WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 16, 1132-1184. Research Article ISSN 2277–7105

FORMULATION AND EVALUATION OF AVOCADO SOYBEAN GEL FOR TOPICAL DELIVERY SYSTEM

Aman Thakur, Puneet Kumar, Jasbir Kumar, Supneet Kaur*, Evinka Barjataya and Naresh Singh Gill

Rayat Institute of Pharmacy, Railmajra, SBS Nagar, PB.

Article Received on
02 July 2024,

Revised on 22 July 2024,
Accepted on 12 August 2024

DOI: 10.20959/wjpr202416-33621

***Corresponding Author**
Supneet Kaur
Rayat Institute of Pharmacy,
Railmajra, SBS Nagar, PB.

ABSTRACT

The aim of the current study is to explore the possibility of formulating a Avocado Soybean topical gel for osteoarthritis. Avocado is a fruit that is known as for its creamy texture and healthy monosaturated fats. Soya is an excellent dietary addition for patients with joint pain. It is rich in omega-3 fatty acids, significant it may reduce inflammation within the body. Carbopol is commonly used in the formulation of herbal gel to improve their consistency, stability and performance. Avocado soybean unsaponifiable are a natural extract made from avocado and soybean oils and it is also responsible for cartilage protection, pain relief, joint function improvement, cardiovascular activity, skin health and anti-inflammatory activity. Preparation of gel is done by incorporation method. percentage drug entrapment was found was 82.20±0.14. particle size of 236.4nm, 21.2%, zeta potential -22.6mV. The gel showed 91.32±0.25 release after 24 hours in a controlled manner.

KEYWORDS: Osteoarthritis (OA), Avocado Soybean, Gel, Bioavailability.

1. INTRODUCTION

Topical administration is an appealing option for both local and systemic treatment. Drug administration to the skin is acknowledged as an effective method of treating local dermatologic disorders. It can penetrate deeper into the skin, resulting in improved absorption.[1]

In the process of creating topical dosage forms, efforts have been made to incorporate drug carriers that guarantee sufficient localization or penetration of the medication through the

skin, either to maximize local effects and reduce systemic effects, or to guarantee sufficient percutaneous absorption. Topical preparation reduces GI discomfort, stops the medicine from being metabolized in the liver, and increases the drug's bioavailability. Drugs have been administered to the human body by a variety of routes during the past few decades, including oral, sublingual, rectal, parental, topical, and inhalation.[2]

Advantages of topical drug delivery systems (TDDS)

- Steer clear of the metabolism on the first pass.
- Practical and simple to use.
- Apply medication more precisely to a designated location.
- Steer clear of gastrointestinal incompatibilities.
- Following the provision of medications with a limited therapeutic window and a short biological half-life increased adherence from patients.
- Offer appropriateness for self-medication

Disadvantages of topical drug delivery systems

- Limited drug penetration through the skin.
- Drugs with bigger particle sizes are more difficult to absorb through the skin's barrier.
- The potential for allergic responses.
- They are only appropriate for use with medications whose mechanisms of action depend on extremely low plasma concentrations.
- The route of certain medications is inappropriate for medications that cause skin irritation or sensitization.

1.1 Anatomy of skin

The skin is the biggest organ in the body. It is composed of three layers. The epidermis is the outermost layer; dermis is the intermediate layer; and hypodermis is the innermost layer. Skin has a normal pH of 4.5 to 6.0. With its 15% of the adult body weight, the skin is the biggest organ in the body. It carries out a multitude of essential tasks, including as preventing the body from losing too much water and aiding in thermoregulation, in addition to providing defense against external physical, chemical, and biological threats. The mucous membranes that line the surface of the body form the continuous skin.[3] The human skin is composed of two different layers: the dermis directly underneath the epidermis. The connective tissue matrix that houses the nerves, hair follicles, pilosebaceous units, and sweat glands makes up

the highly vascular dermis. The stratum corneum, the outermost layer of the avascular epidermis, is made up of dead, keratin-rich epidermal cells known as corneocytes that are encased in a lipid-rich matrix.[4]

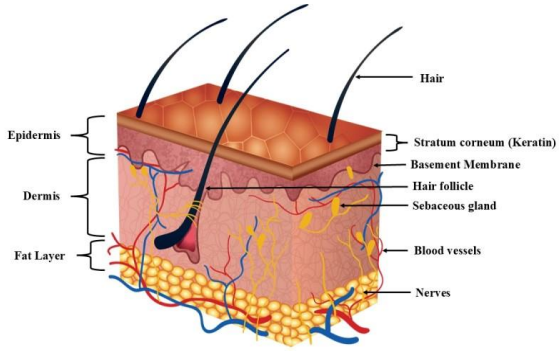


Fig. 1.1: Anatomy of skin (Layers of the skin).

1.1.1 Epidermis

The outermost layer of skin, known as the epidermis, is characterized as a stratified squamous epithelium, mainly made up of keratinocytes at varying stages of development.[5] Keratinocytes are the main cells that make up the epidermis and are responsible for producing the protein keratin. Because the epidermis lacks blood arteries, it is vascular and depends on the dermis underneath it for waste removal and nutrition delivery via the basement membrane. The primary purpose of the epidermis is to serve as a biological and physical barrier to the outside world, stopping allergens and irritants from penetrating. It also keeps the body's internal balance intact and stops water loss.[6,7]

The layers of epidermis are[8]

- Stratum corneum (Horny layer);
- Stratum lucidum (Only found in thick skin - that is, the palms of the hands, the soles of the feet and the digits);
- Stratum granulosum (Granular layer);
- Stratum spinosum (Prickle cell layer);
- Stratum basale (Germinative layer).

- **Stratum corneum (Horny layer)**

Imagine the stratum corneum as a wall made of bricks, with lamellar lipids serving as the mortar and corneocytes as the bricks. Corneocytes are able to draw in and retain water since they are endowed with a natural moisturizing component. Because of their high-water

content, corneocytes swell, maintaining the stratum corneum's elasticity and avoiding the development of fissures and cracks.[9] When administering topical drugs to the skin, this is a crucial factor to take into account. These enter the subcutaneous absorption process, which passes through the epidermal barrier into the underlying tissues and structures before entering the systemic circulation. The amount and pace of percutaneous absorption are controlled by the stratum corneum. The humidity level of the surroundings and the hydration of the skin are two of the key variables influencing this. Medication can only enter the stratum corneum of healthy skin with normal hydration levels by getting past the tight, comparatively dry lipid barrier that separates each cell. Percutaneous absorption will increase in cases of skin illness, excoriations, erosions, fissuring, or prematurity that compromise the normal skin barrier or increase skin moisture.[10]

- **Stratum basale (Germinative layer)**

One melanocyte for every ten basal cells is the ratio of melanocytes to keratinocytes along the basement membrane, which is found in the stratum basale. They create the pigment melanin, which is derived from the amino acid tyrosine. Melanosomes are cellular vesicles that are packed into and supplied into the cytoplasm of keratinocytes.[11] Melanin's primary job is to absorb ultraviolet (UV) light so that we are shielded from its damaging effects. The quantity and size of melanosomes, not the number of melanocytes, influence skin colour. Numerous pigments, including as melanin, carotene, and haemoglobin, have an impact on it. The amount of melanin produced by melanocytes in the stratum basale and taken up by keratinocytes determines the colour of the skin because melanin is transported into the keratinocytes via a melanosome. Melanin occurs in two primary forms:

- Eumelanin – Exists as brown and black;
- Pheomelanin – Provides a red colour. In addition, skin colour is impacted by hormonal, genetic, and UV radiation exposure.[12]

1.1.2 Dermis

The dermis, which makes up the skin's inner layer, is significantly thicker than the epidermis (1-5 mm). The dermis is located between the subcutaneous layer and the basement membrane zone. Its main function is to maintain and nourish the epidermis. The dermis's primary functions are:

- Protection
- Protecting the deeper structures from damage caused by machines

- Providing nourishment to the epidermis
- Having a significant impact on the healing of wounds.

Its main component, the network of interlacing connective tissue, is primarily composed of collagen with a small amount of elastin. Numerous specialized cells and structures, including blood arteries, lymphatics, sweat glands, and nerves, are dispersed throughout the dermis. These cells include mast cells and fibroblasts. Although they are located in the dermis or subcutaneous layers, the epidermal appendages are connected to the skin's surface. The dermis is made up of two layers:

- The dermal layer of the papillary
- The reticular dermis, which is deeper

1.1.3 Classifications of topical drug delivery systems

- **Solid preparation:** Plaster ointments, poultices, and topical powders.
- **Semi solid preparation:** Creams, Poultices, Gels, Pastes, ointment.
- **Liquid preparation:** Solution, tinctures, Emulsions, Lotions, Suspensions, Paints, Liniment.
- **Miscellaneous preparation:** Topical aerosol, liquid cleanser, rubbing alcohol, tapes and gauzes, and transdermal medicine delivery devices.

1.2 Factors affecting topical absorption of drugs

Drug absorption in tropical regions is considered with reference to physiological and physicochemical factors.

Physiological factors

- Skin thickness.
- Lipid content.
- Hair follicle density.
- Sweat gland density.
- The skin's pH.
- The flow of blood.
- Hydration of the skin.
- Skin inflammation.

Physicochemical factors

- Partition coefficient.
- Molecular weight
- Degree of ionization
- Effect of vehicles.[13]

1.2.1 Considerations for selecting a topical preparation

- Ointments and creams containing water and oils have a lower potential for irritation or sensitization overall, but gels irritate. Ointments are inappropriate for you if you are allergic to emulsifiers or preservatives.[14]
- The type of preparation should match the type of lesions.
- Fit the type of preparation for the environment. (A gel or lotion, for instance, for areas that are hairy).[15]

Advantages

- Hydrophobic medications can be effortlessly incorporated into gels by using d/o/w emulsions.
- Enhanced stability.
- There won't be a lot of sonication.
- Avoid using first-pass metabolism.
- More concentrated in one area.
- Avoid consuming items that are not suitable for your digestive system.
- Increasing the patient's inclination to follow instructions.
- It is feasible to self-medicate.
- Using a medication with a limited therapeutic window and a brief biological half-life.
- The capability to discontinue taking medication with ease.
- Administer medication according to the patient's need.
- Easier to prepare than complex to manufacture.
- A greater capacity for loading
- The release is controlled.
- Easy to use and practical.[16]

Disadvantages

- An excessive amount of skin irritation

- The possibility of an allergic reaction.
- Certain medications have a low skin permeability.
- Drugs with large particles are challenging for the skin to absorb.
- Skin irritation is a result of contact dermatitis.
- The formation of a bubble in the emulgel formulation process.[17]

1.3 Gel

Gels are characterized as semi rigid systems in which a three-dimensional network of interlacing particles or solvated macromolecules of the dispersed phase restricts the mobility of the dispersing medium. The term "gel" comes from "gelatin," and the Latin words "gelu," which means "frost," and "gel are," which means "freeze" or "congeal," are the origin of the word's "gel" and "jelly." This source illustrates the basic concept of a liquid setting to a solid-like substance that is elastic and retains some liquid properties but does not flow.

