratios using IPM as the oily phase.

Group 3 : six phase diagrams with different PG and ethanol ratios using R(+)- Limonene as the oily phase.

The systems consisted of oleic acid, IPM, R(+)-Limonene, respectively as the oil phase and 0.1M pregabalin as the aqueous phase with PG as solublizaing agent. 0.1M pregabalin / PG ratio was 1:0, 1:1, respectively, for the mixture of 0.1 M pregabalin : PG. Tween 80 as the surfactant and ethanol absolute as cosurfactant. The surfactant/cosurfactant ratio was 1:1, 2:1, respectively, for the mixture of Tween 80: ethanol absolute as shown on (Table 3.2).

Pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature. The mixture of oils and surfactant/cosurfactant mixture was prepared at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1, respectively. These mixtures were titrated dropwise with 0.1 M pregabalin and 0.1 M pregabalin : PG with ratio (1:1) under gentile stirring on the vortex mixer. After 24 hours the systems were visually characterized after equilibration.

Transparent fluid systems were characterized as microemulsion. All these systems were plotted as ternary or pseudo ternary phase diagram using Origin Pro 8 computer programs.

Group	System	Surfactant/cosurfactant	Oil phase	Aqueous phase
1	A	Tween 80	Oleic acid	0.1M pregabalin
	B	Tween 80 : Ethanol (2:1)	Oleic acid	0.1M pregabalin
	C	Tween 80 : Ethanol (1:1)	Oleic acid	0.1M pregabalin
	D	Tween 80	Oleic acid	0.1M pregabalin: PG (1:1)
	E	Tween 80 : Ethanol (2:1)	Oleic acid	0.1M pregabalin: PG (1:1)
	F	Tween 80 : Ethanol (1:1)	Oleic acid	0.1M pregabalin: PG (1:1)
2	G	Tween 80	IPM	0.1M pregabalin
	H	Tween 80 : Ethanol (2:1)	IPM	0.1M pregabalin
	I	Tween 80 : Ethanol (1:1)	IPM	0.1M pregabalin
	J	Tween 80	IPM	0.1M pregabalin: PG (1:1)
	K	Tween 80 : Ethanol (2:1)	IPM	0.1M pregabalin: PG (1:1)
	L	Tween 80 : Ethanol (1:1)	IPM	0.1M pregabalin: PG (1:1)
3	M	Tween 80	R(+)-Limonene	0.1M pregabalin
	N	Tween 80 : Ethanol (2:1)	R(+)-Limonene	0.1M pregabalin
	O	Tween 80 : Ethanol (1:1)	R(+)-Limonene	0.1M pregabalin
	P	Tween 80	R(+)-Limonene	0.1M pregabalin: PG (1:1)
	Q	Tween 80 : Ethanol (2:1)	R(+)-Limonene	0.1M pregabalin: PG (1:1)
	R	Tween 80 : Ethanol (1:1)	R(+)-Limonene	0.1M pregabalin: PG (1:1)

Table 3.2: The eighteenth phase diagrams that were formulated in this study.

3.3.3. Selection of microemulsion formulations

The criteria for selection of microemulsion formulations for the skin permeation studies was based on formulations having good physicochemical property with the best region, least surfactant concentration and the highest oil content. After selection of the microemulsion formulation a sample from each formula has been chosen to represent this sample in skin permeation studies. The results were illustrated in chapter 4 section 3.

3.3.4. Diffusion study

Diffusion is defined as a process of mass transfer of molecules across a barrier associated with a concentration gradient. This can be depicted by using FDC to simulate the true clinical situation. The FDC is composed of donor cell, membrane and a receiver cell as shown in (Fig. 1.16). A membrane, typically skin or synthetic membrane, is mounted in the diffusion cell, a small amount of formulation is applied to the membrane, and samples are withdrawn from a stirred receiver compartment beneath the membrane as a function of time. An advantage of this method is that, in principle, the amounts of formulated drug applied can reasonably reflect the usage situation. In addition, the cell can be left open to ambient conditions of laboratory for the formulation to undergo the same compositional changes as occurs clinically. Another advantage to this method is that phenomena of topical drug delivery as skin penetration enhancement can be realistically evaluated [41].

3.3.4.1. Preparation of synthetic membrane:

The synthetic membrane was composed of three layers; a PVDF filter membrane layer sandwiched between two layers of dialysis membrane. The two layers of dialysis membrane were soaked for half hour in receiver medium before use. The PVDF filter membrane was soaked in n-octanol for 24 hrs to simulate the lipophilic barrier in the skin. Sandwiching was used to prevent n-octanol from floating or leaving the filter membrane during the diffusion experiments [42].

3.3.4.2. Receptor phase

Water or other aqueous systems are usually used as the receptor phase in skin permeation studies. For most studied, an isotonic solution buffered at pH 7.4 is a suitable and preferred receptor fluid.

3.3.4.3. Donor phase

The donor phase was prepared after selecting the best microemulsion formula.

3.3.4.4. General Diffusion procedure

FDC as shown in (Figure 1.16) was used for permeation in this study. The receiver compartment was filled with phosphate buffer pH 7.4. The temperature was kept 32 ^{o}C ± 0.5 ^{o}C (that is the temperature of the human skin), by using water bath and a pump that supply the water jacket

around the receiver chamber. The synthetic membrane was sandwiched between the donor and the receiver compartments in order to avoid leakage. The two compartments were connected tightly with each other by using a metal clamp. The available membrane area for diffusion was approximately 3.14 cm^2. The donor compartment was filled with 1 ml of the selected microemulsion systems.

The diffusion experiment was done for four hours; the first 1 ml was taken at the first half hour then one ml was taken every 1 hour. The sampled volume (1 ml) was replaced with the same volume of the receiver solution. The receiver fluid was mixed continuously during diffusion experiment using magnetic stirring at 700 rpm in order to have sink condition.

The amount of the diffused drug was analyzed using HPLC. Cumulative amount of permeated drug per unit area was plotted against time and slope of the linear part of the graph (permeation flux) was measured, from which the permeability coefficient using first Fick's Law.

In order to find out the mechanism of pregabalin release from the selected microemulsion formulations, the data obtained from in-vitro release studies were fitted to three kinetic equations:

a) $M_t = k_0 t$ (zero-order equation)

b) $\ln M_t = \ln M_0 - k_1 . t$ (first-order equation)

c) $Mt = K . S . t^{0.5} = k_H . t^{0.5}$ (Higuchi equation)

Here, M_t is the amount of drug release in time per unit area, M_0 is the initial amount of drug, S is the surface area of the drug and k_0, k_1 and k_H are rate constants of zero order, first order and Higuchi equation, respectively [43].

The zero-order model characterizes a drug delivery system that does not disaggregate, the active substance being released slowly, independent of the initial drug concentration. In contrast, the first order model best describes a release process that is directly proportional to the drug concentration embedded in the vehicle. Assuming a homogeneously dispersed drug in a planar matrix and under perfect sink conditions, Higuchi's mathematical model suggests a pure diffusion release mechanism of the active substance from a vehicle, with no occurring erosion or swelling of the matrix [44].

3.3.4.5. Calculation of diffusion parameters (M_t, T_L, D, P, K, J)

3.3.4.5.1. Cumulative amount of diffusant (Pregabalin)

The cumulative amount (Q_t) of penetrant released was determined by following equation:

$$Q_t = \{ C_n V + \sum_{i=1}^{n-1} C_i V1 \} \quad \text{---------- (3.1)}$$

Where,

Q_t = Cumulative amount of penetrant released (mg)

Cn= Concentration of penetrant (mg/ml) determined at nth sampling interval.

V = Volume of individual Franz diffusion cell, 20 ml

$\sum_{i=1}^{n-1}$ = Sum of concentration of pregabalin (mg/ml) in the receptor compartments at the previous sampling times.

V1 = sampling volume, 1 ml

3.3.4.5.2. Diffusion parameters

The cumulative amount of penetrant crossing unit area of membrane (M_t) at time (t) as obtained by dividing the cumulative amount (Q) on the surface area of membrane [38]:

$$M_t = Q_t / A \quad \text{---------- (3.2)}$$

If we plot the M_t, which passes per unit area through the membrane as a function of time we obtain the plot shown in (Fig. 1.17)

The steady state flux (dm/dt) may be expressed mathematically as:

$$Y = ax + b \quad \text{---------- (3.3)}$$

Where, Y is M_t in (mg/cm^2) and x is the time in (hours)

If the steady state plot is extrapolated to the times axis, the intercept obtained is the lag time (T_L) in (hours) that can be calculated from the equation (3) as :

$$T_L = b/ a \quad \text{---------- (3.4)}$$

Where, a: the slop of steady state plot and b: the intercept of steady state plot with (y) axis

Diffusion coefficient (D) in (cm^2/hr) was calculated using the relation derived from Fick's second law as described in equation number (1.16) :

$$T_L = h^2 /6D \quad \text{---------- (1.16)}$$

Where, h: is the thickness of membrane in (cm) and T_L: is the lag time in (hr)

The permeability coefficient (P) [cm/hr] was calculated using the relation derived from Fick's first law of diffusion described in equation :

$$P = J/ C = Slope / C \quad \text{---------- (3.5)}$$

Where, J : is steady state flux in [$mg/cm^2.hr$] and C is the initial concentration of penetrant in the donor

Partition coefficient (K) was calculated by equation :

$$K=P.h/ D \quad \text{---------- (3.6)}$$

3.3.5. HPLC Assay method for Pregabalin microemulsion

A simple, precise and accurate RP- HPLC method was developed and validated for the estimation of pregabalin and methylcobalamin in capsule formulation. The HPLC instrument used was Shimadzu Prominence LC20AT with UV detection. Inertsil ODS 3 C-18 column with dimensions of 250 mm length, particle size of 3 microns and internal diameter of 4.0 mm was used for separation

Materials :

- Pregabalin Reference
- Methanol HPLC grade
- Potassium dihydrogen phosphate
- Potassium phosphate dibasic
- Water HPLC grade
- Buffer solution

The mobile phase consisted of 60 volumes of buffer 0.01 M potassium dihydrogen phosphate and 0.01M potassium phosphate dibasic and 40 volumes of solvent methanol at a flow rate of 0.6 ml/min. The wave length of 210 nm was used for the detection**.**

Sample preparation :

1 ml of sample/ 50 ml mobile phase (20 ml methanol + 30 ml 0.01 M (KH_2PO_4+K_2HPO_4).

Chapter Four

Results and Discussion

4.1. Pseudo-ternary phase diagram

The phase behavior of pregabalin microemulsions was studied on ternary or pseudo-ternary phase diagrams. The percentages (by weight) of the aqueous phase, surfactant and oil that gave a clear transparent and isotopic was represented as a point on the phase diagram. The border line that passed through these points and the area on its right hand side represented a total monophasic microemulsion while the region on the left hand side of that line represents a cloudy dispersion or multi-phase domain. The percentage of the monophasic area relative to the total area of the triangle phase diagram was taken as a measure for the extent of microemulsion formation and was denoted by (A_T%). This term was previously used by other investigators for the same purpose. A_T represents the percentage of the total monophasic and isotropic microemulsion area that encompasses oil-continuous, bi-continuous and water-continues structures. No attempts were made to trace the transition between these three forms of pregabalin microemulsions as a function of increasing aqueous phase content because it was out of the scope of this work.

The area of the formed microemulsion in each system is calculated relative to the whole area of the pseudo-ternary phase diagrams using ZWCAD program.

4.1.2. Pseudo-ternary phase diagrams of Group 1

The pseudo-ternary phase diagram of microemulsion system composed of Oleic acid as the oil phase, Tween 80 as the surfactant with different surfactant: cosurfactant ratios and 0.1 M pregabalin as the aqueous phase with different 0.1M pregabalin : PG ratios are shown in (Fig. 4.1).

The presence of ethanol and/or PG can affect the phase behavior of the system. Accordingly, pseudo-ternary phase diagrams were constructed in the absence and presence of PG with and without increasing concentrations of ethanol.

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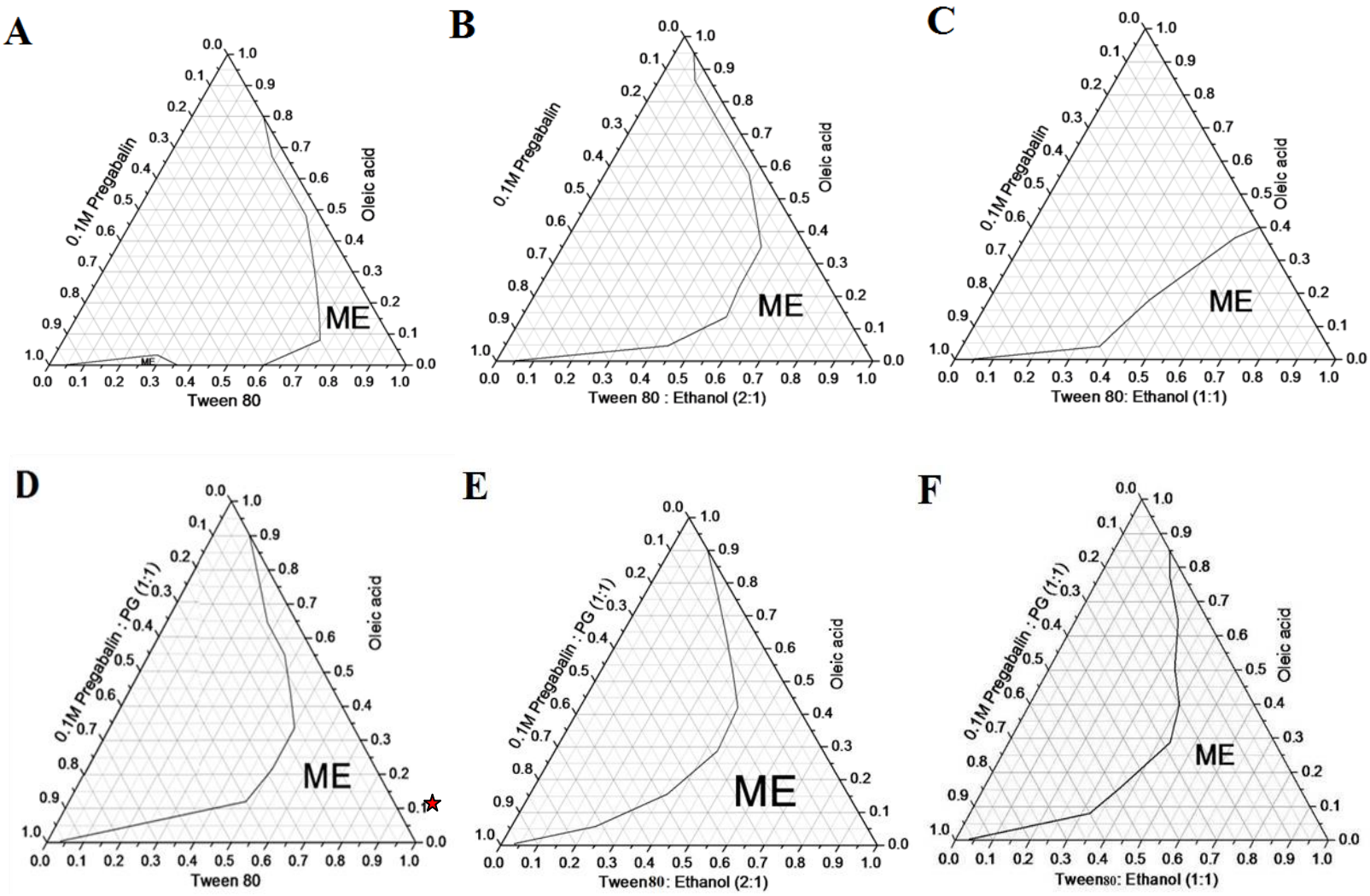


Fig 4.1: Pseudo-ternary phase diagrams of **Oleic acid, 0.1 M pregabalin, Tween 80 : Ethanol ratio** (A) 1:0 , (B) 2:1, (C) 1:1 and **Oleic acid, 0.1M pregabalin : PG (1:1), Tween 80 : Ethanol ratio** (D) 1:0, (E) 2:1, (F) 1:1

System A (Fig. 4.1A) is the simplest phase diagram in group 1 which was constructed using oleic acid as the oil phase, Tween 80 as the surfactant and 0.1M pregabalin as the aqueous phase. This system produced a phase diagram with two ME regions which both occupied approximately 17.0% of the total area of the phase diagram. The two microemulsion regions were separated as it can be observed in (Fig. 4.1A) that because of the turbid region that was formed when the 0.1 M pregabalin content in the system was in the range of 40 to 60% which was insufficient to hydrate the polyoxyethylene groups, which are critical for the swelling of surfactant chains to demonstrate the turbid structure. However, when the 0.1 M pregabalin content was above 60%, the distance between the polyoxyethylene groups increased and destabilized the turbid structure forming another microemulsion region (A_T%) which was approximately 2.0% .

