IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

FORMULATION AND DEVELOPMENT OF CIPROFLOXACIN, MICONAZOLE, AND FLUOCINOLONE ACETONIDE LOADED NANOPARTICLE AND ITS EVALUATION

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Abstract: In the present study, we have developed and characterized Ciprofloxacin, Miconazole, and Fluocinolone Acetonide simultaneously loaded PLGA nanoparticles to enhance the bioavailability of both the agents, to achieve the therapeutic advantage of the combination and to obtain sustained release of the drug. These nanoparticles have been characterized for their size, morphology, drug loading, and entrapment efficiency and optimized at different theoretical drug loadings and also evaluated *in vitro* to see their release from the formulation.

Nanoparticles were prepared by emulsion-diffusion-evaporation method using PLGA (75/25) at three different drug loadings 5%, 10%, and 15%. The % entrapment efficiency was found to increase with the increase in drug loading from (5% to 15%). The % yield was found to increase from 58% to 80%. The particle size of nanoparticles was found to be 222-283 nm with a polydispersity index below 0.312 and a zeta potential of around -6.0 mV was observed. D-trehalose (10% w/v) was selected as cryoprotectant for freeze-dried simultaneously loaded PLGA nanoparticles.

In vitro release study showed that ciprofloxacin, Miconazole, and Fluocinolone Acetonide were released from nanoparticles with theoretical drug loading of 10% w/w. Ciprofloxacin, Miconazole, and Fluocinolone Acetonide showed sustained release for 40 days, while resveratrol showed sustained release for 20 days. These nanoparticles were found to follow the Higuchi diffusion control release model.

Keywords: Nanoparticle, ciprofloxacin, nanotechnology, Miconazole, Fluocinolone, TEM, Particle size.

Introduction:

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.[1]

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.

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.[2]

Nanoparticles formulated with Ciprofloxacin, Miconazole, and Fluocinolone Acetonide show promise in the treatment of various diseases, particularly those involving bacterial, fungal, or inflammatory components. Here's an overview of their potential applications:

1. Antibacterial Efficacy (Ciprofloxacin):

- Ciprofloxacin is a potent antibiotic known for its broad-spectrum activity against bacteria. When formulated as nanoparticles, it offers several advantages:
- Enhanced bioavailability and prolonged release, lead to sustained therapeutic levels in the body.
- Targeted delivery to specific sites of infection, reducing systemic side effects.
- Effective against drug-resistant bacterial strains due to improved penetration and increased drug concentration at the infection site.

2. Antifungal Activity (Miconazole):

- Miconazole is an antifungal agent used to treat various fungal infections. Nanoparticles of miconazole exhibit the following benefits:
- Improved solubility and bioavailability, increasing its therapeutic effectiveness.
- Enhanced adhesion to fungal cells, facilitating more efficient drug delivery.
- Potential for combination therapy with other antifungals, increasing treatment options for complex fungal infections.

3. Anti-Inflammatory Properties (Fluocinolone Acetonide):

- Fluocinolone acetonide is a potent corticosteroid known for its anti-inflammatory and immunosuppressive properties. Nanoparticles of this drug offer unique advantages:
- Targeted delivery to inflamed tissues, minimizing systemic side effects.
- Controlled release, providing a prolonged anti-inflammatory effect.
- Potential for treating inflammatory skin conditions, ocular diseases, and other localized inflammations.

Transdermal Drug Delivery System (TDDS) 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.

Nanoparticles loaded with Ciprofloxacin, Miconazole, and Fluocinolone Acetonide are emerging as a significant innovation in pharmaceuticals, particularly in the formulation of ointments. These nanoparticles offer unique advantages for topical applications:[3,4,5]

1. Enhanced Drug Delivery:

- Nanoparticles provide a high surface area-to-volume ratio, facilitating improved drug penetration into the skin.
- They can reach deeper layers of the skin, ensuring targeted delivery to the site of infection or inflammation.

2. Controlled Release:

- Nanoparticles enable the controlled and sustained release of active pharmaceutical ingredients (APIs), ensuring prolonged therapeutic effects.
- This controlled release can improve patient compliance by reducing the frequency of application.

3. Minimized Systemic Absorption:

- By focusing drug delivery on the skin's surface or specific layers, nanoparticles reduce systemic absorption.
- This minimizes the risk of systemic side effects commonly associated with these medications when taken orally or intravenously.

