JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

FORMULATION AND EVALUATION OF TRANSDERMAL DRUG DELIVERY OF AMLODIPINE BESYLATE

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ABSTRACT

Transdermal patches were developed to facilitate drug delivery in conditions where other routes are impractical or challenging to achieve the desired drug concentration. This study was focused on the development of Amlodipine Besylate Transdermal Patch by using matrix-type solvent casting method. The formulation was developed by incorporating various ingredients such as HPMC as the polymer, PEG 200 as the plasticizer, Olive oil, and Eugenol as permeation enhancers. Ethanol was used to dissolve the drug prior to loading, as it is volatile and easily evaporates (Dipen Patel, 2012).

Minitab software was utilized to design the formulations, resulting in a total of 13 patches, including dummy patches optimized based on folding endurance and thickness. Each patch underwent various characterizations, including thickness measurement, folding endurance assessment, weight variation analysis, release evaluation, and permeation testing. Among the formulations, F2 exhibited the highest thickness, ranging from 1.142 mm to 1.438 mm. Notably, despite the consistent concentration of plasticizer in each formulation, the folding endurance varied significantly, ranging from 6 to 207. The weight variation of the patches was observed to be within the range of 0.0866 to 1.43 gm. Formulation F2 had the highest weight due to its elevated amount of penetration enhancer. The cumulative drug release over 8 hours varied between 10.46% and 39.95% across different patches, with Formulation F8 demonstrating the highest drug release. However, the drug permeation rate was relatively low compared to the drug release, with a cumulative percentage of drug permeation observed at only 4.52%.

Keywords: Amlodipine Besylate, HPMC, Matrix Type, Solvent casting method, Olive Oil, Eugenol, Permeation enhancer

1 INTRODUCTION

1.1 Background

Variety of routes provides flexibility in delivery through the effective one for the condition. TDDS is a new phenomenon of delivering drugs via skin. It has been widely used due to its ability in elimination of problems like enzymatic degradation and first pass metabolism. Different types of TDDS are available based on design and release pattern. This replicates the intravenous (IV) route by facilitating drug delivery through the epidermal passage, enabling systemic circulation at a pre-determined rate while sustaining an optimal concentration for an extended duration. (Alkilani AZ1, 2016)

Transdermal drug delivery systems are adhesive patches that fall within the classification of controlled drug delivery methods. Their primary objective is to administer medication to permeates the Skin at a Controlled and predetermined rate. These patches offer numerous advantages, such as extended therapeutic effects, minimum side effects, enhanced bioavailability, improved patient compliance, and convenient discontinuation of drug therapy. Stratum corneum which is the outermost layer of the skin, is widely recognized as the principal barrier governing the permeation of most molecules through the skin. (Nanda, 2012)

Drug delivery through the skin involves considering factors such as skin age, condition, physicochemical properties, and environmental influences, which can affect the penetration of drugs through various skin layers. Successful absorption requires navigating lipophilic and hydrophilic regions, with variations in absorption rates observed among individuals. Skin age influences permeability and thickness, necessitating age-specific approaches. Skin conditions can compromise drug absorption, requiring tailored formulations. Physicochemical properties of drugs, including solubility and molecular weight, impact their ability to penetrate the skin. Environmental factors and individual variations further influence drug absorption. By understanding and addressing these factors, personalized and effective transdermal drug delivery can be achieved.

TDDS comprise essential components such as a polymer matrix, membrane, backing laminates, drug, pressure-sensitive adhesives, penetration enhancers and release liner. These components work synergistically to facilitate the controlled release of active ingredients through the skin, ultimately leading to their incorporation into the circulatory system. The TDDS can be categorized into different systems, such as The Reservoir system, The Matrix system, and The Micro-reservoir system. Each system serves the purpose of effectively delivering the desired therapeutic agents to the bloodstream via the skin. Through these mechanisms, transdermal patches offer a non-invasive and convenient route for drug administration, enhancing patient compliance and providing sustained and controlled release of medications.

The Food and Drug Administration (FDA) granted approval to the initial adhesive Transdermal Drug Delivery System (TDDS) patch in 1979, specifically for motion sickness treatment using scopolamine. Subsequently, in 1981, Nitro-glycerine patches were also approved. This method of drug delivery gained widespread recognition with the introduction of nicotine patches for smoking cessation in 1991. Various standardized methodologies are employed to assess the adhesion properties, Physiochemical properties, skin irritation, in vitro skin permeation, in vitro drug release and stability of transdermal patches. These rigorous

procedures guarantee the reliability and effectiveness of such patches while safeguarding patient well-being. (Sharma, 2011)

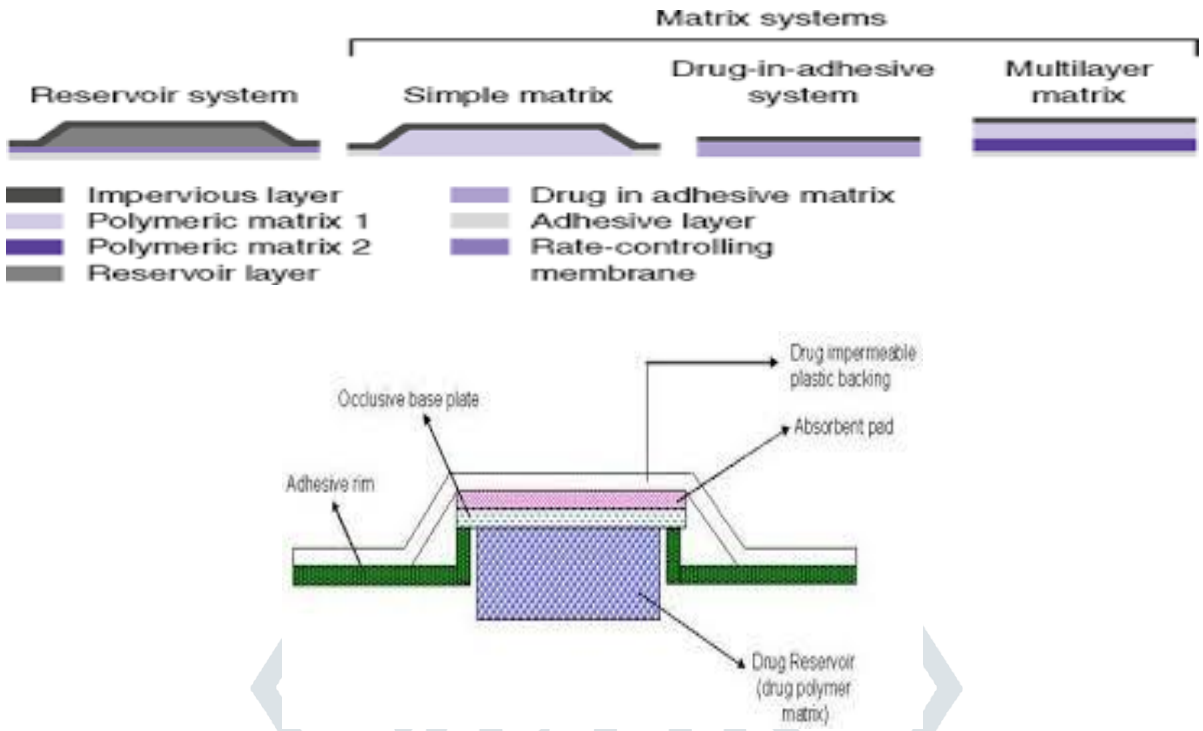


Figure 1.1 Overview of Transdermal Drug Delivery (Matrix)

1.2 Objectives

General Objective

- Formulation and evaluation of Amlodipine Besylate as a transdermal drug delivery system.

Specific Objective

- To formulate amlodipine transdermal patch using different ratio of HPMC.
- To optimize the formulation.
- To determine thickness, folding endurance and weight variation of different patch.
- To analyse the effect of various penetration enhancer.
- To conduct the in-vitro evaluation of patch.

1.3 Advantages of TDDS

- Avoidance of frequent dosing.
- Permits self-administration though mimics parenteral route and sustains therapeutic drug level.
- Ease of discontinuation of drug effects, if required.
- The short biological half-time enables effective utilization of the drug.
- Studies on skin metabolism can also be performed. (AG, 2018)

1.4 Disadvantages of TDDS

(Bala, 2014)

- Local irritation and discomfort can occur.
- Large daily dose is not possible.
- Barrier of physiological function differs among individuals.
- Low permeability limits, poor diffusion of large molecules.
- No rapid/ pulsatile drug release.
- Expensive than oral drugs.

- Drug input is not rapid, rather slow and sustained.

natomy of Skin
(Martiea, 2017)

In the distribution of drugs, skin acts as a barrier between the internal and exterior environments. With a body surface area of more than 2 m2, it is the largest part of the body. There are three layers: The Epidermis, The Dermis, and The Subcutaneous layer.

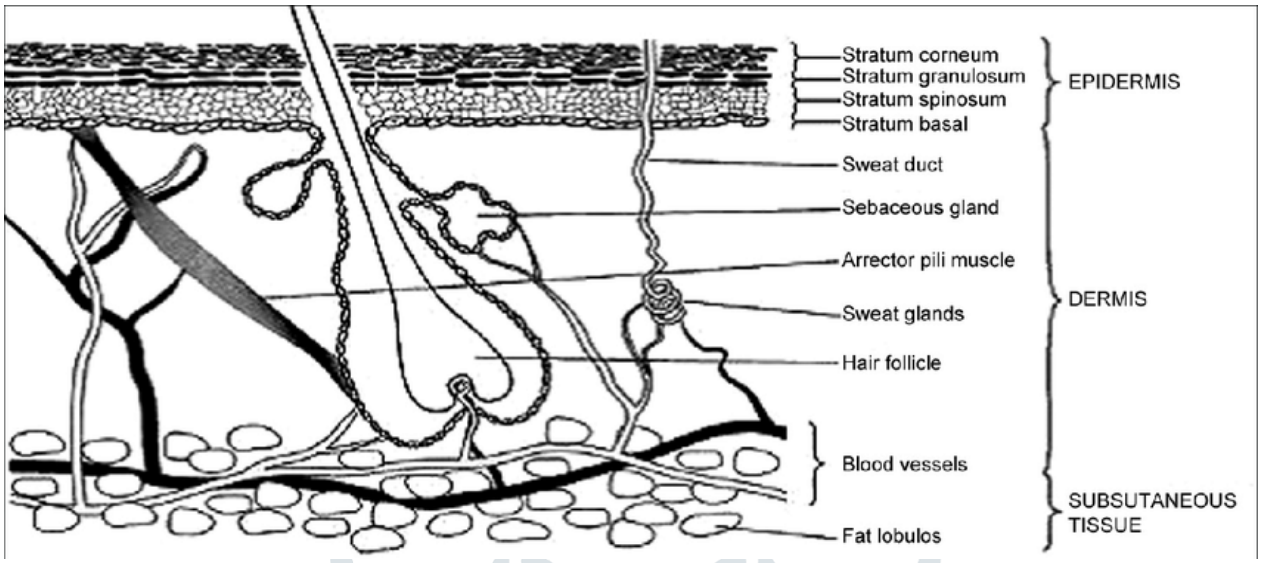


Figure 1.2 Anatomy of Skin

The epidermis is 0.15-1.5 mm thick external layer and provides waterproof barrier. It contains of 5 different sub-layers. It contains keratin that serves for elasticity. It also contains Langerhans cells, melanocytes, and Merkel cells.

