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DEVELOPMENT AND CHARACTERIZATION OF TRETINOIN NANOSPONGE GEL FOR SKIN DELIVERY

***Simranjeet Kaur, Supneet Kaur and Dr. Naresh Singh Gill**

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

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

ABSTRACT

The aim of this research work is to formulate and characterization of tretinoin nanosponge gel for skin delivery. The primary goal of this study was to increase the residence time, bioavailability and onset of action. Acne vulgaris is one of the most common dermatological disorders that effect people in their adolescence. Acne vulgaris are simply known as acne is a human skin disease characterized by skin with scaly red skin, blackheads, whiteheads. Tretinoin block several important inflammatory pathways that activated in acne all like receptors, leukocytes migration AP-1 pathway. It improves scarring texture and fades pigmentation. Nanosponge gel has large and tremendous effector potential to serve as topical drug delivery systems, better antifungal activity, storage and stability. Nanosponges are water soluble; its particle mix with water and use it as transport fluid. Preparation of nanosponge is done by emulsion solvent diffusion method. percentage drug entrapment was found was 99.9±0.01. particle size of 404.6nm, 28.2%, zeta potential -21.6mV. The nanosponge gel showed 85.69±0.003 release after 24 hours in a controlled manner. The outcome of work recognized a unique, simple, and stable product having improved drug entrapment and increased bioavailability thus improved bioavailability at acne vulgaris disease with less adverse actions.

KEYWORDS: Nanosponge, Tretinoin, topical drug delivery, gels.

1. INTRODUCTION

1.1 Topical drug delivery system

Topical drug administration refers to the localized delivery of medication through the skin, vaginal, ophthalmic, and rectal channels to any part of the body. One of the human body's

easiest organs to administer topically is the skin, which serves as the primary route for topical medication delivery systems.[1] The skin is a useful delivery channel for drugs since it avoids many of the problems associated with parenteral, inhalation, and oral routes. Researchers have been interested in it in recent years due to these benefits for the skin.[2]

At least four factors affect the toxicity and efficacy of topical products. These include.

(1) medication or chemical absorption via the skin from a product.

(2) application conditions.

(3) skin physiology in the product user.

(4) Patient and consumer impression of the product.

Advantages of topical drug delivery system

1. Prevents problems with gastrointestinal (GI) drug absorption brought on by GI pH, enzymatic activity, and drug interactions with food, beverages, and other oral medications.
2. It eliminates the difficulty of parenteral therapy, patient acceptability is improved.
3. Avoids the first-pass effect, possibly avoiding the deactivation by digestive and liver enzymes. Reduction of doses as compare to oral dosage forms.
4. Enhances compliance by offering prolonged therapy with a single application.
5. The application of medication can be quickly stopped by removing it from the skin's surface.
6. Less oily and simpler to remove from skin.

Disadvantages of topical drug delivery system

1. Extreme sensitivity of the skin.
2. The possibility of an allergic reactions.
3. Certain medications have a low skin permeability.
4. Drugs with large particles are challenging for the skin to absorb.
5. Skin irritation is a result of contact dermatitis.
6. The formation of a bubble in the emulgel formulation process.[3-5]

1.2 Anatomy and Physiology of Skin

Anatomy of Skin

The largest organ in the body is skin. There are three layers to it. The epidermis is the outermost layer, the dermis is the middle layer, and the hypodermis is the innermost layer.[6]

Structure of Skin

The skin is a thick, multilayered organ that protects the body from chemical and physical harm. Certain accessories, such as mustard gas, nickel ions, and oleoresins from Rhus toxico dendron, or "bane ivy," can penetrate the hedge, but not all of the material can. The skin serves as a thermostat to regulate body temperature, shields the body from microbial invasion, shields against ultraviolet radiation, and aids in blood pressure regulation.[7]

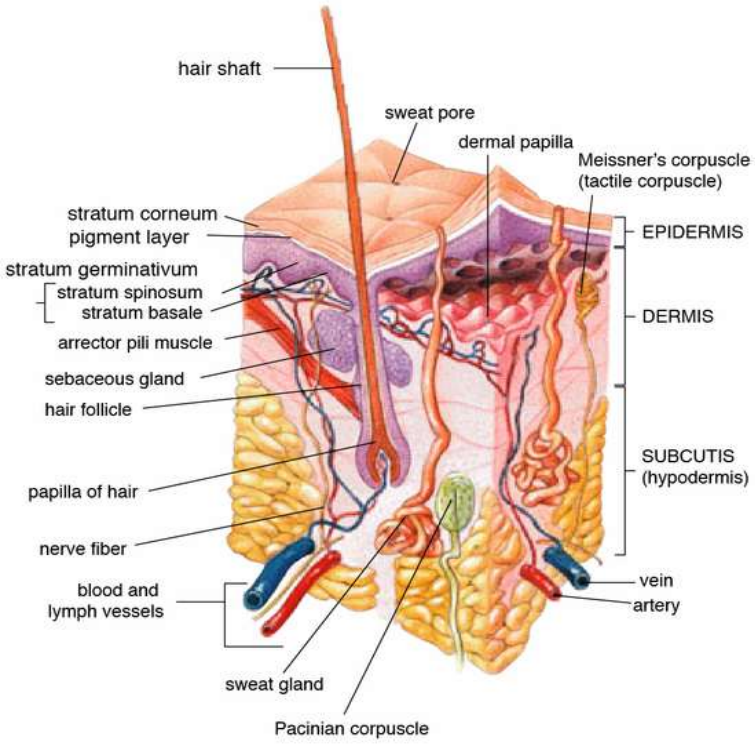


Figure 1.2: Structure of skin.[8]

Layers of Skin

Epidermis

consists of cells called epithelium. There are both dead and living cells among these cells. The older cells are forced upward by the rapid division of these young cells at the base of the epidermis. There is no direct blood vein supply to the epidermis to supply nourishment. It receives nourishment by the diffusion of essential molecules from a dense circulatory network in the dermis underneath. Desmosomes form a very tight connection between epidermal cells. Desmosomes come into contact with the keratin filmates inside cells. Keratin is produced by keratin filmates. It keeps the majority of chemicals from entering or exiting the body.

The five layers of the epidermis are as follows

- stratum germinativum (basal layer)
- stratum spinosum
- stratum granulosum
- stratum lucidum
- stratum corneum, which is the outermost layer. The thickness of the stratum corneum is 10–20 µm when it is dry and 40 µm when it is hydrated and swollen.[9,10]

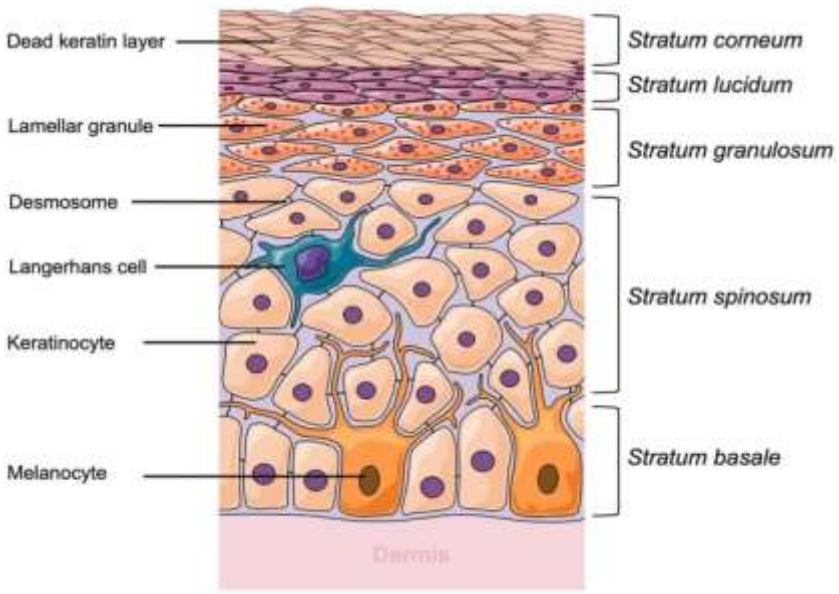


Figure 1.2: Layers of epidermis.[11]

Dermis

The dermis, which lies beneath the epidermis, is distinguished by a high concentration of collagen, which gives the skin strength, and elastin fibers, which give the skin its capacity to stretch. Dermal blood veins supply nutrition to the epidermis as well as the dermis. The dermis is also essential for controlling body temperature. Sensations of pressure and discomfort are produced by the nerves that are located there. The thickness of the dermis is 3–5 mm. The dermis contains elastin fibers, blood arteries, nerves, salt, water, lymphatic cells, and sweet glands in addition to an interfibrillar gel of glycosaminoglycan.[10]

Hypodermis

The epidermis is the skin's innermost layer. It is the layer of skin that comes into contact with the body's underlying tissues, including the muscles and bones. Although they originate in the dermis, sweat glands, sebaceous glands, and hair follicles are encased in the epidermis. A diluted salt solution is secreted onto the skin's surface by sweat glands. Skin cools down as a

result of this solution evaporating, which is crucial for controlling body and skin temperature. The body is covered in sweat glands. The temperature of the surrounding air, the quantity of heat-producing skeletal muscle activity, and a variety of emotional elements all affect how many dilutions (sweets) are created. Sebum is produced by the sebaceous glands. Sebum is an oily substance that is secreted into hair follicles and then reaches the surface of the skin.[12]

Absorption Through the Skin[13]

Transdermal administration is another form of topical administration in which a drug is applied topically and then absorbed by the body to achieve systemic distribution. Similar details are often hydrophobic substances, as hormones found in steroids. Topical specifics are those that are used to treat colored affections on the body. In most cases, a topical medication delivery system is applied topically, where the medication either treats the targeted location or permeates the dermis and enters the bloodstream. The enhancement of skin permeability and retention in the dermis is the primary goal of the topical drug delivery method. The "pores," or openings in the hair follicles and sebaceous glands, allow these molecules to enter the skin and limit the amount of medicine that may be absorbed. Active composites can penetrate the epidermis through three different routes. These are transcellular (intracellular) saturation through the corneocytes and intercellular lipid matrix, and appendageal (intercellular) penetration through the hair follicle or via the sebaceous and sweat glands.

Drug permeation through skin routes[13,14]

Three main pathways for skin absorption.

1. Primary Transcellular: The chemical moieties are transferred into and out of the cell membrane via keratin – packed coenocytes.
2. Secondary Intercellular: In the lipid – rich extracellular space, the molecules are moved around coenocytes.
3. Sebaceous glands, hair follicles, and sweat glands promote the transappendageal transfers.

