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Box-Behken Design Assisted Formulation and Optimization of Microsphere Containing Momentasone Furoate and Clotrimazole and Its Anti-Fungal Activity

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ABSTRACT
This study describes a Box-Behnken experimental design to optimize the formulation of Momentasone furoate and Clotrimazole loaded microspheres and its anti-fungal activity. The prepared microspheres were evaluated for their production yields, particle size distribution, morphology, entrapment efficiency % and drug release characteristics. The pH analysis revealed that Mometasone furoate has an observed pH of 4.6, indicating an acidic nature and Clotrimazole showed a pH of 6.7, which is near neutral and favourable for maintaining stability and compatibility with skin and mucosal membranes. Mometasone furoate exhibited a melting point of 214 °C. Similarly, Clotrimazole showed a melting point of 148 °C. The UV spectroscopic analysis of Mometasone furoate revealed an absorption maximum (λ max) at 247.5 nm and Clotrimazole was determined to be 262.0 nm. The FTIR studies revealed no chemical interaction between the drug molecule and polymers and found that drug was compatible with used polymer. Box-Behnken design produced fifteen formulations containing specified amounts of the independent variables, (Y1) Polymer Eudragit S100 (mg), (Y2) Surfactant tween 80(%) and (Y3) Stirring time (min.), the dependent factors studied were Vesicle size (nm) (Z1), and Entrapment efficiency (%) (Z2). The outcomes revealed that Vesicle size across the formulations ranged from 197.3nm to 947.2nm, while entrapment efficiency varied between 63.07% and 91.76%, indicating that the selected variables had a significant impact on the formulation characteristics. Furthermore, the enhanced in vitro antifungal activity observed with the optimized formulation validates the potential of this dual-drug microsphere system for clinical applications in managing fungal infections with inflammatory symptoms. Overall, the study successfully utilized a quality-by-design approach to develop a controlled-release microsphere formulation with significant potential for treating fungal infections with inflammatory components.

1. INTRODUCTION:

Microspheres allow the controlled release of drug at the target site. Microspheres can be used to achieve the desired release profile by choosing a polymer of appropriate molecular weight and the right method of preparation or by varying copolymer concentration **(Le *et al.*, 2018).** As they have small particle size and hence large surface-tovolume ratio, they can be used for controlled delivery and hence may be used for sustained release of the metformin hydrochloride for the treatment of periodontitis. Chitosan is a biodegradable natural polymer with

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various properties like biocompatibility, high charge density, non-toxicity, and mucoadhesion. Various techniques like interactions with anions, thermal cross-linking, chemicals, solvent evaporation technique, etc can prepare chitosan microspheres **(Iacob *et al.*, 2021).**

Mometasone is a locally acting glucocorticoid with high topical anti-inflammatory activity. It has been used for many years in the treatment of inflammatory airway diseases for the low systemic activity of one of the most common uses of Mometasone is in ulcerative colitis and Crohn's disease. It undergoes extensive, primarily hepatic metabolism after oral administration **(Boardman *et al.*, 2014).** Specifically, mometasone appears to induce phospholipase A2 inhibitory proteins, thereby controlling the release of the inflammatory precursor arachidonic acid from phospholipid membrane by phospholipase A2 **(Giresha, 2021).** Clotrimazole is a member of the class of imidazoles that is 1H-imidazole in which the hydrogen attached to nitrogen is replaced by a monochlorotrityl group. It has a role as an antiinfective agent, an environmental contaminant and a xenobiotic. It is a member of imidazoles, a member of monochlorobenzenes, a conazole antifungal drug and an imidazole antifungal drug **(Szentmáry *et al.*, 2020).** Clotrimazole causes inhibition of ergosterol biosynthesis, an essential constituent of fungal cell membranes. Clotrimazole is a chlorinated imidazole derivative that is used to treat topical fungal, dermatophyte, & yeast infections. While clotrimazole has marked in vitro activity against many fungi, it is of little value in treatment of systemic mycoses **(Tushir *et al.*, 2022).**

The aim of the present research was to assist Box-behken design and formulate and optimized the microsphere develop a controlled drug delivery system of Momentasone furoate and Clotrimazole which exhibit its antifungal activity.

2. MATERIAL AND METHODS:

2.1 Chemicals:

Acetonitrile was procured from Rankem. Mometasone Furoate was received from Glenmark Pharmaceuticals Ltd. Carbopol 934, Methanol, Propylene glycol and Triethanolamine was acquired from Merck. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Loba**,** Sulab, Sigma eldrich and Alkem Laboratories.

2.2 Pre-formulation studies:

Pre-formulation studies of drugs like Mometasone furoate and Clotrimazole are essential preliminary investigations that evaluate the physical, chemical, and mechanical properties of the active pharmaceutical ingredients (APIs). These studies help in understanding the drug's stability, solubility, compatibility with excipients, and ideal storage conditions. Performing pre-formulation studies is crucial because it provides critical data needed for designing a stable, effective, and safe dosage form. For drugs like Mometasone furoate (a corticosteroid) and Clotrimazole (an antifungal), these studies ensure that both drugs remain effective when combined and formulated into products like creams or ointments **(Mijaljica *et al.*, 2022).**

2.2.1 Organoleptic Properties:

Organoleptic properties refer to the sensory characteristics of a drug, such as color, odor, taste, and appearance, which are important for ensuring consistency and patient acceptability **(Hauber *et al.*, 2024).**

2.2.2 Solubility study:

The solubility study of Mometasone furoate and Clotrimazole was performed by adding 1mg of each drug to selected polar solvents (such as water, ethanol, and methanol) and non-polar solvents (such as chloroform and petroleum ether) in separate test tubes. Each mixture was vigorously shaken after the settling period, the solutions were visually inspected for clarity and the presence of un-dissolved drug particles. This method helps determine the solubility profile and guides solvent selection for formulation **(Craveiro *et al.*, 2024).**

2.2.3 pH determination:

The pH of Mometasone furoate and Clotrimazole solutions was determined using a digital pH meter **(Wable *et al.*, 2024).**

2.2.4 Melting Point:

The melting point of Mometasone furoate and Clotrimazole was determined using a melting point apparatus**.**