According to the USP, gels, also referred to as jellies, are semisolid systems that comprise either big organic molecules interpenetrated by a liquid or suspensions made up of minute inorganic particles. Gels that have a network of tiny, distinct particles inside their mass are categorized as two-phase systems. When a two-phase system has a dispersed phase with relatively large particle sizes, the gel mass is referred to magma. Organic macromolecules are evenly distributed throughout a liquid in single-phase gels so that there are no discernible borders between the liquid and the distributed macromolecules. Because gels have a larger density of physical bonds, more covalent crosslinks, or are just less liquid than jellies, they are often thought to be stiffer than jellies.

1.3.1 Structure of gel

The network created by the interlinking of the gelling agent particles gives a gel its stiffness. The composition of the particles and the kind of force forming the connections define the network's structure and the gel's characteristics. Hydrophilic colloid's individual particles might be solitary macromolecules or spherical or isometric clumps of tiny molecules. Strong primary valencies, like in silicic acid gels, to weaker hydrogen bonds and van der Waals forces are examples of the forces of attraction that link the gelling agent particles together. The fact that gel frequently liquefies at very small temperature increases suggests that these latter forces are weaker. Gels have a distinct macroscopic and molecular structure that combines the qualities of liquids and solids. Gels create three-dimensional networks at the

molecular level through the dispersion of colloidal particles in a liquid medium or polymer chains. Long-chain molecules are cross-linked in polymer network gels, either chemically or physically, to provide stability or flexibility in response to external stimuli. Colloidal gels, on the other hand, are based on the aggregation of micro- or nanoparticle-sized particles, which results in formations that vary in their degree of order and disorder. Because of their viscoelastic characteristics, gels can have macroscopic textures that range from soft and elastic to stiff and brittle, and they can look transparent or opaque.[18]

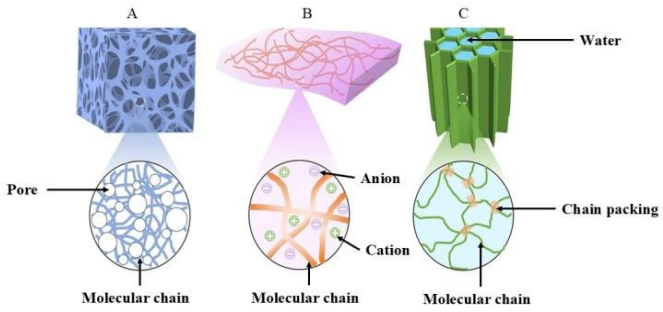


Fig. 1.2: Diagram showing the Structure and Makeup of gels: (A) Aerogel; (B) Ionogel; (C) Hydrogel.

1.3.2 Properties of gel

- A safe, inert gelling agent that doesn't react with other formulation ingredients is ideal for use in pharmaceutical or cosmetic applications.
- When the preparation is stored and exposed to shear forces created by shaking the bottle, squeezing the tube, or applying topically, the gelling agent used in it should provide a reasonable solid-like character that is easily broken.
- To guard against microbial attack, it needs to have an appropriate anti-microbial.
- Tacky topical gel is not what you want.
- Sterile ophthalmic gel is required.

1.3.3 Characteristics of gels

A) Swelling

A sizable portion of the liquid is absorbed by the gelling agent and the volume rises while it is kept in contact with the liquid that solvates it. This is known as the swelling process. As the solvent permeates the matrix, this process takes place. Gel solvent interactions are used in

place of gel-gel interactions. The quantity and strength of the bonds that connect the individual gelling agent molecules determine how much swelling occurs.[19]

B) Syneresis

When left standing, many gels frequently spontaneously contract and release a fluid medium. Syneresis is the term for this phenomenon. When the gelling agent concentration falls, the degree of syneresis increases. Syneresis's recurrence suggests that the original gel was unstable in terms of thermodynamics. The relaxation of elastic stress that arose during the gels' setup has been linked to the contraction process. The solvent is forced out of the interstitial space as a result of the release of these tensions.

C) Ageing

Usually, colloidal systems aggregate slowly on their own. The term "aging" refers to this process. Ageing causes a denser network of the gelling ingredient to gradually accumulate in gels. D) Organization The network created by the gelling agent particles connecting with one another gives a gel its stiffness. the particle's nature and the tension, balancing them out and reducing flow resistance.

D) Rheology

Pseudoplastic solutions, or those with flocculated solid dispersion and gelling agents, display non-Newtonian flow behavior, which is defined by a drop in viscosity with an increase in shear rate. When shear stress is applied, the weak structure of inorganic particles scattered in water is broken up by the breakdown of interparticulate connection, showing a stronger inclination to flow. In a similar vein, applying shear stress to macromolecules causes them to align in the direction of an organic (Single phase system).

1.3.4 Uses

- As drug delivery devices for oral medications.
- To provide topical medication that is given straight to the skin, mucosal membrane, or eye.
- As long-acting intramuscular medication injections.
- As thickeners in oral liquid and suppository bases, protective colloids in suspensions, binders in tablet granulation, and in cosmetics such as shampoos, scent products, dentifrices, and skin and hair care preparations.

1.3.5 Classification of gels[20,21]

Gels can be categorized according to their physical characteristics, rheological attributes, solvent type, and colloidal phases, among other factors.

1.3.5.1 Based on colloidal phases

a) Inorganic (Two phase system)

Such a system is made up of floccules of small particles rather than bigger molecules and gel structure; if the partition size of the dispersed phase is quite large and forms the three-dimensional structure throughout gel, the system is not always stable. They must produce a semisolid thixotropic phase when left in a semisolid state and turn liquid when disturbed.

b) Organic (Single phase system)

These are made up of big organic molecules that are dissolved in a continuous phase and reside on twisted threads. These bigger organic molecules, which can be synthetic or natural polymers, are known as gel formers because they have a tendency to entangle with one another randomly or to be linked together by forces known as Vander Walls.

1.3.5.2 Based on nature of solvent

a) Hydro gels (Water based)

Here, they-such as bentonite magma, gelatin, cellulose derivatives, carpooler, and poloxamer gel-contain water as their continuous liquid phase.

b) Organic Gels (With a non-aqueous solvent)

On their continuous phase, these have a non-aqueous solvent. For instance, metallic stearate dispersion in oils and plastibase (low molecular weight polyethylene dissolved in mineral oil and short-cooled Olag (aerosol) gel).

c) Xerogels

Xerogels are solid gels that have a low solvent content. These are made by freeze-drying or solvent evaporation, which leaves the gel structure behind. When they come into contact with new fluid, they swell and reconstitute. For instance, polystyrene, cyclodextrin, acacia tear β, tragacanth ribbons, and dry cellulose.

1.3.5.3 Based on rheological properties

(a) Plastic gels

E.g. – Aluminum hydroxide flocculated suspensions, known as Bingham bodies, display a plastic flow. The rheogram plot indicates the gels' yield value, over which the elastic gel deforms and starts to flow.

(b) Pseudo-plastic gels

E.g. - Pseudo-plastic flow is seen in the liquid dispersion of tragacanth, sodium alginate, Na CMC, etc. These gels have no yield value and their viscosity falls as the rate of shear increases. A shearing action on the linear polymers' long chain molecules produces the rheogram. With the release of solvent from the gel matrix, the disorganized molecules start to align their long axes in the direction of flow as the shearing stress increases.

(c) Thixotropic gels

These gels have extremely weak particle connections that are easily disrupted by shaking. Because of the particles' collisions and subsequent connecting, the resultant solution will revert to gel (the reversible isothermal gel-solgel change). This creates a scaffold-like structure in a colloidal solution containing nonspherical particles. For example, agar, bentonite, and kaolin.

1.3.5.4 Based on physical nature

(a) Elastic gels

Agar, pectin, guar gum, and alginates gels behave elastically. At the point of junction, the fibrous molecules are joined by relatively weak connections such dipole attraction and hydrogen bonding. Additional bonding occurs by a salt bridge of type COO-X-COO between two neighboring strand networks if the molecule has a free COOH group. Such as carbapol and alginate.

(b) Rigid gels

This can be created from a macromolecule with a primary valence bond linking the framework. For instance, the Si-O-Si-O link that holds silic acid molecules together in silica gel creates a polymer structure with a network of pores.

1.3.6 Preparation of gel

Gels are typically produced in commercial quantities at room temperature. However, a few polymers require particular preparation before processing. Gels can be created using the following ways.

a) Thermal changes

When exposed to heat fluctuations, solvated polymers (lipophilic colloids) form gelatin. Many hydrogen formers are more soluble in hot water than in cold. If the temperature drops, the degree of hydration decreases, and gelatin forms. (Cooling a concentrated hot solution will result in a gel). Examples include gelatin, agar sodium oleate, guar gum, and cellulose derivatives, among others. In contrast, other materials, such as cellulose ether, obtain their water solubility by hydrogen bonding with water. Raising the temperature of these solutions will break hydrogen bonding and limit solubility, resulting in gelation. As a result, this technology cannot be used to make gels on a larger scale.

b) Flocculation

Here, gelation is created by adding just enough salt to cause precipitation in order to create an age state, but not enough to cause total precipitation. Quick mixing is required to prevent significant precipitant concentrations in one area. For example, ethyl cellulose and polystyrene solutions in benzene can be gelled by quickly combining with the right proportions of a non-solvent, like petroleum ether. Seldom is gelation seen when salts are added to a hydrophobic solution; coagulation results. The flocculation process produces gels that behave in a thixotropic manner. Hydrophilic colloids, such acacia, proteins, and gelatin, are only impacted by high electrolyte concentrations; when this happens, the colloidal and gelation processes stop.

c) Chemical reaction

By using this approach, the solute and solvent interact chemically to generate gel. For example, aluminium hydroxide gel can be made by reacting an aluminium salt with sodium carbonate in an aqueous solution; a higher reactant concentration will result in the formation of a gel structure. A few more instances include the chemical reactions that cross-link the polymeric chain: PVA, cyanoacrylates with glycidol ether (Glycidol), toluene diisocyanates (TDI), and methane diphenyl isocyanine (MDI).

1.3.7 Formulation considerations for pharmaceutical gels[22]

a) The choice of vehicle/solvent

Typically, a solvent is used with purified water. Co-solvents, such as alcohol, glycerol, PG, PEG 400, etc., may be employed to promote drug penetration through the skin and/or to increase the solubility of the therapeutic agent in the dosage form.

b) Inclusion of buffers

Aqueous and hydroalcoholic-based gels may contain buffers to regulate the formulation's pH. Buffer salt solubility decreases in hydroalcoholic-based vehicles. For example, citrate, phosphate, etc.

c) Preservatives

Preservatives that work with the hydrophilic polymers used to make gels can lower the amount of free preservative—which is antimicrobially active—in the mixture. Consequently, the starting concentration of these preservatives needs to be increased to make up for it. For instance, phenolics and parabens.

d) Antioxidants

It might be used in the formulation process to strengthen the chemical stability of medicinal substances that are vulnerable to oxidative damage. The type of vehicle utilized to prepare the gel determines its selection. Considering that most gels are aqueous in nature, water-soluble antioxidants are typically utilized. For instance, sodium formaldehyde sulfoxylate, sodium metabisulphite, etc.

e) Flavour/sweetening agent

Only gels intended for administration into the oral cavity—such as those intended to treat infections, inflammations, ulcerations, etc.-contain flavours and sweeteners. Examples of sweeteners include aspartame, glycerol, sucrose, liquid glucose, sorbitol, and saccharin sodium.

