International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Formulation And Evaluation of Ciprofloxacin, Miconazole, And Fluocinolone Acetonide Nanotechnology Based Ointment for Topical Drug Delivery System

Anupam Anand[1], Suresh Chaudhary[2]

[1]Phd Research Scholar, lords University, Alwar
[2]Associate Professor, Lords University, Alwar

ABSTRACT:
Nanotechnology is a multidisciplinary field of science and engineering that deals with the manipulation and application of materials and structures at the nanoscale, typically involving objects with dimensions less than 100 nanometers. This emerging technology has revolutionized various industries, from electronics and medicine to materials science and energy production, by enabling scientists and engineers to work at the molecular and atomic levels. This research fully focused on the nanotechnolgy based drug delivery system.

KEYWORDS: Nanotechnology, formulation, evaluation, topical drug delivery solution.

INTRODUCTION:

At the heart of nanotechnology lies the ability to understand, control, and manipulate matter at the nanoscale, where unique and often unexpected properties emerge. These properties can differ significantly from those exhibited by bulk materials, making nanotechnology a powerful tool for innovation and advancement[1].

Nanotechnology encompasses a wide range of applications and techniques, including:

1. Nanomaterials: The design and synthesis of materials with nanoscale features, such as nanoparticles, nanotubes, and nanowires, that exhibit enhanced properties and functions. These materials find applications in electronics, coatings, and drug delivery, among others.
2. Nanoelectronics: The development of smaller and more efficient electronic components, enabling the creation of faster and more energy-efficient electronic devices, like transistors and memory chips.
3. Nanomedicine: The use of nanoparticles and nanoscale materials for targeted drug delivery, disease diagnosis, and imaging, offering the potential for more effective and less invasive medical treatments.
4. Nanomanufacturing: The creation of nanoscale structures and devices through various fabrication techniques, such as lithography and self-assembly, to produce advanced materials and products.
5. Nanotechnology in Energy: The development of nanomaterials and nanodevices for more efficient energy storage and conversion, such as advanced batteries, solar cells, and fuel cells.

6. Environmental Applications: Nanotechnology plays a role in pollution control, water purification, and remediation of contaminated sites by creating nanoscale materials that can absorb or degrade pollutants.
7. Nanotechnology in Aerospace: In aerospace and materials science, nanotechnology is used to create stronger and lighter materials for aircraft and spacecraft, enhancing their performance and durability.
8. Nanosensors: Nanoscale sensors can detect and measure various physical and chemical properties, making them valuable in areas like environmental monitoring, healthcare, and security.
9. Nanoelectromechanical Systems (NEMS): These are miniaturized mechanical and electromechanical devices that operate on the nanoscale, offering new possibilities for sensors, actuators, and even quantum computing.
10. Ethical and Societal Implications: Nanotechnology raises important ethical and safety considerations, including potential environmental impacts and ethical concerns related to privacy and human enhancement[2].

Nanotechnology has the potential to reshape industries, improve healthcare, enhance energy efficiency, and address some of society's most pressing challenges. However, it also demands careful ethical and safety considerations to ensure responsible development and application. As researchers continue to push the boundaries of what is possible at the nanoscale, the field of nanotechnology promises to drive innovation and create new opportunities across various sectors.

Nanotechnology offers a novel platform for enhancing the bioavailability and stability of drugs, providing sustained release, and promoting targeted delivery to specific skin layers. The combination of Ciprofloxacin, Miconazole, and Fluocinolone Acetonide in a single nanotechnology-based ointment holds significant potential for treating various skin infections and inflammatory conditions with improved efficiency and reduced side effects.

Transdermal Drug Delivery System (TDDS), often referred to as a transdermal patch, is a pharmaceutical technology designed to deliver medication through the skin into the bloodstream for systemic therapeutic effects. This approach offers several advantages over traditional oral or injection-based drug delivery methods. TDDS provides a controlled and continuous release of medication, reduces the need for frequent dosing, minimizes side effects, and improves patient compliance[3].

Here's a brief introduction to the key aspects of Transdermal Drug Delivery Systems:

HOW IT WORKS: Transdermal patches are typically composed of several layers, including an adhesive backing, a drug reservoir, a membrane to control drug release, and a protective outer layer. The medication is formulated in a way that allows it to penetrate the skin and reach the bloodstream steadily over time. The skin acts as a barrier that regulates the rate of drug absorption.

ADVANTAGES:

- **Steady Drug Release:** TDDS provides a constant and predictable release of medication, maintaining therapeutic levels in the bloodstream.
- **Reduced Side Effects:** Because of the controlled release, TDDS can reduce the likelihood of side effects associated with fluctuations in drug concentration.
- **Improved Compliance:** Patients often prefer transdermal patches as they eliminate the need for frequent dosing, injections, or swallowing pills.

- **Avoids First-Pass Metabolism:** TDDS bypasses the liver's first-pass metabolism, which can alter the effectiveness of some drugs when taken orally.

APPLICATIONS: Transdermal drug delivery has been employed in various therapeutic areas, including:

- Pain management (e.g., opioid patches)
- Hormone replacement therapy (e.g., estrogen and testosterone patches)
- Smoking cessation (nicotine patches)
- Motion sickness prevention (scopolamine patches)
- Cardiovascular drugs
- Neurological disorders

CHALLENGES:

- Not all drugs are suitable for transdermal delivery, as they must have specific physicochemical properties to penetrate the skin.
- Some patients may develop skin irritation or allergies at the patch application site.
- The size of the drug molecule and the formulation of the patch can influence the rate of drug absorption.

FUTURE DEVELOPMENTS:

Ongoing research in the field of TDDS aims to improve patch design, enhance drug penetration through the skin, and expand the range of medications that can be delivered transdermally. This includes the development of microneedle patches and innovative formulations.

Transdermal Drug Delivery Systems have transformed the way certain medications are administered, providing a convenient and effective option for patients. As technology advances, it is likely that TDDS will continue to play a significant role in drug delivery, offering new solutions for various medical conditions[4].

An ointment is a homogeneous, viscous, semi-solid preparation, most commonly a greasy, thick oil (oil 80% - water 20%) with a high viscosity, that is intended for external application to the skin or mucous membranes. Ointments have a water number that defines the maximum amount of water that they can contain. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a Degree of occlusion is desired.

Ointments are semisolid preparations that incorporate a lipid or hydrophobic excipient and are intended for external application to the skin or other muco-sal membranes. An ointment Usually contains < 20% water and other volatile ingredients, such as ethanol, and >50% Hydrocarbons, waxes, or polyols. Ointments are designed to soften or melt at body Temperature, spread easily, and have a smooth, no gritty feel. Ointments are typically used As emollients to make the skin more pliable, protective barriers to prevent harmful Substances from coming in contact with the skin, and vehicles for hydrophobic drugs.

Ointments are usually very moisturizing, and good for dry skin. They have a low risk of sensitization due to having few ingredients beyond the base oil or fat, and low irritation risk. There is typically little variability between brands of drugs. They are often disliked by patients due to greasiness.

The vehicle of an ointment is known as the ointment base. The choice of a base depends uponthe clinical indication for the ointment. The different types of ointment bases are:Absorption bases, e.g., beeswax and wool fat

- Emulsifying bases, e.g., cetrimide and emulsifying wax
- Hydrocarbon bases, e.g., ceresine ,microcrystalline wax, hard paraffin, and soft paraffin
- Vegetable oil bases, e.g. Almond oil, coconut oil, olive oil, peanut oil, and sesame oil
- Water-soluble bases, e.g., macrogols 200, 300, 400

TYPES OF OINTMENT BASES

An Ointment bases are classified into four general groups:

- Hydrocarbon bases,
- Absorption bases,
- Emulsion or water-removable bases, and
- Water-soluble bases.

Ointments are used topically on a variety of body surfaces. These include the skin and the mucous membranes of the eye (an eye ointment), chest, vulva, anus, and nose. An Ointment may or may not be medicated.

The major advantage of a semi-solid dosage form is that it can be applied directly to the affected area, it gives prolong the action, and does not need to be administered orally. It is convenient for unconscious patients or patients, such as children and old people, for whom oral administration is difficult.

Semisolid dosage forms are more stable in atmospheric conditions than liquid dosage forms and solid dosage forms. It is suitable for those APIs that are low-density, amorphous hygroscopic. Different drugs and excipients or ingredients can be added together to form a single dose. It gives better adhesion and lasts long after application, hence the medicament give effects, it does not harm the gastrointestinal tract, as it does not need to be taken orally,and the semi-solid dosage form is more convenient for patients than other forms of drug administration. It is suitable for medicines with a bitter taste, as it does not need to be taken orally.

The major disadvantage of the semi-solid dosage form is that it has no dose accuracy and it needs to be applied directly. In general, Pharmaceutical creams, ointments, gels, suppositories, and paste are greasy and they difficult to clean. The semi-solid dosage form is less stable than the solid dosage form. The base used in the dose may be oxidized. The need to apply with the fingers every time can cause contamination. Some patients may become irritation or allergic.Semisolid dosage forms are usually a costly dosage because its manufacturing process is complex.

Semisolid dosage forms are Physio-chemically less stable compared with the solid dosage form. Clothing staining is often associated with the use of creams, ointments, gels, and paste[5].

The skin has several layers. The over laying outer layer is called Epidermis the layer below Epidermis is called Dermis. The dermis contains a network of blood vessels, hair follicle,sweat gland & Sebaceous gland. Beneath the dermis are subcutaneous fatty tissues, bulbs of hair project into these fatty tissues.

Majority the drug absorbed through the skin by two ways. They are namely

A. Trans epidermal Absorption

B. Trans follicular Absorption

Trans epidermal Absorption: It is now generally believed that the trans Epidermal pathway is principally responsible for diffusion across the skin. The resistance Encountered along this pathway arises in the stratum corneum. Permeation by the transepidermal route first involves partitioning into the stratum corneum. Diffusion then takes place across this tissue. The current popular belief is that must substances diffuse across the stratum corneum via the intercellular lipoid route. This is a tortuous pathway of limited fractional volume and even more limited productive fractional area in the plane of diffusion.

However there appears to be another microscopic path through the stratum corneum for extremely polar compounds and icons. Otherwise these would not permeate at rates that are measurable considering their O/W distributing tendencies. When a permeating drug exits at the stratum corneum, it enters the wet cell mass of the epidermis and since the epidermis has no direct blood supply, drug is forced to diffuse across it to reach the vasculature immediately space for icons and polar non electrolyte molecules to diffusionally squeeze through.

Thus, permeation requires frequent crossing of cell membranes, each crossings being a thermodynamically prohibitive event for such water- soluble species extremely lipophilic molecules on the other hand, or thermodynamically constrained from dissolving in the watery regime of the cell (Cytoplasm). Thus the viable tissue is rate determining when non polar compounds are involved. Passage through the dermal region represents a final hurdle to systemic entry.

This is so regardless of whether permeation is transepidermal or by a shunt route. Permeation through the dermis is through the interlocking channels of the ground substance. Diffusion through the dermis is facile and without molecules since gaps between the collagen fibres are far too wide to filter large molecules.

Since the viable epidermis and dermis lack measure physicochemical distinction, they are generally considered as a single shield of diffusion, expect whenpenetrates of extreme polarity are involved as the epidermis offers measurable resistance to such species. The skin appendages offer only secondary avenues for permeation. Sebaceous and eccrine glands are the only appendages, which are seriously considered as shunts by passing the stratum corneam since these are distributed over the entire body, through eccrine glands are numerous, their orifices are tiny and add upto a miniscule fraction of the body's surface.

Moreover, they re either evacuated or so profusely active that molecule cannot diffuse inwardly against the glands output. For these reasons, they are not considered as a serious route for percutaneous absorption.

However, the follicular route remains an important avenue for percutaneous absorption since the opening of the follicular pore, where the hair shaft exits the skin, is relatively large and sebum aids in diffusion of penetrates. Partitioning into sebum, followed by diffusion through the sebum to the depths of the epidermis is the envisionedmechanism of permeation by this route. Vasculature sub serving the hair follicle located in the dermis is the likely point of systemic entry[6].

