CERTIFICATE FROM THE DEAN

This is to certify that research work embodied in this thesis entitled "*Formulation and Evaluation of Levofloxacin Loaded Alginate-Locust Bean Gum Interpenetrating Polymer Network Based Sustained Release Antimicrobial Microspheres*" **submitted to K. R. Mangalam University,** Gurugram, Haryana, for the award of the degree of **M. Pharmacy (Pharmaceutics)** has been carried out by Nidhi Bansal under at Department of Pharmaceutics, School of Medical & Allied Sciences,

K. R. Mangalam University from September 2021 to August 2022.

To the best of my knowledge and belief, this work is original and has not been submitted so far in part or in full for the award of any degree or diploma of any University/ Institute.

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FORMULATION AND EVALUATION OF LEVOFLOXACIN LOADED ALGINATE- LOCUST BEAN GUM INTERPENETRATING POLYMER NETWORK BASED SUSTAINED RELEASE ANTIMICROBIAL MICROSPHERES

Thesis Submitted for the Award of the Degree of

MASTER IN PHARMACY

Pharmaceutics

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INTRODUCTION

1.1 Challenges with conventional drug delivery system

Drug delivery system are engineered technologies for the targeted delivery or controlled release of therapeutic agents. Drug delivery is the method of administrating pharmaceutical compound to achieve a therapeutic effect in humans or animals. It shows a tremendous impact on the treatment of various disease. Drugs delivery systems control the rate at which a drug is released and the location in the body where it is released (1).

The drug delivery system can be further divided into two main types-

1.2 Conventional Drug Delivery System and Novel Drug Delivery System

Conventional drug delivery system are classical methods for delivery of a drug into the body. These methods are used more often when the goal is quickly absorption of a drug; therefore, a quick release of the drug is required. It is designed to give rapid and complete release of the drug contained therein immediately after administration (1). The concentration of the drug varies up and down irregularly in blood plasma and patient typically forgets to take the specific dose at its exact time. Due to the problems mentioned for conventional drug delivery, the necessity of providing novel drug delivery system becomes more apparent.

The examples of these system based on route of delivery include:

i) **Oral Delivery** – Drugs which are delivered by mouth through alimentary canal. It includes tablets, capsules, syrups etc. which are taken through the mouth. The oral administration is very convenient and non-invasive.

ii) **Buccal/Sublingual Delivery** -Buccal delivery is defined as drug administration through the mucosal membranes lining the cheeks (buccal mucosa). The main impediment to the use of many hydrophilic macromolecular drugs as potential therapeutic agents is their inadequate and erratic oral absorption (2). Example – Nitro glycerine, Loratadine, Mirtazapine, Rizatriptan.

iii) **Rectal Delivery** - In this system, suppositories are placed inside rectum and it melts at body temperature to give quick effect. Example- Prednisolone ®, Budesonide, Mesalazine.

iv) **Intravenous Delivery** - Drug in liquid form is administered directly into blood by injecting in vein with the help of sterile injector. Example – Normal Saline (0.9% sodium chloride), lactated Ringer's solution, 5% dextrose in water (3).

v) **Subcutaneous Delivery** – In this system, liquid drug is administered in subcutaneous tissue by injecting with injector. Example- Insulin, Opioids, Heparin, Epinephrine, and allergy medication (4).

vi) **Intramuscular Delivery**- The liquid drug is administered in the muscle tissue. The drug is absorbed slowly, but prolonged effect. Example- Antibiotics- penicillin G benzathine penicillin, streptomycin. Biologicals- immunoglobins, vaccines, and toxoids. Hormonal agents- testosterone, medroxyprogesterone (5).

vii) **Topical**- A topical medication is applied directly to a particular place on or in the body. There are many classes such as cream, foam, gel, ointment, paste, powder etc.

viii) **Ophthalmic**- These are the specialized dosage form designed to be instilled onto the external surface of the eye or administered into the eye cavity.

1.2.1 Novel Drug Delivery System is a combination of advanced techniques and new dosage forms to introduce better drug potency, control drug release, provide greater safety, and a target a drug specifically to a desired tissue (6).

It leads to efficient use of expensive drugs and excipients, and reduce in production cost. It brings better therapy by improved comfort drug delivery devices which increase the standard of living (7).

This system is divided into following classes based on mechanism of drug release-

i) **Prodrug**- In this approach of drug delivery, active ingredients are chemically modified by connecting some special functional groups that will be removed afterwards in the body after administration, releasing parent molecule. Such groups are used to modify the properties of the parent drug to achieve a specific function such as the permeability, solubility, or stability (8).

The prodrug approach has been used for many reasons:

- To change the half-life
- To cross a biological barrier

- To increase retention time
- To target a specific site

ii) **Diffusion- Controlled Delivery Systems-** This method has been utilized in design of controlled release drug delivery systems for several decades. According to this principle, controlled-release drug delivery systems can be designed as a reservoir or matrix system. Drug released from the both systems follow the principle of diffusion, but they show different release patterns (9).

In a **reservoir system,** if the active agent is in a saturated state, the driving force is kept until it is no longer saturated.

In a **matrix system,** because of the changing thickness of the depletion one, release kinetics is a function of the square root of time.

iii) **Dissolution/ Coating – Controlled Delivery Systems** – In this method, the drug is enclosed in a polymer shell or coating. After the dissolution or eroding of the coating, drug become available for absorption (10). Drug molecules are enclosed in beads of different thickness to control the amount and time of drug release.

- **The enclosed particles with thin coatings will dissolve and release the drug first, while**
- **A thicker coating will take longer to dissolve and will release the drug at the later time.**

iv) **Biodegradable/Erodible Delivery Systems** – It contain polymers that degrade into smaller fragments inside thew body to release the drug on a controlled manner (11).

v) **Rate pre-programmed drug delivery system-** In this system, there is a pre-planned release of drug molecule from the delivery system with constant flow rate profile of medicine.

It is further divided into various types:

a) **Polymer membrane permeation-controlled system** – In this system, the drug formulation is totally or partially encapsulated within drug reservoir compartment. The drug release surface is covered by a rate controlling polymeric membrane (12).

b) **Polymer matrix diffusion-controlled system** – In this the drug is prepared by homogeneously dispersing drug particles in rate controlling polymer matrix. The drug

dispersion on the polymer matrix is carried out by two ways- Blending of drug with liquid polymer and mixing of drug with a rubbery polymer.

c) **Micro reservoir partition-controlled system** – The drug reservoirs are a suspension of solid particle in the aqueous solution. It is prepared by applying high dispersion techniques (13).

vi) **Activated modulated drug delivery system-** In this system, the release of drugs from the delivery system is controlled or activated by the some physical, chemical and biological process or by any supplied external energy source (14).

Carrier Based Drug delivery system-

a) **Liposomes-** Liposomes are the structures in the form of vesicles that consist of either many of few of the layers of phospholipids. The polar part of the liposomal core encapsulates polar drug molecules. On the basis of the affinity towards the phospholipids, the amphiphilic and lipophilic molecules are solubilized within the bilayer of phospholipids.

b) **Dendrimers-** These are the structure with symmetrical architecture and are nanometric-sized, heavily branched and monodispersed molecules (15). The core with the internal units identifies the solubilizing properties and surroundings of the nanocavities.

c) **Nanoparticles-**These are the structures ranging from size 10-200 nm are present in the solid from either in amorphous or crystalline. Nanoparticles have an ability to encapsulate a drug entity, thus protecting it from external chemical and enzymatic degradation. Nanomaterials are further classified into various ways such as nanotubes, nanowires, nanocantilever, quantum dots, nano pores, gold nanoparticles (16,17).