1.3 Nanosponges

Nanoscale materials have been developed and used by the pharmaceutical and medical industries to address a variety of physical, chemical, and biological issues related to illness treatment. The science and technology of accurately modifying matter's molecular structure is known as nanotechnology. It is the small-scale application and manipulation of materials. Nanotechnology is the study of creating usable materials, devices, and systems by manipulating matter at the nanoscale length scale and taking advantage of new phenomena

and features. As a result of developments in nanoscience and technology, a wide range of materials and better goods might become accessible with altered physical characteristics when their sizes are reduced. Drug delivery methods based on nanotechnology can also shield medications against deterioration. These characteristics may lessen the number of dosages needed, improve the course of treatment, and save costs. Many nanotechnology-based platforms enable the delivery of insoluble medications, enabling the use of previously rejected medications or medications that are challenging to administer, such as paclitaxel. They technologies are currently mostly employed for fully established off-patent pharmaceuticals that are already on the market; they are the so-called low-hanging fruit of nanotechnology-based delivery. It is incorrect to think of nanotechnology as a singular method that has limited use. It is more of a catch-all word for a science that is useful for hundreds of commercial products as well as the environment and healthcare.[15,16]

The polyester nanosponges are a three-dimensional scaffold, or backbone, or network that can break down spontaneously. To create Nanosponges, these polyesters are combined with a crosslinker in a solution. In this case, polyester degrades somewhat in the body because it is often biodegradable. When the nanosponges' scaffold disintegrates, the drug molecules that are loaded are released in an unfavourable way.[17]

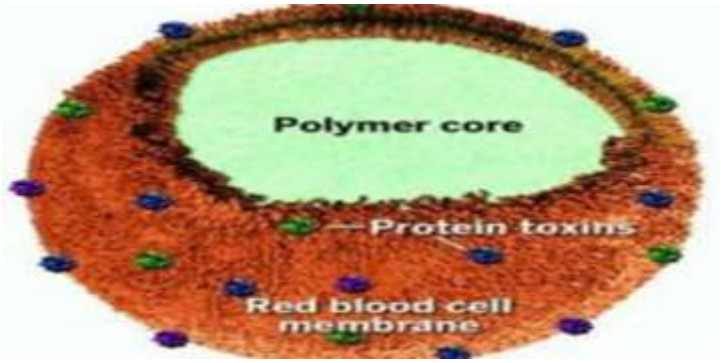


Figure 1.4: Structure of Nanosponge.[18]

Advantages of Nanosponges[19-21]

1. Targeted distribution of medication to precise sites.
2. Capable of disguising unwanted Flavors and solidifying liquids.
3. Less detrimental adverse effects.
4. Because nanosponge particles dissolve in water, hydrophobic medications can be encapsulated in them by mixing them with an adjuvant reagent.
5. Changing the ratio of cross-linker to polymer allows for the manipulation of particle size.

6. Production using click chemistry, a kind of relatively basic chemical
7. Easily scaled up for industrial manufacturing.

Disadvantages of Nansponges

1. Larger molecules cannot be encapsulated by nanosponges; they are only appropriate for small molecules.

2. Sometimes, dose dumping could happen.[22]

Chemicals used for the synthesis of Nanosponges

1. Polymers - Methyl β-cyclodextrin and other hypercross-linked polystyrene derivatives are examples of cyclodextrines. -Cyclodextrins such as Alkyloxy carbonyl, 2-Hydroxy Propyl β, and copolymers such as poly (valerolactone-allylvalerolactone, ethyl cellulose, and PVA).

2. Crosslinkers - Dichloromethane, acetic acid, carboxylic acid dianhydride, dichloridine glutaraldehyde, diphenyl carbonate, dialrylcarbonates, and carbonyldiimidazole.[23]

Preparation of Nanosponge

1. Nanosponges prepared from hyper-cross linked β-cyclodextrins

Made from β-cyclodextrins, these nanosporous materials function as drug delivery carriers. As a result, three-dimensional networks are created, which could resemble a roughly spherical structure the size of a protein with interior channels and pores. using a cross linker, such as di-isocianates, diaryl carbonates, carbonyl di-imidazoles, etc., to react with cyclodextrin. The porosity and surface charge density of sponges determine their size and ability to adhere to various molecules. Depending on the cross linker employed, neutral or acidic nanosponges can be created. They are made up of solid particles that have been transformed into crystals. The ability of nanosponges to encapsulate drugs with varying solubility and shapes. They are used to make medications that aren't very soluble in water more soluble in water.[24,25]

2. Solvent method

By combining the polymer with polar aprotic solvents such as dimethyl sulfoxide (DMSO) and dimethylformamide (DMF), nano sponges are created by the solvent method. Then, in a 1:4 ratio, a crosslinker is added to this mixture. To reflux the solvent's temperature for a duration of one to forty-eight hours, the aforementioned reaction should be carried out at a temperature of 10˚C. After the reaction is finished, the mixture is allowed to cool to room

temperature, and the resulting product is then mixed with bi-distilled water. The product is recovered using vacuum-separated filtration, soxhlet extraction with ethanol, and drying.

3. Ultra-sound assisted synthesis

Crosslinkers and polymers are made to react in a flask without the presence of a solvent. The flask is submerged in a water-filled, ultrasonic bath that has been heated to 90˚C. The mixture is sonicated for five hours. Following the mixture's cooling to room temperature, the finished product is roughly divided into pieces. Finally, the product is cleaned with water to eliminate the non-reacting polymer, and ethanol is used in the soxhlet apparatus to refine the product and create nanosponges.[26]

4. Emulsion solvent diffusion method

Using this method, different ratios or amounts of ethyl cellulose and polyvinyl alcohol are combined to form nanosponges. This method uses both continuous and scattered phases. The medication and ethyl cellulose together make up the dispersed phase, which is subsequently mixed with 20 milliliters of dichloromethane and a small amount of polyvinyl alcohol (PVA) to generate the continuous phase (aqueous). Next, the mixture is stirred at 1000 rpm for about two hours. By filtering, the final product—nanosponges—is produced. The final temperature at which the product is dried in the oven is 400°C.[27]

5. Quasi-emulsion solvent diffusion

The Nanosponges can be made by varying the amounts of polymers and employing a quasi-emulsion solvent diffusion process. Eudragit RS100 was dissolved in a suitable solvent to prepare the inner phase. Subsequently, the fluid can contain medication or an active substance that can be ultrasonically broken down at 350°C. The PVA was filled with the inner phase. After agitating the mixture for 60 minutes, the Nanosponges are removed by filtering. The Nanosponges are drained in an air-heated oven for 12 hours at 40°C.[28]

6. Melt method

This method involves continuously stirring the cross linker and CD at 100°C for three to five hours in order to homogenize and melt them together. The mixer was cooled to room temperature in order to eliminate the reaction byproducts, and the final product was broken up and repeatedly cleaned using a suitable solvent.[29]

7. Microwave assisted synthesis

Microwave irradiation is the most straightforward way to synthesize CDNS and also results in a significant reduction in reaction time. The NS that is produced has more crystallization. The reaction time was cut in half when comparing conventional melt technique to microwave-assisted production. The technique resulted in homogenous particle size distribution and crystallinity.[30,31]

Characterization of Nanosponges

1. Particle size evaluation

The particle sizes of burdened and unburdened NSs were assessed using laser light diffractometry, the Malvern Zeta Sizer, and zeta potential. After each sample was examined three times, the mean value was utilized to take additional measurements.[32,33]

2. Solubility studies

It is the most popular method for studying inclusion complexes and is mostly based on Higuchi and Connor's equation for phase solubility, which also aids in examining how a nanosponge affects a drug's solubility.

3. IR spectroscopy

It is employed to calculate the solid-state drug molecule interaction with nanosponges. Upon complex formation, it frequently varies. If a small portion of the molecule is encapsulated in the complex, less than 25% of the band is assigned to contain part of the other molecule, which is marked by bands of the nanosponges spectrum. The use of IR is restricted to certain medications that have bands or characteristics like sulfonyl or carbonyl groups. Information on hydrogen in different functional groups is involved in infrared investigations. As a result, absorbance bands change to a lower frequency, become more intense, and broaden as a result of the group engaged in hydrogen bond formation stretching vibration.[34,35]

4. Microscopy studies

The active pharmaceutical ingredients (API)/NSs complex can be examined using scanning electron microscopy and transmission electron microscopy to examine the morphology, surface topography, and microscopic details.[36,37]

5. Determination of entrapment efficiency

The medication, which is equal to Nanosponges, was taken in amounts of 0.1 gm and pulverized before being transferred into a 100 ml measuring flask. The flask was then filled to capacity with simulated stomach fluid of pH 1.2 and 10 ml of methanol. The following day, or after twenty-four hours, the solution was filtered using Whatman filter paper, and following the proper dilutions, the absorbance was measured using spectrophotometry.

6. Zeta potential determination

The zeta potential was determined in order to ascertain the speed at which particles move in an electric field. The Zetasizer Nano ZS, manufactured by Malvern Instruments Ltd. in the UK, was utilized to assess the Nanosponges following ten diluting cycles with distilled water.[38]

7. Porosity

The purpose of this study is to verify the composition of the various nanochannels and nanocavities. Since helium gas has the ability to perforate both intra- and inter-particular channels of substances, helium pycnometers are used to check the porosity of nanoscale structures. Eqn specifies the percent porosity:

%Porosity=Bulk volume -True volume/Bulk volume ×100

8. Fourier transform infrared (FTIR) analysis

FTIR analysis is used to examine how the medication and polymer interact chemically. Powder was scanned in the carbon black position and 400–4000/cm range.[39]

9. Thermoanalytical methods

It demonstrates the modifications made to the drug's composition prior to the thermal destruction of nanosponges. The medication may change due to polymeric transition, melting, evaporation, oxidation, or breakdown. Drug substance changes signify the complex's development. DTA and DSC monitored for peak broadening, shifting, and emergence. Changes in weight loss may indicate the development of inclusion complexes.[40]

10. Single crystal X-ray structure analysis

It could be applied to ascertain the precise inclusion structure and interaction style. It is possible to pinpoint the precise geometric relationship and identify the interaction between the host and guest molecules.[51]

11. In Vitro release studies

A rotating cell with many compartments is used for conducting in vitro release kinetics research. The doner compartment is filled with a 1 ml aqueous dispersion of nanosponges containing the drug, and the receptor compartment, which is divided by a hydrophillic dialysis membrane, is filled with either pH 1.2 or 7.4 phosphate buffer. Every experiment lasts for a full day. The receptor buffer is fully removed and replaced with new buffer at predetermined intervals. A suitable analytical method is used to estimate the amount of drug in the medium, and drug release is computed to determine the pattern of release.

11. Permeation studies

To investigate the dissolution release of the manufactured nanosponge across a cellophane membrane, diffusion studies of the nanosponge can be conducted in a Franz diffusion cell. A 0.5g nanosponge sample can be placed on a cellophane membrane, and diffusion studies were conducted at 37 ± 1° with a dissolution media of 250 ml of phosphate buffer (pH 7.4). Every one to eight hours, 5 milliliters of each sample can be taken out and replaced with an equivalent volume of brand-new dissolving medium. After then, the samples' medication content can be determined by using phosphate buffer as a blank.[42]

1.4 Applications of Nanosponge

1. Nanosponges for drug delivery - Class II medications in the Biopharmaceutical Classification System can be effectively carried by nanosponges due to their nanoporous nature. These complexes can be used to conceal off-putting odors, turn liquids into solids, and speed up the rate at which medications dissolve, become soluble, and remain stable. It has been found that β-cyclodextrin-based nanosponges are three to five times more effective than direct injection at delivering the drug to the target site.[43]

2. Nanosponges as chemical sensors - Using nanosponge titania, a form of "metal oxide," nanosponges function as chemical sensors for the extremely sensitive detection of hydrogen. Since there is initially no point of contact in the nanosponge structure, electron transport is less hindered, leading to greater 3D interconnect nanosponges titania that are gas sensitive to H2.[44]

3. Nanosponge in protein drug delivery - The protein known as bovine serum alubin (BSA) is kept in lyophilized form since it becomes unstable in solution. The stability of proteins like BSA was improved by swellable poly (amidoamino) nanosponge based on cyclodextrin.