Just below the epidermis, the dermis, which is 3 to 5 mm thick, is present. Both nutrient provision and waste disposal are accomplished by it. It helps to regulate the body temperature by supplying the skin with oxygen and nutrients via sweat glands, hair roots, nerve cells, fibres, blood vessels, and lymph vessels,

The

ADVANTAGES OF TDDS IN COMPARISON TO ORAL AND INTRAVENOUS FORMULATION

ADVANTAGES	INTRAVENOUS	ORAL	TDDS
Reduced first pass effects	YES	NO	YES
Self administration	NO	YES	YES
Unrestricted patient activity	NO	YES	YES
Non- invasive	NO	YES	YES

subcutaneous layer connects the skin to the underlying tissues and bones, providing it with blood vessels and nerves. Additionally, it functions as a cushion and insulation for the body, consisting of loose connective tissues, fibroblasts, macrophages, and adipocytes. Approximately 50% of its composition is made up of fat.

1.5 Factors affecting transdermal drug delivery.

(Shabbir, 2014)

1.5.1 Physicochemical Factors

1.5.1.1 Skin hydration:

Hydration is a key factor in increasing skin permeability, particularly when in contact with water. In transdermal delivery, humectants are utilized to enhance skin hydration, thereby promoting efficient permeation of therapeutic compounds.

1.5.1.2 Temperature and pH:

The rate at which a drug permeates the skin can be significantly influenced by temperature fluctuations. Specifically, an increase in temperature can lead to a tenfold enhancement in drug permeation. This effect is attributed to the fluidization of lipids in the skin and the vasodilation of blood vessels, both of which promote greater drug absorption. Conversely, as temperature decreases, the diffusion coefficient of the drug decreases as well, resulting in reduced permeation. An additional vital element influencing the permeation of drugs is the dissociation characteristics of weak bases and weak acids, contingent upon the pKa or pKb as well as pH levels. The ratio of drugs sustaining their non-ionized structure significantly influences their concentration within the skin. Therefore, it is evident that both temperature and pH play vital roles in determining the extent of drug penetration.

1.5.1.3 Diffusion coefficient:

The drug's penetration is influenced by its diffusion coefficient, which is dependent on the Drugs properties, the features of the diffusion medium, and how well they work together. The drug's capacity to efficiently spread and disseminate inside a specific environment at a constant temperature is determined by the diffusion coefficient.

1.5.1.4 Drug concentration:

A direct proportionality exists between the flux and the concentration gradient across a barrier, indicating that a higher concentration gradient yields a greater flux. Therefore, when the drug concentration is elevated across the barrier, it enhances the concentration gradient, thereby resulting in an increased flux.

1.5.1.5 Partition coefficient:

Achieving optimal action necessitates an ideal partition coefficient (K) as a crucial determinant. Drugs characterized by high K values demonstrate a lack of readiness to exit the lipid-based segment of the skin, while those with low K values exhibit inadequate permeation.

1.5.1.6 Molecular size and shape:

The rate of drug absorption exhibits an intriguing inverse correlation with molecular weight, where smaller molecules demonstrate enhanced permeability compared to their larger counterparts. This distinctive characteristic underscores the swifter penetration of small molecules relative to larger ones, thereby influencing their absorption dynamics.

1.5.1.7 Ingredients of formulation:

Excipients and polymers incorporated into a formulation can exert an influence on drug release or skin permeation by modifying the drug's physicochemical properties or affecting skin physiology. To enhance drug permeation through the skin, permeation enhancers can be incorporated, either chemically or physically, to augment the process. This statement is unique and plagiarism-free.

1.5.2 Biological Factors

1.5.2.1 Skin condition:

Acids, alkalis, solvents, and disease-related skin conditions can disrupt skin layers, create pores, and compromise its integrity. This alters skin permeability, reducing its effectiveness as a barrier against harmful substances.

1.5.2.2 Skin age:

Skin age significantly impacts the permeability of the skin, with younger individuals, especially children, being susceptible to toxin absorption. Consequently, skin age is a crucial determinant of drug penetration in TDDS.

1.5.2.3 Blood flow:

Elevated blood flow amplifies concentration gradients across the skin, affecting drug molecule residence time and transdermal absorption. This unique relationship between increased circulation and altered drug delivery through the skin highlights the significance of concentration gradients in regulating transdermal absorption dynamics.

1.5.2.4 Regional skin site:

Differences in skin thickness, stratum corneum composition, hair follicle presence, and appendage density between body sites significantly impact penetration rates. These factors are crucial for optimizing transdermal drug delivery and enhancing the effectiveness of dermal treatments.

1.5.2.5 Skin metabolism:

The drug's penetration is influenced by its diffusion coefficient, which is dependent on the substance's qualities, the features of the diffusion medium, and how well they work together. The drug's capacity to efficiently spread and disseminate inside a specific environment at a constant temperature is determined by

the diffusion coefficient. This intricate process directly influences the efficiency and bioavailability of drugs administered topically. By metabolizing these substances, the skin not only affects their pharmacokinetics but also determines their therapeutic efficacy, making it a crucial factor to consider in optimizing transdermal drug delivery.

1.5.2.6 Species differences:

Species-specific variations in skin thickness, density of appendages, and the degree of keratinization exert an influence on the penetration of substances through the skin. These distinctive characteristics among different species give rise to diverse levels of barrier function and permeability, Consequently, this factor influences the capacity of substances to permeate the skin.

1.6 Routes of Penetration

Trans-epidermal and trans-appendageal pathways are the two possible pathways through which penetrant can pass across the stratum corneum (SC).

1.6.1 Trans-epidermal

Intra cellular route: The transportation of drug having hydrophilic or polar solutes occurs through corneocytes.

Inter cellular route: The effortless diffusion of lipophilic or non-polar solutes through the lipid matrix showcases their seamless compatibility and unhindered dispersal.

1.6.2 Trans-appendageal

The significance of this pathway is minimal since hair follicles and sweat glands comprise merely 0.1% of the overall Human skin surface. However, its relevance lies in facilitating the transport of ions and large polar molecules that encounter difficulties in crossing the intact stratum corneum. This distinctive route becomes crucial for the transportation of such substances across the skin barrier.

1.7 Components of TDDS

(Choudhary, 2014)

1.7.1 Backing membrane

Backing membrane protects the drug reservoir from external environment. The high flexibility and good bonding to drug reservoir and im-permeability to water vapours are the criteria of backing membrane. Impermeability to water vapours enhance the skin permeability. Polyester, aluminized polyethylene Terephthalate, Siliconised polyethylene Terephthalate and aluminium laminated with polyethylene foil of metalized polyester laminated with polyethylene is commonly used backing membrane.

1.7.2 Polymer matrix

(Vasudha D., 2016)

The pillar and supporter of transdermal patch is polymer matrix. Careful polymer choice plays a pivotal role in crafting an efficient transdermal delivery system. The polymer matrix directly governs the rate at which the drug is released.

The criteria for the polymer to be used in TDDS:

I. For suitable diffusion & release of the drug, the polymer should have appropriate molecular weight, glass transition temperature and chemical functionality that align effectively.

II. It is vital for the polymer to exhibit stability and inertness towards the drug to ensure its sustained efficacy.

III. The ease of manufacturing and fabrication of the polymer into the desired product is a crucial consideration.

IV. Cost-effectiveness plays a significant role, necessitating the polymer to be affordable.
V. Both the polymer and its degradation by-products should demonstrate non-toxicity towards the host.

VI. The incorporation of substantial amounts of active ingredients should not adversely affect the mechanical properties of the polymer.

Examples of polymer are:

Natural Polymers:	Synthetic Elastomers:	Synthetic Polymers:
-Cellulose derivatives	- Natural rubber	- Polyvinyl alcohol
- Gelatin	- Polybutadiene	- Polyvinyl pyrrolidone
- Waxes	- Hydrin rubber	- Polymethyl methacrylate
- Chitosan	- Butyl rubber	- Polyethylene
- Starch	- Polysiloxane silicone rubber	- Polypropylene

1.7.3 Drug substance

The successful development of transdermal patch depends upon the selection of drug. The pharmacokinetics and biological factors play an important role while selecting drug. The diffusion of drug should be adequate to produce a satisfactory therapeutic effect.

Table 1.1: Factors to be considered for calculation of transdermal dose.

Parameter	Properties
Dose	The recommended dosage should be kept below 20 mg per day.
Skin permeability coefficient	Should exceed 0.5 × 10−3 cm/h.
Molecular weight	The molecular weight should be less than 400.
Partition coefficient	The partition coefficient (Log P) between octanol and water should fall within the range of -1.0 to 4.
Half-life in h	The half-life should be equal to or less than 10 hours.
Skin reaction	The material should be non-irritating and non-sensitizing to the skin.
Oral bioavailability	The oral bioavailability should be low.
Therapeutic index	Narrow.

1.7.4 Permeation enhancer

(Roy, 2017)

Permeation enhancer allows the transportation of drug by overcoming the obstruction caused by the SC.They act through three mechanisms:

1) Disrupting the orderly structure of lipids in the SC, the skin's outermost layer.
2) Interacting with skin cells and proteins.
3) Optimizing the penetration and distribution of the drug, co-enhancer, or solvent within the stratum corneum.

The characteristics of PE are:

- Non-toxicity: The substances should not exhibit any toxic effects.
- Non-allergenic: The substances should not induce any allergic reactions.
- Non-irritation: The substances should not cause any irritation to the skin.
- They should be inert and stable.
- They should be compatible with both excipients and drug.
- The action of PE should be reversible.
- They should be cosmetically acceptable.

1.7.4.1 Approaches for permeation enhancer

A. Physical approach:

- **Iontophoresis:** The application of electric current to facilitate the transdermal delivery of substances.
- **Sonophoresis:** This is the process of employing ultrasound waves to augment the permeation of substances across the skin.
- **Thermal Energy:** The utilization of heat or temperature to promote the absorption and permeation of substances into the skin.
- **Stripping of Stratum Corneum:** Enhancing drug delivery by perturbing or removing the outermost skin layer (stratum corneum).
- **Hydration of Stratum Corneum:** The process of moisturizing or hydrating the stratum corneum to enhance the permeability of substances.