1.3 Based on materials

Polymeric Nanoparticles – These are particles ranged from 1 -1000 nm and they are loaded with active compound which is entrapped onto the polymeric core (18).

Lipidic (Vesicular, Cuboidal and spherical)

Metallic Nanoparticles – Metallic nanoparticles have functional groups attached to them. It is synthesized and can be modified to add other ligands, drugs and antibodies. They have a size range of 1-100 nm. Example- Gold nanoparticle, silver nanoparticle (19).

Inorganic Nanoparticles- Due to their high cellular uptake capacity and low toxicity, inorganic nanoparticles have gained much attention. They have shown multiple properties like

physical, chemical and biological properties from their bulk counterpart's. Calcium phosphate and iron oxide are examples of inorganic nanoparticles (20).

Type of Drug Targeting with NDDS-

By manipulating the pattern of the drug carrier, targeting can be achieved. This is known as passive targeting. By changing the pattern of the drug carrier thereby directing the drug to the specified site. This is known as active targeting.

1. **Passive Targeting-** In this targeting, the particles are administered into the body intravenously will distribute depending on the particle size in different organs. The particles will pass through the heart but with no uptake to the lungs. Particles with size less than 7µm get trapped in the capillary beds and particles with size greater than 7µm enters the systemic circulation (21).
2. **Active targeting-** In this targeting, the quantity of drug delivered is increased to the target cell as compared to free drug (22).

1.4 Microencapsulation-

It is a method by which drug in any form solids, liquids or even gases are encapsulated in microscopic particles forming the thin coatings of wall around it. Various approaches are used for delivering a drug substance to the targeted site (23).

Applications of Microencapsulation-

- Used to mask the taste of the bitter drug. For e.g., Paracetamols, Nitrofurantoin
- For easy handling and storage of drugs, liquids are converted to a pseudo-solid. For e.g., Eprazinone.
- Some substances such as carbon tetrachloride are used in microencapsulation to reduce the volatility and odor.
- This method has been used to give protection to the core materials against the atmospheric effects. E.g., Palmitate.

Some of the problems of conventional therapy can be overcome by a well-designed controlled drug delivery system as it can enhance the therapeutic efficacy of a particular drug. To get maximum therapeutic efficacy, it is mandatory to deliver the drug agent to the target site in the optimal amount in the correct period of time causing no toxicity and minimal side effects (24). New methods of drug delivery are possible nowadays, desired drug release can be provided by rate- controlling membranes or by implanting biodegradable polymers. The major advantage is to administer the medication via such system because microspheres can be tailored for desired release profiles; as they can be injected or ingested (25).

1.5 Microspheres

Microspheres are small solid spherical particles, ranging 1-1000 µm in size. They are also referred to microparticles. Microspheres are made up of polymeric material (natural and synthetic), the drug is dispersed throughout the microsphere matrix (26). The drugs administered in the body interact with target cells along with normal healthy cells with less toxic effects. It requires high concentration to maintain a therapeutic efficacy because of the dilution effect and to achieve systemic administration for the drug (27).

To achieve the biocompatibility, we should opt natural polymers such as chitin, cellulose, chitosan or polymers that are made from naturally occurring monomers or those derived from synthetic monomers show excellent properties. The various factors affecting drug release are controllable, they are confined to some properties like the molecular weight of polymer, along with the size of microspheres, morphology and distribution etc. (28).

1.6 Historical Perspective

This concept came long back in 1932 with the work of Dutch Chemist H.G (29). The term 'coacervate' is used by the chemist which describe droplets which contain a colloid, and is rich in organic compounds; surrounded by layer of water (30).

The very first project employing Microencapsulation was carbonless copy paper, developed by L. Schleicher and B. Green (31). They developed another set by undercoating sheets of paper with microparticles. W.M. Holiday and collaborators patented in year 1970, their first use of microparticles in the pharmaceutical industry (32). The pharmaceutical formulation served as a strategy to minimize the irritant effect of acetylsalicylic acid on gastric mucosa, it reduces the frequency of administration (33).

1.7 Microspheres as Drug Carriers

1.7.1 Microspheres based on Biodegradable Polymers

Certain synthetic as well as natural polymers are used to prepare biodegradable polymers. Many natural polymers such as protein undergo degradation while the synthetic like amide, esters contain hydrolysable linkage and undergo simple hydrolysis (34). The most important requirement of polymer is that degraded product should have low toxicity so that it can enter circulation, or result in tissue deposition.

1.7.2 Criteria of ideal microspheres

- Must have ability to incorporate reasonably high concentration of the drug.
- Susceptibility to chemical modification.
- Stability of the preparation after synthesis with a clinically acceptable shelf line.
- Release of active reagent with a good control over a wide time scale.

1.8 Types of Microspheres-

Bioadhesive Microspheres- The term adhesion refers to sticking of the drug to the membrane by using the adhesion property. Bioadhesive microspheres causes contact with the absorption site and produces good therapeutic action and shows a long residence time at the site of application (35).

Magnetic Microspheres- This type of delivery system is very essential because it localizes the drug to the main site (36). Magnetic microspheres are of two types-

a) **Therapeutic magnetic microspheres-** They are basically used in liver cancer. Some drugs like proteins and peptides can also be targeted through this system (37).
b) **Diagnostic Microspheres-** They are used for determining liver metastases and are employed to differentiate bowel loops from other structures (38,39).

Floating Microspheres- Ketoprofen is the drug given through this method. In this, microspheres remain buoyant in stomach due to bulk density been less than gastric fluid. The drug release is steady but at the desired rate, increases gastric residence and plasma

concentration variability if the system is floating on gastric content. It also minimizes the risk of striking and dose dumping, as well as providing a longer-lasting therapeutic benefit (40).

Radioactive Microspheres- These types of microspheres are injected in the arteries. High radiation dose is delivered to the target site without harming the neighboring tissues. Radio immobilisation therapy microspheres sized10-30 nm is of larger than capillaries and gets tapped in first capillary bed when they come across (41).

Polymeric microspheres -Polymeric microspheres are categorised into biodegradable polymeric microspheres and Synthetic polymeric microspheres (42).

Polymers used in the formulation of microspheres (43)-

1. **Synthetic Polymer**
   - **A) Biodegradable:**
     - Lactides and glycolides and their copolymer
     - Polyalkyl cyno acrylates
     - Polyanhydides
   - **B) Non-biodegradable:**
     - Epoxy Polymer
     - Acrolein
     - Poly methyl methacrylate
2. **Natural Polymer**
   - **A) Carbohydrates**
     - Agarose (Examples)
     - Dextran
     - Sodium Alginate
     - Alginic Acid
     - Starch
     - Chitosan
     - Cellulose
   - **B) Chemically modified/Semi synthetic carbohydrate**
     - Poly (acryl) starch
     - Poly (acryl) dextran

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1. INTRODUCTION

1.1. Novel drug delivery system

The novel drug delivery system development and formulation is an ongoing process in the fields of pharmaceutical research with nature of increasing the therapeutics effect of existing drug [1]. Microemulsions can be defined as these are the isotropic, transparent, thermodynamically association with a co-surfactant. The droplet size in the range between 10-200 nm [2].