The effects of surfactant /cosurfactant ratio were shown in (Fig. 4.1A-C). System B (Fig. 4.1B) composed of Tween 80/ethanol at the ratio of 2:1 showed a microemulsion area (A_T%) was approximately 31.0% which is higher than system A (Fig. 4.1A). The microemulsion area was smaller at the ratio of 1:0 (Fig. 4.1A) due to the absence of ethanol since ethanol is a cosurfactant which can decrease the interfacial tension between oil and water in microemulsion, resulting in a more flexible and dynamic layer system. The cosurfactant enables surfactant to distribute between the aqueous and oil phase thereby altering the relative hydrophilicity/ lipophilicity. At the ratio of 1:1 (Fig. 4.1C), the A_T% was approximately 22.5% which is smaller than system B (Fig. 4.1B) because of the low proportion of surfactant. In conclusion, the ratio of 2:1 was the optimum ratio of surfactant to cosurfactant due to its highest microemulsion area.

System D as shown in (Fig. 4.1D) showed the effect of the solubilization enhancers on the phase behavior and dilutability of pregabalin microemulsions. Changing the aqueous phase from 0.1 M

pregabalin to a mixture of 0.1M pregabalin /PG, in a ratio of (1:1) enhanced to a great extent the formation of pregabalin microemulsion using the same surfactant. Some surfactant molecules are known to selfassemble in polar organic solvents like PG, so PG like water form hydrogen bonds have relatively high dielectric constants, and are immiscible with hydrocarbon solvents. When these solvents are used as water substitutes, their penetration into the surfactant interface leads to smaller or no LC phase regions. The A_T% of system D (Fig. 4.1D) was 36.0% which represent an increase of approximately 112.0% compared with that formulated with 0.1 M pregabalin alone (Fig. 4.1A). The advantage of the microemulsion formulated with 0.1 M pregabalin /PG is that it can be fully diluted with the aqueous phase without converting into cloudy dispersion at surfactant to oleic acid weight ratios 9:1. That was evident from (Fig. 4.1D) that showed that the microemulsion boarder line had reached the 100.0% 0.1M Pregabalin/PG corner of the phase diagram without interruption. One should note that the microemulsion can reach this dilutable nature at surfactant to oleic acid at ratio 9:1, respectively. This ratio was represented by the "red asterisk" on the oleic acid-surfactant arm of the phase diagram of (Fig. 4.1D). PG which is a "diol" -bearing molecule, can arrange itself at the oleic acid-0.1M pregabalin interfacial film along with the original surfactant leading to increase of its elasticity and decrease of the interfacial tension. That behavior can lead to enhancement of the spontaneous film curvature, making its value less than zero which in turn prevents formation of gel phases and facilitates formation of microemulsion.

As shown in systems E and F (Fig. 4.1 E and Fig. 4.1F) the ME zone occupied approximately 45.0% and 44.0%, respectively of the total area of the phase diagram. These system gave the highest microemulsion areas in group 1. The change in phase behavior was caused by the addition of ethanol as cosurfactant and the use of PG with 0.1 M pregabalin as the aqueous

phase. The addition of ethanol makes the interfacial film itself more flexible and increases the single phase area. As shown in (Fig. 4.1E) system E has larger microemulsion area than system D (Fig.4.1D) due to the presence of ethanol as cosurfactant in ratio 2:1 and also larger microemulsion area than system F with the surfactant/cosurfactant ratio 1:1 in (Fig. 4.1F). Finally, in group 1 the best phase diagram with the largest microemulsion area was system E (Fig. 4.1E).

4.1.2. Pseudo-ternary phase diagram of Group 2

The pseudo-ternary phase diagram of microemulsion system composed of IPM as the oil phase, Tween 80 as the surfactant with different surfactant: cosurfactant ratios and 0.1 M pregabalin as the aqueous phase with different 0.1M pregabalin : PG ratios are shown in (Figure. 4.2)

The presence of ethanol and/or PG can affect the phase behavior of the system. Accordingly, pseudo-ternary phase diagrams were constructed in the absence and presence of PG with and without increasing concentrations of ethanol.

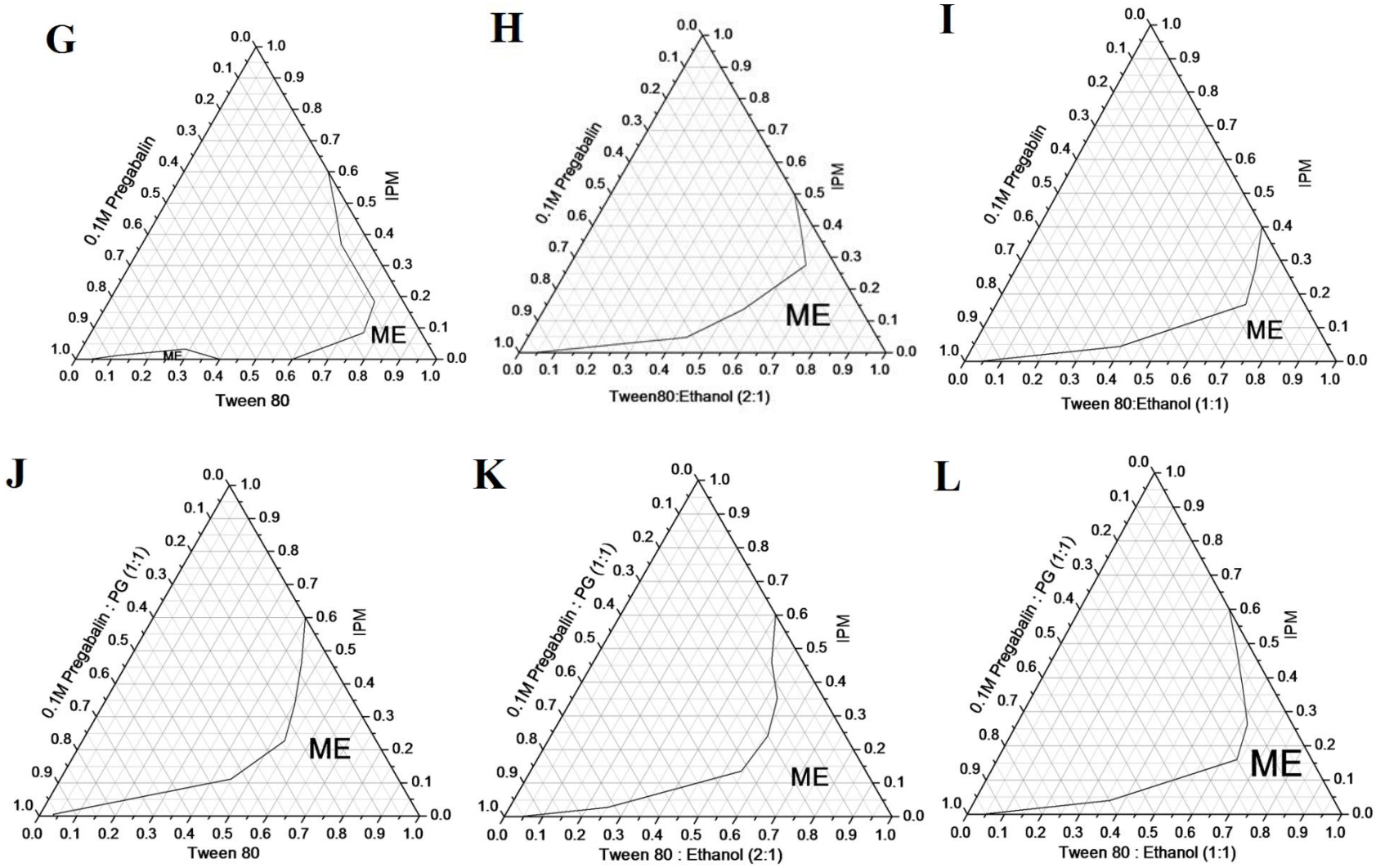


Fig 4.2: Pseudo-ternary phase diagrams of **IPM, 0.1 M pregabalin, Tween 80 : Ethanol ratio** (G) 1:0 , (H) 2:1, (I) 1:1 and **IPM, 0.1M pregabalin : PG (1:1), Tween 80 : Ethanol ratio** (J) 1:0, (K) 2:1, (L) 1:1

As the systems in group 1 systems in group 2 have approximately the same phase behavior .

System G (Fig. 4.2G) is the simplest phase diagram in group 2 which was constructed using IPM as the oil phase, Tween 80 as the surfactant, and 0.1M pregabalin as the aqueous phase. This system produced a phase diagram with two ME regions which both occupied approximately 13.0% of the total area of the phase diagram. This system has the smallest microemulsion area in comparison of the other five systems (H, I, J, K and L) in group 2 .

The (AT%) in system H (Fig. 4.2H) is significantly increased from 13.0% to 21.2% due to the use of ethanol as cosurfactant in the ratio of 2:1 which represent an increase of approximately 63.1% compared with that formulated without cosurfactant (Fig. 4.2G). However, increasing the ratio of the surfactant/cosurfactant to 1:1 in system I (Fig. 4.2I) has decreased the microemulsion area percentage from 21.2.0% to 18.4% which indicates that the ratio of 2:1 was the optimum ratio of surfactant to cosurfactant .

In system J (Fig. 4.2J) the use of PG as solubilization enhancers and changing the aqueous phase from 0.1 M pregabalin to a mixture of 0.1M pregabalin /PG, in a ratio of (1:1) has the same effect on the phase behavior as shown in system D in (Fig. 4.1D). The (AT%) of system J (Fig. 4.2 J) was 31.0% which represent an increase of approximately 138.5% compared with that formulated with 0.1 M pregabalin alone (Fig. 4.2G).

In System K (Fig. 4.2K) the ME zone occupied approximately 28.3% of the total area of the phase diagram. The change in phase behavior was caused by the addition of ethanol as cosurfactant and the use of PG with 0.1 M pregabalin as aqueous phase. However, increasing the ratio of the surfactant/cosurfactant to 1:1 in system L (Fig. 4.2L) has decreased the (AT%) from 28.3. % to 22.5% which also proofs that for these systems the ratio of 2:1 is the optimum

ratio of surfactant to co-surfactant. As shown (Fig. 4.2) system J and K has the largest microemulsion area in comparison of the other four systems (G, H, I and L) in group 2.

4.1.3. Pseudo-ternary phase diagram of Group 3

The pseudo-ternary phase diagram of microemulsion system composed of R(+)-Limonene as the oil phase, Tween 80 as the surfactant with different surfactant: cosurfactant ratio and 0.1 M pregabalin as the aqueous phase with different 0.1M pregabalin : PG ratio are shown in (Fig. 4.3).

The presence of ethanol and/or PG can affect the phase behavior of the system . Accordingly, pseudo-ternary phase diagrams were constructed in the absence and presence of PG with and without increasing concentrations of ethanol.

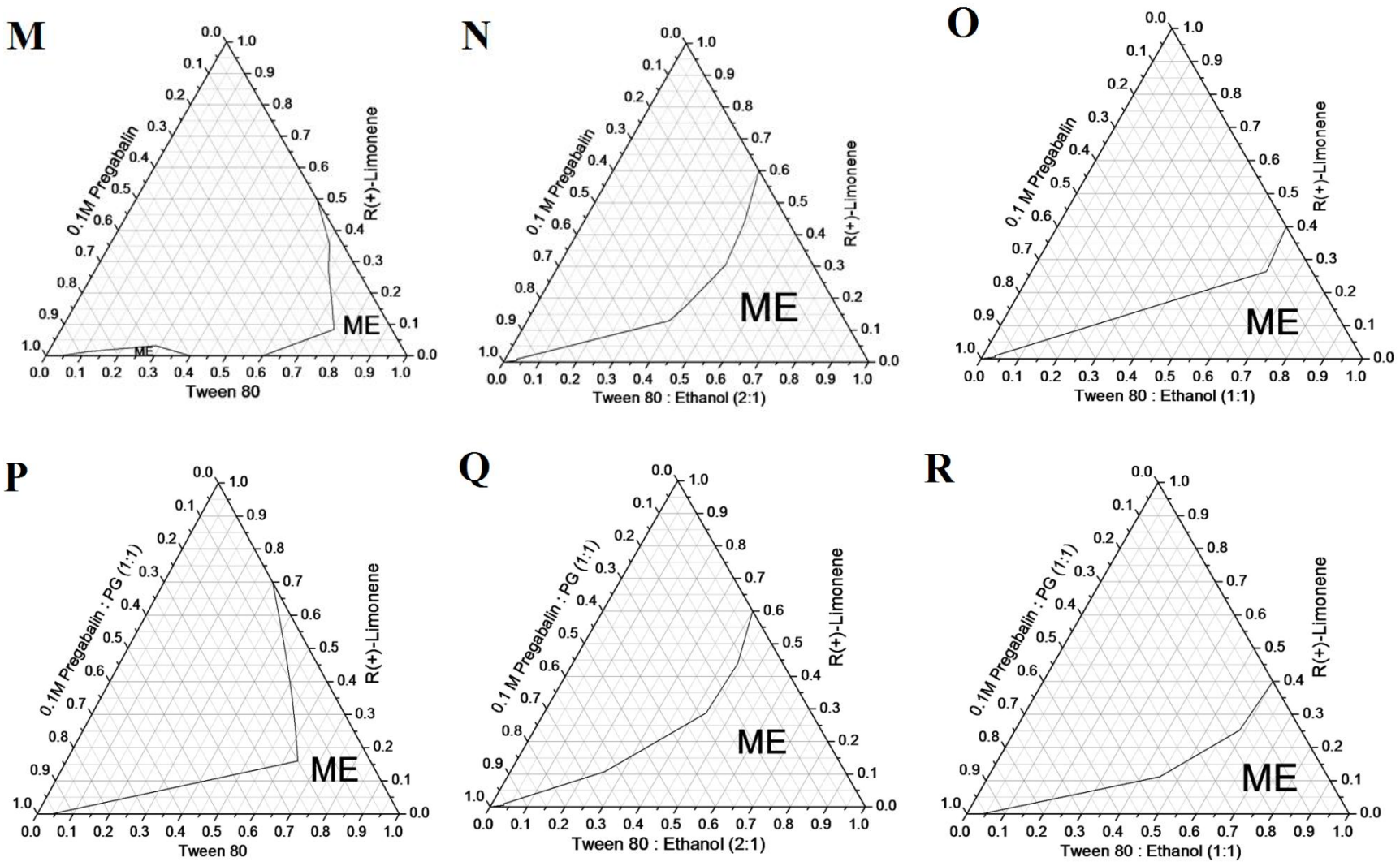


Fig 4.3: Pseudo-ternary phase diagrams of **R(+)-Limonene, 0.1 M pregabalin, Tween 80 : Ethanol ratio** (M) 1:0 , (N) 2:1, (O) 1:1 and **R(+)-Limonene, 0.1M pregabalin : PG (1:1) Tween 80 : Ethanol ratio** (P) 1:0, (Q) 2:1, (R) 1:1

As the systems in group 1and 2 systems in group 3 have approximately the same phase behavior.

System M (Fig. 4.3M) which is the simplest phase diagram in group 3 which was constructed using R(+)-limonene as the oil phase, Tween 80 as the surfactant and 0.1M pregabalin as the aqueous phase. This system produced a phase diagram with two ME regions which both occupied approximately 13.1% of the total area of the phase diagram. System M has the smallest microemulsion area in comparison of the other five systems (N,O, P,Q and R) in group 3 .

The (AT%) in system N (Fig. 4.3N) is significantly increased from 13.1% to 41.0% due to the use of ethanol as cosurfactant in the ratio of 2:1 which represent an increase of approximately 213.0% compared with that formulated without cosurfactant (Fig. 4.3M). However, increasing the ratio of the surfactant/cosurfactant to 1:1 in system O (Fig. 4.3O) has decreased the (AT%) from 41.0% to 31.2% which indicates that the ratio of 2:1 was the optimum ratio of surfactant to cosurfactant.

In system P (Fig. 4.3P) the use of PG as solubilization enhancers and changing the aqueous phase from 0.1 M pregabalin to a mixture of 0.1M pregabalin /PG, in a ratio of (1:1) has the same effect on the phase behavior as shown in system D (Fig. 4.1D) and in system J (Fig. 4.2 J). The (AT%) of system P (Fig 4.3P) was 30.0% which represent an increase of approximately 129.0% compared with that formulated with 0.1 M pregabalin alone (Fig. 4.3M)

System Q (Fig. 4.3Q) has (AT%) approximately 42.2% which is the largest microemulsion area in comparison to the other five systems (M, N, O, P and R) in group 3. The change in phase behavior was caused by the addition of ethanol as cosurfactant and the use of PG with 0.1 M pregabalin as aqueous phase. However, increasing the ratio of the surfactant/cosurfactant to 1:1 in system R (Fig. 4.3R) has decreased the microemulsion area percentage from 42.2% to 27.0% which also proofs that for these systems the ratio of 2:1 is the optimum ratio of surfactant to co-surfactant.

4.2. The effect of surfactant /cosurfactant ratio on the phase behavior.

After studying the phase behavior of the all systems in group 1, 2 and 3 we conclude that the area covered under the microemulsion was found to be larger in presence of higher amount of surfactant than cosurfactant. The surfactant/cosurfactant ratio (2:1) was found to be more efficient than the ratio (1:1) in formation of isotropic region. The efficiency of the surfactant/cosurfactant mixture decreased with decreasing amount of surfactant, clearly evident from (Table 4.1).

Group	System	Surfactant/cosurfactant ratio	A_T%
1	A	1:0	17.0
	B	2:1	31.0
	C	1:1	22.5
	D	1:0	36.0
	E	2:1	45.0
	F	1:1	44.0
2	G	1:0	13.0
	H	2:1	21.2
	I	1:1	18.4
	J	1:0	31.0
	K	2:1	28.3
	L	1:1	22.5
3	M	1:0	13.1
	N	2:1	41.0
	O	1:1	31.2
	P	1:0	30.0
	Q	2:1	42.2
	R	1:1	27.0

Table 4.1: A comparison between the percentage of the monophasic area relative to the total area of the triangle phase diagram(A_T %) for the whole eighteenth systems.