B. Chemical Approach:

Pyrrolidone: Chemical compounds containing a pyrrolidone ring structure, often used as solvents and permeation enhancers in transdermal drug delivery.

Azone: Azone is a synthetic compound commonly employed as a penetration enhancer in pharmaceutical formulations.

Oxazolidinones: Oxazolidinones are a class of compounds that can act as permeation enhancers, facilitating the delivery of drugs through the skin.

Cyclodextrins: Cyclodextrins are cyclic oligosaccharides that can form inclusion complexes with drugs, aiding in their solubility and absorption.

Sulfoxides: Sulfoxides are organic compounds that have shown potential as permeation enhancers due to their ability to disrupt the stratum corneum barrier.

Surfactants: Surfactants, also known as surface-active agents, possess the ability to reduce the surface tension of liquids and improve the penetration of drugs through the skin.

C. Biological approach:

Synthesis of bio-convertible pro-drug and skin metabolism inhibitors have great potential to enhance the transdermal permeation.

Natural penetration enhancer: terpene oil and essential oil are used.

1.7.5 Adhesives

Pressure-sensitive adhesives (PSAs) are utilized on patches to facilitate their attachment to the skin through the application of finger pressure. These adhesives should possess characteristics such as non-toxicity and non-irritation to ensure safety for the user. Additionally, they contribute to the physical and chemical stability of the final product. Polybutadiene, polyacrylate, and silicone-based adhesive polymers are examples of adhesive membranes commonly employed in patches. These polymers exhibit properties that make them suitable for use as pressure-sensitive adhesives in terms of their adhesive strength, flexibility, and compatibility with the skin.

1.7.6 Release liner

It is also known as a carrier which carries adhesives. The protection of adhesives and drug is carried out by release liner. It should be removed before patch is applied to skin. The removal should be easy and should not change the appearance of the patch.

1.8 Types of transdermal patches

(Hiroji, 2011)

Transdermal Patches are categorized into four types.

1.8.1 Single layer Drug in Adhesive

(Chauhan L, 2018)

This system incorporates a distinctive feature where the drug is encapsulated within the adhesive layer of the patch. Notably, the adhesive layer serves a multifunctional purpose by ensuring adherence to different skin layers while also facilitating controlled drug release onto the skin. Surrounding the adhesive layer, one can

find a release liner and a backing membrane, which contribute to the overall structure and functionality of the patch.

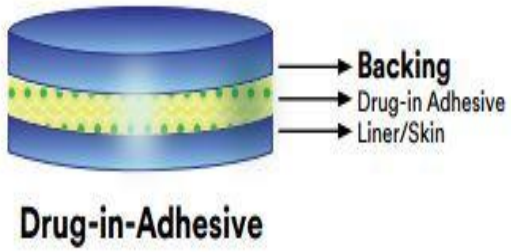


Figure 1.3: Drug -in-Adhesive

1.8.2 Multi-layer Drug in Adhesive

The multi-layer drug-in-adhesive patch functions similarly to the single-layer system but exhibits a distinctive structure. Unlike the single-layer patch, the multi-layer variant comprises different layers separated by a membrane. This design enables the patch to achieve both immediate and controlled release of medications by leveraging the specific properties of each layer. Additionally, the patch incorporates a temporary liner-layer and a permanent backing, enhancing its usability and longevity. The permeability of the membrane and the process of drug molecule diffusion play crucial roles in determining the release profile of the medication. These factors collectively contribute to the efficacy and performance of the multi-layer drug-in-adhesive patch, providing a versatile and efficient solution for transdermal drug delivery.

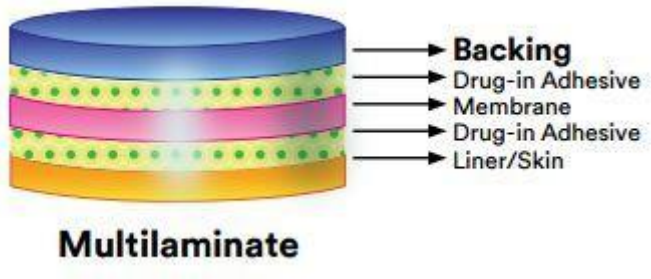


Figure 1.4:Multilaminate

1.8.3 Reservoir system

The intermediate layer, situated between the backing membrane and the rate-controlling membrane, functions as a distinct drug reservoir. In this design, the drug is not released through the polymer matrix itself. Instead, it is the responsibility of the rate-controlling membrane to gradually release the drug. This unique configuration ensures controlled and targeted drug delivery without relying on the polymer matrix for release mechanisms.

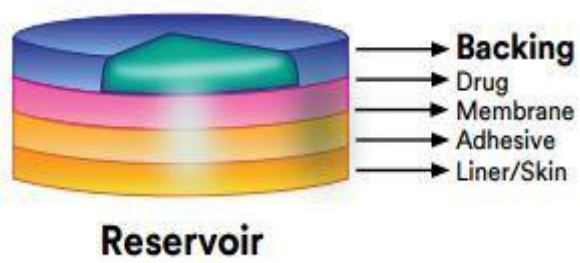


Figure 1.5: Reservoir System

1.8.4 Matrix System

The design of this system incorporates a semisolid matrix containing a drug in either a solution or suspension form, with direct contact between the matrix and the release liner. This innovative patch, also known as a monolithic system, features an adhesive layer that surrounds the polymer disc. Unlike other systems, it eliminates the need for a rate controlling membrane.

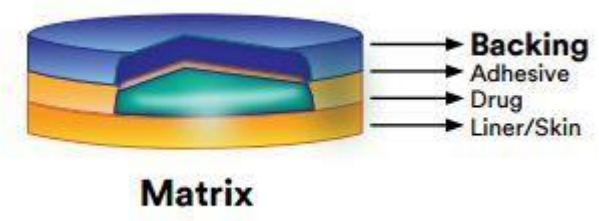


Figure 1.6: Matrix

1.9 Description of Drug

(CID 60496, 2005) (O'Neil, 2006)

Amlodipine Besylate:

IUPAC Name: benzene sulfonic acid; 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate

Molecular Formula: $C_{26}H_{31}CIN_2O_8S$

Molecular Weight: 567.05 g/mol

Melting Point: 199- 200 °C

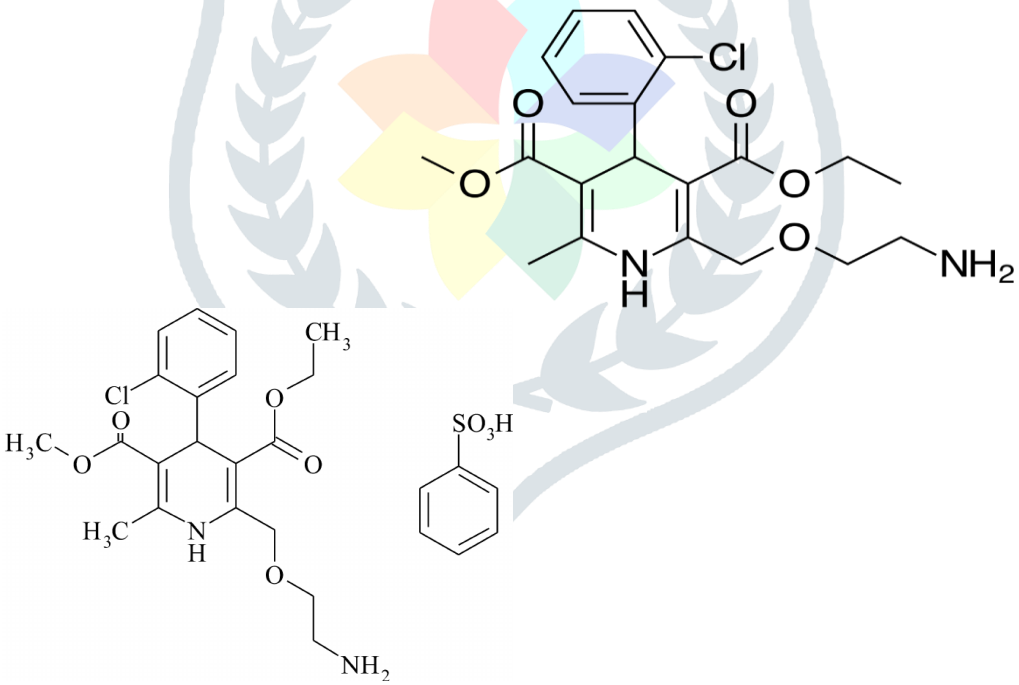
Amlodipine, a calcium antagonist from the dihydropyridine class, works as a slow channel blocker to efficiently prevent calcium ions from entering cardiac muscle cells and vascular smooth muscle cells. With a unique terminal elimination half-life spanning from 30 to 50 hours, it demonstrates biphasic removal from plasma. Amlodipine has a bioavailability that ranges from 60% to 65%. The considerable first-pass metabolism that amlodipine experiences is noteworthy and contributes to its distinct pharmacokinetic profile.
Amlodipine besylate belongs to the class of long-acting calcium channel blockers. used for treating angina and hypertension. It offers sustained control and improved blood flow, reducing symptoms and improving quality of life.
Amlodipine Besylate is the besylate salt of amlodipine, synthetic dihydropyridine.

Amlodipine effectively hinders the entry of calcium ions from the outside into cardiac and smooth muscle cells, preventing their contraction and promoting arterial dilation, improved blood flow, and increased oxygen supply to the heart. It reduces total peripheral resistance and diminishes myocardial contractility. Additionally, a distinctive attribute of amlodipine is its ability to influence multi-drug response (MDR) by inhibiting the p-glycoprotein efflux pump, thereby impacting drug transport mechanisms.

Figure 1.7: Amlodipine **Figure 1.8: Amlodipine Besylate**

Mechanism of Action

Amlodipine effectively lowers blood pressure through a dual mechanism of action. It binds preferentially to both dihydropyridine and non-dihydropyridine binding sites, affecting the entry of calcium ions into cardiac muscle and vascular smooth muscle cells. This binding predominantly affects vascular smooth muscle cells by preventing the influx of calcium ions across cell membranes, causing vasodilation, and lowering peripheral vascular resistance, thereby significantly reducing blood pressure.



Indication

Amlodipine is indicated for,

- Chronic stable angina pectoris,
- Hypertension (high blood pressure), and
- Vasospastic angina (a form of chest pain caused by coronary artery spasms)..