These are the thermodynamically stable liquid systems, spontaneously formed. Due to this reason, these systems have advantages over conventional emulsions, Microemulsions having a wide range of prospective and real utilizations. Because of this reason at present microemulsions are subject of various investigations. These systems propose various advantages for oral administration, including increased absorption by decreasing toxicity and enhanced clinical potency. Microemulsions also shows potential tools as delivery systems, it offers various routes of administration and also offers both type of drug release controlled as well as sustain release [3-5].

1.2. History

Traditionally, the Microemulsion first discovered by Rodewal, in 1928 in the appearance of liquid waxes. The word micro-emulsion introduced by Hoar and Schulman in 1940.Microemulsions defined as these are transparent solutions prepared by titrating a normal coarse emulsion with medium chain alcohols. And, they also defined that these are the simplest carrier system due to their thermodynamic stability, the technology which is used to prepare is very simple [2, 6].

Hoar and Schulman prepared first Microemulsions by dispersing oil in an aqueous surfactants solution and by adding alcohol as co surfactant, by which formation of transparent stable formulation. Hence, Microemulsion can be defined as these are the clear, transparent and thermodynamically stable. These are the dispersions of oils and aqueous phase, and it is stabilized by surfactant repeatedly in the combination with a co-surfactant [7-8].

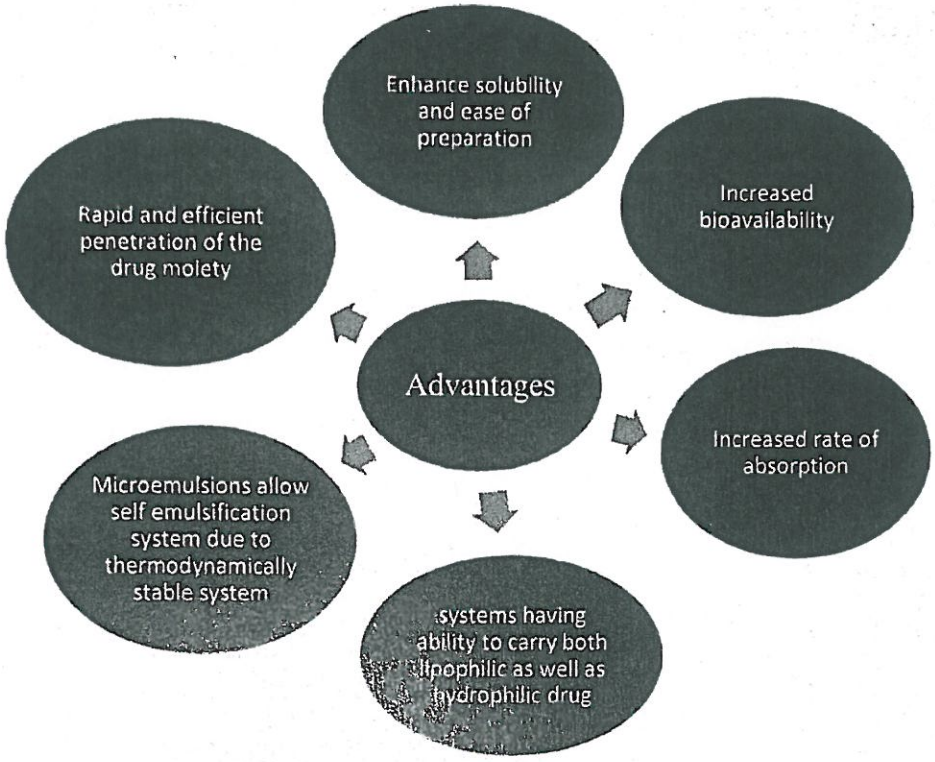

Fig1.2. Advantages of Microemulsions [10-15]

1.5. Disadvantages of Microemulsions [10-12]

1. The stability of micro-emulsion is affected by various environmental factors such as pH temperature, Humidity and light.
2. For stabilizing the droplets, it requires high concentration of surfactants.
3. These systems have limiting solubilizing capacity for substances which have high melting point.
4. Surfactants which are used must be non-toxic for the use of pharmaceutical application [10-12].

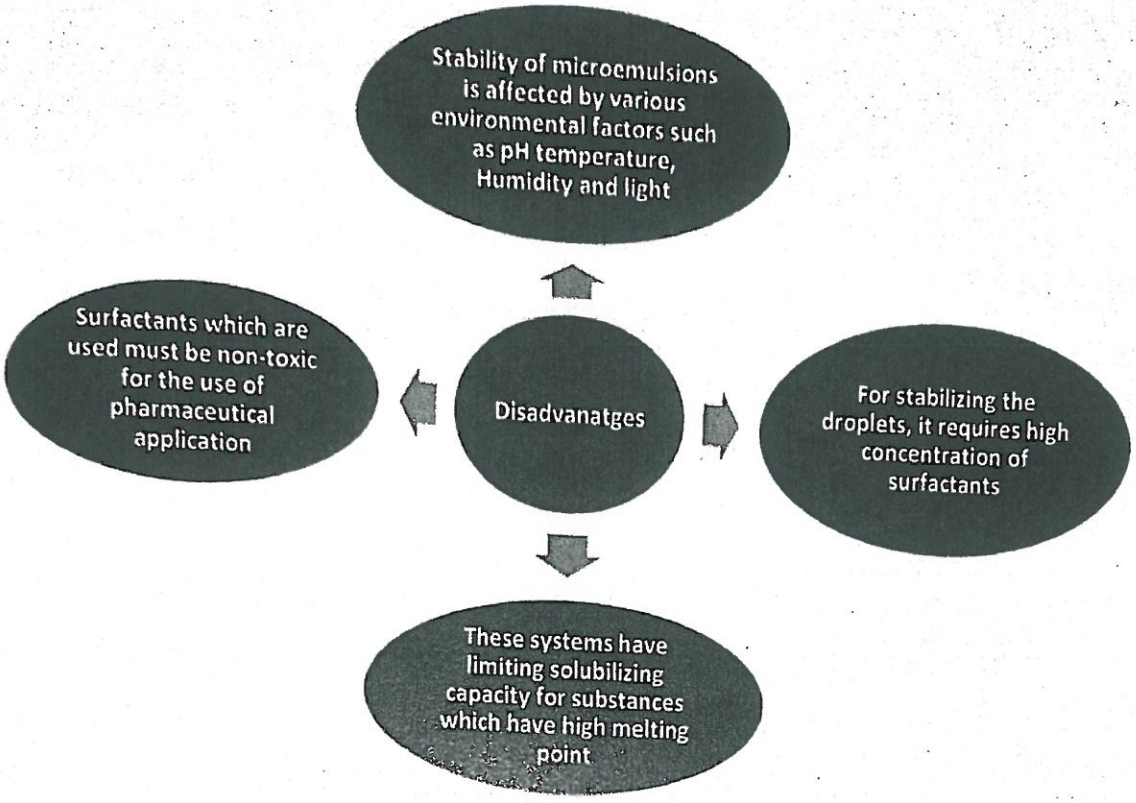

Fig 1.3.Disadvantages of Microemulsions [10-12]

1.6. Types of Microemulsion

Microemulsions are divided into three types which are discussed below:

1.6.1. Oil in Water Microemulsion

These are the microemulsions which are formed when there is a dispersion of oil droplets in the continuous aqueous phase.