The above (Table 4.1) indicates that even thought cosurfactant assists in formation of microemulsions, their presence amounts is more than necessary to influence the interfacial parameters, decrease the solubilization capacity of surfactant molecules. Moreover, We can notice from the (Table 4.1) that the (A_T %) in each system in group 1 is approximately larger

than its match in group 2 and group 3 which indicates that microemulsions containing oleic acid as the oily phase has a better phase behavior than IPM and R(+)-Limonene as the oily phase.

4.3. Selection of microemulsion formulations

In order to assess and compare microemulsions obtained from different systems to select the best PE among the three oils (Oleic acid, IPM, R(+)-Limonene) according to the highest permeability coefficient value (P) which is a significant criteria for comparison of drug permeation, a constant point at surfactant/cosurfactant ratio of 2:1 was selected from the phase diagrams that gave me best microemulsion regions (B,H and N). The selection of this point was based on being the most common point containing the highest amount of the oil phase (14.4 %). The concentration of surfactant /cosurfactant (2:1) was fixed to 57.6% (% surfactant is 38.4% and % cosurfactant is 19.2%) and the aqueous phase was fixed to 28.0 %. The aqueous phase contains 0.44% pregabalin.

On the other hand three other systems (E,K and Q) were selected to choose the system having the highest permeability coefficient (P) in the presence of PG to study the effect of PG on enhancement of penetration through the skin. Therefore, samples were formulated from each system (E,K,Q) contains fixed concentration of oil approximately 14.4 %. The concentration of surfactant /cosurfactant (2:1) was fixed to 57.6% (% surfactant is 38.4% and % cosurfactant is 19.2%) and the aqueous phase was fixed to 28.0 %. The aqueous phase contains 0.22% pregabalin.

4.4. pH of microemulsion.

The solubility of drug and its potential to cause skin irritation depends on pH of system. Skin surface has pH in the range of 4-6. The pH of topical preparations must also be in this pH range. The pH of selected microemulsions was found in the range of (4.5-6.5).

4.5. Drug release kinetics and Diffusion parameters

In order to predict and correlate the *in vitro* pregabalin permeation behavior from these pregabalin-loaded microemulsions through synthetic membrane, it is necessary to fit into a suitable mathematical model. The *in vitro* pregabalin permeation data from microemulsions containing pregabalin through synthetic membrane were evaluated kinetically by various mathematical models like zero-order, first-order and Higuchi model. The results of the curve fitting into these above-mentioned mathematical models indicate the *in vitro* pregabalin permeation behavior of pregabalin-loaded microemulsions perfectly following Higuchi drug release model as the drug release profile is very closest to trend line or regression line and there is highest value of coefficient of correlation.

In this part of the study we investigated the effect of the PEs in microemulsion through diffusion experiments by using diffusion parameters. Diffusion coefficient (D) is a property of the permeant and is a measure of how easily it penetrates a specific membrane expressed in units of area/time, the lag time (T_L) reflects the time required by API to pass through the membrane and reach the receiver compartment. The partition coefficient (K) gives an indication about the ability of API to partition between the hydrophilic and hydrophobic phase. The permeability coefficient (P) gives an indication about the distance passed by the substance within specific period.

4.5.1. Drug release kinetics and diffusion parameters for systems B,H and N

All the samples in systems (B,H and N) were analyzed by HPLC before applying them on the donor of the FDC. The following (Table 4.2) and (Fig. 4.4) illustrate the absorbance of standard pregabalin and the plot of absorbance versus concentration respectively.

Table 4.2 : Absorbance of standard pregabalin for the calibration of systems B,H and N

Serial number	Concentration (ppm)	Absorbance
1	2.075	3.1
2	5.188	6.5
3	10.375	12.7
4	20.750	24.9
5	41.500	49.2
6	83.000	99.5
7	103.750	121.2
8	207.500	241.3

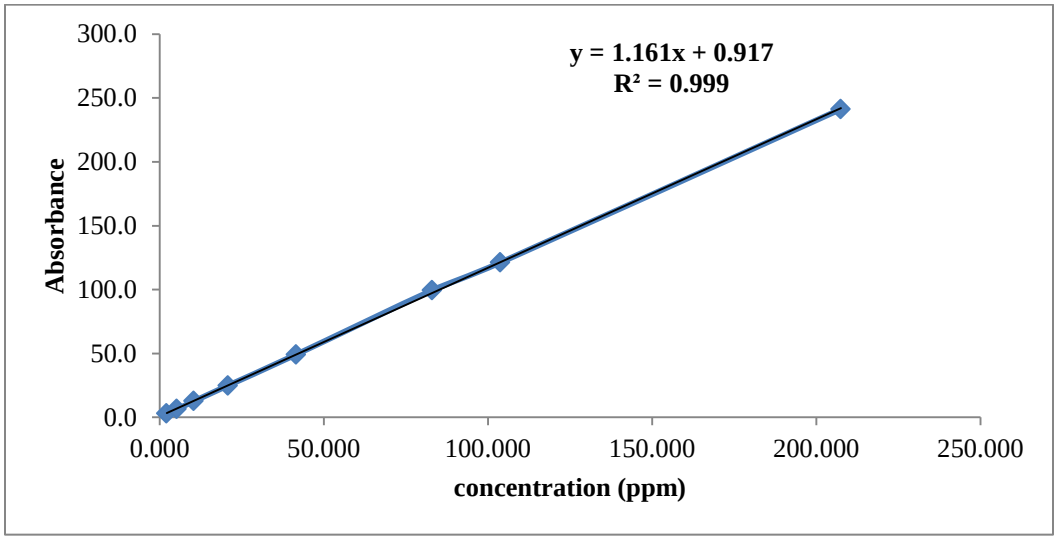


Fig 4.4: Calibration curve of pregabalin for systems B,H and N

The amount of pregabalin in each sample was 4.4 ± 0.05 mg. All samples for systems (B,H and N) were performed in triplicates and the results were represented as mean ± S.D

4.5.1.1. System B

(Table 4.3) summarize the assay results of pregabalin penetration to receiver compartment by time from system B. Area under peaks is presented in duplicates, and the total amount of drug penetrated per unit of membrane area is determined and plotted as a function of time, the linear part of the curve is plotted in (Fig. 4.5).

Table 4.3: Raw data for the diffusion of 0.44% pregabalin from system B in the presence of oleic acid as PE.

	B1				B2			B3			B	
Time (hr)	SQRT	Mean area ± SD	Conc. (mg/ml)	Mt (mg/cm^2)	Mean area ± SD	Conc. (mg/ml)	Mt (mg/cm^2)	Mean area ± SD	Conc. (mg/ml)	Mt (mg/cm^2)	Mean Mt (mg/cm^2) ± SD	Log cumulative drug remaining
0.5	0.7071	1.70 ± 0.00	0.0007	0.0043	12.50 ± 0.28	0.0100	0.0635	2.80± 0.14	0.0016	0.0103	0.0260 ± 0.0326	0.64087
1	1.0000	17.20 ± 1.13	0.0140	0.0895	28.75 ± 0.50	0.0240	0.1558	6.50± 0.28	0.0048	0.0311	0.0921± 0.0624	0.6342
2	1.4142	31.20 ± 0.42	0.0261	0.1707	41.65 ± 0.21	0.0351	0.2341	24.15±0.21	0.0200	0.1294	0.1781± 0.0527	0.6255
3	1.7321	32.20 ± 0.00	0.0269	0.1845	46.20 ± 0.00	0.0390	0.2702	29.50± 0.13	0.0246	0.1651	0.2066± 0.0559	0.6226
4	2.0000	34.0 ± 0.00	0.0285	0.2029	46.65 ± 0.07	0.0394	0.2851	29.95±0.35	0.0250	0.1754	0.2211± 0.0571	0.6211

Release rates from system B were fitted to statistical models like Zero order, First order and Higuchi equation (Fig. 4.5). It was observed that Higuchi was the best model for drug release from microemulsion.

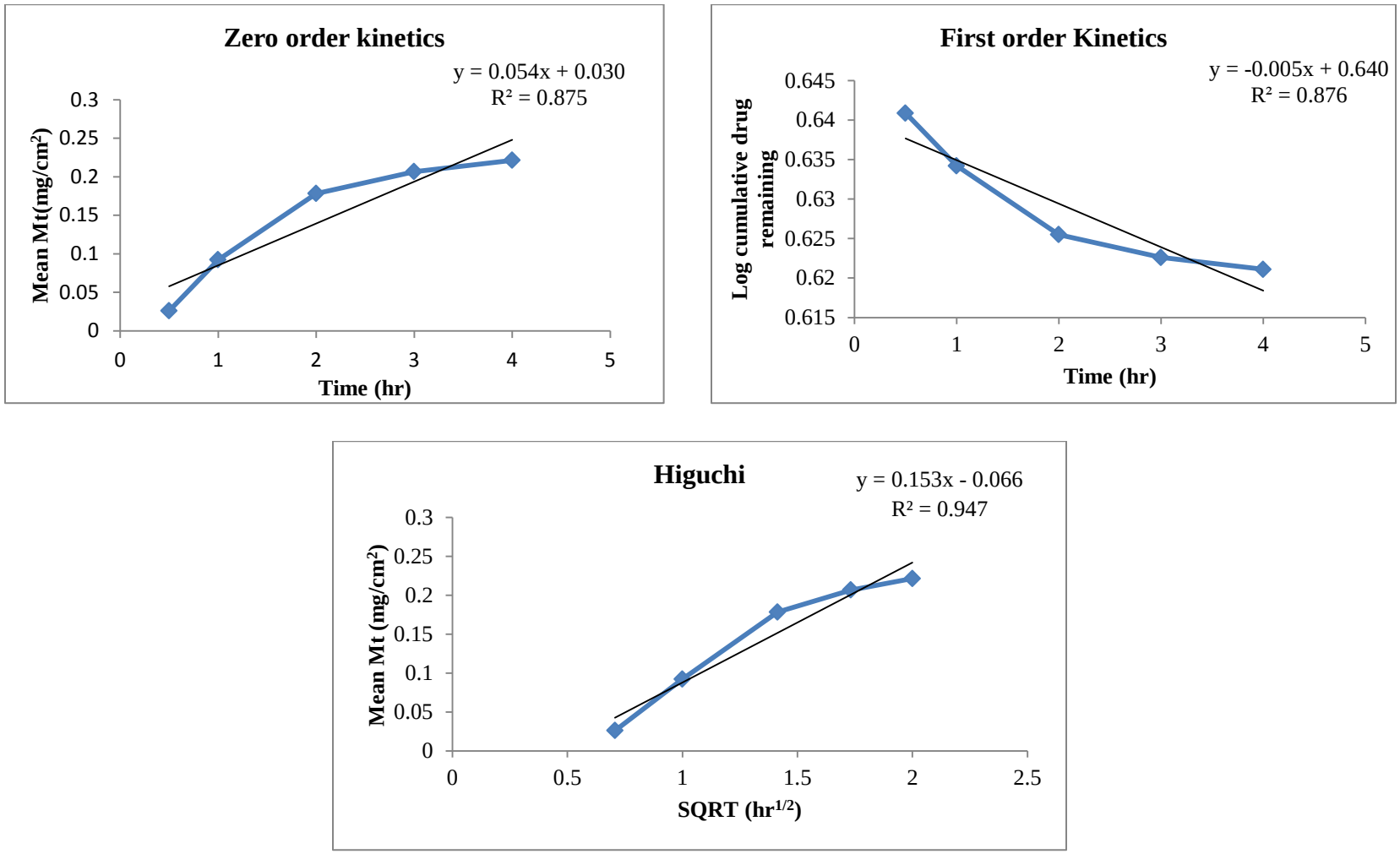


Fig 4.5 : The *in-vitro* drug release kinetic studies on system B: Zero Order plot, First Order plot and Higuchi plot

From the Higuchi plot (Fig. 4.5), lag time (T_L) was calculated from dividing the intercept of the equation of flux profile on the slope from the same equation. The diffusion coefficient (D) was calculated using equation (1.6) :

$$D = h^2/6.T_L \text{.....(1.6)}$$

Where (h) is the thickness of the synthetic membrane, which equals 0.0125 cm.

The permeability coefficient (P) is calculated by dividing the value of slope of the flux profile by the concentration of pregabalin in the donor compartment (4.4 mg/ml). The partition coefficient (K) is obtained from previously mentioned equation (3.6).

The calculated slope, intercept, cumulative amount per unit area (M_t), lag time (T_L), diffusion coefficient (D), permeability coefficient (P) and partition coefficient (K) of pregabalin in system B are in (Table 4.4).

The great value of permeation coefficient in the presence of oleic acid as the oil phase in the microemulsion in system B is due to the formation of separate phase (or as ‘ pool’) within the bilayers lipids. These pools would provide permeability defects within the lipid bilayers thus facilitating permeation of pregabalin through the membrane .

Table 4.4: Diffusion parameters of 0.44% Pregabalin microemulsion in the presence of oleic acid as PE (system B)

Slope	intercept	M_t (mg/cm^2)	T_L [hr$^{1/2}$]	D [cm^2/hr$^{1/2}$]	P [cm/hr$^{1/2}$]	K
0.1530	-0.0660	0.2211± 0.0571	0.4313	6.0379E-05	0.0347	7.1838

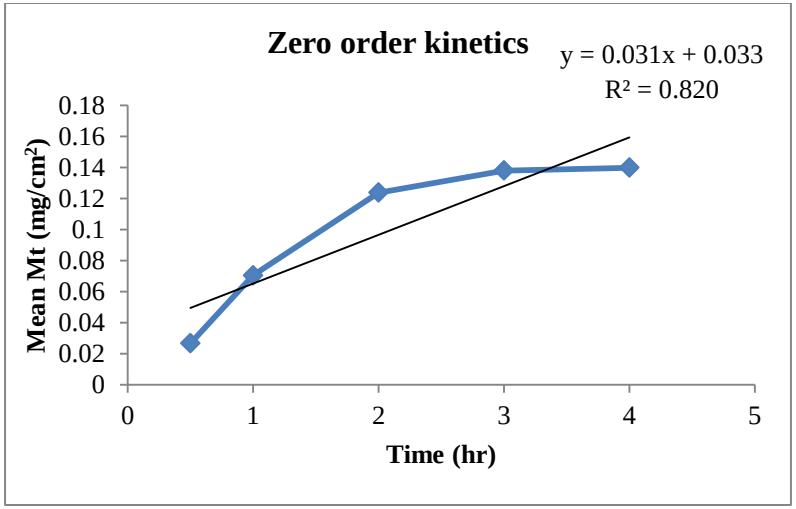
4.5.1.2. System H

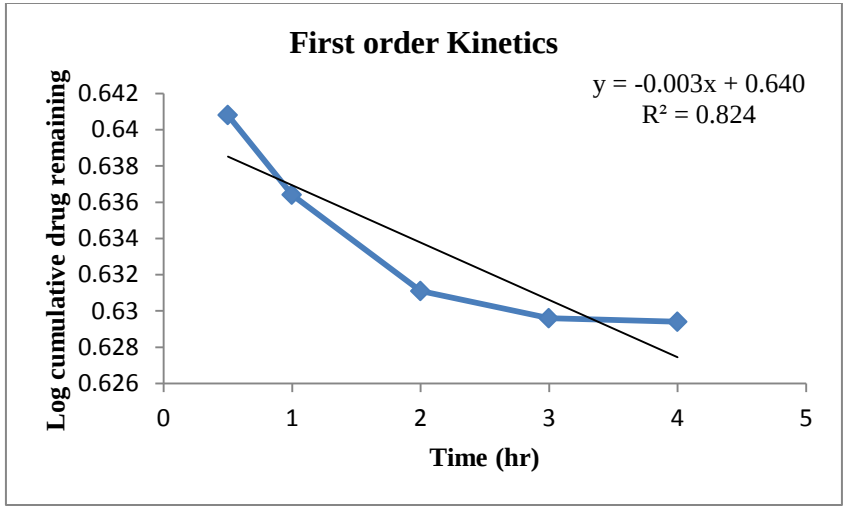
(Table 4.5) summarize the assay results of pregabalin penetration to receiver compartment by time from system H. Area under peaks is presented in duplicates, and the total amount of drug penetrated per unit of membrane area is determined and plotted as a function of time, the linear part of the curve is plotted in (Fig. 4.6)

Table 4.5: Raw data for the diffusion of 0.44% pregabalin from system H in the presence of IPM as PE.