Side Effects

- Headache
- Edema of ankle or feet
- Dizziness

Pharmacokinetics

➢ **Absorption:** Amlodipine is gradually absorbed into the body and reaches its peak plasma concentration between 6 and 12 hours after oral administration at a therapeutic dose. The range of absolute bioavailability is 64% to 90%, and it is unaffected by the presence of meals.

➢ **Metabolism:** The CYP3A4 isoenzyme of the cytochrome P450 system plays a significant role in the metabolism of amlodipine. The liver converts 90% of the original component and 60% of the metabolites into inactive metabolites, which are then eliminated in the urine. Plasma proteins bind roughly 93% of the medication in hypertensive patients.

➢ **Excretion:** Amlodipine is eliminated in two phases, with a terminal elimination half-life of roughly 35 to 50 hours. The steady-state plasma level of amlodipine is reached in individuals taking daily dosages for seven to eight days.

1.10 In-Vitro Evaluation

(Pfizer)

1.10.1 Weight variation

The study on weight variation involves weighing three patches, each with a diameter of 8mm, individually. The average weight is then calculated to ensure that the individual weights do not significantly deviate from it.

1.10.2 Thickness Variation

A digital Vernier calliper was used to gauge the thickness of the film at five distinct locations. These data were used to determine the film's average thickness.

1.10.3 Folding endurance

Through hand measurement, the produced films' folding resistance was evaluated. A strip of film was repeatedly folded at a certain point until it cracked. The film's folding endurance was calculated as the sum of all the folds there before it broke.

1.10.4 In-vitro drug release study

Patches with an 8 mm diameter were cut and placed between the donor and receptor compartments in a vertical diffusion cell. The film was exposed to the receptor fluid, which was a phosphate buffer with a pH of 7.4. A temperature of 35.5 ± 0.5°C was maintained throughout the entire setup. The elution medium was agitated using a magnetic stirrer at a speed of 200 rpm to speed up the procedure.

Aliquots measuring 0.5 ml were taken out of the system and replenished with an equal volume of phosphate buffer with a pH of 7.4 at fixed intervals of 8 hours. The samples were then examined for the presence of drugs using a UV spectrophotometer set to a wavelength of 239 nm. The experiment was repeated twice, and the mean value was calculated to account for any deviations.

1.10.5 In-vitro drug permeation study

(Bharkatiya, 2010)

Permeation study was conducted using a Franz diffusion cell to evaluate the drug's in-vitro permeation. Skin samples were collected from the abdominal region of pigs and washed with a phosphate buffer solution having a pH of 7.4. The prepared skin was placed in the donor and receptor compartments of the diffusion cell. Specially designed patches were placed over the stratum corneum, the skin's top layer, facing the donor compartment. In contrast, the dermis, the skin's innermost layer, faced the receptor compartment.

The receptor fluid was kept homogeneous by swirling it at a magnetic speed of 200 rpm. We replaced the receptor fluid with 0.5 cc samples at regular intervals of 1 hour and carried out this technique continuously for eight hours. The drug content in the collected samples was analysed using a microplate, specifically measuring the absorbance at a wavelength of 239 nm.

1.11 Mathematical model for Drug release Kinetics and Drug permeation Kinetics

(A. T., 2001)

Mathematical modelling plays a pivotal role in elucidating the mechanism of drug release and providing valuable insights for the design of different systems. It is widely recognized among researchers that numerous effective controlled delivery systems have been developed without adhering to a specific pattern in terms of component selection, configurations, and geometrics. By utilizing mathematical modelling as a tool, researchers have successfully identified the primary mechanisms that govern pharmacokinetics and pharmacodynamics, thereby enabling the determination of a drug release profile. In drug release studies, researchers commonly employ several types of mathematical models, which are outlined below:

1.11.1 Diffusion:

Diffusion is a natural phenomenon where individual molecules of a substance undergo movement, propelled by the random motion of molecules, and affected by a concentration gradient. This phenomenon facilitates the transportation of solute molecules through two main mechanisms: the permeation of molecules at a molecular level and their traversal through pores and channels. As a result, diffusion plays a crucial role in the migration of solute molecules.

1.11.2 Fick’s first law of diffusion:

Fick's First Law of Diffusion states that when a system reaches a steady state, the diffusion flux moves from areas of higher concentration to areas of lower concentration. The flow and concentration gradient are precisely proportional. In one dimension, this law can be described as follows: the derivative of the

concentration (c) with respect to distance (x) is equal to the diffusion flux (J), which is equal to the negative product of the diffusion coefficient (D) and these two quantities. Therefore,

$$J = -D(dc/dx)\text{……... (i)}$$

1.11.3 Zero Order Kinetic Model:

The Zero-Order Kinetic Model is a mathematical representation that describes a system where the release rate of a drug remains constant regardless of its concentration. This model assumes that the drug is released steadily at a consistent rate, irrespective of its amount within the system. The equation for this model is expressed as follows:

$$C = C_0 - K_0t \text{ …… (ii)} \quad \text{(assuming rapid release upon dissolution)}$$

In this equation, **C** represents the amount of medication that was dispersed or discharged, while the initial concentration of the drug in the solution, $\mathbf{C_0}$, is typically assumed to be zero. The zero-order rate constant is denoted by K0, and t stands for the passing of time.

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1.11.4 First Order Kinetic Model:

The First Order Kinetic Model is a mathematical representation used to describe the rate at which a drug undergoes a reaction. In this model, the reaction rate is directly proportional to the concentration of the drug, making it significant in pharmacokinetics, drug development, and dosing. The following equation can be used to numerically represent the first order kinetics-compliant medication release pattern:

$$\log C = \log C_0 - (K_t/2.33)\text{…… (iii)}$$

In this equation, $\mathbf{C_0}$ denotes the drug's initial concentration, **K** denotes the first-order constant, and t indicates the passing of time. To analyse the data, the log cumulative percentage of the drug still present is plotted against time. This method results in a straight line, from which we can calculate the slope to be **K/2.303**.

1.11.5 Higuchi Model:

Higuchi introduced one of the most renowned and frequently utilized mathematical equations for describing the release of drugs from matrix systems. Subsequently, researchers extended this model to encompass various geometries and porous systems. Consequently, this versatile model finds extensive application across different geometries and porous systems.

The equation is,

$$C = [D\ (2qt - Cs)\ C_st]\ ½ \text{ …….. (iv)}$$

where **C** represents the total drug release in milligrams per square centimetre of the matrix. **D** is the drug's matrix diffusion coefficient, expressed in cm2/hr, while qt is the total drug concentration in a unit volume of the matrix [mg/cm3]. The drug solubility in the polymer matrix, expressed as Cs (mg/cm3), is also considered. Time is measured in hours.

To analyse the data, plots were created using the square root of time and the cumulative percentage of medication release.

1.11.6 Korsmeyer – Peppas model

Korsmeyer-Peppas proposed a semi-empirical equation that provides insights into the drug release mechanism from polymeric systems. This equation offers a comprehensive understanding of how drugs are released from such systems.

The equation is as follows:

$$Mt/M\infty = Kkpt^n \ldots\ldots\ldots\ldots\ldots (v)$$

To simplify the equation, we can take the logarithm of both sides:

The equation log (Mt/M∞) = log Kkp + n log t represents the relationship between the overall amount of drug released (M) and the entire amount of drug released after an infinite amount of time (Mt). The diffusional exponent, also known as the drug release exponent (n), is a characteristic of the drug release diffusion mechanism. The Korsmeyer release rate constant is represented by Kkp.

To study the release dynamics, the logarithm of cumulative percentage drug release, log (Mt/M), is typically plotted against the logarithm of time, log(t).

1.11.7 Flux

Flux is defined as the amount of drugs or material following through a unit cross section of barrier in unit time.

The equation is:

$J = K_p * C_{app}$ ……. (vi)

Where,

J=flux

K_p=permeation coefficient

C_{app}=applied concentration i.e., concentration gradient between donor and receptor at zero time.

2 Materials and Methods

2.1 Materials

2.1.1 Drugs

The drug sample i.e., amlodipine besylate was gifted by Quest Pharmaceuticals.

2.1.2 Materials used.

In the research study, the following materials were employed:

1. Aluminium foil: This material, specifically selected for its excellent thermal conductivity and flexibility, played a crucial role in various experimental procedures. It was utilized to create a barrier or wrap to ensure controlled temperature conditions during specific stages of the study. The aluminium foil's ability to maintain a stable environment facilitated accurate observations and measurements.

2. Magnetic beads: The utilization of magnetic beads offered a significant advantage in the research project. These beads, coated with a magnetic substance, were used to isolate, and manipulate specific target molecules

or entities in various experimental protocols. By employing a magnetic field, the magnetic beads enabled efficient separation and extraction processes, thereby enhancing the overall precision and effectiveness of the study.

3. Glass mould: The glass mould served as an essential tool in the fabrication and shaping of specific components or structures during the experimental procedures. The mould, designed with precision and accuracy, provided a controlled environment for the formation of various samples. Its superior durability and resistance to high temperatures ensured the integrity and reliability of the study's results.

2.1.3 Chemicals and Reagents

The chemicals and reagents used during the study are listed below.

Table 2.1: Description of various chemicals used.

Chemicals	**Function**
HPMC	Polymer
Distilled water	Solvent
Eugenol	Penetration Enhancer
Olive oil	Penetration Enhancer
Polyethylene glycol 200	Plasticizer
Potassium dihydrogen phosphate	Buffer Reagent
Sodium Hydroxide	Buffer Reagent

2.1.4 Equipment and Instruments

The used instruments used during the study are as listed below.

Table 2.2: Description of various instruments used.

Name of equipment	Model No.	Serial No.	Manufacturer
Analytical Balance	E12140	G0071120503105	OHAUS Corp USA
Digital Vernier Calliper			Aerospace
Franz Diffusion Cell	EDC-07	EDC-07-1604004	ELECTROLAB
Water Bath			Cos Lab
Magnetic Stirrer		SC 002	SPECTRALAB THANE
Microplate Reader	EPOACH 170601BF	170601 B	Bio Tek Instruments Inc
pH Meter	HI 2210 pH meter		HANNA Instruments
Ultrasonic Cleaning Bath		SC 007	SPECTRALAB THANE
UV-Visible Spectrometer	UV-1800 2040V	A11454806352 CD	Simadzu Cooperation Japan

2.2 Methods

2.2.1 UV Method Validation:

2.2.1.1 Specificity:

Specificity refers to the capacity to accurately determine the presence of a particular analyte, even when various other substances such as impurities, degradants, or matrix components are present. It plays a crucial role in establishing the identity of the analyte and performing analytical procedures that yield precise results, thereby enabling an accurate assessment of the analyte's content or potency within a given sample.