1.6.2. Water in Oil Microemulsion

These are the microemulsions which are formed when there is a dispersion of water droplets in the continuous oil phase.

1.6.3. Bi-Continuous Microemulsion

These are the microemulsions which are formed when the water and oil droplets are in similar amount. The interface is balanced by a proper combination of surfactants and co surfactants in all the types of micro-emulsion. Wide range of phases and structures which depend on component proportion are formed from the mixtures of water, oil and surfactants [13-15].The most important factor in this regard is flexibility of the surfactants film and this film allow the formation of various structures such as droplet like shapes, bi-continuous structures, aggregates, and hence expand the range of micro-emulsion. Existence of bi-continuous structures will not

occur if the film is very rigid. Apart from microemulsions, structural evaluation gives the information about the regular emulsions, lamellar structures and cubic phases or anisotropic crystalline hexagonal structures which depends on the component's ratio [16].

1.7. Components of Microemulsion

There are four components of micro-emulsion which are named as oil, surfactant, co surfactants, and water as aqueous phase. Various oils and surfactants are available, used as the components of micro-emulsion system depending upon the toxicity, irritation potential and unclear mechanism of action. The components which are used must be non-toxic, biocompatible, and clinically acceptable and the emulsifier used in the proper concentration range that will gives mild and non-aggressive microemulsions. Hence, excipients used are **"Generally Regarded as Safe Excipients" (GRAS).**

1.7.1. Oil Phase

The oil components affected curvature by its penetration property and the tail group region of the surfactant monolayer swell. The oils which are short chain penetrate the tail group region to a larger level, which results in increased negative curvature (and decreased effective HLB).([36]. Saturated fatty acids such as capric acid, myristic acid, and lauric acid and unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid have properties of penetration enhancing of their own. Ethyl or methyl esters of lauric, myristic and oleic acids are the fatty acid esters and are used as oily phase. The drugs which are lipophilic get solubilized in oil in water micro-emulsion. The oil phase is selected on the basis of the highest solubility of drug in that type of phase.

1.7.2. Surfactants

The role of surfactants in the micro-emulsion is to lower the interfacial tension, which is responsible for its uniform and stable dispersion. They also provide a flexible film that can eagerly collapse in the region of the droplets. For the formulation of Oil-in-water micro-emulsion, surfactants with high HLB value (>12) are chosen; whereas for the formulation of water in oil micro-emulsion, surfactants with low HLB value are chosen. The surfactants which have HLB value more than 20 often need the existence of co-surfactants to decrease their effective HLB to a limit or value within the range of micro-emulsion formation.

1.7.3. Co surfactants

The surfactants which are single chain alone fail to decrease the oil in water interfacial tension adequately to facilitate a microemulsion to form. The existence of cosurfactants allow the interfacial film adequately flexibility to engage various curvatures which are necessary to form microemulsion of different range of composition. Commonly used or added cosurfactants are short to medium chain length alcohols (C3-C8).These cosurfactants decrease the interfacial tension and enhance the fluidity of the interface [17-24].

Table.1.1.Components of Microemulsion [25]

Oils	Surfactant	Co-surfactant
Soyabean Oil	Tween 80	Span 80
Sunflower Oil	Cremophore RH -80	Lauroglycol
Castor Oil	Polyoxy-10-hydrogenated castor oil	Transcutol
Sesame Oil	Labrasol	Capmul
Oleic Acid	Propylene Glycol	Span 20

1.8.Theories of Microemulsion Formulation

These formulations are the formulations which are based on some theories that control and affects their phase behavior and stability.These theories are:

1.8.1. Thermodynamic theory

The formulation and stability of microemulsion depends on the thermodynamic mechanism.The level to which surfactants decreases the surface tension of oil in water interface and alteration in entropy of the system decided the free energy of microemulsion formulation.Therefore,

$$DG f = \gamma DA - T DS$$

Where;

DG f-Free energy of formation,

γ-Surface tension of the oil in water interface,

DA-Change in the interfacial area on microemulsification

DS-Change in entropy of the system which is effectively the dispersion entropy,

T-Temperature

According to Pranjal Kumar Singh and its coworkers, it is established that formation of a microemulsion when DA is changed to a great level because of the huge number of formation of very small droplets.It is required that when the γ value is positive at all times,it is very small and is counterbalance with the help of entropic component.A very huge dispersion entropy which is also called dominant favorable entropic contribution, these are obtained by mixing of one phase to the other in the form of huge number of small droplets. On the other hand, favorable entropic contributions also obtained from differemt type of other dynamic processes named as surfactant diffusion and monomer micelle surfactant exchange in the interfacial layer. When there is more decrease in surface tension set up due to significant favorable entropic change, formation of negative free energy obtained.It follows that the microemulsification is spontaneous and the consequential dispersion is thermodynamically stable.

1.8.2. Solubilisation Theory

The microemulsion formulation is water phase and oil soluble phase by means of micelles or reverse micelles in micellar which is step by step turn into bigger and swelling to a definite size range outcome.

1.8.3. Interfacial Theory

The interface mixed film theory is also called the negative interfacial tension theory. According to Interfacial theory, the microemulsion are able to form immediate and sudden create a negative interfacial tension in the surfactant and cosurfactant in functioning simultaneously.The film which contains surfactants and cosurfactants, that molecule known as a liquid "two dimensional" and the third phase in balance with both water and oil.This type of monolayer called as a duplex film which gives a number of properties on the oil side as well as water side. According to the duplex film theory,interfacial tension γT is expressed by the following equations:

$$\gamma T=\gamma(O/W)---\pi$$

Where;

$\gamma(O/W)a$=Interfacial tension (Which is reduced by the presence of alcohol)

$\gamma(O/W)a$ which is considerably lower than $\gamma(O/W)$ in the absence of alcohol [26-30].

1.9. BCS Classification system:

The major and chief objective of BCS classification is to enhance the drug development's effectiveness.It also helps in gather the challenges of formulation design, it also gives information of in vivo pharmacokinetic performance of drug products with the help of measurement of permeability and solubility.For Biowaiver condition,yielding of in vivo bioequivalence studies.

Class I Drug	Class II Drug
High Solubility High Permeability Examples: Paracetamol, Metoprolol,Propranolol, Verapamil.Theophylline	Low solubility High permeability Example: Carbamezpine,Griseofulvin.Ketoconazole,
Class III Drug	**Class IV Drug**
High solubility Low Permeability Example: Acyclovir,Atenolol,Cemitidine,Rantidine	Low Solubility Low permeability Example: Furosemide, Hydrochlorothiazide.

Table 1.2. The Biopharmaceutical classification system (BCS) of drug [31-34].