DRUG PROFILE

We have used following drug for our research work

CIPROFLOXACIN:

Ciprofloxacin is an antibiotic belonging to the fluoroquinolone class of drugs. It is commonly prescribed to treat a wide range of bacterial infections in various parts of the body. Ciprofloxacin works by

inhibiting bacterial DNA gyrase and topoisomerase IV enzymes, crucial for bacterial DNA replication and repair, leading to the inhibition of bacterial growth and ultimately causing their death.

Physicochemical and pharmacokinetic properties of Ciprofloxacin

Physicochemical Parameters	**Values**
Molecular formula	$C_{17}H_{18}FN_3O_3$
Molecular weight	331.3415
Melting point	293.15–323.15 °C
Log P	1.546 to −0.347
Aqueous solubility	36 mg/ml
Pharmacokinetic Parameters	**Values**
Volume of distribution	2.00-3.04L/kg
Half life	4-6 h
Clearance	0.48-0.60 L/h/kg
Bioavailability	82% +/- 13%

Some of the common infections that ciprofloxacin is used to treat include urinary tract infections (UTIs), respiratory tract infections, skin and soft tissue infections, and certain gastrointestinal infections. It can also be prescribed for the prevention of infections in certain cases, such as in individuals who are exposed to anthrax.

Ciprofloxacin is available in various forms, including oral tablets, extended-release tablets, and intravenous (IV) solutions. The dosage and duration of treatment depend on the type and severity of the infection and the patient's overall health condition.[6]

While ciprofloxacin can be highly effective against bacterial infections, it is essential to use it judiciously to minimize the risk of developing antibiotic resistance. Like all antibiotics, ciprofloxacin may cause side effects, with some of the common ones being gastrointestinal disturbances, dizziness, headache, and skin reactions. It is crucial to follow the healthcare provider's instructions regarding dosage and complete the full course of treatment even if the symptoms improve.

It is important to note that ciprofloxacin can interact with other medications or medical conditions, so it should be taken under the guidance of a healthcare professional. Additionally, certain groups of individuals, such as pregnant women, nursing mothers, and children, may require specific precautions or alternative treatment options.

Several clinical studies have demonstrated the effectiveness of ciprofloxacin ointment in treating bacterial skin infections. One such study published in the Journal of Antimicrobial Chemotherapy (https://academic.oup.com/jac/article/52/5/864/725645) found that ciprofloxacin ointment showed comparable efficacy to oral antibiotics in treating mild to moderate skin infections.:

MICONAZOLE

Miconazole is an antifungal medication used to treat a variety of fungal infections. It belongs to the imidazole class of antifungals and is commonly available in various forms, including creams, powders, sprays, and vaginal suppositories.

Physicochemical and pharmacokinetic properties of *Miconazole*

Physicochemical Parameters	**Values**
Molecular formula	$C_{18}H_{14}Cl_4N_2O$

Molecular weight	416.13 g/mol
Melting point	178-179°C
Log P	4.1
Aqueous solubility	96 mg/ml at 25° C
Pharmacokinetic Parameters	**Values**
Volume of distribution	95 546 L
Half life	1.3-1.7 hours.
Clearance	0.25 mg/1g
Bioavailability	0.01–0.025%

This medication is effective against a wide range of fungal organisms, such as Candida species (responsible for yeast infections) and dermatophytes (causing skin infections like athlete's foot and ringworm). Miconazole works by inhibiting the synthesis of ergosterol, a vital component of fungal cell membranes, leading to the disruption of the fungal cells and their subsequent death.

For topical applications, Miconazole is typically used to treat skin infections and conditions like ringworm, jock itch, and athlete's foot. It is also commonly used for the treatment of vaginal yeast infections. For more severe or systemic fungal infections, Miconazole can be administered intravenously, but this is less common.

Uses: Miconazole ointment is primarily used to treat superficial fungal infections affecting the skin. It is effective against a broad spectrum of fungi, including dermatophytes, yeast, and molds. Some of the common skin conditions treated with miconazole ointment include:

1. **Athlete's Foot (Tinea Pedis):** A fungal infection that affects the feet, particularly between the toes, causing itching, redness, and scaling.
2. **Ringworm (Tinea Corporis):** A contagious fungal infection that forms a circular, red rash on the skin, resembling a ring.
3. **Jock Itch (Tinea Cruris):** A fungal infection that affects the groin area, causing itching, redness, and a rash.
4. **Tinea Versicolor:** A superficial yeast infection that causes discolored patches on the skin, especially in hot and humid conditions.
5. **Vaginal Yeast Infections:** In some cases, miconazole ointment may also be used to treat vaginal yeast infections. However, it is more commonly available in the form of a vaginal suppository or cream.

As with any medication, Miconazole may cause some side effects, such as skin irritation, burning, or itching at the application site. Serious side effects are rare, but if you experience any allergic reactions or unusual symptoms, it is essential to seek medical attention promptly.

It is crucial to follow the prescribed dosage and treatment duration as directed by a healthcare professional to ensure the most effective and safe use of Miconazole. If you suspect a fungal infection or have any concerns about its treatment, consult with a healthcare provider for proper evaluation and management.

FLUOCINOLONE ACETONIDE:

Fluocinolone acetonide is a synthetic corticosteroid medication commonly used in dermatology for its anti-inflammatory and immunosuppressive properties. It is available in various formulations, such as creams, ointments, and intravitreal implants. The primary therapeutic applications of fluocinolone

acetonide include the treatment of various skin conditions, such as eczema, psoriasis, and allergic reactions, as well as certain eye disorders like diabetic macular edema and uveitis.

Physicochemical and pharmacokinetic properties of *Fluocinolone Acetonide*

Physicochemical Parameters	**Values**
Molecular formula	$C_{24}H_{30}F_2O_6$
Molecular weight	452.4882
Melting point	266-268.
Aqueous solubility	0.5 mg/ml
Pharmacokinetic Parameters	**Values**
Volume of distribution	95 546 L
Half life	20-30 hours.
Clearance	1% L/h/kg
Bioavailability	25–30%

The mechanism of fluocinolone acetonide involves binding to cytoplasmic glucocorticoid receptors, leading to the inhibition of pro-inflammatory mediators and the suppression of the immune response. This results in the alleviation of inflammation, itching, and redness associated with skin disorders.

Although fluocinolone acetonide can be effective in managing skin conditions and certain eye diseases, it is essential to use it cautiously due to potential side effects, particularly if used for an extended period or in excessive amounts. Common adverse effects may include skin thinning, irritation, and increased vulnerability to skin infections. Prolonged use of high-potency topical preparations can lead to systemic side effects like adrenal suppression.[4]

Fluocinolone Acetonide Ointment is commonly prescribed by dermatologists to treat a range of inflammatory skin conditions. Some of the key indications include:

1. **Eczema**: Fluocinolone Acetonide is highly effective in managing eczema, also known as atopic dermatitis. It helps in reducing the inflammation, redness, and itching associated with this chronic skin condition, providing much-needed relief to patients.
2. **Psoriasis**: This ointment is also used in the treatment of psoriasis, a chronic autoimmune disorder characterized by the rapid buildup of skin cells, leading to red, scaly patches on the skin.
3. **Contact Dermatitis**: Fluocinolone Acetonide Ointment is useful in treating allergic contact dermatitis, which occurs when the skin comes into contact with an allergen, causing redness, itching, and irritation.
4. **Seborrheic Dermatitis**: This common skin condition, often affecting the scalp and face, can also be managed effectively with Fluocinolone Acetonide Ointment.
5. **Lichen Planus**: Lichen Planus is an inflammatory skin condition that results in itchy, purplish bumps on the skin and mucous membranes. The ointment can provide relief and help in reducing the inflammation associated with this condition.

As with any medication, it is crucial to follow the prescribing doctor's recommendations and use fluocinolone acetonide only as directed. Patients should inform their healthcare provider about any other medications they are taking and any pre-existing medical conditions to prevent potential drug interactions or complications. Regular follow-up visits with the healthcare provider are essential to monitor treatment progress adverse effects.

RESEARCH ENVISAGED, AIM AND PLAN OF WORK

ENVISAGED RESEARCH:

In current research of formulation has been used to its medicinal and therapeutic application. The active chemical constituents (Ciprofloxacin, Miconazole, and Fluocinolone Acetonide) of individual are sufficient to attain the desirable anti-microbial therapeutic effects. But Innanoparticle formulations combining with other drug and constituents in a meticulous ratio, it could enhanced antimicrobial effect. The active constituents used from individual are adequate to provide attractive pharmacological action. There are evidences that drugstogether often have greater potency rather than isolated constituents so novel nanoparticle ointment formulation of drugs provide greater potency than isolated chemical formulation. Hence, it is assumed that similar findings will be obtained from present study.

AIM AND OBJECT

This ointment is a combination of nanoparticle of three drugs, namely Ciprofloxacin, Miconazole and Fluocinolone acetonide. Ciprofloxacin is a broad-spectrum antibiotic that acts against both aerobic (grow in the presence of oxygen) and anaerobic (increase in the absence of oxygen) gram-negative and gram-positive bacteria. It interferes with a bacterial enzyme involved in repairing and replicating bacteria's DNA (genetic material). Thus, it kills bacteria and clears the infection. Miconazole is an antifungal that damages the fungal cell membranes, which are essential for their survival. They prevent unwanted substances from entering into the cells and stop the leakage of cell contents and kill fungi. Fluocinolone acetonide is a corticosteroid that acts inside skin cells and inhibits the release of certain chemical messengers in the body that cause redness, itching, and swelling. When the skin reacts to any allergens, such chemicals are usually released.

The main objective to formulate this ointment is as following:

- Less work conducted
- Innovative
- Cheap & Best
- Better result
- Narrow Therapeutic index
- Less drug-drug And Drug excipients Interaction
- Better permeation Capacity to skin

Plan of Work

1. Selection of drug namely Ciprofloxacin Hcl USP, Miconazole USP,Fluocinolone Acetonide USP.
2. Selection and procurement of ointment base.
3. Analytical method development of drugs Ciprofloxacin Hcl USP,Miconazole USP,Fluocinolone Acetonide USP.
4. Physicochemical properties of drug according to literature reviews,
5. Compatibility study with the excipients and other drugs.
6. Nanoparticle development of drugs.
7. Evaluation of nanoparticles
8. Formulation and evaluation with different process and parameters of Ointment base.
9. Physical observation of the Ointment,
10. In-vitro Quality testing of Ointment

11. Stability Study
12. Antimicrobial Study

MATERIALS AND METHODS

MATERIALS

DRUG AND POLYMER

Ciprofloxacin, Miconazole, and Fluocinolone Acetonide was purchased from Xuang, CHINA. PLGA (75:25) of molecular weight (Mn= 39,440 Da, Mw= 71,330) was used for nanoparticles purchased from Sigma, USA.