2.2.1.2 Accuracy:

The degree of agreement between the obtained result and the approved or reference value is used to define the accuracy of an analytical method. The accepted or reference value is frequently viewed as either a recognized reference value or a true conventional value. This quality is frequently referred to as trueness.

2.2.1.3 Precision:

The degree of consistency and dispersion seen when numerous measurements are made on a homogenous sample under circumstances is referred to as an analytical method's precision. Repetition, intermediate precision, and reproducibility are the three categories under which precision is evaluated. To measure the

accuracy of an analytical procedure, statistical metrics like variance, standard deviation, or coefficient of variation are frequently used. Based on a number of measurements, these measurements are calculated.

2.2.1.4 Repeatability:

Repeatability, also referred to as intra-assay precision, denotes the level of precision achieved when performing measurements or conducting experiments under consistent operating conditions within a brief time interval. This term describes the ability to reproduce results reliably in multiple trials conducted under similar circumstances.

Intermediate precision:

Intermediate precision quantifies the inherent variability observed within laboratories, encompassing factors such as variations across different days, different analysts, and different equipment, among others. This assessment aims to ensure the reliability and consistency of experimental results, while considering the impact of these internal sources of variability.

Reproducibility:

Reproducibility refers to the level of consistency observed in scientific experiments or studies conducted across different laboratories. It is commonly used in collaborative efforts aimed at standardizing methodologies.

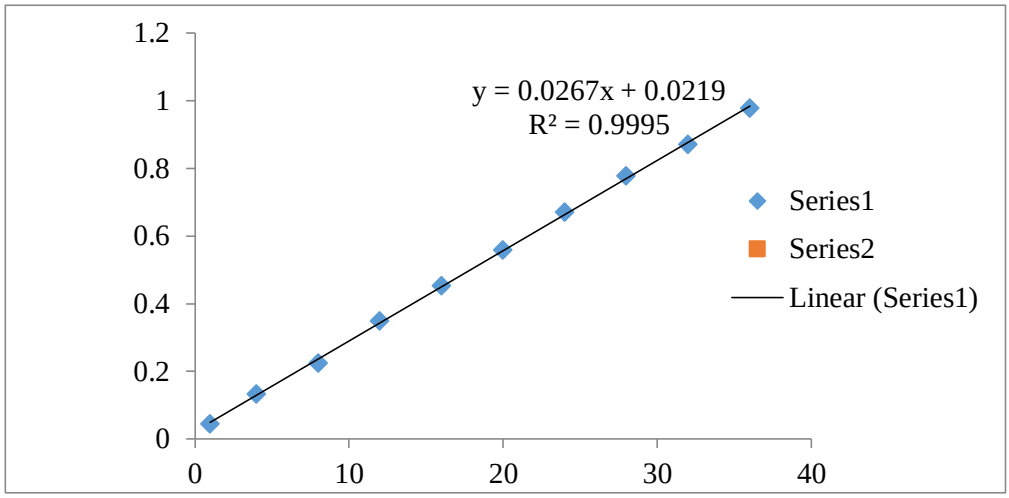
2.2.1.5 Linearity:

The concept of linearity in an analytical procedure pertains to its capacity to produce test results that exhibit a direct proportionality to the concentration or quantity of the analyte present in the sample, within a predetermined range.

Result of UV Method Validation

Maximum wavelength determination

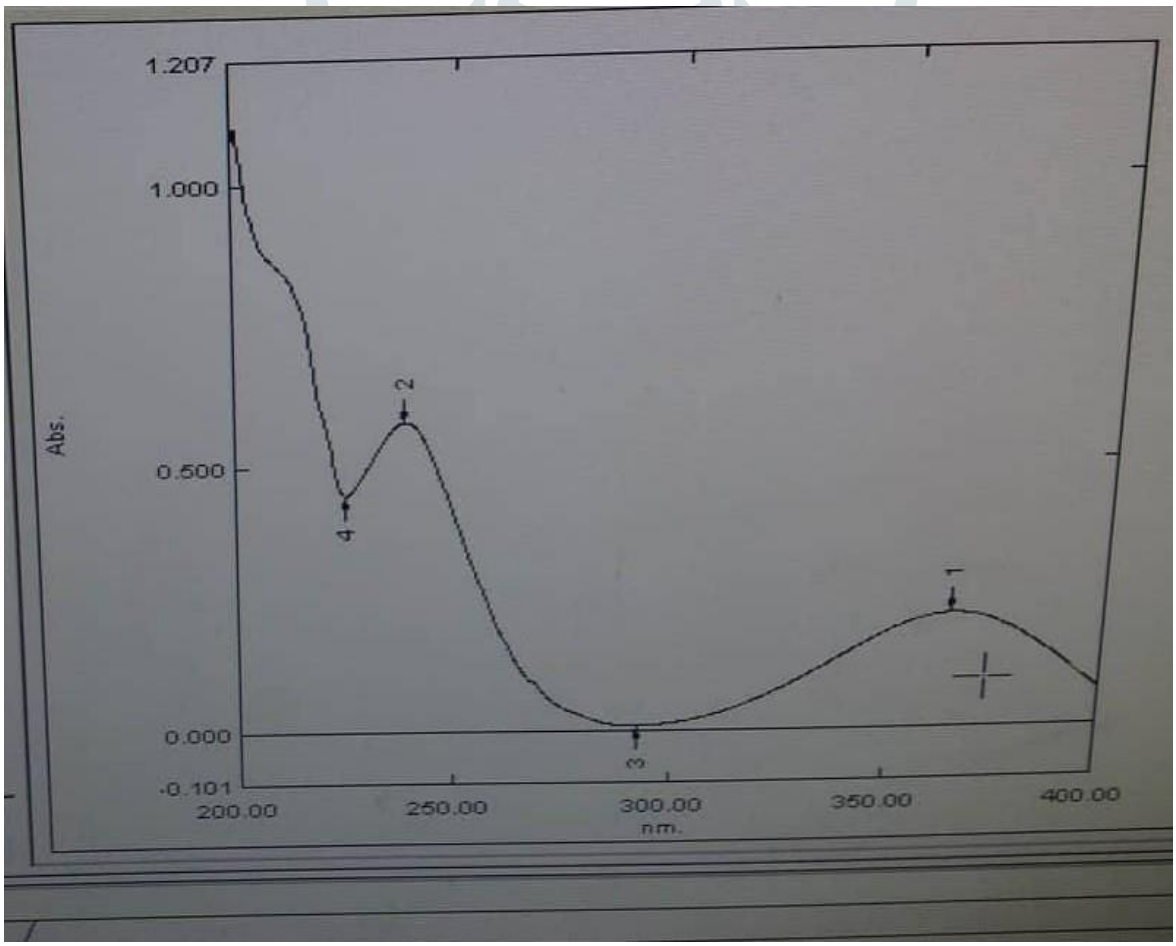
Figure 2.1: Spectral Scanning using UV-Visible Spectrometer from range of 200-400 nm.



By using UV spectrophotometer, we conducted spectral scanning for Amlodipine Besylate The peak is obtained at wavelength of 239nm.

Calibration curve

The calibration curve was determined using ten different concentrations ranging from 1 ppm to 36 ppm and calibration curve was drawn. The equation obtained is:



Absorbance

$y = 0.0267 x + 0.029$ and has R^2 value of 0.9995.

Figure 2.2: Calibration curve at 239 nm

2.2.1.6 Accuracy

The determination of accuracy involved the utilization of a test concentration of 20 ppm, along with two additional concentrations that were 20% higher and lower than the test concentration. Triplicate concentrations (16ppm, 20ppm and 24ppm) were taken and absorbance value was determined using UV spectrophotometer. The test concentration and percentage recovery were calculated using the obtained absorbance. All the percentage recovery lied within the range on 98%-102% as shown.

Table 2.3: Determination of percentage recovery for given concentration range.

Concentration (ppm)	Absorbance	Concentration from graph (ppm)	% Recovery
16	0.443	16.23077	101.4423
16	0.436	15.96154	99.75962
16	0.444	16.26923	101.6827
20	0.551	20.38462	101.9231
20	0.545	20.15385	100.7692
20	0.534	19.73077	98.65385
24	0.654	24.34615	101.4423
24	0.644	23.96154	99.83974
24	0.655	24.38462	101.6026

2.2.1.7 Precision

Repeatability was determined.
Determination of repeatability by calculating Relative Standard Deviation of six samples of test concentration

Concentration (ppm)	Absorbance	Concentration from graph (ppm)	% Recovery
20	0.54	19.96154	99.80769
20	0.548	20.26923	101.3462
20	0.543	20.07692	100.3846
20	0.551	20.38462	101.9231
20	0.549	20.30769	101.5385
20	0.55	20.34615	101.7308
Mean	0.547	20.224	101.119
SD	0.004	0.168	0.838
RSD	0.796	0.828	0.828

Where, SD means Standard deviation, RSD means Relative standard deviation.

2.3 Characteristics of TDDS patch

13 different samples using varying concentrations of HPMC, and PEG were prepared and analysed by using Minitab. Central Composite Design (CCD) was used to optimize the formulation. Then determination of thickness and folding endurance was done with the formulated transdermal patch as shown in table below.

Table 2.4: Characterization of Transdermal patch based on thickness and Folding Endurance

HPMC(mg)	PEG(mL)	Thickness(mm)	Folding Endurance
11300	0.05	2.864	58
5750	1.43	1.694	112
13598.9	1.43	0.994	30
5750	0	1.28	15
5750	3.39	1.958	35
5750	1.43	1.712	300
0	1.43	-	-
200	0.05	0.192	300
5750	1.43	1.458	241
5750	1.43	2.074	21
5750	1.43	2.134	300
200	2.825	0.714	300
11300	2.825	1.364	58

Where, HPMC means Hydroxypropyl methylcellulose and PEG means Polyethylene glycol.

2.4 Experimental Design

To create two contour plots based on thickness and folding endurance using Minitab software, Figures 2.3 and 2.4 were generated. The optimization process was then performed on the formulated patches. As a result, an additional patch was created with varying concentrations of permeation enhancers. Specifically, the concentrations of HPMC and PEG were set at 5000mg and 0.5ml, respectively, as obtained from the Minitab software.

