DEVELOPMENT AND EVALUATION OF EMULGEL LOADED WITH GREEN TEA EXTRACT BASED SILVER NANOPARTICLES AND VITAMIN D FOR WOUND HEALING

Thesis Submitted for the Award of the Degree of

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Pharmaceutics

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2025

DECLARATION

I, hereby declared that the presented work in the thesis entitled "**Development and Evaluation of Emulgel loaded with Green Tea Extract based Silver Nanoparticles and Vitamin D for Wound Healing**" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision Dr. Narendra Kumar Pandey, working as Professor, in the School of Pharmaceutical Sciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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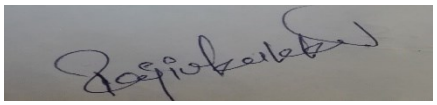
CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**Development and Evaluation of Emulgel loaded with Green Tea Extract based Silver Nanoparticles and Vitamin D for Wound Healing**" submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the School of Pharmaceutical Sciences, is a research work carried out by Rishu Yadav, 41900201, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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(Rishu Yadav)

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LIST OF ABBREVIATIONS

Abbreviations	Full-form
ABS	Activated B cells
API	Active pharmaceutical ingredient
ANOVA	Analysis of variance
Appx.	Approximately
Avg.	Average
CCD	Central composite design
Conc.	Concentration
ºC	Degree Celsius
DoE	Design of experiment
ESVDH	Emulgel loaded with high dose of Vitamin D-3
ESVDL	Emulgel loaded with low dose of Vitamin D-3
EC	Epicatechin
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
e.g.	Example
FDA	Food and Drug Administration
FTIR	Fourier transform infrared spectroscopy
gm	Gram
GTE	Green tee extract
HPLC	High performance liquid chromatography
HQC	High quality control
hrs.	hours
HIV	Human immunodeficiency virus
HPC-3	Human Prostate Carcinoma Cells
ICH	International Council for Harmonization
IU	International unit
IPA	Iso propyl alcohol
et.al.,	Latin et al (and others)
LOD	Limit of detection

LOQ	Limit of quantification
LQC	Low quality control
MQC	Medium quality control
µg	Micro gram
µg/mL	Micro gram per milliliter
MTCC	Microbial Type Culture Collection and Gene
mL	Millimeter
mg	Milligram
mV	Millivolt
min	Minute
nm	Nanometer
NK-FB	Nuclear factor kappa-light-chain-enhancer of
no.	Number
PDI	Poly disparity index
PEG	Polyethylene glycol
PG	Propylene glycol
%RSD	% Relative standard deviation
RRT	Relative retention time
rpm	Round per minute
SEM	Scanning electron microscopy
SNPs	Silver nanoparticles
SD	Standard deviation
SPF	Sun protecting factor
i.e.	That is
UV	Ultraviolet

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Abstract

ABSTRACT

Infections, burns, surgeries, and accidents produce many wounds in daily life. A complex process, wound healing involves inflammation, proliferation, and maturation. Severity and body location determine wound symptoms and kinds. The extended healing time and risk of complications make chronic wounds a burden on patients and healthcare systems. Novel and innovative wound healing aspirant Vitamin D-3 and its combination with silver nanoparticles in one formulation may open several gates for wound healing in current scenario. In this study Vitamin D-3 has addressed as a main active pharmaceutical agent for wound healing and silver nanoparticles for aid in wound healing. The enormous surface area and biocompatibility of nanoparticles make them ideal for targeted medication administration and tissue regeneration. Physical, chemical, and plant extract processes can synthesis metallic nanoparticles. Site specific delivery, simple cellular absorption, and drug delivery versatility are nanoparticle advantages. Drug delivery, gene delivery, cancer treatment, and cosmetics can benefit from nanoparticles. They may improve wound healing results and speed up healing. In this research, we cover the occurrence of wounds, the function of metallic nanoparticle in wound healing, its synthesis, Vitamin D-3 in wound healing and coupled with silver nanoparticles in form of emulgel to reduce wound healing time. In addition, wound healing, antibacterial and anti-inflammatory properties of Vitamin D-3 in the context of wound healing are investigated, as well as the formulation of Vitamin D-3 in emulgel for topical application. In the context of wound care, the notion of emulgel, which is a combination of gel and emulsion dosage forms, is presented as a potentially useful delivery strategy for lipophilic medications. Emulgel formulations that are currently on the market as well as the procedures used to prepare them are also discussed. This research, in its whole, sheds light on the potential of Vitamin D-3 and silver nanoparticles synthesizes form green tea extract and its incorporation in emulgel in the treatment of wounds. As a result of their medicinal characteristics, their exceptionally small size, and their high surface area, silver nanoparticles (SNPs), have emerged as a potentially useful therapeutic agent for the healing of wounds. In this study, the many methods of synthesizing silver nanoparticles (SNPs) are discussed, with a special emphasis on environmentally friendly synthesis that makes use of biological reducing agents like plant extracts. A discussion is held regarding the

utilization of silver nanoparticles (SNPs) in the process of disease targeting, the antibacterial, anti-inflammatory, and anti-tumor activities of SNPs, as well as their inert nature, which is advantageous for wound healing. In addition, this article highlights the significance of natural reducing agents, such as green tea extract, in the production of metallic nanoparticles. This is due to the fact that green tea extract has a greater yield and a synthesis process that only requires one step.

This work develops a Vitamin D-3 formulation and analytical method for estimation of Vitamin D-3, using high performance liquid chromatography (HPLC). Pre-formulation tests determined the active drug's physical and chemical properties. Vitamin D-3 properties were assessed using solubility and melting point. HPLC using methanol and water mobile phase was used to develop a new analytical technique. The International Council for Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH) validation criteria included linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). Silver nanoparticles were synthesized utilizing green tea extract and silver nitrate, optimized using Central composite design for stable zeta potential, particle size, and yield. The evaluation of silver nanoparticles is the primary emphasis of this work. More specifically, the particle size, zeta potential, shape, and surface morphology are all investigated thoroughly. The particle size was determined with the help of a zetasizer, and the suspension was subjected to ultrasonic treatment in order to break up the aggregates. By utilizing the same device, the zeta potential was accurately calculated. The scanning electron microscopy (SEM) technique was utilized in order to thoroughly examine the morphological traits. Through the use of centrifugation and lyophilization of the nanoparticle suspension, practical yield estimations were carried out. Infrared spectra obtained by the Fourier transform were utilized for the purpose of identifying a variety of moieties. The creation of emulgel formulations that incorporate silver nanoparticles is the main element of the study. For the purpose of statistical optimization, a three-level, two-factor central composite design is utilized. Various aspects of the formulations, including their physical features, drug concentration, pH, viscosity, spreadability, *in vitro* drug release, and release kinetics, were examined and evaluated. Testing for toxicity or skin irritation was carried out with the help of the Het-CAM assay, and stability tests were carried out in accordance with the guidelines provided by the ICH. In order to evaluate the efficacy of the formulated emulgel in terms of wound healing, in vivo excision wound models were

generated in rats. An induction of wounds, the application of formulations, the measurement of wound contraction, the determination of the epithelialization time, and a histological inspection are all included in the study. In order to conduct statistical studies, the analysis of variance (ANOVA) was followed by the Dunnett post -test. The findings shed light on the prospect of employing emulgel that contain Vitamin D-3 silver nanoparticles for the purpose of wound healing applications.

Prior to the formulation of a medicinal ingredient into a final dosage form, it is necessary to conduct pre-formulation studies in order to gain a knowledge of the pharmaceutical substance's physical characteristics and qualities. The purpose of this study was to evaluate a number of different pre-formulation factors of Vitamin D-3. A determination was made regarding the physical features, which included the color, texture, odor, and melting point. It was discovered that Vitamin D-3 is a white powder that is crystalline in appearance, has no odor, and has a melting point that ranges from 84°C to 85°C. Vitamin D-3 was found to be insoluble in water, mildly soluble in ethylene glycol, dimethyl sulphoxide (DMSO), and propylene glycol, and soluble in methanol and iso propyl alcohol (IPA), according to the results of investigations on its solubility. Additionally, in accordance with the guidelines established by the International Council for Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH), an analytical method validation analysis of Vitamin D-3 was carried out using the high-performance liquid chromatography (HPLC) method. Optimization was performed on the selection of the mobile phase, the preparation of the stock solution, and the standard curve in order to achieve accurate analysis. A correlation coefficient (R^2) of 0.999 was observed across a concentration range of 0.25-1.25 µg/mL, indicating that the approach exhibited a high degree of linearity. Evaluations were conducted to determine the accuracy, precision, robustness, and system compatibility aspects of the approach in order to guarantee its dependability and reproducibility. The limit of detection (LOD) and limit of quantification (LOQ) of Vitamin D-3 were found to be 0.01 µg/mL and 0.05 µg/mL, respectively, after carrying out the necessary experimentation. In general, the pre-formulation investigations and the validation of the analytical method provide useful insights into the physical features and analytical parameters of Vitamin D-3. These are essential for the formulation of Vitamin D-3 and for quality control in pharmaceutical applications. This investigation is centered on the optimization of silver nanoparticles by the utilization of a three-level, three-factor central composite

design. The objective of this study is to achieve a stable zeta potential, polydispersity index (PDI), optimal vesicle size, and the largest production of nanoparticles. For the purpose of optimization, the concentration of green tea extract, the concentration of silver nitrate, and the reaction time were selected as the independent variables. These three factors particle size, zeta potential, and percentage yield were considered to be dependent variables. The software known as Design Expert® was utilized in order to carry out the optimization process. This software was responsible for the generation of response surface plots and contour plots, which were utilized to analyze the impacts of the independent variables on the dependent variables. Increases in the concentration of green tea extract and silver nitrate, as well as an extension of the reaction time, were shown to have substantial effects on the particle size, zeta potential, and % yield of silver nanoparticles, as demonstrated by the findings. Particle size, zeta potential, PDI and % yield was found 79.46 ±1.1 nm, -12.6±0.9 mV, 0.261±0.002 and 72.1±2.1% respectively. Several methods, such as Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM), were utilized in order to carry out additional analysis of the silver nanoparticles that had been optimized. SEM photos revealed the spherical shape and smooth texture of the nanoparticles, while FTIR analysis proved the presence of functional groups involved in the synthesis of silver nanoparticles. Both of these findings were provided by the nanoparticles. This research was conducted not only optimized silver nanoparticles, but it also optimized an emulgel formulation that contained Vitamin D-3 by utilizing the Design expert® programme. An evaluation of the emulgel formulation was conducted to determine its *in vitro* cumulative drug leaching, pH, and viscosity. Optimized emulgel was having 1.5 % carbopol as gelling agent has shown *in vitro* cumulative drug leaching, pH and viscosity, 89.47±0.48%, 5.81±0.1 and 1373.5±11cps respectively. The findings revealed that increasing the concentration of carbopol had a substantial impact on these parameters. Wound healing activity of emulgel was performed on eight group of animals contains six animals in each group. The normal control group was not created for surgery and kept only for histopathological examination and to establish the relationship between the improved structure of the injured cells and the normal cell structure. The highest percentage of wound contraction was observed in the marketed formulation (Silvex contains silver sulfadiazine 1% w/w) and ESVDH (emulgel loaded with high dose Vitamin D-3 and silver nanoparticles), i.e., 103.725±1.12 and 100.5±1.7 respectively, which is very

similar to the marketed one. An emulgel containing only 0.006 %w/w Vitamin D-3 and 0.002 %w/w silver nanoparticles showed a dose-dependent improvement, in contrast the marketed formulation contained 1% w/w silver sulfadiazine, a much higher concentration has showed same % wound contraction. The ESVDH-treated and marketed preparation also showed epithelialization on day 15^{th} of the experiment. No other group of animals has shown this phenomenon. The remaining groups slowly recovered but not showed complete wound contraction. Emulgel with a small dose of Vitamin D-3 and a high dose of SNP showed a much higher rate of wound contraction but no complete healing till 16^{th} day of experiment, it was found that both groups had the same concentration of silver nanoparticles either ESVDH or ESVDL, but the concentration of Vitamin D-3 changed, so it seems that the Vitamin D-3 has a dose-dependent wound healing ability and can heal the wound even at low concentrations. These studies indicated the efficacy of the ESVDH formulations in inducing wound contraction and epithelialization. Histopathological examination confirmed the existence of viable cells and increased collagen fiber in the wound tissue, which was corroborated by *in vivo* investigations using an excision wound model. The formulations were shown to have maintained their stability over a period of six months, according to other stability investigations. In general, the optimized silver nanoparticles and Vitamin D-3 loaded emulgel formulation demonstrated features that gave rise to optimism regarding their prospective uses in the field of wound healing.

Chapter -1

1. Introduction

1.1 Wound

It is the injury occurred in the body or any part of the body. There are several causes for wound like accident, by extreme heat, during surgery or fighting etc [1-2]. Wound can be divided into two categories one is superficial wound and another is deep wound [3]. Superficial wounds take place only upper layer of skin and other hand deeper wound take place deeper layer of skin till endodermis and sometimes it reaches till capillaries and veins and fat tissues also. [4-5]. Wound healing is body's defence mechanism and also a complex phenomenon and it takes around 3 days to 12 months in healing on the basis of severity and deepness of wound [6-8]. There are three steps involved in wound healing and given below [9-10].

- Inflammation phase
- Proliferation phase
- Maturation or remodeling phase

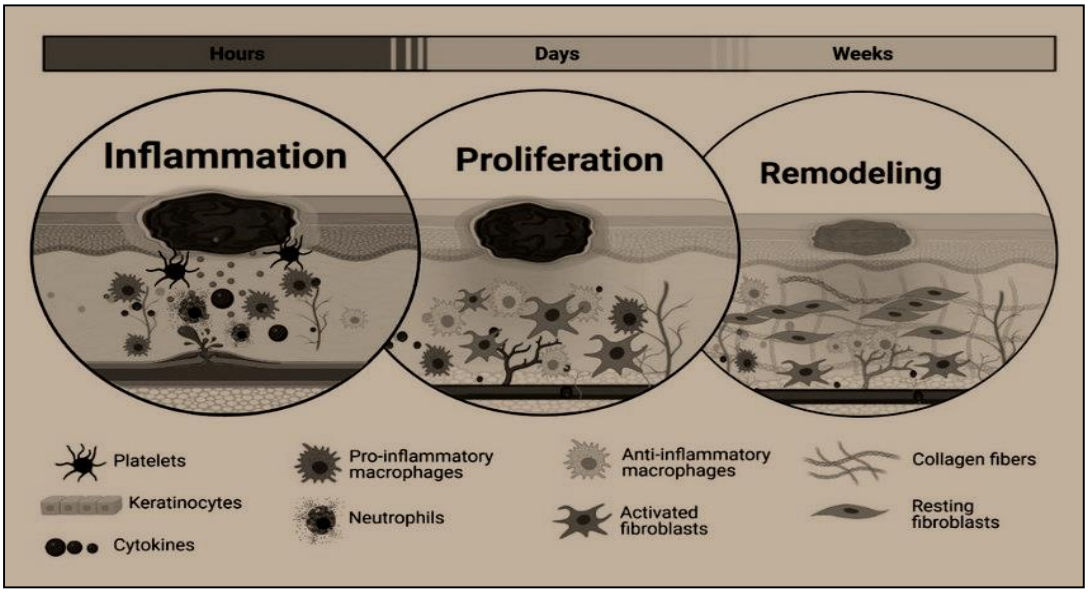


Figure 1.1 Different phases of wound healing

1.2 Symptoms of wound

The commonest characteristics of wounds are to produce pain, fluid deposition and bleeding from affected area [11]. On the basis of the severity and kinds of injury, some severe wounds produce high intensity of pain, hurts a lot, get more bleeding and create erythema more than others [12-15]. Some minor wounds such as cuts on skin by knife, scrapes, bruises and scratches, are very common and usually don't require any treatment as well as medical attention these get healed by self but major

wounds including accidental wounds, burn wounds, surgical wounds as well as infected wounds, requires medical treatment to prevent it any type of functional error of affected area and also prevents complications [16-18]. Symptoms of an infected wound is very common as normal wound like soreness, redness, sharp pain at injured site, inflammation, oozing and pus cells in the wound [17-18].

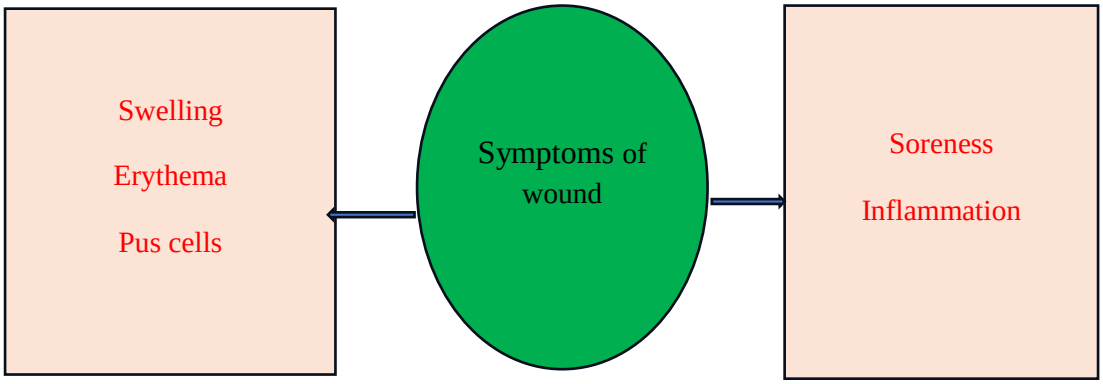


Figure 1.2 Symptoms of wounds

1.3 Types of wounds

A wound is a type of injury that causes the skin or other tissues to break or open. Wounds vary in severity, extent, and etiology, and can be classified in different ways. Here are some basic facts about the wound is mentioned below [19-20].

- **Cut wounds:** Caused by a sharp object, resulting in a clean, straight result.
- **Tearing:** Irregular and mottled scars due to tearing or breaking forces.
- **Puncture wounds:** Caused by a jagged material, leaving a small, deep hole.
- **Injury:** A wound or injury in which a blood vessel ruptures without scarring.
- **Abrasions:** Superficial injuries that require removal of the surface layer of skin, commonly known as scrapes.

1.3.1 Classification of wound on the basis of its depth is described below [21].

- **Superficial wounds:** Affect only the outer layer of skin.
- **Partial thickness wounds:** Penetrate the skin layer but do not penetrate the entire thickness [22].
- **Fully complex wounds:** Extend to all layers of the skin and may involve muscles, tendons, or lower organs [23].
- **Trauma:** An injury resulting from an accident, fall, or physical force.
- **Surgical wounds:** Deliberately cut during treatment [24].
- **Burn:** Damage to tissue due to thermal, chemical, electrical, or radiation exposure [25].

1.3.2 Classification of wound on the basis of their various causes is described below:

- **Excision wound:** It created during surgery for particular opening and done by medical surgeon. Ex: surgery of gall bladder, kidney, excision during baby birth. These wounds are not permanent and healed after a period of time [26-28].

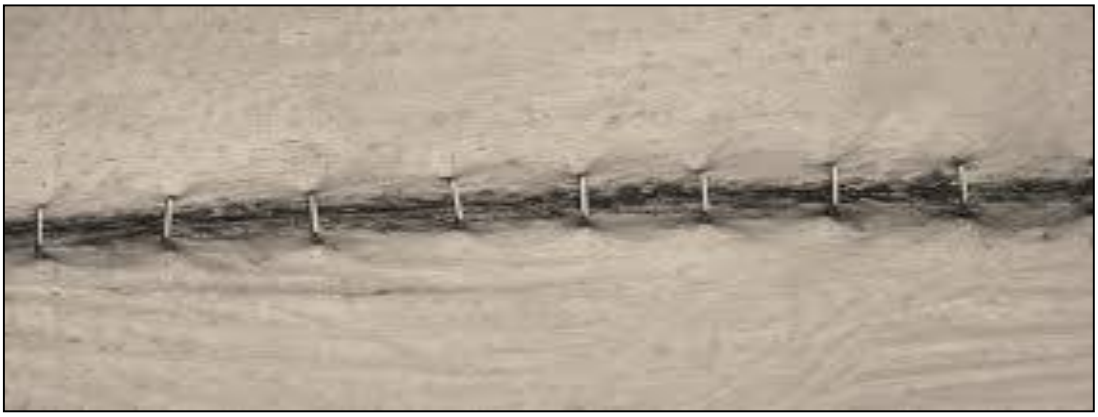


Figure 1.3 Excision wound

- **Accidental wound:** These wounds occurred accidently, by roads accident, during not proper handling of chemicals etc., according to their deepness these can be severe or not, some wounds may lead to death some are heals [29-30].

Figure 1.4 Accidental wound

- **Chronic wound:** These types of wounds can be with patient throughout their life, if left untreated it can also convert into metastasis like cancer, diabetic foot syndrome etc. [31-32].

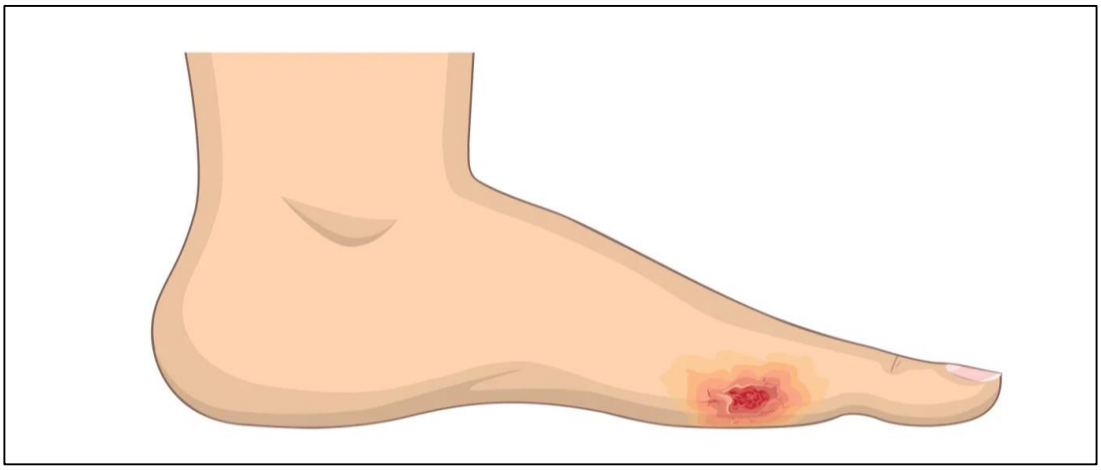


Figure 1.5 Diabetic foot syndrome

- **Burn wound:** These types of wounds occurred by burning injury, it can be accidental or intentional. On the basis of severity and deepness of wounds these are classified in several categories. In the case of 90% burn patient usually not survive because in this type of cases burn reaches to the deepest layer of skin and usually mucosa and fluid get burned [33-35].

Figure 1.6 Burn wound

1.3.3 Phases of wound healing

Wounds can be open, which exposed body tissue and contains broken skin, or closed wounds are found when there is damage in tissue under the skin or deepest layer of skin [36- 37]. Closed wounds are caused by trauma, or internal injury where internal tissue is not exposed, there can be internal loss of blood and damage deepest muscle, major organs or tissue or it may be in the tiny or larger bones. Time scale and description of these phases are given below [39].

- **Inflammatory phase:** Burn, surgical and traumatic wounds cause shape changes and haemorrhage etc. Initially wound fills by blood and activation of Hageman factor take place. These procedures take place in 0-5 days [40].

- **Proliferative phase**: In this phase granulation take place, in granulation several factor like fibroblast, cellular elements are involved, this phase takes around 3-14 days [41].
- **Maturation phase:** In this phase collagen synthesis get started, and other factors like metalloproteinase, enzyme are get activated and aid to wound healing, this phase take place around day 7-1 year depends upon deepness of wound [42].

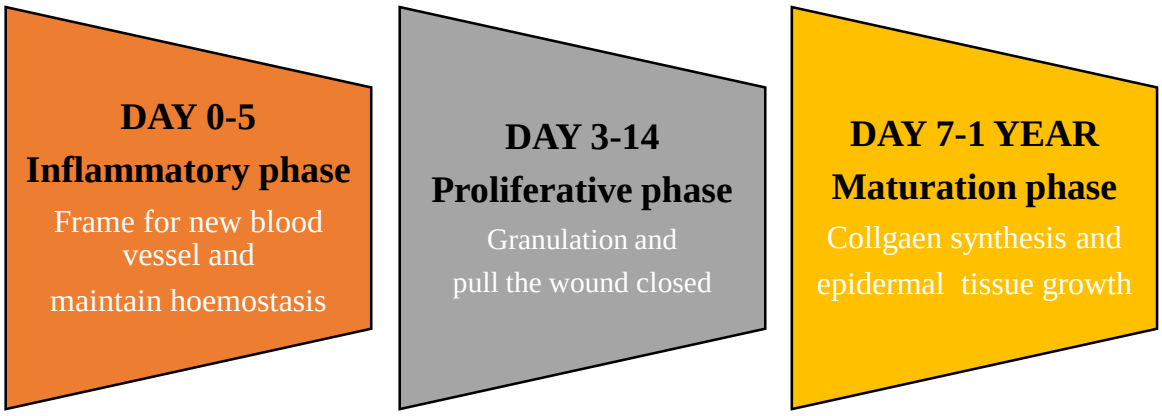


Figure 1.7 Wound healing process

1.4 Prevalence of wound

It was found that almost all population experience any type of wound in their life and 1-2% of population will get exposed by chronic wound in future [43-45]. Normal wound heals in almost one week but in case of chronic wound it almost takes months to years and it is totally depending on the deepness of wound in skin layers, severity of wound can lead to the death of patient some of the examples are acid attacked victims and burn wounds [46-49]. Chronic wounds can create infection trough out the body, some wounds create eczema gangrene etc. [50-51]. If the wound left untreated it can convert into several severe disease like cancer [52-53]. Patients suffering from wounds and infection can leads to death and it is common in the case of burn wound [54-55]. Family of patient feels financial burden, stress, anxiety, hypertension etc [56-58]. In the previous data it was revealed that there is no proper estimation of patients suffering from wound in particular years [59-61].

1.5 Effect of nanotechnology in the treatment of wound

Nanotechnology is the technology in which we study, the synthesis of devices and structure in between range of nanometre size [62-65]. These nano-sized material e.g., “nanoparticles”, have new properties and functions that made them differ from conventional method of preparation of dosage and according to some author carboxylic group in plant extract only responsible for reduction and formation of

nanostructure [66-69]. Their small size, large surface area, higher solubility, and multi-functionality may open many doors in biomedical applications like targeting in case of cancer tumours ae well as in wound [70-75]. Its smaller size is very effective to reach in the deepness of wound area [76-77]. Some metallic nanoparticles are proven beneficial in healing of wound itself without loading any active pharmaceutical ingredients such as silver nanoparticles, gold nanoparticles, ferrite nanoparticles etc. [78-81].