Additionally, proteins can be encapsulated in nanosponge materials for regulated delivery and stabilization of the enzymatic material.[45]

4. Nanosponge for oral delivery - When applied orally, it creates a nanosponge system with pores that speed up the solubilization of medications that aren't very soluble in water and trap the medication inside the pores. The nanosize shape and faster rate of solubilization increase the surface area.[46]

5. As absorbent in treating poison in blood - By absorbing the toxin, nanosponges can remove harmful substances from our blood. By injecting nanosponges into the bloodstream, we can absorb toxins instead of employing antidotes. The nanosponge imitates a red blood cell in the bloodstream, deceiving toxins into attacking it before absorbing them. The toxin determines how many poison molecules each nanosponge can absorb.[47]

6. Nanosponges for cancer therapy - The administration of anticancer drugs is one of the most difficult tasks in the pharmaceutical industry these days due to their low solubility. According to one report, direct injection is three times less effective than nanosponge complex at slowing down tumor growth. The drug-loaded nanosponge combination exposes a targeting peptide that firmly bonds with the tumor receptor's radiation-induced cell top layer. Upon encountering a tumor cell, nano sponges adhere to its surface and initiate the release of medication molecules. Targeting drug delivery has the benefit of maximizing therapeutic impact at the same dose while minimizing side effects.[48]

7. Nanosponges as protective agent from light or degradation - A blend of ferulic acid ester, gamma-oryzanol has garnered a lot of attention lately as a natural antioxidant. It is typically used to stabilize food and pharmaceutical raw materials, as well as a sunscreen in the cosmetics industry. Due to its high instability and photodegradation, its applicability is restricted. Encased in nanosponges, gamma-oryzanol demonstrated strong resistance to photodegradation. The nanosponges that were loaded with gamma-oryzanol were used to create a gel and an O/W emulsion.[49]

8. Nanosponges as a carrier for biocatalysts and release of enzymes, proteins, vaccines and antibodies - It covers industrial processes that are related to operational conditions. Non-specific reactions result in low yields and need high temperatures and pressures, which use a lot of energy and cold water in the downstream process. The disadvantages can be overcome

by employing enzymes as biocatalysts since they function well in moderate conditions and at high reaction speeds.[50,51]

9. Antiviral application - Nanosponges used in nasal, pulmonary route of administration. It provides specificity to deliver antiviral drug on RNA to lungs or nasal route through nanocarriers for targeting virus which may cause infection to RTI such as influenza virus, rhinovirus. Drugs used as nanocarriers are- Zidovudine, Saquinavir.[52,53]

1.5 Gels

Topical gels are semi-solid, homogenous formulations used for both skin disease treatment and prevention. Gels' hydrophilic properties allowed the medication or active ingredient to be delivered fast. Two components make up a gel: a three-dimensional, cross-linked material with enough liquid in it to form a sufficiently rigid network to trap the liquid continuous phase. To form the structural network of gel, both inorganic particles and organic macromolecules are used. In contrast to physical topical gels, which feature weaker and reversible Vander Waal forces, electrostatic interactions, hydrophobic contacts, and permanent covalent bonding holding the particles together, chemical gels have secondary intermolecular forces.[54]

1.10.1 Structure of gels

A gel consists of a natural or synthetic polymer forming a three-dimensional matrix throughout a dispersion medium or hydrophilic liquid. As soon as the liquid is applied, it evaporates, leaving the medication contained in a thin layer of the gel-forming matrix that physically covers the skin.[89] The flexibility of a gel is caused by the existence of a network created by the interlocking of gelling agent particles. The composition of the particles and the kind of forming that creates the links influence the network's structure and the gel's properties.[55]

Ideal properties of topical gels

- The gel ought to be uniformly transparent.
- When shear or force is applied during shaking the container, the gel should break easily.
- The gel need to possess inert properties.
- The gel ought not to be tackey.
- There should never be any interaction between the gel and other formulation elements.
- The gel needs to be steady.

- The skin or whatever area the gel is administered to shouldn't get irritated.
- It should have antimicrobial properties and have the ideal viscosity.[56]

Method of preparation of gel

Gel preparation can be done in three different ways.

1. Fusion method: In this technique, the medication, vehicles, gelling agents, and additives are mixed at a high temperature to prevent the formation of a semi-solid texture.

2. Cold method: In this procedure, every component—aside from the medication or active pharmaceutical ingredient—is heated and blended at the same time. The formulation's temperature is then lowered, the drug is added, and the blending process is repeated until the gel is not formed.

3. Dispersion method: In this technique, the drug is dissolved in the medium and mixed in with the gelling agent after it has been agitated with water till it swells up. If necessary, add buffer solution to the gel to change its pH.[57]

Evaluation parameters of topical gels[58-63]

pH measurement: The pH of various gel compositions is measured using a digital pH monitor. One gram of gel is combined with 100 milliliters of freshly prepared distilled water, and it is left to dissolve for two hours. The pH of each formulation is measured three times, and the average values are calculated.

Measurement of Viscosity: A digital viscometer made by Brookfield can be used to measure the viscosity of gel formulations that have been manufactured. The gels rotate at 0.3, 0.6, and 1.5 revolutions per minute. For every speed, the appropriate dial reading is indicated. The viscosity of gel can be calculated by multiplying the dial measurement by a factor found in the Brookfield viscometer catalogues.

Spreadability: The spreadability of a gel is the area to which it readily spreads after application. It is assessed using a wooden block measurement tool and a glass slide. Spreadability is the time it takes for two slides to separate from a gel placed in their interstices when subjected to a particular force. It is measured in seconds. Shorter intervals between two slides result in better spreadability. To calculate spreadability, use the following formula.

S = M.L / T

S stands for spreadability

M = Top slide tide's weight

L is the length of the glass slide

T is the time required to completely isolate one slide from the others.

Homogeneity: All of the gels are visually inspected to assess their uniformity after formation.

Grittiness: All gel formulations are microscopically examined to see whether any particle matter is present.

Extrudability: Collapsible tubes are filled with gel compositions that have been placed in containers. The extrudability of gel compositions is measured by the weight in grams required to extrude a 0.5 cm ribbon of gel in 10 seconds.

Screening for stability: One method for examining stability is freeze-thaw cycling. For a duration of one month, the product is heated to 40 degrees, followed by 25 degrees and finally 40 degrees. Syneresis is present. After exposing the gel to room temperature, distinct liquid exudates are seen.

Drug content: To 100 milliliters of an appropriate solvent containing the medication, 1g of gel is added. After a suitable dilution at max nm, absorbance is measured using a UV spectrophotometer.

Drug Diffusion Study in Vitro: The Franz diffusion cells are utilized in in vitro drug release investigations. 0.5 grams of gel are contained in a cellophane membrane. Diffusion tests are conducted at 37°C to 10°C using a 250 mg phosphate buffer with a pH of 7.4 as the dissolving media. One millilitre of sample is collected every hour, and new buffer solution is added in its stead. The gathered samples are appropriately evaluated.

Skin irritation test: For a skin irritation test, ten volunteers—ten men and ten women—who were in good health were chosen. A 2 cm area on the inside surface of the upper arm received 100 mg of gel application for 6 hours, after which it was wrapped with a cotton bandage. Following a six-hour period, the locations were cleaned using acetone, and measurements were taken using Draize's scale. Not irritated: 0 Mild annoyance: 1 Two points of irritation.

In-vivo Study: Mercury paleothermometer is used to investigate the inhibition of carrageenan-induced rat paw edema in male wistar arabino rats. The experimental animals' unilateral hind paw volume is assessed both before and after carrageenan is administered. % There is an inhibition.

2. Preformulation Study

It is the study of a drug substance's physical and chemical characteristics both on its own and in combination with excipients. Preformulation testing's main goal is to produce data that formulators can use to create stable, bioavailable dosage forms that are easy to mass manufacture. Preformulation studies are intended to provide all relevant information, particularly about the physicochemical, physico-mechanical, and biopharmaceutical characteristics of medicinal ingredients, excipients, and packaging materials.

OBJECTIVES

- Prior to developing a dosage form, it is crucial to comprehend the physical characteristics of a drug ingredient in order to create elegant dosage forms that are safe, stable, and effective.
- It is the first stage in the methodical process of developing a drug's dosage form before developing the dosage form itself.[64]

2.1 Organoleptic properties (API) Visual observations were used for the organoleptic research, which included overall appearance such as nature, color and odor among others.

- Colour: A small amount of medication was placed on butter paper and observed in an area with good lightning.
- Odor: Very little medication was smelled in order to detect the odor. The aforementioned experiments were all completed visually, without the need for any specialized equipment.

2.2 Melting point Determination

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.[65]

2.3 Determination of Maximum absorption wavelength

A stock solution of 100µg/ml of tretinoin was made by dissolving 10 mg of the medication in 100 ml of ethanol in order to determine the drug's wavelength of maximum absorption (λmax). Using ethanol as a blank, a spectrophotometer was used to scan a drug solution (10µg/ml) in ethanol within the 400–200 nm wavelength range. The resultant spectra revealed the drug's distinctive absorption maxima at 352 nm on the absorption curve.

2.4 Preparation of standard stock solution To create the standard stock solution, tretinoin was dissolved in ethanol until a final concentration of 10µg/ml was reached. Ethanol was used to dilute various aliquots from the stock solution, resulting in a range of concentrations from 1 to 12 µg/ml. In ethanol, the λmax was discovered to be 352 nm. Ethanol served as a blank when the absorbance was measured at 352 nm. Plotting the absorbance vs tretinoin concentration allowed for the preparation of the calibration curve.

2.4.1 Linearity

This method's linearity was assessed at concentration ranges of 2µg/ml to 12µg/ml. It was discovered that the range of the absorbance v/s concentration of tretinoin was linear. Beer's law held true within this range of concentrations.[66]

2.5 Solubility of tretinoin in solvents

By dissolving an excess of tretinoin in 2 ml of solvents and other components with a stirrer at 37 °C ± 0.5 for 72 hours, the solubility of tretinoin was ascertained in several solvents, including ethanol, methanol, chloroform, DCM, and Ph 6.8. To extract the remaining medication, the equilibrated samples were centrifuged for 30 minutes at 15,000 rpm. After dilution with ethanol at 352 nm, the filtrate was analyzed spectrophotometrically using nano spectrophotometry to ascertain the solubility of tretinoin.[67]

2.6 Partition coefficient of drug

An indicator of a drug's lipophilicity or hydrophilicity and its capacity to cross cell membranes is its partition coefficient (oil/water). Its definition is the proportion of unionized medication that is evenly split between the aqueous and organic phases at equilibrium. The partition coefficient offers a way to describe how lipophilic or hydrophilic the medication is. Drugs with P values significantly more than 1 are considered lipophilic, whereas hydrophilic drugs are indicated by values significantly lower than 1. An oil phase consisting of water and

n-octanol is frequently used to calculate the partition coefficient. In the instance of water and n-octanol.

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

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**

With the shake flask method, the partition coefficient determination investigation was carried out. Overdosage of the medication (aspirin) dissolved in 10 ml of n-octanol: water (1:1) mixture and left for 24 hours. The two layers were separated and centrifuged for 15 minutes at 15,000 rpm after a 24-hour period. After the proper dilution, the absorbance was measured in a UV spectrophotometer at the corresponding λ max.[68]

2.7 FTIR of Drug FTIR (Spectrum Two; Perkin Elmer, USA) was used to examine the functional groups of the medication and polymer as well as any potential interactions between them at room temperature. The wavelength range for this analysis was 500–4000 cm^{-1}.[69]

3. MATERIAL AND METHODS

Table 3.1: Active Pharmaceutical Ingredients (API)

Sr. No.	Active Pharmaceutical Ingredients (API)	Source/Manufacturer
1.	Tretinoin	Enaltec Labs, Maharashtra

Table 3.2: List of Instruments used during the experimental work.