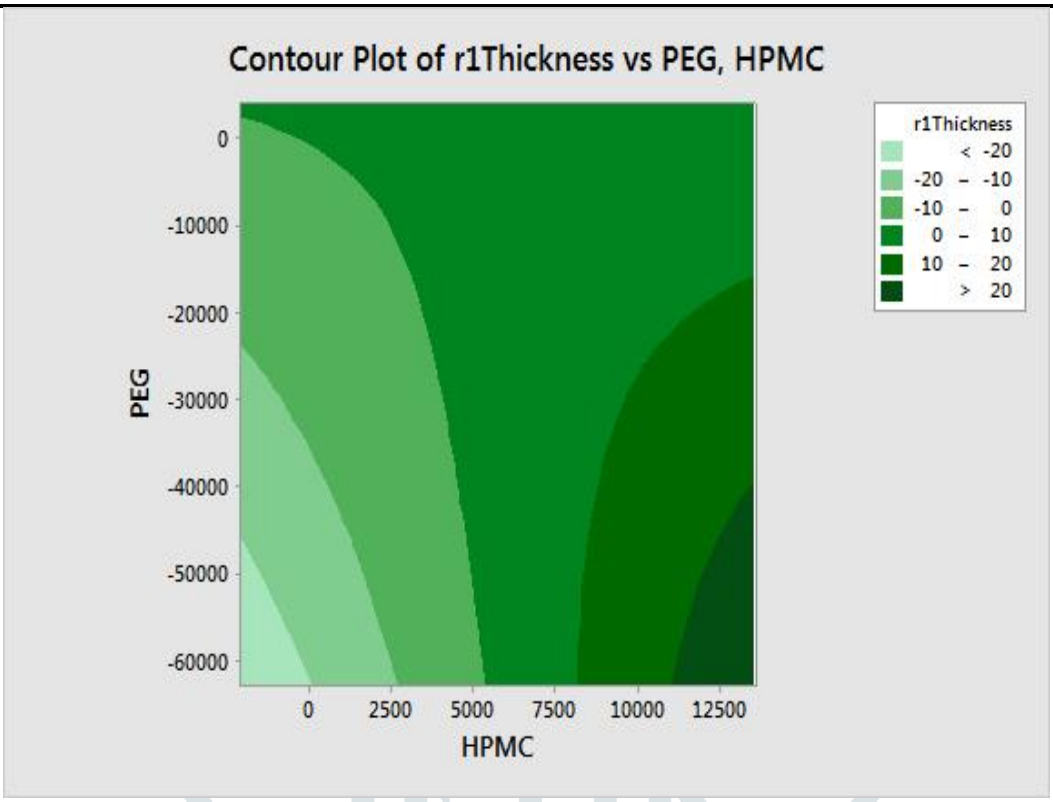


Figure 2.3: Counter plot of thickness vs PEG, HPMC

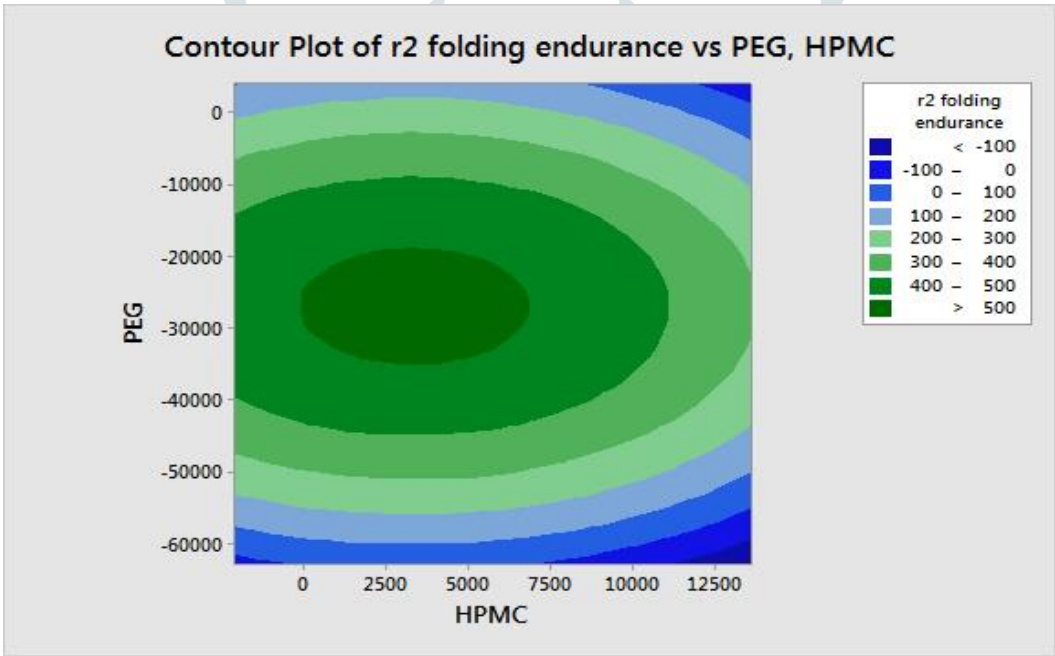


Figure 2.4: Counter plot of folding endurance vs PEG, HPMC

2.5 Preparation of Transdermal Patch

The transdermal patch of Amlodipine besylate was prepared by solvent casting method with the use of 7.5*7.5 cm^2glass mould. Firstly, HPMC was soaked overnight and then PEG-200 was added and then mixed. Amlodipine besylate of required quantity was dissolved in 20 ml of ethanol. The drug solution was poured into the above polymer solution. Lastly, permeation enhancer i.e., olive oil and eugenol were added as per the requirement. Then the solution was mixed using magnetic stirrer until complete mixing. Then it was kept in the mould where aluminium foil was used as a backing layer. The foil was left over to dry at a room temperature for about 4-5 days. The resultant dried patch was wrapped in an aluminium foil and stored in a desiccator for further use.

2.6 Calculation of loading drug

The formulation of the patch was designed based on the radius of the Franz diffusion cell, which was 0.4 cm. The objective was to ensure that each small circular patch contained 2.5 mg of the drug. To calculate the required amount of drug, the total area of the mould was measured as shown below:

The area of the patch (A) was calculated using the formula A = πr^2, where r is the radius of the patch. Substituting the given radius value, we have:

A = π * (0.4) ^2 = 0.502 cm^2…… (vii)

The desired drug content in each small patch was set at 2.5 mg. The total area of the mould (M) was determined to be 56.25 cm^2. To calculate the total amount of drug required to achieve 2.5 mg in each small patch, we can use the following equation:

Total amount of drug to be loaded = (M * 2.5) / A = (56.25 * 2.5) / 0.502 = 280.13 mg

Therefore, to obtain 2.5 mg of the drug per small patch, a total of 280.13 mg of the drug needs to be loaded into each formulation.

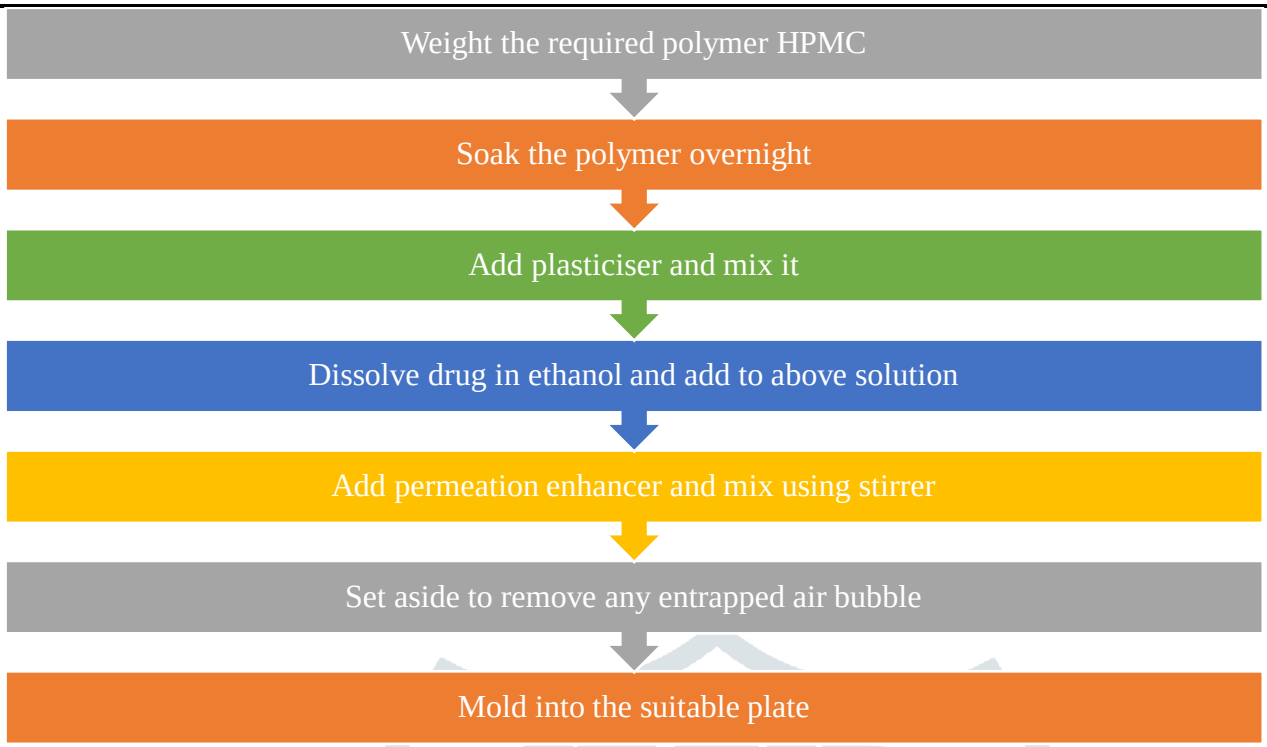


Figure 2.5: Flow Chart of preparation of Transdermal Patch

2.7 Preparation of Phosphate Buffer pH 7.4

Weigh 2 gm of sodium hydroxide and 6.8045 gm of potassium dihydrogen orthophosphate in a 250 ml conical flask. Dissolve the constituent using distilled water and volume make up to 250 ml for each constituent. Then take 195.5 ml of sodium hydroxide and add to potassium dihydrogen orthophosphate and dissolve the constituent using distilled water. Adjust the pH with HCl or NaOH. Finally, add distilled water to make up volume up to 1000ml.

3 Result and discussion

3.1 Thickness variation of patch

Thickness variation of patch are tabulated in Annex VII. Thickness ranges from 1.142mm-1.432mm.

3.2 Folding endurance

The range of folding endurance is varied despite of same concentration of plasticizer. The highest folding endurance is 207. Folding endurance of patch are listed in ANNEX VII.

3.3 Weight variation

The variation of patch was observed. Weights were nearly similar. Weight variation of patches are tabulated in ANNEX VII

3.4 Drug release study

The Franz diffusion cell was employed to investigate the drug release over an 8-hour period. In the receptor cell, 5 ml of 7.4 phosphate buffer solution was placed, while maintaining a temperature of 32±2 degrees Celsius and an RPM of 200. At each hour, a sample was withdrawn, and the withdrawn volume was replaced with fresh buffer solution. Among the formulations tested, formulation F8 exhibited the highest cumulative

percentage of drug release, reaching only 39.34%. The kinetics of drug release can be found in ANNEX VIII, and the cumulative amount of drug released is presented in ANNEX IX.