1.6 Methods for synthesis of metallic nanoparticles

There is variety of nanostructures or nanocarriers that has been synthesized for delivery of variety of drugs [82-84]. The most common form of nanocarriers is niosomes, polymeric nanoparticles, solid lipid nanoparticles and liposome [85-89]. Metallic nanoparticles which have antibacterial property like silver and gold nanoparticles are the newest approach to open new tracks to fight and prevent various infectious diseases [90-92]. Metallic nanoparticles can be produced by several methods like physical such as condensation, evaporation and laser ablation, chemical approach using reducing agent like ascorbic acid, citric acid sodium borohydrate along with some plant extracts like green tea [93-95].

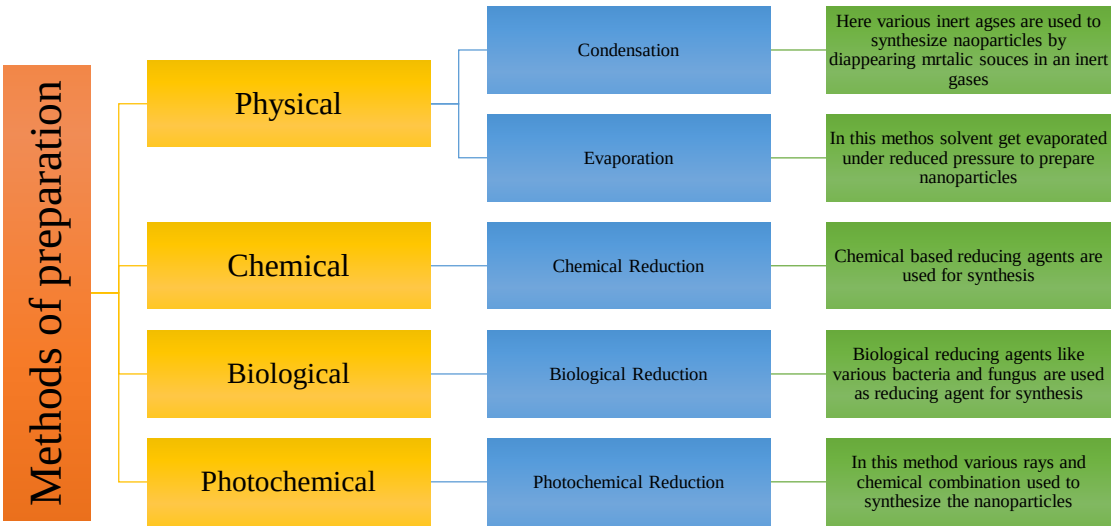


Figure 1.8 Method of preparation for nanoparticles

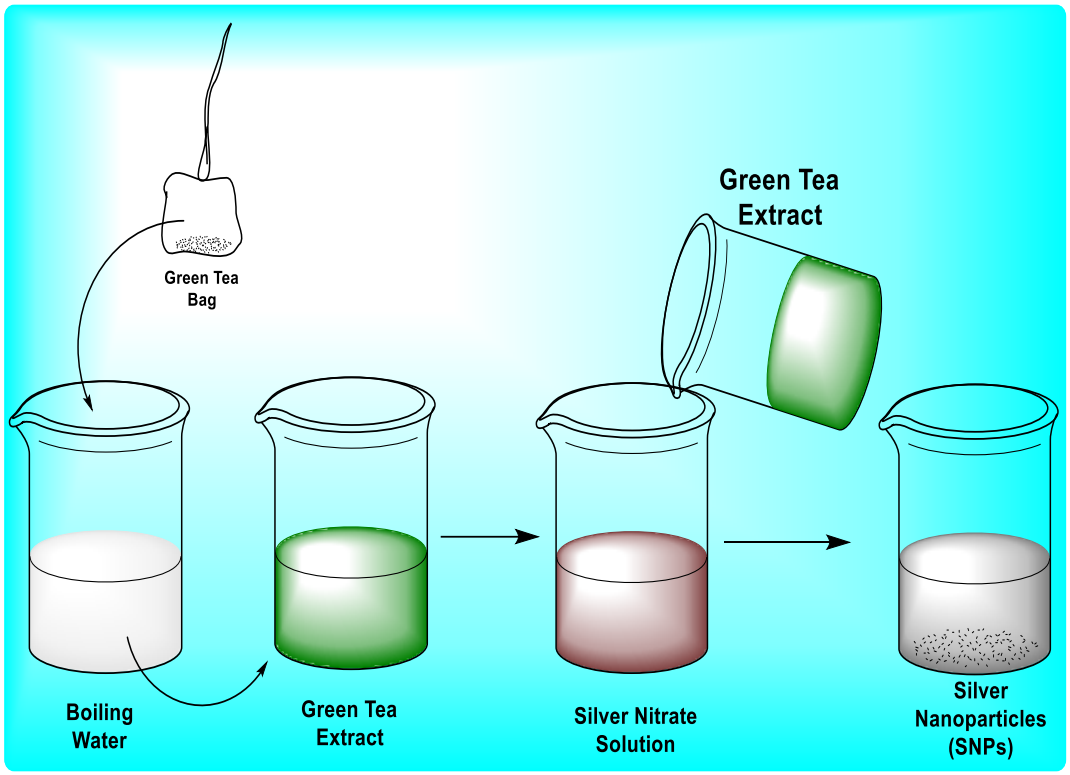


Figure 1.9 One pot synthesis of silver nanoparticles

1.7 Advantages of nanoparticles [96-98]

- Silver nanoparticles has proven beneficial in wound healing.
- Because of their smaller size they can easily reached at inflammatory sites and can load large quantity of drug to accelerate the wound healing.
- These nanoparticles also contain anti- bacterial property and these characteristics may aid in healing.
- They can also use in wound dressing to prevent infection and promote healing.

1.8 Application of nanoparticles in biomedicals [99-101]

- Excision Wound healing
- Burn wound healing
- Anti-inflammatory effect
- Anti- bacterial effect
- Anti- fungal effect

1.9 Silver nanoparticles a boon for wound healing therapy

Medicinal, preservative, and antibacterial, action of silver has been proven for many years and it was also using in the ancient time for topical treatment. Since the nineteenth century, silver-based formulations have been used for wound healing, burns and bactericidal property [102-103]. Silver has been also used in India in the

form of ornaments [104-105]. Since some decades silver nanoparticles are synthesizing due to their medicinal property, tiny size and higher surface area [106-108].

Different biological methods are continuously using for the production of silver nanoparticles (SNPs) because these have multiple applications such as drug delivery and diagnosis [109-111]. The use of plant extracts in the green synthesis of nanoparticles leads as a cost effective, environmental-friendly and produce stable materials [112-113]. Different methods have been used for the green synthesis of nanoparticles using different biological object in the form of reducing agents such as bacteria, marine organisms and algae, several fruit extract, microorganism-fluids, and plant decoction [114-115].

1.10 Synthesis of silver nanoparticles using green tea extract as reducing agent

In the synthesis of metallic nanoparticles, one reducing agent is required [116-119]. There are several types of reducing agents are available like physical, chemical, biological and natural [120-121]. Best example for the natural reducing agent is green tea extract [122]. It produces higher yield of nanoparticles as well as it is a one-step synthesis [123-124]. Green tea (*Camellia sinensis*) is the most regularly used beverage, following water and it also has anticancer property *in vivo* [125-126]. Polyphenols in green tea may be responsible for the anti-cancer effects as well as cardiovascular diseases [127]. Catechins are polyphenolic flavonoids basically found in green tea and also act as reducing agent [128]. There are many types of catechin such as epicatechin, epicatechin gallate and epigallocatechin gallate are also found in green tea which has an antioxidant as well as anti-inflammatory property so basically whole green tea extract can use for its medicinal value as well as green reducing agent for synthesis of variety of nanoparticles. Possible applications of green tea for producing silver nanoparticles. Bioactive compounds in green tea such as polyphenols have been utilized to synthesize nanoparticles, including silver nanoparticles. Here is an overview

- **Reduction agent:** Silver nanoparticles can be synthesized using green tea extracts as reducing agent [129]. The presence of catechins particularly polyphenols in green tea has been found to be effective for reducing silver ions into silver nanoparticles [130].

- **Stabilizing Agent**: Synthesized silver nanoparticles using essential components of green tea may also possess stabilizing properties to prevent agglomeration and precipitation [131]. Nanoparticles stability could be provided by capped –polyphenols derived from green leaf elements [132].
- **Antimicrobial properties**: Silver nanoparticles are popular because they are antimicrobial. Green tea synthesized silver nanoparticles possessed these features, which means they might have other applications like being used in making anti-microbial coatings or wound dressings.

1.11 Applications of silver nanoparticles

- **Bacterial inhibition:** Experts have suggested that this discovery of silver nanoparticles can help to stop and treat infection as silver nanoparticles have been proven to possess high antimicrobial properties against bacteria, fungi and viruses. [133].
- **Anti-Inflammatory:** It has been reported that silver nanoparticles possess anti-inflammatory properties as they help to suppress inflammation at the site of the wound thus leading to more controlled healing process and less pain to the patient [134].
- **Wound healing promotion:** Silver nanoparticles may boost fibroblasts and keratinocytes proliferation as well as migration which are important for wound healing. This can speed up the wound closure [135].
- **Regulation of collagen synthesis:** Studies suggest that silver nanoparticles could potentially modulate collagen synthesis thereby resulting in less scarring during recovery.
- **Preventing biofilm formation:** A solution that can disrupt the formation of biofilms on the surface of a wound is silver nanoparticles; these are colonies of bacteria stuck in a surrounding, it slows down the process of healing.
- **Incorporation into dressings**: Introducing silver nanoparticles into dressing materials for wounds and other delivery systems, ensure regulated and continuous discharge of silver ions at the affected area to foster wound healing.
- **Synergies with other agents:** In general, silver nanoparticles may be employed together with other therapeutic agents/technologies to improve overall wound healing outcomes.

1.12 Vitamin D-3 as a wound healing agent

Increased awareness of Vitamin D-3 has for decades been used in treating certain diseases like tuberculosis and HIV because of its antimicrobial and anti-inflammatory properties. It does this by providing assistance
in the prevention of several inflammatory mediators. [136-137] For Vitamin D-3 product, FDA has given approval for off-label use to have topical application in several cutaneous diseases. Vitamin D-3 can help reduce swelling, which can aid the body's natural recovery process after an injury. When the skin is damaged, inflammation occurs as part of the healing. But too much inflammation can slow wound repair. Maintaining the right amount of inflammation is important for healing wounds properly, Vitamin D-3 supports this balance by increasing cathelicidin synthesis, which indirectly promotes the collagen synthesis and accelerate wound healing [138]. Oil soluble vitamins like Vitamin D-3 can use for the formulation of nano emulsion, in this formulation O/W type of emulsion was prepared because it has good solubility in oil [139]. Topically ointment of Vitamin D-3 on corneal walls wound promotes wound healing [140]. Vitamin D-3 plays dual role nutritionist as well as anti-inflammatory factor. Orally intake of Vitamin D-3 boosts the immune system and can also be used in skin disease like *Psoariasis* [141]. Vitamin D-3 can affect how cells grow and develop, which are important processes for healing and renewing tissues. The vitamin may influence whether cells divide into new ones or mature into specialized forms. Maintaining adequate Vitamin D-3 levels could support the body's natural mending and rejuvenation abilities [142-143]. It also induces wound healing in diabetic rat by inhibiting NF-KB inflammatory mediators [144-145]. Two types of Vitamins found in human body i.e., Vitamin D-3 (cholecalciferol) and Vitamin D-2 (ergosterol). Vitamin D-3 direct synthesized in human body from sun light and Vitamin D-2 obtained from dietary sources. Vitamin D-3 has various medicinal property and can convert into various dosage forms for their therapeutic effect [146-150]. Vitamin D-3 helps in nourishment, bone strengthening, and one major role to increase cathelicidin synthesis, which is an antimicrobial peptide, and elevates collagen synthesis in human body. Collagen is required for the production of the new tissue and rejuvenation and hence support a wound healing [151-155].

Figure 1.10 Mushroom (Source of Vitamin D-2**)**

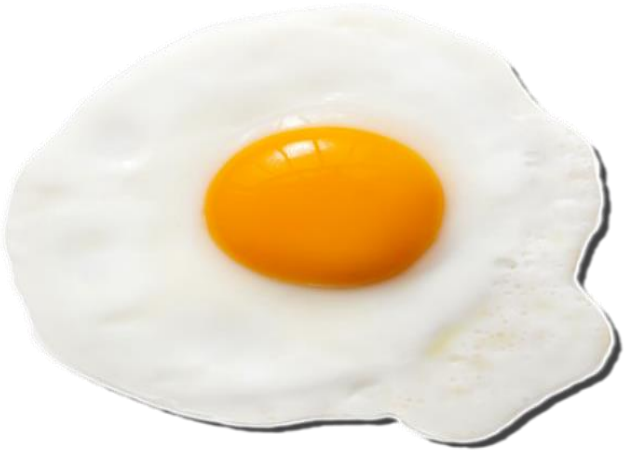


Figure 1.11 Egg Yolk (Source of Vitamin D-3)

Vitamin D-2 (Ergocalciferol)	• Mushrooms • Meat
Vitamin D-3 (Cholecalciferol)	• Fish • Fish liver oil • Egg yolk

Figure 1.12 Types and sources of Vitamin D-3

Table 1.1 Some marketed formulation of Vitamin D-3

Sr. No.	Brand name	Application
1	Vitals tablets	Support to strong bone and muscle
2	True basics	Immunity booster
3	Vitamin D-3 oral spray	Support to strong bone and muscle
4	Vitamin D-3 cream	Nourish skin
5	TOP-D	Vitamin D-3 deficiency

1.13 Limitations of semi solid conventional dosage forms

There are many medications that can be applied topically to the skin or mucous membrane to improve quality or bring back basic function. Topical goods, such as lotions, ointments, and creams, frequently present problems including low spreading coefficients, stickiness, and stability problems. In response to these constraints, gels, which utilize emulsion-based methods are becoming more and more popular as a means of incorporating hydrophobic medications into pharmaceutical and cosmetic formulations. This eliminates the limits that were previously associated with semisolid formulations [155].

1.14 Rationale of emulgel in topical drug delivery

Emulgel is basically combination of two dosage form i.e. gel and emulsions. An "emulgel" refers to a kind of medicine or beauty item that mixes qualities of both an emulsion and a gel. Let's break down the parts of the term [156].

- An emulsion is a blend of two liquids that don't mix, like oil and water, kept together with an agent. In medicines and beauty items, this regularly includes mixing oil and water to make a steady and uniform blend.
- A gel is a semi-solid, jelly-like substance that has properties of both solids and liquids. Gels are regularly utilized as a base for different topical items because of their capacity to give a smooth and effectively spreadable consistency.
- Along these lines, "emulgel" consolidates the properties of an emulsion and a gel. This blend is particularly helpful in topical applications, similar to creams and salves, where water dissolvable and oil dissolvable dynamic fixings need to be incorporated. The emulsion part assists with conveying both water and oil-base fixings.
- Oil in water system used for the lipophilic drugs in contrast for hydrophilic drugs water is used in continuous phase while oil is used in non -continuous

phase base. These are bio-friendly and have higher spreadability. Emulgel is used to prepare for enhance the solubility of hydrophobic drugs and also gives site specific delivery.

- Emulgel are the gifted agent for the delivery of lipophilic drugs by using different permeation enhancer like iso propyl alcohol (IPA). Emulgel has the option to work as a promising carrier for several lipophilic drugs to be delivered topically, apart from enhancing the therapeutic effects and duration of retention time to the skin; however, the market currently offers only a limited range of emulgel products. First one is Voltaren emulgel contains diclofenac and dimethylamine second is Miconaz-H contains miconazole nitrate and hydrocortisone and day by day the formulations are getting prepared [157].

Figure 1.13 (a) Voltaren emulgel

Figure 1.13 (b) Miconaz- H emulgel

Table 1.2 Marketed formulation of emulgel

Sr. no.	Brand name	Active constituents	Manufactured by
1.	Voltaren	Diclofenac sodium	GSK
2.	Lotus white glow gel crème	Grape extract and mulberry extract	Lotus herbal
3.	Iodex	Clove oil, menthol, eucalyptus oil	GSK
4.	Dove cooling gel crème	Dimethicone, glycerin and carbomer	Hindustan uniliver ltd
5.	Clinagel	Clindamycin phosphate	Stiefel Pharma
6.	Diclon	Diclofenac dimethylamine	Med Pharma
7.	Cloben	Neomycin, Clotrimazole, Beclomethasone dipropionate	Indoco Remedies

An emulgel, a pharmaceutical and cosmetic mixture that combines traits of both emulsion and gel, reveals a growing interest in its development, optimization, and applications. Emulgel is versatile systems with unique properties, allowing them to be suitable for a variety of topical and transdermal uses. The various research provides an overview of key aspects related to emulgel. Emulgel can be stabilized using a gelling agent and made as either water-in-oil (w/o) or oil-in-water (o/w). It offers a reliable and efficient platform for integrating medications that are not very soluble in water [158]. Gels have many benefits, but they have trouble delivering drugs that are hydrophobic. This problem is solved by an emulsion-based method, which allows even hydrophobic substances to take advantage of the special qualities of the gel. Emulgel having two phases aqueous and non-aqueous allow it to hold both hydrophilic and lipophilic medicines. It also functions as a controlled-release formulation by utilizing a biphasic method to improve the stability and drug-loading capability [159]. It functions as a dual control release system and possesses both gel and emulsion properties. Recently, this kind of innovative formulation has been created for topical medication distribution, and it has proven to be appropriate [160].

1.14.1 Formulation and composition of emulgel

Various mixtures of emulsifiers, thickening agents, and other ingredients to achieve stable and effective emulgel formulations. Choosing the oil part, water part, and thickening agent plays a crucial role in determining the physical and chemical qualities of the emulgel. Composition of an emulgel is listed below [161]

- **Drug:** The active pharmaceutical ingredient (API) has high pKa value or partition coefficient between 0.5-10 are suitable for emulgel preparation. Lipophilic drugs are suitable for preparation of emulgel to enhance their solubility as well as to control the drug release.
- **Emulsifiers:** Emulsifying compounds are used to control stability during a shelf life as well as to prepare the emulsion by emulsification procedure. Some of the examples for emulsifying agents are tween 80, span 20, detergents, sodium mono stearates etc.
- **Gelling agents:** These agents can be utilized as thickening agents as well as being used to improve the consistency of any dosage form. They contribute significantly to the preparation of emulgel by giving the formulation viscosity, stability, and rheological characteristics. They contribute to the emulsion's transformation into a semi-solid gel matrix, improving the drug's administration and therapeutic efficacy by improving the emulsion's spreadability, adherence, and retention on the skin.
- **Penetration enhancers:** Drug delivery methods are enhanced by penetration enhancers, which make it easier for medications to pass through biological barriers including mucous membranes and skin. These enhancers improve the therapeutic results of topical and transdermal medications by raising drug bioavailability and efficacy. Some of the penetration enhancers are oleic acid, polyethylene glycol (PEG), lecithin etc.

1.14.2 Method for preparation of emulgel

There are different ways to prepare emulgel depending on the ingredients and desired results.: It is worthwhile noting that the choice of ingredients, emulsifiers, and gelling agents should be thoughtfully considered to accomplish the planned stability, feel, and therapeutic qualities of the emulgel. Furthermore, suitable quality control steps and adherence to good production practices are critical during the planning procedure. The gel must be examined to make sure it has the right consistency and properties before it is filled into tubes, bottles or other packing. Testing helps confirm the gel meets specifications and standards of quality [162]. Above mentioned guidelines carefully help produce effective emulgel that delivers the required benefits

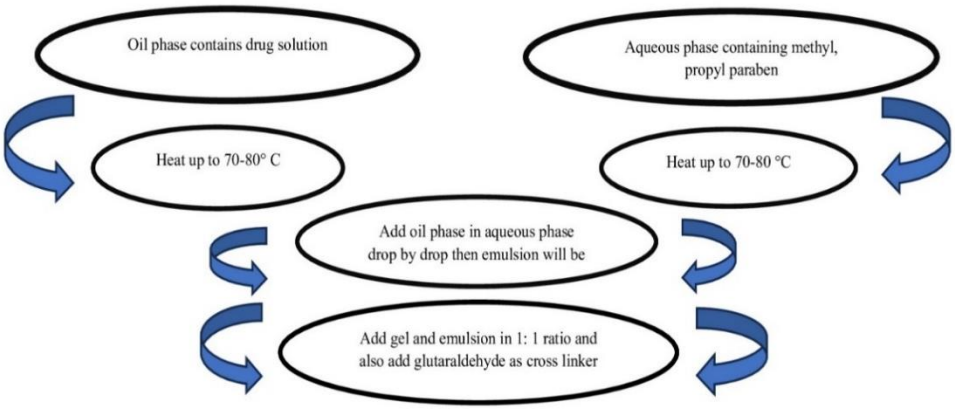


Figure 1.14 Schematic diagram representing preparation of emulgel

In this schematic diagram

- The water phase in an emulgel contains water and any water-soluble ingredients.
- A gelling agent like carbopol forms the gel like structure. It holds the emulsion together.
- An emulsifying agent such as tween 80 stabilizes the mixture of oil and water. It stops the oil from separating out.
- Active ingredients provide the intended benefits, whether therapeutic effects from drugs or effects on the skin.
- The oil phase carries the lipophilic or fat loving part, like oils. It is crucial for forming and maintaining the emulsion over time.

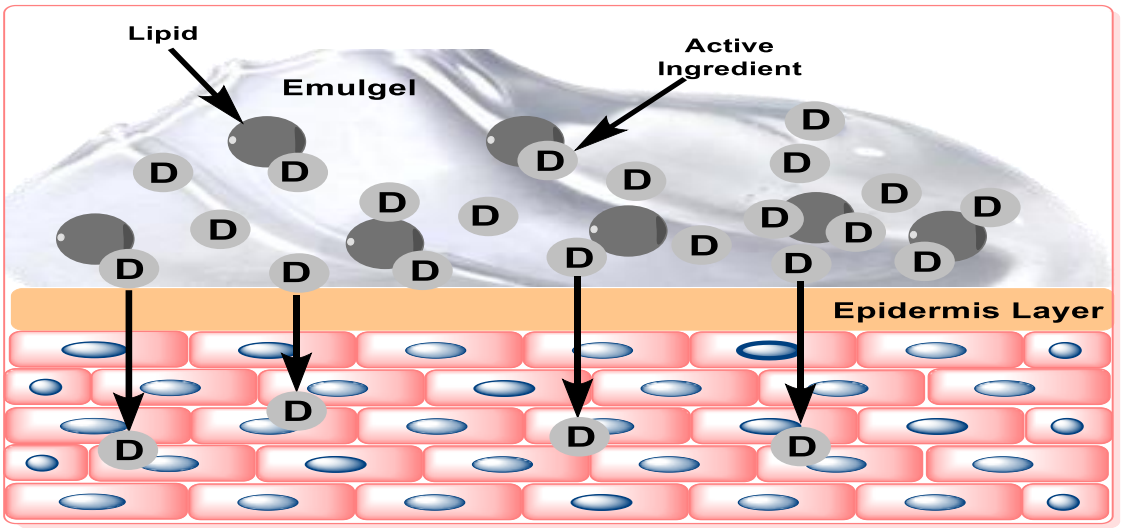


Figure 1.15 Drug delivery through emulgel

1.14.3 Drug delivery through emulgel

Drug particles are trapped in the internal phase of the emulsion and subsequently travel through the exterior phase to the skin, where they are absorbed gradually. This mechanism allows for a controlled release of drugs. The internal phase of the skin serves as a reservoir for the medicine and allows it to enter the external phase of the skin in a controlled manner. The gel's cross-linked network helps it to trap tiny drug particles and release the drug in a regulated way. Its mucoadhesive qualities also allow the medication to stay in touch with the skin for longer duration. Emulgel functions as a dual control release system since it has the qualities of both gel and emulsion [163-164].

1.14.5 Various applications of emulgel

There are many different types of emulgel on the market; some are used as cosmetics, while others are used as analgesics. Patients and general public alike are beginning to adopt it more extensively because of its unique constancy. Patients with muscle soreness, low back pain, back discomfort, etc. frequently utilize Votaren emulgel. Lotus herbal gel creme, which has an SPF 30, is readily accepted by both men and women as an alternative to regular sunscreen use because it works well for all skin types, including mixed ones. Some other uses of emulgel are listed below [165].

- **Topical drug delivery:** Emulgel find wide usage in pharmaceuticals for topical drug delivery. They have benefits such as sustained release, enhanced skin penetration and stability improvement of active pharmaceutical ingredients (APIs). Pain relief gels, anti-inflammatory and dermatological formulations commonly contain them.
- **Cosmetics:** Cosmetic products like creams, lotions and gels use emulgel. They give a smooth texture, good spreadability and can encapsulate both hydrophilic as well as lipophilic ingredients. Moisturizers, sunscreens and anti-aging creams have emulgel among their components.
- **Personal care products:** In personal care products such as shaving gels, toothpaste gels and hair styling gels emulgel are employed. Desired consistency stability along with enhanced delivery of active ingredients is provided by them.
- **Veterinary formulation:** Now a days emulgel are widely used in the veterinary formulation. It provides the control release of drug. There are variety of topical

emulgel are available for the treatment of wound, act as analgesic as well as bacterial and fungal treatment in animals.

Chapter -2

2.1 Literature review on Vitamin D-3 and silver nanoparticles

An extensive literature review was undertaken. The salient features of the same are:

Bikle D. D. (2023) stated that Vitamin D-3 is beneficial in epidermal wound healing by several pathway, it reduces the wound healing time due to its special mode of action on epidermal tissue [166]. In 2023 Abdellatif A. *et al* stated that silver nanoparticles made from ethyl cellulose are useful in the treatment of wound and it also suggested their antibacterial property [167]. Franceschinis G. *et al.,* (2023) suggested newly developed green method for the synthesis of nanoparticles using aloe maculate extract. The nanoparticles synthesized from this kind of method was found therapeutically more effective in the topical wound treatment [168]. Kragballe K *et al*., (2018) stated that Vitamin D-3 and its related substitutes can use in the treatment of *Psoriasis* and several infectious diseases of skin. *Psoriasis* is the disease in which skin get infected and delayed healing. Vitamin D-3 increased the production of cathelicidin which inhibit the growth of bacteria as well as increase collagen synthesis [169]. Liang *et al*., (2017) found that silver nanoparticles (SNPs) are anti-cancer in nature. These enter in to the cells by endocytosis and kill the cancerous cell and also help to stop the cancerous cell migration to other body organ. Silver nanoparticles inhibit the growth of tumorous cells in the body as well as prevent the chances of cancer cell kinetics from distal metastasis [170].