1.10. Factors influencing Microemulsions

The following elements will influence the production of microemulsion:

1.10.1. Packing ratio

The type of microemulsion is determined by the surfactant's HLB. Molecular packing and film curvature are affected. Curvature of film analysis for surfactant interaction that leads to microemulsion creation

1.10.2. Surfactant properties, oil phase, and temperature

The nature of the surfactant determines the type of microemulsion. Surfactant contains a lipophilic tail group and a hydrophilic head group. These are the areas of these group, which is a measurement of the water's differential tendency to swell at the head. When it comes to specialised formulation, having a group and oil to inflate the tail area is crucial. Calculating the surfactant HLB in a specific system.

When a high concentration of surfactant is applied, or when the surfactant is in the presence of salt, the degree of polar group dissociation is reduced, and the resulting system may be w/o type. Water diluting can promote dissociation and result in an o/w system. Temperature has a big impact on ionic surfactants. Increased surfactant counter ion dissociation is the key effect. The capacity of the oil component to enter and so swell the tail group region of the surfactant monolayer also effects curvature. Short chain oils penetrate deeply into the lipophilic group region, resulting in enhanced negative curvature.

The effective head group size of nonionic surfactants is highly dependent on temperature. They are hydrophilic at low temperatures and create a typical o/w system. They are lipophilic at higher temperatures and form w/o systems. Microemulsion coexists with surplus water and oil phases at an intermediate temperature, forming a bicontinuous structure.

1.10.3. Co-surfactant chain length, kind, and nature

Alcohols are commonly utilised as co-surfactants in microemulsions. Shorter chain co-surfactant has a positive curvature effect because alcohol swells the head region more than the tail region, making it more hydrophilic and favouring the o/w type, whereas longer chain co-surfactant favours the w/o type because alcohol swells the chain region more than the head region [35]

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This is to certify that research work embodied in this thesis entitled "Formulation and Evaluation of Azithromycin Loaded Alginate-Gum Ghatti Interpenetrating Polymer Network Based Sustained Release Antimicrobial Microspheres" submitted to K. R. Mangalam University, Gurugram, Haryana, for the award of the degree of **M. Pharmacy (Pharmaceutics)** has been carried out by Nikita Yadav under at Department of Pharmaceutics, School of Medical & Allied Sciences, K. R. Mangalam University from September 2021 to August 2022.

To the best of my knowledge and belief, this work is original and has not been submitted so far in part or in full for the award of any degree or diploma of any University/ Institute.

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FORMULATION AND EVALUATION OF AZITHROMYCIN LOADED ALGINATE-GUM GHATTI INTERPENETRATING POLYMER NETWORK BASED ANTIMICROBIAL MICROSPHERES

Thesis Submitted For the Award of the Degree of

MASTER IN PHARMACY

Pharmaceutics

By

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INDIA

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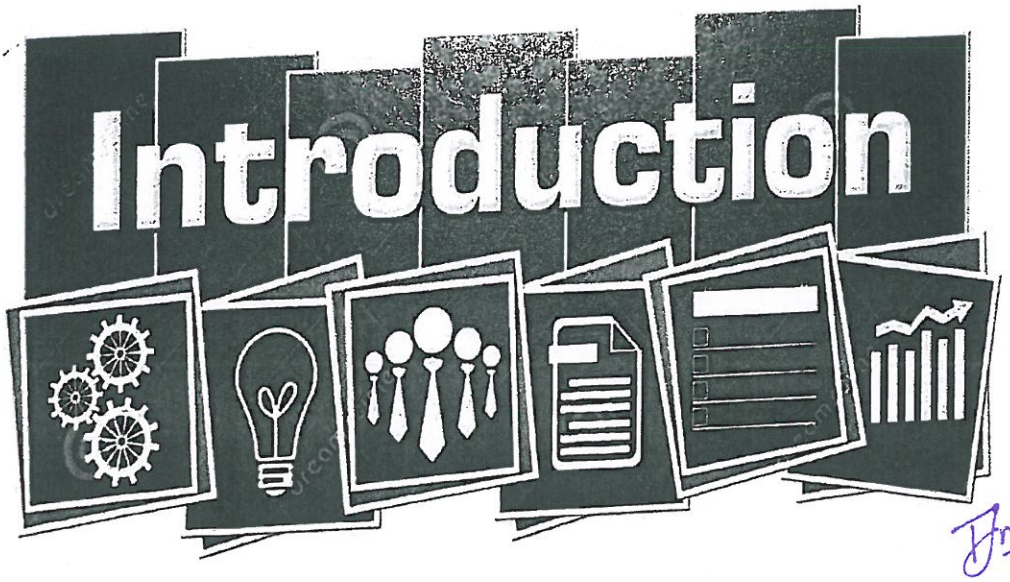
Chapter 1

INTRODUCTION



INTRODUCTION

1.1.Definition of Novel Drug Delivery System (NDDS)

Drug delivery refers to the process of administering a pharmacological or medical medication to achieve the desired therapeutic outcome. Regarding a drug's effectiveness, the delivery method is important. Products that combine medication and devices or are medical devices are examples of new drug delivery systems. In the creation of new drug delivery systems, polymer science, pharmaceutics, and molecular biology are all utilised (NDDS). A medication's effectiveness can be significantly impacted by how it is given. Some drugs have a range of optimal concentrations within which they work best, while doses outside of this range may be toxic or have no therapeutic benefit at all [1].

There are various advantages of novel drug delivery system over conventional dosages [2]. These systems are basically to achieve controlled or targeted release of drug and provides maximum therapeutic efficacy and cause less side effects, so it becomes essential to deliver the drug at the target site in proper amount at a proper period of time [3]. And therefore, carrier technology provides an innovative method for delivery of drug by attaching the drug with carrier particles like liposomes, nanoparticles, implants, etc which enhances absorption as well as release properties of the drug and microsphere also from this carrier drug delivery system [4,5].

1.2. Advantages of novel drug delivery system: - [6]

- Provides protection from physical and chemical degradation.
- Provides sustained delivery.
- Protects from toxicity.
- Improves stability and enhance bioavailability.
- Improved tissue macrophages distribution.
- Improves stability and solubility.

1.3. Microspheres in drug delivery system

Microspheres are free flowing particles having solid spherical shape and size in the range of 1-1000µm and sometimes the word microparticles is also used as synonym for microspheres [7]. These are consisted of synthetic polymers or proteins having biodegradable nature and are used for targeted delivery of antibiotics, hormones, vaccines and drugs and holds an easier valuation of mass transfer behaviour and diffusion and offers large surface area [8]. Microspheres for oral use have also come in market to enhance absorption of drug. Two types of microspheres are: -

1. Micromatrices - entrapped matter is dispersed all over the matrix of microspheres.
2. Microcapsules – entrapped matter is particularly enclosed by distinct capsule wall.

Solid biodegradable microspheres have the capacity for controlled release of drug which is dispersed through the particle matrix. These are formed from waxy, polymeric and some protective materials, which is, altered natural products and synthetic polymers which are biodegradable [9].

1.4. Ideal properties of microspheres

1. Reduces toxicity
2. Biocompatible
3. It can integrate high concentration of drug
4. Long shelf life
5. Enhances therapeutic efficacy
6. Sterilizability
7. Release of active reagent with a good control over a broad time scale [10].