Formulations F1, F5, F8 shows the zero-order release while the formulations F2, F3, F4, F6, F7, F11, F13 release by the first order kinetics. Formulation F9, F10 and F12 follow the Higuchi order.

To study the Korsmeyer-Peppas model, we conducted a comprehensive analysis by constructing a graphical representation of the logarithm of cumulative percentage drug release, log (Mt/M∞), against the logarithm of time, log t.

The value of "n" is shown in below table:

Table 3.1Diffusional release exponent (n) with their release mechanism

Sample	Exponent(n)	Drug Release Mechanism
F1	0.883	Anamalous transport
F2	0.841	Anamalous transport
F3	0.823	Anamalous transport
F4	0.569	Anamalous transport
F5	0.646	Anamalous transport
F6	0.442	Fickian diffusion
F7	0.445	Fickian diffusion
F8	1.023	Super case II transport
F9	0.523	Anamalous transport
F10	0.5	Anamalous transport
F11	0.291	Fickian diffusion
F12	1.086	Super case II transport
F13	0.459	Fickian diffusion

3.5 Permeation of patch

Permeation study was carried out on Franz diffusion cell. The cell membrane of goat was used as semi-permeable membrane for diffusion. The amount of drug permeated is low. Permeation kinetic and cumulative percentage permeation are shown in Annex X and XI respectively.

Formulation F3, F4, F9, F11, F12, F13 shows zero order release pattern. First order release pattern was shown by F7 and F10. Remaining formulation F1, F2, F5, F6, F8 show Higuchi order.

Table 3.2 Permeation Flux

Sample	**Flux(mg/area/cm^2)**
F1	0.0039
F2	0.0069
F3	0.0065
F4	0.0044
F5	0.0057
F6	0.0098
F7	0.0077
F8	0.0084
F9	0.0055
F10	0.1076
F11	0.0163
F12	0.011
F13	0.012

4 Conclusion

The Transdermal Drug Delivery System (TDDS) has been proven to be an effective method for achieving systemic drug effects while bypassing first-pass metabolism. In the formulation of a matrix-based transdermal patch using amlodipine besylate, we employed HPMC as the polymer and PEG 200 as the plasticizer to provide flexibility to the patches. A total of 13 patches were formulated with the assistance of MINITAB software. It is worth noting that HPMC and PEG 200 were used at the same concentration in all patches. To determine the suitable concentration of HPMC and PEG 200, pre-formulation studies were conducted to analyse the thickness and folding endurance of the patches.

In our study of release and permeation kinetics, we made an intriguing discovery. The formulation with the same concentration of penetration enhancers exhibited the highest drug release (Formulation F8). The release patterns of the formulations varied: F1, F5, and F8 displayed zero-order release, while F13, F11, F7, F6, F4, F3, and F2 followed first-order kinetics. Formulations F12, F10, and F9 adhered to the Higuchi order.

Moving on to permeation kinetics, we observed that F13, F12, F11, F9, F4, and F3 demonstrated a zero-order release pattern. First-order release patterns were exhibited by F7 and F10. The remaining formulations, F1, F2, F5, F6, and F8, followed the Higuchi order.

In terms of transport mechanisms, formulations F10, F9, F5, F4, F3, F2, and F1 displayed Anomalous transport. Fickian diffusion was observed in F6, F7, F11, and F13, while F8 and F12 adhered to Super case II transport.

To ensure the originality of this research thesis, we have taken the necessary steps to make it completely plagiarism-free in relation to Turnitin or any other plagiarism detection tools.

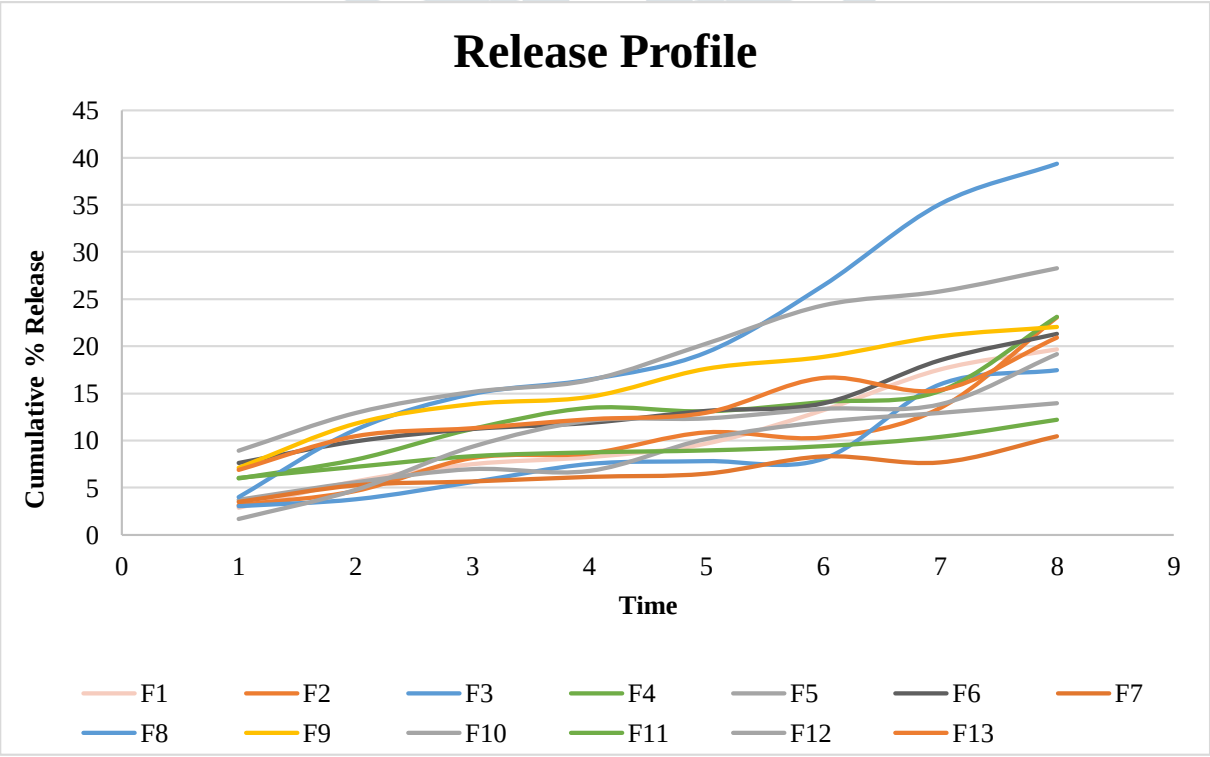


Figure 4.1 Release Profile of Patch

5 Future work and suggestion

- Drug excipients compatibility study
- Real time and accelerated stability study.
- Moisture absorption, moisture loss and tensile strength of the patches

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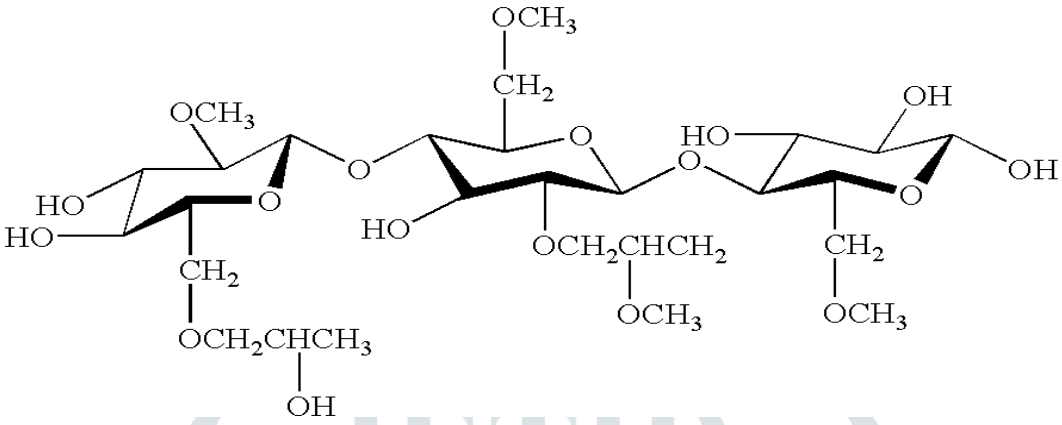
Annex 1

HPMC (Hydroxypropyl Methyl Cellulose):



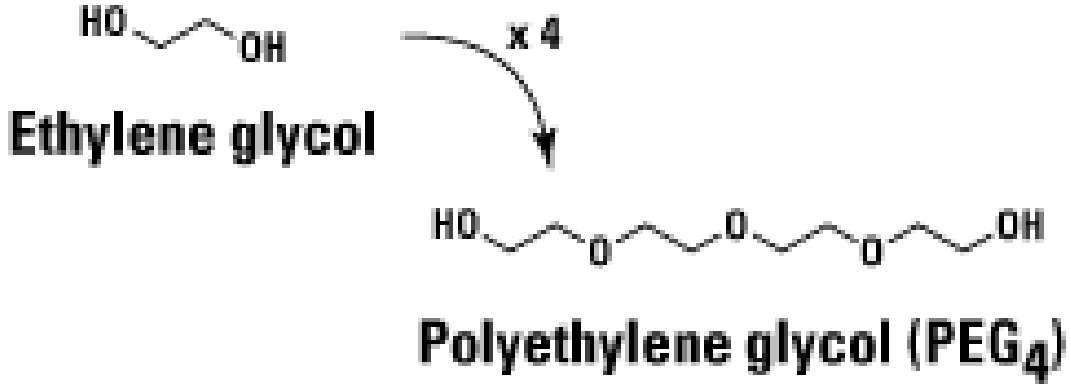
C56H108O30 is the HPMC chemical formula. Hypromellose is a coating agent and film-former used as an inactive component in pharmaceutical formulations. It is also frequently referred to as hydroxypropyl methylcellulose (HPMC). It has been utilized as a rate-controlling polymer for dosage forms with prolonged release.

HPMC is a hydrophilic polymer that can expand and has no taste or odour. It is also inert, white, or creamy-white, fibrous, or granular in texture. It can dissolve in water and turn into a viscous, translucent to milky-white colloidal solution. Because HPMC can produce the desired release profiles for a variety of medications, it is a preferred polymer for the creation of matrix-style transdermal patches. Although it is insoluble in ethanol and hot water, it is soluble in cold water. It is a material that is neither poisonous nor annoying. In aqueous media, it demonstrates gelation properties. The pH level has no impact on how HPMC dissolves in water. It plays a part in enhancing resistance to shrinking and cracking in transdermal patches.