S. No.	INSTRUMENTS	MANUFACTURER
1	UV/VIS Spectrophotometer,	Shimadzu, Japan
2	Weighing balance, (CY220)	Shimadzu, Japan
3	Magnetic stirrer	Remi Scientific Instruments, Mumbai
4	Vortex mixer	Remi Scientific Instruments, Mumbai
5	Hot air oven	Narang Scientific Works, New Delhi, India
6	pH Meter	Labindia Ltd, Mumbai, India
7	Melting Point Apparatus	Remi Scientific Instruments, Mumbai
8	Water Bath Shaker	Perfit, Mumbai
9	Infrared red spectrophotometer (FTIR)	Perkin elmer
10	Ultrasonicator	PCi analytics, India
11	Eppendorf tubes	Tarsons Products Pvt. Ltd., Kolkata, India

12	Vaccum pump	Suguna single phase, Chennai, India
13	Vials	Tarsons Products Pvt. Ltd., Kolkata, India
14	Microcentrifuge	Remi Scientific Instruments, Mumbai

Table 3.3: List of Chemicals.

S. No	Materials	Source
1	Tretinoin	Enaltec Labs, Maharashtra
2	Potassium dihydrogen Orthophosphate	Fischer Scientific, Mumbai
3	Sodium dihydrogen orthophosphate	Fischer Scientific, Mumbai
4	Methanol	SD Fine-Chem Ltd, Mumbai
5	Ethanol	SD Fine-Chem Ltd, Mumbai
6	DCM	SD Fine-Chem Ltd, Mumbai
7	Chloroform	SD Fine-Chem Ltd, Mumbai
8	n-octanol	SD Fine-Chem Ltd, Mumbai
9	Propylene glycol	Fischer Scientific, Mumbai
10	Ethyl cellulose	Fischer Scientific, Mumbai
11	PVA	Hi media
12	Carbopol934	Lubrizol Advanced Materials, India

3.1 Methods of Formulation of Nanosponges

Emulsion Solvent Diffusion Method: Different ratios of polyvinyl alcohol and ethyl cellulose were directly generated using the emulsion solvent diffusion method to create nanosponges. This method had two phases: the dispersion phase contained a certain quantity of ethyl cellulose dissolved in 20 mL dichloromethane and a set dose of Tretinoin (25 mg). The continuous phase contained a predetermined amount of polyvinyl alcohol in 100 mL water. The reaction mixture was agitated at 100 rpm for three hours using a magnetic stirrer after the organic dispersed phase was gradually introduced to the aqueous continuous phase at 35°C. To guarantee that all remaining solvent was removed, the produced nanosponges were gathered, dried for 24 hours at 40°C in an oven, and then stored in a desiccator.[70]+

Table 3.4: Composition of Different Formulation Tretinoin.

Formulation code	Tretinoin(mg)	PVA (mg)	Ethyl cellulose (mg)	DCM (ml)
F1	25	200	100	20
F2	25	200	200	20
F3	25	200	300	20
F4	25	200	400	20
F5	25	100	400	20
F6	25	200	400	20
F7	25	300	400	20
F8	25	400	400	20

3.2 Evaluation of formulated Tretinoin Nanosponge

1. Percentage yield

The nanosponges were weighed once they had dried. The following method was used to calculate the % yield value:[71]

Yield percentage = Weight of nanosponges × 100/Weight of total solids

2. Drug Entrapment Efficiency

A vortex mixer was used to precisely weigh 10 mg of nanosponges and 5 ml of ethanol in a volumetric flask, which was then shaken for one minute to determine the entrapment effectiveness. The 10 ml volume was added. After filtering and diluting the mixture, the concentration of tretinoin was measured using spectrophotometry at a wavelength of 352 nm.[72]

Loading Efficiency = Theoretical Drug Content / Actual Drug Content in nanosponge ×100

3. Determination of Particle size

The size of particles is maintained during polymerization for the formation of free-following powders having fine aesthetic attributes. Particle size analysis of loaded and unloaded nanosponges performed by laser light diffractometry or Malvern zeta sizer. The cumulative graph is maintained or plotted as particle size against time to study effect of particle size on drug release The size of particles is maintained during polymerization for the formation of free-following powders having fine aesthetic attributes. Particle size analysis of loaded and unloaded nanosponges performed by laser light diffractometry or Malvern zeta sizer. The cumulative graph is maintained or plotted as particle size against time to study effect of particle size on drug release The size of particles is maintained during polymerization for the formation of free-following powders having fine aesthetic attributes. Particle size analysis of loaded and unloaded nanosponges performed by laser light diffractometry or Malvern zeta sizer. The cumulative graph is maintained or plotted as particle size against time to study effect of particle size on drug release The size of particles is maintained during polymerization for the formation of free-following powders having fine aesthetic attributes. Particle size analysis of loaded and unloaded nanosponges performed by laser light diffractometry or Malvern zeta sizer. The cumulative graph is maintained or plotted as particle size against time to study effect of particle size on drug release Using a Nano ZS-90 (Malvern Instruments limited, UK) at a fixed angle at 25°, photon correlation spectroscopy (PCS) was used to measure the average particle size of

tretinoin nanosponges. The sample was examined for particle size after being diluted ten times with distilled water.[71,73]

4. Zeta Potential

In order to ascertain the particle charge and movement velocity of the particles in an electric field, the zeta potential was measured. In the current study, Zetasizer used Laser Doppler Micro electrophoresis (Zetasizer nano ZS, Malvern instruments Ltd., UK) to examine the nanosponges after diluting them ten times with pure water.[71,73]

3.3 Formulation of tretinoin loaded nanosponge topical gel

Different concentrations of gelling chemicals, such as Carbopol 934, were dissolved and steeped overnight in insufficient amounts of water to prepare the topical gel. After a full day, add propylene glycol—the last ingredient—as a permeability enhancer. The optimized batch (F8) formulation of nanosponges was dissolved in water in a different beaker. This was put into the beaker that held the other excipients earlier. Triethanolamine was then added to balance the formulation's pH.[74]

Table 3.5: Composition of Different Formulation Tretinoin nanosponge gel.

Sr. No	Composition	Formulation Code		
		F8A1	F8A2	F8A3
1	Tretinoin nanosponge (mg)	5	5	5
2	Carbopol 934 (%w/v)	1%	1.5%	2%
3	H_2O	QS	QS	QS

3.4 Characterization of nanosponge gel

Physical appearance

Visual observation of every gel composition was used to complete the process.

pH measurement

The pH of gel formulations was determined using digital pH meter. One gram of gel was dispersed in 100 ml of distilled water and the pH was recorded using a pH meter by bringing it in contact with the gel and allowing it to equilibrate for 1 min. pH was measured in triplicate and average values were calculated.[75]

Spreadability

A glass slide device and a hardwood base block are used to evaluate it. Samples were distributed between two slides and compacted to a consistent thickness using a 1000 gm

weight for five minutes in order to detect spreadability. 50 grams or so in weight was put to the pan. Next, calculate how long it takes to separate one slide from the others. Spreadability was therefore respected as the amount of time needed to separate the upper glass slide from the lower plates. The spreadability of gels was tested using the formula below.

$S = m l / t$ ……Eq (1)

where M is the weight applied to the upper slide and S is the spreadability. L is the length moved on the glass slide, and T is the amount of time needed to separate the slides.[76]

Viscosity Viscometer was employed in rheological research. A T-4 spindle spinning at five revolutions per minute was used to measure the dial reading after the sample (30 g) had been in a beaker for five minutes to acclimate. At the speed, the matching dial reading of the viscometer was recorded. For every spindle speed drop, the dial reading that matched it was noted. Every measurement was conducted thrice, and the mean ± standard deviation is given.[77]

Drug content

A gel formulation containing 100 mg of tretinoin was dissolved in 100 milliliters of methanol, filtered, and then the volume was adjusted to 100 milliliters using methanol. The drug content was ascertained by utilizing a Shimadzu-1700 UV Visible spectrophotometer to measure the absorbance at 352 nm after ten times diluting the resultant solution with methanol.[78]

Transmission Electron Microscopy (TEM) Analysis

The TEM analysis of nanosponge was performed for morphological characterization and visualization of nanosponge droplets. Nanosponge 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.[79]

In -Vitro Drug Release Studies

In Franz-type diffusion cells, the permeabilization of a nanosponge gel containing tretinoin through a dialysis membrane was carried out. Phosphate buffer (pH 6.8) was used as the receptor medium, and it was continuously shaken at 100 rpm using a tiny magnetic bar. A

recirculating water jacket kept the receiver compartment at 37±0.2◦C. In the donor compartment, a quantity of nano sponge gel equal to the required dosage of medication was added. At 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, and 24 samples were taken out of the receptor compartment using the sampling portion and promptly replaced with an equivalent amount of brand-new receptor solution. For every investigation, experiments were carried out in triplicate, and sink conditions were consistently upheld. Every sample was examined using UV spectrophotometry at λ max = 352 nm of medication to determine the amount of tretinoin.[80-82]

Drug release kinetics

In order to determine the % drug release and the release kinetics of an optimal formulation, raw data from in vitro release tests was evaluated in the current study and fitted to several equations and kinetics models. The Higuchi model, the Korsmeyer-Peppas equation, the zero-order equation, and the first-order equation were the kinetic models employed.[65,83,84]

Zero Order Model

It can be applied to various modified release pharmaceutical dosage forms, such as some transdermal systems, matrix tablets containing poorly soluble pharmaceuticals in coated forms, osmotic systems, etc., to characterize how the drug dissolves. Zero order release can be represented as follows in its most basic form:

Q0 - Qt = K0t

where Q0 is the starting drug concentration in the solution (usually equal to 0), Qt is the amount of drug dissolved in time t, and K0 is the zero-order release constant given in concentration/time units. Plotting the cumulative amount of drug released against time was done using data from in vitro drug permeation tests to analyze the release kinetics. The following equation would predict a zero-order release.

K 0 t – A 0 = A t

Where: Drug release at time "t" = A t

A 0 = Starting dose of medication

K 0 = Rate constant of zero order (hour - 1)

If the data obeys zero-order release kinetics and the plot is linear when expressed as a percentage of drug release versus time, the slope is equal to K 0.

First Order Model

The following equation would predict a first-order release.

Log C is equivalent to Log C 0 – K t /2.303

Where:

C = Amount of medication left at time 't'

C 0 = Initial Drug Amount

K= Rate constant of first order (hr - 1)

A straight line is produced when the data is displayed as the log percent of medication remaining vs time, suggesting that first-order kinetics govern the release. You may find the constant 'K' by multiplying 2.303 by the slope values.

Higuchi's Model

Higuchi's classical diffusion equation has been used to characterize drug release from matrix devices by diffusion.

$Q = [(2A – \varepsilon C s) / \tau D\varepsilon] C s t ½$

Where: Q = Drug release amount at time't'

D= The drug's diffusion coefficient within the matrix

A = Total quantity of medication in a matrix's unit volume

C s = The drug's solubility in the diffusion media.

ε = The matrix porosity

τ = Tortuosity t = Time (hours) at which a drug of quantity 'Q' is released.

The equation can be made simpler by assuming that D, C, and A are constants. Equation then becomes Q=K.