Barrea L. *et al*., (2017) proposed that Vitamin D-3 can also be used in the healing of wound along with skin diseases like *Psoriasis.* They also concluded that it gets easily dissolves in oils and play an important role in nutrition as well as anti-inflammatory factor. Orally intake of Vitamin D-3 boosts the immune system and can also be used in skin disease like *Psoriasis,* fungal infection, bacterial infection etc. It was also observed that it promotes wound contraction in rat suffering from diabetes by inhibiting and binding with inflammatory mediators [171]. Reins R.Y. *et al.,* (2016) proposed that Vitamin D-3 helps in corneal wound healing by acting as a main wound healing agent. Vitamin D-3 shows anxiolytic effects in tuberculosis and HIV by having antimicrobial, anti-inflammatory action. This vitamin plays the role of regulating some inflammatory factors. Several formulations of Vitamin D-3 and its existing forms are now available in the market covering nutraceuticals, pharmaceuticals along with cosmetic products. Vitamin D-3 is fat soluble and gives higher penetration in the skin tissue. Vitamin D-3 contains cremes and lotions are

available in the market as fairness creme, moisturizing creme, rejuvenating creme etc. [172].

Britto H. *et al*., (2016) suggested a newly developed method for the synthesis of silver and gold metallic colloids, instantaneously using ascorbic acid as reducing agent. This method is allowed the instant synthesis of nanoparticles at room temperature without using even a single instrument or sophisticated instrument [173]. Tawfik A. Saleh *et al.,* (2016) proposed the various methods for synthesis of gold and silver nanoparticles. Since some era, silver nanoparticles are synthesizing and using widely due to its pharmacological property, smaller size

and larger surface area to achieve prolonged release. Different biological methods are continuously using for the synthesis of silver nanoparticles because these have multiple applications such as drug delivery as well as diagnosis aid in various disease. The use of plant extracts is a cost effective, environmental-friendly and produce stable materials. Metallic nanoparticles can be produced by several methods like physical such as condensation, evaporation and laser ablation, chemical approach by using reducing agent like ascorbic acid, citric acid sodium boro hydrate and some plant extracts like green tea [174]. Mazeed *et al*., (2016) stated that *Penicillium brevicompactum* (MTCC-1999) is a fungus which was used for the green synthesis of silver nanoparticles. These nanoparticles were evaluated for *in vitro* as well as *in vivo* characterization to determine antibacterial effect followed by synergistic effects with antibiotics like tetracycline, penicillin, and azithromycin etc. These nanoparticles were also evaluated for their anti-cancer activity against the breast cancer cell line MCF-7 using *in vitro* MTT assay [175]. Saleh *et al.,* (2016) stated that nanoparticles are widely used in the field of biotechnology as well biomedical. Due to its larger surface area, physical properties, enhanced permeability, and retention time make them as promising agent in diagnosis and therapy in various diseases. The gold and silver nanoparticles have proven very beneficial and safest carrier for drug applications [176].

Malassis *et al*., (2016) revealed that metallic gold and silver nanoparticles can be prepared by one step procedure using vitamin c chemically known as ascorbic acid. This vitamin was acted as reducing as well as stabilizing agent. Beside this other herbal extract like green tea, black tea, white tea, turmeric and citrus etc. are also used for synthesis of metallic nanoparticles. This is safe and economical method. Now a day's herbal or green methods are used by industry for synthesis of materials [177]. Khan *et al.,* (2013)

proposed that the green synthesis or herbal synthesis of metallic nanoparticles like silver and gold has gained a lot of attention in recent years because these economic and environmentally friendly than other reported methods of synthesis. These methods are simple and less man power required for synthesis [178]. Khan M. *et al.,* (2013) proposed the eco-friendly production of silver nanoparticles using extract of *Pulicaria grutinosa.* This extract has an anti-inflammatory, anti-migratory, antiphagocytic as well as anti -oxidant in nature. Its anti-oxidant property is very strong can also use in prevention of cancer as well as good reducing agent for herbal nano synthesis [179]. Tran H.Q. *et al.,* proposed the different method for synthesis of sliver nanoparticles in the year 2013. Nanoparticles can prepare varieties of methods like physical, chemical, biological and physiochemical. Synthesis of metallic nanoparticles using plant based reducing agent is meant to be safe due to it simple and one pot synthesis [180].

2.2 Literature review on green tea extract

Sharma P. *et al.,* in 2022 revealed higher uptake of green tea catechins reduce the chances of hepatotoxicity, liver diseases, stomach ulcer, Stomach pain etc [181]. Raygeart C. was found that catechins extracted from green tea could be use in treatment and prevention of infectious disease. Green tea catechins are responsible for benefits in several diseases and also prevent from infection due to its anti-microbial activity in 2021 [182]. Miyata Y. *et al.,* (2018) revealed that green tea catechin and green tea polyphenols can be used in bladder cancer treatment. Its polyphenolic compounds are responsible for neutralizing the effect of free radical, reduce cell division, reduce the risk of cancer etc. in bladed cancer it is found very effective in treatment *in vivo* [183]. Tsai Y.J. *et al.,* was observed in the year 2016 that the catechin extracted from green tea can inhibit the prostate cancer cell growth PC-3 by reacting with free radicals. Green tea (*Camellia sinensis*) is the most regularly used beverage, following water and it also has anti-cancer property *in vivo*. Green tea catechins neutralizes the free radicals and prevent the cells from cell damage which can leads to cancer in future. So, consumption of green tea is a beneficial from the medicinal point of view [184]. Forester S. C. *et al.,* (2013) finds and revealed that daily intake of green tea in morning can fight with chronic disease such as cancer. Cancer is fatal disease which can leads to death. Free radicals generate throughout the body during metabolism, it is required to neutralize these free radicals to reduce the chances of cancer. Green tea contains catechins and flavonoids which are responsible to

neutralize the effect of free radicals, as well as it is used in synthesis of metallic nanoparticles as a reducing agent and also found safest method for synthesis [185].

Asadi Y. *et al.,* (2013) revealed that green tea extract has an antioxidant as well as anti-inflammatory property. Green tea extract contains catechins and flavonoids which are responsible to neutralize the effect of free radicals, so consumption of green tea can reduce the chance of cancer. Green tea contains, epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin (EC) and flavonoids etc. These are most powerful anti-oxidant than other herbal plants contains so it is good for health as well as in prevention of several metabolic disease [186]. Zhong Y. *et al.,* (2011) proposed that the EGCG have antiviral potential as well as strong antioxidant activity. Epigallocatechin gallate (EGCG) is a strong anti-oxidant agent present in green tea extract also confirms the reduction rate of cancer in humans as they reduce the free radicals moving throughout the body, which are mainly responsible for metastasis. These polyphenolic compounds play an excellent role in metabolic function. These polyphenols are strong reducing agent, nanoparticles synthesized from green tea extract do not requires any stabilizing agent. Oral uptake of green tea reduces free radicals in the body and prevent from various metabolic disorders [187].

2.3 Literature review on emulgel

An extensive literature review was undertaken. The salient features of the same are:

Lanjekar D. *et al.,* (2024) stated that emulgel based drug delivery was found effective in diabetic induced wound in albino wistar rat. Diosgenin was used for the wound healing potency and it was found that it has produced desired therapeutic effect in the treatment of wound [188]. Gupta P *et al.,* (2024) proposed the novel method for the synthesis of emulgel. Researcher explained that emulgel was prepared using *Propolis* extract and *Passiflora edulis* seed oil and it was evaluated against excision wound model and also identified the cytokines level in rat [189]. Permana AD. *et al.,* (2023) revealed metronidazole is hydrophobic drugs hence the drug pose problem in topical drug delivery due to its low solubilization, to overcome this factor emulgel of metronidazole was prepared [190]. B.A. Khan *et al.,* stated that *Ocimum bacilimum* based emulgel was found beneficial in the treatment of wound, it increases the collagen synthesis during proliferation phase of healing and hence accelerate the wound healing in 2021 [191]. N.P. Lakshmi *et al.,* in the year 2020 revealed that *Hibiscus* leaf extract was used for the formulation of emulgel. On quality control test it was observed that *Hibiscus* leaf extract has given the exact gelling property as well

as medicinal value to the emulgel resulted powerful tool for wound healing [192]. Sohail M. *et al.,* (2018) proposed the synthesis of lycopene based emulgel, which will enhance the stability. Emulsion is thermodynamically unstable formulation; to increase the stability of emulsion emulgel is prepared. Due to its gelling structure, it remains stable in hot and cold temperature and does not allow phasing change of emulsion [193]. Jagdale S. *et al.,* revealed that ofloxacin emulgel gives site specific target delivery. Emulgel is a novel formulation meant to use for targeting of disease due to entrapment of oil globules in gel form, polymer is responsible for sustained or controlled delivery on the topical surface for longer duration of time and can be beneficial in several disease and also increase patient compliance in the year 2017 [194]. Vishnubhaktula *et al*., was done research and concluded in 2017 that the use of formulations based on hydrogels made from carbomers are useful in melanoma skin cancer and delivers the drug in constant and controlled manner a delivery system of anti-proliferative this formulation over comes all the problems over drug delivery system like conventional [195].

Ashara K. *et al.,* (2016) stated that emulgel has dual control drug delivery system from the formulation i.e., gel and emulsion. Oleaginous base in water system used for the oil loving drugs in contrast for hydrophilic drugs water in oil emulsion are used these are biofriendly and have higher spreadability. Emulgel is used to prepare for enhance the solubility of hydrophobic drugs and also gives site specific delivery. Nano emulgel is suitable for delivery of hydrophobic drugs due its globule size in nano range [196]. In 2016 Sultana S.S *et al.,* revealed that herbal emulgel prepared from *Lantana camara* leaves was found useful in the treatment of diabetic induced wound [197]. Kumar D. *et al.,* (2016) proposed various uses of emulgel. Emulgel is a novel formulation which contains two moieties like hydrophilic as well as hydrophobic. Gel and emulsion combination are now trending in the market in the form of sunscreen, analgesic creme, face creme as well as in the form of hydra-facial. HydraFacial is a patented facial in the field of cosmetics [198]. Bhura M.R.G. *et al., (*2015) stated that nano emulgel made up of adapalene found useful in reducing the side effects created by other drugs and also increase the penetration of drug. Acne treated with emulgel of adapalene has shown less skin discoloration and also maintained the equal tone of skin [199]. Khullar R. *et al.,* proposed that emulgel release the drug in sustained and dual manner in topical application, it is also a promising and very useful formulation

for delivery of both type of drugs whether lipophilic as well as hydrophilic in the year 2016 [200].

2.4 Research gap, title and objectives

2.4.1 Research gap

Green tea extract is an antioxidant and an excellent reducing agent. Preparing silver nanoparticles with green tea extract is a cost-effective, green, and environmentally friendly one-step synthesis. Vitamin D promotes wound healing and can easily be included into the oil phase of an emulgel due to its lipophilic nature. When combined with silver nanoparticles, it is thought to have a stronger therapeutic impact, due to antibacterial effect of silver. Emulgel-based topical medication delivery is needed in this work because a silver nanoparticles and Vitamin D-3 coupled emulgel is not yet available.

2.4.2 Title

Development and Evaluation of Emulgel loaded with Green Tea Extract based Silver Nanoparticles and Vitamin D for Wound Healing

2.4.3 Objective

- Synthesis of silver nanoparticles by using green tea extract
- Preparation of silver nanoparticles and Vitamin D loaded emulgel.
- Evaluation of proposed formulation.
- Stability studies as per ICH guidelines
- *In vivo* study

Chapter -3

3.1 Materials

3.1.1 Vitamin D-3(*Cholecalciferol*)

It is fat soluble vitamin and also known as cholecalciferol. IUPAC name and molecular formula of Vitamin D-3 are (3β,5Z,7E)-9,10-secocholesta-5,7,10(19)-trien-3-ol, $C_{27}H_{44}O$ respectively. It is obtained from various plant as well as animal source and used for nourishment and bone strengthening. Some researcher has also explained its wound healing potential. Vitamin D-3 is highly lipophilic in nature and soluble in various organic solvent like dimethyl sulphoxide (DMSO), methanol, dimethyl formamide (DMF), iso propyl alcohol (IPA) and ethanol. Log P as well as melting range of Vitamin D-3 is 8.80 and 83-86 °C respectively [201]. Vitamin D-3 was purchased from Loba Chemicals, Mumbai, India

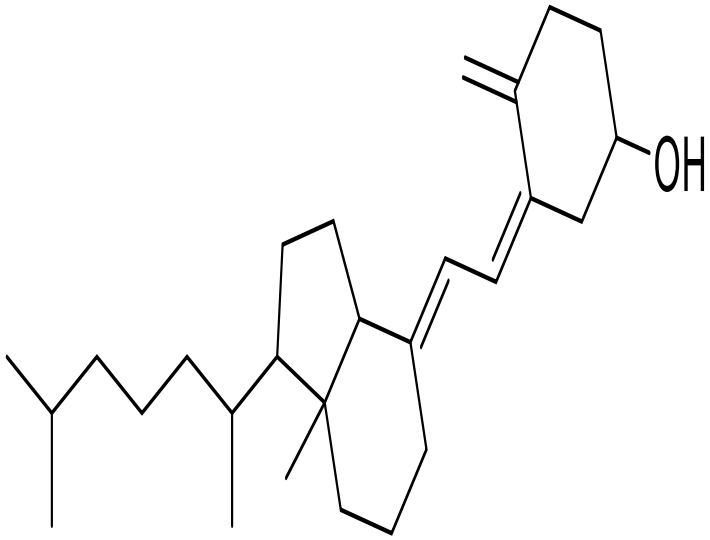


Figure 3.1 Chemical structure of Vitamin D-3

3.1.2 Silver nitrate

Its molecular formula is $AgNO_3$ and only precursor for synthesis of colloidal nanoparticles of silver. It is hydrophilic in nature and get easily dissolve in water to give white colour solution. It is used in the photographic films and staining agent in laboratory. Silver nitrate having melting point range and molecular weight 209.7±1.1°C and 169.87 respectively [202]. Silver nitrate was purchased from Loba Chemicals, Mumbai, India

3.1.3 Green tea

Catechins are polyphenolic flavonoids basically found in green tea and act as strong reducing agent. There are many types of catechin such as epicatechin (EC),

epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) are found in green tea which has an antioxidant as well as anti-inflammatory property so basically whole green tea extract can use for their medicinal value as well as green reducing agent for synthesis of variety of nanoparticles [203]. Green tea was purchased from local market of H.P.

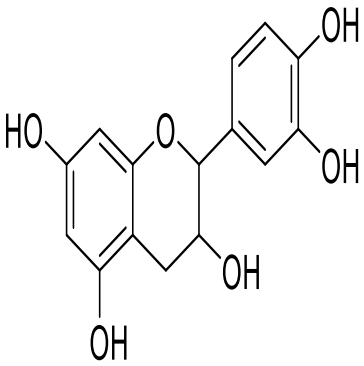


Figure 3.2 (A)

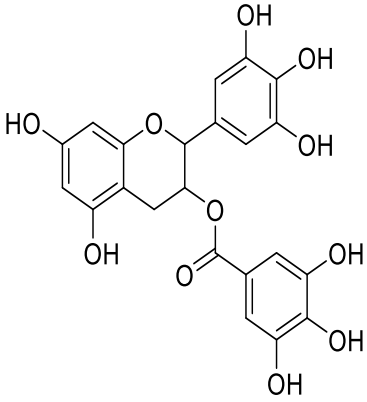


Figure 3.2 (B)

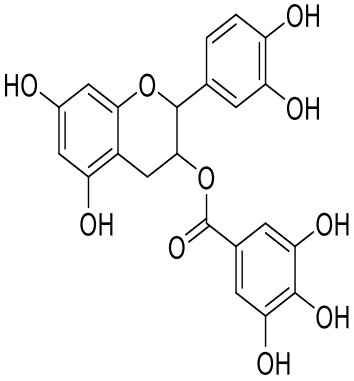


Figure 3.2(C)

Figure 3.2 (A) Epicatechin, (B) Epicatechin gallate, (C) Epigallocatechin gallate

3.1.4 Other chemicals and excipients

Methanol, ethylene glycol, dimethyl sulphoxide (DMSO), polyethylene glycol (PEG), iso propyl alcohol (IPA), carbopol 940, liquid paraffin, tween 80, span 20, propylene glycol, triethanolamine, methyl paraben, propyl paraben and glutaraldehyde were procured from in campus laboratory.

Analytical grade chemicals were used for pre formulation study, formulation development and evaluations.

3.2 Methods

3.2.1 Pre-formulation studies

3.2.1.1 Organoleptic properties

Pre formulation studies are carried out for identification of the physical and chemical properties of active drug. It includes physical appaearance like color, odour, physical state etc. [204].

3.2.1.2 Solubility determination by shake flask method

It is the determination of amout of solute (drug) get dissolved in specific amount of solvents, and a very important as well as primary parameter for formulation development, all the procedure was perforemed at 25±2 °C. Step by step procedure

for estimation of solubility by shake flask method is described here. Taken a flask and added 10 mL of the solvent. An excess amount of drug is added to the solvent. The added amount of drug should be enough to make saturated solution. Prepared solution should be kept in incubator shaker for up to 24 hrs at 37°C ±2. After 24 hrs of equilibrium, filtration method was used to separate two phases, then analysis of saturated solution was done by UV spectrophotometer at 264 nm. Data was compared with the previously reported data to ensure the solubility of drug sample [205].

3.2.1.3 Melting point determination by capillary rise method

It is the range of temperature from which any substance convert from solid to liquid. Melting point instrument, Labcare, LB-MPS8 was used for obtabinig range. All the procedure was performed at 25±2°C. Taken a glass capillary melting point tube that is sealed at one end and open at the other. Placed the capillary tube on the pile of the sample to be tested and then, invert the capillary tube and lightly tap it on the countertop for proper fixing of sample. Placed the sample containing capillary tube behind the viewfinder of a melting point device. The sample must be free from moisture for accurate results. To get the solid to fall to the closed end, inverted the capillary tube and lightly tap it the the countertop. Placed the sample containing capillary tube behind the viewfinder of a melting point device. Turned on the apparatus and adjust the setting to an appropriate heating rate.The rate of heating is frequently experimental, and it should be fine tuned by keeping a close eye on the thermometer on the equipment [206]. With the arrival of the first visible drop of liquid, recorded the first temperature of the melting range till the complete melting of Vitamin D-3.The melting point range of Vitamin D-3 was found to be 84 -85 ºC.

3.2.1.4 Standard curve preparation by UV method

Accurately weighed 10 mg of Vitamin D-3 and transferred in 100 mL of volumetric flask and then added 100 mL of 1:1 mixture of methanol and water and dissolved it to obtained a clear solution. Further taken 10 mL from above solution and diluted up to 100 mL with same solvent to prepare 10 µg/mL solution. That was known as parent solution, then further dilutions were prepared using above solution (P.S.). Taken 2 mL of P.S. diluted up to 10 mL to prepare 2 µg/mL dilution. Withdrawn 4 mL of P.S. diluted up to 10 mL to prepare 4 µg/mL dilution. Transferred 6 mL of P.S. diluted up to 20 mL to prepare 6 µg/mL dilution. Taken 8 mL of P.S. diluted up to 10 mL to prepare 8 µg/mL dilution. Firstly, scanned the sample solution to get the maximum absorption wavelength (λ_{max}) in UV spectrophotometer (Labtronics, LT-2010). Then

placed the sample in UV spectrophotometer to get absorption for different dilutions. Recorded the absorbance in triplicate [207-210]. All the procedure was performed at 25±2 °C

3.2.1.5 Analytical method development for estimation of Vitamin D-3 in formulation using HPLC

Several methods are available for estimation of Vitamin D-3 in formulations but those were not much precise, costly, time consuming and sometimes accuracy is not up to the mark. Here a novel method was developed for estimation of Vitamin D-3. This newly developed method is economical, precise and can quantify the minute concentration of Vitamin D-3 in formulation. This newly developed method is economical and found more accurate upon validation, so this method may prefer over previous methods of HPLC. In this method water and methanol were used as mobile phase. ICH guidelines for analytical method development and validation (Q2 R1) were followed throughout the procedure. Whole procedure was performed at 25±2 °C [211-213].

3.2.1.5.1 Preparation of standard solution

Taken 50 mL of volumetric flask, added 25 mg of Vitamin D-3 and 50 mL of methanol, then withdrawn 5 mL from above solution and diluted up to 50 mL. Withdrawn 1 mL from above solution and diluted up to 100 mL. Final concentration of standard solution was found 0.5 µg/mL.

3.2.1.5.2 Preparation of stock solution

25 mg of Vitamin D-3 added in 50 mL of volumetric flask and made up the volume 50 mL with methanol, then prepared various dilutions using this parent solution (P.S). Withdrawn 5 mL from stock solution and diluted up to 50 mL (P.S.). Taken 1 mL of P.S. diluted up to 200 mL to prepare 0.25 µg/mL. Transferred 1 mL of P.S. diluted up to 100 mL to prepare 0.5 µg /mL. Withdrawn 3 mL of P.S. diluted up to 200 mL to prepare 0.75 µg/mL. Taken 2 mL of P.S. diluted up to 100 mL to prepare µg/mL. Withdrawn 5 mL of P.S diluted up to 200 mL to prepare1.25 µg/mL.

3.2.1.5.3 Preparation of mobile Phase

After many trials methanol and water were selected used for as mobile phase. 3 volumes of water + 97 volumes of methanol were added in a beaker and the solution was filtered with 0.45 µ filtration assembly. Waters HPLC (L185CH361G), pore size 0.35µ and C18 column was selected with flow rate 1mL/min followed by UV detector

(264 nm). Sample injector volume was taken 50 µL with run time 15 mins. Linearity and range, accuracy, precision, robustness, limit of detection (LOD) and limit of quantification (LOQ) these parameters were used for method analytical method development and validation of Vitamin D-3 in the formulation as per guidelines of ICH.

- **Linearity and Range**: It is an important parameter of method validation. Linearity refers as it is the ability of any newly developed method which is directly proportional to the concentration of analyte to be tested at a specific concentration range, where r^2 value is nearest to 1. For linearity and range identification, calibration graph was prepared between mean area occupied by dilutions of drug and the concentration of drug. r^2 value was calculated for linearity by straight line equation [214].
- **Accuracy:** The accuracy of newly developed method is checked in the term of % drug recovery as how much amount of drug can be recovered. The accuracy of developed method was validated by % drug recovery. Limit accepted for % RSD is below 2 % [215].
- **Precision:** The precision assures that the method will produce the same result at specific circumstances, it is also categorized as inter assay as well as intra assay precision**.** The precision of developed method was evaluated by SD and % RSD (relative standard deviation) for inter-day as well intraday. Limit accepted for % RSD is below 2 % [216].
- **Robustness:** The robustness data assures that the method will produce the same result upon changing the various HPLC conditions. Robustness was evaluated for developed method in terms of change in flow rate and mobile phase ratio. Limit accepted for % RSD is below 2 % [217].
- **LOD (Limit of detection) and LOQ (Limit of quantification):** These values were determined by slope of calibration curve and standard deviation of response. LOD is the amount of drug which can detect by HPLC but not quantify and LOQ is the amount of drug which can quantify by the HPLC [218]. Formula for calculation of LOD and LOQ $LOD: 3.3\ \delta/S\ and\ LOQ: 10\frac{\delta}{S}$ respectively [219-220].

3.2.2 Optimization and development of silver nanoparticles using central composite design (CCD) in Design expert software®

To optimize the formulation of silver nanoparticles, a three-level, three-factor central composite design was employed using Design expert software®. Stable zeta potential, PDI, optimal vesicle size, and maximum

nanoparticles yield were the targets for statistical optimization [221-223]. To achieve this, three independent and three dependent variables were chosen, as stated in the central composite design described in the table

Table 3.1 Central composite design for optimization of silver nanoparticles

Factors	**Level**		
Factors (Independent variables)	**Low**	**Medium**	**High**
Conc. of green tea extract (mL)	10	20	30
Conc. of silver nitrate (M)	3	3.5	5
Stirring time (hrs.)	2	4	6
Responses (Dependent variables)		Constraints	
Particle size (nm)	Optimize		
Zeta potential (mV)	Optimize		
Percentage yield (%)	Maximize		

3.2.2.1 Procedure for synthesis of silver nanoparticles

Silver nitrate solution was prepared in different molar concentration. Green tea bag was infused in hot water for 10 mins and then removed it to prepare green tea infusion (10 mg/mL). Different volume of green tea extract infusion was added in fixed volume (10 mL) silver nitrate solution of various molar concentration and allowed the reaction mixture to stand in hours according to design of experiment suggested by Design expert software®. Milky white solution of silver nitrate turned into brown colour; it indicates that silver nanoparticles has been prepared [224-226].

3.2.3 Evaluation of silver nanoparticles

3.2.3.1 Particle size, PDI and zeta potential

The particle size of nanoparticles were measured with the instrument named zetasizer (Malvern, U.K. model ver 7.13) First, turn on the zetasizer instrument, it took 20 mins to warm up so it started before 20 mins of experiment. Next, ensured that the instrument is properly calibrated. Prepared sample according to the specific

requirements. This might involve diluting the sample to an appropriate concentration range to ensure accurate measurements. Also, made sure the sample is properly dispersed to avoid any aggregation or settling of particles during measurement. Carefully loaded the prepared sample into the appropriate cuvette. Nanoparticles suspension was kept at ultrasonic water bath for disruption of aggregates present in the suspension to be sure there are no air bubbles present in the sample. Cuvvette was placed in sample holder and made sure there should not be noise, it may create hindrence in estimation of size. 25±2°C temperature was maintaned through out the procedure. All the data was recorded in triplicates [227-229].

3.2.3.2 Shape and surface morphology of silver nanoparticles

The scanning electron microscopy (Schaefer SEE. Srl) was used to evaluate the morphological character of nanoparticles. The nanoparticles was placed directly on the scanning sample holder which was attached with

moisturized stage. Then electron beam was fired form electron gun which was passing from different interface and reached to smaple. X ray detector was attached with whole set up. Sample was emmited the secondry electron and detected by secondry electron detector, amplifies by amplifire and cretated a image of nanoparticles on desktop attached with SEM [230].