2.2.5 Preparation of Mometasone furoate and Clotrimazole standard stock solution in methanol

A standard solution of Mometasone furoate and Clotrimazole was prepared by accurately weighing 10 mg of each drug and dissolving them in 5 mL of methanol in a 10 mL volumetric flask. To obtain a 1000 µg/mL stock solution, methanol was added to the flask to make the final volume 10 mL. For the preparation of a 100 µg/mL standard solution, 1 mL of the stock solution was further diluted to 10 mL with methanol as the solvent **(Merey *et al.*, 2016).**

2.2.6 Lambda max

To prepare a 20 µg/mL stock solution, 2 mL of the sample containing Mometasonefuroate and Clotrimazole was transferred to a 10 mL volumetric flask, and methanol was added to reach the final

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volume. The working standard solution of the drugs was then scanned in the UV range of 200 to 400 nm, using methanol as the blank. The obtained spectra were analyzed to identify the absorption maxima (λmax) for each drug **(Unde and Kurup 2021).**

2.2.7 Linearity and Calibration Curve

The working standard solution (100 µg/mL) was diluted to concentrations of 2, 4, 6, 8, 10, and 12 µg/mL for Mometasone furoate, and 5, 10, 15, 20, 25, and 30 µg/mL for Clotrimazole. To prepare these solutions, the appropriate volume of the stock solution was transferred into a series of 5 mL volumetric flasks, and methanol was added to reach the final volume. The absorbance of the resulting solutions was measured at 247.5 nm for Mometasone furoate and 262 nm for Clotrimazole, using distilled water as the blank. A five-point calibration curve was generated for both Mometasone furoate (concentrations ranging from 5 to 25 µg/mL) and Clotrimazole (concentrations ranging from 40 to 80 µg/mL).

2.2.8 Functional group identified by FTIR

A disc was prepared by mixing 1 mg of Mometasone furoate and Clotrimazole with 100 mg of spectroscopic-grade KBr, followed by drying under infrared light. The mixture was then compressed under hydraulic pressure to form the pellet, which was subsequently placed in the FT-IR chamber for analysis **(Siddique, 2024).**

2.3 Preparation of Mometasone furoate and Clotrimazole microsphere Formulation

Microsphere formulations using Eudragit S100 as a carrier polymer were prepared using emulsion solvent evaporation technique. Desired quantity of Eudragit S100 polymer was dissolved in 10 ml of chloroform to form a homogenous polymer solution. The resulting mixture was then added to 250 ml of aqueous mucilage of sodium CMC (0.5%) containing 0.5 to 1.5 % v/v tween 80, while stirring at 1000 rpm for emulsification. Chloroform was used as the polymer solvent, aqueous mucilage of sodium CMC as microencapsulating vehicle and tween 80 as dispersing agent. During stirring period, chloroform was completely removed by evaporation. Microspheres were collected by vacuum filtration, washed repeatedly with distilled water and petroleum ether and dried at room temperature for 24 h to get free flowing microspheres **(Verma *et al.*, 2015).**

Table 1: Independent variables

Independent variables	Levels used, actual(coded)		
	Low (-1)	Medium (0)	High (1)
A= Polymer Concentration (mg)	100	200	300
B= Surfactant concentration (%)	0.5	1	1.5
C= Stirring Time (Min)	10	30	90

Table 2: Composition of microsphere Formulation

Formulation	Polymer Eudragit S100 (mg)	Mometasone furoate and Clotrimazole (%)	Surfactant Tween 80 (%)	Distilled water (ml)	Sodium CMC (%)	Chloroform (ml)	Stirring time (min)
MS1	1	0.1 -1%	1	250.0	0.5	10.0	0
MS2	0	0.1 -1%	-1	250.0	0.5	10.0	1
MS3	0	0.1 -1%	1	250.0	0.5	10.0	-1
MS4	0	0.1 -1%	0	250.0	0.5	10.0	0
MS5	1	0.1 -1%	0	250.0	0.5	10.0	1
MS6	-1	0.1 -1%	0	250.0	0.5	10.0	-1
MS7	1	0.1 -1%	0	250.0	0.5	10.0	-1
MS8	1	0.1 -1%	-1	250.0	0.5	10.0	0
MS9	0	0.1 -1%	1	250.0	0.5	10.0	1
MS10	-1	0.1 -1%	0	250.0	0.5	10.0	1
MS11	-1	0.1 -1%	1	250.0	0.5	10.0	0
MS12	0	0.1 -1%	0	250.0	0.5	10.0	0
MS13	0	0.1 -1%	-1	250.0	0.5	10.0	-1
MS14	-1	0.1 -1%	-1	250.0	0.5	10.0	0

2.3.1 Values of variables

Table 4: - Values of variables

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Polymer Conc.	mg	Numeric	-1.0000	1.0000	-1 ↔ -1.00	+1 ↔ 1.00	0.0000	0.7845
B	Surfactant Conc.	%	Numeric	-1.0000	1.0000	-1 ↔ -1.00	+1 ↔ 1.00	0.0000	0.7845
C	Stirring time	min.	Numeric	-1.0000	1.0000	-1 ↔ -1.00	+1 ↔ 1.00	0.0000	0.7845

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2.4 Evaluation parameter of Mometasone furoate and Clotrimazole loaded microspheres formulation:

2.4.1 Physical properties: The physical properties of the Mometasone furoate and Clotrimazole-loaded microsphere cream formulation can be initially evaluated by visual inspection to assess clarity, color, and homogeneity

2.4.2 Vesicle size determination: Vesicle size is a critical parameter for characterizing Mometasone furoate and Clotrimazole-loaded microsphere formulations, as it directly influences drug release, stability, and skin penetration. The Vesicle size was measured using the Malvern Zeta Sizer (Malvern Instruments)

2.4.3 Zeta potential: The zeta potential was measured to evaluate the surface charge and stability of the Mometasone furoate and Clotrimazole-loaded microsphere formulation, as it reflects the particles' movement velocity in an electric field and helps predict their colloidal stability. In this study, the microsphere samples were diluted tenfold with distilled water and analyzed using the Malvern Zetasizer **(Singh *et al.*, 2021).**