The drug was released by a diffusion mechanism, as shown by the straight line that results from plotting the data using the equation, which is drug released versus square root of time. 'K' is the slope's equal.

Korsmeyer – peppas model

The following equation can be used to describe the release rates from controlled release polymeric matrices.

Q = K 1 t n

Where

Q = Drug release percentage at time't'

K = Kinetic constant, which incorporates geometric, structural, and the diffusion exponent that represents the release mechanism.

n = 0.45 for Fickian release, 0.45–0.89 for anomalous (non-Fickian) transport, and 0.89 for zero-order release features, and "n" is 0.89.

4. RESULT AND DISCUSSIONS

Preformulation Studies

The aim of preformulation studies is to investigate the physical and chemical properties of a drug substance. The selected drug Tretinoin was subjected for investigation of physical characterization parameters such as.

- Organoleptic properties
- Melting point
- UV-visible spectra
- Solubility
- Partition coefficient
- FT-IR spectra

Organoleptic Properties

Organoleptic properties of Tretinoin were found to be as per literature. The Organoleptic properties of Tretinoin were found to the given in **Table 4.1**.

Table 4.1: Organoleptic Properties of Tretinoin.[85]

Sr. No.	Properties	Inferences
1.	Colour	Yellow
2.	Form	Solid
3.	Taste	Slightly bitter

Melting Point

The melting point of a substance is the temperature at which the solid phase gets converted to liquid phase under the one atmosphere of pressure. The melting point determination implies the purity of drug. Melting point of Tretinoin was determined by capillary tube method and was found to be quite similar to the reported melting point as shown in **Table 4.2.**[86]

Table 4.2: Melting Point of Tretinoin.

Drug	Reference M.P.	Observed M.P.
Tretinoin	178-184°C	178.3±0.577°C

Discussion: The melting point of Tretinoin was found to be 178.3±0.577°C which is in the range of the pure drug. Hence drug sample was free from any type of impurities.

UV Spectroscopy

Determination of absorption maxima in ethanol

A double beam UV-visible spectrophotometer was used for quantitative analysis of the drug. A 12 µg/ml solution of Tretinoin in ethanol was scanned in the range of 200-400 nm. The result of UV spectrum of Tretinoin is shown in **Figure.**

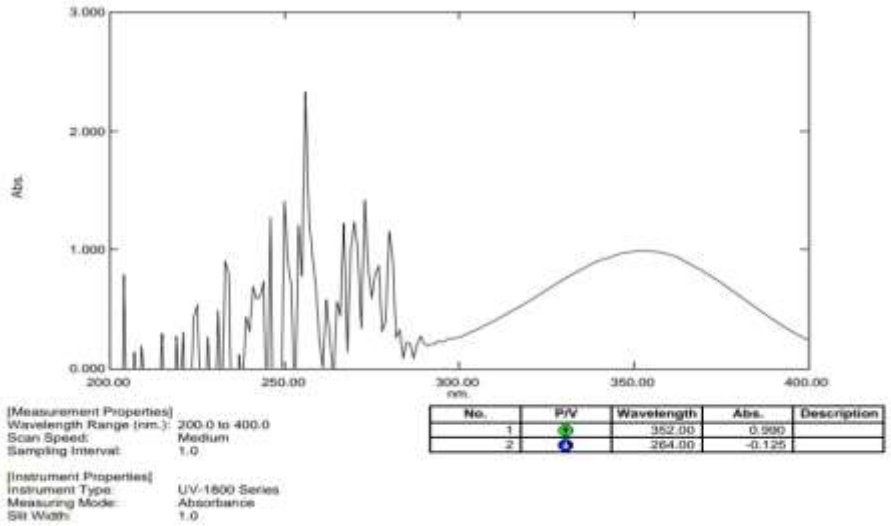


Figure 4.1: UV Spectrum of Tretinoin in ethanol.

Table 4.3: Absorption maxima (λ_{max}) of Tretinoin in ethanol.

Name of drug	Absorption maxima (λ_{max})	
	Observed	Reference
Tretinoin	352	352

Discussion: The maximum wavelength of Tretinoin was observed at 352 nm similar to literature (**Table 4.3**).[87]

Preparation of standard curve of Tretinoin in ethanol

Table 4.4: Calibration curve of Tretinoin in ethanol (λ_{max}= 352 nm)

Sr. No.	Concentration(µg/ml)	Mean±SD
1	0	0
2	2	0.192±0.001
3	4	0.353±0.001
4	6	0.513±0.001

5	8	0.681±0.001
6	10	0.816±0.001
7	12	0.990±0.00

Value is expressed as mean ± SD; n = 327

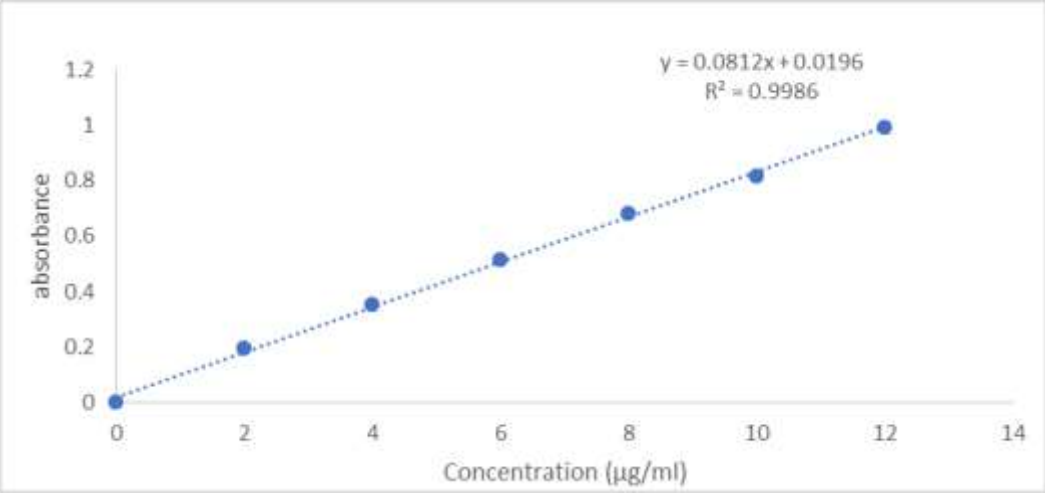


Figure 4.2: Standard calibration curve of Tretinoin in ethanol.

Table 4.5: Result of regression analysis of UV method.

Statistical parameters	Results
λ_{max}	352nm
Regression equation (y =mx+ c)	y = 0.0812x - 0.0196
Slope (m)	0.0812
Intercept (C)	0.0196
Correlation coefficient (R^2)	0.9986

Discussion: - The calibration curve for Tretinoin was obtained by using the 2 to 12 µg/ml concentration of Tretinoin in ethanol. The absorbance was measured at 352 nm. The calibration curve of Tretinoin as shows in graph indicated the regression equation y = 0.0812x + 0.0196 and R^2 value 0.9986, which shows good linearity as shown in **Table 4.5** and **Figure 4.2**.

Solubility studies

Solubility of drug in various solvents, were carried out in order to screen for the components to be used for formulation development. Analysis of the drug was carried out on UV Spectrophotometer at 352 nm.[88]

Table 4.6: Solubility studies of Tretinoin for different solvents.

Sr. No.	Name of Solvents	Solubility (mg/ml)
1	Methanol	0.703±0.004
2	DCM	36.09±0.25
3	Chloroform	55.591±0.123
4	PH6.8	0.002±0.001
5	Water	0.002±0.001
6	Ethanol	4.441±0.188

Value is expressed as mean ± SD; n = 3

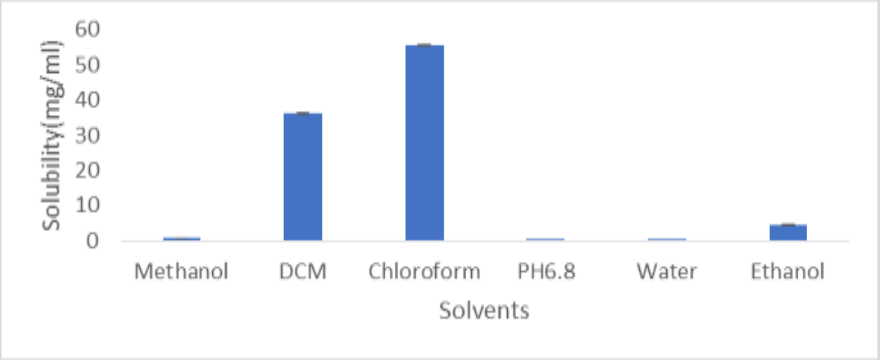


Figure 4.3: Solubility study of drug in different solvents.

Discussion: From the above data, it is clearly seen that Tretinoin is freely soluble in Chloroform and soluble in DCM, Ethanol and methanol. (**Figure 4.3** and **Table 4.6**).

Partition coefficient determination

Partition coefficient of the Tretinoin 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 lipophilicity and purity of drug.

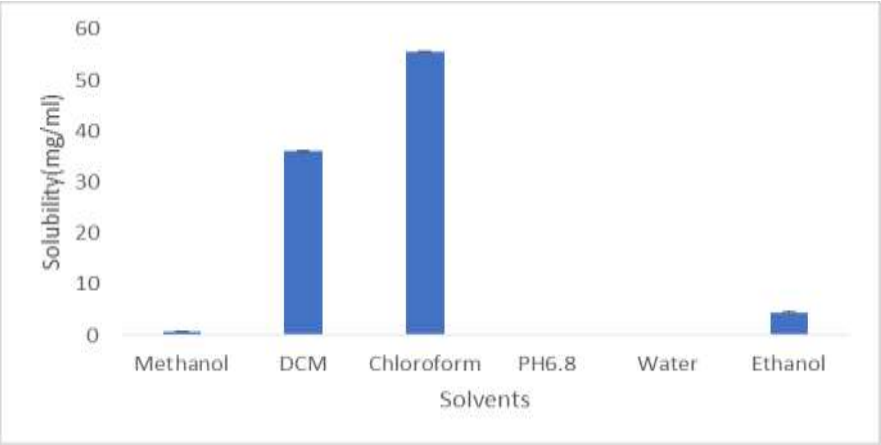


Fig 4.4: Partition coefficient determination of Tretinoin.

Table 4.7: Partition coefficient of drug.

Partition coefficient of drug	Solvent	Log P Values	Reference
Tretinoin	Oil: water	6.248±1.407	6.3

Value is expressed as mean ± SD; n = 3

Discussion: The partition coefficient of Tretinoin in n-octanol: water was found to be 6.248±1.407, this indicates that the drug is lipophilic in nature (**Table 5.7**) which is similar to the literature.[88]

FTIR study of Tretinoin[89]

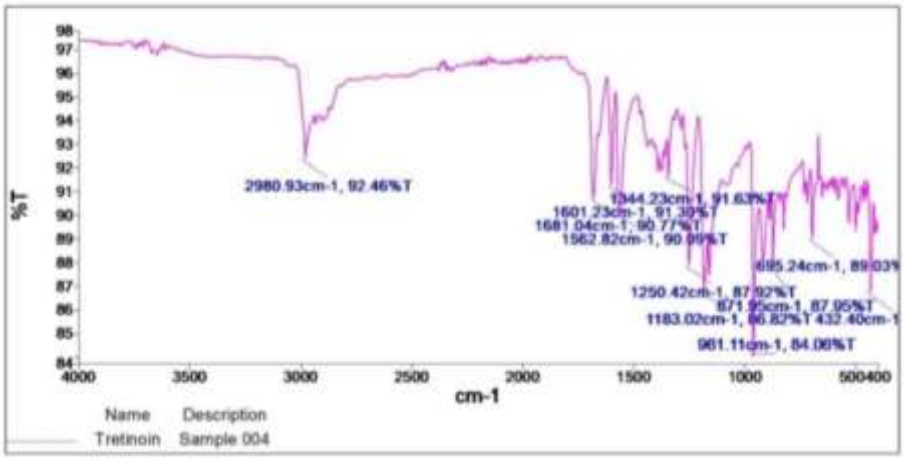


Figure 4.5: FTIR spectrum of Tretinoin.