3.2.3.3 Percentage yield

Nanoparticles suspension were allowed to centrifuged (Remi R-4C) at 5000 rpm for 30 mins. After centrifugation supernatant was collected and pellet transferred into a beaker to lyophilized until dry. Then the nanoparticles yield was calculated using below mentioned formula [231].

$$Percentage\ yield: (weight\ of\ product\ /weight\ of\ precursure\ ) \times 100$$

3.2.3.4 Confirmation of synthesized silver nanoparticles using fourier transfrom infrared spectroscope (FTIR) spectra

Fourier transfrom infrared spectroscope (Perkin Elmer, version 10.6.10) was used to record the spectra. It is the spectroscopic spectra based on the wavenumber for identification of various moieties, functional groups and hydrocarbons etc, it is primary confirmatorty spectroscopic technique for further formulation development. FTIR spectra was performed for identifaction and confiration of silver nanoparticles. In this instrument, sample was placed in sample holder it can be solid, liquid or mixture of both. Detector is connented to detect the signal in form of spectra. This is completely an automatic process in which computer is allowed to analyze and identify

the peak signals. FTIR recoreded the spectra between percentage transmittance and wavelength of functional group in cm $^{-1}$ of silver nanoparticles and confirmed with previously reported data [232].

3.2.4 Preparation of oil and aqueous phase for preparation of emulsion

Oil phase was prepared adding liquid paraffin and span 20 in a beaker and heated it up to 80°C. On other hand aqueous phase was prepared using tween 80, propylene glycol (PG) and methyl paraben in water and it also heated up to 80°C separately [233].

3.2.4.1 Formulation of emulgel by incorporation gel in to emulsion

First, gel phase was formed using carbopol 940. Carbopol 940 was mixed with previously heated distilled water to yield a thick gel, then silver nanoparticles were incorporated in the gel base and pH adjusted to 5.5-6.5 using triethanolamine. Solution of the drug (Vitamin D-3) was made using methanol and added into oil phase. Methylparaben was mixed with propylene glycol and then with aqueous phase. Each phase was heated separately to 70–80 °C for 10 mins and cooled down to room temperature. The oil and water phases were combined at room temperature (25±2 °C), which indicated the emulsion formation after cooling. Emulsion along with carbopol gel (loaded with silver nanoparticles) were mixed in ratio 1:1 followed by crosslinking using glutaraldehyde. Here hot method was used for emulgel formulation [234].

3.2.4.2 Optimization of emulgel using Design expert software®

Central composite design explores relationships between multiple variables and responses. It's a design of experiments method used when optimizing processes or systems with various factors. Central composite design helps minimize experiments needed. Factors are variables examined or manipulated. Independent variables you control have levels representing different settings or values, like low, high, or in between. Factors are the adjustable variables. They are independent variables which can be manipulate. Levels indicate different

settings or conditions for each factor. Low, high, and sometimes intermediate levels exist. Factors, these can have various levels like low, high, or somewhere in between. These levels represent different settings or conditions. Centre points, are experiments done at the middle values of factors. They estimate pure error and check if the model fits properly. Factorial points are experiments at the extreme factor combinations. They allow estimation of main effects and factor interactions [235]. To optimize the emulgel formulation, statistical optimization was done using a three-level, two-factor

central composite design by Design expert software®. It led to the required pH, viscosity and controlled *in vitro* release of Vitamin D-3 from the formulation. In this research study, different formulation was prepared by use of many variables for making the said formulation and it was discovered that some independent variables produced significant results while others did not. Only two independent variables, carbopol concentration (940) and stirring time, as well as three dependent variables, % cumulative drug release, pH, and viscosity were chosen [236]. The following table shows the central composite design.

Table 3.2 Central composite design for optimization of emulgel

Factors	**Level**		
Factors (Independent variables)	**Low**	**Medium**	**High**
Conc. of carbopol 940 (%w/w)	1	1.5	2
Stirring time (mins)	10	20	30
Responses (Dependent variables)		Constraints	
% Cumulative drug release	Highest		
pH	Optimize		
Viscosity(cps)	Optimize		

Table 3.3 Various formulation of emulgel contains different amount of carbopol along with stirring time suggested by Design expert software®

Run	**Factor 1**	**Factor 2**
	Conc. of carbopol (%w/w)	**Stirring time (mins)**
F1	2	30
F2	1.5	20
F3	1.5	20
F4	0.7	20
F5	1	30
F6	2	10
F7	1.5	20
F8	1.5	20
F9	1.5	20
F10	1.5	34
F11	1	10

F12	1.5	5.8
F13	2.2	20

3.2.5 Evaluation of emulgel

3.2.5.1 Physical evaluation of emulgel

Formulation was checked physically for its appearance, color, texture, phase separation and homogeneity [237].

3.2.5.2 Drug content

1 gm of formulation was taken and transferred into a 100 mL beaker containing buffer solution (50 ml) simulated with skin pH (5.5), allowed it to stirred on magnetic stirrer. Then 5 mL of sample was withdrawn using pipette and checked the absorbance at 264 nm using UV spectrophotometer. All the data were taken in triplicate and calculated with the help of previously prepared calibration curve. [238]**.**

3.2.5.3 pH determination

Digital pH meter (Systonic, S-901) was used to record pH. Made sure to calibrate pH meter properly with pH 4.0 and pH 9.0 buffer solutions. Taken a small amount of emulgel sample, about 1 gram. Used a spatula or pipette to transfer it into a clean container. Added some distilled water to the sample. Mixed well until it's homogenous. Let the formulation reach room temperature, if it was stored differently. Rinsed the pH electrode with distilled water gently. Blot it with a clean tissue to remove extra water. Immersed the pH electrode into the sample fully. Made sure that the electrode tip is submerged and not touching the container's bottom. Let the pH reading settled down. It may take a couple seconds or up to a minute, depending how steady the formulation is. Noted down what the pH meter reading. All the readings were taken in triplicate and maintained room temperature (25±2 °C) thorough out the procedure [239].

3.2.5.4 Viscosity

Digital viscometer (Labtronics-LT 730) was used to determine viscosity of formulation. Viscosity of optimized emulgel was checked at room temperature (25±2 °C) with spindle no. 6 at 100 rpm**.** All the readings were taken in triplicate [240].

3.2.5.5 Spreadability

Accurately weighed 1 gm of emulgel and placed it on the glass plate and marked the diameter of 1cm with marker. Another glass plate of the same size was placed and 100 gm of weight was applied over it [241]. After waiting for 10 minutes when there

is not further spreading between glass plate spreading area of circle was calculated using standard formula.

$$S = \frac{ml}{t}$$

Where: S is spreadability, m is the weight of the upper plate and rested on it, l is the diameter of the spreading emulgel (cm), and t is the time taken (min)

3.2.5.6 *In vitro* cumulative drug release study

The estimation of *in vitro* cumulative drug release of Vitamin D-3 from emulgel was done using a Franz diffusion cell that had dialysis membrane with a diameter of 1.5 cm and volume 60 mL. Preparation of the phosphate buffer was done with different salts which was used to fill receptor compartment simulating pH of the skin with magnetic bead having 5.5 pH. In donar compartment of franz diffusion assembly, 100 mg of emulgel was put.The buffer was kept stirred at definite time of interval with magnetic stirrer at 50 rpm and maintained the temperature at 37 ºC ±0.5, simulated with skin physiology, and inlet outlet water supply was maintained to keep the temperature constant. 5 mL was withdrawn using pipette through the receptor compartment at specific time intervals up to 12 hrs. and 5 mL fluid was replaced with phosphate buffer after every sample withdrawal to maintain the sink condition. Samples were analyzed using UV visible spectrophotometer (Labtronics, Model LT-2010) at 264 nm in triplicates. Calculated the drug release from the formulation using pre prepared calibration curve [242].

3.2.5.7 Release kinetics study

In the present study various pharmacokinetics mdels was used to determine the drug release pattern of formulation and plotted the graph between time and % cumulative drug release to determine the straight line equation as well as R^2 value. Drug release kinetics were calculated by puttiing the obtained data in zero order, first order, Korsemeyer and Peppas model as well in Higuchi's model equation to get the best fit model of drug release from emulgel. Lets explain about various kinetics model.

- **Zero order kinetics**

This model is used to determine the type of formulation either it is concentration dependent or not. Zero order model defines concentration independent release of drug from formulation. It is obeyed by controlled release formulation. To plot zero order kinetics graph, on x axis took time and on y axis took % cumulative release of drug [243].

- **First order kinetics**

This model is also used to determine the type of formulation either it is concentration dependent or not. First order model defines concentration dependent release of drug from formulation. It is obeyed by sustained release formulation. To plot first order kinetics graph, on x axis took time and on y axis took log % cumulative release of drug [244].

$$\log C = \log C_{\circ} - Kt / 2.303$$

where $C_{\circ}$ is the initial concentration of drug, k is the first order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time.

- **Higuchi model**

A Higuchi plot is one of the graphical presentations that has been developed to study the release kinetics of a drug from the dosage forms knowingly in those matrices or films which exhibit release controlled by diffusional process. Higuchi's model consents that the release of a drug from a dosage from occurs by diffusion which takes place through an open planar surface of the matrix. According to the Higuchi equation, the cumulative amount of drug released (Q) is proportional to the square root of time (t). According to the Higuchi equation, the cumulative amount of drug released (Q) is proportional to the square root of time (t) [245].

$$Q = kt1/2$$

Q is the amount of drug release at time t, Kt is the Higuchi's release rate constant

- **Korsmeyer-Peppas model**

Korsmeyer *et al.,* (1983) derived a simple relationship between the polymeric system and drug. This equation easily describes the drug release from membrane of polymer.

$$Mt / M\infty = Ktn$$

where Mt / M∞ is a ratio of drug release at time t, k is the release rate constant and n is the release exponent. The n value is describing the release of drug from cylindrical shape structures [246].

3.2.5.8 Physical accelerated stability study along with, drug content, pH and % *in vitro* cumulative drug release

Checking a semi solid product's quality over time is crucial. Creams, gels, lotions, or ointments must undergo stability studies to ensure they remain safe and effective. Choose the key features and traits to monitor during these studies. Physical aspects

like- colour, smell, and texture are important, as well as chemical makeup (e.g. active ingredient levels, pH) and microbial growth prevention. Picked appropriate storage conditions mimicking real world scenarios. Common options: room temperature (around 25°C), accelerated testing (40 ±2 °C with 75% humidity), and refrigeration (between 2-8°C). If the product targets different climates, test stability under those conditions too**.** Figure out how many months you want the stability test to last. This could be different for some products. Some common lengths are 3 months, 6 months, 12 months, or even longer. Make a plan for taking samples at set times during the test. The samples need to represent the whole batch.

Look at things like colour, smell, and texture. Also check active ingredients, pH levels, *in vitro* drug release and most important spreadability. Use the right tests for your product. Here ICH Q1 R2 guidelines was followed for accelerated stability testing of optimized formulation**.** Formulation was kept in suitable container in stability chamber (NSW-75, Model HCS-2) for 6 months at 40±2°C temperature and 75±5% RH. Every month formulation was checked for drug content, pH, % cumulative drug release, and physical evaluation like phase separation, creaming and viscosity [247].

3.2.5.9 Het CAM assay for irritation testing

A HET-CAM assay can be used to determine the toxicity or irritation of formulation in place of Draize test (Rabbit eye test), it is economical and quick test. In this test fertilized egg were purchased from a poultry farm, kept these eggs in an incubator at 37°C for proper incubation insuring that air sac is upright. Using sterile method albumin of the egg was removed out from th egg at 3rd day of experiment and closed the egg opening by using parafilm. Allowed the development of the chorioallantoic memebrane(CAM). At 10th day when the membrane developed completely, made an casement of 2*2 cm^2 and 50 mg of emulgel inserted over the CAM , after 20 sec rinsed the membrane with 5ml of warm saline. Examined the membrane vascular drainage, haemorrhagic damge and coagulation for upto 300 sec. After getting all the data placed in below mentioned formula and calculated the score. Then evaluated the score from standard data and revealed that either formulion is irritant or not [248].

$$\boldsymbol{Scores} = [\mathbf{301} - \boldsymbol{H}/\mathbf{300}] * \mathbf{5} + [\mathbf{301} - \boldsymbol{L}/\mathbf{300}] * \mathbf{7} + [\mathbf{301} - \boldsymbol{C}/\mathbf{300}] * \mathbf{9}$$

Where H= Haemorrhage (Sec), L=Lysis Time(Sec), C= Coagulation Time(Sec)

Table 3.4 Scoring chart for Het CAM test

Score for toxicity	Level of irritation
0-0.9	Not irritant
1-4.9	Slightly irritant
5-8.9	Moderate irritant
>9	Strong irritant

3.2.6 *In vivo* excision wound model

There are variety of animal model are used to evaluate the formulation *in vivo*. These models help to evaluate the doses, side effects adverse effects of newly developed drugs. These models are also helpful to learn about biological processes and create cures for diseases. These are an important part of research. Skin is the largest body organ and plays a vital role in against stimulant like heat, touch cold etc. when the skin got damaged from any reason it becomes red, irritates, nutritional loss, burning sensation and also converts in the wound if left untreated. Researchers use animal wound healing models to study tissue repair in living things. These models help understand how wounds heal. They also help to develop new treatments. In this study excision wound model was used. Creating a wound by cutting out skin is called an excisional wound model. It lets scientists study swelling, tissue changes, and scars during healing. Excisional wounds can be made on rodents like mice and rats. The model is simple yet useful for research. Sometimes these wounds are can be created accidently like burn or some time intentionally like surgery. Wound healing is a repair mechanism of body regulated by several cells and it consist of three phases like inflammation, proliferation and remodeling [249].

3.2.6.1 Preparation of animals before experiment

The animals were housed in an animal house with controlled temperature and humidity levels of 26± 2 °C and 44–56%, respectively. The animals were subjected to cycles of light and dark, lasting 10 hrs. and 14 hrs., respectively, and a specified atmospheric condition was maintained for seven days prior to and during the experiments. The animals were housed in large, hygienic cages and fed a standard rodent pellet diet. Throughout the experiment, all of the animals were given unrestricted access to their cages to obtain food and drink. Throughout the experiment,

the necessary diet and ventilation were maintained to ensure adequate nutrition requirements [250]. The Pinnacle Biomedical Research Institute in Indore conducted this animal study in accordance with protocol no. PBRI/IAEC/PN-2304. Eight groups in total, with six animals in each group, were chosen for the experiment; the table below details each group.

Table 3.5 Groups of animals as per dose of formulations

Groups	Route	Dose of Formulation (per gram of emulgel)	%w/w (per 100 gm of emulgel)
I. Normal Control	--	--	--
II. Experimental control	No treatment	No treatment	--
III. Blank emulgel	Topical	1 gm	--
IV. Marketed Silver sulphadiazine (silvex)	Topical	20 mg/g	1%w/w
V. ESVDL	Topical	300 IU=0.75µg/g	0.00075 % w/w
VI.ESVDH	Topical	2500 IU=62.5 µg/g	0.0062 % w/w
VII. Emulgel with SNPs Low dose	Topical	20 µg/g	0.002 % w/w
VIII. Emulgel with SNPs High dose	Topical	10 µg/g	0.001 % w/w

*ESVDH (emulgel with high dose of Vitamin D-3and silver nanoparticles, ESVDL (emulgel with low dose of Vitamin D-3 and silver nanoparticles), SNPs (Silver nanoparticles)

3.2.6.2 Procedure for creation of excision wound

Ketamine was administered to the animals to make them anaesthetized in the dose of 30 mg/kg using intravenous route and then the animals were observed to be unconscious. Firstly, applied the epilator on the skin to shaven the back dorsal thoracic of the rat then put a conventional ring, on the rat back area measuring 200 mm^2 and depth 1 mm to create a mark on the rat's skin. In relation to the wound and the area around this wound, there was checking for sign of bacterial infection including the sign of inflammation or any other sign of an infection, and if there was, the infected rat was changed with a healthy one. In this study, the marketed as well as test formulation of various concentration was used to treat the wound of the particular group animal up to 16 days using certain dose. Accompanying the marker, the area of the wound was outlined on the transparent sheet in the day that the wound was created and also in the 4^{th} day, 8^{th} day, 12^{th} day, and 16^{th} day of this experiment. Evaluation

of the reduction of size was made by definite interval, and calculated % wound contraction using standard formula [252].

$$\%\,\boldsymbol{Wound\ contraction} = \frac{Wound\ \ area\ on\ initial\ day - Wound\ area\ \ on\ test\ day * 100}{Wound\ Area\ on\ Initial\ day}$$

- **Epithelialization period**

It was evaluated by noticing the number of days required for the covering of wound surface with epithelial tissue.

3.2.6.3 Histopathology

Skin samples were taken from animals that had been sacrificed and stored in formalin solution (10%) for the purpose of histopathology. The skin tissues were cleaned with distilled water, the water content was eliminated using various grades of alcohol, toluene was added to completely remove the tissue, and the tissues were then immersed in melted paraffin wax for a predetermined amount of time. Freshly melted paraffin wax was used to embed processed tissues, which were then left to solidify. To see the general features of tissue structure, tissue microsections were cut, dried on a hot plate for 15 mins, and then stained with 1% aqueous eosin and hematoxylin. Stained tissue was examined under a microscope with 10X magnification [251].

3.2.6.4 Statistical analysis of excision wound model

Analysis of the data were carried out using statistical software, Prism (version 10. 1. 0). All the *in vivo* related data has undergone statistical analysis using one way ANOVA followed by Dunnet post- test. $P < 0.01$ has been considered as the level of significance or lesser for the purpose of grouping the data sets. [252].

Chapter -4

4. Pre-formulation studies

4.1 Physical state of Vitamin D-3: It is also known as cholecalciferol and found in solid crystals.

4.2 Color: It exist in white to off -white in color.

4.3 Texture: These are fine crystals.

4.4 Odor: It is odourless compound.

4.5 Melting point

Capillary rise method was used to determine melting point of Vitamin D-3 using melting point apparatus and it was found in the range of 84-85 ºC.

4.6 Solubility

Solubility of Vitamin D-3 was determined using shake flask method. It was revealed that Vitamin D-3 was insoluble in water, slightly soluble in ethylene glycol, dimethyl sulphoxide (DMSO), propylene glycol, polyethylene glycol (PEG) and soluble in methanol and IPA [253].

Table 4.1 Solubility profile of Vitamin D-3

Name of the solvent	Amount (mg/ml)	Solubility expression as USP
Water	<0.1	Insoluble(<0.1/mL)
Ethylene glycol	8.41±1.1	Slightly soluble (1-10 mg/mL)
DMSO	31.5±1.2	Sparingly soluble (10-33 mg/mL)
Propylene Glycol	32.9±0.9	Sparingly soluble (10-33 mg/mL)
PEG 400	31.62±1.3	Sparingly soluble (10-33 mg/mL)
Methanol	97.5±1.2	Soluble (33-100 mg /mL)
IPA	95.5±1.6	Soluble (33-100 mg/mL)

Values are expressed as n=3, mean ±SD

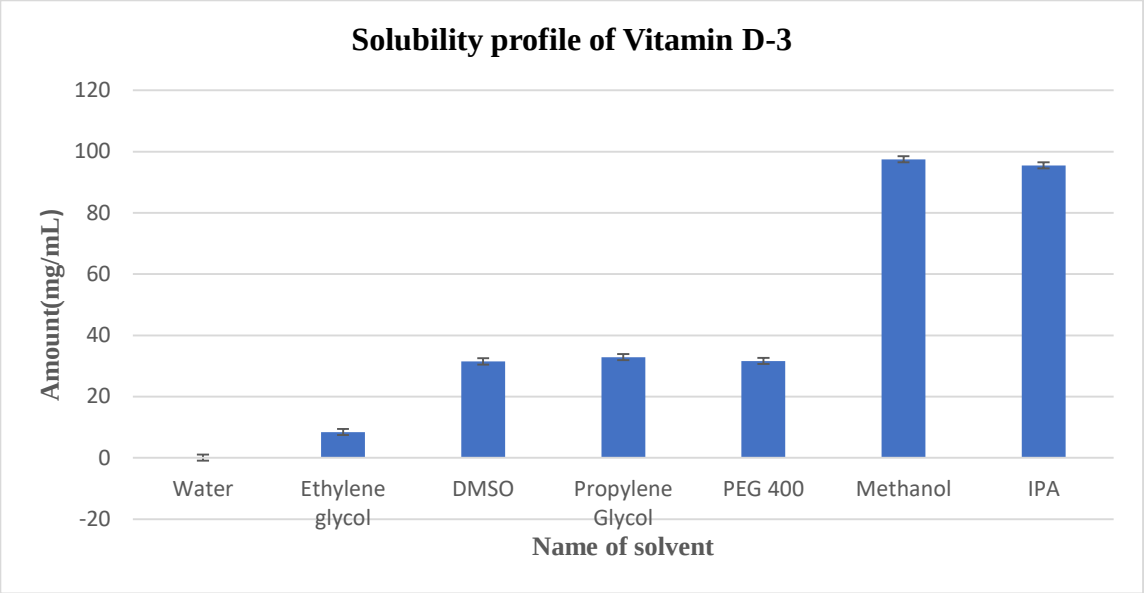


Figure 4.1 Solubility graph of Vitamin D-3 in different solvents

4.7 Standard curve of Vitamin D-3 by UV method

λ_{max} of Vitamin D-3 was found to be 264 nm. Standard curve of Vitamin D-3 was prepared using various dilutions and it was found linear from the range 2-10 µg/mL. R^2 value was observed 0.9991, this indicates that graph is linear.

Table 4.2 Standard curve data of Vitamin D-3 using UV method

Sr. No.	Conc.	Abs.	Abs.	Abs.	Avg.	S.D.
1	2 µg/mL	0.044	0.046	0.048	0.046	0.001
2	4 µg/mL	0.092	0.091	0.093	0.092	0.001
3	6 µg/mL	0.133	0.138	0.135	0.135	0.001
4	8 µg/mL	0.187	0.190	0.192	0.190	0.001
5	10 µg/mL	0.233	0.235	0.231	0.233	0.002

Values are expressed as n=3, mean ±SD

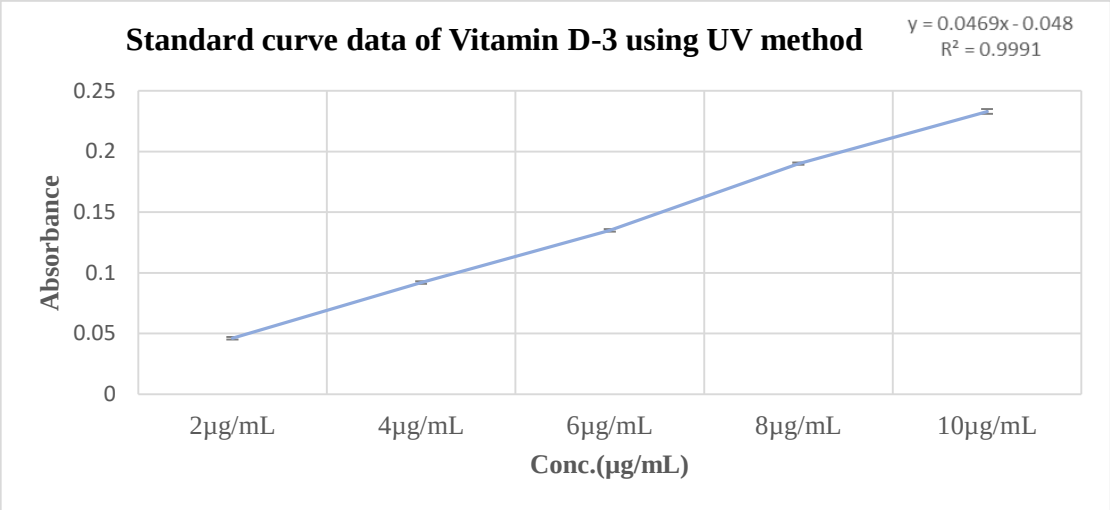


Figure 4.2 Standard curve of Vitamin D-3 using UV method

4.8 Analytical method validation of Vitamin D-3 by HPLC

Method was developed according to the ICH Q2 R1 guidelines of method validation.

4.8.1 Selection of mobile Phase

Several trials were performed for detection of suitable mobile phase for example methanol, methanol and water in several ratios, DMSO and phosphate buffer. Then according to parameter like peak sharpness, retention time, and resolution methanol and water in 97:3 ratio was selected as mobile phase for method validation of Vitamin D-3. In different trials it was found that peak was not sharp, somewhere HPLC was giving no peak but after several trials chromatogram of Vitamin D-3 was found sharp when methanol and water was used as mobile phase on this basis methanol and water in 97:3 ratio was selected as mobile phase for best result.

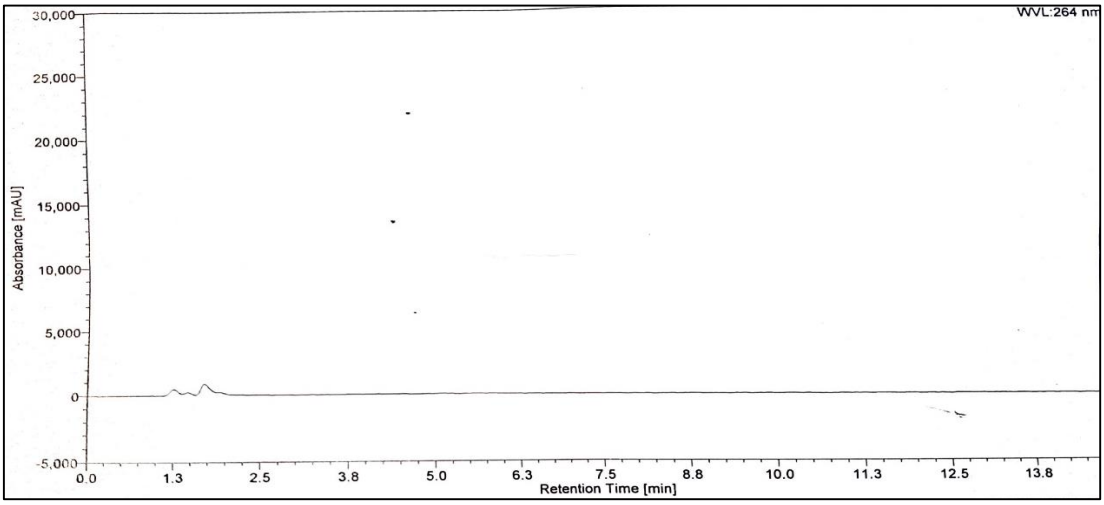


Figure 4.3 Chromatogram having no peak (Blank injection)

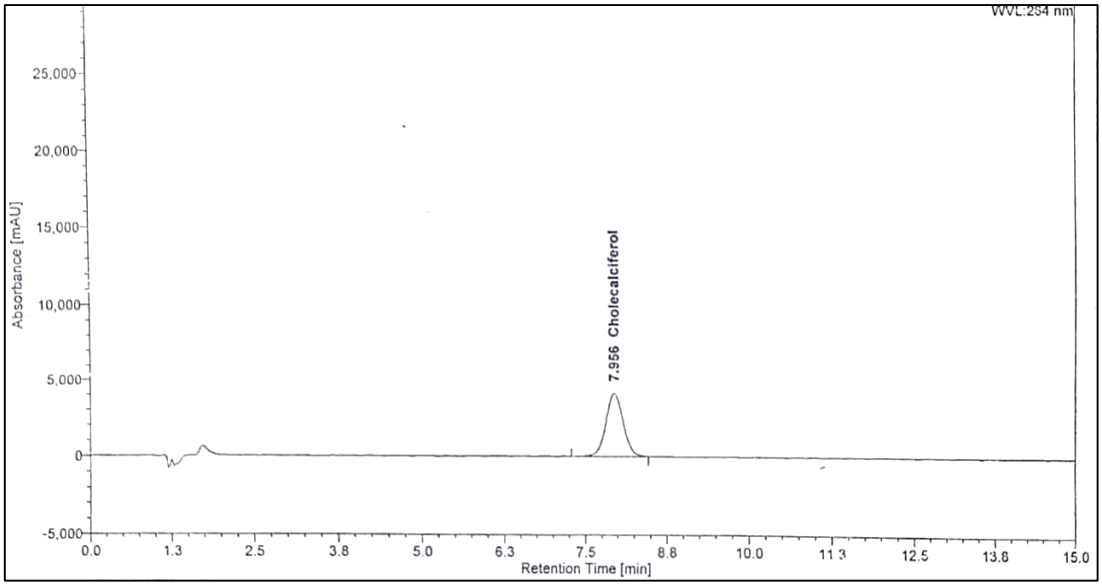


Figure 4.4 Chromatogram showing Vitamin D-3 peak

4.8.2 Preparation of stock solution and standard curve

Waters HPLC model no L185CH361G having pore size 0.35µ with flow rate 1mL/min was used. Octadecylsilane column was selected for validation. 50µL sample was injected with flow rate 1mL/min, with run time of 15 minutes. Atmospheric condition was suitable for method validation of Vitamin D-3. 25 mg of Vitamin D-3 was added in 50 ml of volumetric flask and made up the 50 ml volume with methanol, then different dilutions like 0.25, 0.5,0.75,1.0 and 1.25 µg/mL were prepared using methanol and HPLC grade water was used as mobile phase. Maximum linearity was obtained in the range of dilution from 0.25-1.25 µg/mL and graph was plotted between area obtained in HPLC and concentration of sample to get straight line equation. All the data were taken in triplicates.