2.4.4 Surface morphology (SEM): The morphological characteristics of the optimized Mometasone furoate and Clotrimazole loaded microspheres were examined using a scanning electron microscope (SEM). Prior to imaging, the microsphere samples were coated with a thin metal layer (2–20 nm) of gold, palladium, or platinum using a sputter coater under vacuum conditions. Electrons scattered at a 90° angle from the interaction between the electron beam and the microsphere surface were detected and processed using principles based on Rutherford and Kramer's Law to produce detailed images revealing the surface morphology and topography of the microspheres **(Jiang, 2015).**

2.4.5 % Entrapment Efficiency: Entrapment efficiency was assessed using an indirect method. The Mometasone furoate and Clotrimazole-loaded microsphere formulation was subjected to centrifugation at 1500 rpm for 30 minutes using a REMI Ultra Centrifuge. The absorbance values obtained were compared against a previously established calibration curve to determine the concentration of the free drug. Entrapment efficiency was then calculated using the following equation: **(De Leo *et al.*, 2018).**

Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc. ×100

2.5 Formulation of microsphere Cream

For preparation of microsphere cream formulation firstly heat the liquid paraffin and beeswax in a china dish at 75^0C and maintain that heating temperature (Oil phase). In another china dish dissolve borax, methyl paraben in distilled water and heat this beaker to 75^0C to dissolve borax and methyl paraben and to get a clear solution (Aqueous phase). Then slowly add this aqueous phase to the oily phase in a mortar and pestle and stir in a single direction to avoid lumps. Then add the microsphere of drugs to the cream base and mix it. Add few drops of Lavender oil as a fragrance to impart the aroma and mix all the ingredients properly **(Badwaik *et al.*, 2022).**

Table 5: Composition of microsphere loaded Cream

Ingredients	Formulation (Qty.)	Uses
Microsphere (mg)	500	API
Beeswax	4	Emulsifier, thickener
Liquid paraffin	15ml	Moisturizing agent, skin softener
Methyl Paraben	0.03g	Anti-bacterial agent, Preservative
Essential oil (lavender oil)	3- 4 drop	Fragrance, glowing skin
Borax	0.4g	Buffering agent, preservative

2.6 Evaluation parameter of Mometasone furoate and Clotrimazole loaded microspheres cream formulation

2.6.1 Appearance: The prepared Mometasone furoate and Clotrimazole-loaded microsphere cream formulation was evaluated for key sensory attributes. **(Yasir Siddique *et al.*, 2021).**

2.6.2 pH determination: A digital pH meter was used to measure the pH of the Mometasone furoate and Clotrimazole-loaded microsphere cream formulation **(Greive and Barnes 2016).**

2.6.3 Viscosity: The viscosity of the Mometasone furoate and Clotrimazole-loaded microsphere cream formulation was measured using a Brookfield viscometer (Rai and Ravikumar 2016).

2.6.4 Spreadability: The spreadability of the Mometasone furoate and Clotrimazole-loaded microsphere cream formulation was evaluated using two glass slides, each 7.5 cm in length. A 350 mg sample of the cream was carefully placed on one slide, and the second slide was positioned 5 cm above it. A 5-gram weight was then placed on the upper slide to allow the cream to spread uniformly. After 1 minute, the diameter of the spread cream circle was measured. The spreadability was calculated using the following formula:

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S= M×L/T

Where, S-Spread ability, g.cm/s M-Weight put on upper glass L-Length of glass slide T-Time for spreading cream in sec **(Khanum, 2017).**

2.6.5 Drug release study: Initially, the formulation was dissolved in a phosphate buffer solution with pH 6.8. This solution was then placed in the donor compartment of a Franz diffusion cell, ensuring that the drug-release surface faced the receptor compartment, which contained 50 mL of phosphate buffer (pH 6.8) maintained at 37 ± 0.5°C. The entire setup was positioned on a magnetic stirrer, and continuous stirring of the receptor medium was carried out using a magnetic bead at 50 rpm. At predetermined time intervals, 5 mL samples were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh phosphate buffer (pH 6.8) to maintain sink conditions. The collected samples were analyzed for drug content using a UV spectrophotometer.

2.6 Antifungal activity of prepared Mometasone furoate and Clotrimazole loaded microspheres cream formulation

2.6.1 Sabouraud Dextrose Agar (SDA) medium Preparation

To prepare 100 mL of Sabouraud Dextrose Agar (SDA) medium, 6.5 grams of SDA powder was precisely weighed using a digital balance. The powder was gradually mixed into 100 mL of distilled water in a conical flask with constant stirring to achieve complete dissolution. The flask was loosely sealed with a cotton plug and subjected to sterilization in an autoclave at 121 °C for 15 minutes fewer than 15 psi pressures. After autoclaving, the medium was cooled to approximately 45–50 °C and then poured into sterile petri dishes under aseptic conditions. The filled plates were allowed to stand undisturbed until the agar solidified and were subsequently stored in a refrigerator at 2–8 °C until needed **(Adadey, 2014).**

2.6.2 Well Diffusion Assay against *Candida albicans*

After preparing the Sabouraud Dextrose Agar (SDA) plates, a Well Diffusion Assay was conducted to evaluate the antifungal activity of the formulation. A standardized suspension of *Candida albicans* was uniformly spread over the surface of the solidified agar using a sterile cotton swab to ensure even microbial growth. Wells of consistent 6 mm diameter were aseptically created in the agar using a sterile cork borer. Each well was then filled with 50–100 µL of the Mometasone furoate and Clotrimazole-loaded microsphere cream formulation. A positive control (such as a standard antifungal cream) and a negative control (placebo or blank cream) were also added to separate wells for comparison. The plates were incubated at 28–30 °C for 24–48 hours. Post-incubation, the antifungal activity was determined by measuring the diameter of the zone of inhibition surrounding each well, indicating the formulation's effectiveness in suppressing fungal growth **(El Khetabi *et al.*, 2021).**