4. Synergistic Effects:

- Combining Ciprofloxacin, Miconazole, and Fluocinolone Acetonide in a single ointment can address a range of skin conditions, including infections (bacterial and fungal) and inflammatory responses.

- The nanoparticles can potentially enhance the synergistic effects of these drugs, improving treatment outcomes.

Applications:

- These nanoparticle-based ointments find applications in dermatology and ophthalmology.
- They are effective in treating skin infections, fungal conditions (like athlete's foot), eczema, psoriasis, and ocular diseases.

Materials and methods

Materials

5.1 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.

5.2 Chemical and reagents

The sources of chemicals and reagents have been given in table

Table
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

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

6.4.1 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.

6.4.2 Emulsion-diffusion-evaporation method

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.

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.

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.

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.

Results and discussion

Preparation and characterization 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 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 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)

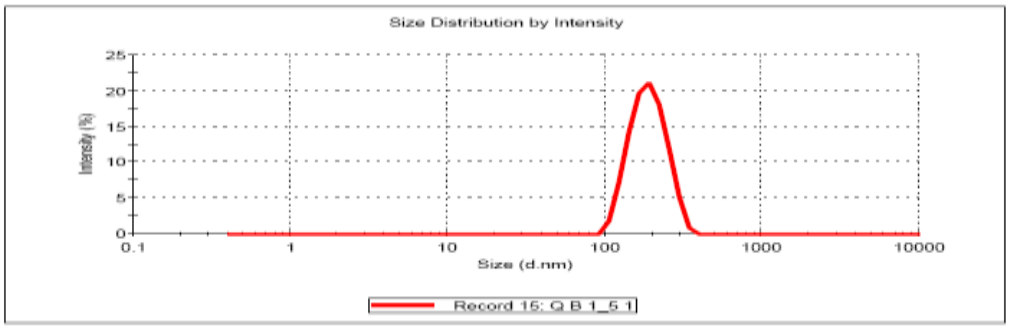


Fig. Size distribution of Ciprofloxacin Nanoparticles

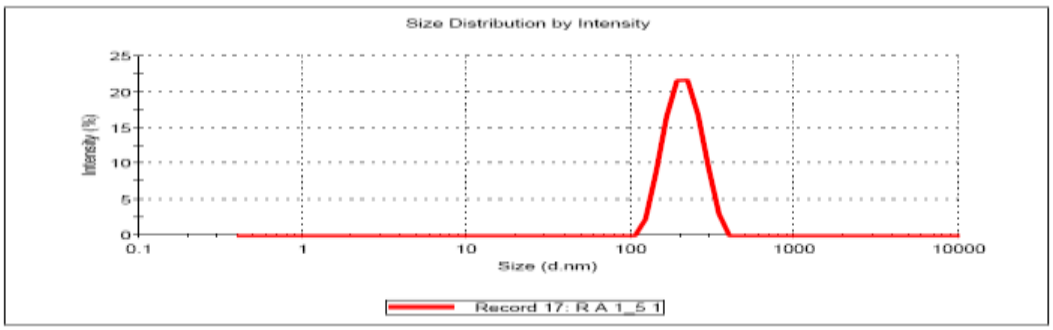


Fig. Size distribution of Fluocinolone Nanoparticles

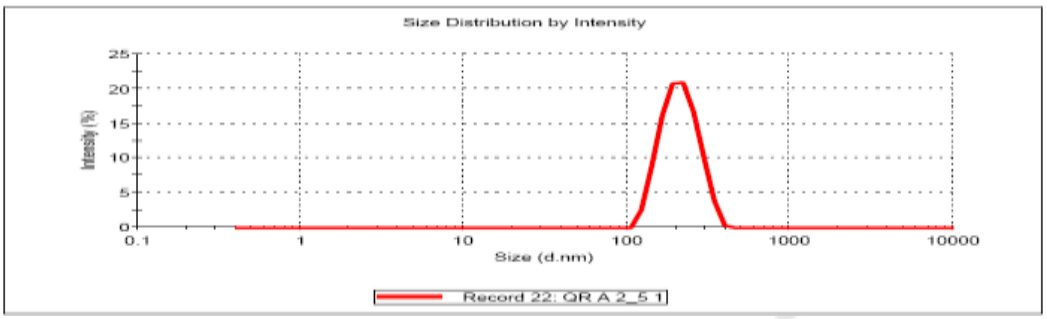
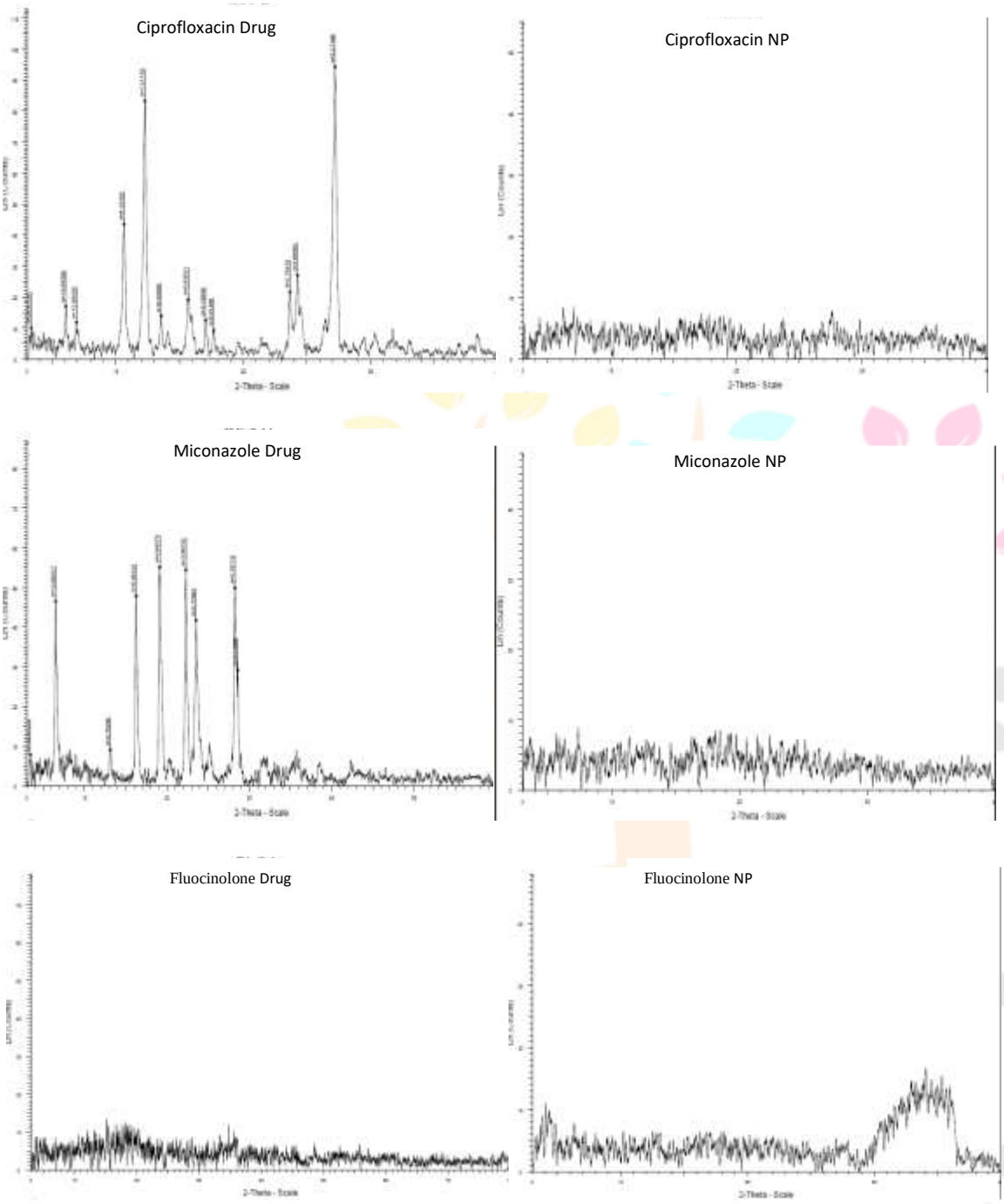