4.8.3 Linearity and range

After several trial standard curve was prepared with 0.25, 0.5,0. 0.75, 1.0 and 1.25µg/mL concentration against the area calculated by HPLC of Vitamin D-3. These concentrations were showing maximum linearity and R^2

value was also found 0.999, which is approximately near to 1, due to good linearity these dilutions were selected for further analysis. 0.75µg/mL was selected as MQC (Medium quality Control) according to ICH guidelines another two dilutions were prepared i.e., 80% of Medium Quality Control having 0.6µg/mL (Low Quality Control) and 120% of MCQ having 9µg/mL (High Quality Control). Data of standard curve is mentioned below

Table 4.3 Standard curve data of Vitamin D-3 using HPLC method

Conc.(µg/mL)	Peak area			Avg.	SD
0	0	0	0	0	0
0.25	38102	38107	38109	38104.66	3.60
0.5	76205	76208	76211	76208	3
0.75	114305	114301	114299	114301.7	3.05
1	156300	156305	156295	156300	5
1.25	190508	190509	190503	190505.7	3.21

Values are expressed as n=3, mean ± SD

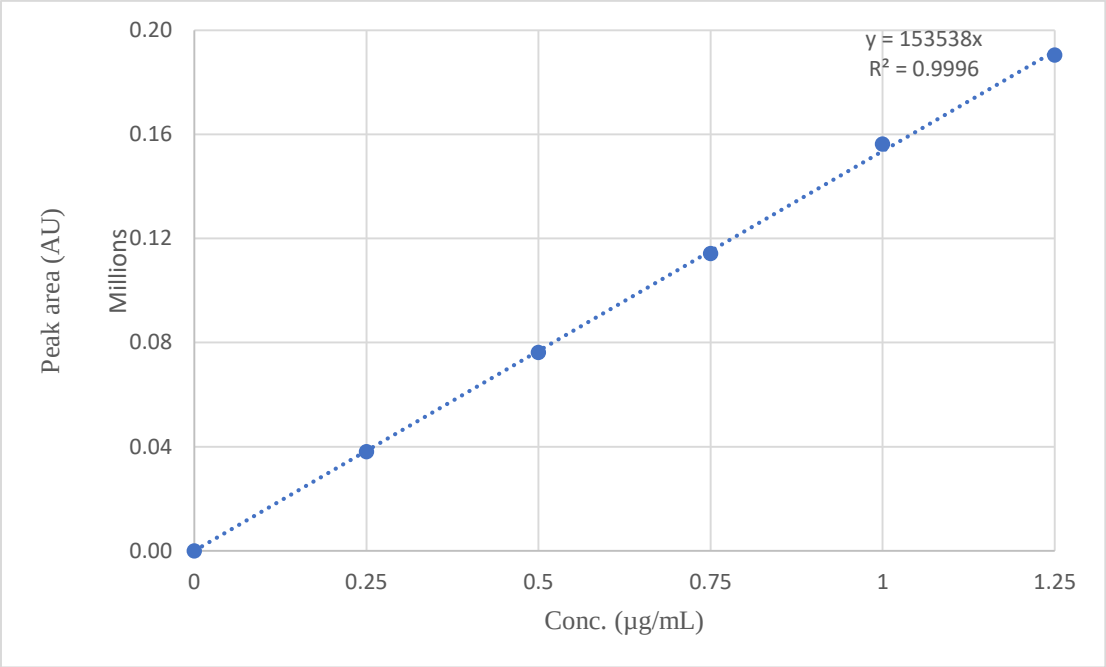


Figure 4.5 Standard curve of Vitamin D-3 using HPLC

4.8.4 Accuracy

The accuracy of developed method was checked by in terms of percentage drug recovery followed by relative standard deviation for detection of these parameters all three quality control standards i.e., LQC, MQC and HQC were injected three times in HPLC and data was recorded. Limit accepted for percentage drug recovery is 95-105% as well as % RSD must be below 2%. Method was verified by evaluation these

data; all the readings were found in accepted range as well as below 2% RSD. All data is given below in table

Table 4.4 Repeatability data for MQC

Repeatability data for MQC					
Conc. µg/mL	**Peak area**			**Avg.**	**SD**
0.75	114307	114306	114305	114306	0.81

Values are expressed as n=3, mean ±SD

Table 4.5 Accuracy data

Conc. µg/mL	**Area**			**Mean**	**Recovered conc. µg/mL**			**Mean**	**SD**	**% RSD**	**% Drug recovery**
0.6	91445	91443	91449	91445.67	0.592	0.591	0.595	0.592	0.002	0.337	98.66
0.75	114301	114305	114307	114303.3	0.747	0.749	0.748	0.748	0.0005	0.066	99.73
0.9	137161	137165	137160	137160	0.896	0.897	0.892	0.895	0.002	0.223	99.44

Values are expressed as n=3, mean ±SD

Table 4.6 Precision data (Intra-day)

Intra-day precision data								
Conc. µg/mL	**Peak area**					**Mean**	**SD**	**% RSD**
0.6	91442	91441	91449	91439	91446	91443.4	3.61	0.004
0.75	114306	114309	114300	114308	114311	114306.8	3.76	0.003
0.9	137155	137162	137159	137154	137158	137158	2.87	0.002

Values are expressed as n=5, mean ±SD

Table 4.7 Precision data (Inter-day)

No. of days/ analyst	Inter-day precision								
	Conc. µg/mL	Peak area					Mean	SD	% RSD
Day 1	0.6	91443	91442	91439	91432	91443	91439.8	4.16	0.004
	0.75	114309	114307	114299	114301	114298	114302.8	4.41	0.003
	0.9	137154	137160	137165	137162	137159	137160	3.63	0.002
Day 2	0.6	91439	91443	91432	91431	91443	91437.6	5.22	0.005
	0.75	114302	114306	114300	114305	114299	114302.4	2.72	0.002
	0.9	137166	137160	137164	137162	137158	137162	2.82	0.002
Day 3	0.6	91442	91440	91430	91433	91442	91437.4	4.96	0.005
	0.75	114308	114308	114298	114306	114299	114303.8	4.44	0.003
	0.9	137166	137169	137164	137168	137160	137165.4	3.25	0.002
Analyst 1	0.6	91441	91443	91439	91432	91443	91439.6	4.07	0.004
	0.75	114304	114301	114299	114306	114298	114301.6	3.00	0.002
	0.9	137154	137160	137165	137162	137159	137160	3.63	0.002
Analyst 2	0.6	91445	91441	91432	91435	91440	91438.6	4.58	0.005
	0.75	114307	114305	114297	114302	114299	114302	3.68	0.003
	0.9	137152	137162	137164	137161	137150	137157.8	5.67	0.004

Values are expressed as n=5, mean ±SD

4.8.5 Robustness

Robustness is evaluated in terms of change in flow rate as well as change in ratio of mobile Phase and percentage relative standard deviation was calculated, all the data were in specified limit and justified the validation.

Table 4.8 Robustness data by change in flow rate

Flow rate	Conc. µg/mL	Retention time			Mean	SD	%RSD
0.8	0.75	8.1	8.2	8.1	8.13	0.05	0.701
1	0.75	7.92	7.95	7.9	7.94	0.02	0.262
1.2	0.75	7.12	7.16	7.22	7.16	0.05	0.702

Values are expressed as n=3, mean ±SD

Table 4.9 Effect on retention time by changing mobile phase ratio

Retention time							
Mobile phase	**Conc. µg/mL**	**I**	**II**	**III**	**Mean**	**SD**	**% RSD**
95:5	0.75	8.12	8.2	8.1	8.14	0.05	0.650
97:3	0.75	7.92	7.95	7.9	7.94	0.02	0.262

Values are expressed as n=3, mean ±SD

Table 4.10 Effect on peak area by changing mobile phase ratio

Mobile Phase	**Conc. µg/mL**	**Peak Area**					**SD**	**Mean**	**% RSD**
95:5	0.75	76267	76144	76243	76157	76155	57.2643	76193.2	0.075
97:3	0.75	76167	76188	76175	76167	76177	8.671793	76174.8	0.0113

Values are expressed as n=5, mean ±SD

4.8.6 System suitability

At its time of use, system suitability involves the performance measures for a method mainly it indicates that the method has been established and executed properly. That the method, as set up, can perform at the same level it did during its qualification. For the system suitability, tailing factor and theoretical plate parameters were estimated and it was found that developed method is suitable for validation.

Table 4.11 System suitability parameters (254)

Parameters	**Value**	**Reference value**
Theoretical plate	4362	>2000
Tailing factor	1.45	>1 but below 2 (1-1.5 is highly acceptable)

4.8.7 Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ was calculated as per standard formula. It is indicated as signal to noise ratio**.** LOD and LOQ value of Vitamin D-3 was found to be 0.01 µg/mL and 0.05 µg/mL respectively. LOD is the amount of drug which can easily detected by detectors but not quantify. LOQ is defined as amount of drug which can be quantified by the UV detectors in HPLC along with all the concentration which are above from this value can quantify easily.

4.9 Green tea extract

Table 4.11 (a) Organoleptic properties of green tea

Sr. no.	Organoleptic properties	Description
1	Color	Greenish brown
2	Odor	Characteristic
3	Texture	Coarse powder

4.10 Silver Nitrate

Table 4.11 (a) Organoleptic properties of silver nitrate

Sr. no.	Organoleptic properties	Description
1	Color	White
2	Odor	No odor
3	Texture	Crystalline powder

4.10.1 Central composite design (CCD) for optimizing silver nanoparticles

Optimization of silver nanoparticles was done using three factor and three level central composite design. It was done using Design expert software® and also utilized for statistical data analysis of silver nanoparticles formulation. The statistical optimization was performed to achieve stable zeta potential, PDI, desired vesicle size and highest yield of nanoparticles. Various nanoparticles standard was suggested by software and data is mentioned below in table:

Table 4.12 Particle size, zeta potential and percentage yield data of various silver nanoparticles

Run	Conc. of Silver nitrate	Reaction time (hrs.)	Conc. of green tea extract	Size (nm)	Zeta potential (mV)	Yield (%)
SNPs 1	3.5	4	32.5	122±4.3	-8.46±0.8	52.92±3.9
SNPs 2	3.5	4	32.5	122±4.3	-8.46±0.8	52.92±3.9
SNPs 3	3.5	0.6	32.5	230±7.3	-6.9±0.4	51.76±3.5
SNPs 4	5	6	50	87±2.1	-11.85±1.0	72.56±4.8
SNPs 5	3.5	4	3.0	125±4.7	-8.44±0.7	45.23±3.6
SNPs 6	0.9	4	32.5	86±2.2	-16.4±1.2	65.89±4.5
SNPs 7	5	2	15	120±3.9	-14.4±1.0	41.32±2.3
SNPs 8	2	2	15	90±2.7	-11±0.9	41.37±2.3
SNPs 9	3.5	4	32.5	122±4.3	-8.46±0.8	52.92±3.9
SNPs 10	*3.5*	*4*	*32.5*	122±4.3	-8.46±0.8	52.92±3.9
SNPs 11	5	2	50	102±3.2	-10.3±1.0	69.31±4.4
SNPs 12	2	6	50	78±1.9	-13.1±1.1	72.67±4.7
SNPs 13	2	6	15	90±2.7	-12±0.9	49.32±3.1
SNPs 14	3.5	4	32.5	122±4.3	-8.46±0.8	52.92±3.9
SNPs 15	3.5	4	61.9	60±1.7	-16.2±1.2	76.81±5.2
SNPs 16	3.5	7.3	32.5	123±3.7	-8.9±0.8	52.92±3.9
SNPs 17	2	2	50	125±3.9	-7.6±0.5	72.51±4.9
SNPs 18	5	6	15	101±3.1	-8.23±0.7	41.76±2.3
SNPs 19	3.5	4	32.5	122±4.3	-8.46±0.8	52.9±3.9
SNPs 20	6.0	4	32.5	98±2.7	-8.3±0.7	72.31±4.8

Values are expressed as n=3, mean ± SD

4.10.2 Optimization of silver nanoparticles using Design Expert® software

4.10.2.1 Effect of independent variables on particle size and zeta potential

One way ANOVA was used for statistical analysis of particle size and zeta potential of silver nanoparticles. Suggested nanoparticles have shown particle size and zeta potential of silver in the range of 60±1.7-122±4.3 nm and -6.4±0.4 -16.4±1.2 mv respectively. Particle size, zeta potential and PDI of optimized nanoparticles was

found to be 99.45±1.1 nm, -12.6±0.9 mV and 0.173±0.002 respectively. It was observed that silver nitrate and green tea extract was creating a significance change in particle size and zeta potential and revealed that higher the concentration of green tea extract and silver nanoparticles produced smaller the size of nanoparticles simultaneously zeta potential was also increased. P value was found 0.0159 and 0.0336 for particle size and zeta potential respectively which is less than 0.0500 indicates model are significant.

Final Equation in Terms of Coded Factors for particle size

+122.69+3.45A-19.11B-8.57C+1.62AB-6.88AC-5.38BC-5.09A2+14.79B214.78C2

Final Equation in Terms of Coded Factors for zeta potential

+8.47+0.6352A-0.3840B-0.7521C+1.39AB-0.2275AC-1.53BC-1.71A^2+0.2716B^2 -1.29C^2

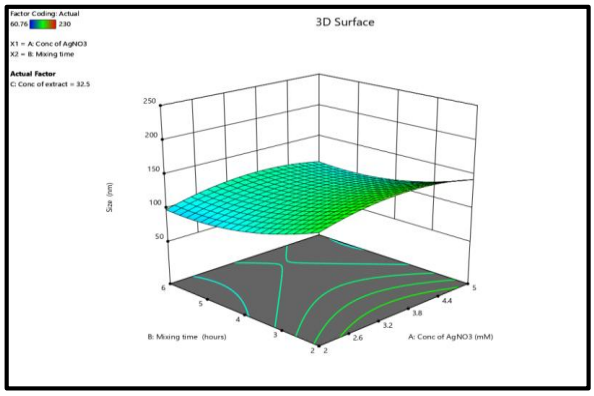


Figure 4.6 (A)

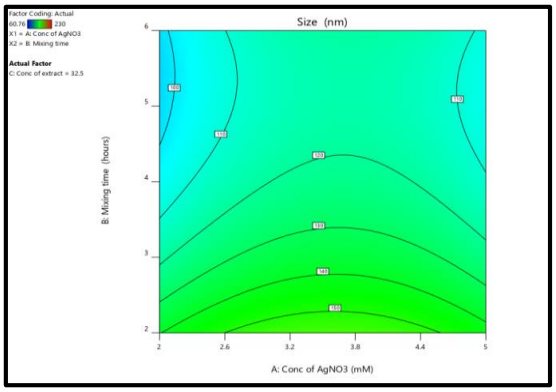


Figure 4.6 (B)

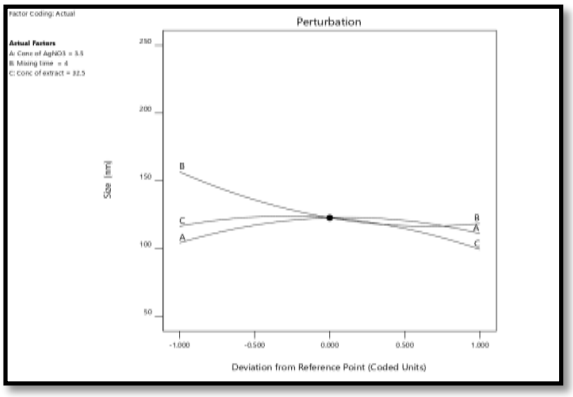
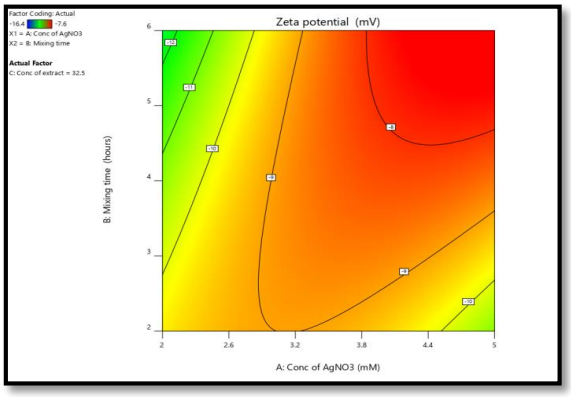


Figure 4.6 (C)



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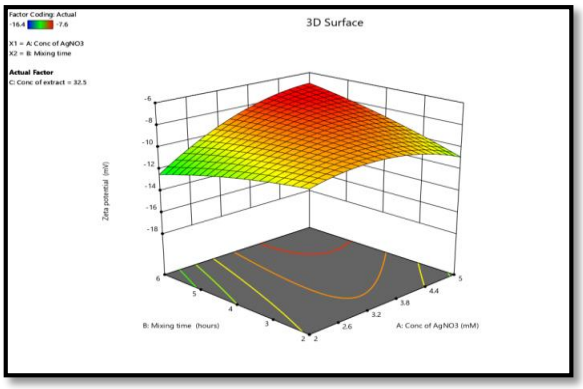

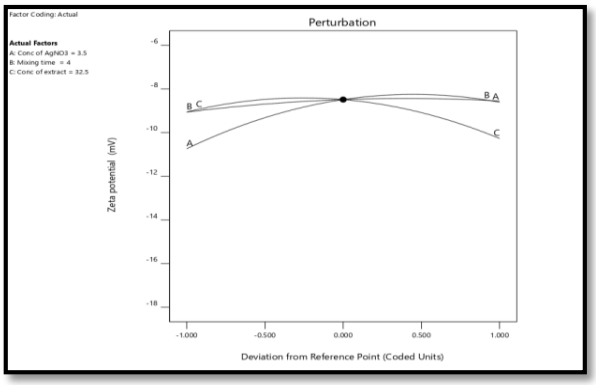

Figure 4.6 (E) **Figure 4.6** (F)

Figure: 4.6 (A) 3-dimensional response surface plot showing effect of conc. of extract and mixing time on particle size **(B)** 2-dimensional surface plot, effect of conc. of extract and mixing time on particle size **(C)** perturbation plot for showing effect on particle size all by all three independent variables. **(D)** 3-dimensional response surface plot showing effect of conc. of extract and mixing time on zeta potential **(E)** 2-dimensional surface plot showing effect of conc. of extract and mixing time on zeta potential **(F)** perturbation plot for showing effect on zeta potential by all three independent variables.

4.10.2.2 Effect of independent variables on percentage yield

The data was estimated by one way ANOVA. Percentage yield of nanoparticles was found in the range of 41.67±2.3 to 76.81±5.2 %. It was also predicted that upon increasing the concentration of green tea extract and silver nitrate solution, the percentage yield of nanoparticles has also increased. Percentage Yield of optimized nanoparticles was found to be 72.1±2.1%. P-values was found < 0.0001 which is less than 0.0500 indicate model is significant.

Final Equation in Terms of Coded Factors

$+53.04-0.0090A+1.01B+12.18C-0.5525AB+0.5375AC-0.6625BC+4.94A^2-0.985B^2+2.08C^2$

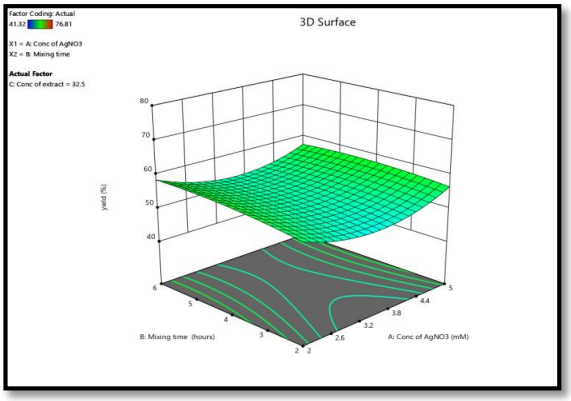

Figure 4.7 (A)

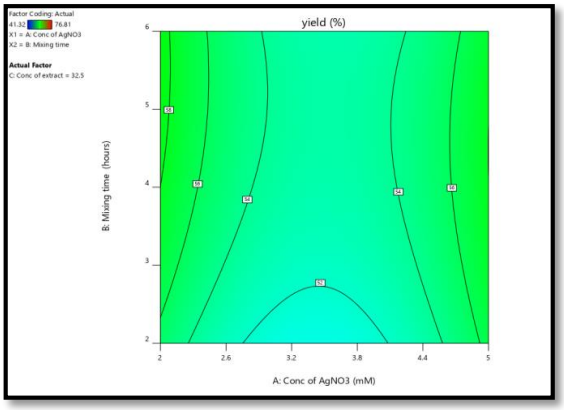

Figure 4.7 (B)

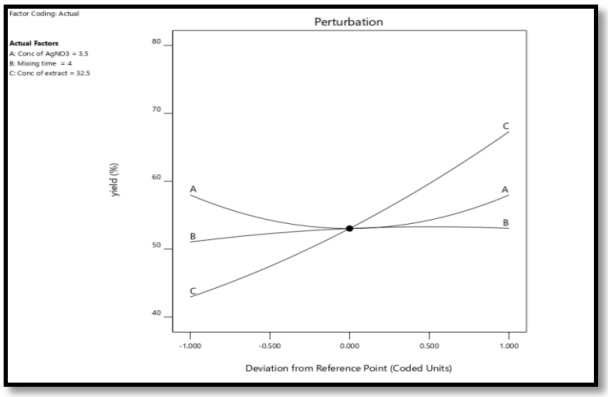

Figure 4.7 (C)

Figure 4.7 (A) 3-dimensional response surface plot sowing effect of conc. of extract and mixing time on % yield **(B)** 2-dimensional surface plot showing effect of conc. of extract and mixing time on % yield **(C)** perturbation plot showing effect on % yield all by all three independent variables

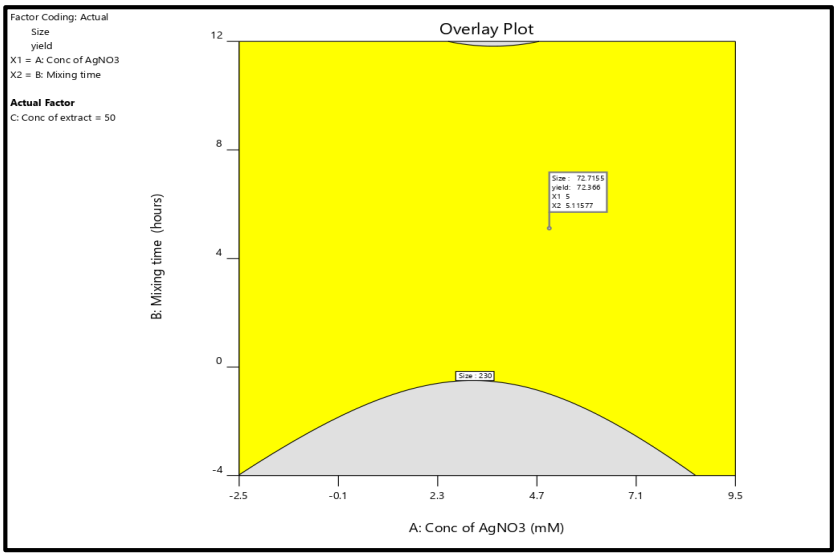

Figure 4.8 Overlay plot for optimization of silver nanoparticles

As per above data Design Expert software suggested values of independent variables data for preparation of optimized silver nanoparticles, and also predicted particle size, zeta potential and % yield with their mean. Optimized nanoparticles were prepared and evaluated further. It was revealed that observed value of dependent variables were found near the predicted value and data is mentioned below in the form of over lay plot followed by ANOVA analysis.