Flavours: Wintergreen mint, butterscotch, apricot, peach, vanilla, cherry, mint, anise, citrus tastes, and raspberry.

1.3.8 Evaluation parameters of the formulated gels[23]

a) Measurement and pH

Digital pH meters were used to measure the pH. 1g of gel should be dissolved in 100 ml of purified water and kept for two hours. measured the pH three times and determined the average results.

b) Drug content

1g of the gel and 100 ml of an appropriate solvent were mixed. The stock solution should be filtered. Next, make aliquots with varying concentrations using appropriate dilutions, and calculate the absorbance. The equation used to compute the drug content was obtained using linear regression analysis of the calibration curve.

c) Viscosity study

A Brookfield viscometer is used to perform the procedure. the gels were rotated at 0.3, 0.6, and 1.5 rpm. At each speed, record the matching dial reading. By dial reading the × factor provided in the Brookfield viscometer catalogues, the viscosity was determined.

d) Spreadability

It shows the size of the region that gel easily spreads when applied to the skin or other affected area. The dissemination of value also influences the therapeutic potency. Spreadability is defined as the amount of time, in seconds, it takes for two slides to separate from gel that is positioned between them when a specific stress is applied. Better spreadability results from separating two slides in less time. The following formula is used to calculate the spreadability:

Spreadability (S) = M × L / T

Where,

M = weight tied to upper slide

L = length of glass slides

T = Duration of time needed to separate the slides

e) Extrudability

After being placed in the container, the formulations are filled into the collapsible tubes. Extrudability is measured by the weight in grams needed to extrude a gel ribbon that is 0.5 cm in length in 10 seconds.

f) Skin irritation study

Guinea pigs (400–500g; either sex) were employed in the skin irritation study. The animals were fed normal animal feed and had unrestricted access to water. The animals were housed in conventional settings. The back had been shaved clean. At 1, 2, 3, 5, 6, 7, and 8 hours, five milliliters of each sample were taken out and replaced with an equivalent volume of brand-new dissolving medium. The samples were then examined for drug content using phosphate buffer as test subjects. A 4-centimeter area was marked blank on both sides; one side was used as a control and the other as a test. For seven days, the guinea pig received 500 mg of the gel twice a day, and the site was monitored for any signs of sensitivity or reaction. It received a grade of:

0	No reaction
1	Minor patchy erythema
2	Tiny but cohesive or small but sporadic erythema
3	Severe erythema with or without edema

g) In vitro diffusion studies

To investigate the dissolution release of gels across a cellophane membrane, diffusion experiments of the produced gels can be conducted in a Franz diffusion cell. A 0.5g gel sample was placed in a cellophane membrane, and 250 ml of phosphate buffer (pH 7.4) was used as the dissolving medium for the diffusion tests, which were conducted at 37 ± 1°. Every one, two, three, four, five, six, seven, and eight hours, five milliliters of each sample were taken out and replaced with an equivalent volume of brand-new dissolving media. Phosphate buffer was then used as a blank in the drug content analysis of the samples.

h) Stability

Through freeze-thaw cycling, stability investigations were conducted for each gel composition. Here, syneresis was seen after the product was heated to 4°C for one month, 25°C for one month, and 40°C for one month. Following this, the gel is left at room temperature, and it is observed that the liquid exudate separates.[24]

i) Homogeneity

All generated gels were visually inspected to ensure homogeneity after they were placed in the container. Their appearance and the existence of any aggregates were examined.

j) Grittiness

Every formulation was examined under a light microscope to determine whether any significant particle matter was present. Therefore, it is evident that the gel preparation satisfies the necessary conditions of being devoid of specific material and grittiness for any topical application.

1.4 Avocado (*Persa americana)*

The tropical fruit known as avocado, or *Persa Americana* Mill, is a member of the Lauraceae family. Avocados are widely recognized for their rich nutritional value and potent medicinal properties. Compared to other fruit kinds, avocados have a higher oil content and are rich in mono unsaturated fatty acids, including oleic, linoleic, palmitic, and palmitoleic acids plus stearic and palmitic acids.[25,26] Additionally, the majority of the fruit's carotenoids and phytochemicals are preserved in this oil. Avocado oil also has a high level of lecithin, B-sitasterol, polyphenol, flavonoids, and other vitamins and minerals. Because of these components, avocado extract has antibacterial, antioxidant, and sunscreen properties, among many other medicinal uses.[27,28] Regarding avocado extract's ability to act as a sunscreen, it has been found that avocado oil absorbs quickly through the skin and works well in products that require lubrication and penetration, such as muscle oils and tissue and massage creams. One of the most absorbing oils for makeup that is available is avocado oil.[29] Apart from avocado extract's antibacterial properties, avocado rhizobacteria release chemical compounds that have antifungal properties to combat fungal infections.[30] They are a well-liked option for those who are health-conscious because they are full of beneficial monounsaturated fats, vitamins, and minerals. Grown all over the world, avocado trees are farmed in subtropical regions and come in a variety of sizes, shapes, and skin textures. Avocado oil is extracted for its cosmetic and therapeutic benefits, demonstrating the fruit's flexibility beyond culinary applications. An essential and cherished fruit in many cultures' cuisines and wellness rituals, avocados can be eaten as guacamole, spread over toast, or pureed into smoothies. The absence of genotoxic activity in vivo provides evidence that *Persa americana* seed extract may find application in food, cosmetic, or medicinal formulations as a material for topical dosage forms.[31]

Fig. 1.3: Avocado (*Persa americana).*

1.4.1 Uses of avocado

- **DNA Damage Protection**

Similar to the phytochemicals found in avocados, xanthophylls may have antioxidant and DNA-protective properties as well as potentially protective effects against healthy aging, according to a number of clinical trials. Eighty-two male airline pilots and frequent travelers were the subjects of one study. They were exposed to high amounts of cosmic ionizing radiation, which is known to damage DNA and may hasten the aging process.[32] The incidence of chromosome translocation, a biomarker of cumulative DNA damage, was significantly and inversely correlated with the intake of vitamin C, beta-carotene, β-cryptoxanthin, and lutein-zeaxanthin from fruits and vegetables ($p < 0.05$). In a different experiment, plasma xanthophyll levels and lipid peroxidation (8-epiprostaglandin F2a) had an inverse relationship.[33]

- **Osteoarthritis**

Most people get osteoarthritis (OA) as they age or gain weight or obesity. OA is defined by a progressive loss of joint cartilage and function with accompanying disability.[34,35] The degeneration of the extracellular matrix in the joint and an imbalance in biosynthesis can result in joint deterioration and loss of function. This can be caused by oxidative and inflammatory stress.[36,37,38,39,40] According to a cross-sectional study, fruits and vegetables high in avocado's principal carotenoids, zeaxanthin and lutein, are linked to a lower risk of cartilage abnormalities, an early sign of osteoarthritis (OA).[41]

- **Eye health**

The area of the eye where light is focused on the lens is called the macula, and it is here that lutein and zeaxanthin are specifically absorbed.[42] (According to Johnson *et al.*, 2010,

females have lower levels of lutein and zeaxanthin than males do, and these intakes decline with age.[43] Mexican Americans are among the biggest avocado consumers in the country and have the greatest intake of lutein and zeaxanthin of any ethnic group. Low dietary intake and plasma concentration of lutein may exacerbate age-related visual impairment, according to observational studies.[44,45, 46,47]

- **Cancer**

A variety of bioactive phytochemicals found in avocados, such as phenols, glutathione, D-mannoheptulose, terpenoids, carotenoids, and persenone A and B, have been shown to have anti-carcinogenic qualities[48,49,50] Certain of these phytochemicals may have therapeutic value based on their quantities in avocados (Jones DP *et al.*, 1992). Since all of the data is based on in vitro research using human cancer cell lines, direct avocado anti-cancer efficacy is still in its very early stages. Dietary carotenoids exhibit biological actions that may protect against breast cancer, such as antioxidant activity, apoptosis induction, and mammary cell growth suppression**.**[51]

1.5 Soybean

The legume soybean (Glycine max) is a popular crop that is used for both food and non-food purposes, as well as an oilseed and animal feed. Many techniques can be used to make soybean oil. But the most common method used in commercial production is to dry, temper, crack, dehull, and crush the soybeans. Next, the oil solvent is extracted using a variety of techniques, most frequently hexane.[52]

Other contaminants are eliminated using a variety of refining techniques, including steam stripping. Triglycerides, phospholipids, sterols, tocopherols, hydrocarbons, and free fatty acids are all complexly mixed together in unrefined soybean oil[53] Triglycerides make up more than 99% of refined soybean oil.[54] Since the chemical and physical properties of soybean oil can vary greatly, the common triglyceride linoleic acid is utilized as a stand-in for the features of soybean oil.[55] Since their introduction from East Asia, soybeans have grown to become one of the world's most significant crops due to its high protein content and wide range of industrial uses. They are essential to agriculture, used in cooking oils, animal feed, and as the main source of plant-based protein that is suitable for human consumption. As a result of a process known as nitrogen fixation, which occurs naturally, soybeans are also essential to sustainable farming methods. Soybeans are a mainstay in cuisines around the

world due to their nutritional value and capacity to adapt to many climates. They also play a key role in economic stability and food security.

According to Wang 2002, the main fatty acids in soybean oil are linoleic acid (53.2%), oleic acid (23.4%), palmitic acid (11.0%), and linolenic acid (7.8%). The primary saturated fats are stearic (C18:0) and palmitic (C16:0) acids, while oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids are unsaturated (Wang 2002). Free fatty acids typically make up less than 1%, while 1.5–4% of the mixture is made up of phospholipids, mainly the emulsifier lecithin[56] Vacuum drying is typically used to recover lecithin (Hasenhuettl GL *et al.*, 2000). Tocopherols, sitosterols, and stigmasterols make up the remaining 0.8% (Merck 2015).

Fig. 1.4: Soybean.

- **Nutritive value of refined soy bean oil**

A unique food is soybeans because of its high nutrient content. Vegetable protein, oligosaccharides, dietary fiber, phytochemicals (Particularly isoflavones), carbs, lipids, and minerals can all be found in soy beans. (Aparicio *et al.*, 2008).

Protein

One food that is high in protein is soy beans. Its protein content is from 20 to 25 percent on average. Methionine stands out among the low sulfur amino acids found in soy bean protein. Trypsin inhibitors, fenolics, and phytic acid are some of the naturally occurring substances found in soyabeans that reduce the amount of protein.

Fat

Oilseeds called soybeans are used to make soybean oil. Fat makes up around 18% of the dry weight, mostly in the form of polyunsaturated and monounsaturated fatty acids, with a small

amount of saturated fat as well. About half of the total fat content in soybeans is made up of linoleic acid, which is the most prevalent type of fat.

Carbohydrates

Whole soybeans have a low glycemic index (GI), a measure of how meals affect the rise in blood sugar following a meal, because they are low in carbohydrates. Because of their low GI, soybeans are perfect for diabetics.