2.7 Stability studies

The Mometasone furoate and Clotrimazole-loaded microsphere cream formulation was packed and placed in a stability testing chamber, where it was evaluated over a period of three months under accelerated conditions at 25 ± 2°C with 60 ± 5% RH and 40 ± 2°C with 70 ± 5% RH. Stability assessments were conducted at regular intervals 30, 45, 60, and 90 days to monitor any changes in the formulation's viscosity and pH. The study was performed in accordance with the International Conference on Harmonization (ICH) guidelines for accelerated stability testing. The data obtained at each time point were compared with the initial characteristics of the formulation (day 0) to detect any significant variations, ensuring the formulation's stability, consistency, and shelf life during storage **(González-González *et al.*, 2022).**

3. RESULTS AND DISCUSSION:

3.1 Pre-formulation study of drug

3.1.1 Organoleptic properties of Mometasone furoate and Clotrimazole

Table 6: Organoleptic properties of Mometasone furoate and Clotrimazole

Drug	Organoleptic properties	Mometasone furoate	Clotrimazole
Mometasone furoate and Clotrimazole	Color	White to off-white	Typically a white
	Odor	Characteristic	Odourless
	Appearance	Powder or as crystals.	White, crystalline
	State	Solid	Crystalline solid

3.1.2 Solubility study

Table 7: Solubility study

Drug	Solvents	Mometasone furoate	Clotrimazole
Mometasone furoate and Clotrimazole	Water	Practically insoluble	Soluble
	Ethanol	Slightly soluble	Freely soluble
	Methanol	Freely Soluble	Freely soluble
	Chloroform	Soluble	Soluble
	DMSO	Freely Soluble	Freely soluble

3.1.3 pH and melting point determination

Table 8: pH and Melting point of both drugs

Drugs	Observed (pH)	Observed (Melting Point)	Reference (Melting Point)
Mometasone	4.6	214 °C	212.0 to

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furoate			216.0 °C
Clotrimazole	6.7	148 °C	147.0 to 149.0 °C

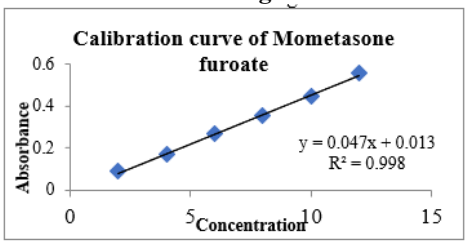
3.2 Determination of λ max by UV spectroscopy

3.2.1 Lambda max Mometasone furoate and Clotrimazole

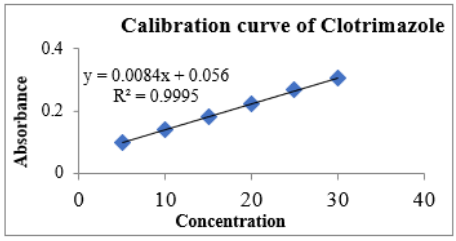
Table 9: Lambda max of Mometasone furoate and Clotrimazole

Drugs	Lambda max of Mometasone furoate	Lambda max of Clotrimazole	Centre point (Both Drug)
Mometasone furoate and Clotrimazole	247.5 nm	262.0 nm	258.0 nm

3.3 Calibration curve of both drugs

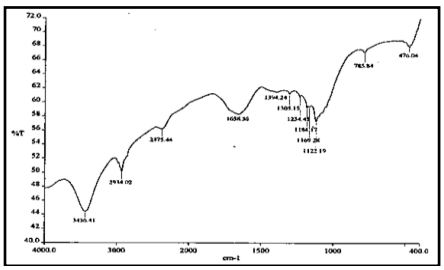


Graph 4: Calibration curve of Mometasone furoate

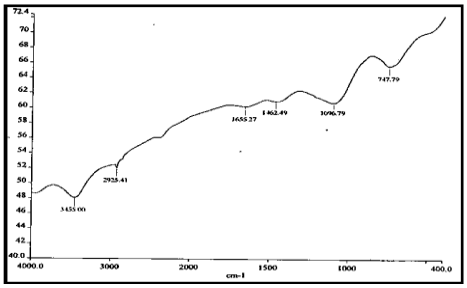


Graph 5: Calibration curve of Clotrimazole

3.4 Functional group identified by Fourier transform infrared (FTIR) study



Graph 6: FTIR of Mometasone furoate



Graph 7: FTIR of Clotrimazole

Table 10: Interpretation of IR spectrum of Mometasone furoate

Peak obtained	Reference peak	Functional group	Name of functional group
3436.41	3500- 3400	N-H Stretch	Primary amine
2934.02	3000-2800	N-H Stretch	Amine salt
1658.36	1690-1640	C=N Stretch	Imine / oxime
1394.24	1415-1380	S=O Stretch	Sulphate
1234.45	1275-1200	C-O Stretch	Alkyl aryl ether

Table 11: Interpretation of IR spectrum of Clotrimazole

Peak obtained	Reference peak	Functional group	Name of functional group
3455.00	3500- 3400	N-H stretch	Primary amine
2925.41	3000-2840	C–H stretch	Alkane
1655.27	1658-1648	C=C stretch	Alkene
1462.49	1470–1450	C–H bend	Alkanes
1096.79	1124-1087	C-O stretch	Secondary alcohol

3.5 Optimization of formulation by design of expert (DOE) software:

The formulation study involved a specific combination of components, leading to the preparation of 14 distinct batches of microspheres. Each batch was assessed based on the targeted responses. Analysis of data from these 14 experimental runs indicated that a linear model offered the best fit for both dependent variables. To determine the statistical relevance of this model in comparison to other potential models, an Analysis of Variance (ANOVA) was performed. All response values were documented across the 14 batches, and the tables demonstrate the relationship between the independent variables and the resulting responses.