	H1				H2			H3			H	
Time (hr)	SQRT	Mean area ± SD	Conc. (mg/ml)	Mt (mg/cm^2)	Mean area ± SD	Conc. (mg/ml)	Mt (mg/cm^2)	Mean area ± SD	Conc. (mg/ml)	Mt (mg/cm^2)	Mean Mt (mg/cm^2) ± SD	Log Cumulative drug remaining
0.5	0.7071	5.40 ± 0.28	0.0039	0.0246	6.25 ± 0.21	0.0046	0.0292	5.65 ± 0.07	0.0041	0.0259	0.0266 ± 0.0024	0.6408
1	1.0000	14.00 ± 0.14	0.0113	0.0729	14.90 ± 0.00	0.0120	0.0781	11.70 ± 0.84	0.0093	0.0604	0.0705 ± 0.0091	0.6364
2	1.4142	28.05 ± 0.35	0.0234	0.1536	15.60 ± 0.14	0.0126	0.0858	24.15 ± 0.07	0.0200	0.1316	0.1237 ± 0.0346	0.6311
3	1.7321	30.9 ± 0.57	0.0258	0.1766	16.15 ± 0.35	0.0131	0.0928	25.3 ± 0.42	0.0210	0.1443	0.1379 ± 0.0423	0.6296
4	2.0000	29.45 ± 0.50	0.0246	0.1769	16.00 ± 0.14	0.0130	0.0962	24.45 ± 1.20	0.0203	0.1463	0.1398 ± 0.0408	0.6294

Release rates from system H were fitted to statistical models like Zero order, First order and Higuchi equation (Fig. 4.6). It was observed that Higuchi was the best model for drug release from microemulsion.





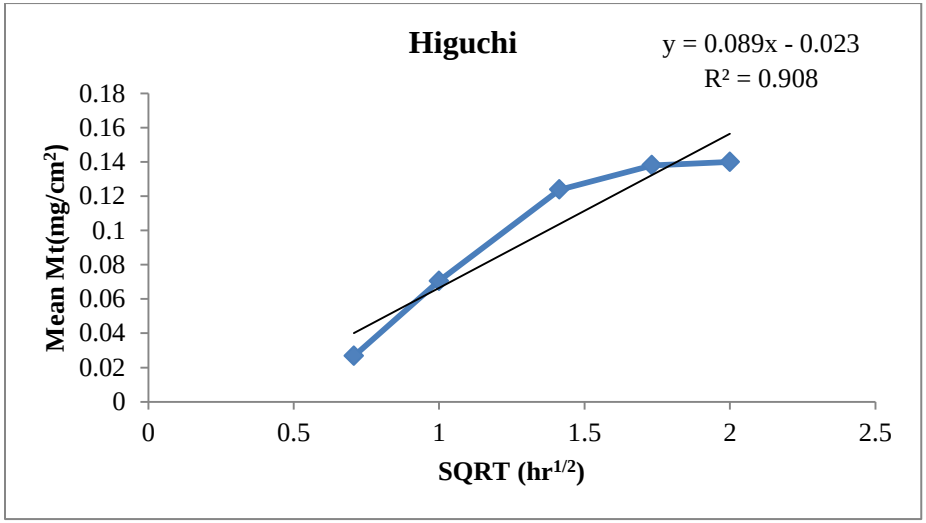


Fig 4.6: The *in-vitro* drug release kinetic studies on system H: Zero Order plot, First Order plot and Higuchi plot

Depending on the data obtained from the Higuchi model (Fig. 4.6),the calculated diffusion parameters are shown in (Table 4.6).The rate of diffusion of pregabalin in the presence of IPM is slower than when it is with oleic acid (system B), this is indicated by the value of permeability coefficient.

Table 4.6: Diffusion parameters of 0.44% Pregabalin microemulsion in the presence of IPM as PE (system H)

Slope	intercept	M_t (mg/cm^2)	T_L [hr$^{1/2}$]	D [cm^2/hr$^{1/2}$]	P [cm/hr$^{1/2}$]	K
0.0890	-0.0230	0.1398 ± 0.0408	0.2584	1.0078E-04	0.0202	2.5055

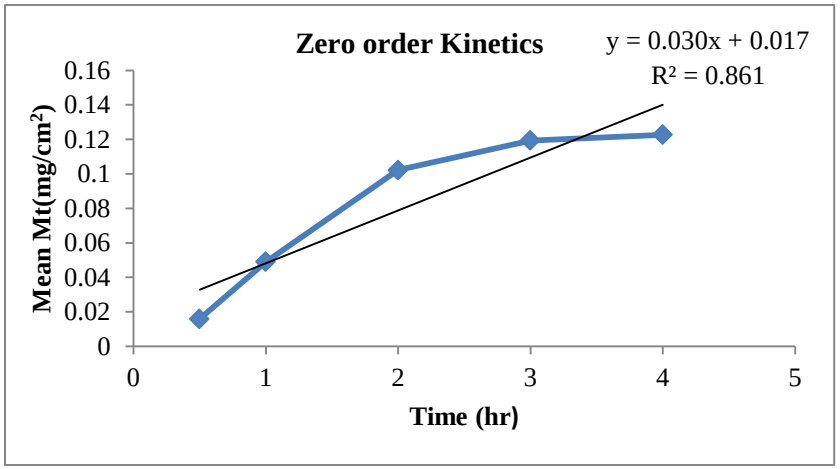
4.5.1.3. System N

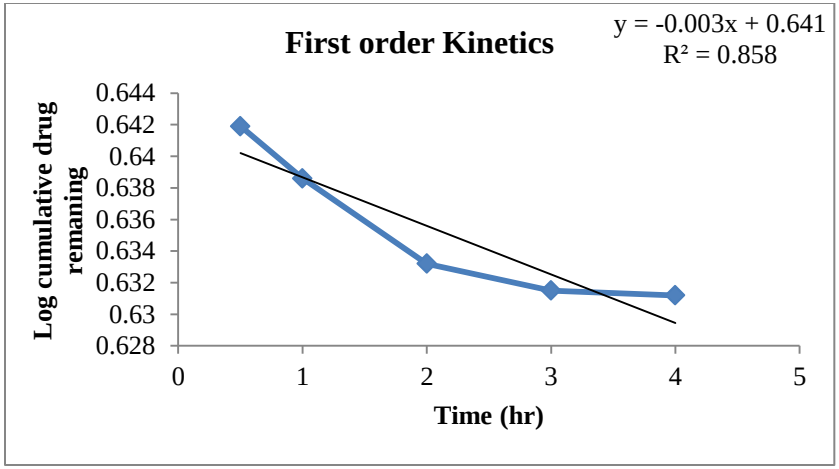
(Table 4.7) summarize the assay results of pregabalin penetration to receiver compartment by time from system N. Area under peaks is presented in duplicates, and the total amount of drug penetrated per unit of membrane area is determined and plotted as a function of time, the linear part of the curve is plotted in (Fig. 4.7).

Table 4.7: Raw data for the diffusion of 0.44% pregabalin from system N in the presence of R(+)-Limonene as PE.

	N1				N2			N3			N	
Time (hr)	SQRT	Mean area ± SD	Conc. (mg/ml)	Mt (mg/cm^2)	Mean area ± SD	Conc. (mg/ml)	Mt (mg/m^2)	Mean area ± SD	Conc. (mg/ml)	Mt (mg/cm^2)	Mean Mt (mg/cm^2) ± SD	Log cumulative drug remaining
0.5	0.7071	3.75 ± 0.35	0.0024	0.0155	3.10 ± 0.00	0.0019	0.0120	4.60 ± 2.39	0.0032	0.0202	0.0159 ± 0.0041	0.6419
1	1.0000	8.75 ± 0.07	0.0067	0.0437	8.80 ± 0.00	0.0068	0.0438	11.60 ± 6.12	0.0092	0.0596	0.0490 ± 0.0091	0.6386
2	1.4142	19.8 ± 0.42	0.0163	0.1064	18.35 ± 0.07	0.0150	0.0983	18.75± 0.67	0.0153	0.1017	0.1022 ± 0.0041	0.6332
3	1.7321	19.2 ± 0.14	0.0157	0.1083	25.85 ± 0.07	0.0215	0.1442	18.55± 8.99	0.0152	0.1055	0.1193 ± 0.0216	0.6315
4	2.0000	21.1 ± 0.00	0.0174	0.1238	23.8 ± 0.00	0.0197	0.1398	17.5 ± 7.80	0.0143	0.1046	0.1227 ± 0.0176	0.6312

Release rates from system N were fitted to statistical models like Zero order, First order and Higuchi equation (Fig. 4.7). It was observed that Higuchi was the best model for drug release from microemulsion.





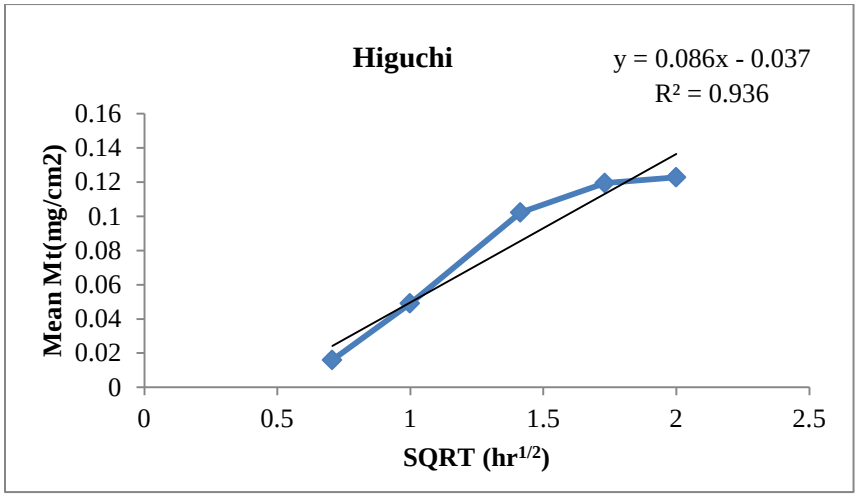


Fig 4.7: The *in-vitro* drug release kinetic studies on system N: Zero Order plot, First Order plot and Higuchi plot

Depending on the data obtained from the Higuchi model (Fig. 4.7), the calculated diffusion parameters are shown in (Table 4.8). The rate of diffusion of pregabalin in the presence of R(+)-Limonene as PE is slower than when it is with oleic acid (system B) and with IPM (System H). This is indicated by the value of permeability coefficient.

Table 4.8: Diffusion parameters of 0.44% Pregabalin microemulsion in the presence of R(+)-Limonene as PE (system N)

Slope	intercept	M_t (mg/cm^2)	T_L [hr$^{1/2}$]	D [cm^2/hr$^{1/2}$]	P [cm/hr$^{1/2}$]	K
0.0860	-0.0370	0.1227 ± 0.0176	0.4302	6.0563E-05	0.0195	4.0343

4.5.1.4. Selection of the best PE between Oleic acid, IPM and R(+)-Limonene.

The following (Table 4.9) summarize the diffusion parameters of 0.44% Pregabalin microemulsion in the presence of different penetration enhancer

Table 4.9: The summary of the diffusion parameters of 0.44% Pregabalin microemulsion in the presence of different PEs.

System	Penetration enhancer	Slope	intercept	M_t (mg/cm^2)	T_L [hr$^{1/2}$]	D [cm^2/hr$^{1/2}$]	P [cm/hr$^{1/2}$]	K
B	Oleic acid	0.1530	-0.0660	0.2211± 0.0571	0.4313	6.0379E-05	0.0347	7.1838
H	IPM	0.0890	-0.0230	0.1398± 0.0408	0.2584	1.0078E-04	0.0202	2.5055
N	R(+)-Limonene	0.0860	-0.0370	0.1227± 0.0176	0.4302	6.0563E-05	0.0195	4.0343

From (Table 4.9) The permeability coefficient value (P) was found to be in the following order :

System B > System H > System N

We conclude from the above relation that among the three systems (B,N and H), which the only difference between them is the oil phase, oleic acid is the best PE since system B has a permeability coefficient (P) (0.0347 cm/hr$^{1/2}$). So the order of the PEs can be found as the following :

Oleic acid > IPM > R(+)-Limonene

Which indicates that the permeation flux of microemulsions containing oleic acid as oil phase was comparatively greater than that of microemulsions containing IPM or R(+)-Limonene as oil phase.

Moreover, The lag time which is the time it takes to permeate through the membrane and diffuse into the receptor fluid and then finally reach a steady state of diffusion was found to be in the following order:

System B > System N > System H

From the above relation we can see that while **system B** showed the highest lag time between the two other systems (0.4313 hr $^{1/2}$) which is 11.16 minutes and the lowest diffusivity (D), while **System N** had the second highest lag time (0.4302 hr $^{1/2}$) which is approximately 11.10 minutes. On the other hand, **system H** showed lowest lag time (0.2584 hr $^{1/2}$) which is 4.01 minutes and the highest diffusivity (D). It indicates that the lag time is a permeation parameter depending mainly on the diffusivity of drug through the skin with the lag time being reduced and the diffusivity increased.

Finally, the partition coefficient (K), which is the ability of API (pregabalin) to partition between the hydrophilic and the hydrophobic phase was found to be in the following order:

System B > System N > System H

From the above relation we can conclude that the presence of oleic acid as PE in system B enhance the ability of pregabalin to partition between the hydrophilic and the hydrophobic phase more than with IPM and R(+)-Limonene as PEs.

4.5.2. Drug release kinetics and diffusion parameters for systems E,K and Q in the presence of PG

All the samples in systems (E,K and Q) were analyzed by HPLC before applying them on the donor of the FDC. The following (Table 4.10) and (Fig. 4.8) illustrate the absorbance of standard pregabalin and the plot of absorbance versus concentration respectively.

Table 4.10: Absorbance of standard pregabalin for the calibration of systems E,K and Q

Serial number	Concentration (ppm)	Absorbance
1	2.075	2.4
2	5.188	6.3
3	10.375	11.8
4	20.750	24.9
5	41.500	50.1
6	83.000	98.8
7	103.750	122.4
8	207.500	248.0

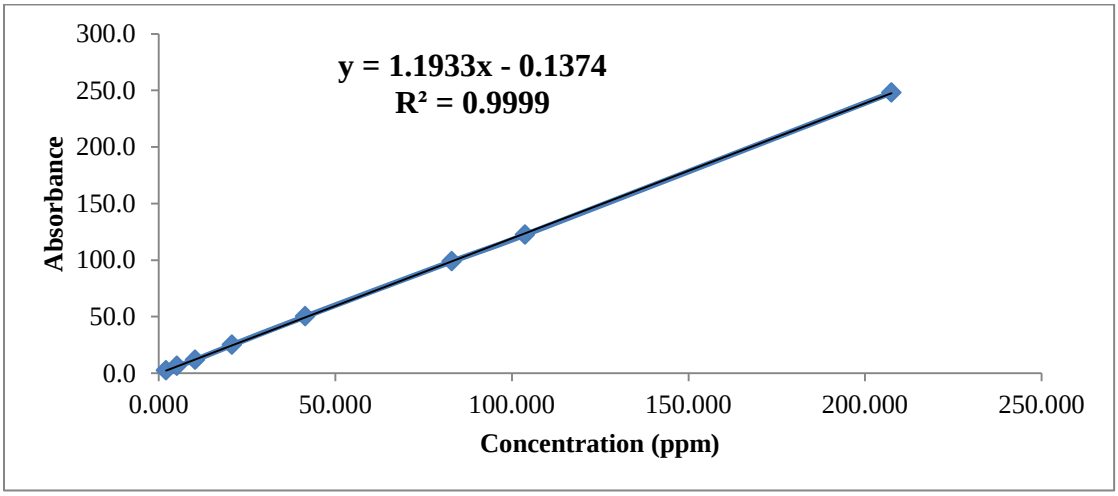


Fig 4.8: Calibration curve of pregabalin for systems E,K and Q

The amount of pregabalin in each sample (E,K and Q) was 2.2 ± 0.01 mg

All samples for systems (E,K and Q) were performed in duplicates and the results were represented as mean ± S.D.

4.5.2.1. System E

(Table 4.11) summarize the assay results of pregabalin penetration from system E to receiver compartment by time. Area under peaks is presented in duplicates, and the total amount of drug penetrated per unit of membrane area is determined and plotted as a function of time, the linear part of the curve is plotted in (Fig. 4.9).

Table 4.11: Raw data for the diffusion of 0.22% pregabalin in the presence of PG from system E.

	E1				E2			E	
Time (hr)	SQRT	Mean area ± SD	Conc. (mg/ml)	Mt (mg /cm^2)	Mean area ± SD	Conc. (mg/ml)	Mt (mg /cm^2)	Mean Mt (mg /cm^2) ± SD	Log cumulative drug remaining
0.5	0.7071	1.00 ± 0.00	0.0001	0.0005	1.50 ± 0.00	0.0005	0.0032	0.0018 ±0.0019	0.3421
1	1.0000	1.95 ± 0.49	0.0009	0.0057	2.35 ± 0.07	0.0012	0.0080	0.0068 ± 0.0016	0.3410
2	1.4142	2.65 ± 0.07	0.0015	0.0098	3.20 ± 0.00	0.0020	0.0131	0.0114 ± 0.0023	0.33957
3	1.7321	3.75 ± 0.21	0.0024	0.0163	3.90 ± 0.00	0.0026	0.0175	0.0169 ± 0.0009	0.3390
4	2.0000	3.50 ± 0.00	0.0022	0.0157	3.80 ± 0.00	0.0025	0.0178	0.0168 ± 0.0015	0.3391

Release rates from system E were fitted to statistical models like Zero order, First order and Higuchi equation (Fig. 4.9). it was observed that Higuchi was the best model for drug release from microemulsion.