Fiber

The soluble and insoluble fiber content of soybeans is high. Alpha-galactosides make up the majority of the insoluble fibers, which might cause gas and diarrhea in sensitive people. This may exacerbate the symptoms of irritable bowel syndrome (IBS). Although certain people may have negative side effects from soluble fibers in soybeans, overall, soluble fibers in soybeans are thought to be safe. When bacteria in your colon digest them, short-chain fatty acids (SCFAs) are created, which can improve gut health and reduce your risk of colon cancer.

Table 1.1: Physical and Chemical properties of soybean oil.

S. No.	Property	Characterstic/value	Source
1	Percent composition	Triglycerides of saturated acids make up 14%, linoleic acid triglycerides: 26%, linolenic acid triglycerides: 11%, and linoleic acid triglycerides: 49%. Phospholipids: 0.8% for tocopherols, stigmasterols, and sitosterols; lecithin: 1.5–4%	(Merck et al., 2015)
2	Physical state at 25^0C/1Atm	Liquid	(Merck et al., 2015)
3	Color	Pale yellow to brownish yellow	(Merck et al., 2015)
4	Odor	Slight chararcterstic odor	(Merck et al., 2015)
5	Density/Specific gravity	0.916-0.922	(Merck et al., 2015)
6	Melting point	-10 – 16 ^{0}C	(Merck et al., 2015)
7	Boiling point	Crude: 185°CRefined & Bleached: 228-234°C	(Pryde et al., 1980)
8	Solubility	When in contact with water, emulsifies. It is soluble in hexane and other non-polar hydrocarbon-	(Pryde et al., 1980; Matthews et al., 2010)

		based solvents and just slightly soluble in water and alcohol.	
9	Vapor pressure	2.61×10^{-17}mm Hg	(Yuan et al., 2005)
10	pH	7.5	(Matthews et al., 2010)
11	Octanol/Water(K_{ow}) coefficient	22.65	(EPI 2012)
12	Viscosity	60n $Pa^{-1}S$	(Hasenhuettl et al., 2000)
13	Miscibility	Miscible with carbon disulfide, petroleum ether, ether, pure alcohol, and chloroform	(Merk et al., 2015)
14	Flammability	Flash point: 328°CFire point: 363°C	(Pryde et al., 1980)
15	Storage stability	Stable	(Matthews et al., 2010)
16	Corrosion characterstics	Not found	
17	Air half life	0.229 hrs	(EPI 2012)
18	Soil half life	1800 hrs	(EPI 2012)
19	Water half life	900 hrs	(EPI 2012)
20	Persistence	1060 hrs	(EPI 2012)

1.5.1 Applications of soybean components

- **Antioxidant effects**

Scientific research have demonstrated that soy milk, an essential soy product, can lower oxidative stress in people with Type 2 Diabetes Mellitus (T2DM). The study's findings showed that fermented soy milk is beneficial for controlling levels of the total antioxidant, oxidized glutathione, 8-isoprostaglandin F2, glutathione peroxidase, malondialdehyde, and reduced glutathione (GSH) as well as for lowering oxidative stress in type 2 diabetes.[57] Research has also been done on the creation of fermented soy meals with potent biological activity and good nutritional values.[58] reported on hydroxyl radicals, DPPH, and ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), as well as changes in the nutritional components (fatty acids, isoflavones, and amino acids) and antioxidant activity of fermented soybean. Furthermore, variations in -glucosidase effect glucosidase inhibitory activity, and total phenolic contents were examined.

- **Anti-obesity effects**

Isoflavones included in soy meals probably work with intracellular oestrogen receptors to decrease the distribution of adipose tissue and the production of fat. Soy meals and their

constituents have been shown in numerous studies to have an anti-obesity impact. Soy isoflavones and their derivatives have been demonstrated to exhibit estrogenic effects and a high affinity for binding to oestrogen receptors due to their structural resemblance to 17-estradiol (E2). Together with other cell and organ types, adipose tissues express oestrogen receptors, which is important for controlling metabolism and fat distribution.[59]

- **Antidiabetic effects**

Products made from soy that are high in isoflavonoids have been shown to have anti-diabetic properties. Additionally, it has been demonstrated that soybean extract works well to stop glucose from being absorbed into vesicles in the brush border membrane.[60] Diabetes can be treated using a variety of diets generated from phytocompounds.[61] Jayachandran M and Xu B 2019 have shown that soy products and soybeans are one of the foods that can effectively prevent diabetes mellitus (DM). Dietary soy has been shown to be important and has a significant impact on individuals with chronic kidney disease. Diabetes mellitus and serious renal problems are most often associated. In type 1 diabetes, replacing animal proteins with soy proteins reduces both proteinuria and glomerular filtration rate (GFR).

- **Anticancer effects**

According to Banerjee S *et al.*, (2008), soy has also demonstrated promise in reducing the incidence of a number of cancer types.[62] Because breast and prostate cancers are particularly sensitive to sex steroid hormones, this is particularly relevant. The cancer-prevention properties of soy are due to the isoflavones. It has become clear that other ways of acting could be involved. Isoflavones have been shown to affect apoptosis, cell signaling, the cell cycle, differentiation, proliferation, and growth. Soy isoflavones have been found to lower cardiovascular disease risk markers in men[63] Sathyapalan T *et al.*, 2017 and to help improve cardiovascular disease risk markers in women during the early stages of menopause.[64]

2. Preformulation Studies

Preformulation studies look into a pharmacological substance's chemical and physical characteristics.

The selected drug Avocado was subjected for investigation of physical characterization parameters such as:

- Organoleptic properties
- Melting point

- UV-visible spectra
- Solubility
- Partition coefficient
- FT-IR spectra

2.1 Organoleptic properties

Organoleptic properties of Avocado were found to be as per literature. The Organoleptic properties of Avocado were found to the given in **Table 2.1**.

Table 2.1: Organoleptic properties of avocado.

Sr. No.	Properties	Inferences
1	Colour	Light green
2	Form	Powder
3	Taste	Grass taste

2.2. Melting point[65]

The temperature at which a substance transitions from the solid to the liquid phase under one atmosphere of pressure is known as its melting point. The drug's purity is implied by the melting point measurement. Melting point of Avocado was determined by capillary tube method and was found to be quite similar to the reported melting point as shown in **Table 2.2**

Table 2.2: Melting point of avocado.

Drug	Reference M.P.	Observed M.P.
Avocado	184°C	182°C

Discussion: The melting point of Avocado was found to be 182°C which is in the range of the pure drug. As a result, the medication sample was devoid of all contaminants.

2.2. UV Spectroscopy

2.2.1. Determination of absorption maxima in avocado[66]

For the drug's quantitative analysis, a double beam UV-visible spectrophotometer was employed. A 21 µg/ml solution of Avocado in water was scanned in the range of 200-600 nm. The result of UV spectrum of avocado is shown in **Figure 2.1**.

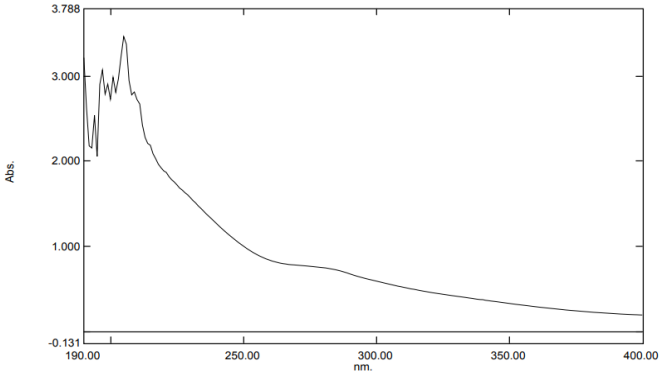


Figure 2.1: UV Spectrum of avocado in water.

Table 2.3: Absorption maxima (λ_{max}) of Avocado in water.

Name of drug	Absorption maxima (λ_{max})	
	Observed	Reference
Avocado	280	280

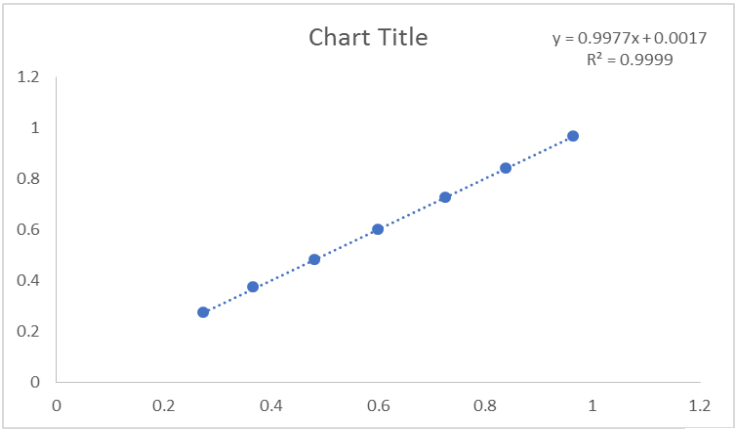
Discussion: The maximum wavelength of avocado was observed at 280 nm similar to literature (**Table 2.3**)

2.2.2. Preparation of standard curve of Avocado in water

Table 2.4: Calibration curve of Avocado in water (λ_{max}= nm).

Sr. No.	Concentration (µg/ml)	Mean±SD
1	3	0.274±0.001
2	6	0.373±0.001
3	9	0.481±0.001
4	12	0.601±0.001
5	15	0.724±0.001
6	18	0.839±0.001
7	21	0.965±0.001

The value is given as mean ± SD for n = 3.



Graph 2.1: Standard calibration curve of avocado in water.

Table 2.5: Result of regression analysis of UV method.

Statistical parameters	Results
λ_{max}	280 nm
Regression equation (y =mx+ c)	y = 0.9977x + 0.0017
Slope (m)	0.9977
Intercept (C)	0.0017
Correlation coefficient (R^2)	0.9989

Discussion: - The calibration curve for Avocado was obtained by using the 3 to 21 µg/ml concentration of Avocado in water. The absorbance was measured at 280 nm. The calibration curve of Avocado as shows in graph indicated the regression equation y = 0.9977x + 0.0017and R^2 value 0.9989, which shows good linearity as shown in **Table 2.5** and **Graph 2.1**.

2.4. Solubility studies[67]

Drug solubility tests in a range of solvents were performed to identify the components that would be used in the formulation creation process. Analysis of the drug was carried out on UV Spectrophotometer at 280 nm.

Table 2.6: Solubility studies of avocado for different solvents.

S. No.	Name of Solvents	Solubility (mg/ml)	Solubility
1	Water	86.830±0.100	freely soluble
2	Methanol	3.558±0.012	Slightly soluble
3	Ethanol	2.342±0.006	Slightly soluble
4	pH 7.4	1.005±0.010	Very Slightly soluble
5	Chloroform	0.106±0.002	Practically insoluble

The value is given as mean ± SD for n = 3

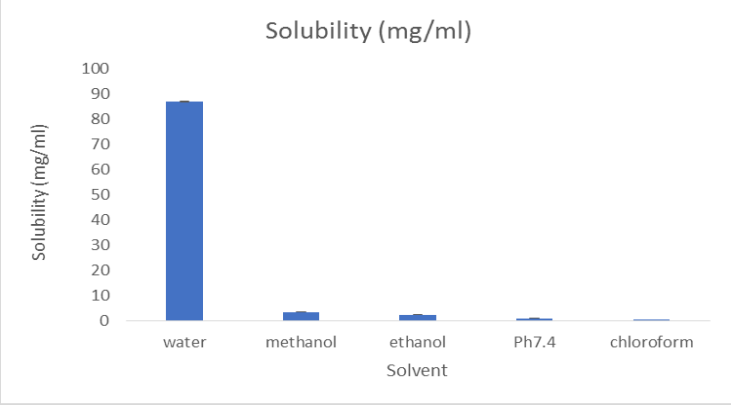


Figure 2.2: Solubility study of drug in different solvents.