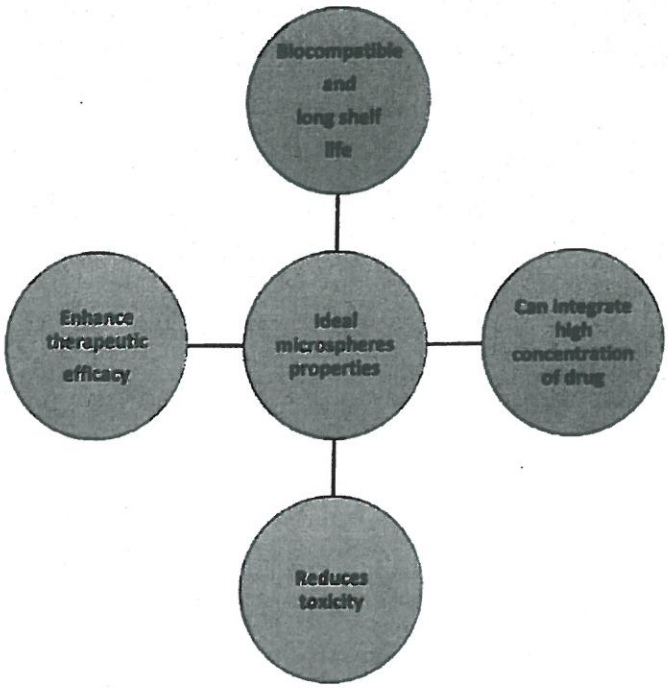


Fig 1.1: Ideal properties of microspheres

1.5. Advantages of microspheres

- High patient compliance by reducing dosing frequency.
- Reduction of dose and risk.
- Protects the gastrointestinal tract from the opioid irritants.
- It provides prolonged and constant therapeutic effect.
- Reduction in size enhances the surface area and the strength of poorly soluble substances.
- Convert the liquid in solid form and mask the unpleasant taste.
- Enhances the bioavailability and absorption of drug and provides controlled and targeted delivery of drugs [11,12].

1.6. Disadvantages of microspheres

- There is a release rate difference from one dose to another.
- These type of dosage forms should not be chewed or cracked.
- The transformed release from the preparations.
- Controlled release preparations basically have high load of dose.

The rate of release of controlled dose fluctuates from factors such as transfer level through gut and diet [13].

1.7. Types of microspheres

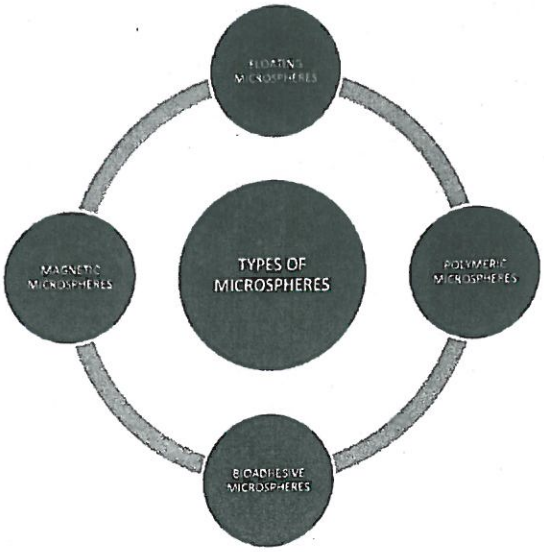


Fig 1.2: Types of microspheres

1.7.1. Bioadhesive microspheres- adhesion may be described as drug adherence to the membrane by the help of adhesion characteristics of polymers which are soluble in water [14]. The delivery system which utilises the bio adhesive property of few of the polymers which adhere during hydration and hence provides extended residence time at the target site and therefore provides better patient compliance and improves bioavailability [15-18].

Ye Zhang et.al formulated chitosan coated alginate/ gelatin microspheres loaded with Berberine hydrochloride and evaluated them for their pharmaceutical characteristics and pharmacokinetics. These bio adhesive microspheres were prepared by emulsification method. Three batches were prepared and then evaluated for stability and are used for sustained delivery to treat duodenal and benign gastric ulcers. https://doi.org/10.1155/2016/4235832

1.7.2. Floating microspheres– These microspheres are tiny and hollow having no center and are free flowing cells which differ in range from 1-1000 µm. The bulk density in this type of microsphere is less than gastric fluid so these stay buoyant in stomach without disturbing the gastric emptying rate [19]. The drug is released deliberately at the desired rate, if the system is floating on

gastric content and therefore increase variation in plasma concentration and gastric residence time [20]. It decreases the chances of dumping of dose and diminishes the dosing frequencies and offers extended therapeutic effect [21,22].

Patel A et al. (2006) prepared and evaluated the floating microspheres of metformin hydrochloride and optimized drug release pattern to match target release profile. By the use of non-aqueous emulsification solvent evaporation method using ethyl cellulose floating microspheres were formulated and which extended the release of drug for at least 8 hours in stomach hence enhancing the patient compliance and bioavailability.

1.7.3. Magnetic microspheres– These are molecular particles which localises the drug to the target site and are very short enough to cross the capillaries without creating an oesophageal obstruction [23]. In this system freely circulating drug present in huge amount can be substituted by a small volume of magnetically targeted drug. Magnetic responses to a magnetic field are received from magnetic carriers from the incorporated substances which are used in magnetic microspheres are dextran, chitosan and many more [24,25]. There are two types of magnetic microspheres which are: - 1. Diagnostic microspheres 2. Therapeutic magnetic microspheres

Fengxia Li et al., formulated magnetic polylactic acid microspheres loaded with curcumin by O/W emulsion solvent evaporation method to obtain a targeted drug delivery system. FTIR was used to characterize functional groups. Scanning electron microscopy was used to check morphology of microspheres while dynamic light spectroscopy for size distribution of microspheres. The microspheres were found spherical with smooth surface with a diameter of 0.55–0.75 μm and showed sustained release effect on in vitro drug release.

https://doi.org/10.1016/j.jmmm.2011.05.045

1.7.4. Polymeric microspheres– These microspheres are of two types and are classified as follows: - [26]

1. Biodegradable polymeric microspheres
2. Synthetic polymeric microspheres

Fajun Zhao et al., in a low-permeability reservoir by using polymeric microspheres to improve in-depth profile control. Distillation precipitation was utilised to create polymeric microspheres with a nanometer-sized particle size. Infrared spectroscopy, scanning electron microscopy, thermogravimetry, high-pressure and high-temperature rheometry, and dynamic light scattering were employed to test and analyse the structure, apparent pattern, thermal endurance, particle size, hydration, and swelling capability of the microspheres. With a centred size distribution, the synthesised polymeric microspheres were all uniformly round. https://doi.org/10.1155/2020/5279608

- **BIODEGRADABLE POLYMERIC MICROSPHERES-** Starch which is a natural polymer is used with the perception as they are biocompatible, biodegradable, and bioadhesive. These biodegradable polymers extend the residence time when they interact with the mucous membrane as it has great swelling properties with the aqueous media which further results in the development of gel. By the concentration of polymer, the extent and rate of drug is controlled in sustained way. The drug loading efficiency of biodegradable microspheres in clinical use is very complicated and is problematic to control the drug release and this is the major disadvantage of these microspheres [27,28].
- **SYNTHETIC POLYMERIC MICROSPHERES** – These microspheres generally have clinical applications and also used as fillers, embolic particles, and bulking agents. These can also be used as drug delivery vehicles. These are safe and biocompatible. But their major drawback is that they have a habit of migrating away from the site of injection and leads to potential risk which results in organ damage [29].