CHEMICAL AND REAGENTS

The sources of chemicals and reagents have been given in table

Table of Chemicals/Reagents

Chemicals/Reagents	Supplier
Acetone	sd Fine-CHEM, Mumbai
Acetonitrile (HPLC grade)	J.T. Baker
Acetic acid (glacial)	Qualigens Fine Chemicalss, Mumbai
Ammonium acetate	Merck Ltd. Mumbai
DCM	sd-Fine CHEM, Mumbai
Dextrose	Purac, Germany
D- trehalose	Sigma, USA
EDTA	Loba chemie pvt Ltd. Mumbai
Ethyl acetate (HPLC grade)	J.T. Baker
Glycolide	Purac, Germany
HCl	RFCL Ltd, New Delhi
Heparin injection	Gland Pharma Ltd, Hyderabad
Hexane	J.T.Baker, USA
Hisperetin	Sigma, USA
Isopropyl alcohol	Merck Ltd, Mumbai
Mannitol	Loba chemie pvt Ltd, Mumbai
Methanol (AR grade)	sd Fine-CHEM, Mumbai
Methanol (HPLC grade)	J.T. Baker
PVA (MW 30,000-70,000)	Sigma, USA
Potassium dihydrogen ortho-phosphate	Merck, Mumbai
Potassium hydroxide	sd-Fine CHEM, Mumbai
Sodium phosphate dibasic	Ranbaxy, Fine chemicals Ltd. New Delhi
Sodium phosphate monobasic	Loba chemie pvt Ltd. Mumbai
Sodium chloride	sd Fine-CHEM, Mumbai
Sorbitol	Lobachemie, Mumbai
Stannous Octate	Sigma, USA

Tween 80	sd Fine-CHEM, Mumbai

METHODOLOGY

Method

HPLC analytical method development and validation for simultaneous in vitro analysis of Ciprofloxacin, Miconazole and Fluocinolone

HPLC analytical method was developed for simultaneous quantitative determination of Ciprofloxacin, Miconazole and Fluocinolone from in vitro drug release study samples. The combined stock solution of Ciprofloxacin, Miconazole and Fluocinolone was prepared in phosphate buffer containing 5% tween 80. The chromatogram of Ciprofloxacin, Miconazole and Fluocinolone has been shown in Fig. Validation parameters are shown in table. Accuracy and precision for HPLC analytical method was within limit of ICH guidelines.

PREPARATION OF NANOPARTICLES

Ciprofloxacin loaded PLGA nanoparticles have been prepared, by emulsion-diffusion-evaporation method, using ethyl acetate as an organic phase. So, we tried the same method for preparing Miconazole, loaded nanoparticles, and Fluocinolone Acetonide loaded nanoparticles but problem encountered with this method was early precipitation of drug Miconazole than polymer PLGA, due to more solubility of the later in ethyl acetate. Therefore this method was not followed.

To optimize Miconazole loaded Nanoparticles with respect to size, drug loading and encapsulation efficiency, following methods were tried

Nanoprecipitation method

Emulsion-diffusion-evaporation method

NANOPRECIPITATION METHOD

This is the reported method for Miconazole-loaded nanoparticles. In this method, 5 mg of Miconazole, and 45 mg of polymer (to give 10% of theoretical drug loading) were dissolved in 2 ml of acetone. After that, this solution was added dropwise to 40 ml of purified water under constant stirring. The precipitated nanoparticles were then separated by ultracentrifugation at 50,000 rpm for 2 hours. The separated nanoparticles were then washed with water and analyzed further for drug loading and entrapment efficiency. The same method was tried using DMSO as a solvent.

EVAPORATION METHOD DIFFUSION EMULSION

In this method, 5 mg of Miconazole and 45 mg of polymer were dissolved in 3 ml mixture of acetone: DCM (2:1). Then this solution was added dropwise to 5 ml of 1% PVA solution under constant stirring at 700 rpm. Then, this primary emulsion was then homogenized at 30,000 rpm for 7 minutes. Then, it was added dropwise to 20 ml of purified water under constant stirring at 700 rpm. The organic solvent was then allowed to evaporate for 3 hours at room temperature. After the complete evaporation of the organic solvent, the nanoparticles were separated from the aqueous dispersion by centrifugation at 10,000 g for 5 minutes. Nanoparticles were then washed twice with the purified water. The supernatant was then analyzed for drug loading and encapsulation efficiency. Similar procedure was followed for the preparation of Ciprofloxacin loaded, Fluocinolone Acetonide and triple agents loaded (Ciprofloxacin, Fluocinolone Acetonide and Miconazole) nanoparticles.

The emulsion diffusion evaporation method as mentioned above was selected and followed for further optimization of Ciprofloxacin loaded, Fluocinolone Acetonide loaded, Miconazole loaded and Ciprofloxacin, Fluocinolone Acetonide and Miconazole simultaneously loaded PLGA nanoparticles. The optimization of the nanoparticles each of Ciprofloxacin, Fluocinolone Acetonide, Miconazole and the combination of both was done with the different theoretical drug loadings like 5%, 10%, and 15% w/w was done. The parameters like particle size, drug loading, and entrapment efficiency were determined.

CHARACTERIZATION OF NANOPARTICLES

PARTICLE SIZE AND SIZE DISTRIBUTION

The evaluation of particle size and size distribution was conducted using the dynamic light scattering technique, employing a zeta sizer (NanoZS, Malvern Instruments, Worcestershire, UK), and the data were analyzed using the 'DTS Nano' software. To ensure accurate measurements, all formulations were appropriately diluted with Millipore water and vigorously shaken to achieve an adequate count rate. Subsequently, the average particle size and the polydispersity index (PDI) were recorded for all the formulations.

ENTRAPMENT EFFICIENCY OF NANOPARTICLES

Entrapment efficiency was assessed by analyzing the drug content present in the supernatant, which was obtained after the centrifugation of nanoparticles at 12,000 rpm for 30 minutes. The analysis for RPM was conducted using reverse phase HPLC (RP-HPLC) on a Shimadzu UFLC model, employing a C-18 column Inertsil (Octadecylsilane [ODS]-3 V) with dimensions of 4.6 x 250 mm. The mobile phase used was methanol–water (90:10 v/v) in an isocratic mode, with a flow rate of 1 ml/min, an analytical wavelength of 278 nm, and a 20 ml injection volume (Farah et al., 2013; Khan et al., 2013). Similarly, for PIP, the analysis was performed using RP-HPLC (Shimadzu UFLC model) on a C-18 column Inertsil (ODS-3 V) with dimensions of 4.6 x 250 mm. The mobile phase consisted of acetonitrile–water (60:40 v/v) in an isocratic mode, with a flow rate of 1.5 ml/min, an analytical wavelength of 343 nm, and a 20 ml injection volume (Chen et al., 2007). Calibration curves were generated within the concentration range of 0.1–50 mg/ml for RPM and 0.25–50 mg/ml for PIP, respectively. The entrapment efficiency was calculated using the formula provided below:

$$\text{Entrapment Efficiency} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug taken}} \times 100$$

TRANSMISSION ELECTRON MICROSCOPY

To investigate the morphology of the developed nanoparticles, we conducted Transmission Electron Microscopy (TEM) analysis. Carbon-coated grids were initially treated with the nanoparticle suspension for a period of 5–10 minutes. Following this, a thorough washing step was performed, and 2% uranyl acetate was utilized to stain the particles. Subsequently, the grids were left to air dry. The particles were observed at various magnifications, using a Hitachi H-7500 TEM, and TEM images were captured for further examination and analysis.

FREEZE THAW STUDY

A freeze-thaw study was conducted by exposing the nanoparticle dispersion to three freeze-thaw cycles. Each cycle involved freezing the nanoparticle dispersion at -20°C for a duration of half an hour in a deep freezer, followed by thawing at 30°C. The particle size and Polydispersity Index (PDI) were measured both before initiating the freeze-thaw cycles and after completing them using a zeta sizer. This measurement serves as a parameter to assess the physical stability of nanoparticles when subjected to abrupt temperature fluctuations.

XRD

The crystalline state of the drug i.e Ciprofloxacin Fluocinolone Acetonide and Miconazole before and after nanoparticle formation was evaluated by using an XRD instrument. From, XRD images it was observed that the drug was present in the crystalline state before that nanoparticle formation, but after the nanoparticles the drug changed from its crystalline to an amorphous state. This confirmed that, after nanoparticle formation drug gets molecularly dispersed in the polymeric matrix. The XRD images of Ciprofloxacin, Fluocinolone Acetonide, Miconazole, PLGA, Ciprofloxacin NPs, Miconazole NPs, Fluocinolone Acetonide NPs CFM-NPs have been shown in Fig.

IN VITRO RELEASE FROM NANOPARTICLES

The in vitro release of RPM and PIP from the nanoparticles was conducted using the dialysis bag method. Initially, pellets of nanoparticles obtained after centrifugation were resuspended in a release media (0.5 ml) to create a nanoparticle suspension equivalent to 0.5 mg of both drugs, comprising RPM nanoparticles, PIP nanoparticles, and co-encapsulated nanoparticles.

The resulting suspension was placed inside a dialysis bag and immersed in 9.5 ml of release media (composed of Saline and Isopropanol [IPA] in a ratio of 90:10) to maintain sink conditions. This setup was then subjected to continuous agitation at 100 rpm at a temperature of 37°C, following the procedure described by Khan et al. in 2013.

At predetermined time intervals, 8 ml of the release medium was withdrawn and replaced with an equal volume of fresh release medium. Subsequently, these samples were analyzed using HPLC. The same procedure was employed to assess the release profiles of the free drugs, specifically RPM and PIP.

DEVELOPMENT OF ANTIMICROBIAL FORMULATION

Certainly, a basic ointment is typically composed of an active pharmaceutical ingredient (API) suspended or dissolved in a suitable base or vehicle. Here's a simple formula for a general-purpose ointment base:

Ointment Base Formula:

Petrolatum: 80% (w/w) - This is the main ingredient in many ointment bases. It provides an occlusive barrier, which helps to retain moisture and protect the skin.

Mineral Oil: 20% (w/w) - Mineral oil is often added to ointment bases to improve their consistency and spreadability.

5.2.5.1 Method of Preparation of Ointment:

- Measure the required amount of petrolatum and mineral oil according to the desired batch size. For example, if you want to make 100 grams of ointment, you would use 80 grams of petrolatum and 20 grams of mineral oil.

- Place the petrolatum and mineral oil in a clean, heat-resistant container.
- Heat the mixture gently using a double boiler or microwave until the petrolatum melts and the two ingredients are thoroughly combined. Be cautious not to overheat the mixture.
- Stir the mixture while it cools to ensure even distribution of the ingredients.
- Once the ointment base has cooled and solidified, you can add your active pharmaceutical ingredient (API) if desired. Follow the specific instructions for adding your API, as the process may vary depending on the API's properties and solubility.
- Mix the API into the ointment base until it is uniformly distributed.
- Transfer the ointment into suitable containers, ensuring they are clean and sterile if intended for medical use.
- Label the containers with the ointment's name, API concentration, usage instructions, and any other required information.

This is a basic ointment base formula, and it can serve as a starting point for creating various ointments by incorporating different active ingredients as needed. Keep in mind that the specific formulation and processing may vary depending on the properties of your active ingredient and any regulatory requirements that apply to your product. Consulting with a qualified pharmacist or pharmaceutical formulation expert is advisable when developing ointments for medical or therapeutic purposes.

FORMULA FOR THE OINTMENT

1. Wool Fat (Lanolin): Lanolin is a natural emollient and moisturizer. The percentage of lanolin in the ointment can vary based on the desired consistency and properties but typically ranges from 15% to 40%.
2. Hard Paraffin: Hard paraffin is used to increase the ointment's consistency and make it thicker. It can be included at around 10% to 30% of the total formulation.
3. White Soft Paraffin: White soft paraffin acts as an emollient and helps in spreading the ointment smoothly. It can be added at approximately 10% to 35%.
4. Cetostearyl Alcohol: Cetostearyl alcohol serves as an emulsifier and stabilizer. It can be used at around 2% to 5% to help blend the water and oil components.
5. Cetyl Palmitate: Cetyl palmitate is used as a thickening agent and skin-conditioning agent. It can be included at about 3% to 5% to give the ointment a smooth texture.

Additives:

6. Propylparaben: Propylparaben can be used as a preservative to extend the shelf life of the ointment. Typically, it is added at around 0.1% to 0.5% of the total formulation.
7. Perfume: The fragrance or perfume can be added to the ointment for a pleasant scent. The amount can vary based on personal preference but is usually kept low, around 0.1% to 0.5%.

CHARACTERIZATION OF OINTMENT

Characterization of an ointment involves a series of tests and analyses to determine its physical, chemical, and pharmaceutical properties. This characterization is essential to ensure the quality, stability, and performance of the ointment. Here are some common aspects to consider when characterizing an ointment:

➢ Physical Appearance and Texture:

- Visual inspection for color, odor, and consistency.