Discussion: From the above data, it is clearly seen that Avocado is freely soluble in methanol and soluble in ethanol, chloroform and acetone. (**Figure 2.2** and **Table 2.6**).

2.5. Partition coefficient determination

Partition coefficient of the Avocado was determined using n-octanol and water. Log P greater than one indicates that the drug is lipophilic in nature, whereas those with partition coefficients less than one are indicative of a hydrophilic drug. This suggested that the medication was pure and lipophilic.

Table 2.7: Partition coefficient determination of avocado.

Drug partition coefficient	Solution system	Values of Log P
Avocado	n-octanol: water	1.66±0.010

The value is given as mean ± SD for n = 3

Discussion: The partition coefficient of Avocado in n-octanol: water was found to be 1.66±0.010, this indicates that the drug is lipophilic in nature (**Table 2.7**)

2.6. FTIR Studies

2.6.1 FTIR study of avocado[68]

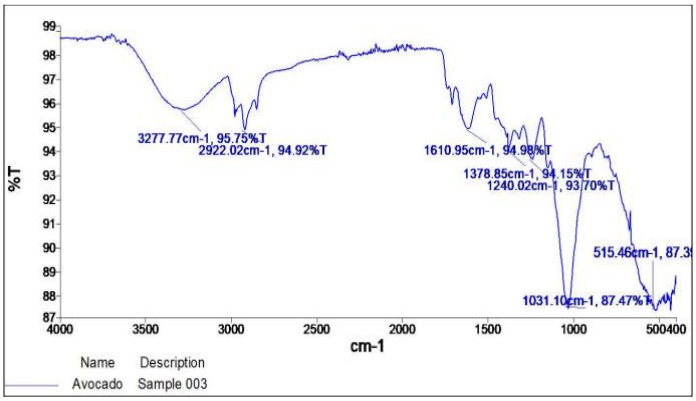


Figure 2.3: FTIR spectrum of avocado.

Table 2.8: FTIR interpretation of avocado.

Sr. No.	Characteristics Peak	Reference (cm^{-1})	Observed (cm^{-1})
1.	O - H stretching	3164 – 3308	3277.77
2.	C - H stretching	2831- 2962	2922.02
3.	C–O stretching	1033	1031.10

Discussion: The FTIR spectra of Avocado were shown in the **Figure 2.3; Table 2.8**. The principal IR absorption peaks of Avocado at 3277.77cm^{-1}(O-H stretching), 2922.02cm^{-1}(C-H), 1031.10cm^{-1}(C–O stretching) were all observed in the spectra of Avocado. These observed principal peaks confirmed the purity and authenticity of the Avocado.

2.7 Preformulation studies

- Preformulation studies look into a pharmacological substance's chemical and physical characteristics. The selected drug Soybean was subjected for investigation of physical characterization parameters such as:
- Organoleptic properties
- Melting point
- UV-visible spectra
- Solubility
- Partition coefficient
- FT-IR spectra

2.7.1. Organoleptic properties[69]

Organoleptic properties of Soybean were found to be as per literature. The Organoleptic properties of Soybean were found to the given in **Table 2.9**

Table 2.9: Organoleptic properties of soybean.

Sr. No.	Properties	Inferences
1	Colour	Pale yellow
2	Form	Liquid
3	Taste	Slight characteristic odor

2.7.2. Melting point

Melting point of Soybean was determined by capillary tube method and was found to be quite similar to the reported melting point as shown in **Table 2.10**

Table 2.10: Melting point of soybean.

Drug	Reference M.P.	Observed M.P.
Soybean	-10°C to -16°C	-9°C to -15°C

Discussion: The melting point of Soybean was found to be which -9°C to -15°C is in the range of the pure drug. As a result, the medication sample was devoid of all contaminants.

2.7.3. UV Spectroscopy[70]

2.7.3.1. Determination of absorption maxima in Methanol

The medication was quantitatively analyzed using a twin beam UV-visible spectrophotometer.

A 100µg/ml solution of Soybean in methanol was scanned in the range of 200-400 nm. The result of UV spectrum of Soybean is shown in **Figure 2.4**.

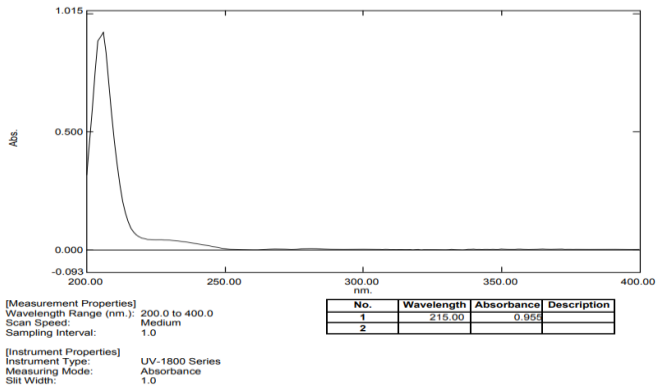


Figure 2.4: UV Spectrum of soybean in methanol.

Table 2.11: Absorption maxima (λ_{max}) of Soybean in Methanol.

Name of drug	Absorption maxima (λ_{max})	
	Observed	Reference
Soybean	215	216

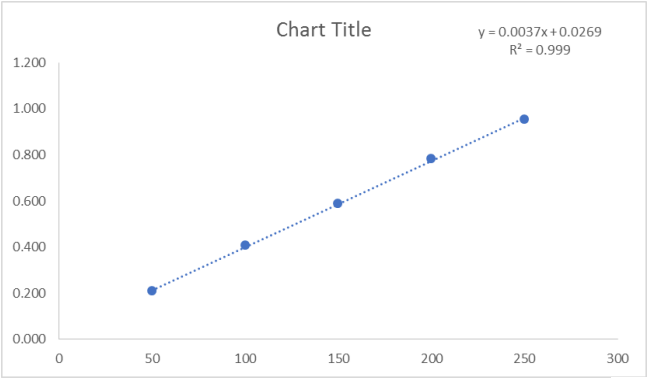
Discussion: The maximum wavelength of Soybean was observed at 215 nm similar to literature (**Table 2.11**)

2.7.3.2. Preparation of standard curve of soybean in methanol

Table 2.12: Calibration curve of Soybean in Methanol (λ_{max} = 215 nm).

Sr. No.	Concentration(µg/ml)	Mean ± SD
1	50	0.208±0.001
2	100	0.406±0.002
3	150	0.587±0.002
4	200	0.782±0.002
5	250	0.955±0.001

The value is given as mean ± SD for n = 3.



Graph 3.2: Standard calibration curve of soybean in methanol.

Table 2.13: Result of regression analysis of UV method.

Statistical parameters	Results
λ_{max}	215 nm
Regression equation (y =mx+ c)	y = 0.0037x + 0.0269
Slope (m)	0.0037
Intercept (C)	0.0269
Correlation coefficient (R^2)	0.999

Discussion: - The calibration curve for Soybean was obtained by using the 50 to 250 µg/ml concentration of Soybean in methanol. The absorbance was measured at 215 nm. The calibration curve of Soybean as shows in graph indicated the regression equation y = 0.0037x + 0.0269 and R^2 value 0.999, which shows good linearity as shown in **Table 2.13** and **Graph2.2**.

2.7.4. Solubility studies

Drug solubility tests in a range of solvents were performed to identify the components that would be used in the formulation creation process. Analysis of the drug was carried out on UV Spectrophotometer at 215 nm.

Table 2.14: Solubility studies of soybean for different solvents.

Sr. No.	Name of Solvents	Solubility (mg/ml)	Solubility
1	Hexane	54.8±0.68	Soluble
2	Chloroform	29.4±0.16	Sparingly soluble
3	Methanol	9.49±0.27	Slightly soluble
4	Ethanol	8.14±0.27	Slightly soluble
5	Water	4.62±0.27	Slightly soluble

The value is given as mean ± SD for n = 3.

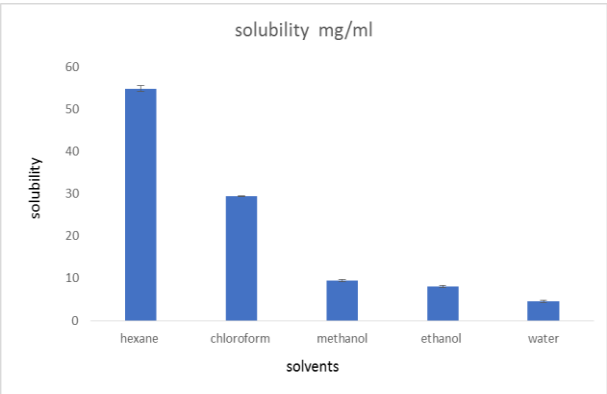


Figure 2.5: Solubility study of drug in different solvents.

Discussion: From the above data, it is clearly seen that Soybean is freely soluble in methanol and soluble in ethanol, chloroform and water. (**Figure 2.5** and **Table 2.14**).

2.7.5. Partition coefficient determination

Partition coefficient of the Soyabean oil was determined using n-octanol and water. Log P greater than one indicates that the drug is lipophilic in nature, whereas those with partition coefficients less than one are indicative of a hydrophilic drug. This indicated the hydrophilicity and purity of drug.

Table 2.15: Partition coefficient determination of Soybean oil.

Partition coefficient of drug	Solvent system	Log P Values
Soyabean oil	n-octanol: water	0.84±0.010

The value is given as mean ± SD for n = 3.

Discussion: The partition coefficient of Soybean oil in n-octanol: water was found to be 1.66±0.010, this indicates that the drug is lipophilic in nature (**Table 2.15**)

2.7.6. FTIR studies

2.7.6.1. FTIR Study 0f Soybean Oil[71,74]

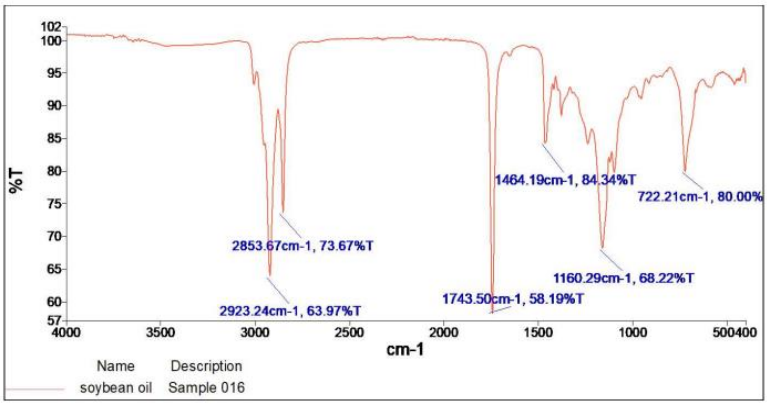


Figure 3.6: FTIR Spectrum of soybean oil.