1.8. Methods of preparation of Microspheres are-

- Emulsion cross linking method
- Emulsion solvent evaporation technique
- Multiple emulsion method
- Spray-drying method
- Emulsion solvent diffusion technique

1.8.1. Emulsion cross linking method- In this method, the medication was dissolved in an aqueous gelatine solution that had been heated at 40°C for 1 hour. The solution was added drop by drop to liquid paraffin while swirling at 1500 rpm for 10 minutes at 35 degrees Celsius, resulting in a w/o emulsion, which was then stirred for another 10 minutes at 150 degrees Celsius. Thus, the microspheres were washed three times with acetone and isopropyl alcohol, then air dried and dispersed in 5mL of aqueous glutaraldehyde saturated toluene solution at room temperature for three hours for cross linking, and then treated with 100mL of 10mm glycine solution containing 0.1 percent w/v of tween 80 at 370 C for ten minutes to block unreacted glutaraldehyde. Example of this technique is Gelatin A microspheres.

Somasree Ray *et al.,* developed glutaraldehyde crosslinked interpenetrating network microspheres of gum ghatti loaded with Glipizide by emulsion cross linking method. The drug entrapment efficiency was found to be 92.85±1.5%. The development of IPN structure was confirmed by FTIR. By controlling the concentration of cross-linking agent, the release of drug can be extended for about 7 hours. To recognize the phenomena of water penetration through the microspheres swelling studies and diffusion coefficient of water transport were carried out. Within the first 3 hours of administration, the initial percentage of reduction of blood glucose level was slow in the case of rats treated with Glipizide loaded IPN microspheres. Thus, it was confirmed that these microspheres were suitable for sustained delivery. **DOI:** 10.2174/1567201816666191017154719

1.8.2. Emulsion solvent evaporation method- According to this method, the medication is first dissolved in polymer that has previously been dissolved in chloroform, and the resulting solution is then added to an aqueous phase that contains sodium PVP at a concentration of.2 percent as an emulsifying agent. The mentioned mixture was stirred at 500 rpm, and the drug and polymer (eudragit) were then converted into tiny droplets. These droplets then solidified into rigid microspheres as a result of solvent evaporation, and they were collected by filtration, washed with demineralized water, and dried at room temperature for 24 hours [30].

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THE COMPLETE WORLD OF EDUCATION

CERTIFICATE

This is to certify that the dissertation entitle ***"Design and development of odorless cream of Allium cepa for topical application"*** submitted to K.R. Mangalam University in partial fulfilment of the requirement for the award of Degree of **MASTER IN PHARMACY** in Pharmaceutics, embodied the original research work carried out by **Ms. Garima soni** at **K.R. Mangalam University** under our supervision and guidance.

It is further stated that no part of this dissertation has been submitted, either in part or full for any other degree of K.R. Mangalam or any other university/institution.

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DESIGN AND DEVELOPMENT OF ODORLESS CREAM OF ALLIUM CEPA FOR TOPICAL APPLICATION

Thesis Submitted to

K.R.Mangalam University in partial fulfilment of the requirement for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

By

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ABSTRACT

Skin is the human body's outermost organ. As a result the appearance of their skin is well understood and highly sensitive. Skin is also aesthetic important. A centuries-old quest of human was the desire to have beautiful and healthy skin. The skin on our feet is naturally dry, unlike the skin on the rest of the body. The skin on our feet has no oil glands, so it relies on hundreds of thousands of sweat glands to keep our feet moisturized. Negligence towards feet can lead to different disorders generally due to improper footwear, and one can suffer from infection because of the external penetration of the dirt, fungi, bacteria through these cuts and wounds.

Since the time of the Vedas, various herbs have been used to treat various diseases and skin conditions. Onion (Allium cepa L.) is a well-known medicinal herb eaten for generations for its suspected health and nutritional advantages. This vegetable is frequently worldwide. Herbal medicines are a key source of health care and traditional herbal practice is regarded an intrinsic component of the community. Allium Cepa has several active compounds that are physiologically active, have excellent anti-inflammatory, immune modulatory, and wound healing actions.

The objective of this research is to produce herbal cream containing extract of Allium cepa L. bulb and it was then formulated to cream using the fusion method. The formula was evaluated for various physical evaluations such as physical appearance, odour, after feel, pH, spreadability, viscosity, cream texture and Microbial Growth Test. The extract and cream were then subjected for Stability tests for 1 month at 25 °C /60% RH. The study has showed that the formulated herbal cream showed that the cream had a cosmetically appealing appearance and has a good texture. The study has also reported a significant reduction in the concentration of microorganisms i.e., E. coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans.

Key words: Allium cepa L., Herbal Cream, Formulation, Herbal Phytomedicines

1 INTRODUCTION

1.1 Skin

Skin is the human body's outermost organ. As a result the appearance of their skin is well understood and highly sensitive. Skin is also aesthetic important. A centuries-old quest of humans was the desire to have beautiful and healthy skin. Skin with a higher complexion and a smoother surface is seen to be healthier and appealing (Igarashi et al. 2007).

The skin's main role is to protect the body against the external environment and maintain homeostasis between the internal and external body. The skin and hair appearance is the 'first image' of us. The task of modern cosmetology is to interact with physiology in order to maintain "good condition" with personal expression changes with changes of condition of our hair and skin. (Celleno & Tamburi 2009)

1.1.1 Layers of Skin and their Functions

Human skin composed of three layers firstly named as epidermis, which is an outermost layer, secondly beneath epidermis is called as dermis and thirdly as the hypodermis or subcutaneous layer. The epidermis is keratinized in nature and composed of cuboidal shape squamous stratified epithelial cells. It is avascular in nature, providing protection to cells. Skin is composed of four layers refereed as thin skin representing Stratum Basale, Stratum Granulosum, Stratum spinosum and Stratum corneum and thick skin referred as Stratum Lucidum. The second layer referred to as the dermis, vascularized and provides support and flexibility to the tissues and composed of a dense network of connective tissues having blood vessels, sweat glands and hair follicles. The innermost layer called as hypodermic composed loose connective tissues. Adipocytes having lipid storage effects, serves as energy reserves, regulate temperature and provide insulation in the body. This layer is highly vascularized in nature (https://opentextbc.ca/anatomyandphysiology/chapter/5-1-layers-of-the-skin). The different layers of skin are explained in figure 1.1.