- Texture analysis, which may include measurements of firmness, spreadability, and stickiness.

➢ pH Value:

- Determination of the pH level to ensure it falls within the appropriate range for skin application.

➢ Rheological Properties:

- Rheological testing to assess the flow and viscosity of the ointment, which is important for application and stability.

➢ Microscopic Examination:

- Microscopic analysis to observe the uniformity of the ointment, the presence of any particulate matter, and the distribution of any drug particles or nanoparticles.

➢ Particle Size Distribution:

- If applicable, measurement of the particle size distribution of any drug-loaded nanoparticles in the ointment.

➢ Drug Content and Uniformity:

- Quantitative analysis to determine the concentration of the active pharmaceutical ingredient (API) or drug-loaded nanoparticles in the ointment.

➢ Stability Studies:

- Accelerated stability testing to assess how the ointment performs under various environmental conditions (e.g., temperature, humidity) over time.

➢ Spreadability

- The spread ability of ointment formulations was determined by measuring the Spreading diameter of 1g of ointment between two horizontal plates.

IN-VITRO RELEASE OF FORMULATION

Cellophane membrane was pre-soaked in distilled water for 24 h (1 g)spread over cellophane membrane was mounted on Franz-type diffusion cells with a receptor compartment volume of 33.2 ml and an effective diffusion area of 3.14 cm2. The receptor fluid was selected as phosphate buffer (pH 7.4) containing 25% (v/v) ethanol to maintain sink conditions. During the experiments, the receptor phase was kept at 37C and continuously stirred at 600 rpm. At certain time intervals, 1 ml samples were withdrawn from the receiver compartment and replaced with an equal volume of fresh receptor fluid.

Data Analysis via Drug Release Kinetics study

Results of in-vitro release profile obtained for all the formulations were plotted
in kinetic models as follows,

1. Cumulative of drug released versus time (zero order kinetic model).
2. Log cumulative percent drug remaining to be absorbed versus time (First order model)
3. Cumulative amount of drug release versus square root of time (Higuchi model)
4. Log cumulative drug released versus log time (Korsmeyer-Peppas model)

➢ **Zero order release kinetics:**

Zero order release would be predicted by the following equation,

$Q_t = Q_O + Kt$

Where, Qt is the amount of drug dissolved in time t,

Q_0 is the initial amount of drug in the solution (most times, $Q_0=0$) and K is the zero order release constant.

➢ **First order release kinetics:**

First order release would be predicted by the following equation,

$$Q_t = Q_o e^{-K_1 t}$$

Where, Qt is the amount of drug released in time t,
Q_0 is the initial amount of drug in the solution and
K is the first order release constant.

➢ **Higuchi kinetics**

A plot of the fraction of drug released against root of time will the linear if the release obeys Higuchi Equation. This equation describes drug release as a diffusion process based on the Flick's Law, Square root time dependent.

$Q=Kt^{1/2}$

Q=Amount of drug release per unit area in time t,
K=release rate constant

➢ **Peppas & Korsemayer equation**

The amount of drug released at time t (Mt) with respect to the total amount of drug released (M_∞), can be expressed in terms of an exponential expression as follows:

$$M_t / M_\infty = kt^n$$

Where, M_t / M_∞ = The fraction of drug released at time t, K = Constant incorporating the structural and geometrical characteristic of the drug /polymer system.
n = diffusion exponent related to the release

N	Mechanism
0.45	Fickian diffusion
0.45 < n < 0.89	Anomalous(Non-Fickian) diffusion
0.89	case II transport
Above 0.89	Super case II transport

The model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved. 'n' value could be used to characterize different release mechanism.

STABILITY STUDY (STORAGE STABILITY)

A stability study for an antimicrobial ointment is essential to ensure that the product maintains its quality, efficacy, and safety throughout its shelf life under various storage conditions. These studies are typically conducted in accordance with regulatory guidelines and include monitoring physical, chemical, and microbiological parameters. Here's an overview of how to conduct a stability study for an antimicrobial ointment:

- Clearly define the objectives of the stability study, such as determining the ointment's shelf life, identifying storage conditions, and assessing the impact of temperature and humidity.

- Select storage conditions based on the intended market and regulatory requirements (e.g., ICH guidelines).

Sample Preparation:

- Prepare multiple batches of the antimicrobial ointment, ensuring that they represent the final commercial product.
- Document the formulation, including ingredients, concentrations, and manufacturing processes.

Storage Conditions:

- Place samples of the ointment in containers suitable for long-term storage (e.g., tubes, jars).
- Store the samples under controlled conditions, including accelerated stability conditions (e.g., elevated temperature and humidity), long-term stability conditions (e.g., room temperature), and any relevant stress conditions (e.g., freeze-thaw cycles).

Sample Testing Schedule:

- Define a testing schedule at predetermined time points (e.g., 0, 3, 6, 9, 12 months) for analysis.
- Testing should include a range of physical, chemical, and microbiological tests.

Physical and Chemical Testing:

- Physical appearance: Assess color, odor, texture, and homogeneity.
- pH measurement: Check if the pH remains within the specified range.
- Viscosity: Measure the viscosity to ensure consistency.
- Microscopic examination: Examine for changes in particle size, shape, or crystallinity.
- Melting point: Monitor for changes in the melting point if applicable.
- Chemical stability: Perform assays to determine the concentration of active ingredients and degradation products.
- Compatibility with packaging: Evaluate the interaction between the ointment and its packaging material.

Microbiological Testing:

- Perform microbiological tests to assess the ointment's microbial stability. This includes testing for the presence of microorganisms and ensuring that preservatives are effective.

Stability-Indicating Methods:

- Develop or use stability-indicating methods for chemical assays to detect degradation products or changes in the active ingredients.

Data Analysis:

- Analyze the data collected during the stability study, comparing results at different time points to initial values.
- Use statistical analysis to identify trends or significant changes.

Shelf-Life Determination:

- Based on the data obtained, determine the estimated shelf life of the antimicrobial ointment under recommended storage conditions.

Reporting:

- Prepare a comprehensive stability report that summarizes the study, including objectives, methods, results, and conclusions.
- Include recommendations for storage conditions, shelf life, and any necessary product labeling changes.

Regulatory Compliance:

- Ensure that the stability study is conducted in compliance with relevant regulatory guidelines and standards, such as ICH guidelines for stability testing.

Ongoing Monitoring:

- After the initial stability study, continue to monitor the product's stability periodically throughout its shelf life to ensure ongoing compliance.

A well-designed stability study for an antimicrobial ointment is critical to ensuring product quality and safety. It helps manufacturers make informed decisions regarding product labeling, storage recommendations, and product lifecycle management. Additionally, stability data may be required as part of regulatory submissions for marketing authorization.

ANTI-MICROBIAL ACTIVITY

Antimicrobial Activity of Ciprofloxacin-Loaded Nanoparticle Preparations

Determining the antimicrobial activity of an ointment involves assessing its ability to inhibit the growth or kill microorganisms, such as bacteria, fungi, or other pathogens.

Here are the general steps to determine the antimicrobial activity of an ointment:

- **Ointment Samples**: Prepare samples of the ointment for testing.
- **Microorganisms**: Choose relevant microorganisms (bacteria, fungi, etc.) that you want to test the ointment against. These may include clinical isolates or standard strains.
- **Culture Media**: Use appropriate culture media for the growth and maintenance of the chosen microorganisms.
- **Incubator**: To maintain controlled temperature conditions for microbial growth.
- **Petri Dishes**: For agar plate cultures.
- **Sterile Swabs or Inoculating Loops**: For inoculating microorganisms onto agar plates.
- **Antimicrobial Controls**: Positive control (standard antimicrobial agent) and negative control (no treatment).

Procedure:

- **Preparation of Microbial Cultures**:
  - Inoculate agar plates with the selected microorganisms and incubate them until visible colonies form. This will be your microbial lawn for testing.
- **Sample Application**:
  - Apply a known amount of the ointment onto sterile disks or onto the agar surface in the form of streaks, wells, or discs.
- **Incubation**:
  - Incubate the agar plates with the ointment samples at the appropriate temperature (e.g., 37°C for bacteria, 25°C for fungi) for a defined period (e.g., 24-48 hours).
- **Measurement and Observation**:
  - After incubation, observe the agar plates for zones of inhibition (clear areas) around the ointment sample application sites. A clear zone indicates antimicrobial activity.

- **Measurement of Zone of Inhibition**:
  - Measure the diameter of the zones of inhibition in millimeters. This provides a quantitative measure of the antimicrobial activity. A larger zone typically indicates stronger antimicrobial activity.
- **Comparison with Controls**:
  - Compare the results of the ointment samples with positive and negative controls. The positive control should show antimicrobial activity, while the negative control (no treatment) should not.
- **Repeat and Statistical Analysis** (if needed):
  - For reliable results, repeat the experiment multiple times.
  - Perform statistical analysis to determine if the observed differences in zone size are statistically significant.
- **Data Analysis and Reporting**:
  - Present your findings in a clear and organized manner.
  - Report the diameter of the zones of inhibition for each ointment sample and control.
  - Discuss the significance of the antimicrobial activity and its implications.

6. RESULTS AND DISCUSSION

6.1. ANALYTICAL METHOD DEVELOPMENT

Analytical method was developed and Validated for simultaneous *in vitro* analysis of Ciprofloxacin, Miconazole and Fluocinolone by HPLC

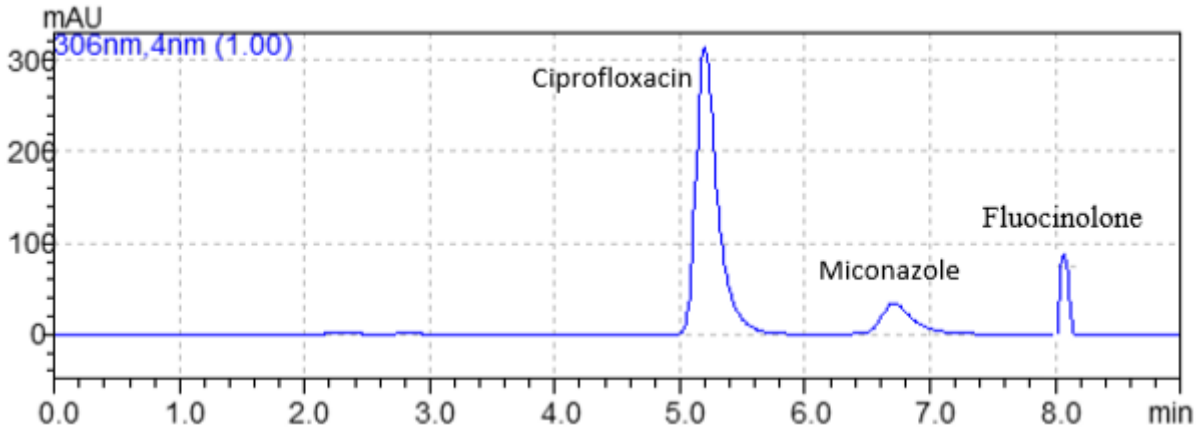


Fig. HPLC chromatogram of drugs.