Table 4.13 Observed and predicted value of optimization of silver nanoparticles

Solution 1 of 78 Response	**Predicted**	**Observed**	**Std Dev**	**n**	**SE Pred**	**95% PI low**	**95% PI high**
Size(nm)	72.6787	74.96±1.1	20.9647	1	25.1902	16.5515	128.806
Zeta potential(mV)	-12.0215	-12.6±1.1	1.92145	1	2.30872	-17.1657	-6.87738
%Yield	72.3752	72.1±2.1	4.15776	1	4.99576	61.244	83.5065

4.10.3. Particle size, zeta potential and PDI of optimized nanoparticles

Optimized nanoparticles size, zeta potential of particles as well as PDI of nanoparticles were found in the range 74.96±1.1 nm, -12.6±0.9 mV and 0.261±0.002 respectively, which was much similar to the predicted data by Design expert software®

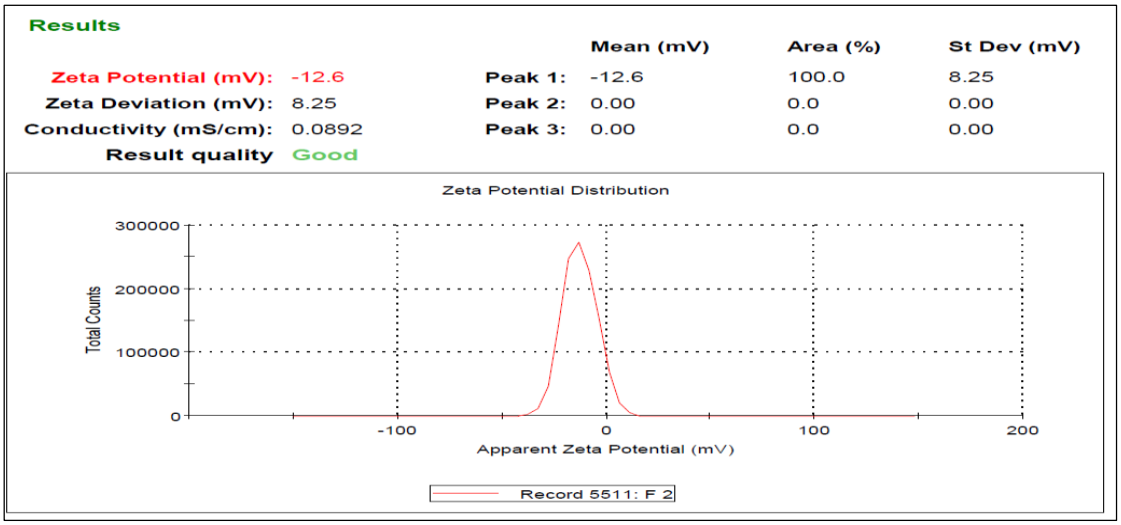

Figure 4.9 Zeta potential of silver nanoparticles

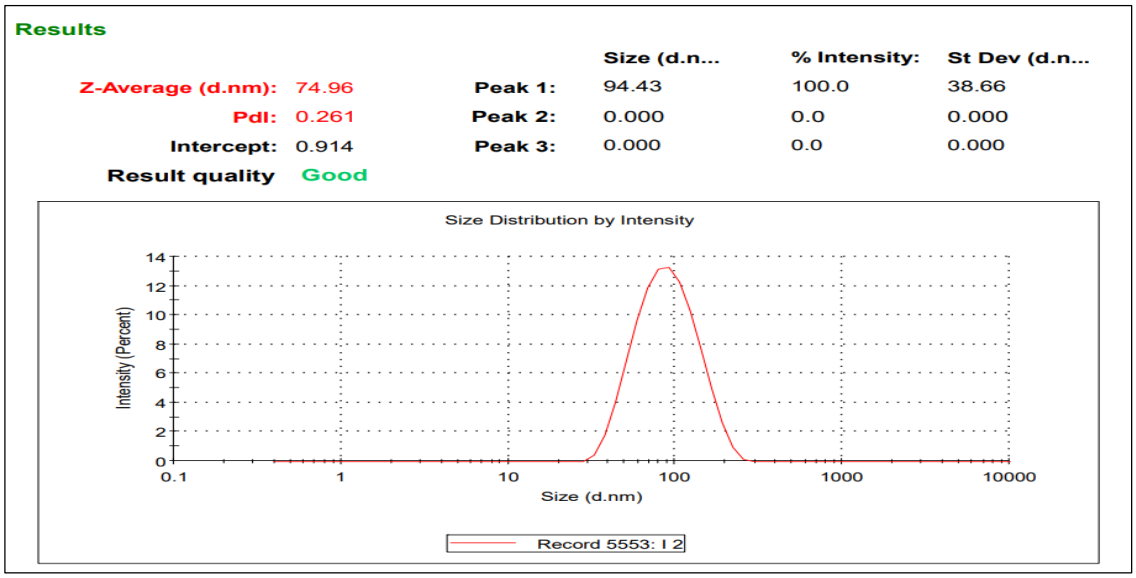

Figure 4.10 PDI of silver nanoparticles

Figure 4.11 (A)

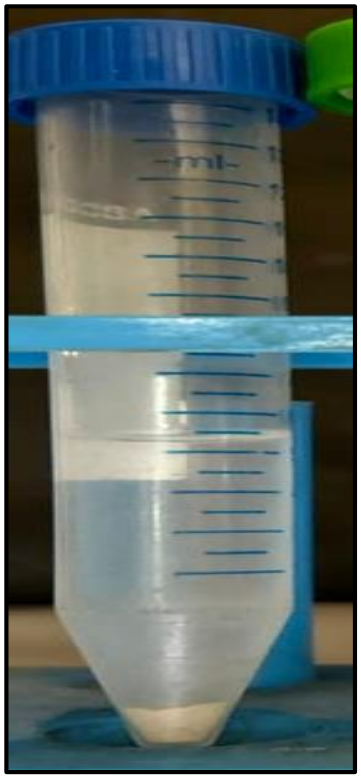

Figure 4.11 (B)

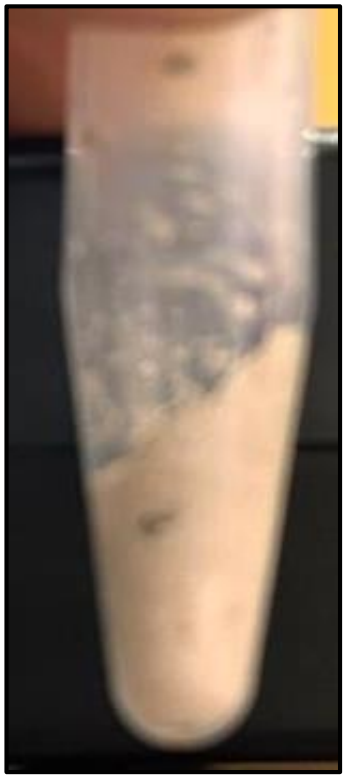

Figure 4.11 (C)

Figure 4.11 (A) SNPs before centrifugation, (B) SNPs pellets, (C) SNPs after filtration

4.10.4 FTIR spectra of silver nanoparticles

The likely components participated in and served as reducing agents for producing Ag nanoparticles using extract of green tea as reducing agent. FTIR spectrum was utilized to confirm the synthesis of the nanoparticles. Four strong peaks were identified and listed in the table.

Table 4.14 FTIR interpretation data [255]

% Transmittance	Functional Group
3319 cm^{-1}	Vibration of O-H stretching
2124.20 cm^{-1}	C≡N Stretching
1635.17 cm^{-1}	Stretching C=O in amide group, existence of protein as capping agent
576.16 cm^{-1}	Aromatic bending vibration

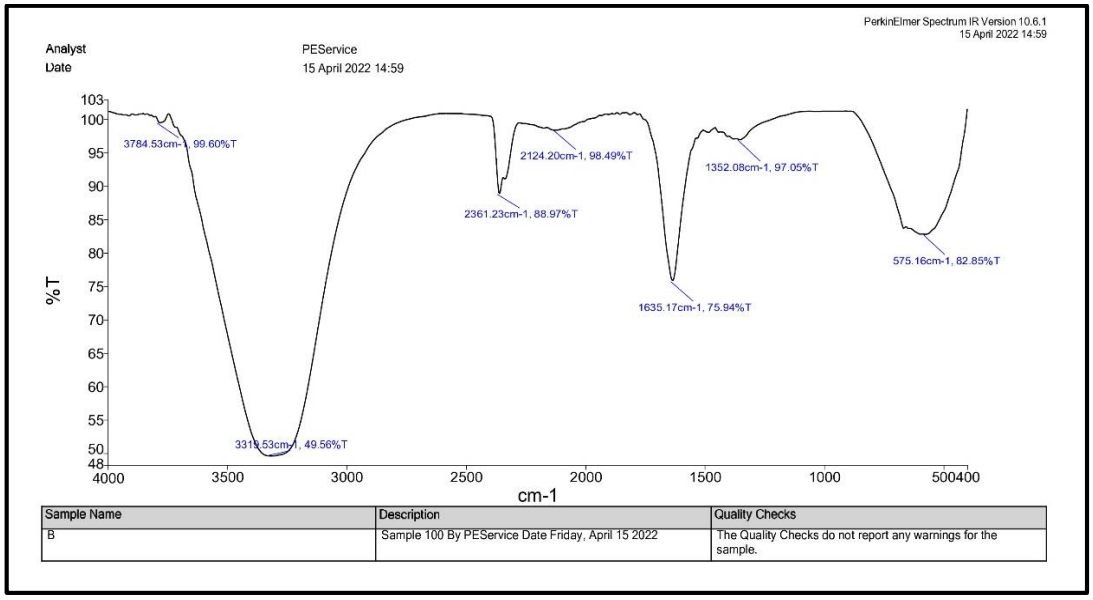

Figure 4.12 FTIR spectra of silver nanoparticles

4.10.5 Shape and surface morphology of nanoparticles

Morphological character of silver nanoparticles was best described by scanning electron microscopy. Nanoparticles was spherical in shape and smooth in texture, almost all the particles were in defined size range. There were no cavities or pores were found within particles. All particles were capturing their separate entity.

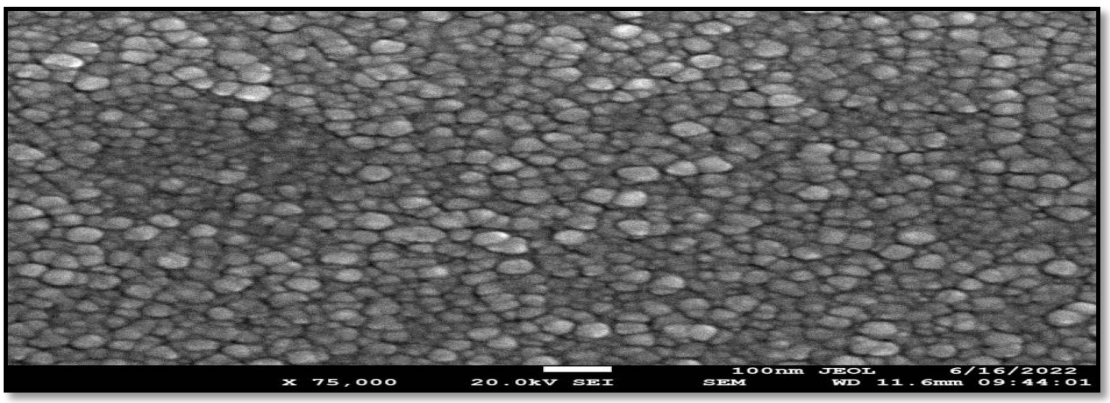

Figure 4.13 SEM image of silver nanoparticles

4.11 Optimization of emulgel using Design Expert® Software

4.11.1 Effect of independent variables on *in vitro %* cumulative drug release

Software called Design Expert® was used to estimate the data. The range of Vitamin D-3 release from emulgel *in vitro was* determined to be 55±1.7-94.5±1.6%. Figure 4.13 (A, B, and C) demonstrated that the independent variable had a noteworthy impact on the release profile of the API from emulgel. It was discovered that release duration increased along with an increase in carbopol% concentration. P value for in vitro release was determined to be 0.016, which is less than 0.0500, indicating that the model is significant.

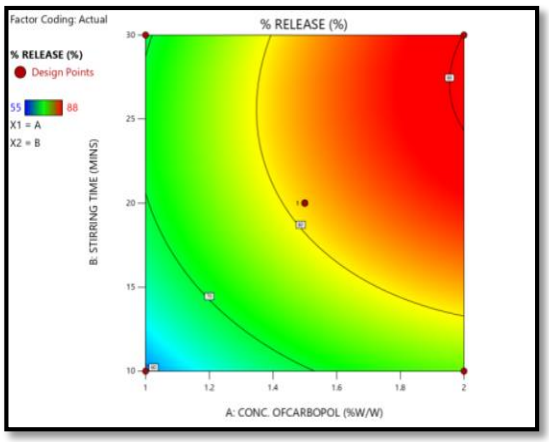

Figure 4.14 (A)

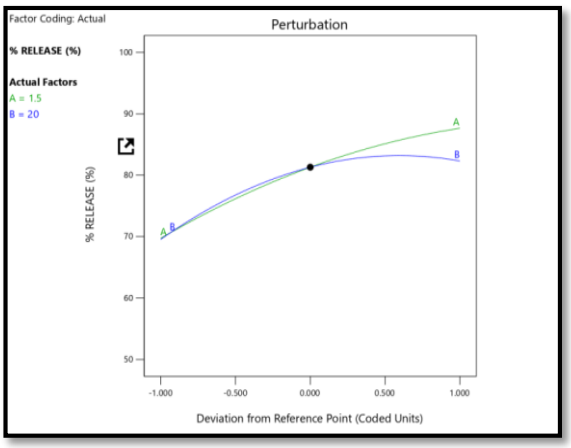

Figure 4.14 (B)

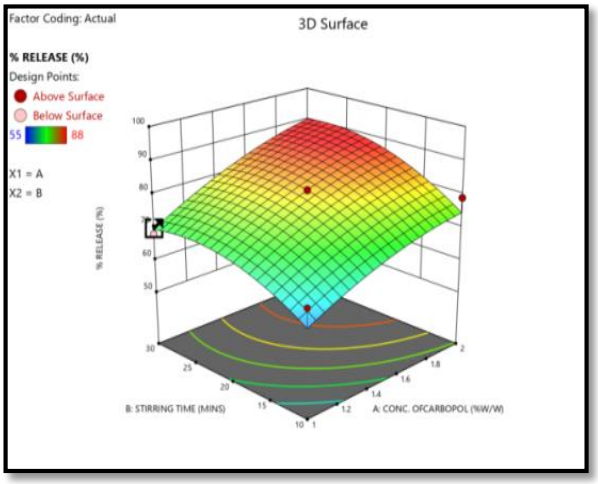

Figure 4.14 (C)

Figure 4.14 (A) 2- dimensional response surface plot presenting effect of conc. of carbopol and mixing time on % drug release **(B)** 3-dimensional surface plot presenting effect of conc. of carbopol and mixing time on % drug release **(C)** perturbation plot presenting effect on all by all two independent variables.

4.11.2 Effect of independent variables on pH of formulation

Data were evaluated using the Design Expert® software. The pH of the emulgel was found to be between 4.4 ± 0.7 and 6.1 ± 0.5%. The contour plot (Figure 4.15 A, B, C) showed that the independent variables had an effect on the pH of the emulgel. It was found that as the concentration of carbopol increased, the pH of the formula also increased. The p-value for the pH study was found to be 0.0275, which is less than 0.0500, indicating that the model is significant.

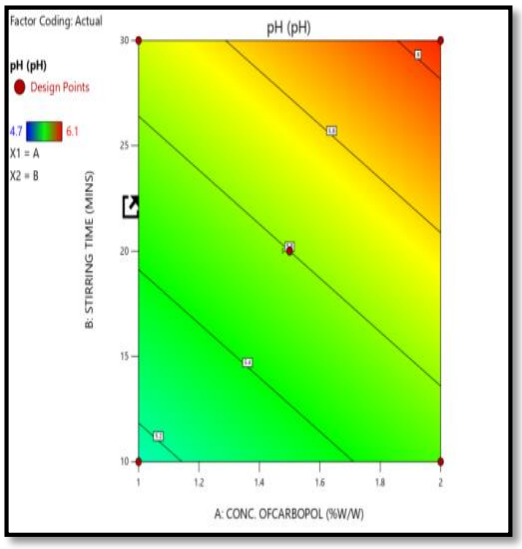

Figure 4.15 (A)

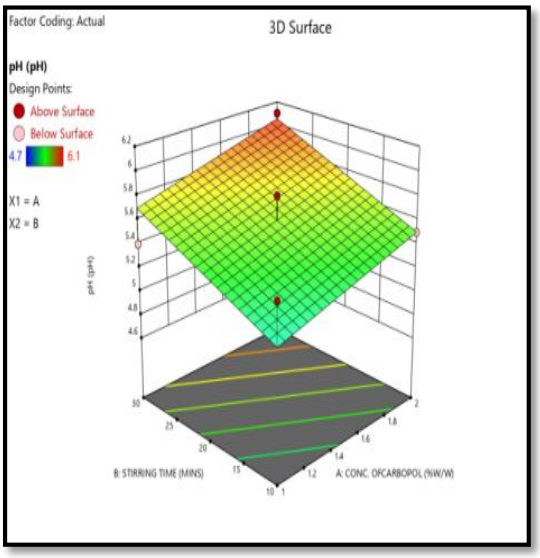

Figure 4.15 (B)

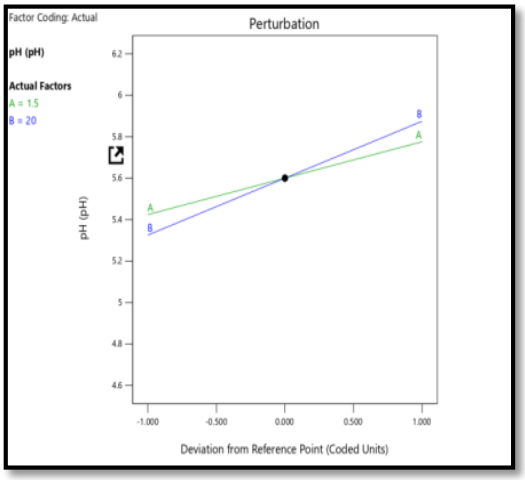

Figure 4.15 (C)

Figure 4.15 (A) 2-dimensional response surface plot showing effect of conc. of carbopol and mixing time on pH of formulation **(B)** 3-dimensional surface plot showing effect of conc. of carbopol and mixing time on pH **(C)** perturbation plot showing effect on formulation by all two independent variables.

4.11.3 Effect of independent variables on viscosity of emulgel

Data were evaluated using the Design Expert® software. The viscosity of the emulgel was found to be between 1034±6.6 and 1674±8. The contour plot (Figure 4.16 A, B, C) showed that the independent variables had an effect on the viscosity of the emulgel. It was shown that the viscosity increased as the concentration of carbopol increased. The P-value was found in the viscosity study was 0.0349, which is less than 0.0500, indicating that the model is significant.

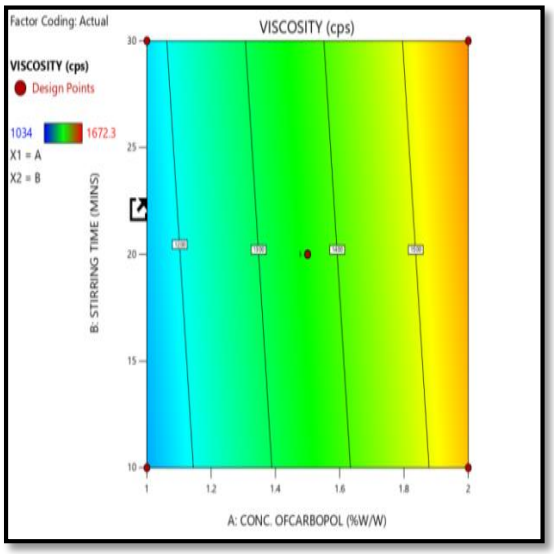

Figure 4.16 (A)

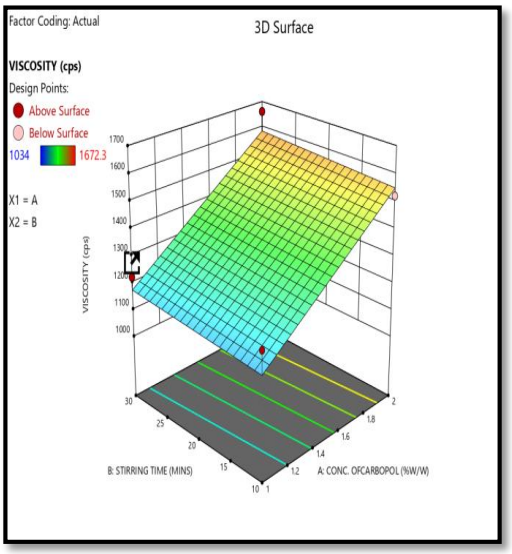

Figure 4.16 (B)

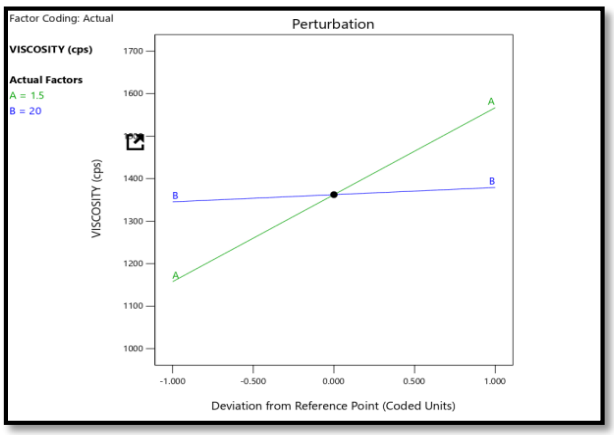

Figure 4.16 (C)

Figure 4.16 (A) 2-dimensional response surface plot showing effect of conc. of carbopol and mixing time on viscosity of formulation **(B)** 3-dimensional surface plot showing effect of conc. of carbopol and mixing time on viscosity **(C)** perturbation plot showing effect on formulation by all two independent variables.

Table 4.15 Various formulations of emulgel

Run	Factor 1	Factor 2	Response 1	Response 2	Response 3
	Conc. of carbopol (%w/w)	Stirring time (mins)	% *in vitro* cumulative drug release	pH	Viscosity (cps)
F1	2	30	87.5±4.1	6.1±0.2	1661±31
F2	1.5	20	81.3±5.2	5.8±0.1	1339±34
F3	1.5	20	81.3±3.5	5.8±0.4	1339±45
F4	0.7	20	61.1±2.7	5.1±0.2	1034±36
F5	1	30	68.2±3.6	5.4±0.3	1223±41
F6	2	10	79.1±2.3	5.5±0.5	1521±39
F7	1.5	20	81.3±4.5	5.8±0.4	1339±40
F8	1.5	20	81.3±4.5	5.8±0.1	1339±40
F9	1.5	20	81.3±4.5	5.8±0.3	1339±40
F10	1.5	34	83.2±4.2	5.9±0.2	1338±42
F11	1	10	65.3±4.3	5.5±0.3	1225±35
F12	1.5	5.8	55.2±4.8	4.7±0.2	1340±38
F13	2.2	20	88.2±4.1	6.2±0.4	1672±33

Values are expressed as n=3, mean ± SD

As per above data Design Expert software suggested values of independent variables data for preparation of optimized formulation, and also predicted % release, pH and viscosity value with their mean. Optimized formulation was prepared and evaluated further and it was revealed that observed value of % release. pH and viscosity were found near the predicted value and data mentioned below in the table

Table 4.16 Predicted and observed value of optimized emulgel

Analysis	Predicted Mean	Observed	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean
% In -vitro drug release	81.3	89.47±0.58	4.15693	1.85904	76.9041	85.6959
pH	5.86	5.81±0.1	0.284451	0.0788925	5.42422	5.77578
Viscosity	1362.3	1373.5±11	48.1603	13.3573	1332.54	1392.06

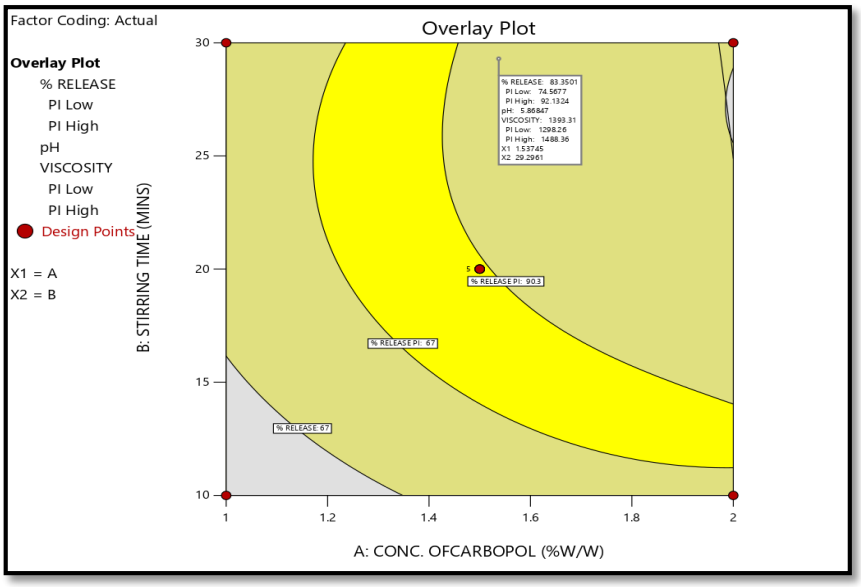

Figure 4.17 Overlay plot for formulation of emulgel

4.12 Evaluation of optimized emulgel [256-257]

4.12.1 Physical evaluation of emulgel

Emulgel is a mixed formulation of gel and emulsion and its physical evaluation is much important. Emulgel was found stable there was no phase separation was observed. It was homogeneous, white in color and smooth in texture.

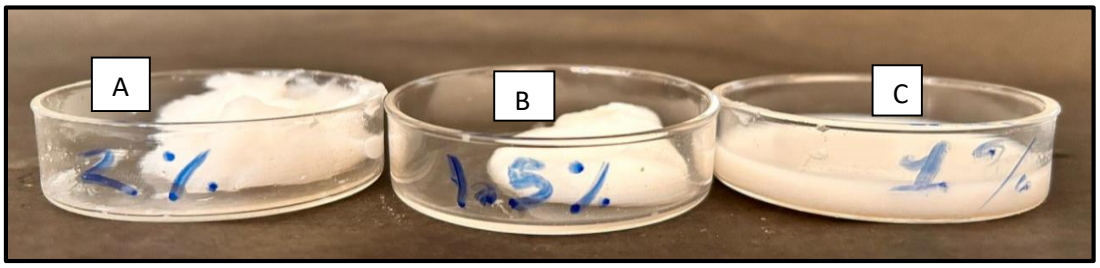

Figure 4.18 (A) Emulgel having 2% gelling agent (B) 1.5% gelling agent, (C) 1 % gelling agent

4.12.2 Drug content

The content of the drug in optimized formulation was found to be 97.4±0.6%. It is revealed that formulation has drug concentration in the specified range between 95-105%

4.12.3 pH determination

pH of the optimized formulation was found to be 5.81±0.1. It was revealed that formulation has desired optimum pH for topical delivery of drug.

4.12.4 Viscosity

Viscosity of the formulation was found to be 1373.5±11 cps. It is revealed that formulation has an optimum viscosity to deliver the drug topically.