3.5.1 Build Information:

Table 12 Build information of DOE software

File Version	12.0.1.0		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	14
Design Model	Linear model and Quadratic	**Blocks**	No Blocks

3.5.2 Independent and Dependent variables

Table 13 Independent and Dependent variables

Coding	Independent Variables

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Y1	(Y1) Polymer Eudragit S100 (mg)
Y2	(Y2) Surfactant tween 80(%)
Y3	(Y3) Stirring time (min.)
Coding	**Dependent Variables**
Z1	Vesicle size (nm)
Z2	Entrapment efficiency (%)

3.5.4 Formulation trials as per Box–Behnken design

Table 14 Formulation trials as per Box–Behnken design

Formulation	Polymer Eudragit S100 (mg)	Mometasone furoate and Clotrimazole (%)	Surfactant Tween 80 (%)	Distilled water (ml)	Sodium CMC (%)	Chloroform (ml)	Stirring time (min)	Vesicle size (PS)	Entrapment efficacy (EE)
MS1	1	0.1-1%	1	250.0	0.5	10.0	0	563.4	68.23
MS2	0	0.1-1%	-1	250.0	0.5	10.0	1	258.6	90.46
MS3	0	0.1-1%	1	250.0	0.5	10.0	-1	776.2	74.35
MS4	0	0.1-1%	0	250.0	0.5	10.0	0	543.8	78.44
MS5	1	0.1-1%	0	250.0	0.5	10.0	1	197.3	89.26
MS6	-1	0.1-1%	0	250.0	0.5	10.0	-1	808.2	68.11
MS7	1	0.1-1%	0	250.0	0.5	10.0	-1	947.2	71.09
MS8	1	0.1-1%	-1	250.0	0.5	10.0	0	523.7	72.28
MS9	0	0.1-1%	1	250.0	0.5	10.0	1	227.1	90.88
MS10	-1	0.1-1%	0	250.0	0.5	10.0	1	219.5	91.76
MS11	-1	0.1-1%	1	250.0	0.5	10.0	0	423.6	84.39
MS12	0	0.1-1%	0	250.0	0.5	10.0	0	347.6	83.76
MS13	0	0.1-1%	-1	250.0	0.5	10.0	-1	806.2	63.07
MS14	-1	0.1-1%	-1	250.0	0.5	10.0	0	522.0	65.28

3.5.5 Limits of Variables (Constraints)

Table 15 Variables operating range for microsphere formulation

Name	Goal	Lower Limit	Upper Limit	Importance
A:Polymer Conc.	is in range	-1	1	3
B:Surfactant Conc.	is in range	-1	1	3
C:Stirring time	is in range	-1	1	3
Vesicle size	none	197.3	947.2	3
Encapsulati	non	63.07	91.76	3

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on efficiency	e			

3.5.6 Fit Summary

Table 16 Response 1: Particle size

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.9564**	**0.9178**	**0.8980**	**Suggested**
2FI	0.5988	0.9461	0.9087	0.8817	
Quadratic	0.6276	0.9319	0.8921	0.7566	
Cubic	0.9319		0.6881		Aliased

3.6 Effect of formulation variables on Vesicle size (ANOVA for Linear model)

3.6.1 Response 1: Vesicle size

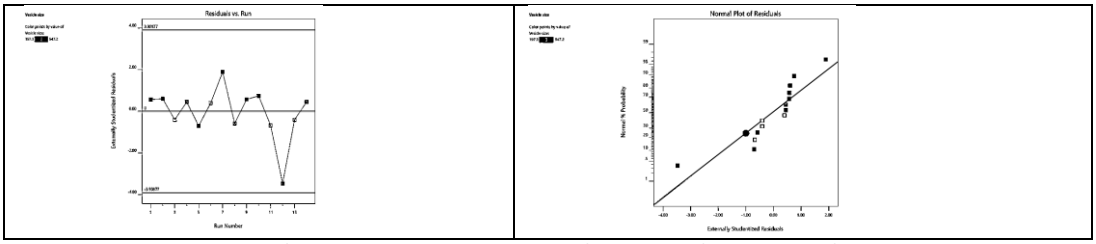
Table 17: Response 1: Vesicle size (ANOVA for Linear model)

Source	Sum of Squares	Mean Square	F-valu	p-valu	
			e	e	
Model	7.515E+05	2.505E+05	49.40	< 0.0001	significant
A-Polymer Conc.	8339.22	8339.22	1.64	0.2286	
B-Surfactant Conc.	1806.31	1806.31	0.3562	0.5639	
C-Stirring time	7.413E+05	7.413E+05	146.19	< 0.0001	
Residual	50710.68	5071.07			
Lack of Fit	31463.46	3495.94	0.1816	0.9564	not significant
Pure Error	19247.22	19247.22			
Cor Total	8.022E+05				

The **Model F-value** of 49.40 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

3.6.2 Final Equation in Terms of Coded Factors

Vesicle size (Z1) +511.74 Intercept +32.29 AY1 -15.03 B Y2 -304.41 C Y3



Graph 8: Graphical representation of Residuals vs run, Normal plot of Residuals of microsphere formulation on Vesicle size

7.6.3 Predicted value and actual value of all formulations

Table 19: Predicted value and actual value of Particle size and Entrapment efficiency

Formulations	Actual Value of Particle size	Predicted Value of Particle size	Actual Value of % entrapment efficiency	Predicted Value of % entrapment efficiency
MS 1	563.40	529.00	68.23	69.02
MS 2	258.60	222.36	90.46	89.78
MS 3	776.20	801.13	74.35	75.03
MS 4	543.80	511.74	78.44	81.10
MS 5	197.30	239.62	89.26	88.32
MS 6	808.20	783.87	68.11	69.05
MS 7	947.20	848.44	71.09	69.62
MS 8	523.70	559.06	72.28	73.91
MS 9	227.10	192.30	90.88	91.04
MS 10	219.50	175.04	91.76	93.23
MS 11	423.60	464.43	84.39	82.77
MS 12	347.60	511.74	83.76	81.10
MS 13	806.20	831.18	63.07	62.91
MS 14	522.01	494.48	65.28	64.50

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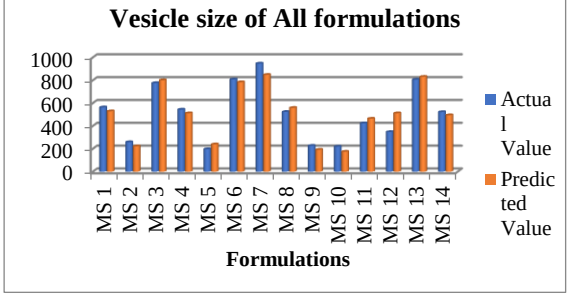
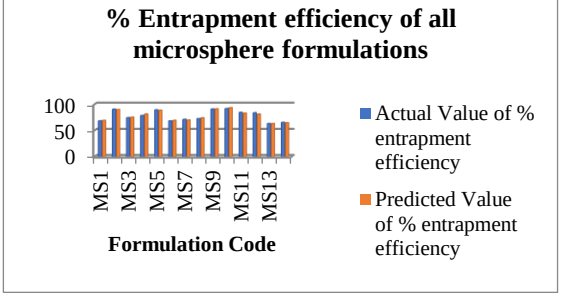