Table 3.16: FTIR interpretation data of Soybean oil.

Characteristic peak	Reported (cm^{-1})	Observed (cm^{-1})
symmetric CH2 stretching and the asymmetric CH3 and CH2 stretching	2853.67	2850.01
C=O stretch	1743.50	1749.64
O-CH2-C asymmetric axial stretching	1160.29	1111

Discussion: The main infrared peaks of the Soybean oil are as follows: The FTIR spectra of Soybean oil are given in the **Figure 2.6**. The FTIR spectra showed the prominent peaks of the various bonds between the groups present in the soybean oil, chemical structure **Table 2.16**.

The prominent peaks for various groups are symmetric CH2 stretching and the asymmetric CH3 and CH2 stretching at 2853.67 cm^{-1}, C=O stretch at 1743.50cm^{-1} O-CH2-C asymmetric axial stretching at 1169.29 cm^{-1}. The observed FT-IR spectrum confirmed and identified the presence of functional groups and purity of the water Extract.

2.7.6.2. Physical mixture of Avocado Soyabean gel

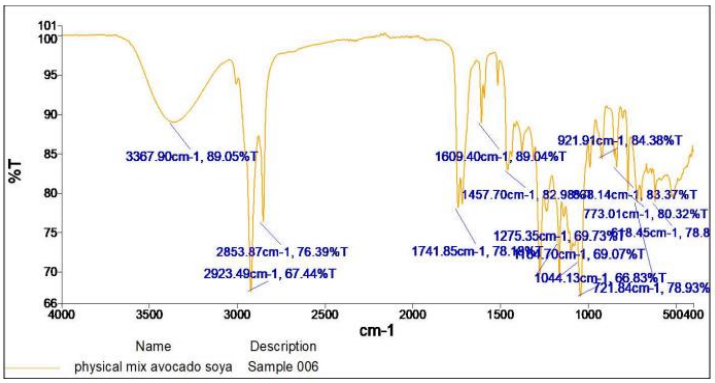


Figure 3.12: Physical mixture of Avocado Soybean gel.

2.7.6.3. FTIR OF Avocado Soybean gel

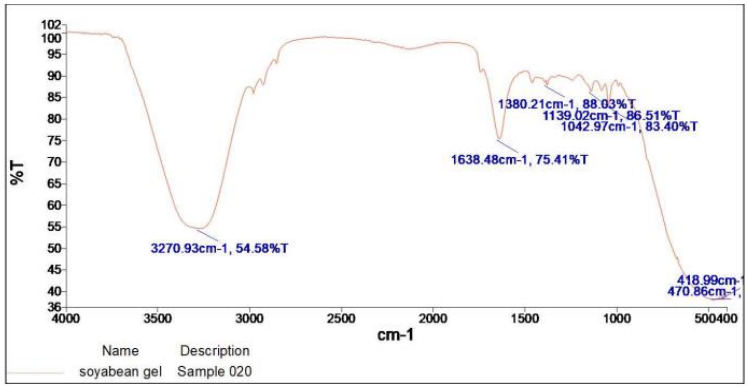


Figure 3.13: FTIR OF Avocado Soybean gel.

2.7.7. Percentage drug entrapment

Percentage yield and drug entrapment of Avocado soybean gel were given a table no 2.22.

Table 2.22: Percentage drug entrapment of different Avocado soybean gel formulations.

S. No.	Formulation Code	Percentage drug entrapment
1	F1	73.32±0.14
2	F2	76.33±0.14
3	F3	78.0.5±0.14
4	F4	82.20±0.14
5	F5	74.33±0.14
6	F6	79.33±0.14
7	F7	80.77±0.14

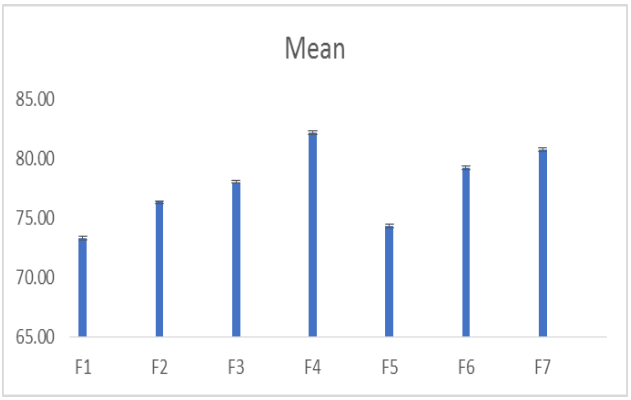


Figure 2.14: Percentage drug entrapment different gel formulations.

Discussion: From figure no.3.14, it was found that increase the concentration of lipid percentage yield will increase and in case of percentage drug entrapment, entrapment of drug in polymer will increase on increasing concentration of polymer, but this increase and drug entrapment will follow a certain concentration of polymers after that no percentage entrapment will increase on increasing concentration of polymer. Maximum percentage yield and percentage drug entrapment was found of formulation F4 that was 82.20±0.14.

2.7.8. Particle size of avocado soybean gel

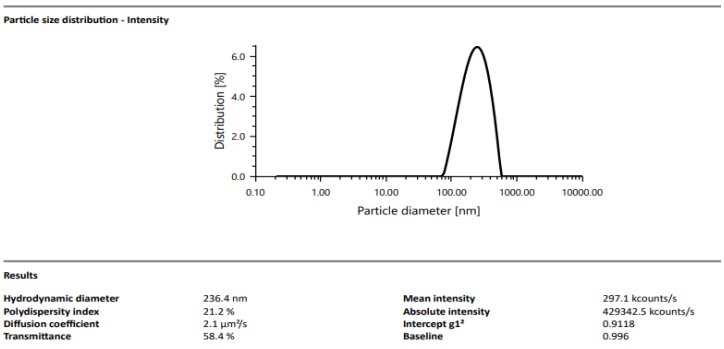


Figure 3.15: Particle size of avocado soybean gel.

2.7.9. Zeta potential of Avocado Soyabean gel

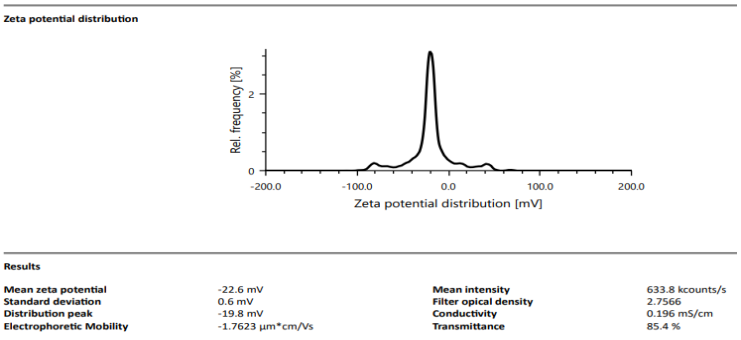


Figure 3.16: Zeta potential of avocado soyabean gel.

2.7.10. TEM (Transmission electron microscopy) result of avocado soybean gel

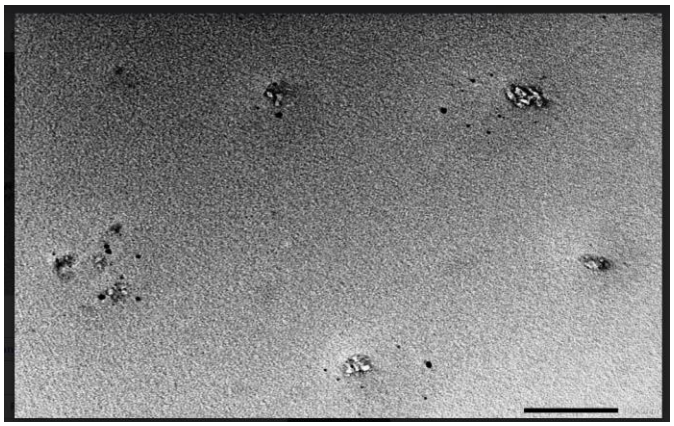


Figure 2.17: TEM result of Avocado Soybean Gel.

3. Materials and Equipment

Table 3.1: List of Instruments.

S. No.	Instruments	Manufacturer
1	UV/VIS Spectrophotometer,	Shimadzu, Japan
2	Weighing balance, (AUX220)	Shimadzu, Japan
3	Ultrasonic Bath	PCI
4	Vortex mixer	Remi (SLM-VM-3000), Bangalore
5	Melting Point Apparatus	Remi Equipment, Mumbai
6	Infrared red spectrophotometer (FTIR)	Perkin
7	Particle size analyzer	Anton Paar
8	pH Meter	Ohaus
9	Water bath shaker	NSW India
10	Rotary Evaporator	Perfit, India
11	Heating plate	IKA, India
12	Franz diffusion cell assembly	Orchid scientific

Table 3.2: List of chemicals.

S. No	Materials	Source
1	Avocado	MG Naturals, cholambedu road, krishnapuram, Tamil nadu.
2	Carbopol (934)	Fisher Scientific India Pvt. Ltd.
3	Polysorbate (Tween) 20	Thermo fisher Scientific India Pvt. Ltd.
4	Soyabean oil	Cargill India Private Limited
5	Methyl paraben	Fisher Scientific India Pvt. Ltd.
6	Propyl paraben	Lipoid GmbH, Germany
7	Propylene glycol	Avantor Performance Materials India Ltd.
8	Water	HPLC Water
9	Methanol	SD Fine-chem Ltd, Mumbai
10	Ethanol	Fisher Scientific India Pvt. Ltd., Mumbai
11	Chloroform	Fisher Scientific India Pvt. Ltd., Mumbai
12	Span20	Thermo fisher Scientific India Pvt. Ltd.

3.1. Pre-formulation studies[75,76]

Pre-formulation is an integral part of the entire development process. It is the study of the physical and chemical properties of the drug prior compounding process. These studies focus on those physicochemical properties of the drug that could affect its performance and development of an efficacious dosage form. A thorough understanding of these properties may ultimately provide a rationale for formulation design, or support the need for molecular modification. In the simplest case, these pre-formulation investigations may merely confirm that there are no significant barriers to the compound's development. These studies are indispensable protocol for development of safe, effective and stable dosage form. The obtained drug sample was identified by various analytical techniques such as IR spectroscopy, UV spectroscopy, melting point etc.

3.1.1 Organoleptic characteristics

The drug sample was characterized for the physical characterization like color, appearance and odor.

3.1.2 Melting point[77]

We determine the drug's melting point using the capillary fusion technique. A tiny amount of medication was inserted into a capillary that had been fused at one end and left open at the other. The temperature range at which the medication melts was measured using the melting point test instrument with this filled capillary inside. Three readings in duplicate were averaged, and the results were compared to literature value.

3.1.3 Solubility studies[78]

The spontaneous interaction of two or more substances to form a homogenous molecular dispersion is called solubility.

For quantitative solubility study, excess amount of drug was taken in thoroughly cleaned culture flasks containing 5 ml of different solvents (Methanol, Ethanol, Chloroform, pH 7.4 phosphate buffer saline, water) and test tubes were tightly closed. These test tubes were shaked on water bath shaker for 24 hrs at room temperature. After 24 hrs, each sample was centrifuge for 15 minutes at 15,000 rpm and was suitably diluted and determined spectrophotometrically.