Table.Validation parameters for simultaneous *in vitro* analysis of Ciprofloxacin, Miconazole and Fluocinolone by HPLC

Validation parameter	Ciprofloxacin	Miconazole	Fluocinolone
Range	2 – 50 µg/ml	2 – 50 µg/ml	2 – 50 µg/ml
Linearity (R^2)	0.99 ± 0.0006	0.996 ± 0.0004	0.992 ± 0.0002
Slope	34284.67± 376.2	56959.33 ± 2493.42	43672.54± 3481.11
Intercept	4101.667± 858.38	16951.67 ± 5878.20	15457.52 ± 4254.40
LOQ	0.92	1.47	1.32
LOD	0.30	0.49	0.37

(Data expressed as mean ± SD, n=3)

Table. Inter and intra-day accuracy, precision and specificity for Ciprofloxacin

Conc. (µg/ml)	**Intraday**			**Interday**		
	% Accuracy	Precision	% Recovery*	% Accuracy	Precision	% Recovery*
2.5	103.03 ± 2.05	0.853	100.90 ± 1.53 ±	101.47± 0.87±	2.024	97.96 ± 0.72
15	102.58 ± 1.75	0.878	100.47 ± 1.53	101.02 ± 0.90	0.562	99.00 ± 0.55
40	101.76 ± 0.56	0.831	99.77 ± .91	100.28 ± 0.84	1.753	98.03± 0.88

*Specificity in drugs samples spiked with polymer (PLGA) and PVA

(Data expressed as mean ± SD, n=3)

Table. Inter and intra-day accuracy, precision and specificity forMiconazole

Conc. (µg/ml)	**Intraday**			**Interday**		
	% Accuracy	Precision	% Recovery*	% Accuracy	Precision	% Recovery*
2.5	98.90 ± 1.44	1.637	99.33 ± 0.74	102.29 ± 1.61	1.41	99.33 ± 2.05
15	99.96 ± 1.73	2.32	101.80 ± 0.91	102.99 ± 2.32	1.68	101.80 ± 0.83
40	99.63 ± 1.25	1.29	99.22 ± 1.23	101.47± 1.29	1.23	99.22 ± 1.74

*Specificity in drugs samples spiked with polymer (PLGA) and PVA

(Data expressed as mean ± SD, n = 3)

Table. Inter and intra-day accuracy, precision and specificity forFluocinolone

Conc. (µg/ml)	**Intraday**			**Interday**		
	% Accuracy	Precision	% Recovery*	% Accuracy	Precision	% Recovery*
2.5	96.70 ± 2.35	1.85	96.21 ± 0.86	101.80 ± 1.82	1.38	98.45 ± 2.15
15	97.55 ± 1.75	2.50	96.60 ± 0.75	101.40 ± 1.40	1.89	99.70 ± 0.72
40	97.63 ± 1.50	2.89	97.56± 1.35	102.35± 1.85	1.75	97.42 ± 1.44

*Specificity in drugs samples spiked with polymer (PLGA) and PVA

(Data expressed as mean ± SD, n = 3)

PREPARATION OF NANOPARTICLES

Ciprofloxacin-loaded nanoparticles (Cip NPs), Fluocinolone Acetonide loaded nanoparticles (Flu NPs), Miconazole-loaded nanoparticles (Mic NPs), and Ciprofloxacin Fluocinolone Acetonide and Miconazole simultaneously loaded PLGA nanoparticles (CFM-NPs) were fabricated using the emulsion-diffusion-evaporation method. In this method, an organic phase comprised of an Acetone: DCM mixture in a 2:1 ratio was used, while water served as the aqueous phase.

Previously in our laboratory, we successfully prepared Ciprofloxacin-loaded PLGA nanoparticles and fluocinolone Acetonide PLGA nanoparticles using the same emulsion-diffusion-evaporation method, but employing ethyl acetate as the organic phase. When we attempted to prepare Miconazole-loaded nanoparticles using this method, we encountered a challenge. The issue was the premature precipitation of Miconazole due to its higher solubility in ethyl acetate compared to the PLGA polymer. Consequently, this method was deemed unsuitable for Miconazole encapsulation.

We then turned to the nanoprecipitation method, a technique previously reported for Miconazole nanoparticle preparation. However, this method presented its own set of challenges, including the production of very small nanoparticles (less than 100 nm), necessitating ultracentrifugation for nanoparticle separation. Additionally, there were issues with low drug loading (<1%) and poor encapsulation efficiency (<12%). These problems were likely linked to Miconazole's high solubility in the solvent.

To mitigate the solubility issue, we substituted DMSO for acetone as the organic solvent. This alteration resulted in nanoparticles of the desired size range (150-200 nm) and slightly improved drug loading. However, these nanoparticles exhibited limited long-term stability.

The primary reason for this short-term stability was the absence of a surfactant. Surfactants coat the nanoparticles, imparting a charge to their surface. As a result, all nanoparticles acquire a similar charge, which leads to repulsion between them and enhances their stability in aqueous dispersion. Therefore, we revisited the emulsion-diffusion-evaporation method, which typically incorporates surfactants. As mentioned earlier, we had previously used PVA as a surfactant in this method for nanoparticle preparation.

However, the choice of an appropriate organic solvent was crucial. After experimenting with different acetone: DCM ratios (1:1 and 2:1), we found that the 2:1 ratio of acetone: DCM yielded better results, including smaller particle size (<250 nm), higher entrapment efficiency (~65%), and increased drug loading (~10%). Consequently, we selected acetone: DCM in a 2:1 ratio and 0.5% PVA as the surfactant for nanoparticle preparation. Detailed experimental data for drug loading and encapsulation efficiency of Miconazole nanoparticles are provided in Table. This same methodology was subsequently employed for the preparation of Ciprofloxacin-loaded nanoparticles Fluocinolone Acetonide nanoparticles and Ciprofloxacin, Fluocinolone Acetonide PLGA nanoparticles and Miconazole simultaneously loaded nanoparticles

Experiments done to improve the stability and encapsulation efficiency of NPs

Sr. No.	**Method**	**Organic solvent**	**Ratio (Org:Aq.)**	**PVA conc. (% w/v)**	**Drug loading (% w/w)**	**Encapsulation efficiency(%)**
1	Nanoprecipitation	Acetone	1:20	-	0.71 ± 0.15	11.98 ± 0.85
2	Nanoprecipitation	DMSO	1:20	-	1.85 ± 0.34	22.78 ± 1.35
3	Nanoprecipitation	Acetone: DCM (1:1)	1:10	-	1.41 ± 0.26	25.4 ± 1.65
4	Emulsion-Diffusion-Evaporation	Ethyl acetate: DCM (2:1)	1:10	0.5	4.16 ± 0.95	35.16 ± 1.89

5	Emulsion-Diffusion-Evaporation	Acetone: DCM (1:1)	1:10	1	5.86 ± 0.27	48.54 ± 2.25
6	Emulsion-Diffusion-Evaporation	Acetone: DCM (1:1)	1:10	0.5	7.64 ± 0.76	54.92 ± 2.79
7	Emulsion-Diffusion-Evaporation	Acetone: DCM (2:1)	1:7	0.5	**9.53 ± 0.85**	**64.72 ± 2.21**

(Data expressed in Mean ± SD, n=3)

OPTIMIZATION OF THE NANOPARTICLES

Optimization of the Ciprofloxacin nanoparticles

Theoretical drug loading (% w/w)	Size (nm)	Practical Drug loading (% w/w)	Encapsulation efficiency (%)	Yield (%)
5 %	222.56 ± 8.5	6.63 ± 0.51	80.39 ± 2.98	58.26 ± 0.92
10 %	235.66 ± 9.5	10.72 ± 0.27	90.05 ± 0.59	61.72 ± 0.59
15 %	266.7 ± 6.97	14.92 ± 0.37	95.54 ± 0.7	68.43 ± 0.6

(Data expressed in Mean ± SD, n=3)

Optimization of Fluocinolone Acetonide nanoparticles

Theoretical drug loading (% w/w)	Size (nm)	Practical Drug loading (% w/w)	Encapsulation efficiency (%)	Yield (%)
5 %	232.20 ± 4.5	8.28 ± 0.41	70.30 ± 2.24	68.16 ± 0.62
10 %	239.16 ± 5.5	12.72 ± 0.20	80.05 ± 1.59	71.32 ± 0.54
15 %	256.10 ± 8.97	18.82 ± 0.32	96.34 ± 1.2	58.53 ± 0.65

Optimization of Miconazole nanoparticles

Theoretical drug loading (% w/w)	Size (nm)	Practical Drug loading (% w/w)	Encapsulation efficiency (%)	Yield (%)
5 %	233.86 ± 9.16	4.98 ± 0.25	59.66 ± 3.54	76.83 ± 0.35
10 %	246.2 ± 8.48	9.93 ± 0.27	69.65 ± 2.80	80.85 ± 0.37
15 %	278.83 ± 10.66	14.81 ± 0.24	81.11 ± 2.24	82.2 ± 0.65

(Data expressed in Mean ± SD, n=3)

Optimization of Ciprofloxacin, Fluocinolone Acetonide and Miconazole simultaneously loaded nanoparticles

Theoretical drug loading (% w/w)	Size (nm)	Practical Drug loading (% w/w)	Encapsulation efficiency (%)	Yield (%)

		Ciprofloxacin	*Miconazole*	*Ciprofloxacin*	*Miconazole*	
5 %	***226.7 ± 6.89***	***5.13 ± 0.27***	***3.20 ± 0.20***	***82.40 ± 3.67***	***55.34 ± 1.16***	***58.23 ± 0.87***
10 %	***257.16 ± 9.45***	***9.88 ± 0.20***	***6.86 ± 0.29***	***97.35 ± 0.44***	***54.27 ± 0.44***	***72.33 ± 0.85***
15 %	***283.5 ± 7.9***	***13.15 ± 0.22***	***9.21 ± 0.22***	***97.29 ± 0.68***	***53.18 ± 0.68***	***80.36 ± 0.77***

(Data expressed in Mean ± SD, n=3)

CHARACTERIZATION OF NANOPARTICLES

SIZE AND ZETA POTENTIAL

Characterization of Cip NPs, Flu NPs, Mic NPs and CFM-NPs at 10% w/w theoretical drug loading

Drug NPs	**Size (nm)**	**PDI**	**Zeta potential (mv)**
Cip NPs	225.5± 10.5	0.192 ± 0.05	- 6.17 ± 1.42
Flu NPs,	232.5± 8.5	0.152 ± 0.05	- 4.17 ± 1.12
Mic NPs	232.5 ± 20.3	0.209 ± 0.14	- 7.77 ± 0.39
CFM-NPs	245.7 ± 35.6	0.222 ± 0.11	- 5.83 ± 1.51

(Data expressed as mean ± SD, n=3)