Fig. Size distribution of miconazole nanoparticles

XRD Images

XRD images shows the drug nature of nanoparticle and alone



In vitro evaluation of the formulation

Drug release study

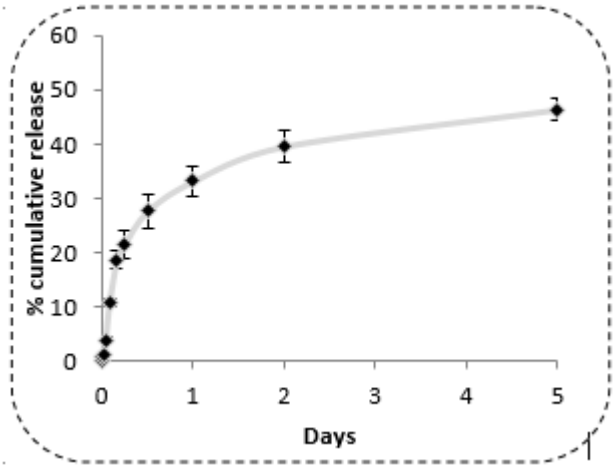
Release of drug form each of the nanoparticles i.e Ciprofloxacin NPs, Fluocinolone Acetonide, Miconazole NP, Ciprofloxacin Fluocinolone Acetonide and Miconazole combined NPs was evaluated. Ciprofloxacin, Fluocinolone Acetonide and Miconazole showed a biphasic release pattern from the nanoparticles. Ciprofloxacin 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

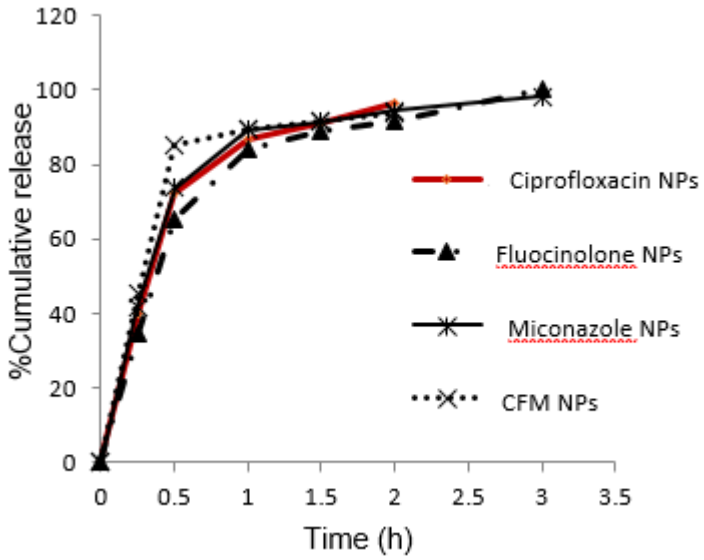
from Ciprofloxacin NPs and 75% drug of Fluocinolone Acetonide NPs, 74% released from Miconazole while 66.5 % of Ciprofloxacin 72% drug of Fluocinolone Acetonide and 71% of Miconazole was released from combination NPs. To further mass balance the drug remaining in the nanoparticles.

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





TEM Image

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.

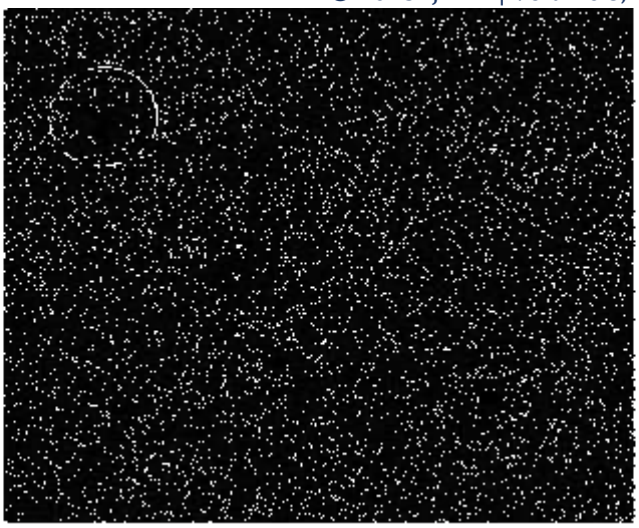


Fig. TEM image of CFM-NPs (inside image showing the magnified view of a single nanoparticle.) (scale bar = 200nm)

Freeze thaw study

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 in particle 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

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.

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