3.1.4 Partition coefficient of drug[79]

Partition coefficient (oil/water) is a measure of a drug's lipophilicity/hydrophilicity and an indication of drug's ability to cross cell membranes. It is defined as the ratio of unionized drug distributed between the organic and aqueous phases at equilibrium. Partition coefficient provides a means of characterizing the lipophilic/hydrophilic nature of the drug. Drugs having values of P much greater than 1 are classified as lipophilic, where as those with values much less than 1 are indicative of a hydrophilic drug. The partition coefficient is commonly determined using an oil phase of n-octanol and water. In the case n-octanol and water:

$P_{o/w} = C_{n\text{-octanol}}/C_{water}$

The partition coefficient ($P_{o/w}$) therefore is the quotient of two concentrations of drug in n-octanol ($C_{n\text{-octanol}}$) and water (C_{water}) respectively and is usually given in the form of its logarithm to base 10 (log P).

- **Shake flask method**

The partition coefficient determination study was performed by using shake flask method. Excess amounts of the drugs dissolved in 5 ml of two solvents (n-octanol: Water) together (1:1) and placed for 24 hrs. After 24 hrs, the two layers were separated and centrifuge for 15 minutes at 15,000 rpm. The absorbance was taken in UV spectrophotometer at the respective λ_{max} after appropriate dilution.

3.1.5 FTIR of Avocado[80]

Fourier transform infrared Spectroscopies of different compounds were performed for identification of that particular compound. FT-IR Spectroscopy of pure drugs was done using Avocado Powder. Various peaks in FT-IR Spectrum were interpreted for identification of different group in the structure of pure drugs. FT-IR Spectroscopy can also be used to investigate and predict any physicochemical interactions between different components.

3.2 Preparation of gel[81]

Gel preparation was carried out in three steps

3.2.1. Preparation of gel base

The get bases were prepared by dispersing required amount of Carbopol 934 in Hplc water separately. The mixture was constantly stirred with the help of magnetic stirrer at moderate speed to form uniform mixture.

3.2.2. Preparation of emulsion

The oil phase of emulsion was prepared by dissolving measured amount of Span 20 in Soyabean oil. The aqueous phase was prepared by dissolving Tween 20 in distilled water. Methyl and propyl parabens were dissolved in propylene glycol and mixed with the aqueous phase. Avocado added in oil phase. Permeation enhancer was also added in oil phase. Both the oily and aqueous phases were separately heated so that all the components get properly mixed. Then the oily phase was added to the aqueous phase slowly with continuous stirring to prepare emulsion.

3.2.3. Preparation of gel using 1:1 Ratio of Gel Base and Emulsion

To prepared emulsion was mixed with the gel bases i.e. Carbopol gel bases respectively in 1:1 ratio to form gel. The composition of different formulation has been shown in Table 3.3

Table 3.3: Composition of different formulation Avocado soybean gel with different polymers.

Ingredients % (w/w)	Formulation Batches							
	F1	F2	F3	F4	F5	F6	F7	F8
Avocado	1	1	1	1	1	1	1	1
Carbopol 934	1	1	1	1	1	1	1	1
Tween 20	0.5	1	1.5	1	0.5	1	0.5	1
Span 20	0.5	0.5	1	1	1.5	1.5	2	2
Soyabean oil	5	7.5	5	7.5	5	7.5	5	7.5
Methyl Paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl Paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Propylene Glycol	5	5	5	5	5	5	5	5
Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

3.3 Transmission Electron Microscopy (TEM) Analysis[82]

The TEM analysis of topical gel was performed for morphological characterization and visualization of gel droplets. Gel formulation was diluted with deionized water and mixed by gentle shaking. A drop of sample obtained after dilution was placed on copper grids, stained with 1% phosphotungstic acid solution for 30s, and finally kept under electron microscope to visualize the particle morphology.

3.4 Incorporation of avocado soyabean gel into gel

The Avocado soyabean gel was prepared by incorporating the optimized Carbopol 934 in the dispersion of solid dispersion kept for 24 h at room temperature.

Table 3.4: Composition of different solid dispersion gel.

Sr. No.	Composition Carbopol 934 (%w/v)	Formulation Code
1	0.5	F4(G1)
2	1	F4(G2)
3	1.5	F4(G3)
4	2	F4(G4)

3.5 Evaluation of gels

3.5.1 Visual appearance[83]

Visual inspection of the freshly made gels and physical inspection of the produced formulations were both performed.

3.5.2 pH determination[84]

The pH of the gel mixtures used to make the Piperine microsponges was measured using a digital pH meter. A pH meter in contact with the gel, one gram of gel dissolved in 100 ml of distilled water, and one minute of equilibration time were used to measure the pH in triplicate. Average readings were then calculated.

3.5.3 Rheological studies[85]

Rheological tests were performed using a viscometer. Before measuring the dial reading with a T-4 spindle revolving at five revolutions per minute, the sample (30 g) was placed in a beaker and given five minutes to acclimatize. The viscometer's dial reading matched the speed (50 RPM), which was noted. Each decrease in spindle speed was matched with a dial number, which was noted. Three readings were taken in total.

3.5.4 Spreadability Study[86]

The spreadability of gel was evaluated using a lab-made apparatus consisting of two glass slides, the bottom slide attached to a wooden plate and the top slide attached to a balance by a hook. One gram of gel was applied to the lower slide, but weight was applied to the upper slide. The higher slide displaced linearly in the weight's direction when weight was added, and the time it took for the upper slide to entirely move was recorded. The weight required for displacement spreadability was calculated using Equation.

$1\ S = m\ l / t$

S represents spreadability, m represents the weight affixed to the top slide, l represents the length of the glass slide, and t represents processing time.

3.5.5 Drug content[87]

The drug content of each sample of the Piperinemicrosponges gel formulation was determined individually by mixing 1 g of the gel with 100 mL of water. The resulting solutions were filtered through a 0.45 ml filter to produce transparent solutions. The drug concentration was measured spectrophotometrically using water as a blank.

3.6 In vitro drug release studies[88,89]

Membrane treatment: Durapore HVLP synthetic membrane was used as the semi-permeable membrane. It was soaked in the medium for 10 to 15 min before the study.

Method: Weighed quantity of gel formulation was taken in the donor compartment. Pre-hydrated membrane was mounted between donor and receptor compartment. The receptor compartment was filled with the phosphate buffer (pH 6.8). The two compartments were clamped together. All openings in the donor and receptor arm were then occluded with parafilm to prevent evaporation. Air bubbles were also removed by tilting cells if any. The receptor compartment was maintained by stirring with a magnetic bead at 500 rpm and the temperature was maintained at 37±2°C. The study was carried out for 4 h. Then samples were withdrawn from receptor compartment at predetermined time intervals (15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min) and an equal volume of buffer was replaced to maintain sink condition. The drug concentration was determined by UV spectrometry at 280 nm.

3.7 Drug release kinetics[90,91]

It is possible to apply a variety of mathematical techniques to describe the release profile, including those that form the basis for model-dependent tactics. The produced model parameters are then used to assess the release profiles after the appropriate function has been chosen. The data processing techniques indicated below were used to display the results of the ex vivo permeation investigations:

- Zero Order model
- First Order model
- Korsmeyer-Peppas model
- Higuchi's model

3.7.1 Zero order kinetics

It can be used to describe how various dosage forms of medications with modified release dissolve, including osmotic systems, coated matrix tablets, specific transdermal systems, and low soluble drug coatings. The following is an explanation of a zero order delivery:

Q0 - Qt is the same as K0t.

Where Qt is the amount of drug dissolved over time t, Q0 is the initial concentration of the drug in the solution (typically, Q0=0), K0 is the zero-order release constant represented in units of concentration/time. To analyze the release kinetics, data from in vitro drug permeation studies were presented as the total amount of drug released vs. time.

3.7.2. First order kinetics

It may be used to describe how medications break down in dose forms, such as those that include medications that are soluble in water in porous matrices. The following equation can be used to characterize the medication's first order kinetics-based discharge:

$C = \log C_0 - K.t / 2.303$

Where k is the first order rate constant, C_0 is the starting drug concentration, and t is the passage of time. Plotting the data as log cumulative % of medication remaining vs. time yields a straight line with a slope of K/2.303.

3.7.3. Higuchi's model

The drug release in the matrix system was anticipated by this model. It was typically considered for planar systems before being applied to various geometrical and porous systems. The following are the fundamental hypotheses for this model: Drug particles are much smaller than the thickness of the system, matrix swelling and dissolution are minimal, drug diffusivity is constant, and optimal sink conditions are always attained in the release environment. Only one dimension is involved in drug diffusion (the edge impact must be minimal).

Higuchi was the first to develop an equation to represent the release of a drug from an insoluble matrix using a time-dependent mechanism based on Fickian diffusion. The following are the Higuchi education levels:

$Q_t = K_H (t)\ 0.5$

Where Q_t is the drug's release rate constant based on the Higuchi model, and KH is the drug's release rate constant over time. A straight line is produced when the cumulative drug release is plotted against the square root of time, which shows that the drug was released by a diffusion mechanism. The slope's value is K_H.

3.7.4. Korsmeyer-Peppas Model[92]

Korsmeyer made a clear connection to explain the drug release from a polymeric framework. To describe the release rates from controlled release polymeric matrices, one can utilize the equation developed by Korsmeyer et al.

Q, which is equal to $K.t^n$, represents the amount of the drug released at time t'.

The tablet's geometrical and structural properties are taken into consideration by the kinetic constant K, and the release process is indicated by the diffusional exponent "n".

For Fickian release, n is equal to 0.45; for anomalous (non-Fickian) transit, it ranges from 0.45 to 0.89; and for zero order release, it equals 0.89.

4. Incorporation of gel

The gel of optimized F4 formulation was prepared by dispersing the formulation successfully in 0.5%, 1%, 1.5% and 2% Carbopol 934P and then subjected for characterization**.**

4.1 Evaluation of avocado soybean gel

4.1.1 Visual appearance

The visual appearance of solid dispersion gel formulation F4(G1-G4) is given below in table no 19:

Table 19: Visual appearance of avocado soyabean gel.

Sr. no.	Formulation code	Visual appearance
1	F4(G1)	Less viscous Gel Formed
2	F4(G2)	Uniform Gel Formed
3	F4(G3)	Uniform Gel Formed
4	F4(G4)	Sticky Gel Formed

Result: The prepared gel were examined visually for their consistency and found to possess smooth appearance. Out of three developed gel formulations batches F4(G2) were showed good homogeneity with absence of lumps. So those batches were used in further study.

4.1.2 pH of avocado soybean gel

The pH of avocado soyabean gel was shown in table 6.17.

Table 6.17: pH data of avocado soybean gel.