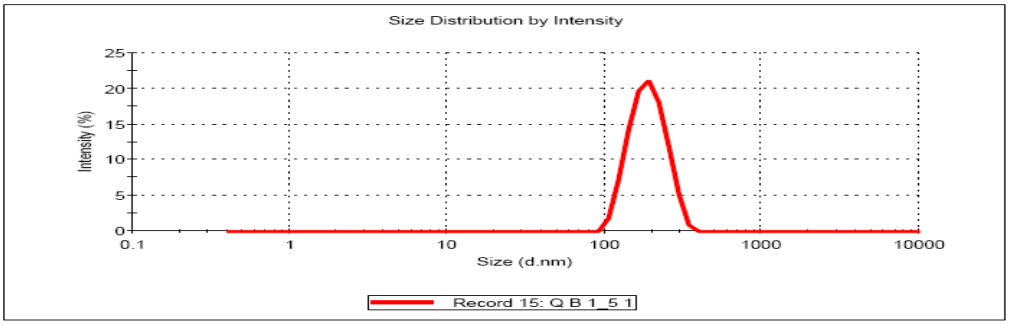


Fig. Size distribution of Ciprofloxacin Nanoparticles

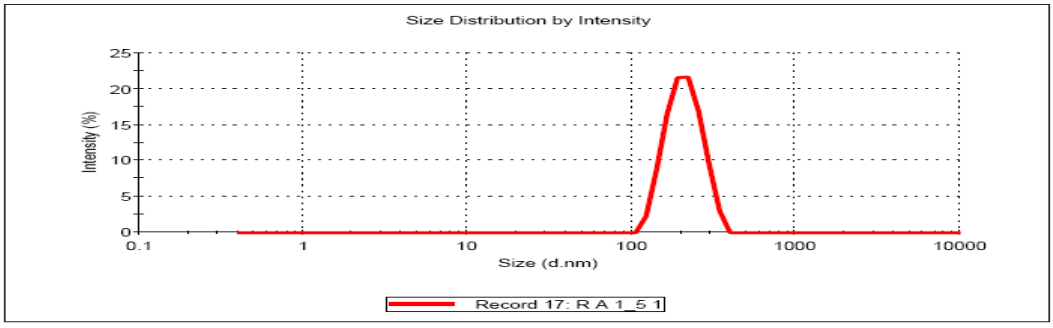


Fig. Size distribution of Fluocinolone Nanoparticles

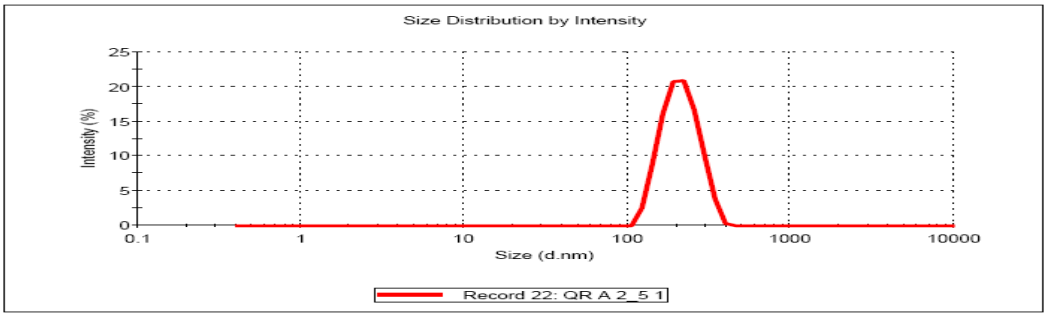


Fig. Size distribution of miconazole nanoparticles

XRD ANALYSIS

XRD images shows the drug nature of nanoparticle and alone

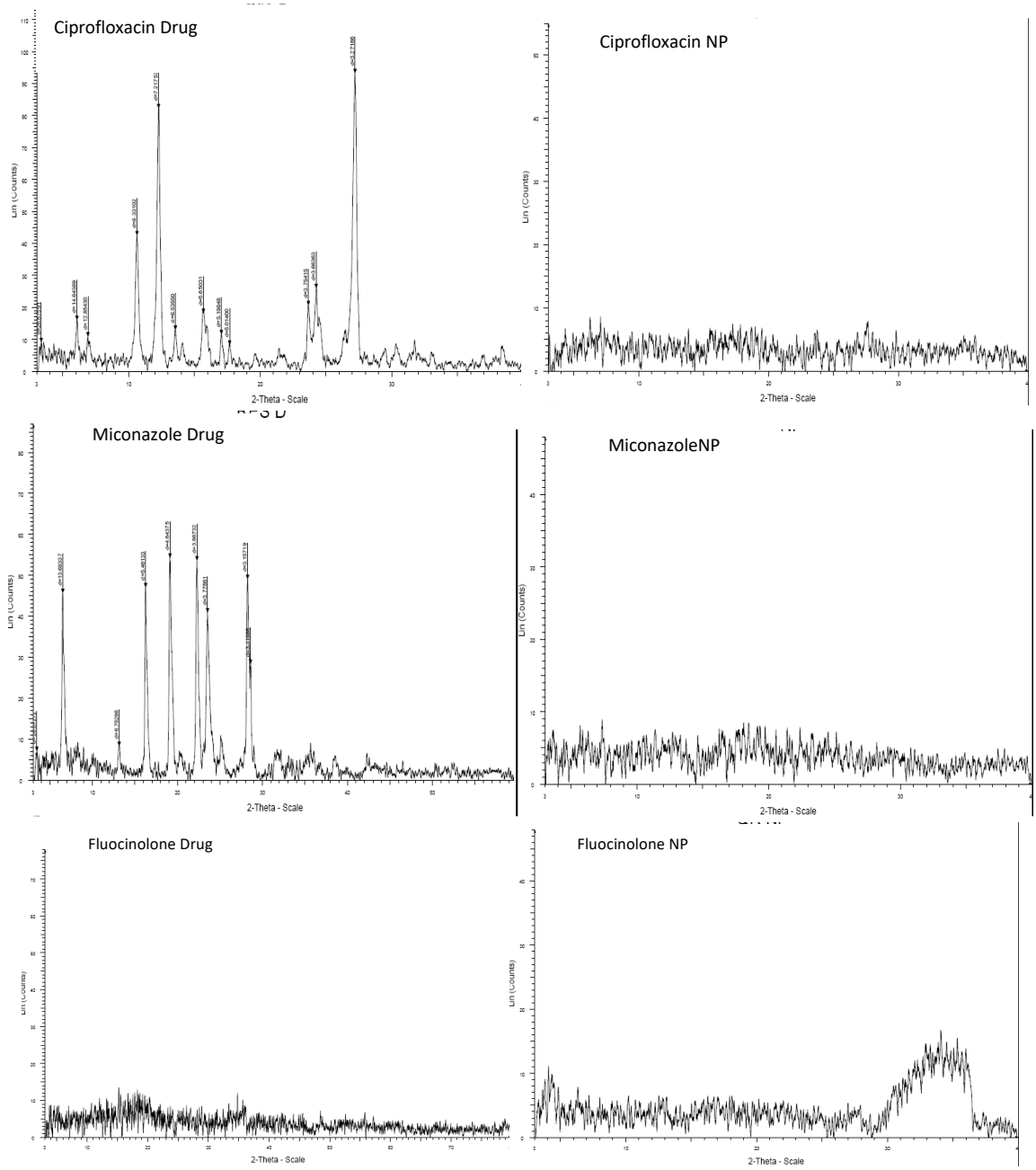


Figure XRD Images of Drugs and Nanoparticles

TEM ANALYSIS

Surface morphology of the nanoparticles was studied by using Transmission Electron Microscopy (TEM) (FEI, TECNAI, Netherland). TEM image showed spherical nanoparticles with smooth surface. The TEM image of QR-NPs has been shown in Fig.

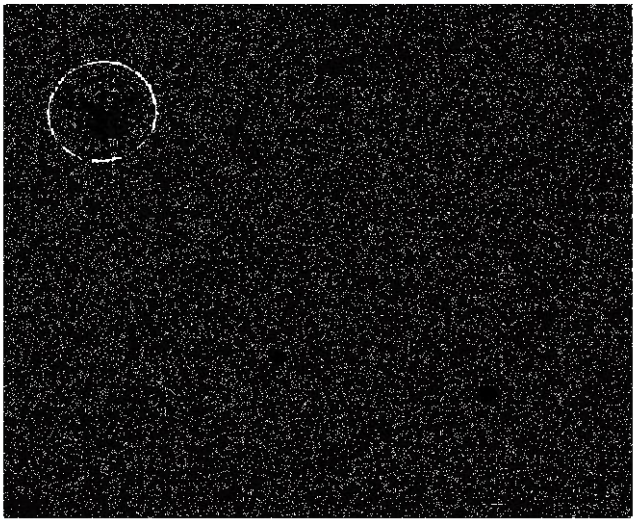


Fig. TEM image of CFM-NPs (inside image showing the magnified view of a single nanoparticle.) (scale bar = 200nm)

Freeze thaw Analysis

Sucrose, lactose, mannitol, sorbitol, inulin, dextrose and d-trehalose were screened as lyoprotectants in freeze drying of nanoparticles at 10% w/v concentration. The effect of different lyoprotectants was studied on cake formation, particle size and redispersibility. An increase inparticle size was observed in nanoparticles without lyoprotectant. Collapsed cake was formed where dextrose, lactose and sucrose were used. Intact fluffy cake was observed in case of d- trehalose, inulin, mannitol and sorbitol.

The ratio of final particle size (S_f) to initial particle size (S_i) was found to be greater than 1 in case of lyoprotectants such as glucose, sucrose, dextrose, and sorbitol. The increase in ratio (S_f/S_i) indicates that there is increase in particle size when these lyoprotectants were used. There was no change in particle size after freeze drying when d-trehalose was used as a lyoprotectant.

Screening of lyoprotectants used in freeze drying

Cryoprotectants	Ratio (S_f/S_i)	Cake formation	Redispersibility
No	1.48	Intact fluffy cake	_
Lactose	0.98	Collapsed cake	+
Trehalose	1.00	Intact fluffy cake	+++
Inulin	1.01	Intact fluffy cake	++
Mannitol	1.01	Intact fluffy cake	+++
Dextrose	1.04	Collapsed cake	+
Sorbitol	1.03	Intact fluffy cake	+++
Sucrose	1.09	Collapsed cake	_

S_f= Particle size after freeze drying
S_i= Particle size before freeze drying

DEVELOPMENT OF OINTMENT

The physical appearance and texture of an antimicrobial ointment containing ciprofloxacin, miconazole, and fluocinolone acetonide can vary based on the specific formulation and manufacturing process.
The exact proportions may need to be adjusted based on the specific requirements of ointment, as well as any regulations or standards applicable to cosmetic or pharmaceutical products in your region.

Ingredient /Formulations	F1	F2	F3	F4	F5	F6
Drug loaded Nanoparticles	5	5	5	5	5	5
Wool Fat	35	25	30	35	30	25
Hard paraffin	25	25	25	25	25	25
Cetostearyl alcohol	5	15	5	10	10	10
Cetyl palmitate	4	4	4	4	4	4
White soft paraffin	25	25	30	20	25	30
Propylparaben	0.5	0.5	0.5	0.5	0.5	0.5
Perfume	0.5	0.5	0.5	0.5	0.5	0.5

METHOD OF PREPARATION:

- In a separate container, melt the hard paraffin, cetostearyl alcohol, cetyl palmitate, and white soft paraffin together to form the oily phase. Heat gently until fully melted, and mix well.
- Add the wool fat (lanolin) to the oily phase and continue mixing until a homogenous mixture is obtained.
- Allow the oily phase to cool down to a temperature where it is still liquid but not hot.
- Carefully incorporate the drug-loaded nanoparticles into the cooled oily phase. Mix thoroughly to ensure even distribution.
- Add the propylparaben (preservative) and perfume (fragrance) to the mixture and stir well.
- Continuously mix the ointment while it cools to room temperature, ensuring that it remains well-blended.
- Once the ointment has reached room temperature and solidified, transfer it into suitable containers or tubes for storage and use.
- Label the containers with the ointment name, ingredients, and any necessary usage instructions.

CHARACTERIZATION OF OINTMENT

1. Physical Appearance:

The color of the ointment may vary depending on the formulation, but it is typically a cream or white color. The color can be influenced by the active ingredients and any excipients or additives used.
The ointment is a semi-solid dosage form, often with a smooth and uniform appearance. It should not have visible clumps, crystals, or particles.

2. Texture:

The texture of the antimicrobial ointment is usually smooth and homogeneous. It should spread easily when applied to the skin.

It is a semi-solid with a consistency that allows for easy application without being too runny or too stiff. When scooped out of its container, it should hold its shape but still be pliable and easily spreadable. The texture should not be gritty, lumpy, or contain any solid particles that can cause discomfort when applied to the skin.

3. pH Value

The pH value of an antimicrobial ointment containing ciprofloxacin, miconazole, and fluocinolone acetonide can vary depending on the specific formulation and the pH of the individual ingredients used. The pH of an ointment is an important parameter as it can affect the stability, efficacy, and skin compatibility of the product. Typically, ointment isformulated to have a pH that is close to the pH of the skin, which is around 4.5 to 5.5.

4. Viscosity of ointment

Viscosity was measured by viscometer

pH of the formulation was measured and results are shown in the table below.