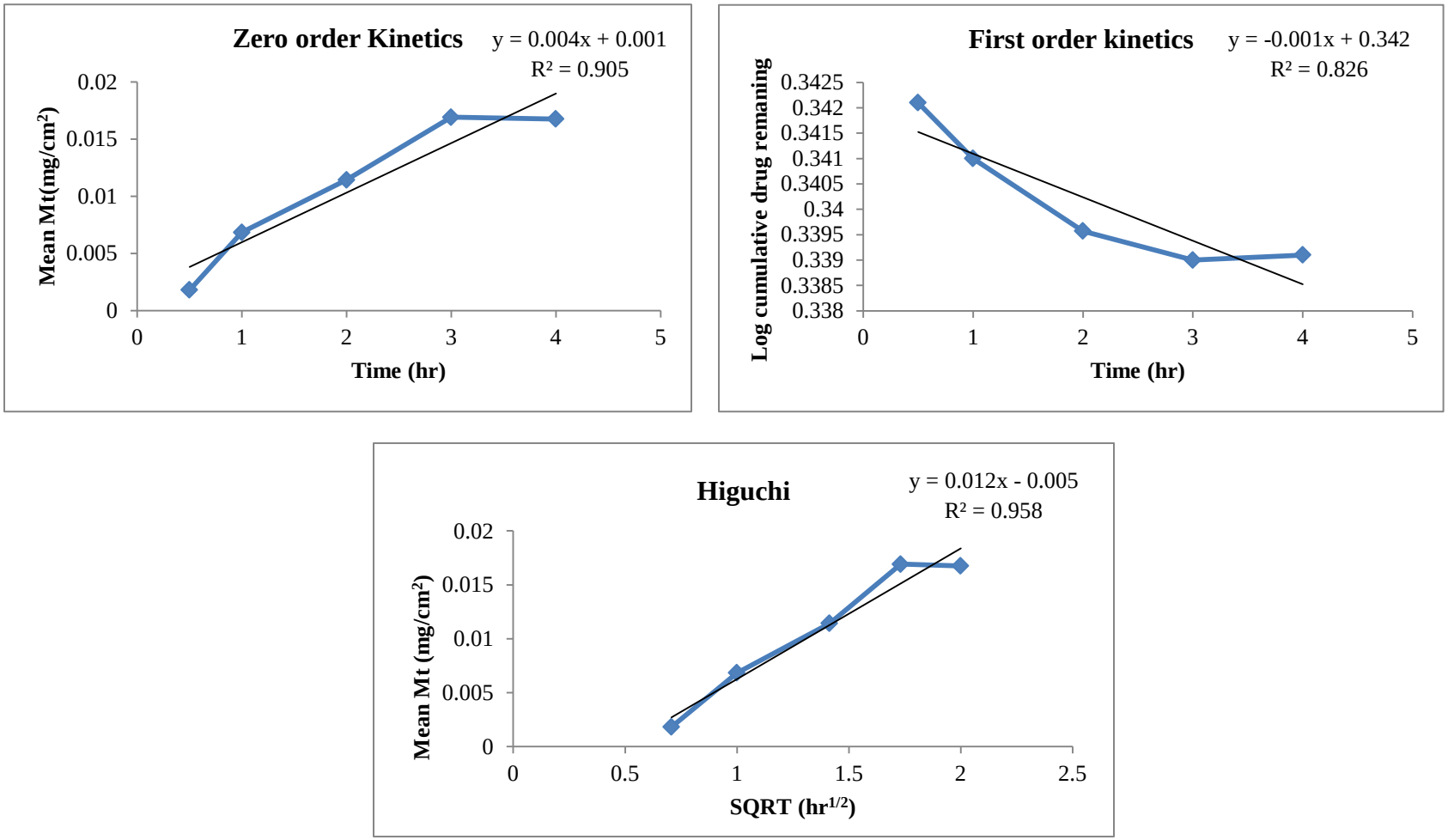


Fig 4.9 : The *in-vitro* drug release kinetic studies on system E: Zero Order plot, First Order plot and Higuchi plot

Depending on the data obtained from the Higuchi model (Fig. 4.9), the calculated diffusion parameters are shown in (Table 4.12). The rate of diffusion of pregabalin in the presence of PG is low. This is indicated by the value of permeability coefficient.

Table 4.12: Diffusion parameters of 0.22% pregabalin in the presence of PG (system E)

Slope	intercept	M_t (mg/cm^2)	T_L [hr$^{1/2}$]	D [cm^2/hr$^{1/2}$]	P [cm/hr$^{1/2}$]	K
0.0120	-0.0050	0.0168 ± 0.0015	0.4166	6.2510E-05	0.0055	1.0998

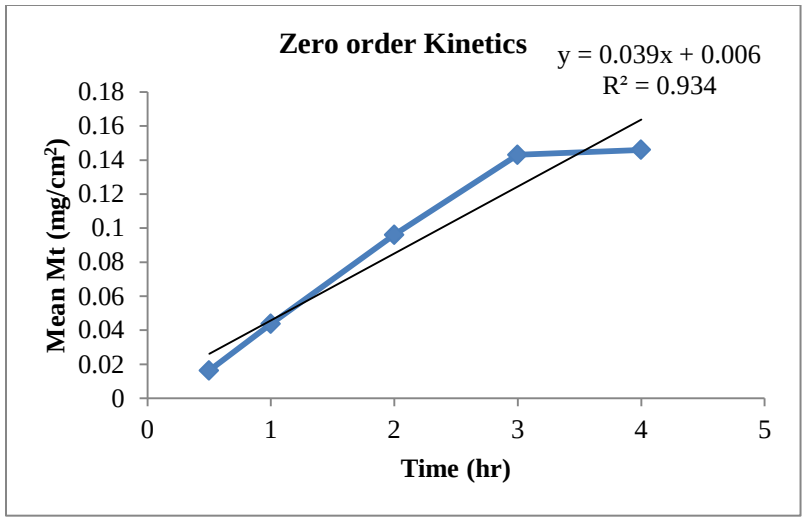
4.5.2.2. System K

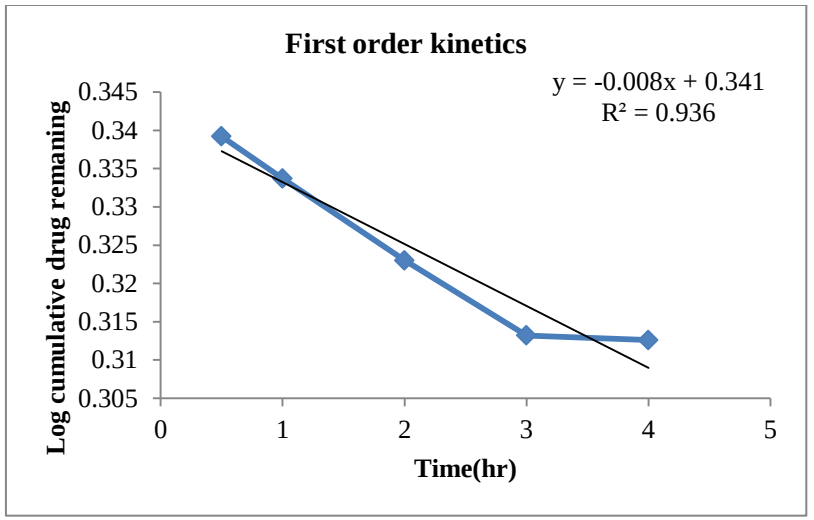
(Table 4.13) summarize the assay results of pregabalin from system K penetration to receiver compartment by time. Area under peaks is presented in duplicates, and the total amount of drug penetrated per unit of membrane area is determined and plotted as a function of time, the linear part of the curve is plotted in (Fig. 4.10)

Table 4.13: Raw data for the diffusion of 0.22% pregabalin in the presence of PG from system K .

	K1				K2			K	
Time (hr)	SQRT	Mean area ± SD	Conc. (mg/ml)	Mt (mg /cm^2)	Mean area ± SD	Conc. (mg/ml)	Mt (mg /cm^2)	Mean Mt (mg /cm^2) ± SD	Log cumulative drug remaining
0.5	0.7071	2.65 ± 0.07	0.0023	0.0149	3.20 ± 0.00	0.0028	0.0178	0.0163 ± 0.0021	0.3392
1	1.0000	6.90 ± 0.00	0.0059	0.0383	8.90 ± 0.00	0.0076	0.0491	0.0437 ± 0.0077	0.3337
2	1.4142	11.95 ± 0.07	0.0101	0.0671	22.65 ± 0.07	0.0191	0.1249	0.0960 ± 0.0409	0.3230
3	1.7321	16.05 ± 0.07	0.0136	0.0923	34.45 ± 0.07	0.0290	0.1940	0.1431 ± 0.0719	0.3132
4	2.0000	15.20 ± 0.00	0.0129	0.0920	33.85 ± 0.07	0.0285	0.2000	0.1460 ± 0.0764	0.3126

Release rates from system K were fitted to statistical models like Zero order, First order and Higuchi equation (Fig. 4.10). It was observed that Higuchi was the best model for drug release from microemulsion.





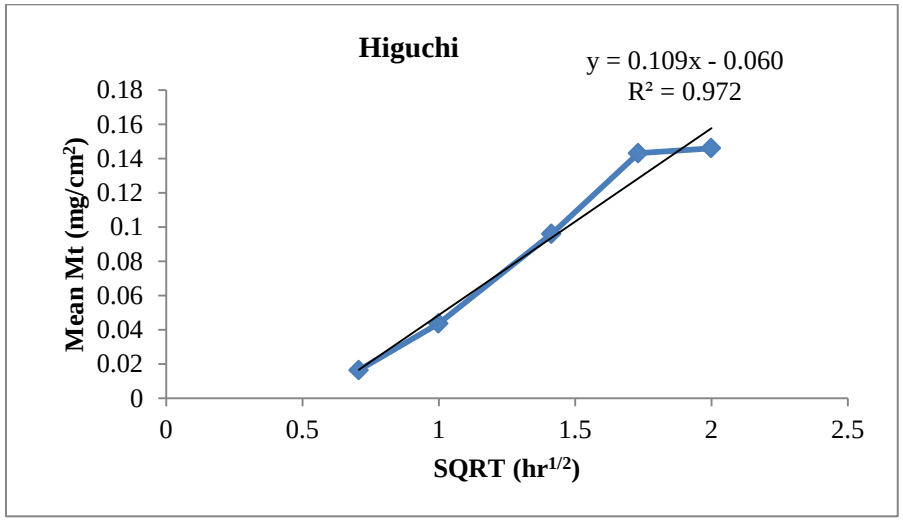


Fig 4.10: The *in-vitro* drug release kinetic studies on system K: Zero Order plot, First Order plot and Higuchi plot

Depending on the data obtained from the Higuchi model (Fig. 4.10), the calculated diffusion parameters are shown in (Table 4.14). The rate of diffusion of pregabalin in the presence of PG in system K is greater than in system E. This is indicated by the value of permeability coefficient.

Table 4.14: Diffusion parameters of 0.22% pregabalin in the presence of PG (system K)

Slope	intercept	M_t (mg/cm^2)	T_L [hr$^{1/2}$]	D [cm^2/hr$^{1/2}$]	P [cm/hr$^{1/2}$]	K
0.1090	-0.0600	0.1460 ± 0.0764	0.5504	4.7314E-05	0.04955	13.0895

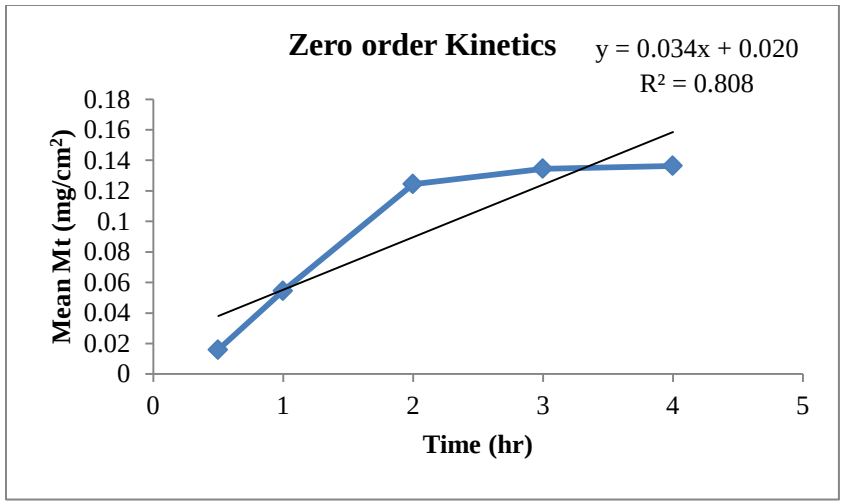
4.5.2.3 System Q

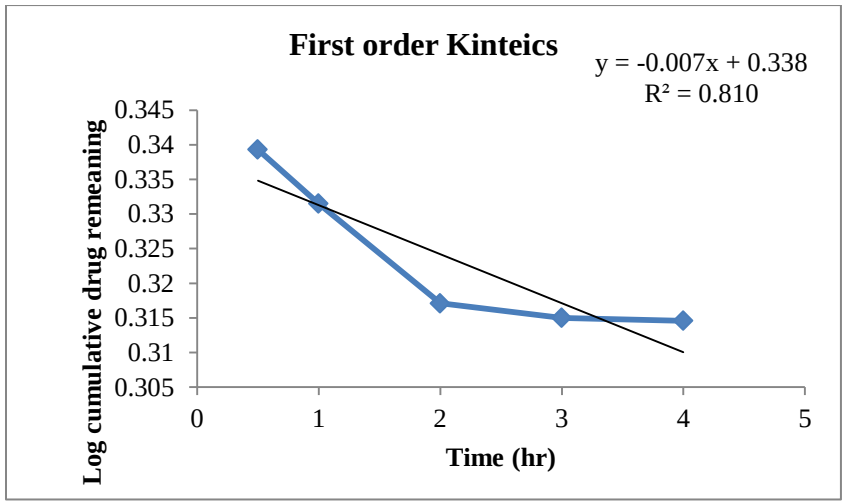
(Table 4.15) summarize the assay results of pregabalin penetration from system Q to receiver compartment by time. Area under peaks is presented in duplicates, and the total amount of drug penetrated per unit of membrane area is determined and plotted as a function of time, the linear part of the curve is plotted in (Fig. 4.11)

Table 4.15: Raw data for the diffusion of 0.22% pregabalin in the presence of PG from system Q.

	Q1				Q2			Q	
Time (hr)	SQRT	Mean area ± SD	Conc. (mg/ml)	Mt (mg /cm^2)	Mean area ± SD	Conc. (mg/ml)	Mt (mg /cm^2)	Mean Mt (mg /cm^2) ± SD	Log cumulative drug remaining
0.5	0.7071	2.95 ± 0.21	0.0026	0.0165	2.75 ± 0.07	0.0024	0.0154	0.0159 ± 0.0008	0.3393
1	1.0000	10.10 ± 0.00	0.0086	0.0555	9.75 ± 0.07	0.0083	0.0535	0.0545 ± 0.0014	0.3315
2	1.4142	25.50 ± 0.00	0.0215	0.1404	19.55 ± 0.07	0.0165	0.1085	0.1244 ± 0.0226	0.3171
3	1.7321	27.80 ± 0.00	0.0234	0.1595	18.75 ± 0.07	0.0158	0.1095	0.1345 ± 0.0354	0.3150
4	2.0000	27.00 ± 0.14	0.0227	0.1627	17.9 ± 0.14	0.0151	0.1100	0.1363 ± 0.0373	0.3146

Release rates from system Q were fitted to statistical models like Zero order, First order and Higuchi equation (Fig. 4.11). It was observed that Higuchi was the best model for drug release from microemulsion.





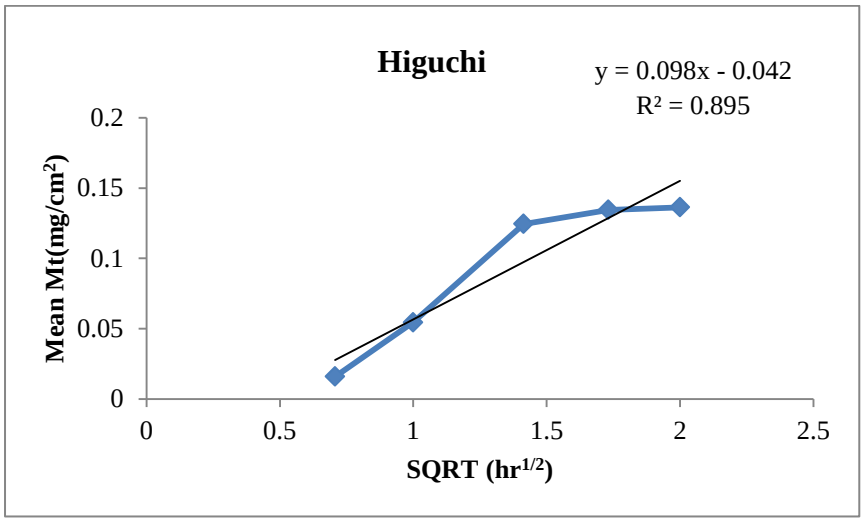


Fig 4.11: The *in-vitro* drug release kinetic studies on system Q: Zero Order plot, First Order plot and Higuchi plot

Depending on the data obtained from the Higuchi model (Fig. 4.11),the calculated diffusion parameters are shown in (Table 4.16). The rate of diffusion of pregabalin in the presence of PG in system Q is less than in system K but higher than system E. This is indicated by the value of permeability coefficient.