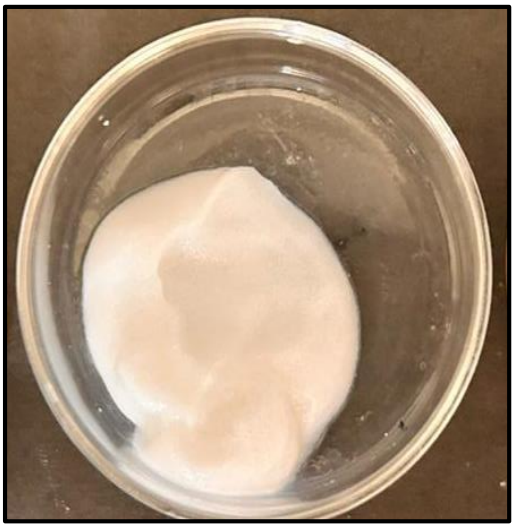

Figure 4.19 Optimized formulation of emulgel

4.12.5 Spreadability

Spreadability of final formulation was found between 22.5±0.9 gm.cm/min. It is revealed that formulation has good and desired spreadability for topical application.

4.12.6 *In vitro* % cumulative drug release from emulgel

% cumulative release of drug from optimized formulation was found to be 89.47±0.58 up to the 12 hrs. of study and best explained by zero order kinetics model showing maximum linearity and N value >1 also showing non- fickian diffusion.

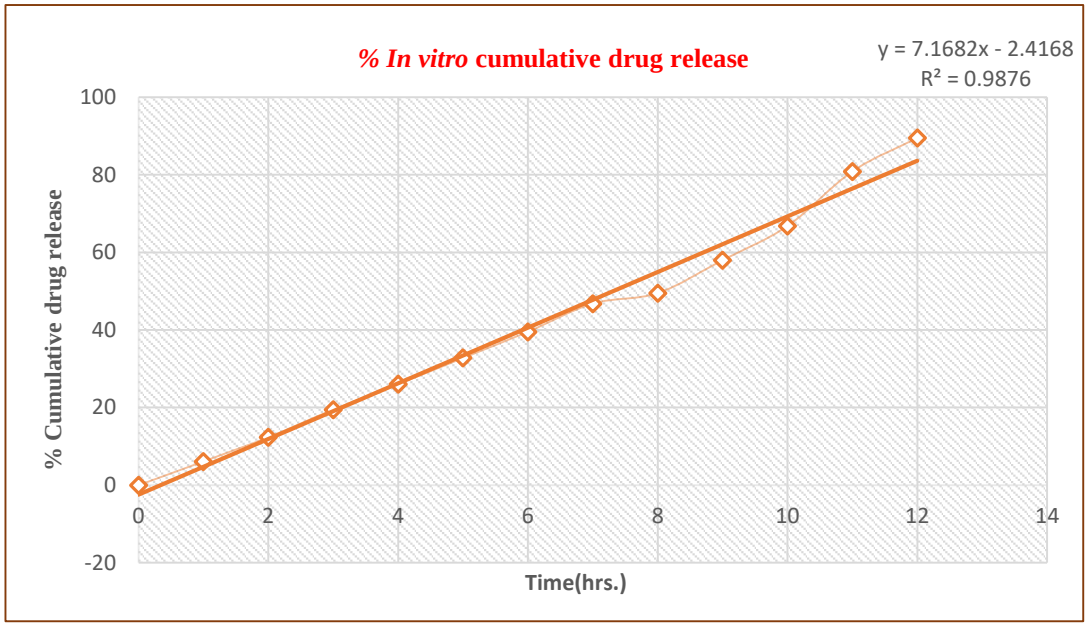

Figure 4.20 *In vitro* cumulative drug release profile of Vitamin D-3 from emulgel

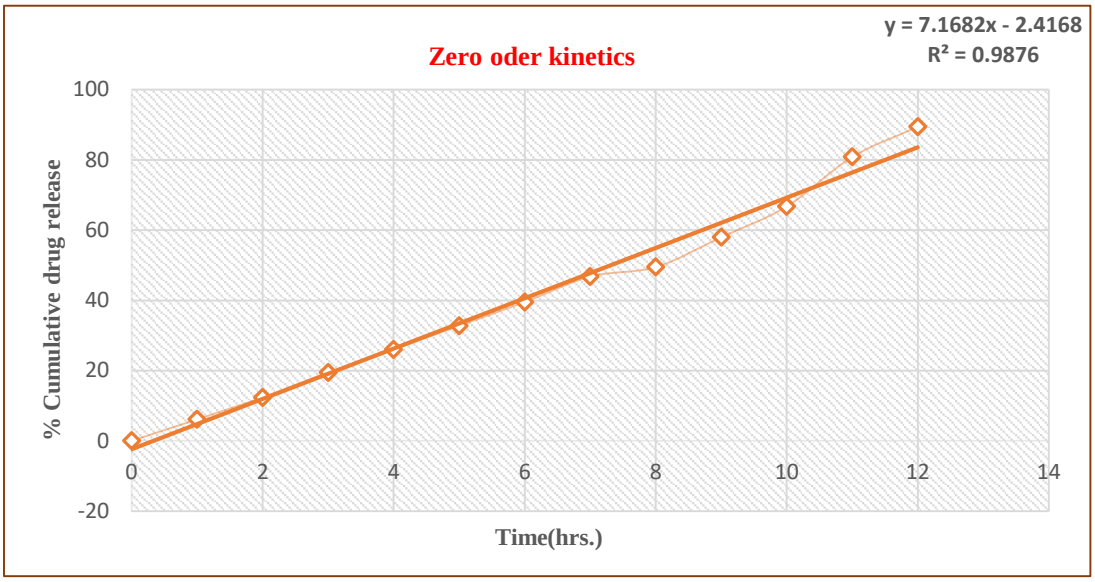

Figure 4.21 Zero order kinetics

Figure 4.22 First order kinetics

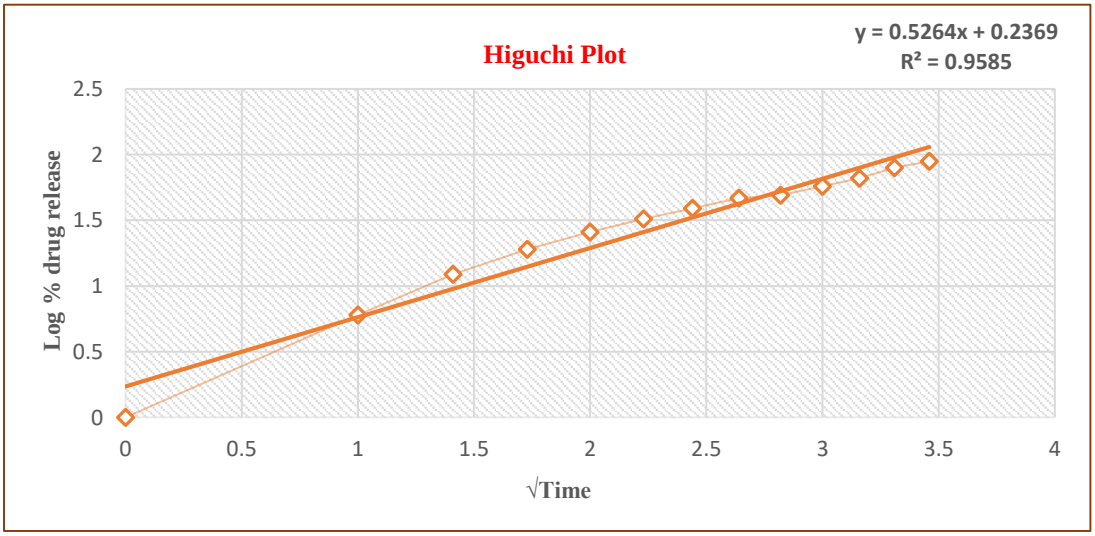

Figure 4.23 Higuchi plot

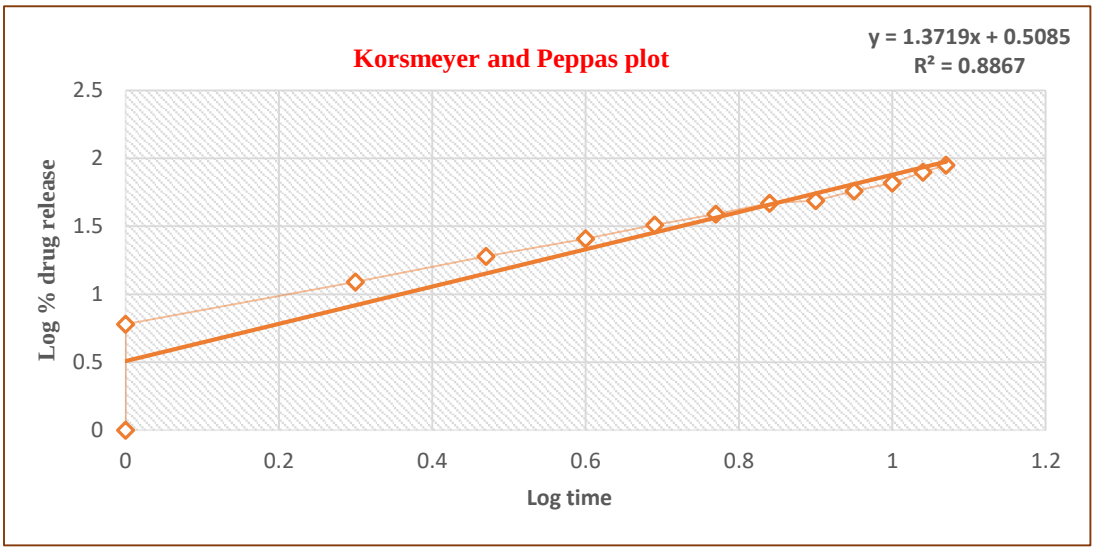

Figure 4.24 Korsmeyer and Peppas plot

Table 4.17 Various kinetic model data of emulgel formulation

Zero order	First order	Higuchi	Korsmeyer and Peppas
K = 7.16	K =0.122	K=26.73	N=1.05
R^2=0.987	R^2=0.883	R^2 = 0.958	R^2=0.886

4.12.7 Physical stability along with pH, drug content and *in vitro drug* release

There was no phase separation and creaming were found upon storage. Formulation was showed no significant changes in ph. No significant change was found in drug content, viscosity and *in vitro* release respectively. All the data were calculated after the completion of respective months. Data of stability study is mentioned below:

Table 4.18 Accelerated stability data of optimized formulation

Time period	pH	Drug content	*In vitro* release	Phase separation	Creaming	Viscosity (cps)
0	5.6±0.1	98.5±0.5	89.56±05	N	N	1375.5±5
1st month	5.6±0.3	98.5±0.5	88.56±0.1	N	N	1366.6±6.1
2nd month	5.5±0.1	98.5±0.6	89.62±0.9	N	N	1371±4.3

3rd month	5.6±0.2	97.5±0.5	89.56±0.2	N	N	1375±4.2
4th month	5.6±0.5	97.2±0.6	89.69±0.7	N	N	1376±5.5
5th month	5.6±0.4	97.3±0.5	88.51±0.1	N	N	1376.5±2.1
6th month	5.6±0.2	97.5±0.8	89.36±0.8	N	N	1374.1±2.1

*N= No (All the data were taken in triplicate n=3, mean ±SD

4.12.8 Het CAM irritation testing

Basically, three mechanism was used to described by Het CAM assay like haemorrhage, cell lysis and coagulation of blood on the membrane of fertilized egg after the interval of 300 seconds. There were no significant changes was observed in the CAM of chicken egg after 300 seconds of formulation application and irritation score was found to be 0.69±0.05, which was in the non-irritation range of score chart. This score revealed that optimized formulation has not created any irritation or cytotoxic effect and found non-irritant.

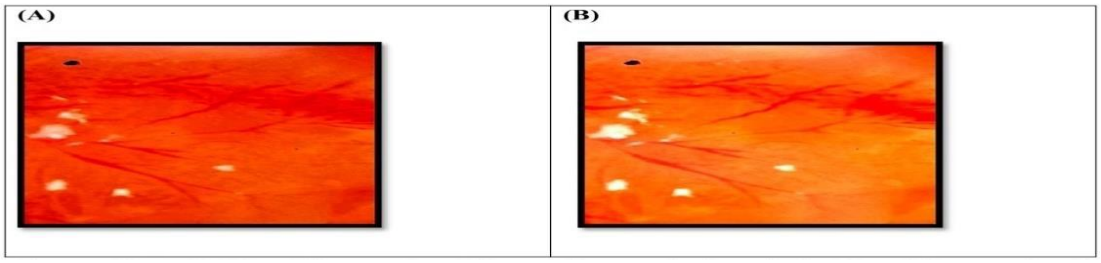

Figure 4.25 (A) **Figure 4.25** (B)

Figure 4.25 (A)Initial image of CAM **Figure 4.25** (B) Image after 300 seconds of formulation application

4.13 *In vivo* excision wound model

The % wound contraction was found highest in marketed formulation (Silvex having silver sulphadiazine 2% w/w) as well as in ESVDH (emulgel with high dose of Vitamin D-3 and silver nanoparticles) i.e., 103.725± 1.12 and 100.5± 1.7 respectively which is much similar to marketed. Dose dependent healing has shown in emulgel because it contains only 0.006% w/w Vitamin D-3 and 0.002 % w/w silver nanoparticles but in marketed formulation silver sulphadiazine was embedded in 1%

w/w which is extremely higher concentration. ESVDH treated and marketed formulation has also shown epithelialization on the day 15th of experiment. This phenomenon was not shown by any other group of animals. Remaining groups was healing slowly. Normal control group was not created excision, and only kept for histopathological study and to do correlation between the healed wound cell structure and normal cell structure. Blank emulgel and experimental control group has shown almost equal wound contraction, it was revealed that emulgel base do not contain any wound healing property. Emulgel with high dose of SNPs and low dose has shown dose dependent healing, as higher the concentration of nanoparticles in emulgel has healed larger area. Emulgel with low dose of Vitamin D-3 and high dose of SNPs has shown much higher % wound contraction, it was observed that in both group either ESVDH or ESVDL silver nanoparticles concentration was equal but Vitamin D-3 concentration was changed, so it reveals that Vitamin D-3 has dose dependent potency of wound healing and can heal the wound even in minute concentration. One way ANOVA was used for statistical analysis along with Dunnett post-test. The results showed that the overall P value for the test and market was less than 0.01. While comparison to the control group, marketed formulation and Emulgel loaded with high dose of Vitamin D-3(ESVDH) has shown significant changes on the day 0 to 16th day of experiment (**= $p<0.01$) denotes statistically significant. This demonstrated the significance of the model because the experimental control group was used for every comparison. All of the group's percentages of wound contraction are listed in the table below, with values stated as n = 6 mean ±SD.

Table 4.19 % Wound contraction area (mm^2) in various groups of animals

Groups	**Route**	**%Wound contraction area**			
		Day			
		4	**8**	**12**	**16**
I. Normal control	--	**--**	**--**	**--**	**--**
II. Experimental control	No treatment	9.3±2.6	21.5±2.4	24.3±3.5	29±1.8
III. Blank emulgel	Topical	8.2±2.9	20.5±1.2	24.3±1.9	28.9±2.3
IV. Marketed	Topical	29.2±3.2	58.9±2.8	85.1±2.9	103.7±1.1**

V. ESVDL	Topical	14.5±3.6	40.3±3.9	52.4±2.5	62.9±2.5
VI.ESVDH	Topical	22.7±1.9	67±1.9	84.5±1.9	100.5±1.7**
VII. Emulgel with SNPs Low dose	Topical	14.5±1.7	23.9±3.7	28.9±4.2	34.8±2.4
VIII. Emulgel with SNPs High dose	Topical	17.5±2.7	26.4±2.4	31.4±1.4	40.3±1.1

Values are expressed as n=6 mean ±SD

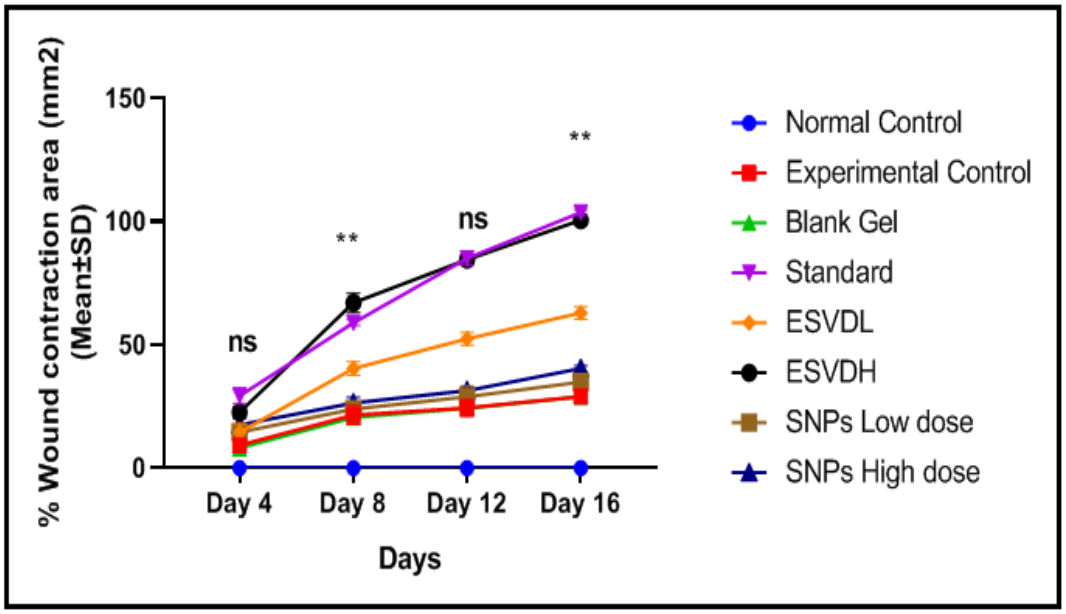

Figure 4.26 % Wound contraction area, in comparison to the control group, marketed formulation and Emulgel loaded with high dose of Vitamin D-3(ESVDH) has shown significant changes on the day 0 to 16th day of experiment (**= $p<0.01$) denotes statistically significant. Using One Way ANOVA followed by Dunnett Post Test. SNPs (silver nanoparticles); ESVDH (emulgel of silver nanoparticles-high dose of Vitamin D- 3); and ESVDL (emulgel of silver nanoparticles-low dose of Vitamin D-3). The values are given as mean ±SD, n = 6

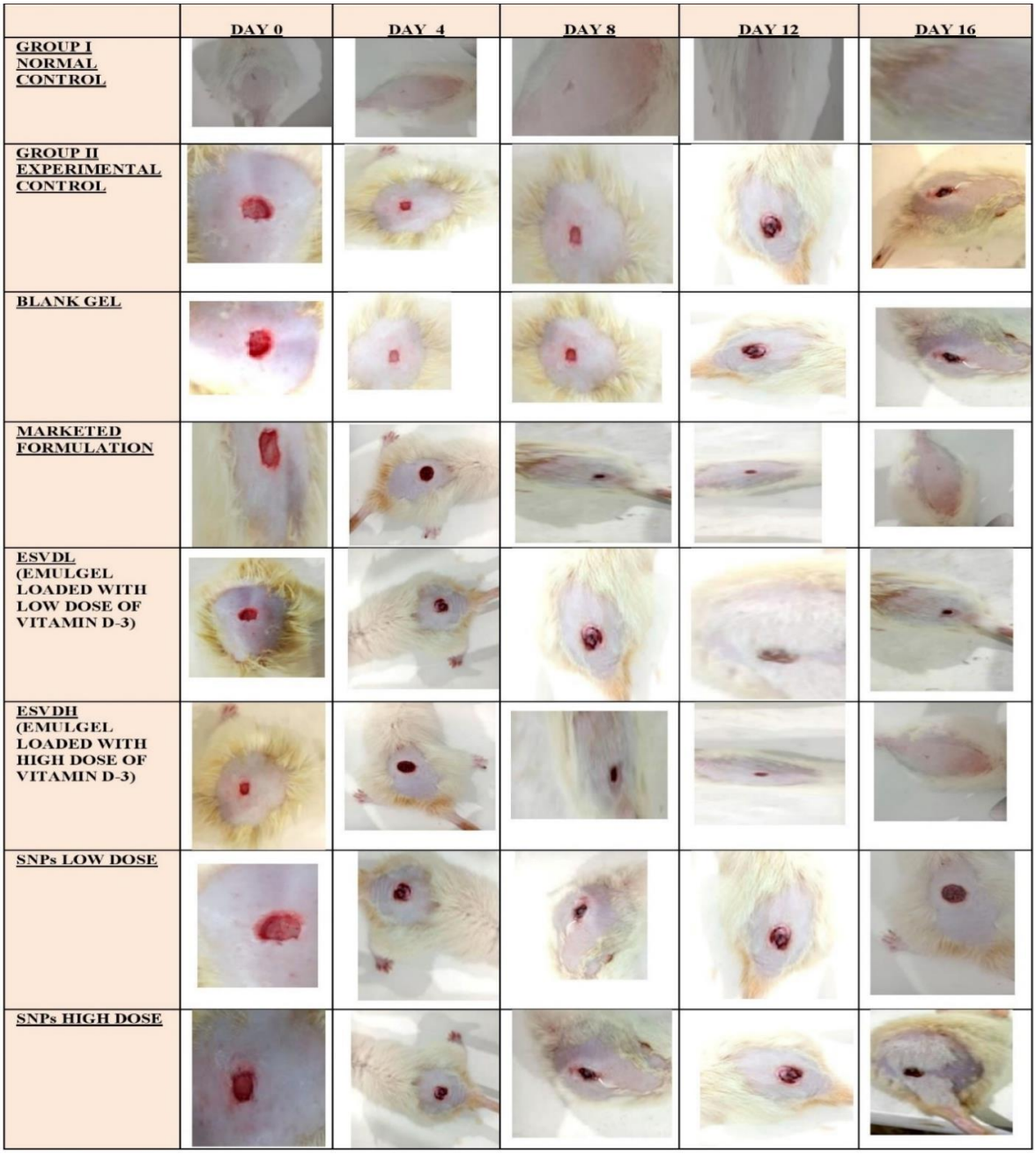

Figure 4.27 Pictorial presentation of wound contraction on specific day

4.13.1 Epithelialization

It's the amount of time a wound needs to generate new epithelial tissue. On day 15th day, the only formulations that exhibited epithelialization were the commercially available formulation treated group and the ESVDH (emulgel of silver nanoparticles-Vitamin D-3 high dose) formulation treated group.

4.13.2 Histopathology

On the 16th day of the animal study, the animals' histopathology was completed and assessed for a number of factors that affect wound healing. A total of eight groups underwent histopathology evaluations. There were live cells with blood vessels in

Group I (Normal Control Group); there were no inflammatory or dead cells present. The second group, known as the experimental control group, exhibited evident scar tissue, non-viable cells, decreased collagen fibre content, and inflammatory cells. Moreover, inflammatory cells have been seen in Group III (Blank emulgel). Group VI (ESVDH) and Group IV (Marketed treatment) demonstrated complete epithelialization, more collagen fibre, blood vessels, and fewer inflammatory cells. The remaining group displayed visible blood vessels, scars, and dead cells.