Table 4.8: FTIR interpretation of Tretinoin

Sr. No.	Characteristics Peak	Reference (cm^{-1})	Observed (cm^{-1})
1.	stretching vibrations of CH_2	2942	2980.93
2.	stretching vibrations of the C-O-C group	1000–1300	1250.42
3.	trans vinyl (CH=CH) groups	960	961.11

Discussion: The FTIR spectra of Tretinoin were shown in the **Figure 4.5; Table 4.8**. The principal IR absorption peaks of Tretinoin at 2980.93cm^{-1}s Stretching vibrations of CH_2, 1250.42cm^{-1} stretching vibrations of the (C-O-C) group and Trans vinyl (CH=CH) groups 961.11cm^{-1} were all observed in the spectra of Tretinoin. These observed principal peaks confirmed the purity and authenticity of the Tretinoin.

FTIR Study of PVA[90]

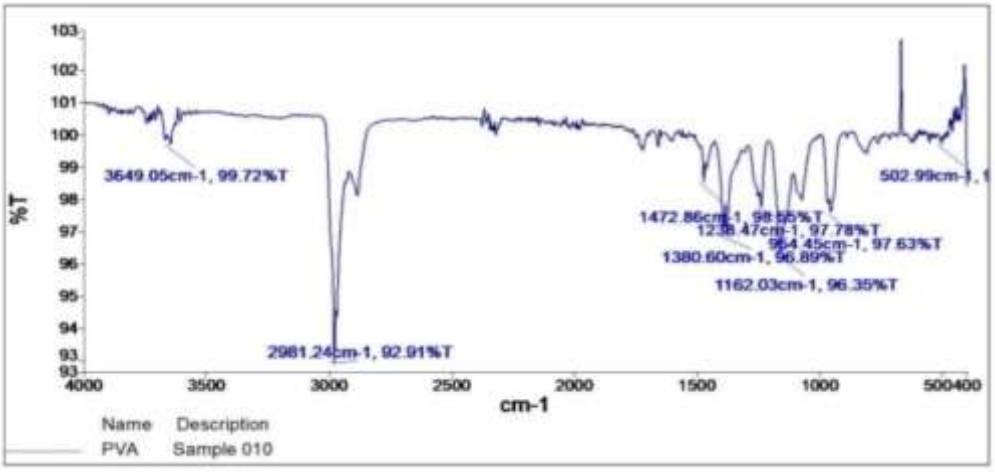


Figure 4.6: FTIR spectrum of PVA.

Table 4.9: FTIR interpretation of PVA.

Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
CH_2 Stretching	2936	2981.24
CH_2 bending	1325	1380.60
C-O Stretching	1138	1162.03
CH_2 Rocking	916	954.45

The FTIR spectra of PVA were shown in the **Figure 4.6; Table 4.9**. The principal IR absorption peaks of PVA at 2981cm^{-1} (O–H stretching band), 1380.cm^{-1} (CH_2 bending), 1162.03cm^{-1} (Shoulder stretching of C-O band) and 954.45cm^{-1} (CH_2 stretching band) were all observed in the spectra of PVA. These observed principal peaks. This observation confirmed the purity and authenticity of the PVA.

FTIR study of Ethyl cellulose[91]

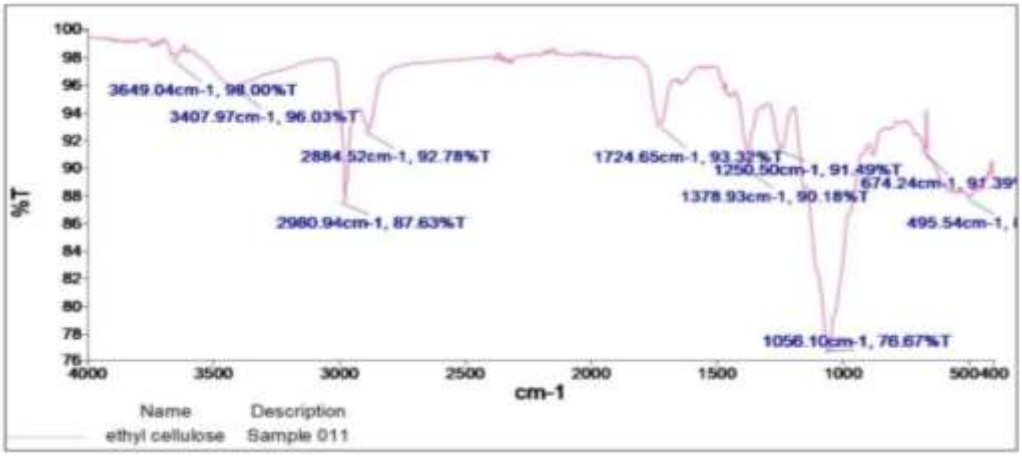


Figure 4.7: FTIR spectrum of Ethyl cellulose.

Table 4.10: FTIR interpretation of Ethyl cellulose.

Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
(C-H) Stretching	2855	2884.52
(C-O-C) Stretching	1300	1378.93
(C-O) Stretching	1200	1250.50
(O-H) Stretching	3300	3407.97

The FTIR spectra of Ethyl cellulose were shown in the **Figure 4.7; Table 4.10**. The principal IR absorption peaks of Ethyl cellulose at 2884.52cm^{-1} (C–H stretching band), 1378.93cm^{-1} (C-O-C stretching), 1250.50cm^{-1} (C-O stretching band) and 3407.97cm^{-1} (O-H stretching band) were all observed in the spectra of Ethyl cellulose. These observed principal peaks. This observation confirmed the purity and authenticity of the Ethyl cellulose.

FTIR study of physical mixture

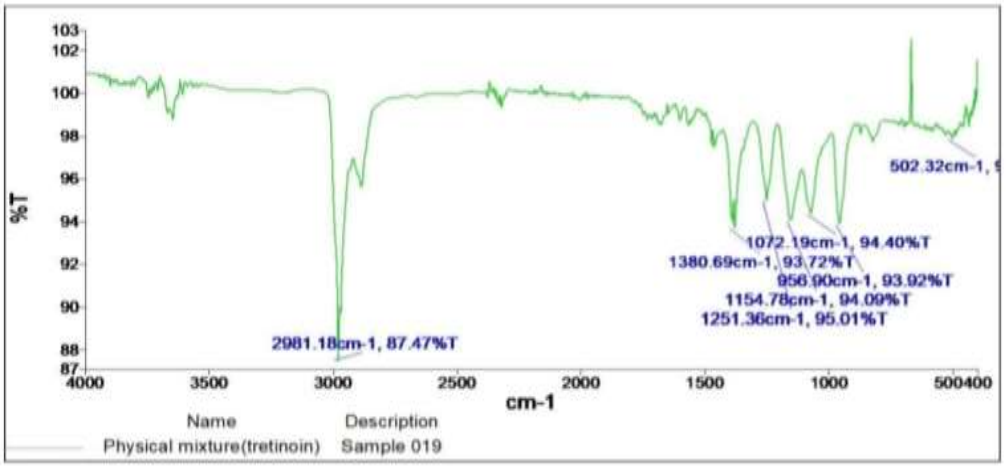


Figure 4.8: FTIR spectrum of physical mixture.

Table 4.11: FTIR interpretation of physical mixture.

Sr.No	Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
1	(O-H) Stretching	2981.18	2981.18
2	(C-O-C) Stretching	1378.93	1380.69
3	(C-O) Stretching	1250.50	1251.36
4	CH_2 Stretching	954.45	956.90

Discussion: FTIR of Pure drug and physical mixture studies **(Figure 4.8; Table 4.11)** were carried out to eliminate the possibility of interaction between drug and excipients. All the spectrum peaks revealed that corresponding peaks of drugs are present in the above spectra along with excipients peaks. Hence no interaction was observed in this mixture.

4.2 Evaluation of Nanosponge

Particle Size[92]

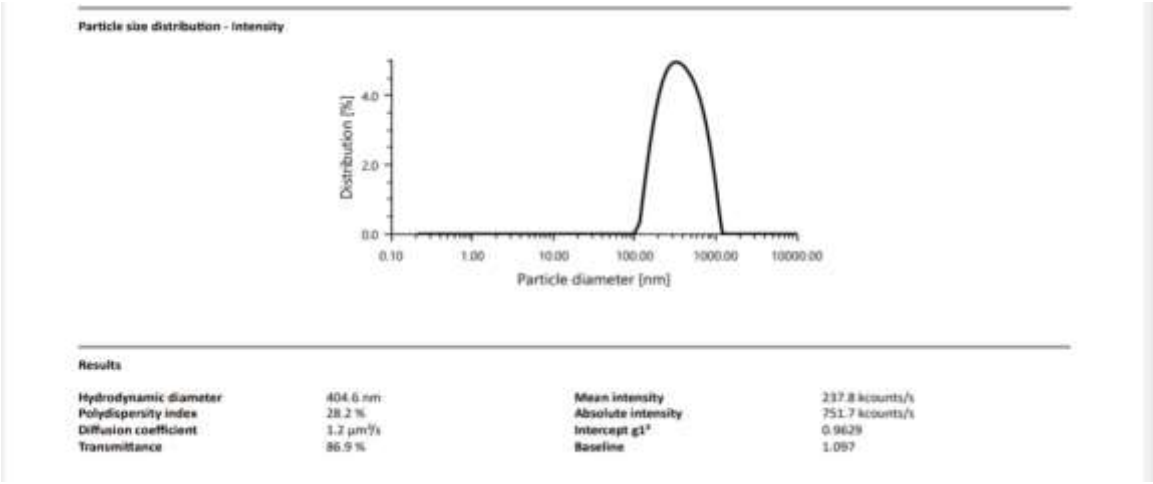


Figure: 4.9 Particle size of Nano sponge (F8)

Discussion: From the figure 4.9 it was concluded that particle size 404.6 nm. Thus, the results show that the particle size of the formed nano sponge gel was in required range therefore a transparent nano-sponge gel was analysed to determine whether it is in the nano-sponge i.e. more than 100nm.