Table 4.16: Diffusion parameters of 0.22% pregabalin in the presence of PG (system Q)

Slope	intercept	M_t (mg/cm^2)	T_L [$hr^{1/2}$]	D [$cm^2/hr^{1/2}$]	P [$cm/hr^{1/2}$]	K
0.0980	-0.0420	0.1363 ± 0.0373	0.4286	6.0760E-05	0.0445	9.1549

4.5.2.4. Selection the system having the highest permeability coefficient (P) in the presence of PG

The following (Table 4.17) summarize the diffusion parameters of 0.22% pregabalin in the presence of PG in systems E, K and Q.

Table 4.17: The summary of the diffusion parameters of 0.22% pregabalin in the presence of PG

System	Slope	intercept	M_t [mg/cm^2]	T_L [$hr^{1/2}$]	D [$cm^2/hr^{1/2}$]	P [$cm/hr^{1/2}$]	K
E	0.0120	-0.0050	0.0168 ± 0.0015	0.4166	6.2510E-05	0.0055	1.0998
K	0.1090	-0.0600	0.1460 ± 0.0764	0.5504	4.7314E-05	0.0495	13.0895
Q	0.0980	-0.0420	0.1363 ± 0.0373	0.4286	6.0760E-05	0.0445	9.1549

From the above (Table 4.17) we can conclude that among the three systems (E,K and Q) the value permeability coefficient (P) was found to be in the presence of PG in the following order:

System K > System Q > System E

Since the only difference between these system (E,K and Q) is the oil phase we can conclude that PG works synergistically with IPM (system K) in enhancing the drug permeability since it has the highest permeability coefficient value (0.0495 $cm/hr^{1/2}$). System Q which have the R(+)-Limonene as the oily phase is the second highest permeability coefficient (0.0445 $cm/hr^{1/2}$). However, system E gave the least permeability coefficient (0.0055 $cm/hr^{1/2}$) in the presence of PG .

The lag time in the presence of the PG is in the following order:

System K > System Q > System E

From the above relation we can see that **system K** showed highest lag time between the two other systems (0.5504 $hr^{1/2}$) which is 18.18 minutes and the lowest diffusivity (D), while **system Q** had the second highest lag time (0.4286 $hr^{1/2}$) which is approximately 11.02 minutes. On the other hand, **system E** showed the lowest lag time (0.4166 $hr^{1/2}$) which is approximately 10.41 minutes and the highest diffusivity (D). That indicates that the lag time is a permeation parameter depending mainly on the diffusivity of drug through the skin with the lag time being reduced diffusivity increased.

Finally, the partition coefficient (K), which is the ability of API (pregabalin) to partition between the hydrophilic and the hydrophobic phase was found to be in the following order:

System K > System Q > System E

From the above relation we can conclude that the presence of PG with IPM in system K enhance the ability of pregabalin to partition between the hydrophilic and the hydrophobic phase more than with the other systems (Q and E) in the presence of PG.

4.6. Comparison between the literature and the of results of this study

We can notice from the literature that there was a lot of attempts to form microemulsion as topical drug delivery because of its characteristic qualities. In this study the results showed that the microemulsion can be potentially used for improved topical drug delivery of pregabalin.

As we can see from the literature that pregabalin can be found as tablet, capsules and solution. All of these dosage forms are orally administrated and there is a few attempts to form topical pregabalin formulation to overcome its orally administrated problems .

In 2014 Dr. Fukasawa and his colleagues studied the ability of forming transdermally simple solution containing pregabalin for the treatment of NP in animal models. However, they only addressed the ability of pregabalin of increasing the pain thresholds in response to mechanical stimuli in nerves of different animal models. There was lake of use of penetration enhancers in their study that definitely can enhance the penetration ability of pregabalin that can increase the pain thresholds. While in this study there was several microemulsion systems which were formulated with different penetration enhancer such as oleic acid in group 1, IPM group 2 and R(+)-limonene in group 3. Moreover, the use of ethanol and PG with different ratios in each group that also have a role in penetration enhancement.

In 2012 Dr. Bhatia and his colleagues formulate transdermally pregabalin patch for the treatment of NP. However, any transdermal patch needs seven main component: liner, drug ,adhesive, membrane, backing, permeation enhancer and matrix filler. Transdermal patches also needs to evaluate their thickness, weight variation, folding endurance, tensile strength, moisture uptake and moisture content. However, in this study formulation of pregabalin microemulsion for the treatment of NP is more easily to prepare, low cost and thermodynamically stable .

4.7. Conclusion

The present project was an attempt to achieve the possibility of preparation of topical microemulsions of pregabalin with increased permeation through the skin. The results indicate that microemulsion formulation can be used as a feasible alternative to conventional formulations of pregabalin with advanced permeation characteristics for improved topical drug delivery. It represents an easy way to manufacture thermodynamically stable system with improved topical availability of the drug and a transparent and elegant appearance.

• The stable microemulsion formulations of oleic acid (group1), IPM (group 2) and R(+)-Limonene (group 3) were successfully prepared with ease of fabrication from a pseudo-ternary phase diagram.

• Optimized formulations containing ethanol as cosurfactant in the surfactant/cosurfactant ratio (2:1) and PG as solublizaing enhancer in the ratio PG: 0.1 M Pregabalin (1:1) were successful in delivering the drug across the skin in 4 hours with Higuchi model kinetics.

• Selection of best penetration enhancer was based on its diffusion parameters especially on the value of permeability coefficient (P) which was found to be in the following order :

Oleic acid > IPM > R(+)-Limonene oil

• The permeability coefficient (P) values of systems (E, K and Q) in the presence of PG were in the following order :

System K > System Q > System E

4.8. Future work

• The application of synthetic membrane can be used for comparative studies during topical drug formulation development.

• It is recommended to investigate the effect of molecular weight of dialysis membrane on permeation to suit the real composition of human skin.

• The present work required to be supported by further studies involving in-vivo pharmacodynamic and pharmacokinetic studies in animal and human models.

References

1. Songkro, S.(2009). An overview of skin penetration enhancers: penetration enhancing activity, skin irritation potential and mechanism of action. *Journal of Science and Technology*, *31* (3), 299-321.

2. Mbah, C., Uzor, P., & Omeje, E. (2011). Perspectives on Transdermal Drug Delivery. *Journal of Chemical and Pharmaceutical Research*, 3(3), 680-700.

3. Arora, R., SL, H., & Geeta, A. (2012). Microemulsion System In Role Of Expedient Vehicle For Dermal Application. *Journal of Drug Delivery and Therapeutics, 2*(4), 23-28.

4. Raut, S., Nemade,L., Desai,M., Bonde, S., & Dongare,S.(2014) .Chemical penetration enhancer for transdermal drug delivery systems. *International Journal of Pharmacy Review & Research, 4*(1), 33-40.

5. Trommer, H., & Neubert, R. (2006). Overcoming the Stratum Corneum: The Modulation of Skin Penetration. *Skin Pharmacology and Physiology, 19*(2), 106-121.

6. Prashar, M., Aggarwal, G., & Harikumar, S. L. (2014). Synergistic Action Of Penetration Enhancers In Transdermal Drug Delivery. *Journal of Drug Delivery and Therapeutics, 4*(3), 45-51.

7. Pathan, I. B., & Setty, C. M. (2009). Chemical Penetration Enhancers for Transdermal Drug Delivery Systems. *Tropical Journal of Pharmaceutical Research, 8* (2), 173-179.

8. Kanikkannan, N., Kandimalla, K., Lamba, SS., & Singh, M. (2000). Structures activity relationship of chemical penetration enhancers in transdermal drug delivery. *Current Medicinal Chemistry, 7*(6), 593-608

9. Pandey,A., Mittal, A., Chauhan, N., & Alam,S.(2014). Role of Surfactants as Penetration Enhancer in Transdermal Drug Delivery System. *Molecular Pharmaceutics & Organic Process Research, 2* (2), 1-10.

10. Syed, H., & Peh, K. (2014). Identification of phases of various oil, surfactant/ co-surfactants and water system by ternary phase diagram. *Acta Poloniae Pharmaceutica, 71*, 301-9

11. Vikas,S., Seema,S., Gurpreet,S., C,R., & Baibhav, J. (2011).Penetration enhancers: a novel strategy for enhancing transdermal drug delivery. *International Resaerch Journal of pharmcy*, *2* (12), 32-36.

12. Saini,S., Chauhan, S., & Agrawal,S.(2014). Recent development in Penetration Enhancers and Techniques in Transdermal Drug Delivery System. *Journal of Advanced Pharmacy Education & Research,4*(1), 31-40.

13. Kogan, A., & Garti, N. (2006). Microemulsions as transdermal drug delivery vehicles. *Advances in Colloid and Interface Science ,123–126* , 369–385.

14. Yuan, S. (2009). *Linker-based lecithin microemulsions as transdermal drug delivery systems. Department of Chemical Engineering and Applied Chemistry* (Doctoral dissertation). University of Toronto, Canada.

15. Sahu, G., Sharma, H., Gupta, A., & Kaur, C. (2015). Advancements in Microemulsion Based Drug Delivery Systems for Better Therapeutic Effects. *International Journal of Pharmaceutical Sciences and Developmental Research,1*(1)8-15.

16. Khodakiya, A., Chavada ,J., Jivani1, N., Patel, B., Khodakiya, M., & Ramoliya, A.(2012). Microemulsions as Enhanced Drug Delivery Carrier: An Overview . *American Journal of Pharm Tech Research, 2*(4) , 206-226.

17. Talegaonkar, S., Azeem, A., Ahmad , F., Khar, R.,, Pathan, S., & Khan, Z. (2008).Microemulsions: a novel approach to enhanced drug delivery.. *Recent Patents on Drug Delivery & Formulation,2*(3), 238-257

18. Baron, R., Binder, A., & Wasner, G. (2010). Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *The lancet neurology journal, 9*(8),807-819.

19. Jongen, .J, Hans, G., Benzon, H., Huygen, F., & Hartrick, C.(2014). Neuropathic pain and pharmacological treatment. Pain Practice Journal, *14*(3),283-95.

20. Mackey, S., & Feinberg, S. (2007). Pharmacologic Therapies for Complex Regional Pain Syndrome. *Current Pain and Headache Reports, 11*(1), 38–43.

21. Haanpää, M., Gourlay, G., Kent, J., Miaskowski, C., Raja, S., Schmader, K., & Wells, C.(2010). Treatment considerations for patients with neuropathic pain and other medical comorbidities. *The Mayo Foundation for Medical Education and Research,85*(3),15-25.

22. Tassone, D., Boyce, E., Guyer, J., & Nuzum, D. (2007).Pregabalin: A Novel gamma - Aminobutyric Acid Analogue in the Treatment of Neuropathic Pain, Partial-Onset Seizures, and Anxiety Disorders. *Clinical therapeutics journal,29*(1), 26-48.

23. Gajraj, N. (2007). Pregabalin: Its Pharmacology and Use in Pain Management. *Anesthesia & Analgesia Journal,105*(6), 1805-1815.

24. Martinotti, G., Nicola, M. D., Tedeschi, D., Andreoli, S., Reina, D., Pomponi, M., & Janiri, L. (2009). Pregabalin versus naltrexone in alcohol dependence: A randomised, double-blind, comparison trial. *Journal of Psychopharmacology*, 24(9), 1367-1374.

25. Modi, P. B., & Shah, N. J. (2016). In Vitro release Techniques for Topical Formulations. *Indo American Journal of Pharmaceutical Research*, 6(5), 5634-5640

26. Ng, S., Rouse, J., Sanderson, D., & Eccleston, G.(2010). A Comparative Study of Transmembrane Diffusion and Permeation of Ibuprofen across Synthetic Membranes Using Franz Diffusion Cells. *Journal of Pharmaceutics, 2*(2), 209-223.

27. Mahato, R., & Narang, A. (2012). Pharmaceutical Dosage Forms and Drug Delivery . Second Edition. *CRC Press* 33-52.

28. Benson, H. A., & Watkinson, A. C. (2012). Transdermal and topical drug delivery: Principles and practice. *Hoboken, NJ: Wiley.*

29. Callender, S. P., Mathews, J. A., Kobernyk, K., & Wettig, S. D. (2017). Microemulsion utility in pharmaceuticals: Implications for multi-drug delivery. *International Journal of Pharmaceutics,526*(1-2), 425-442.

30. Malakar, J., Sen, S. O., Nayak, A. K., & Sen, K. K. (2011). Development and Evaluation of Microemulsions for Transdermal Delivery of Insulin. *ISRN Pharmaceutics, 2011*, 1-7.

31. Dong, X., Ke, X., & Liao, Z. (2011). The microstructure characterization of meloxicam microemulsion and its influence on the solubilization capacity. *Drug Development and Industrial Pharmacy, 37*(8), 894-900.

32. Cui, Y., Li, L., Gu, J., & Li, Z. (2011). Investigation of microemulsion system for transdermal delivery of ligustrazine phosphate. *African Journal of Pharmacy and Pharmacology, 5*(14), 1674-1681.

33. Singh, M. K., Chandel, V., Gupta, V., & Ramteke, S. (2010). Formulation Development and Characterization Of Microemulsion For Topical Delivery Of Glipizide. *Der Pharmacia Lettre,2*(3), 33-42.

34. Dhamankar, A. K., Manwar, ,. V., & Kumbhar, D. D. (2009). The Novel Formulation Design of O/W Microemulsion of Ketoprofen For Improving Transdermal Absorption. *International Journal of PharmTech Research, 1*(4), 1449-1457.

35. Sharma, P., Mittal, A., & Bajpai, M. (2009). A study of oleic acid oily base for the tropical delivery of dexamethasone microemulsion formulations. *Asian Journal of Pharmaceutics, 3*(3), 208.

36. Fukasawa, H., Muratake, H., Nagae, M., Sugiyama, K., & Shudo, K. (2014). Transdermal Administration of Aqueous Pregabalin Solution as a Potential Treatment Option for Patients with Neuropathic Pain to Avoid Central Nervous System-Mediated Side Effects. *Biological and Pharmaceutical Bulletin, 37*(11), 1816-1819.

37. Mbah, C. J., & Nnadi, C. O. (2014). Transdermal Delivery of Gabapentin: Effect of Cosolvent and Microemulsion on Permeation through the Rat Skin. *Pharmacology & Pharmacy, 05*(05), 471-478.

38. Okeke, N. C. (2012). *Transdermal Delivery of Gabapentin and Glipizide: Effects of Cosolvent Systems and Microemulsions* (master's thesis). University of Nigeria.

39. Bhatia, C., Sachdeva, M., & Bajpai,M. (2012). Formulation and evaluation of transdermal patch of pregabalin. *International Journal of Pharma Sciences and Research,3*(2), 569-575.

40. Plaza-Villegas, F., Heir, G., Markman, S., Khan, J., Noma, N., Benoliel, R., Patel, J., & Eliav, E. (2012). Topical pregabalin and diclofenac for the treatment of neuropathic orofacial pain in rats. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, 114*(4), 449-456.

41. Abu Hanieh, M .(2009). *Prepration of topical NSAID Celecoxib Spray and Gel and Investigation the Effects of Different Penetration Enhancers on Drug Permeation rate*(master's thesis). Alquds University, Palestine.

42. Kayali, I., Al Hindi, F., Malkieh, N., & Habis, M. (2007). Studying in Vitro Release of Dexchlorpheniramine Maleat from Aqueous Phases and Water/Oil Emulsions (creams) with various Penetration Enhancers. *Pharmaceutical chemistry Journal, 41*(6), 327-331

43. Dash, S., Murthy P.N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica, 67*(3), 217-23.

44. Siepmann J., Peppas N.A. (2001). Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Advanced Drug Delivery Reviews, 48*, 139-157.