Graph 9: graphical representations of Predicted value and actual value of all microsphere formulations



Graph 10: graphical representation of Predicted and actual value of Entrapment efficiency

Figure 1: Response surface plot showing combined effect of polymer and surfactant on vesicle size of microsphere

3.6.4 Effect of formulation variables on Entrapment efficiency

Table 20 Response 2: Entrapment efficiency (Fit Summary)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0031	0.4381	0.9766	0.9812	
2FI	0.1948	0.4820	0.7392	0.3405	
Quadratic	**0.0363**	**0.8248**	**0.9348**	**0.9020**	**Suggested**
Cubic	0.8248		0.8670		Aliased

3.7ANOVA for linear model:

3.7.1 Response 2: EE (ANOVA Quadratic):

Table 21 Response 2: Encapsulation efficiency:

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	1355.47	150.61	21.73	0.0047	significant
A-Polymer Conc.	9.42	9.42	1.36	0.3086	
B-Surfactant Conc.	89.51	89.51	12.91	0.0229	
C-Stirring time	918.92	918.92	132.55	0.0003	
AB	134.10	134.10	19.34	0.0117	
AC	7.51	7.51	1.08	0.3568	
BC	29.48	29.48	4.25	0.1082	
A^2	53.66	53.66	7.74	0.0497	
B^2	63.65	63.65	9.18	0.0388	
C^2	29.77	29.77	4.29	0.1070	
Residual	27.73	6.93			
Lack of Fit	13.58	4.53	0.3198	0.8248	not significant
Pure Error	14.15	14.15			
Cor Total	1383.20				

3.7.3 Final Equation in Terms of Coded Factors

Entrapment efficiency EE **(Z2)** = +81.10(Intercept) -1.09 A Y1 +3.34 B Y2 A2 +10.72C Y3-5.79 AB Y1 Y2 -1.37 AC Y1 Y3 -2.71 BC Y2 Y3 -4.09 A^2 $Y1^2$ -4.46 B^2 $Y2^2$ +3.05 C^2Y3^2.

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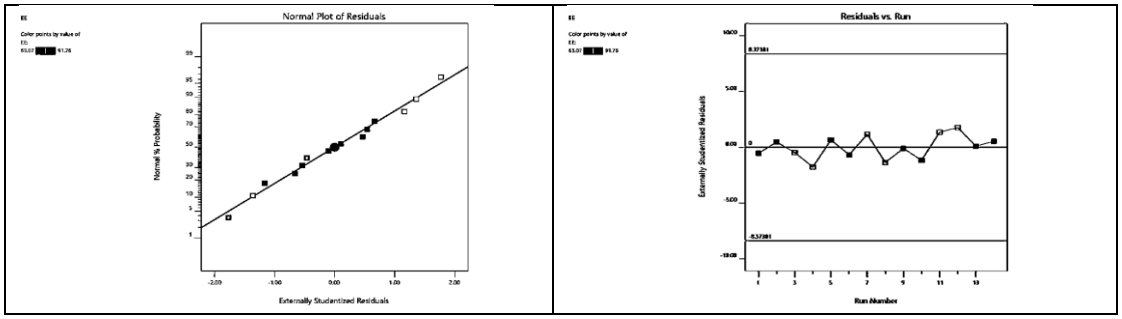

Figure 2: Graphical representation of Residuals vs run, Normal plot of microsphere formulation on Entrapment efficiency

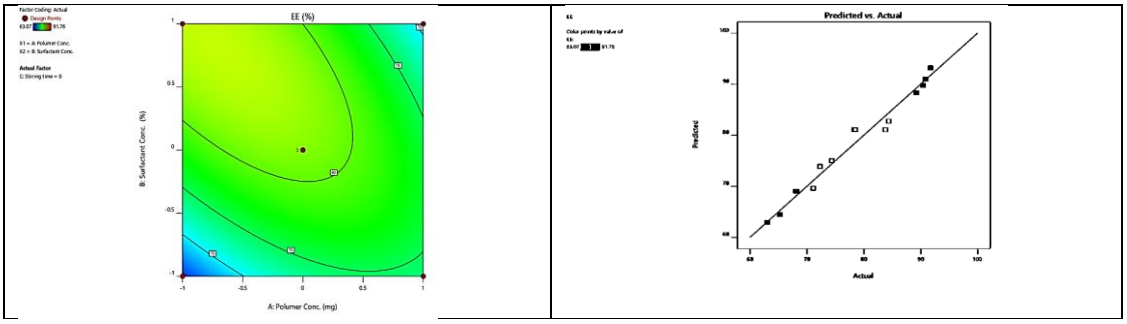

Figure 3: Two-dimensional contour plots for the effect of polymer and surfactant concentration on % entrapment efficiency and predicted vs. actual

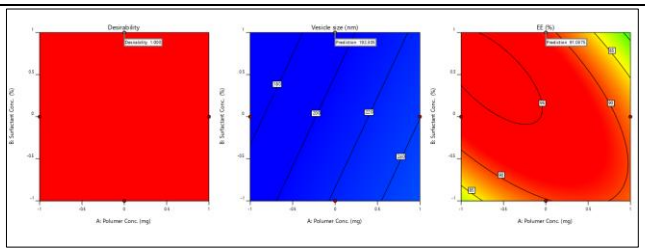

Figure 4: Response surface plot showing prediction data for optimization

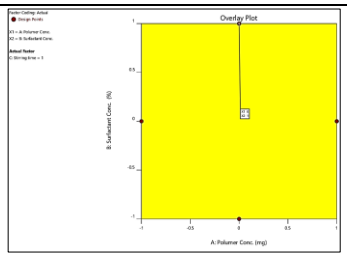

Figure 5: Overlay plot for optimization formulation

3.7.5 Optimized formula of microsphere formulation

Table 23: Optimized formula of microsphere formulation

Polymer Eudragit S100 (mg)	Surfactant tween 80(%)	Stirring time (min.)	Vesicle size (nm)	EE (%)	Desirability	
100.000	1.000	10.000	783.870	69.052	1.000	
200.000	**1.5**	**90.000**	**192.305**	**91.037**	**1.000**	**Selected**
200.000	1.5	10.000	801.130	75.032	1.000	