Sr. no.	Formulation code	pH value
1	F4(G1)	6.6±0.2
2	F4(G2)	7.4±0.1
3	F4(G3)	7.3±0.1
4	F4(G4)	7.9±0.23

Value is expressed as mean ± SD; n=3

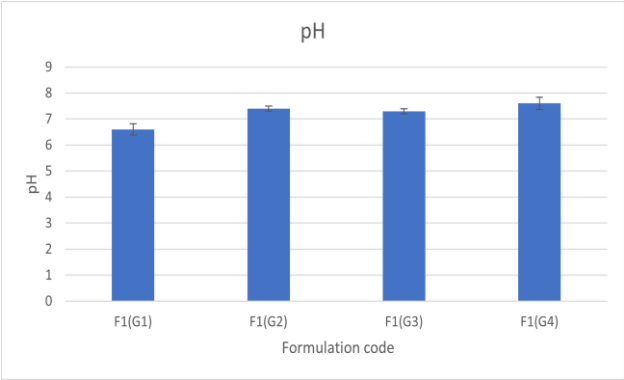


Figure 7.37: pH of avocado soyabean gel.

Discussion: From the Table 20 & fig. 20, it was found that pH of all formulation was found to be in a range 6.6±0.2 to 7.6±0.23.

4.1.3 Viscosity of avocado soybean gel

The viscosity of avocado soybean gel is shown in table 6.17.

Table 6.17: Viscosity of avocado soybean gel.

Sr. No.	Formulation code	Viscosity(cPs)
1	F4(G1)	2468±12
2	F4(G2)	1264±17
3	F4(G3)	3260±20
4	F4(G4)	4351±9

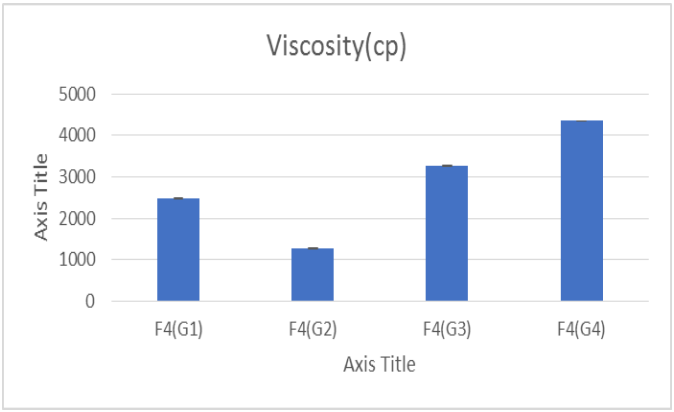


Figure 7.37: Viscosity of avocado soybean gel.

Discussion: The Viscosity of the gel at different formulation F4(G1)-F4(G4) was found to be in the range from 2468±12 to 4351±9.

4.1.4 Spreadability of avocado soybean gel

The spreadability of Avocado Soybean Gel is shown in table 6.19.

Table 6.19: Spreadability of avocado soybean gel.

Sr. No.	Formulation Code	Spreadibility (cm)
1	F4(G1)	7.3±0.1
2	F4(G2)	6±0.15
3	F4(G3)	6.6±0.1
4	F4(G4)	5.7±0.1

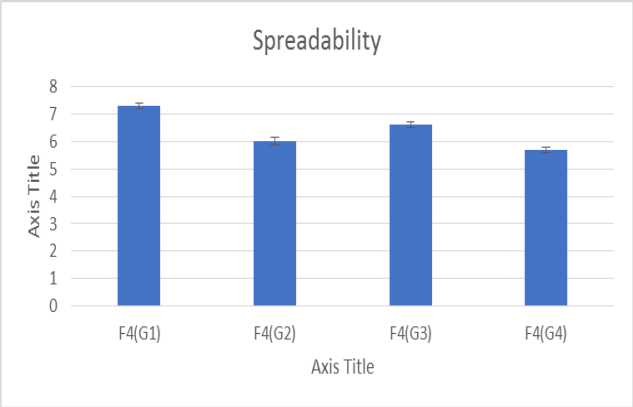


Figure 7.38: Spreadability of avocado soyabean gel.

Discussion: Spreadability was an important property of topical formulation from patient compliance point of view. The diameter was found to be 7.3 cm which is indicative of good spreadability.

4.1.5 Percentage drug content of avocado soyabean gel

The percentage drug content of Avocado soyabean gel was shown in table 6.20.

Table 6.20: % Drug content of avocado soybean gel.

Sr. No.	Formulation code	% Drug content
1	F4(G1)	93.54±0.4
2	F4(G2)	96.82±0.57
3	F4(G3)	94.67±1.74
4	F4(G4)	93.39±0.57

Discussion: The drug content of formulations was found to be96.82±0.57 and93.39±0.57 %, respectively. The percentage drug content of all formulations was found to be satisfactory. Hence, the method adopted for formulations was found to be suitable.

4.2 In-vitro Drug release study[70]

The in-vitro drug release of pure drug & Formulation F4(G2)in phosphate buffer (pH 7.4) was given in a table 6.20. Results of the dissolution efficiencies upto 8hrs are shown in Table 6.22.

Table 6.22: In-vitro drug release study of avocado soybean gel.

Time (Hr)	% drug release of formulation F4(G2)
0.25	6.39±0.4
0.5	18.73±0.07
1	25.46±0.07
2	35.25±0.36
4	45.98±0.36
6	56.64±0.21
8	65.53±0.36
10	78.55±0.36
12	86.53±0.36
24	91.32±0.36

Figure 7.41: In-Vitro drug release of avocado soybean gel.

Discussion: Drug release graphs for control gel & formulation F4(G2) was shown in Figure 6.19 were significantly different from the profile of control gel. In the control gel 91.33% was released within 1hr. On the other hand, the release of formulation F4(G2) was release up to AR.34% within 24 hr. followed by sustained manner. The in-vitro drug release of formulation F4(G2) and Pure drug was given in a **Table 6.21.**

4.3 In-vitro drug release kinetic

In-vitro drug release kinetic study data of formulation F4 has been given below.

4.3.1 Zero order

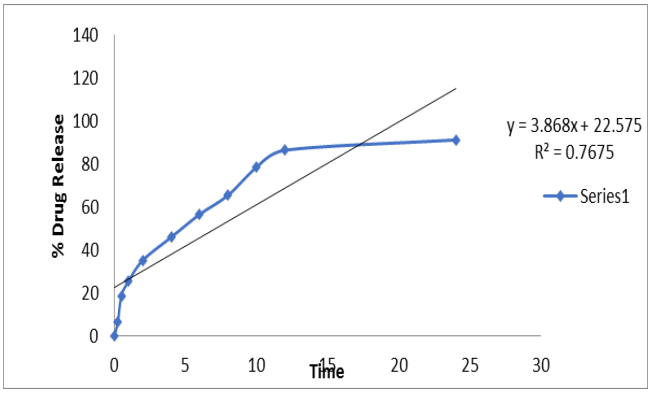


Figure 26: Zero order graph of formulation F4.

4.3.2 First order

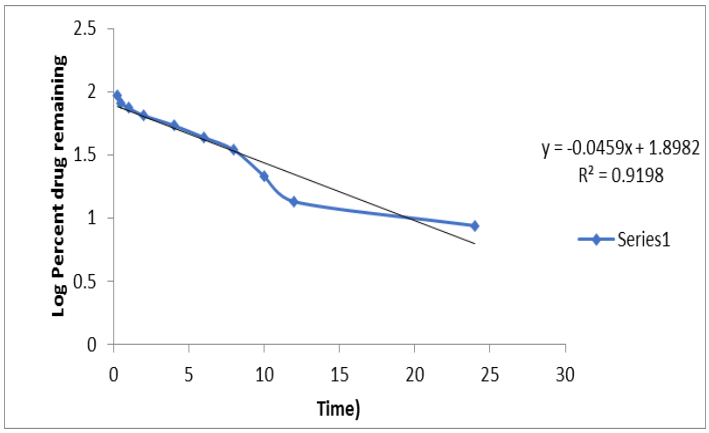


Figure 27: First order graph of formulation F4.

4.2.3 Higuchi model

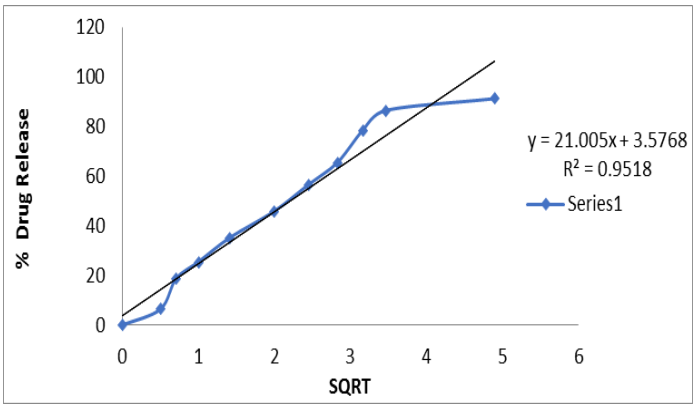


Figure 28: Higuchi order graph of formulation F4.

4.2.4 Korsmeyer peppas model

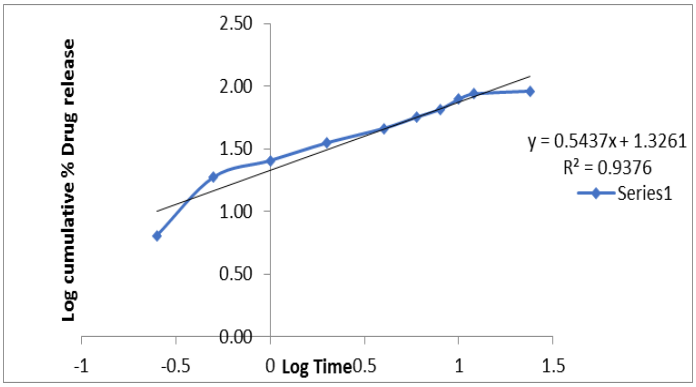


Figure 29: Korsmeyer Peppas order graph of formulation F4.

Table 25: Kinetic equation parameter of formulation F4.

Formulation Code	Zero order		First order		Higuchi		K. Peppas	
	K0	R2	K0	R2	K0	R2	K0	R2
F4	3.868	0.7675	0.0459	0.9198	21.005	0.9518	0.5437	0.9376

Mathematical models are commonly used to predict the release mechanism and compare release profile. For the optimized formulation, the % drug release vs. time (zero order), log percent drug remaining vs. time (first order), log per cent drug release vs. square root of time (Higuchi plot), and log of log % drug release vs. log time (Korsmeyer and Peppas Exponential Equation) were plotted. In each case, R2 value was calculated from the graph and reported in Table 25 and Figure 26 to Figure 29. Considering the determination coefficients, Higuchi's model was found (R2=0.9518) to fit the release data best. It could be concluded from the results that the drug was released from mometasone furoate loaded solid dispersion gels by a control mechanism.

CONCLUSION

The objective of the study was to prepared gel of Avocado Soybean, using Carbopol 934 as a gelling agent. Preformulation study of Avocado soybean was carried out all the parameter for Organoleptic characteristics, Melting point determination, Ultraviolet absorption maxima, partition coefficient, solubility study. The drug excipient interaction analysis revealed that there is no chemical interaction between the drug and the polymer. The formulations were evaluated for pH determination, rheological studies, spreading coefficient studies, extrudability studies, in vitro drug release and stability study. Gel have emerged as one of the most interesting topical delivery systems as it has dual release control system i.e. They have several favourable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, long shelf-life biofriendly, transparent and pleasing appearance. Thus, designing of Avocado soyabean gel topical drug delivery would efficacy of drug.

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