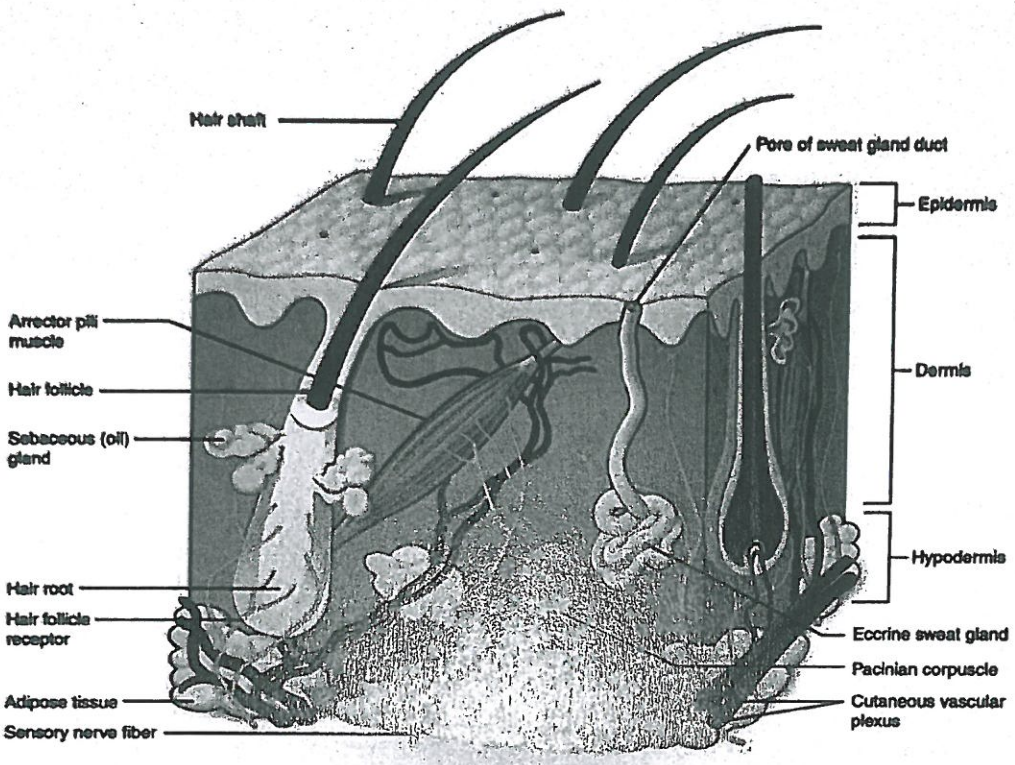

Figure 1.1: Different layers of skin

Skin is composed of three primary layers:

- The Epidermis, a barrier of infection, which offers waterproofing.
- The dermis, used as a site for skin appendages.
- The subcutaneous layer of adipose hypodermis.

Layers of Epidermis:

The epidermis is split into multiple layers in which cells originate in the innermost layers by mitosis. Keratinocytes are the predominant type of epidermis and come from the basal layer, generate keratin and form an epidermal water barrier through lipid production and secretion. The activation of UVB light cholesterol precursors also regulate the calcium absorption by the form of vitamin D. The keratinization process occurs in a few weeks. The epidermis is made up of 25 to 30 layers of dead cells. Epidermis does not include a blood vessel and therefore it depends fully on dermis to supply nutrient to the underground membrane and dispose of waste. Epidermis' main

function is to be physical and the external biological barrier for the environment. (Yousef H et al. 2017)

Sub-layers

The following 5 sub-layers or strata divide the epidermis:

- Stratum corneum
- Stratum lucidum
- Stratum granulosum
- Stratum spinosum
- Stratum germinativum

DERMIS

Dermis is a tissue-connective layer between the epidermis and the hypodermis. The Dermis layer constitutes the bulk of the skin and protects the bulk.

The dermis is made up of two layers: The more superficial papillary dermis and the deeper reticular dermis. The thinner layer of the papillary dermis is a loosely-connected tissue containing capillaries, elastic fibres and collagen. A thicker layer of dense connective tissue contains larger blood vessels closely interlaced with elastic bundles of collagen. The reticular dermis contains thicker collagen bundles. (Yousef H et al. 2017)

HYPODERMIS

The hypodermis consists mainly of fat, the subcutaneous layer below the dermis and is referred to as subcutaneous fascia. It is the deepest skin layer, contains adipose lobules, together with certain additives to the skin, such as hair follicles, sensory neurons, and blood vessels. It provides the skin's main structural support and protects the body from cold and helps to absorb shocks. The blood vessels and the nerves interlink it with. (Yousef H et al. 2017)

1.1.2 MECHANISM OF DRUG ABSORPTION THROUGH SKIN

The dosage form releases the drug and a series of steps governs its path towards the dermis for treatment of skin disorders. Therapeutic Phytoconstituent partition and diffuses into the dermis after penetration through epidermal layer. The phytoconstituent reaches systemic circulation and

used in the treatment of skin disorders. A graphical representation of its mechanism (Barry et al., 1991) is summarized in Figure 1.2.

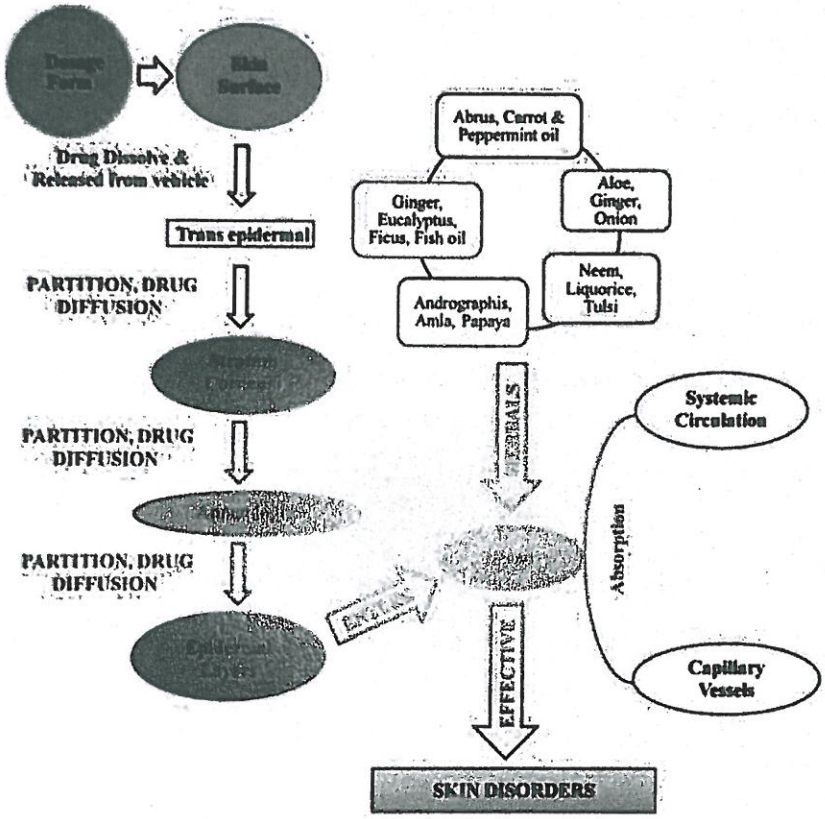

Figure 1.2: Mechanism of drug adsorption through skin

1.1.3 SKIN OF THE FEET

The skin of the feet has a structure and function in other areas of the body with certain significant differences. The skin on the foot is thicker than that of the rest of the body; the most outside epidermis on the sole is hard and may grow to 5 mm in thickness, 20 times the thickness of most of the other areas of the body as a result of the stress and strain adsorbed in an upright position.

With this heavily compressed type of structure, it is clear that, compared to other cosmetics, the skin of the feet, in particular the sole, demands specific products with greater water binding levels. (Celleno L et al. 2009)