Table 24: Final Composition of optimized Microsphere formulation as per Design of experiment approach

Formulation	Polymer Eudragit S100 (mg)	Surfactant Tween 80 (%)	Distilled water (ml)	Sodium CMC (%)	Chloroform (ml)	Stirring time (min)
MSOF	200	1.5	250.0	0.5	10.0	90.00

3.8 Characterization of optimized microsphere formulation

3.8.1 Particle size

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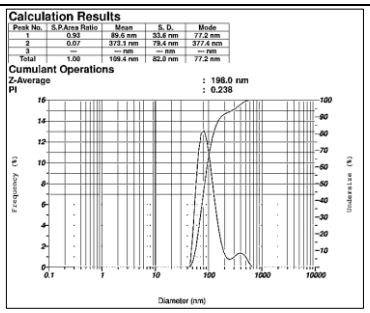
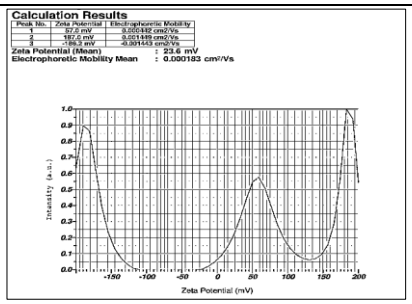


Graph 11: Particle size of optimized formulation



Graph 12: Zeta potential of optimized formulation

3.8.2 Zeta potential, particle size and Entrapment efficacy

Table 25: Particle size and zeta potential

Formulation	Vesicle size (Predicted value)	Vesicle size (Actual value)	Entrapment efficacy (Predicted value)	Entrapment efficacy (Actual value)	Zeta potential
Microsphere	192.30 nm	198.0 nm	91.03%	90.27 %,	23.6 mV

3.8.4 SEM analysis

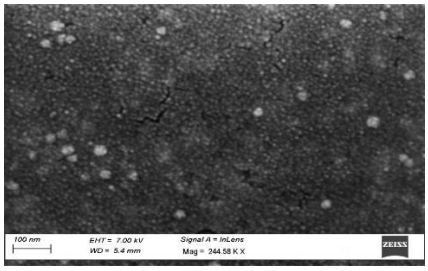


Figure 6 SEM analysis of optimized formulation

3.9 Evaluation of Prepared microsphere loaded cream

3.9.1 Organoleptic properties

Table 28: Organoleptic properties of microsphere loaded cream

Parameters	Results
Physical appearance	Semisolid cream
Colour	Slightly yellowish cream
Homogeneity	Absence of aggregates

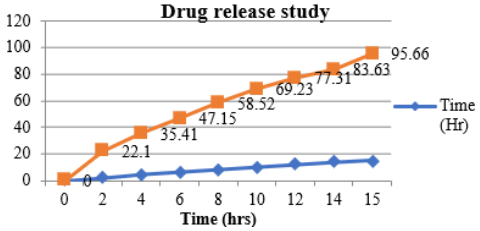
3.6.2 Measurement of pH and viscosity of microsphere loaded cream

Table 29: pH and viscosity determination

Formulation	pH	Results (cps)	Results (gm.cm/sec)
Drug-loaded microsphere cream	5.9	4023±0.34	17.58

Table 30 *In-vitro* drug release studies

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
2	22.1	77.9	1.414	1.892	0.301	1.344
4	35.41	64.59	2.000	1.810	0.602	1.549
6	47.15	52.85	2.449	1.723	0.778	1.673
8	58.52	41.48	2.828	1.618	0.903	1.767
10	69.23	30.77	3.162	1.488	1.000	1.840
12	77.31	22.69	3.464	1.356	1.079	1.888
14	83.63	16.37	3.742	1.214	1.146	1.922
15	95.66	4.34	3.873	0.637	1.176	1.981



Graph 13: *In-vitro* drug release studies

Table 31: Correlation value (R^2 value)

Formulation	Model	Kinetic parameter values
Microsphere cream	Zero Order	$R^2 = 0.978$
	First Order	$R^2 = 0.883$
	Higuchi	$R^2 = 0.971$
	Korsmeyerpeppas	$R^2 = 0.819$

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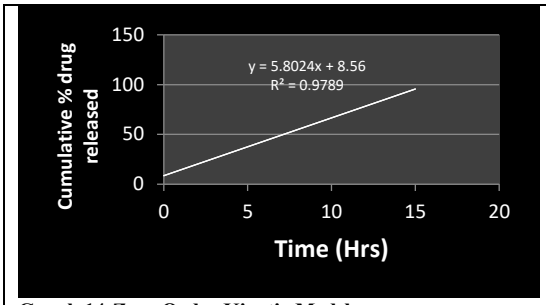
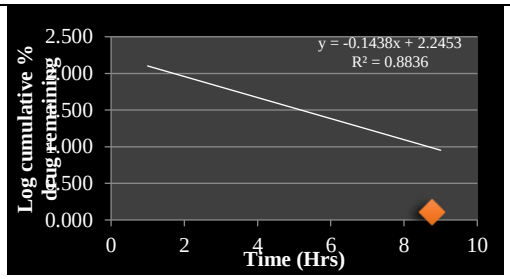
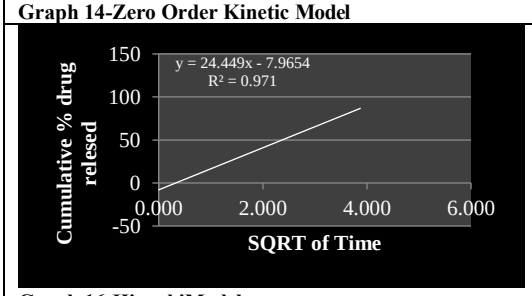