Table pH and viscosity of the ointment

S. no.	Formulation Code	pH	Viscosity (cps in 100 rpm)
1	F1	5.1	2850±20
2	F2	4.4	2940±35
3	F3	5.0	2900±25
4	F4	5.3	2850±45
5	F5	4.5	2650±23
6	**Reference F6**	4.8	2655±22

5. Particles in Ointment

Table no. Results of evaluation of F3

S . No.	**Hard and sharp edged particles**	**Fineness**
1	Absent	passes
2	Absent	passes
3	Absent	Passes
4	Absent	Passes
5	Absent	Passes
Reference grade	Absent	Passes

6. Consistency Color and Odour of Ointment

Table Evaluation of physical characteristics of Ointment

Formulation	**Consistency**	**Colour**	**Odour**
F1	Semi solid	Light yellow	Mood elevating

F2	Semi- solid	Light yellow	Mood elevating
F3	Semi- solid	Light yellow	Mood elevating
F4	Semi- solid	Light yellow	Mood elevating
F5	Semi- solid	Light yellow	Mood elevating
Reference F6	Semi- solid	White	Mood elevating

Ointment formulated as semi-solid in consistency, ointment was light yellow in colour and fragrance was mood elevating and very pleasant in taste.

IN VITRO EVALUATION OF THE FORMULATION

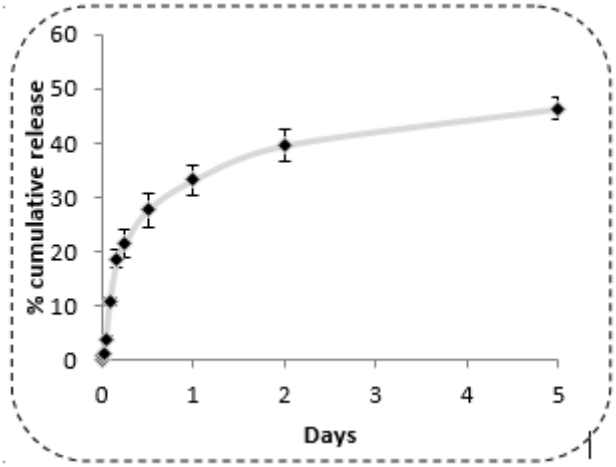
1. Drug release study

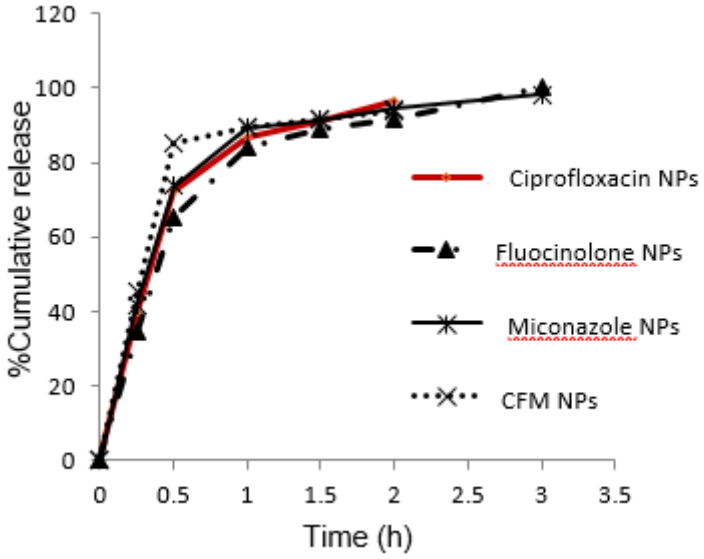
Release of drug form each of the nanoparticles i.e Ciprofloxacin NPs, Fluocinolone Acetonide, Miconazole NP, Ciprofloxacin Fluocinolone Acetonide and Miconazole combined NPs was evaluated. Ciprofloxacin, Fluocinolone Acetonide and Miconazole showed a biphasic release pattern from the nanoparticles. Ciprofloxacin and Fluocinolone Acetonide showed initial fast release followed by sustained release for 40 days, while Miconazole showed initial fast release followed by sustained release for 20 days. About 67% of drug was released from Ciprofloxacin NPs and 75% drug of Fluocinolone Acetonide NPs, 74% released from Miconazole while 66.5 % of Ciprofloxacin 72% drug of Fluocinolone Acetonide and 71% of Miconazole was released from combination NPs. To further mass balance the drug remaining in the nanoparticles.

The release profiles were fitted in different kinetic models and were characterized by correlation coefficient values which are denoted in table 20. The release profile of Ciprofloxacin, Fluocinolone Acetonide and Miconazole from nanoparticles was found to follow Higuchi diffusion controlled release model.

Model fitting of *in vitro* drug release profile of nanoparticles using R^2 values

Drug NPs	**R^2 value**			
	Zero order	**First order**	**Higuchi**	**Hixon Crowell**
Ciprofloxacin	0.710	0.822	0.874	0.438
Fluocinolone Acetonide	0.750	0.662	0.854	0.490
Miconazole	0.428	0.568	0.638	0.238
CFM- NPs				
Ciprofloxacin	0.847	0.928	0.966	0.859
Fluocinolone Acetonide	0.952	0.861	0.934	0.590
Miconazole	0.469	0.619	0.666	0.246





2. Antimicrobial Study of Ointment

Drugs were evaluated for its anti-microbial activity and isolation of active ingredients, were performed and after that ointment were prepared and evaluated. Various observations and results obtained from evaluation are discussed in this chapter.

Screening of Antimicrobial activity of CFM nanoparticle

The screening of anti-microbial activity was performed with the help of disc diffusion method. Following tables shows anti-microbial activity of nanoparticles at given concentration against gram negative bacteria, gram positive bacteria and fugai (Candida albicans)

Table Zone of inhibition for various concentrations of *CFM nanoparticles* compared to reference drugs: activity against gram negative bacteria.

Micro-Organism ▼	Staphylococcus aureus	Candida albicans	Streptococcus pyogenes

Name of drug	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %
Ciprofloxacin (10 mg/ml)	17.67±1.47	100	16.88±0.87	100	19.34±0.68	100
CFM Nanoparticles (10 mg/ml)	26.33±0.33	149	20.45±0.87	121	23.56±0.63	121

, Mean value of diameter of inhibition zone with standard error.

As the diameter of paper disc used was 6 mm, 6 mm diameter included in the table is indicative of no activity.Percent was calculated after subtracting disc diameter (6mm) from all observations.

* indicates significant activity at $p<0.05$

*CFM nanoparticles*have shown the significant activity against bacteria on the same concentration of reference drug ciprofloxacin.

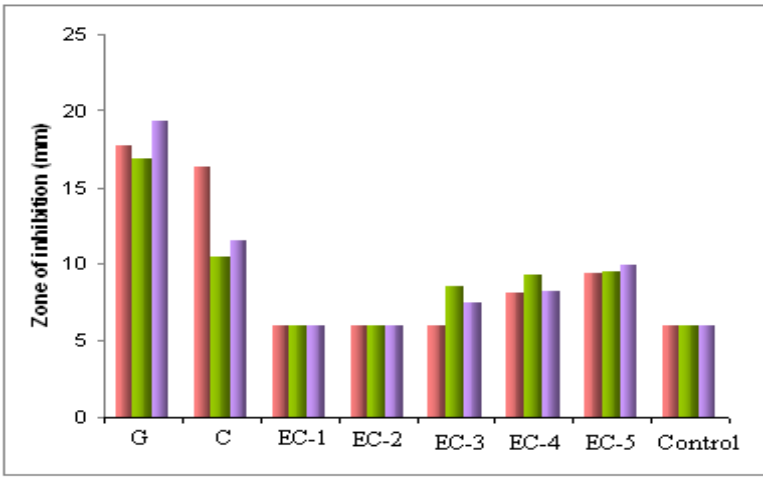


Fig –Zone of inhibition for various concentrations of CFM nanoparticlescompared to reference drugs: activity against gram negative bacteria.

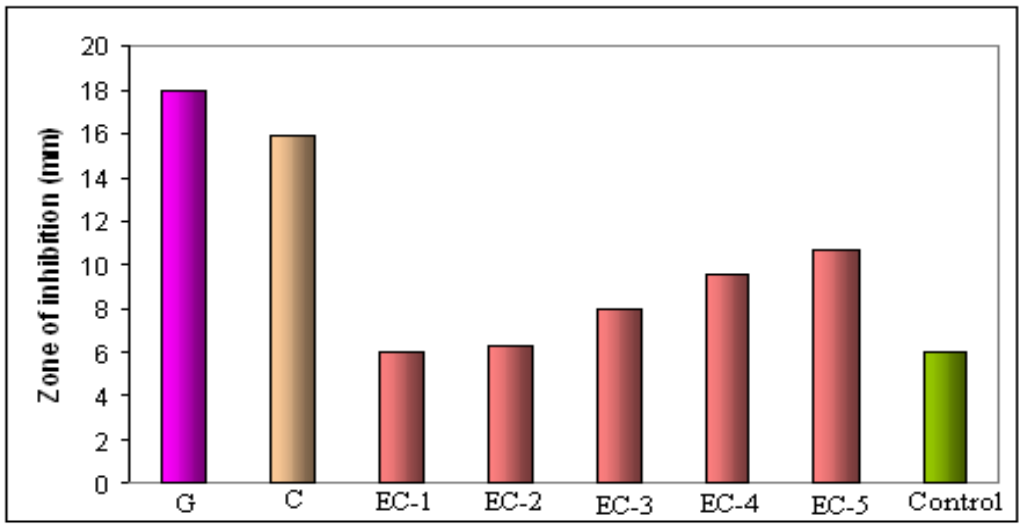


Fig –Zone of inhibition for various concentrations of *Blue berry* compared to reference drugs: activity against gram positive bacteria.

Table no. Zone of inhibition of CFMcompared to reference drugs: activity against Gram-positive bacteria.

Micro-Organism ▼ Name of drug ↓	***Staphylococcus aureus zone of inhibition***	***Candida albicans zone of inhibition***	**Streptococcus pyogenes *zone of inhibition***
	In mm Mean	In mm Mean	In mm Mean
Miconazole (10 ug/ml)	27.67±1.47	25.88±0.87	19.00±0.68
Fluocinolone (10ug/ml)	25.33±0.33	24.45±0.87	17.56±0.63
Ciprofloxacin (10 ug/ml)	25.66±1.88	24.00±0.68	18.00±0.48
CFM nanoparticle (10ug/ml)	35.40±0.44	37.42±0.64	43.00±0.52

Conclusion

In conclusion, the development of nanoparticles containing Ciprofloxacin, Miconazole, and Fluocinolone Acetonide represents a promising avenue for disease treatment. Their enhanced drug delivery, targeted action, and reduced side effects make them a valuable addition to the arsenal of therapies for various infections and inflammatory conditions. However, further research and clinical trials are essential to validate their efficacy and safety profiles for widespread clinical use.

The integration of nanoparticles loaded with Ciprofloxacin, Miconazole, and Fluocinolone Acetonide into ointment formulations represents a promising advancement in topical therapy. These formulations offer targeted, controlled, and effective treatment options for a wide range of skin-related conditions while minimizing systemic side effects. However, further research and clinical trials are needed to establish their safety and efficacy profiles and to make them widely accessible in clinical practice

References

1. Amir, M., Seyed, A.S., Ebrahim, V.F., 2008. 5-Fluorouracil - Loaded BSA Nanoparticles: Formulation Optimization and *In vitro* Release. AAPS Pharm. Sci. Tech. 9, 1092-1096.
2. Arias, J.L., Gallardo, V., Gómez-Lopera, S.A., Delgado., 2005. Loading of 5-fluorouracil to poly(ethyl-2-cyanoacrylate) nanoparticles with a magnetic core. J. Biomed. Nanotech. 1, 214-223.
3. Linares-Molinero, F., Gallardo, V., Delgado, A.V., 2008. Study of carbonyl iron/poly(butylcyanoacrylate) (core/shell) particles as anticancer drug delivery systems. Loading and release properties. Eur. J. Pharm. Sci. 33, 252-261.
4. Ruiz, M.A., López-Viota, M., Delgado, Á., 2008. Poly (alkylcyanoacrylate) colloidal particles as vehicles for antitumour drug delivery: A comparative study. Colloids and Surf B Biointerfaces 62, 64-70.