Zeta Potential

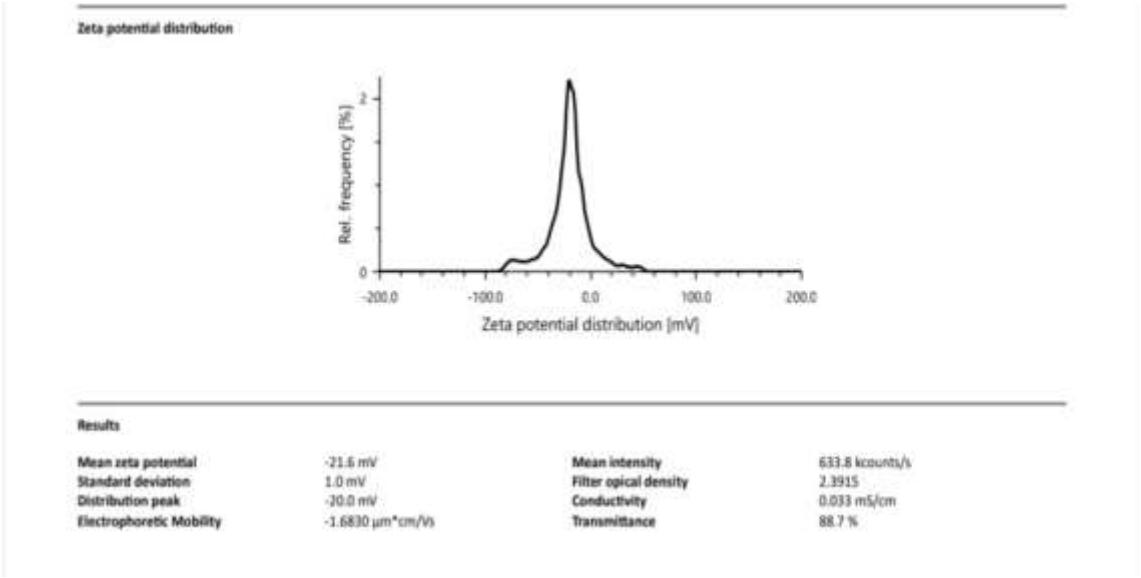


Figure 4.10: Zeta potential graph of Nano sponge (F8)

Discussion: Figure 4.10 demonstrated zeta potential of F8 formulation was -21.6 mV represents stability of nanosponge.

4.3 Evaluation of Nano sponge gel

Physical Appearance

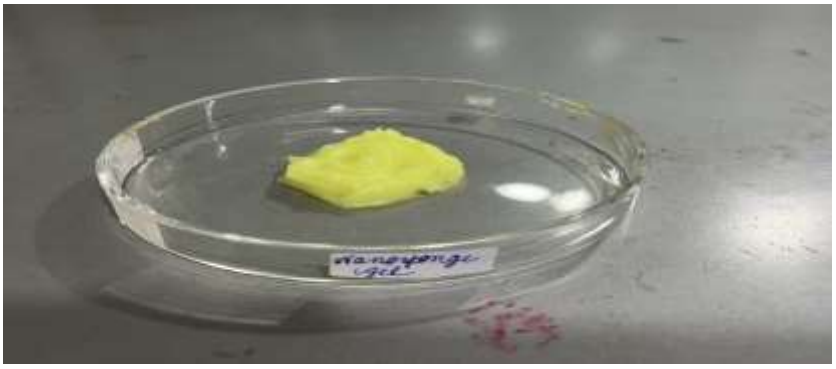


Figure 4.11: Visual Appearance of nano sponge gel.

Table 4.12: Visual Appearance of nano-sponge gel.

Sr. no.	Formulation code	Visual Appearance
1	F8A1	Gel not formed
2	F8A2	Smooth gel without lump formation
3	F8A3	Smooth gel without lump formation

Discussion: The prepared gel were examined visually for their consistency and found to smooth appearance. Out of three developed gel formulations batches F8A2 and F8A3 were showed good homogeneity with absence of lumps. So those batches were used in further study.

pH determination

Table 4.13: pH study of gel[93]

Sr. no.	Formulation code	pH (Mean ± S.D)
1	F8A2	6.2±0.2
2	F8A3	6.83±0.152

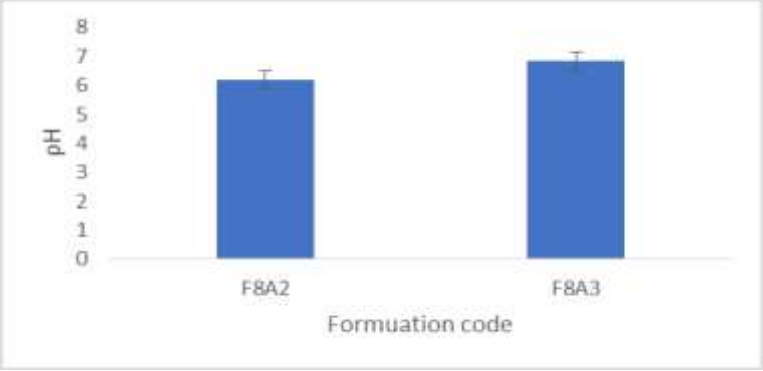


Figure 4.12: pH of gel (F8A2-F8A3)

Discussion: From the Table 4.13 & fig. 4.12, it was found that pH of all formulation was found to be in a range 6.2±0.2, 6.83±0.152.

Rheological studies

Table 4.14: Viscosity Study of formulations (F8A2-F8A3).[94]

Sr. no.	Formulation code	Viscosity (Mean ± S.D)
1	F8A2	2885.3±6.11
2	F8A3	2892.3±1.15

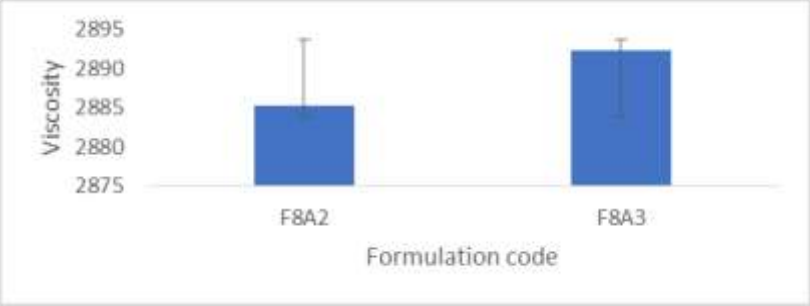


Figure 4.13: Viscosity Study of formulations (F8A2-F8A3)

Discussion: The viscosity (Visco QC100) of all the formulation was in range of 2885.3±6.11 and 2892.3±1.154 (Table 4.14 and Figure 4.13).

Spreadability Study

Table 4.15: Spreadability Study of formulations (F8A2-F8A3)[93]

Sr. no.	Formulation code	Spreadability (g.cm/sec) (mean ± SD)
1	F8A2	0.43±0.152
2	F8A3	0.5±0.1

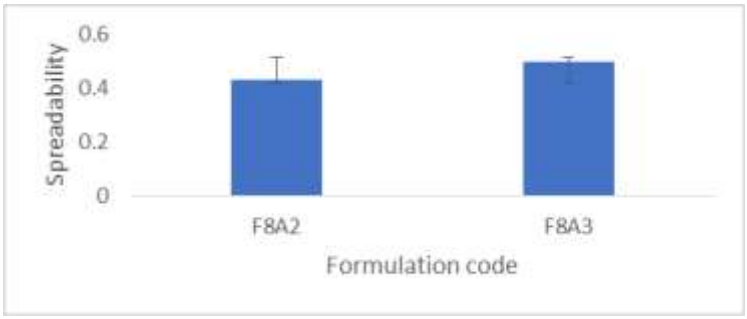


Figure 4.14: Spreadability Study of formulations (F8A2-F8A3)

Discussion: The Spreadability of all the formulation was in range of 0.43±0.152and 0.5±0.1 (Table 4.15 and Figure 4.14).

Drug content

Table 4.16: Drug content of formulations (F8A2-F8A3)

Sr. no.	Formulation code	% Drug Content
1	F2	90.545±0.075
2	F3	95.832±0.147

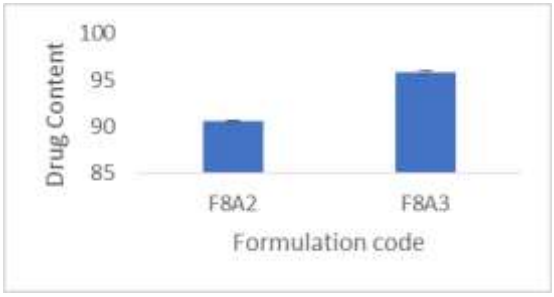


Figure 4.15: Drug content of formulations (F2-F3)

Discussion: The drug content gels were found to be 90.545±0.075to 95.832±0.147% respectively. The percentage drug content of all formulations was found to be satisfactory. Hence, the method adopted for gel formulations was found to be suitable.

FTIR Study[95,96]

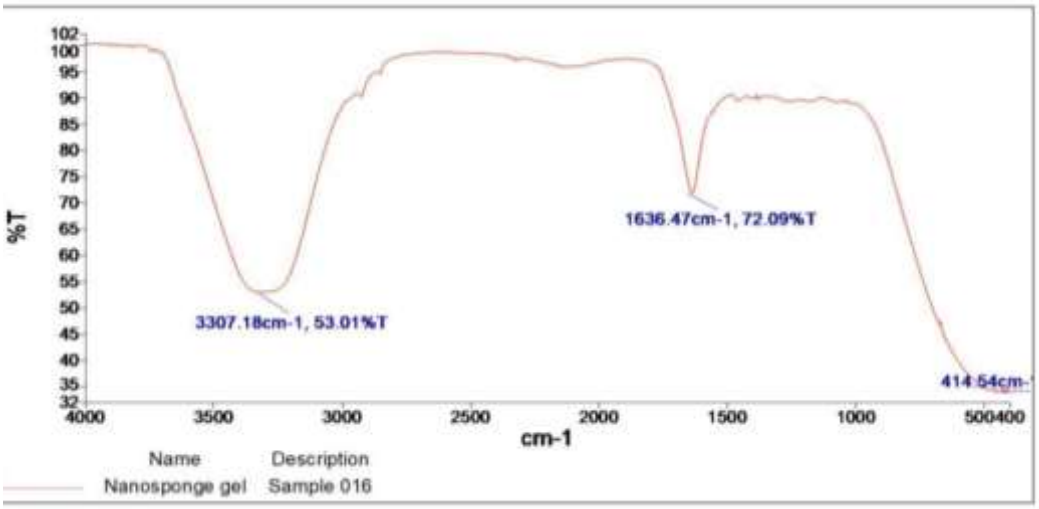


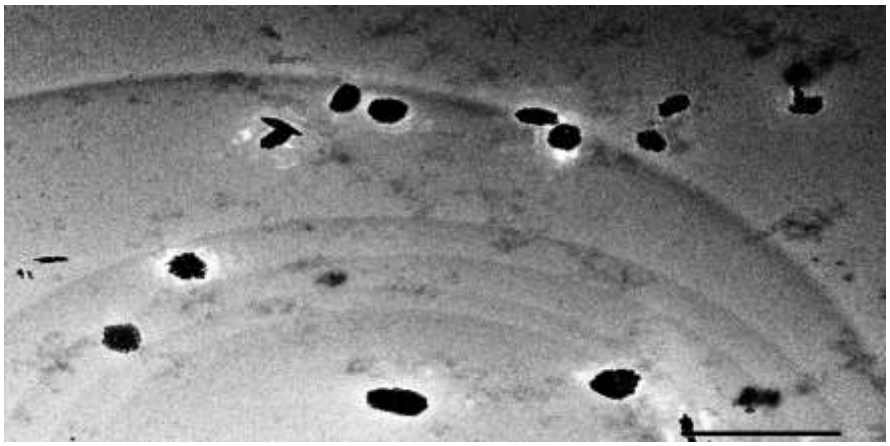
Figure No. 4.16: FTIR Spectra of Formulation F8A3.

Table 4.17 FTIR interpretation of Nanosponge gel.

Sr.No	Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
1	(C=O) Stretching [1]	1673.85	1636.47
2	(OH) Stretching [2]	3362	3307.18

Discussion: The FT-IR spectra of final formulation (F8A3) indicate that characteristic peak of tretinoin was not visible in the Nano sponge gel spectra which indicates that oil was completely encapsulate in the nano-sponge gel.