List of Appendix

Appendix 1 : Certificate of Analysis of Pregabalin

Alembic — Touching Lives over 100 years

QUALITY CONTROL DEPARTMENT

CERTIFICATE OF ANALYSIS

Product	**Pregabalin**		
Batch No.	**1402000112**	A.R. No.	80000021629
Mfg. Date	JAN-2014	Retest Date	DEC-2016
Batch size	187.16 Kg	Date of Analysis	31/03/2014

SR. NO.	TESTS	RESULTS	SPECIFICATIONS
01	Description	White crystalline powder	White to off white crystalline powder.
02	Solubility	Complies	Sparingly soluble in water.
03	Identification by A) IR [KBr]	Comparable	Absorption spectrum of the test sample should be concordant with that of (S)-Pregabalin standard.
	B) HPLC	Complies	The retention time of main peak of test sample should match with the retention time of peak of (S)-Pregabalin standard in the test of related substances.
	C) XRD	Complies	The XRD pattern of test sample should match with the XRD pattern of (S)-Pregabalin standard and should match with 2θ values at 9.5, 12.2, 16.6, 18.2, 18.3, 19.0, 22.1, 23.1, and 23.4 (±0.2 θ)
04	Water by KF	0.03 % w/w	Not more than 0.50 % w/w
05	Residue on ignition	0.04 % w/w	Not more than 0.10 % w/w
06	Specific optical rotation(°) (on anhydrous basis) (c=1.06 in water, at 23°C)	+11.5°	+10.0 to +12.0
07	Bromide content	Less than 50 ppm	Not more than 50 ppm
08	Heavy Metals	Less than 10 ppm	Not more than 10 ppm

COA Date: 22/04/2014		Prepared By	Checked By	Approved By
	Sign.			
	Date	22/04/14	22/04/14	22/04/14
	Name	Bharat Bhoi	Kirti Ladani	Tumma Ilaiah
	Designation	Asst. Manager-QC	Dy. Manager-QC	Manager-QC

(Page 1 of 2)

ALEMBIC PHARMACEUTICALS LIMITED

API UNIT – III, KARAKHADI

REGD. OFFICE: ALEMBIC ROAD, VADODARA – 390 003, Gujarat State, INDIA • TEL: (0265) 2280550 • FAX: (0265) 2284729
Website: www.alembic-india.com • E-mail : alembic@alembic.co.in

FACTORY : SURVEY NO. 842, 843 VILL. KARAKHADI, TAL. PADRA, DIST. VADODARA-391 450 • TEL : 02662-300700, 300701 FAX : 02662-300732
E-mail : karakhadi.api@alembic.co.in

CIN-L24230GJ2010PLC061123

Alembic
—Touching Lives over 100 years

QUALITY CONTROL DEPARTMENT

CERTIFICATE OF ANALYSIS

Product	Pregabalin		
Batch No.	1402000112	A.R. No.	80000021629
Mfg. Date	JAN-2014	Retest Date	DEC-2016
Batch size	187.16 Kg	Date of Analysis	31/03/2014

SR. NO.	TESTS	RESULTS	SPECIFICATIONS
09	Assay (By chemical) (on anhydrous basis)	100.3 % w/w	98.0%w/w to 102.0%w/w
10	R-Isomer (By HPLC)	0.01 %	Not more than 0.15 %
11	Related substances by HPLC		
	Impurity-III	Not detected	Not more than 0.15 %w/w
	Impurity-IV	Not detected	Not more than 0.15 %w/w
	Any other impurity	0.06 % w/w	Not more than 0.10 %w/w
	Total impurities	0.11 % w/w	Not more than 0.50 %w/w
12	Residual solvents by GC		
	Cyclohexane	Not detected	Not more than 1000 ppm
	Toluene	6 ppm	Not more than 500 ppm
	Ethyl acetate@	Not detected	Not more than 5000 ppm
	Methanol	Not detected	Not more than 1800 ppm
	Chloroform	Not detected	Not more than 60 ppm
	Isopropyl alcohol@	Not detected	Not more than 5000 ppm
	Ethanol@	Not detected	Not more than 5000 ppm
	Total of class 3 solvents	Nil	Not more than 5000 ppm
Additional test :-			
13	Particle size (By Malvern) D(0.9)	364 µm	For information.

Batch complies with respect to above specification No. API/30000503/H-U-03. @: Class 3 solvents.

COA Date: 22/04/2014		Prepared By	Checked By	Approved By
	Sign.			
	Date	22/04/14	22/04/14	22/04/14
	Name	Bharat Bhoi	Kirti Ladani	Tumma Ilaiah
	Designation	Asst. Manager-QC	Dy. Manager-QC	Manager-QC

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Website: www.alembic-india.com • E-mail : alembic@alembic.co.in

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E-mail : karakhadi.api@alembic.co.in

CIN-L24230GJ2010PLC061123

Appendix 2 : HPLC analysis of pregabalin standards

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\003-0301.D
Sample Name: STD1- Pregabalin (0.002 mg/ml)

Acq. Operator	: Ramzi Muqedi	Seq. Line	: 3
Acq. Instrument	: Instrument 1	Location	: Vial 3
Injection Date	: 4/11/2018	Inj	: 1
Injection Time:	2:17:50 PM	Inj. Volume (Method):	50 µl
		Act. Inj. Vol. from Sequence:	50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

Analysis Method : C:\CHEM32\1\METHODS\PREGABALIN\PREGABALIN.M
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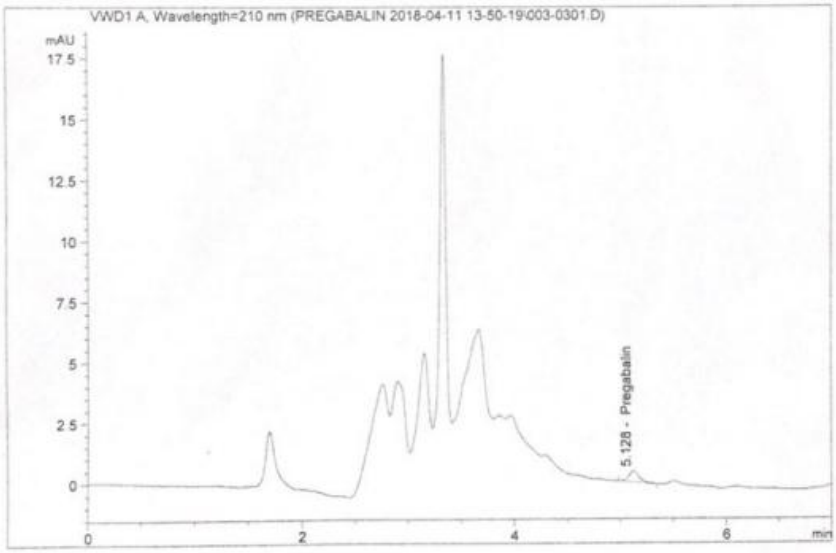
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

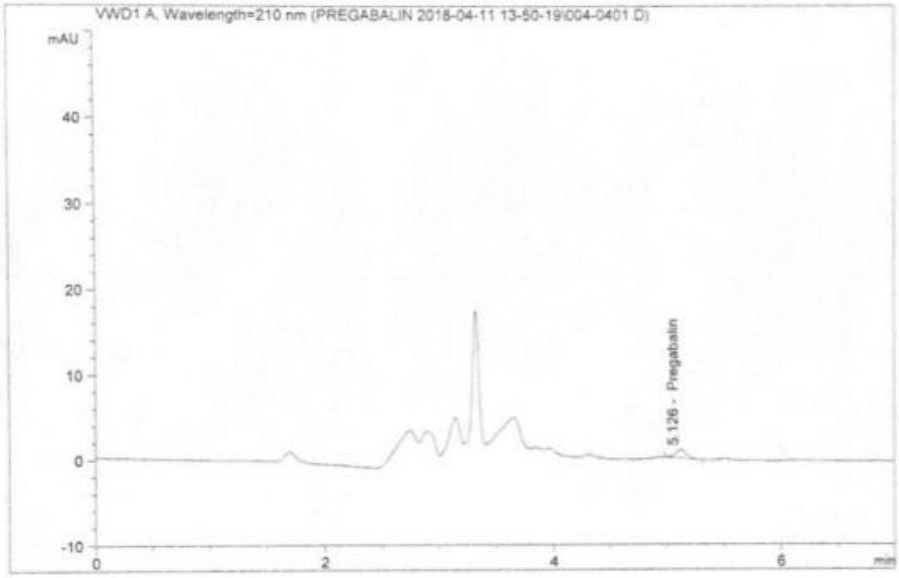
RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.128	Pregabalin	3	0.097	0.862	15415	

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\004-0401.D
Sample Name: STD2- Pregabalin (0.005 mg/ml)

Acq. Operator : Ramzi Muqedi Seq. Line : 4
Acq. Instrument : Instrument 1 Location : Vial 4
Injection Date : 4/11/2018 Inj : 1
Injection Time: 2:35:29 PM Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Sequence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

Analysis Method : C:\CHEM32\1\METHODS\PREGABALIN\PREGABALIN.M
Last changed : 4/12/2018 4:07:19 PM (modified after loading)
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.126	Pregabalin	6	0.098	0.825	15037	

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\005-0501.D
Sample Name: STD3, Pregabalin (0.01 mg/ml)

Acq. Operator : Ramzi Muqedi
Acq. Instrument : Instrument 1
Injection Date : 4/11/2018
Injection Time: 2:53:11 PM
Seq. Line : 5
Location : Vial 5
Inj : 1
Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Sequence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

Analysis Method : C:\CHEM32\1\METHODS\PREGABALIN\PREGABALIN.M
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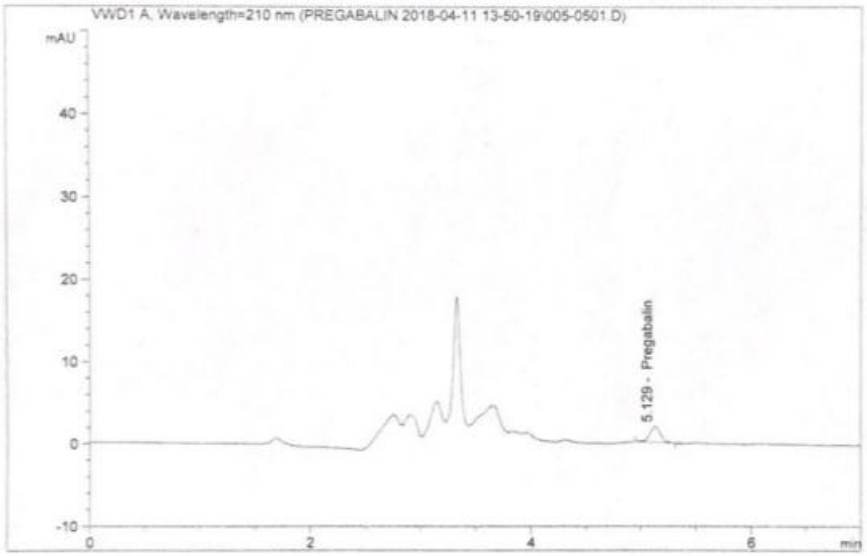
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.129	Pregabalin	13	0.101	0.798	14348	

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\006-0601.D
Sample Name: STD4- Pregabalin (0.02 mg/ml)

Acq. Operator : Ramzi Muqedi
Seq. Line : 6
Acq. Instrument : Instrument 1
Location : Vial 6
Injection Date : 4/11/2018
Inj : 1
Injection Time: 3:10:54 PM
Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Sequence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

Analysis Method : C:\CHEM32\1\METHODS\PREGABALIN\PREGABALIN.M
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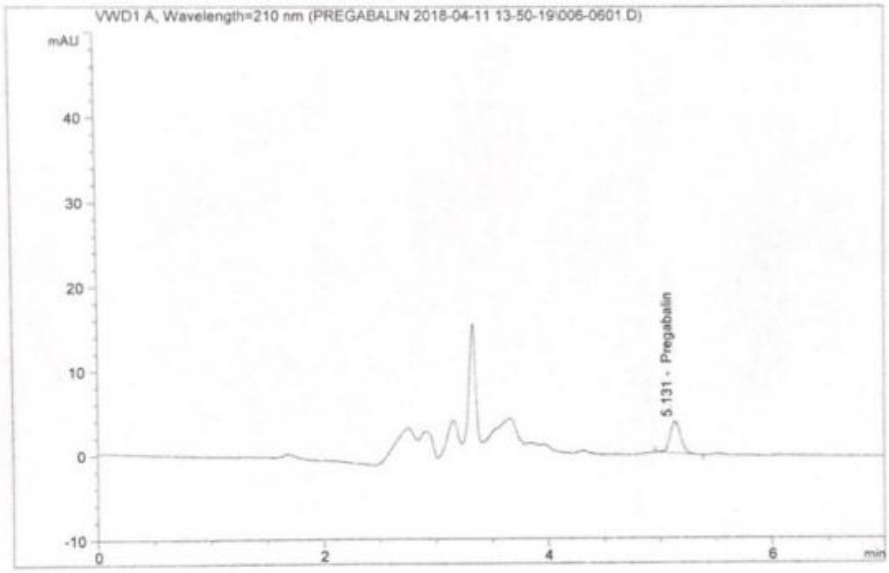
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.131	Pregabalin	24	0.099	1.017	14762	

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\007-0701.D
Sample Name: STD5- Pregabalin (0.04 mg/ml)

Acq. Operator : Ramzi Muqedi
Acq. Instrument : Instrument 1
Injection Date : 4/11/2018
Injection Time: 3:28:46 PM
Seq. Line : 7
Location : Vial 7
Inj : 1
Inj. Volume (Method): 50 µl
Act. Inj, Vol. from Sequence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

Analysis Method : C:\CHEM32\1\METHODS\PREGABALIN\PREGABALIN.M
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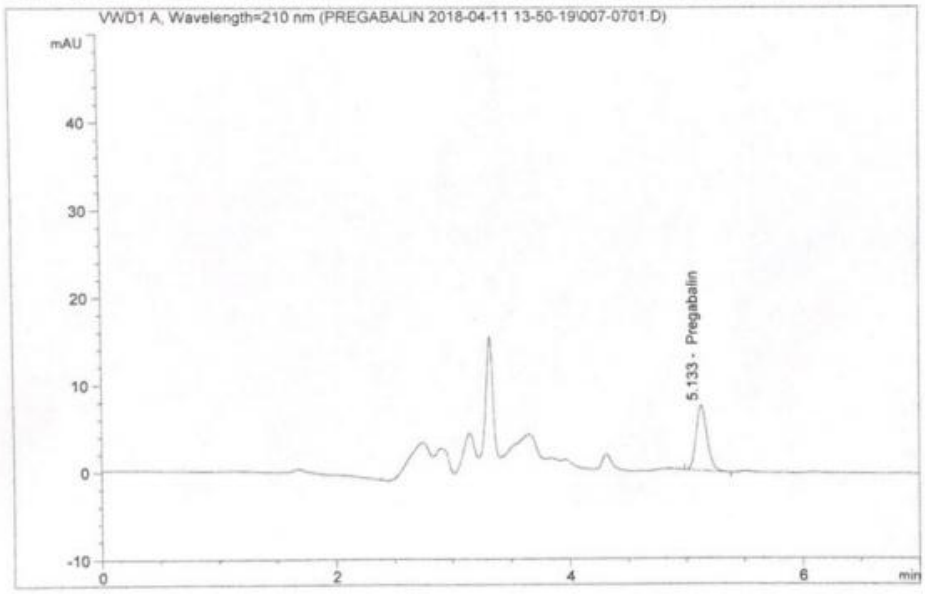
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.133	Pregabalin	48	0.099	1.141	14999	

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\008-0801.D
Sample Name: STD6- Pregabalin (0.08 mg/ml)

Acq. Operator : Ramzi Muqedi Seq. Line : 8
Acq. Instrument : Instrument 1 Location : Vial 8
Injection Date : 4/11/2018 Inj : 1
Injection Time: 3:46:30 PM Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Sequence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

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Method Info:
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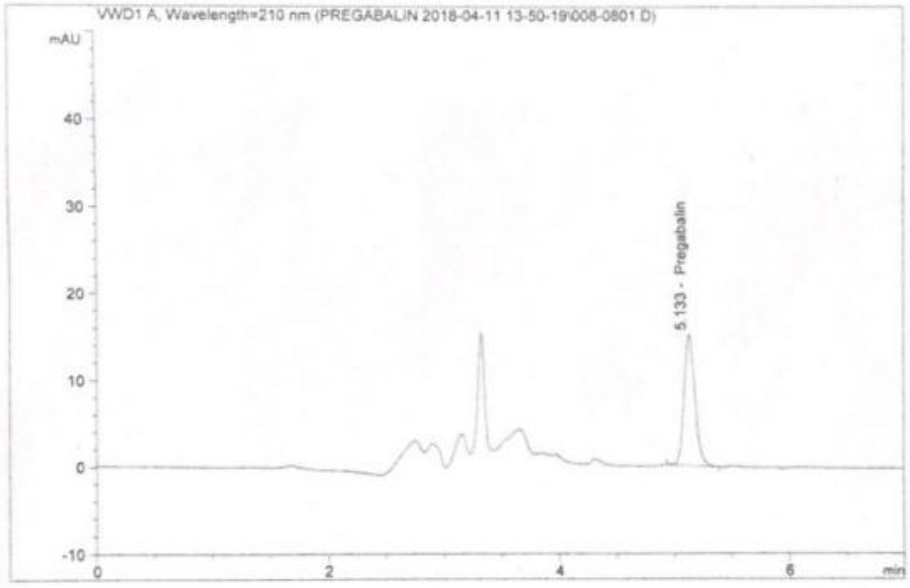

Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.133	Pregabalin	100	0.101	1.154	14367	

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\009-0901.D
Sample Name: STD7- Pregabalin (0.10 mg/ml)

Acq. Operator : Ramzi Muqedi Seq. Line : 9
Acq. Instrument : Instrument 1 Location : Vial 9
Injection Date : 4/11/2018 Inj : 1
Injection Time: 4:04:17 PM Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Sequence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

Analysis Method : C:\CHEM32\1\METHODS\PREGABALIN\PREGABALIN.M
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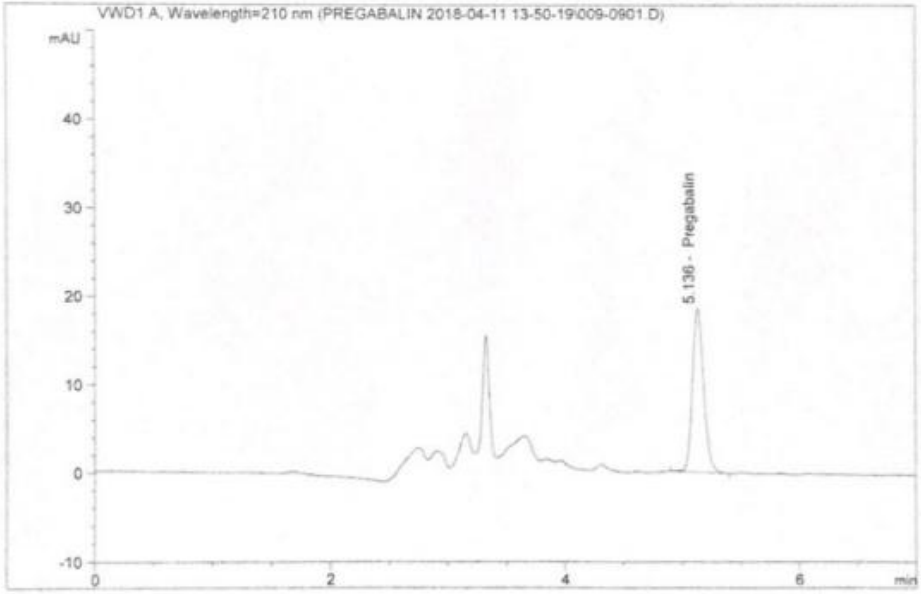
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.136	Pregabalin	122	0.100	1.142	14564	