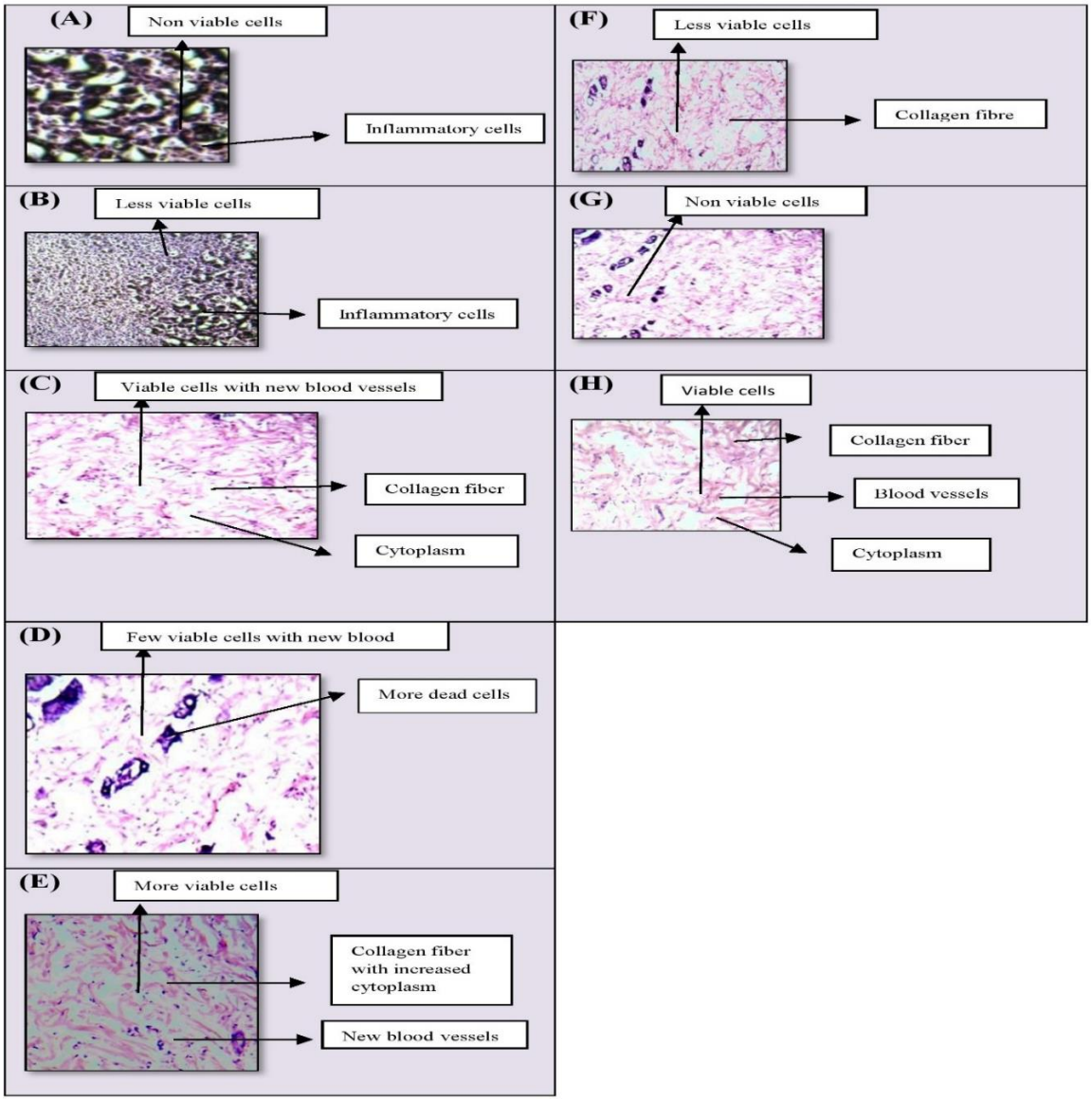

Figure 4.28 Histopathology data (A) Experimental control group, (B) Blank gel, (C) Marketed formulation (Silvex), (D) ESVDL, (E) ESVDH, (F) SNP s low dose, (G) SNPs high dose, (H) Normal control group

Chapter -5

Summary

Emulgel is a system contains property of gel and emulsion and found novel and effective agent for delivery of many oil-soluble drugs. It gives better, controlled and dual release delivery system. Emulsion is thermodynamically unstable aa well as gels are limited for hydrophilic drugs to overcome such kind of drawback emulgel formulation has prepared. Vitamin D-3 potentiate wound healing and nanosized silver also aid in wound healing and prevent the wound from infection. The aim of this study was to preparation and evaluation emulgel loaded with Vitamin D-3 and SNPs. It was envisaged to formulate the nanoparticles of silver reduced with green tea extract. All the ingredients which are selected for formulation have been reported for their characteristic feature like silver has an anti -inflammatory as well as anti -bacterial which may aid in wound healing and Vitamin D-3 which is fat soluble vitamin and help in wound healing by increase the collagen synthesis and also increase the synthesis of cathelicidin which is responsible for keratinocyte cell production. It was also envisaged to formulate an emulgel which has controlled release as well and dual release drug delivery system and also promote wound healing. The drug was analyzed and identified by various identification tests given in various literatures like solubility, melting point and standard curve preparation by UV. Analytical method was also developed for quantification of Vitamin D-3 in formulations, this method was not yet developed and found reproducible and accurate upon validation. The solubility of drug was performed using shake flask method and the Vitamin D-3 was found soluble in methanol and has shown linearity in the range of 0.25-1.25 µg/mL and the λ_{max} was found to be 264 nm. The correlation coefficient was found to be 0.999 indicating good linearity using HPLC method. The silver nanoparticles were prepared by green tea extract using herbal reduction method using silver nitrate as precursor. Formulation of silver nanoparticles was done using central composite design and optimization was done using one way ANOVA by Design expert software®. Optimized nanoparticles have shown particle size (74.96±1.1 nm), Zeta potential (-12.6±0.9 mV) and PDI (0.261 ±0.002) along with higher yield. This size was found better for topical delivery otherwise it may cause nanotoxicity. Zeta potential represents stability of particles as higher the negative charges on particle will make them separate to each other and will not allow to clump together, these charges will make sure to maintained the stability of nanoparticles. PDI indicates the uniformity of particle size distribution as PDI value

less than 1 indicates mono dispersion of the particles. Same procedure was used for formulation and optimization of Emulgel, there three independent and dependent variables were selected for optimizing best formulation. Firstly, Vitamin D-3 emulsion was prepared and nanoparticles incorporated in gelling system was added in emulsion in 1:1 ratio to prepare emulgel. Various evaluation parameters for emulgel like viscosity, drug content, pH, and spreadability were used to obtain the optimized formulation. The drug content of formulation was within the range and ensures uniformity of drug content. Formulation showed sustained release of drug for a period of 12 hrs. The consistency of the formulation was found satisfactory. The pH of optimized formulation was found in range of 5-6, which is desirable for topical delivery. Formulation was found stable for 6 months of period on storage and all the stability studies was performed using ICH guidelines of stability studies. *In vivo* studies were done included various parameters for wound healing evaluation and it was found that emulgel having high dose of Vitamin D-3 and silver nanoparticles (0.006% Vitamin D-3 and 0.002% silver nanoparticles) has shown almost similar wound contraction to marketed silvex creme (1% w/w silver sulphadiazine) and epithelialization was also observed only in ESVDH (High dose of Vitamin D-3 and silver nanoparticles) treated and silvex treated group. As the data revealed Vitamin D-3 and silver nanoparticle based emulgel has healed the wound in minute concentration upon comparison with marketed formulation. Histopathological studies of tissue concluded that ESVDH (High dose of Vitamin D-3 and silver nanoparticles) treated groups showed more viable cells, new blood vessels and amino acids along with higher amount of collagen upon microscopic examination. It indicates that wound Vitamin D-3 promotes healing by collagen synthesis. Over all the *in vitro* as well as *in vivo* evaluation of emulgel was found satisfactory and also showed desired effect.

Conclusion and future perspective

Emulgel is a new carrier for lipophilic drugs in topical since it combined physicochemical properties of both emulsion and gel systems. This is due to the fact that it is not a single component but possesses two components hence delivering a better and controlled and dual release system. Silver nanoparticles in the emulgel that was prepared from green tea extract and Vitamin D-3 helps to decrease the healing time and also enhances the efforts of each step of wound healing. Vitamin D-3; it potentiates wound healing and nano sized silver; which has an antibacterial and anti-

inflammatory proprieties may be useful in aiding the wound to heal without getting infected. Emulgel was found stable, and it has gone through all the investigations both in *in vitro* and *in vivo*. Vitamin D-3 and silver nanoparticles loaded emulgel is not yet available in the market being totally novel as well as it also has not been used in the wound healing treatment till now. It may replace several semi-solid formulations for its Vitamin D-3 based, intended for topical application for wound healing because if the supplement has the capability of healing the wound on a topical application particularly with less side effects. It will tend to be force for the treatment of wound and may prescribe this by the medical practitioner by replacing some wound healing cremes in the future.

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Appendix

Rishu thesis final

ORIGINALITY REPORT

6%	4%	4%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Simsek, A.. "Response surface methodological study on HMF and fluorescent accumulation in red and white grape juices and concentrates", Food Chemistry, 2007 Publication	<1%
2	repository-tnmgrmu.ac.in Internet Source	<1%
3	mdpi-res.com Internet Source	<1%
4	storage.googleapis.com Internet Source	<1%
5	Submitted to Student Paper	<1%
6	idr.mnit.ac.in Internet Source	<1%
7	T Mohan, S Prabhakara, K Anbazhagan, Krishnan Pavithra, Basavaraj Bhandare, H. Sahjahanand. "DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF VITAMIN-D3	<1%

LETTER OF CANDIDACY

LOVELY PROFESSIONAL UNIVERSITY

Centre for
Research Degree Programmes

LPU/CRDP/PHD/EC/20210107/000705

Dated: 17 Sep 2020

KM Rishu Yadav
Registration Number: 41900201
Programme Name: Doctor of Philosophy (Pharmaceutics)

Subject: Letter of Candidacy for Ph.D.

Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on 17 Sep 2020 by accepting your research proposal entitled: "Development and Evaluation of Emulgel loaded with Green Tea Extract based Silver Nanoparticles and Vitamin D for Wound Healing"

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

We wish you the very best!!

In case you have any query related to your programme, please contact Centre of Research Degree Programmes.

Head

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Ph : +91-1824-444594 E-mail : drp@lpu.co.in website : www.lpu.in

CPCSEA CERTIFICATE

Ref. No.: PBRI/CER/23122

Date: 16th Feb. 2023

CERTIFICATE

It is hereby certified that animal experimentation of research work entitled **"Development and evaluation of emulgel loaded with green tea extract based silver nanoparticles and vitamin D for wound healing"** of **Rishu Yadav** (Research Scholar – Pharmaceutics, Registration No. 41900201), School of Pharmaceutical Sciences, LPU, Punjab is approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal (Reg. No. 1824/PO/ERe/S/15/CPCSEA).

Protocol approval reference number is **PBRI/IAEC/PN**-23024.

Bhopal
PBRI, Bhopal

(Research Co-ordinator)
Research Co-ordinator
Pinnacle Biomedical Research Institute
Bhopal

Pinnacle Biomedical Research Institute (PBRI)
Bharat Scout and Guide Campus, Near Depot Square, Shanti Marg, Shamla Hills, Bhopal (M.P.) – 462 003
Contact No. – 0755-2665174; +91 94258-90029 **e-mail.** – *pbrinstitute@gmail.com* **Web:** www.pbri.in

RESEARCH ARTICLE

Design, development and improvement of an emulgel containing silver nanoparticles and Vitamin D-3 for its potential to accelerate the healing of wound in International Journal of Applied Pharmaceutics (IJAP), Vol 16, Issue 3, May 2024

International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Issue 3, 2024

Original Article

DESIGN, DEVELOPMENT AND IMPROVEMENT OF AN EMULGEL CONTAINING SILVER NANOPARTICLES AND VITAMIN D-3 FOR ITS POTENTIAL TO ACCELERATE THE HEALING OF WOUND

RISHU YADAV[1], NARENDRA KUMAR PANDEY[1*], RAJIV KUKKAR[2]

[1]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India. [2]School of Pharmaceutical Sciences, Dr. K. N. Modi University, Nawai, Rajasthan, India

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Received: 11 Jan 2024, Revised and Accepted: 27 Mar 2024

ABSTRACT

Objective: The aim of this research work was to prepare a topical emulgel based dosage form incorporated with vitamin D-3 and silver nanoparticles to reduce the wound healing time in any kind of wound.

Methods: Central Composite Design (CCD) was applied for the optimization of emulgel by using *Design expert software*. Three responses (pH, viscosity, and *in vitro* drug release) and two factors (Carbopol concentration and stirring duration) were chosen, and Statistical Analysis of Variance (ANOVA) revealed that all the factors were significantly affecting the responses. Silver Nanoparticles (SNPs) was prepared with Green Tea Extract (GTE) and evaluated for particle size, Poly Dispersity Index (PDI), zeta potential and Fourier Transform Infra-red (FTIR) spectroscopy and revealed that SNPs of desired range and stability have been synthesized. Here excision wound model was used to evaluate the wound healing activity of formulation *in vivo*.

Results: Maximum *in vitro* release 88.2±2.1 has shown by the optimized formulation F13, pH and viscosity were also found in optimum range i.e., 6.2±0.4 and 1672±33 respectively, followed by Korsmeyer and Peppas model. Total eight groups were designed for animal study and silver sulphadiazine was used as marketed formulation. F13 formulation was further evaluated for *in vivo* data, it was revealed that emulgel loaded with high dose of vitamin D-3 along with silver nanoparticles has shown 100.5±1.7% wound contraction, while marketed formulation has shown 103.7±1.1% wound contraction, which was much similar with test formulation. Cytotoxic cell study was done using assay on chicken egg, formulation has not shown any cytotoxic behaviour like haemolysis and cell damage on chick embryo's blood vessels. Accelerated stability study of the optimized formulation was also performed to check whether the formulation was stable or not and it was revealed that optimized formulation

REVIEW ARTICLE

Emulgel A Reliable System for Topical Delivery of Lipophilic Drugs in Present Scenario: Review in **Research Journal of Pharmacy and Technology** (RJPT) in June 2022

Research J. Pharm. and Tech. 15(6): June 2022

ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

REVIEW ARTICLE

Emulgel A Reliable System for Topical Delivery of Lipophilic Drugs in Present Scenario: Review

Rishu Yadav[1,3], Narendra Kumar Pandey[1], Rajiv Kukkar[2], Deepika Dutta[3], Preeti Avasthi[3] Monika Rana[3], Swati Modgil[3]

[1]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India.
[2]Raffles University, Neemrana, Rajasthan, India.
[3]Maharaja Agrasen University, Baddi, H.P. India.
*Corresponding Author E-mail: **rishu.yadav789@gmail.com**

ABSTRACT:

Topical drug delivery can be defining as good delivery system for drugs, in which drug directly get interact with skin and give its pharmacological effect to desired site to cure and treat variety of disorder. Gel has major disadvantage because it cannot prepare for hydrophobic drugs this type of limitation can be defeated by emulgel. Emulgel have dual property like emulsion and gel and gives dual release system. Primary disadvantage of lipophilic drug that it does not dissolves in aqueous solvent and cause problem in drug delivery. Emulgels gives a good advantage over gel that hydrophobic drugs are easily dissolve in oil phase and then added in aqueous phase to form w/o type emulsion. These emulgel then incorporate into gel to form emulgel. Emulgel are good carrier for topical delivery of lipophilic drugs and have several advantages over different drug delivery system. Emulgel give better patient compliance and promote controlled pattern of drug delivery due to presence of cross linked structure of gelling agent. Emulgels formulations can be used for anti-inflammatory, analgesic, antifungal, acne, skin disorder and also in form of cosmetic products.

KEYWORDS: Topical, Emulgel, Lipophilic Drugs, Hydrophobic Drugs W/O Emulsion.

Participated as Oral Presenter in a International Conference conducted by IEC University, H.P

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THIS IS TO CERTIFY THAT

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PARTICIPATED IN THE CONFERENCE AND PRESENTED POSTER/ORAL PRESENTATION ON

A Review on Emulgel

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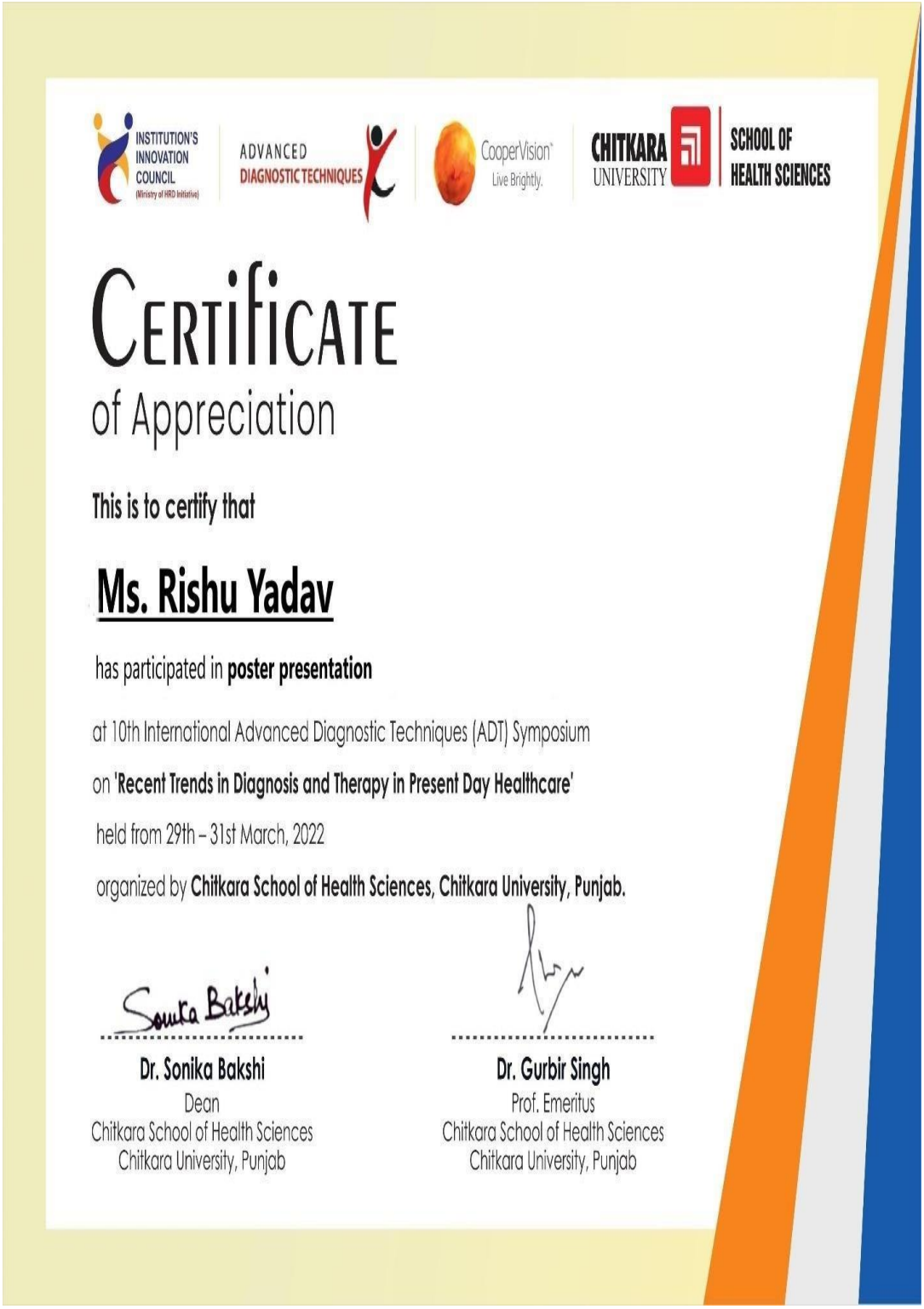

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Dean
Chitkara School of Health Sciences
Chitkara University, Punjab

Dr. Gurbir Singh
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Presented an oral presentation at Maharaja Agrasen University, Baddi, H.P.

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and secured 3rd position held on 17th May, 2022,
Baddi, Himachal Pradesh.

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and got BEST POSTER/ORAL PRESENTATION AWARD on recommendation of

the evaluation panel in National Conference

held during 25th - 26th November, 2022

at

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Dr. Rakesh Yadav
Dean (UCC, Academics, NFSU-Tripura Campus)
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Honoured with Young Researcher Award by VD GOOD PROFESSIONAL Organization

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International Conference for Women Researchers (ICWOR 2024)

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This is to certify that

Ms. Rishu Yadav

Assistant Professor, Department of Pharmaceutics,
Maharaja Agrasen University,
Baddi, Himachal Pradesh, India.

Has participated

And has been Awarded as Young Researcher Award In the International Conference for Women Researchers, held on 08-Jun-2024, Online, Organized by VDGOOD Professional Association, India.

Authority Signature

Association Reg.no:221907T2019NPL127500

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LIST OF PATENTS

Sr. no.	Tittle of patent	Publication year
1	**NOVEL HERBAL ANATACID**	2024
2	**FORMULATION AND EVALUATION OF NATURAL PRODUCT BASED FAIRNESS CREAM**	2024
3	**AGELESS A NOVEL FORMULATION TO REDUCE THE MARK OF AGEING**	2024
4	**EMULSIFIED CLAY BASED TOPICAL FORMULATION**	2024
5	**NATURAL OIL BASED EMULSION HAVING SKIN CLEANSING AND ANTIBACTERIAL PROPERTY**	2022

TITLE: EMULSIFIED CLAY BASED TOPICAL FORMULATION

12) PATENT APPLICATION PUBLICATION

19) INDIA

22) Date of filing of Application :26/04/2024

(21) Application No.202411033506 A

(43) Publication Date : 17/05/2024

54) Title of the invention : EMULSIFIED CLAY BASED TOPICAL FORMULATION

51) International classification	:A61P0029000000, A61P0031040000, A61K0009500000, A61K0031192000, A61K0045060000
86) International Application No Filing Date	:NA :NA
87) International Publication No	: NA
61) Patent of Addition to Application Number Filing Date	:NA :NA
62) Divisional to Application Number Filing Date	:NA :NA

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2)Kriti Sharma

3)Pooja Thakur

4)Anjana devi

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Address of Applicant : NA

(72)**Name of Inventor :**

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Address of Applicant :MAHARAJA AGRASEN UNIVERSITY ATALSHIKSHA KUNJ, KALUJHANDA, BADDI Baddi ----------

TITLE: AGELESS A NOVEL FORMULATION TO REDUCE THE MARK OF AGEING

(54) Title of the invention : AGELESS A NOVEL FORMULATION TO REDUCE THE MARK OF AGEING

(51) International classification	:A61Q0019000000, A61Q0019080000, A61K0031496000, A61K0008978900, C12Q0001000000	(71)**Name of Applicant :** **1)Rishu Yadav** Address of Applicant :352/1 N.L.C. KIDWAI NAGAR ---------- ---------- **2)Mona Piplani** **3)Pankaj Bhateja** **4)Preeti Devi** **Name of Applicant : NA** **Address of Applicant : NA**
(86) International Application No Filing Date	:NA :NA	
(87) International Publication No	: NA	
(61) Patent of Addition to Application Number Filing Date	:NA :NA	
(62) Divisional to Application Number Filing Date	:NA :NA	(72)**Name of Inventor :** **1)Rishu Yadav** Address of Applicant :352/1 N.L.C. KIDWAI NAGAR ---------- ---------- **2)Mona Piplani** Address of Applicant :Maharaja Agrasen University Baddi ---------- ---------- **3)Pankaj Bhateja** Address of Applicant :Maharaja Agrasen University Baddi ---------- ---------- **4)Preeti Devi** Address of Applicant :Maharaja Agrasen University Baddi ---------- ----------

(57) Abstract :
This novel emulsion has property to reduce the fine lines on skin. It comprises natural oils and fruit enzymes in the form of nanoparticles. The main aim of this formula is to increase the surface area of oils and enzymes so that they can cover and penetrate the skin and can show maximum effect. Due to its novel combination and being herbal, it has lesser side effect. Various in vitro and skin toxicity studies have been performed, and it has shown acceptable results. The formulated anti -ageing cream is useful for both dry as well as oily skin. Its future perspective is to commercialize it on a large scale at a low cost so that every people can afford this formulation.

No. of Pages : 26 No. of Claims : 7

TITLE: NOVEL GEL FOR TREATMENT OF ACIDITY

(12) PATENT APPLICATION PUBLICATION

(19) INDIA

(22) Date of filing of Application :03/06/2024

(21) Application No.202411043118 A

(43) Publication Date : 14/06/2024

(54) Title of the invention : NOVEL GEL FOR TREATMENT OF ACIDITY

(51) International classification	:A61P0001040000, A61K0033100000, A61K0047360000, A61B0017340000, A61K0009000000
(86) International Application No Filing Date	:NA :NA
(87) International Publication No	: NA
(61) Patent of Addition to Application Number Filing Date	:NA :NA
(62) Divisional to Application Number Filing Date	:NA :NA

(71)**Name of Applicant :**

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Name of Applicant : NA

Address of Applicant : NA

(72)**Name of Inventor :**

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Address of Applicant :Faculty of Health Sciences School Of Pharmaceutical Studies Dr.K.N.Modi University, NIwai Rajasthan Niwai ---------- ----------

3)Kshama Srivastava

Address of Applicant :School of Pharmaceutical Sciences, Maharishi University of Information Technology, Noida, UP Noida ---------- ----------

4)Saik Rahana Parveen

Address of Applicant :Gland Institute of Pharmaceutical Sciences Sy. No: 551, Shangri-la, Kothapet (village), Shivampet (Mandal), Medak, Telangana 502220 Shivampet ---------- ----------

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7)Sunaina Sharma

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8)Parina Gill

Address of Applicant :Sunder Deep Pharmacy College, Ghaziabad Ghaziabad ---------- ----------

9)Nishant Singh

Address of Applicant :Faculty of Health Sciences School Of Pharmaceutical Studies Dr.K.N.Modi University, Niwai, Rajasthan Niwai ---------- ----------

(57) Abstract :

In this scenario every second people is suffering from acidity and acid reflux, and uses OTC chemical-based medicine to reduce excess amount of HCl secreted by stomach linings. Here novel formula was developed to reduce the acidity which is totally herbal and prepared from plant source. In this formula herbal seed extract as antacid property and polysaccharides was used as gelling/thickening agent. This novel formula was proven beneficial in neutralizing acid content in stomach, also prevent the stomach to get basic as other antacid do In vitro test has confirmed its activity and also found economical

No. of Pages : 12 No. of Claims : 5

TITLE: FORMULATION AND EVALUATION OF NATURAL PRODUCT BASED FAIRNESS CREAM

!) PATENT APPLICATION PUBLICATION (21) Application No.202411040932 A

)) INDIA

!) Date of filing of Application :27/05/2024 (43) Publication Date : 07/06/2024

)) Title of the invention : FORMULATION AND EVALUATION OF NATURAL PRODUCT- BASED FAIRNESS CREAM

|) International ssification :A61Q19/02, A61K8/02, A61K8/92, A61K8/97, A61K36/22

5) International Application :NA
Filing Date :NA

7) International Publication : NA

|) Patent of Addition to plication Number :NA
Filing Date :NA

!) Divisional to plication Number :NA
Filing Date :NA

(71)**Name of Applicant :**
1)Rishu Yadav
Address of Applicant :352/1 N.L.C. KIDWAI NAGAR ---------- ----------
2)Mona Piplani
3)Pankaj Bhateja
4)Lucky Rajput
Name of Applicant : NA
Address of Applicant : NA
(72)**Name of Inventor :**
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Address of Applicant :MAHARAJA AGRASEN UNIVERSITY, ATALSHIKSHA KUNJ KALUJHANDA, BADDI, SOLAN H.P. Baddi ---------- ----------
2)Mona Piplani
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Address of Applicant :MAHARAJA AGRASEN UNIVERSITY ATALSHIKSHA KUNJ, KALUJHANDA, BADDI Baddi ---------- ----------
4)Lucky Rajput
Address of Applicant :MAHARAJA AGRASEN UNIVERSITY ATALSHIKSHA KUNJ, KALUJHANDA, BADDI Baddi ---------- ----------

7) Abstract :
smetics are used to enhance/modify the beauty of human beings. Now a day, herbal plants are widely used in cosmetic products. smetic preparations are used to treat the various skin disorders like skin pigmentation, skin ageing, skin wrinkles, acne as well as beautification. Herbal fairness cream, which has various herbal components added to it, may naturally lighten skin, clean up spots, d give fair skin with an even tone. It results in a lovely complexion which looks healthy. This herbal based novel invention is found be beneficial in skin lightening as the demand of such kind of product is increasing day by day to overcome the barrier of toxicity or ıer side effects caused by chemicals. Herbal novel invention has been investigated as a potent carrier system for several armacological and cosmetic active substances. The primary goal of present work was to develop a fairness cream with dry fruit as base to lessen greasiness and essential oil's solid lipid nanoparticles (SLNs) for improved absorption through skin. Essential oil-sed nanoparticle and dry-fruit cream base reduces the greasiness and also increases the surface area for better topical absorption.

. of Pages : 19 No. of Claims : 7

TITLE: NATURAL OIL BASED EMULSION HAVING SKIN CLEANSING AND ANTIBACTERIAL PROPERTY

(12) PATENT APPLICATION PUBLICATION

(19) INDIA

(21) Application No.202111054946 A

(22) Date of filing of Application :27/11/2021

(43) Publication Date : 25/11/2022

(54) Title of the invention : NATURAL OIL BASED EMULSION HAVING SKIN CLEANSING AND ANTIBACTERIAL ACTIVITY

(51) International classification :A61Q0019000000, A61K0008970000, A61K0008979400, A61K0008920000, A61Q0001140000

(86) International Application No :NA
Filing Date :NA

(87) International Publication No : NA

(61) Patent of Addition to Application Number :NA
Filing Date :NA

(62) Divisional to Application Number :NA
Filing Date :NA

(71)**Name of Applicant :**
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Address of Applicant :Maharaja Agrasen University kalujhanda barotiwala solan ---------- ----------
2)Dr. Yogendra SIngh
3)Dr. Mona Piplani
4)Pankaj Bhateja
Name of Applicant : NA
Address of Applicant : NA
(72)**Name of Inventor :**
1)Rishu Yadav
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