5. Babu, V.R., Sairam, M., Hosamani, K.M., Aminabhavi, T.M., 2006. Development of 5-fluorouracil loaded poly (acrylamide-co-methylmethacrylate) novel core-shell microspheres: *In vitro* release studies. Int. J. Pharm. 325, 55-62.
6. Bhadra, D., Bhadra, S., Jain, S., Jain, N.K., 2003. A PEGylated dendritic nanoparticulate carrier of fluorouracil. Int. J. Pharm. 257, 111-124.
7. Hetty Lendora Maha, Kasmirul Ramlan Sinaga, Masfria (16 August 2017, Revised and Accepted: 11 December 2017)
8. Ahmed Adel Ali Youssef, Chuntian Cai, Narendar Dudhipala,(2021 Mar; 14(3): 210)
9. Mangesh R. Bhalekar, Varsha Pokharkar, Ashwini Madgulkar, Nilam Patil, (2009 Mar; 10(1): 289–296)
10. Mohammed Elmowafy , Mohammad M. Al-Sanea (Volume 29, Issue 9, September 2021, Pages 999-1012)
11. Tejashree Waghule, Vamshi Krishna Rapalli, Srividya Gorantla, (Volume 26, Issue 36, 2020,Page: [4569 - 4579])
12. Sameer S. Katiyar, Eameema Muntimadugu, Towseef Amin Rafeeqi, Abraham J. (Drug Deliv, 2016; 23(7): 2608–2616)
13. Tejashree Waghule, Vamshi Krishna Rapalli, Srividya Gorantla, Ranendra Narayan Saha, Sunil Kumar Dubey Volume 26, Issue 36, 2020 Page: [4569 - 4579]
14. Ankita Dadwal, Neeraj Mishra and Raj K. Narang, (Volume 14, Issue 3, 2021, 28 June, 2020 Page: [399 - 411])
15. Hemant A. Ranpise, Kishore N. Gujar, Sachin C. Pawar, Rajendra Awasthi, Kamal Dua , Dyandevi Mathure and Jyotsana R. Madan (Volume 10, Issue 1, 2020 Page: [61 - 71])
16. Sadhana Raut, Nupur Gargate, Sharddha Ugale, Kishor N. Gujar and Harshad Kapare (Volume 11, Issue 4, 2023, 19 April, 2023 Page: [344 - 354])
17. Pierre A. Hanna, Mamdouh M. Ghorab* and Shadeed Gad (Volume 18, Issue 1, 2019,Page: [26 - 44])
18. Sweta Garg, Ashish Garg (March 2018International Journal of Pharmaceutical Sciences and Research 9(3)1160-66)
19. J P Diamond, L White, J P Leeming, H Bing Hoh (Br J Ophthalmol. 1995 Jun; 79(6): 606–609.)
20. M S Osato 1, H G Jensen, M D Trousdale, J A Bosso, L R Borrmann, J Frank, (Am J Ophthalmol. 1989 Oct 15;108(4):380-6.)
21. Veronica Volpe a, Monica N. Giacomodonato b, Daniel O. Sordelli b, Matías Insausti a, Fernanda R. Buzzola b, Marcos Grünhut (Journal of Drug Delivery Science and Technology, Volume 57, June 2020, 101705)
22. Lalit Prakash, Shivani, Manpreet Kaur, Kamlesh Sharma (Journal of Research in Medical and Dental Science 2022, Volume 10, Issue S1, Page No: 85-88)
23. Rabab Kamel a, Nahla A. El-Wakil (Journal of Drug Delivery Science and Technology Volume 64, August 2021, 102553)
24. M.–K. Kim; P. Botelho; C. Zhang; J. Dick; N. Lee (ARVO Annual Meeting Abstract,Volume 47, Issue 13 May 2006)
25. Yasir E. N. Al-Khashab,Yassir M. K. Al-Mulla Hummadi,Salim (Al-Mustansiriyah Journal of Pharmaceutical Sciences (AJPS) volume 8,Issue 2. 2010)

26. Hassan,F.A. Mohammed,E.A. Sabour (May 2003S.T.P. Pharma Sciences 13(3):195-201)
27. Chukwuebuka E Umeyor Franklin Kenechukwu John Ogbonna (January 2012Journal of Microencapsulation 29(3):296-307)
28. Anthony A. AttamaC E Okafor, Philip Builders (November 2009Drug Delivery 16(8):448-57)
29. K.M. HosnyS.M. RamboM.M. Al-Zahrani (June 2013International Journal of Pharmaceutical Sciences Review and Research 20(2):306-310)
30. J.M. Van Cutsem; D. Thienpont (Chemotherapy (1972) 17 (6): 392–404.)
31. Pietro Nenoff ,D Koch (March 2017Mycoses 60(8))
32. Mikael Alsterholm, Nahid Karami (Acta Derm Venereol 2010; 90: 239–245)
33. Tiffany H. Khaw, S.N. Michael Wong,(ACS Omega 2020, 5, 37, 23951–23959 Publication Date:September 8, 2020)
34. Wichuda Phiphitphibunsuk ,Rapee Sukthong chaikool (Pharmaceutics 2023, 15(6), 1637)
35. Singh S, Patil VM Masand N (Pharm Nanotechnol. 2023 Aug 18)
36. Ayatollahi Mousavi SA, Mokhtari A (Heliyon. 2023 Aug; 9(8))
37. Beijing Da Xue Xue Bao Yi Xue Ban (2023 Aug 18; 55(4):748-754.)
38. Nadia Shaukat, Umar Farooq, Kashif Akram, Afshan Shafi, Zafar Hayat, Ambreen Naz2, Ayesha Hakim, Khizar Hayat 1/6Asian J Agric & Biol. xxxx(x).Asian J Agric & Biol. (x).DOI: 10.35495/ajab.2020.03.188
39. Waghule, Tejashree; Rapalli, Vamshi K.; Gorantla, Srividya; Saha, Ranendra Narayan; Dubey, Sunil Kumar; Puri, Anu; Singhvi, Gautam Nanostructured Lipid Carriers as Potential Drug Delivery Systems for Skin Disorders Volume 26, Number 36, 2020, pp. 4569-4579(11)
40. PradeepKumar Bolla,CarlosA. Meraz,VictorA. Rodriguez ,Isaac DeagueroMahimaSingh,VenkataKashyapYellepeddiandJwalaRenukuntla Clotrimazole Loaded Ufosomes for Topical Delivery: Formulation Development and In-Vitro Studies,*Molecules* 2019, *24*(17), 3139; Published: 29 August 2019
41. IsaacG. Deaguero,MdNurulHuda,VictorRodriguez,JadeZicari,TaslimA. Al-Hilal,AbuZayedMdBadruddoza andMdNurunnabi Nano-Vesicle Based Anti-Fungal Formulation Shows Higher Stability, Skin Diffusion, Biosafety and Anti-Fungal Efficacy In Vitro*Pharmaceutics* 2020, *12*(6), 516; Published: 5 June 2020
42. MadhulikaPradhan[a]DeependraSinghS. NarasimhaMurthy[c]ManjuRawatSingh[ac]Design, characterization and skin permeating potential of Fluocinolone acetonide loaded nanostructured lipid carriers for topical treatment of psoriasis, Volume 106, February 2016, Pages 93
43. Sung MinPyo[a]DavidHespeler[a]Corneli a M.Keck[b]RainerH.Müller[a]Dermal miconazole nitrate nanocrystals – formulation development, increased antifungal efficacy & skin penetration, International Journal of Pharmaceutics, Volume 531, Issue 1, 5 October 2017, Pages 350-359
44. Salman J. Latif*1 and Dr. Shubhangi C. Daswadkar development and evaluation of fluocinolone acetonide and neomycin sulphate nanomiemgel for topical drug delivery system ,World Journal of Pharmaceutical Research SJIF Impact Factor 8.074 Volume 8, Issue 6, 1279-1293. Research Article ISSN 2277– 7105
45. Surajit Das, Wai Kiong Ng and Reginald B H Tan Sucrose ester stabilized solid lipid nanoparticles and nanostructured lipid carriers: II. Evaluation of the imidazole antifungal drug-loaded nanoparticle dispersions and their gel formulations Published 14 February 2014 • © 2014 IOP Publishing Ltd Nanotechnology, Volume 25, Number 10

46. Efstathios Rallis, Elma Koumantaki-Mathioudaki Treatment of Mycobacterium marinum cutaneous infections , Dec;82007 (17):2965-78
47. Chandra Pratap S, Pawan Kumar M, Surya Prakash G. Design and Formulation of Tridax procumbens based Poly herbal Cream for Wound Healing Potential. Der Pharmacia Lettre. 2016; 8(12): 15-21.
48. Shilpa S, Komal S, Rajanigandha J et al. Evaluation of poly herbal ointment for wound healing activity in Wistar rats. Journal of Drug Delivery & Therapeutics. 2018; 8(6): 26- 31.
49. Sorg H, Tilkorn DJ, Hager S et al. Wound Healing: An Update on the Current Knowledge and Concepts. European Surgical Research. 2017; 58(2): 81–94.
50. J.S. Kulkarni, A.P.Pawar, V.P. Shedbalkar, “Pharmaceutics and Pharmacokinetics” Ist Edition 2006; 40.
51. Yie.W.Chien, “Novel drug delivery system” IInd Edition 2005; 1-2.
52. N.k Jain, “Pharmaceutical product development” Ist Edition 2006; 420-432.
53. J.N.Khandare, G.Madhavi, B.M. Tamhankar, “Niosomes Novel Drug Delivery System” The Eastern Pharmacist – March, 1994; 61.
54. Blanco, M.D., Sastre, R.L., Teijón, C., Olmo, R., Teijón, J.M., 2005. 5-Fluorouracil-loaded microspheres prepared by spray-drying poly(D,L-lactide) and poly(lactide-co-glycolide) polymers: characterization and drug release. J. Microencapsul. 22, 671-682.
55. Chandy, T., Das, G.S., Rao, G.H.R., 2000. 5-Fluorouracil-loaded chitosan coated polylactic acid microspheres as biodegradable drug carriers for cerebral tumours. J. Microencapsul. 17, 625-638.
56. Chang, S.J., G.C.-C, N., Kuo, S.M., Chen, S.F., 2006. Preparation and preliminary characterization of concentric multi-walled chitosan microspheres. J. Biomed. Mat. Res. 81, 554-566.
57. Dong, Y., Feng, S.-S., 2004. Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs. Biomaterials 25, 2843–2849.
58. Elorza, B., Elorza, M.A., Frutos, G., Chantres, J.R., 1993. Characterization of 5-fluorouracil loaded liposomes prepared by reverse-phase evaporation or freezing-thawing extrusion methods: study of drug release. Biochim. Biophys. Acta. 1153, 135-142.
59. Faisant, N., Akiki, J., Siepmann, F., Benoit, J.P., Siepmann, 2006. Effects of the type of release medium on drug release from PLGA-based microparticles: experiment and theory. Int. J. Pharm. 314, 189-197.
60. Fresta, M., Villari, A., Puglisi, G., Cavallaro, G., 1993. 5-Fluorouracil : various kinds of loaded liposomes : encapsulation efficiency, storage stability and fusogenic properties. Int. J. Pharm. 99, 145-156.
61. Ghoroghchian, P.P., Levine, D.H., Davis, K.P., Bates, F.S., Hammer, D.A., Therien, M.J., 2006. Bioresorbable vesicles formed through spontaneous self-assembly of amphiphilic poly(ethylene oxide)-block-polycaprolactone. Macromolecules 39, 1673
62. Huang, X., Wei, R.l., 2008. Preparation and *in vitro* evaluation of fluorouracil nanoparticles for ophthalmologic usage. Dier Junyi Daxue Xuebao. 29, 1227-1229.
63. Lo, C.L., Lin, K.-M., HsiueT, G.H., 2005. Preparation and characterization of intelligent core-shell nanoparticles based on poly(d,l-lactide)-g-poly(N-isopropyl acrylamide-co-methacrylic acid). J. Control. Release 104, 477–488.