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\099-1001.D
Sample Name: STD8▾ Pregabalin (0.20 mg/ml)

Acq. Operator : Ramzi Muqedi Seq. Line : 10
Acq. Instrument : Instrument 1 Location : Vial 99
Injection Date : 4/11/2018 Inj : 1
Injection Time: 4:22:17 PM Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Seguence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

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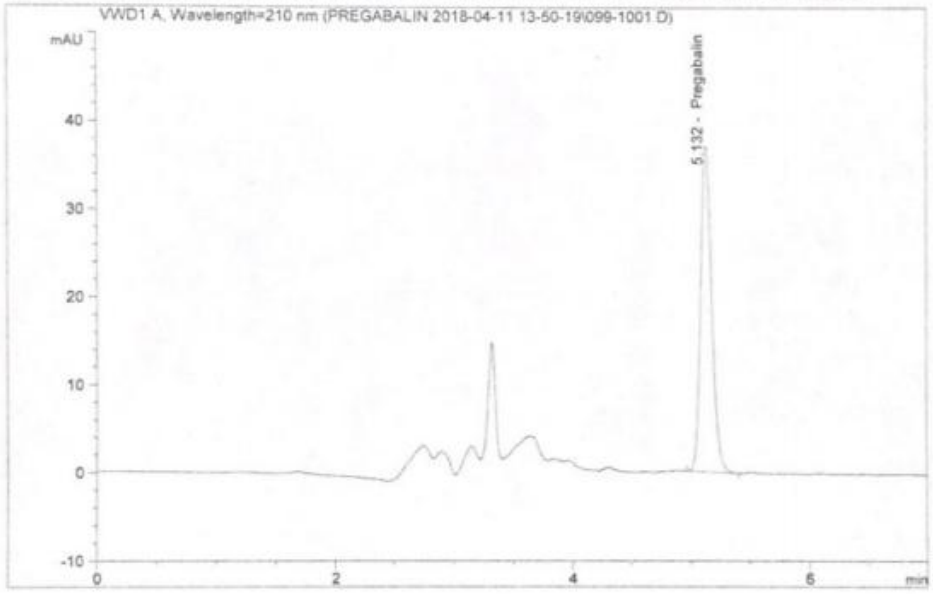
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.132	Pregabalin	241	0.099	1.158	14740	

Appendix 3 : HPLC analysis of samples before applying them on the donor of the FDC.

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\011-1201.D
Sample Name: SA- ASSAY B2

Acq. Operator : Ramzi Muqedi
Acq. Instrument : Instrument 1
Injection Date : 4/11/2018
Injection Time: 5:06:56 PM
Seq. Line : 12
Location : Vial 11
Inj : 1
Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Sequence: 50 µl

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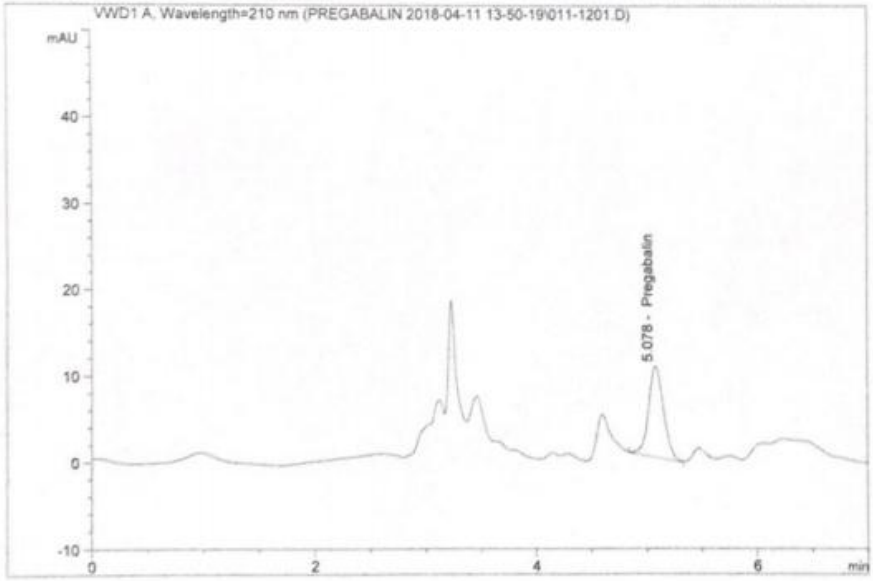
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.078	Pregabalin	99	0.141	1.020	7194	

Instrument 1 4/16/2018 10:24:43 AM Page 1 of 1

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\013-1401.D
Sample Name: SA- ASSAY H1

Acq. Operator : Ramzi Muqedi
Acq. Instrument : Instrument 1
Injection Date : 4/11/2018
Injection Time: 5:59:55 PM
Seq. Line : 14
Location : Vial 13
Inj : 1
Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Sequence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

Analysis Method : C:\CHEM32\1\METHODS\PREGABALIN\PREGABALIN.M
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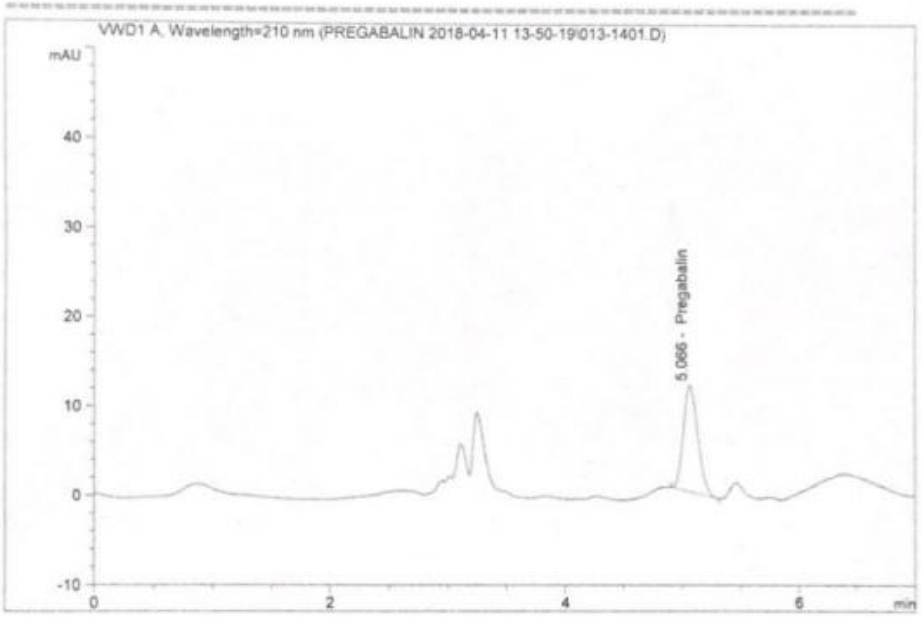
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

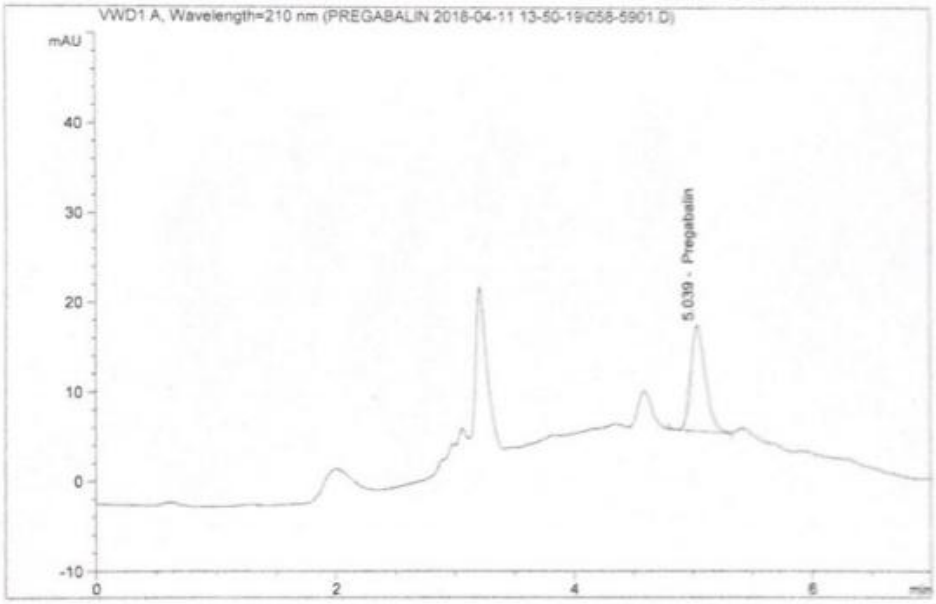
RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.066	Pregabalin	101	0.131	1.324	8267	

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\058-5901.D
Sample Name: SA- ASSAY (N1)

Acq. Operator : Ramzi Muqedi
Acq. Instrument : Instrument 1
Injection Date : 4/12/2018
Injection Time: 7:45:12 AM
Seq. Line : 59
Location : Vial 58
Inj : 1
Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Sequence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

Analysis Method : C:\CHEM32\1\METHODS\PREGABALIN\PREGABALIN.M
Last changed : 4/12/2018 4:07:19 PM (modified after loading)
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

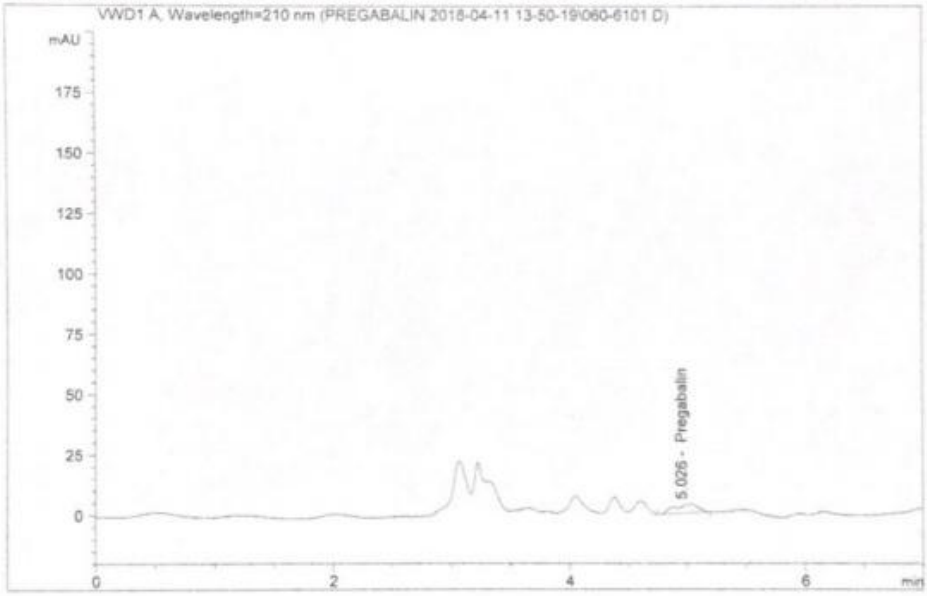
RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.039	Pregabalin	102	0.133	1.243	7974	

Data File: C:\CHEM32\1\DATA\PREGABALIN 2018-04-11 13-50-19\060-6101.D
Sample Name: SA- ASSAY (E1)

Acq. Operator : Ramzi Muqedi Seq. Line : 61
Acq. Instrument : Instrument 1 Location : Vial 60
Injection Date : 4/12/2018 Inj : 1
Injection Time: 8:38:45 AM Inj. Volume (Method): 50 µl
Act. Inj. Vol. from Sequence: 50 µl

Acq. Method ->C:\Chem32\1\DATA\PREGABALIN 2018-04-11 13-50-19\PREGABALIN.M

Analysis Method : C:\CHEM32\1\METHODS\PREGABALIN\PREGABALIN.M
Last changed : 4/12/2018 4:07:19 PM (modified after loading)
Method Info:
Pregabalin



Customized Report: Performance Report per Signal
This report template has been designed for uncalibrated methods.

Available Signals:
VWD1 A, Wavelength=210 nm

Signal: VWD1 A, Wavelength=210 nm

RetTime [min]	Name	Area [mAU*s]	Halfh. Width [min]	USP Tail.	Plates	Resolution
5.026	Pregabalin	50	0.353	0.820	1120	

تحضير بريغابالين موضعي لعلاج آلام الأعصاب

إعداد الطالبة : حليمة أحمد ابراهيم ذويب

اشراف : د. أبراهيم كيالي

الملخص

البريغابالين هو دواء مضاد للاختلاج يستخدم لعلاج الصرع وآلام الأعصاب المرتبطة بالاعتلال العصبي السكري و الأَلَمُ العَصَبِيُّ التِالي للهَرْبِس تم تسويقه عبر شركة فايزر تحت العلامة التجارية ليريكا (Lyrica®) . هذا الدواء متوفر على شكل كبسولات، محلول فموي و أُقراص ممتدة الاطلاق و هذا الدواء مرتبط بعدد من الآثار الجانبية غير المرغوب فيها وخاصة على الكلى.

هدف هذه الرسالة هو تطوير بريغابالين على شكل مستحلب ذو جزيئات بالغة الصغر (microemulsion) موضعي يستخدم لعلاج الأعصاب للتغلب على المشاكل المرتبطة بإعطاء الدواء عن طريق الفم والبحث بتأثير مختلف محسنات النفاذية على معدل نفاذ الدواء.

لقد تم تقسيم هذه الدراسة التجريبية إلى ثلاثة مراحل :

المرحلة الأولى تم فيها بناء رسم بياني للطور شبه الثلاثي باستخدام طريقة المعايرة المائية. المستحلب ذو جزيئات بالغة الصغر (microemulsion) تم تحضيره باستخدام زيوت مختلفة للطور الزيتي مثل حمض الأولييك المعروف باسم (oleic acid)، و أيزوبوبيل ميرستات المعروف باسم (IPM)، و زيت الليمونين (الأيسومر R) المعروف باسم (R(+)–limonene) التي تستخدم ايضا كمحسنات نفاذية. تم استخدام البريغابالين بتركيز 0.1 مولار للطور المائي مع بروبلين جلايكول بنسب مختلفة . و تم استخدام Tween 80 كمنشط للسطح (cosurfactant) والايثانول كمساعد للسطوح المنشطة (cosurfactant)بنسب مختلفة.

المرحلة الثانية هي اختيار صيغ المستحلب ذو جزيئات بالغة الصغر (microemulsion): ثلاثة انظمة (B و H و N) تم اختيارهم لمعرفة أفضل محسن نفاذية بين الزيوت الثلاثة (حمض الأولييك ، و أيزوبوبيل ميرستات ، و زيت الليمونين (الأيسومر R)) اعتماداً أعلى قيمة لمعامل النفاذية (P) وثلاثة انظمة أخرى (E و K و Q) تم اختيارها لمعرفة افضل نظام بوجود البروبلين جلايكول اعتماداً أعلى قيمة لمعامل النفاذية (P).

المرحلة الثالثة هي تحديد قيم النفاذية للأنظمة المختارة. القيم التي تم تحديدها هي : الكمية المتراكمة التي للدواء التي نفذت في وحدة الزمن ، الفترة الزمنية التي استغرقتها المادة الفعالة قبل النفاذ ، معامل الانتشار ، معامل النفاذية ، ومعامل التجزئة .

من بين الأنظمة الثلاثة (B وH وN) كان حمض الأوليك أفضل محسن نفاذية من بين الزيوت الثلاثة حيث أنه يحتوي على أعلى معامل نفاذية .من ناحية أخرى من بين الأنظمة الثلاثة الأخرى (E وK وQ) فأن نظام K لديه أعلى قيمة لمعامل النفاذية (P) في وجود بروبيلين جلايكول مما يشير إلى أن البروبلين جلايكول يعمل بشكل تآزري مع أيزوبوبيل ميرستات في النظام K من اجل تعزيز نفاذية الدواء.

تشير النتائج انه يمكن استخدام تركيبة المستحلب ذو جزيئات بالغة الصغر (microemulsion) كبديل عملي للتركيبات التقليدية لدواء البريغابالين مع خصائص متميزة لتحسين توصيل الدواء بشكل موضعي. وقد لوحظ أن فترات التأخر الزمني قصيرة و مستقلة تقريبا عن مكونات المستحلب ذو جزيئات بالغة الصغر (microemulsion) و / أو تغيرات تدفق الدواء ، مما يشير إلى أن ظروف الحالة شبه المستقرة قد تحققت بسرعة في جميع الحالات.