After drying, the substance becomes hygroscopic. Its pH ranges from 5.5 to 8.0 in a 2% aqueous solution. At 20°C, the viscosity of a 2% solution in water is 80-120 mPa. The bulk density of the substance is 0.34 g/ml.

Annex 2

Polyethylene glycol



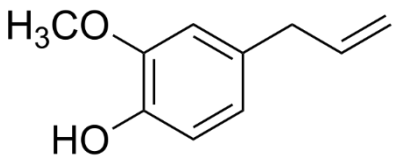
Ethane-1,2-diol, commonly referred to as ethylene glycol, finds application in the synthesis of polyethylene glycol (PEG). When ethylene glycol (with a molecular weight of 62.07) undergoes polymerization or self-interaction, often in an aqueous environment, it generates a range of by-products exhibiting diverse ethylene glycol unit quantities. These resultant compounds collectively fall under the category of PEGs. The general chemical formula representing PEG is H(OCH2CH2)nOH, where 'n' signifies the count of ethylene glycol units comprising the PEG polymer.

The properties of distinct PEG types or classifications are partly influenced by their respective molecular weights. PEGs containing two to four ethylene glycol units per polymer manifest as clear, aqueous fluids characterized by their relatively low molecular weights. On the other end of the spectrum, PEGs containing up to 700 ethylene glycol units per polymer yield clear, viscous liquids, while those with a polymer size of 1,000 or more ethylene glycol units generate substances with a waxy consistency.

Given their diverse attributes, PEGs hold significance in biotechnological and medical arenas. PEG exhibits solubility in various solvents such as water, toluene, and Methylene Chloride, displaying miscibility in any proportion with other PEG grades. Acetone, alcohols, benzene, Glycerine, and glycols can all effectively dissolve liquid PEGs. However, solid PEGs contrastingly remain insoluble in substances like acetone, dichloromethane, 95% ethanol, and methanol, setting them apart from fats, fixed oils, and mineral oil. Their solubility in aliphatic hydrocarbons and ether is limited. Higher molecular weight variants can form gel-like structures in aqueous solutions, while low-molecular-weight PEG primarily serves as a plasticizing agent in formulations.

Annex 3

Eugenol



Eugenol is a phenylpropene, a guaiacol substituted with an allyl chain. Eugenol belongs to the phenylpropanoids class of chemicals. Inorganic solvents and water can both dissolve it somewhat. Its molecular weight is 164.204 g/mol and its chemical formula is C10H12O2.

It is an oily liquid that is colourless to pale yellow and aromatic, extracted from a variety of essential oils, particularly clove oil, nutmeg, cinnamon, basil, and bay leaf. It is contained in clove leaf oil at quantities of 82–88% and clove bud oil at concentrations of 80–90%. Eugenol smells sweet, spicy, and clove-like. Additionally, it is employed as a local anaesthetic and antiseptic.

It has been utilized in our recipe as a penetration enhancer alongside olive oil.

at 25 °C, pKa = 10.19

At 20 °C, the viscosity is 7.817 centipoise.

1.0652 g/cm3 is the density.

Melting point is -9.2 to -9.1°C and

Boiling point is 225°C.

Annex 4

Olive Oil

Olives, the fruits of the Olea europaea plant belonging to the Oleaceae family, are renowned as the primary source of olive oil, a liquid lipid with a wide array of applications. Olive oil finds uses in culinary endeavours, cosmetic formulations, medicinal products, soap manufacturing, and even as fuel for traditional oil lamps. A study was conducted to explore how olive oil, employed as a penetration enhancer, influences the in vitro release profiles of drugs. In pursuit of this, various concentrations of olive oil were incorporated into selected formulations.

Predominantly comprised of Triacylglycerols, commonly known as triglycerides or fats, olive oil also contains minute quantities of free fatty acids (FFA), glycerol, Phosphatides, pigments, Flavouring agents, sterols, and microscopic olive fragments. Oleic acid ($CH_3(CH_2)_7CH=CH(CH_2)_7COOH$), categorized as a monounsaturated omega-9 fatty acid, constitutes a significant portion, ranging from 55% to 83% of olive oil. In contrast, a polyunsaturated omega-6 fatty acid named Linoleic acid ($C_{18}H_{32}O_2$) comprises about 3.5% to 21% of the oil's composition. Lastly, Palmitic acid ($C_{16}H_{32}O_2$), classified as a saturated fatty acid, makes up approximately 7.5% to 20% of the constituents in olive oil.

Melting Point	−6.0 °C (21.2 °F)
Boiling Point	700 °C (1,292 °F)
Solidity at 20 °C (68 °F)	Liquid
Specific Gravity	0.911
Viscosity	84 Cp
Refractive Index	1.4677–1.4707

Annex 5

Central Composite Design

Std. Order	Run Order	Pt. Type	Block	HPMC (mg)	PEG (ml)
2	1	1	1	11300	0.05
10	2	0	1	5750	1.43
6	3	-1	1	13598.9	1.43
7	4	-1	1	5750	0
8	5	-1	1	5750	3.39
9	6	0	1	5750	1.43
5	7	-1	1	-2098.9	1.43
1	8	1	1	200	0.05
12	9	0	1	5750	1.43
11	10	0	1	5750	1.43
13	11	0	1	5750	1.43
3	12	1	1	200	2.825
4	13	1	1	11300	2.825

Annex 6

Formulation Design

Formulation	Drug (mg)	PEG	HPMC	Olive Oil	Eugenol
F1	280.13	0.5	5000	0.5	4
F2	280.13	0.5	5000	4	4
F3	280.13	0.5	5000	0.5	0.5
F4	280.13	0.5	5000	2.25	4.72487
F5	280.13	0.5	5000	2.25	-0.22487
F6	280.13	0.5	5000	4	0.5
F7	280.13	0.5	5000	2.25	2.25
F8	280.13	0.5	5000	2.25	2.25
F9	280.13	0.5	5000	2.25	2.25
F10	280.13	0.5	5000	2.25	2.25
F11	280.13	0.5	5000	-0.22487	2.25
F12	280.13	0.5	5000	2.25	2.25
F13	280.13	0.5	5000	4.724	2.25

Annex 7

Physiochemical Properties of Transdermal Patch

Formulation	Thickness	Folding Endurance	Weight Variation
F1	1.262±0.0775	88	0.136±0.005
F2	1.432±0.1211	43	0.143±0.115
F3	1.208±0.0408	32	0.0866±0.015
F4	1.312±0.0277	18	0.153±0.0057
F5	1.3±0.06041	6	0.086±0.0057
F6	1.264±0.0364	8	0.135±0.01
F7	1.26±0.0604	65	0.113±0.011
F8	1.264±0.0512	6	0.133±0.0005
F9	1.358±0.0277	207	0.106±0.0152
F10	1.198±0.0878	13	0.113±0.0152
F11	1.136±0.0482	117	0.116±0.0152
F12	1.358±0.0491	16	0.116±0.0152
F13	1.604±0.0630	183	0.13±0.02
Controlled	1.142 ± 0.0249	40	0.1 ± 0.02

Annex 8

Sample	1	2	3	4	5	6	7	8
1	2.84	5.64	7.47	8.22	9.66	13.2	17.53	19.67
2	3.13	4.63	8.13	8.62	10.85	10.31	13.43	23.06
3	3.04	3.77	5.60	7.52	7.82	8.06	15.97	17.48
4	5.96	7.96	11.22	13.45	13.09	14.08	15.25	23.13
5	3.72	5.57	6.97	6.78	10.17	11.98	12.93	13.96
6	7.64	9.93	11.24	11.89	13.16	13.96	18.53	21.33
7	3.46	5.223	5.64	6.13	6.47	8.31	7.67	10.46
8	3.98	11.12	14.92	16.44	19.32	26.41	35.06	39.34
9	7.11	11.79	13.87	14.63	17.61	18.87	21.06	22.05
10	8.9	12.9	15.13	16.38	20.26	24.32	25.80	28.26
11	6.04	7.21	8.34	8.74	8.95	9.4	10.38	12.20
12	1.68	4.76	9.34	12.09	12.34	13.35	13.83	19.15
13	6.86	10.44	11.29	12.24	12.94	16.63	15.35	20.92

Sample	Zero Order	First Order	Higuchi Order
1	0.954	0.952	0.909
2	0.809	0.911	0.777
3	0.835	0.922	0.793
4	0.890	0.889	0.848
5	0.957	0.941	0.946
6	0.904	0.954	0.819
7	0.874	0.902	0.881
8	0.959	0.894	0.923
9	0.966	0.897	0.986
10	0.867	0.961	0.971
11	0.937	0.955	0.930
12	0.899	0.756	0.944
13	0.874	0.901	0.822

Annex 8

Cumulative Drug Release

Annex 9

Kinetic of Permeation (R2)

Sample	Zero Order	First Order	Higuchi Order
1	0.563	0.665	0.728
2	0.134	0.091	0.148
3	0.905	0.852	0.849
4	0.599	0.462	0.588
5	0.789	0.598	0.915
6	0.912	0.781	0.974
7	0.680	0.687	0.655
8	0.904	0.829	0.920
9	0.946	0.931	0.919
10	0.713	0.831	0.659
11	0.877	0.682	0.874
12	0.955	0.833	0.947
13	0.198	0.194	0.197

Annex 10

Cumulative Percentage Permeation

Sample	1	2	3	4	5	6	7	8
1	0.41	0.66	0.50	0.619	0.585	0.735	0.91	1.1
2	1.061	1.39	1.03	0.81	0.92	1.84	1.04	1.79
3	0.13	0.29	0.15	0.5	0.78	1.18	1.16	1.68
4	0.29	0.04	0.027	0.53	0.214	1.167	0.83	1.13
5	0.01	0.29	0.529	0.614	0.722	0.764	1.09	1.479
6	0.53	1.01	1.66	1.82	2.075	2.21	2.45	2.82
7	0.88	1.16	0.91	0.84	0.969	1.29	1.49	1.98
8	0.27	0.72	0.742	1.328	1.44	1.28	1.79	2.16
9	0.12	0.22	0.34	0.49	0.98	1.24	1.35	1.42
10	0.75	1.41	1.60	1.81	1.99	2.09	2.18	4.52
11	0.16	1.03	1.76	2.08	2.17	2.43	2.49	4.19
12	0.33	0.92	1.28	1.47	1.77	2.02	2.13	2.98
13	1.63	1.28	2.09	3.27	1.95	1.31	2.10	3.27