TEM of Nanosponge gels



Discussion: From the figure 4.17 it was concluded that the prepared nano sponge gel of the optimized formulation (F8A3) was found to be spherical to oval in shape.

4.4 In vitro drug release studies

Table 4.18: In vitro drug release of loaded nano-sponge gels & control gel formulation (λ max= 352 nm)

Sr. No.	Time (hr)	Drug Release of control gel formulation (%)	Drug Release of Formulation F8A3(%)
1	0	0	0
2	0.25	43.180±0.031	12.01±0.003
3	0.5	64.75±0.047	15.10±0.003
4	1	79.38±0.031	30.50±0.003
5	2	99.18±0.031	37.26±0.003
6	3	-	42.18±0.002
7	4	-	48.84±0.003
`8	6	-	59.00±0.005
9	8	-	62.11±0.003
10	10	-	68.28±0.004
11	12	-	83.55±0.003
12	24	-	85.69±0.003

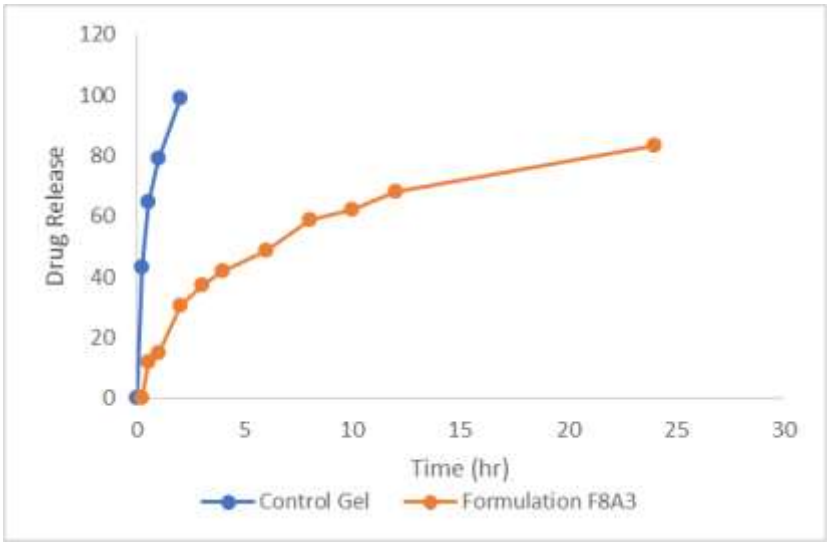


Figure 4.18: Percentage drug release of Tretinoin loaded nano-sponge gels & control gel formulation.

RESULT

The in-vitro drug release of loaded nano- sponge gels & control gel formulation was given in a Table 4.18 and figure 4.18. The release from control gel shows 99.18±0.031% within 2hrs. On the other hand, the release of drug in gel formulations F8A3 showed 85.69±0.003% respectively in the phosphate buffer saline (pH 6.8) with in 24hr. Drug loaded gel formulation F8A3 (containing 2% Carbopol 934) demonstrated maximum drug release up to 81.200±0.267%, within 24hr check absorbance in UV spectrophotometer range at (λmax= 352 nm).

Drug release kinetic studies

Zero order kinetics

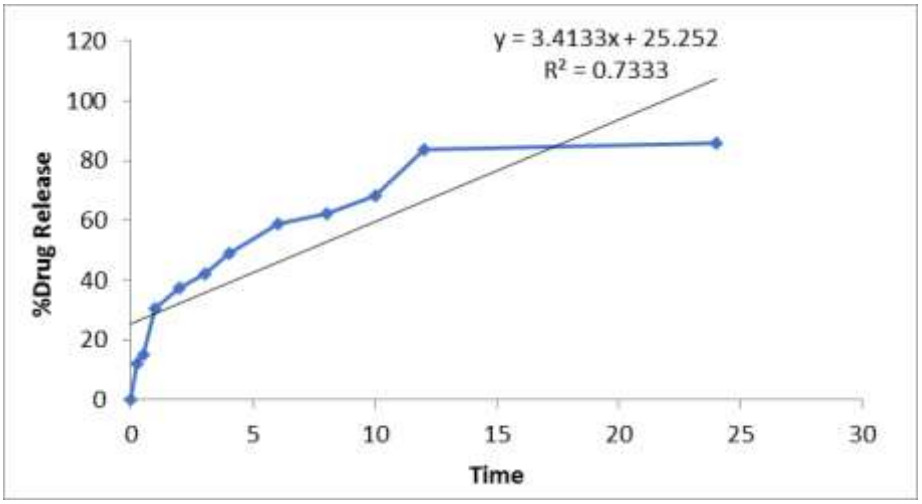


Figure 4.19: Zero order graph of formulation F8A3.

First order kinetics

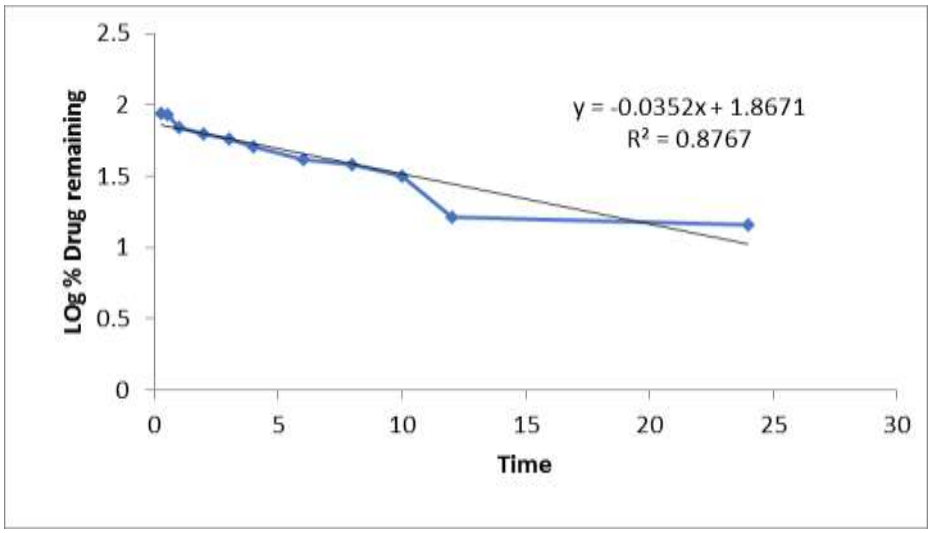


Figure 4.20: First order graph of formulation F8A3.

Higuchi's Model

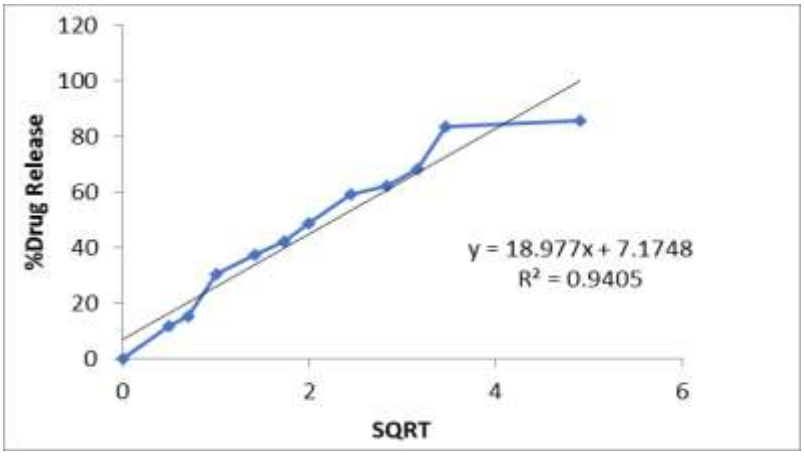


Figure4.21: Higuchi order graph of formulation F8A3.

Korsmeyer-Peppas Model

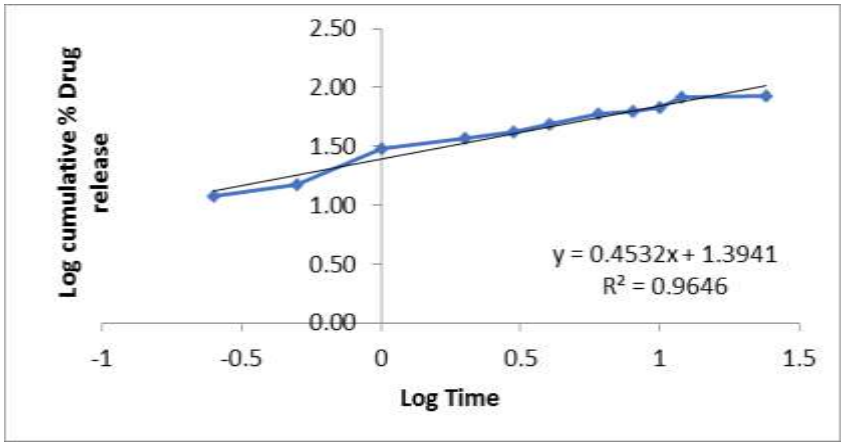


Figure 4.22: Korsmeyer-peppas order graph of formulation F8A3.

Table 4.19: Kinetic equation parameter of formulation F8A3.

Formulation Code	Zero-order		First-order		Higuchi		K. Peppas	
	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2
F8	3.413	0.733	-0.035	0.876	18.977	0.94	0.453	0.964

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, R^2 value was calculated from the graph and reported in Table 4.19 and Figure 4.19 to Figure 4.22. Considering the determination coefficients, K.Peppas was found (R^2=0. 0.964) to fit the release data best. It could be concluded from the results that the drug was released from loaded nanosponge gels by a sustain mechanism.

5. CONCLUSION

The main aim of study was to increase the residence time, bioavailability and onset of action. On physicochemical evaluation, melting point of tretinoin was found to be in range of 178.3±0.577°C respectively. On UV spectrophotometer analysis absorption maxima of tretinoin were found to be 352 in solvent (ethanol). The partition coefficient of tretinoin in n-Octanol: Water was found to be 6.248±1.407. This indicates that the drug tretinoin is lipophilic nature. As far as solubility profile is concerned, tretinoin was found soluble in DMSO, chloroform, slightly soluble in methanol and ethanol and practically insoluble in water. On FTIR spectroscopy analysis there was no interaction between drug and excipients.

The nano sponge of tretinoin was prepared. The method of preparation of was found to be simple and reproducible. The physical appearance of all the nanosponge was evaluated. The selected nanosponge F1-F8 revealed that F8 was having maximum tretinoin Entrapment efficiency that was found to be 99.9±0.01.

The optimal final nanosponge F8 was selected to preparation of topical gel with Carbapol 934P in different concentration. The pH of the prepared topical gel F8A3 with 2% carbopol was found to be 6.83±0.152. Upon spreadibility study, the gels were found to have optimum results. Percentage drug content was obtained in all formulations with gel formulation was found to be 95.832±0.142%. The drug release from the topical gel prepared in formulation

F8A3 achieved respective amount of drug release contributing 85.69±0.003% within 24 hrs and in marketed gel (tretinoin gel) it was 99.18±0.031% within 2 hrs.

The best fit model has Korsmeyer-peppas per high regression coefficient value. Hence from all aspects; we concluded that the release of drugs can be sustained release by proper designing of the nanosponge and selection of a suitable method of preparation. In this study we successfully produced sustained released drug delivery systems for the drug release of tretinoin.

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