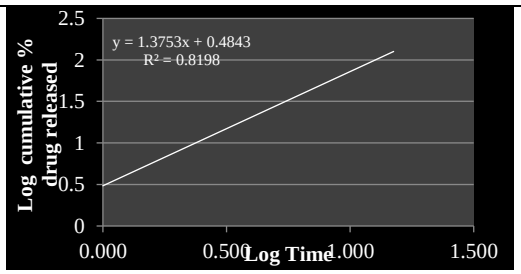
Graph 14-Zero Order Kinetic Model



Graph 15–First Order Kinetic Model



Graph 16-HiguchiModel



Graph 17-KorsmeyerPeppas

11 Results of anti-fungal activity of Microsphere cream formulation:

7.11.1 Anti-fungal activity of Microsphere cream

Table 32: Anti-fungal activity of Microsphere cream *against Candida albicans* fungus

Sample Name (mg/ml)	Zone of Inhibition (mm) of *Candida albicans*
Control	00 mm
Placebo or blank cream	2 mm
Microsphere cream (1mg/ml)	5 mm
Microsphere cream (1.5mg/ml)	13 mm

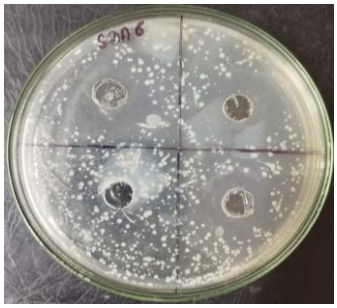


Figure: 7 Anti-fungal activity of Microsphere cream against *Candida albicans* fungus

7.12 Stability Study of optimized formulation (microsphere cream)

Table 33: Stability Study of optimized formulation (microsphere cream)

Time (Days)	30^{0}C±2 ^{0}C and 60 ± 5% RH		40^{0}C±2 ^{0}C and 70 ±5% RH	
	pH	Viscosity	pH	Viscosity
0	5.9	4023	5.9	4023
30	5.5	4020	5.4	4025
45	5.8	4030	5.7	4032
60	5. 3	4028	5. 1	4030
90	5. 7	4022	5. 5	4028

4. CONCLUSION:

In conclusion, the use of Box-Behnken design in the formulation of microspheres containing mometasone furoate and clotrimazole proved to be an effective strategy for optimizing drug delivery parameters and enhancing therapeutic outcomes. The final optimized formulation demonstrated favourable characteristics such as uniform particle size, high encapsulation efficiency, and sustained drug release, which are critical for effective topical or localized antifungal therapy. The combination of a corticosteroid and an antifungal agent within a single delivery system not only ensured better control of inflammation and infection simultaneously but also reduced the need for multiple applications or treatments. The statistical design approach provided a comprehensive understanding of how formulation variables interact and allowed for precise control over the desired properties of the microspheres. Furthermore, the enhanced in vitro antifungal activity observed with the optimized formulation validates the potential of this dual-drug microsphere system for clinical applications in managing fungal infections with inflammatory symptoms. This research lays the groundwork for future investigations into advanced, patient-compliant, and targeted drug delivery systems using a rational design and formulation approach.

5. REFERENCES:

1. Le, M. Q., Violet, F., Paniagua, C., Garric, X., & Venier-Julienne, M. C. (2018). Penta-block copolymer microspheres: Impact of polymer characteristics and process parameters on protein release. *International Journal of Pharmaceutics*, *535*(1-2), 428-437.
2. Iacob, A. T., Lupascu, F. G., Apotrosoaei, M., Vasincu, I. M., Tauser, R. G., Lupascu, D., ... & Profire, L. (2021). Recent biomedical approaches for chitosan based materials as drug delivery nanocarriers. *Pharmaceutics*, *13*(4), 587.

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 621-633
DoI-10.004687/1000-9035.2025.085

3. Boardman, C., Chachi, L., Gavrila, A., Keenan, C. R., Perry, M. M., Xia, Y. C., & Sharma, P. (2014). Mechanisms of glucocorticoid action and insensitivity in airways disease. *Pulm*
4. Giresha, A. S. (2021). Secretory phospholipase A2 group IIA: a potential therapeutic target in inflammation. *Current Research and Trends in Medical Science and Technology. Lucknow (Uttar Pradesh, India): Department of Ortho KGMU*, *1*, 34-85.
5. Szentmáry, N., Shi, L., Daas, L., & Seitz, B. (2020). Diagnostics and management approaches for Acanthamoeba keratitis. *Expert Opinion on Orphan Drugs*, *8*(7), 227-236.
6. Tushir, R., Chauhan, A., Bansal, R., Dalal, A., & Kumar, P. (2022). A descriptive review on pharmacokinetics and pharmacodynamics profile of an antifungal agent: Clotrimazole. *Eur J Pharm Med Res*, *9*, 204-216.
7. Mijaljica, D., Spada, F., & Harrison, I. P. (2022). Emerging trends in the use of topical antifungal-corticosteroid combinations. *Journal of Fungi*, *8*(8), 812.
8. Hauber, B., Hand, M. V., Hancock, B. C., Zarrella, J., Harding, L., Ogden-Barker, M., ... & Watt, S. J. (2024). Patient acceptability and preferences for solid oral dosage form drug product attributes: a scoping review. *Patient preference and adherence*, 1281-1297.
9. Craveiro, R., Rocha, Â., Fernandes, C., Duarte, A. R. C., Marto, J., & Paiva, A. (2024). Determination of mometasone furoate solubility, using deep eutectic systems, and topical formulation-experimental and computational studies. *Sustainable Chemistry and Pharmacy*, *42*, 101783.
10. Wable, S. A., Pingale, P. L., Shinkar, D. M., Boraste, S. S., & Amrutkar, S. V. (2024). Formulation, Optimization, and Evaluation of Transdermal Patches of Clotrimazole and Betamethasone Dipropionate for Candidiasis. *Research Journal of Pharmacy and Technology*, *17*(9), 4267-4274.
11. Merey, H. A., El-Mosallamy, S. S., Hassan, N. Y., & El-Zeany, B. A. (2016). Spectrophotometric and chemometric study for the simultaneous determination of mometasone furoate and miconazole nitrate in the presence of pharmaceutical dosage form additive. *Analytical Chemistry Letters*, *6*(1), 70-85.
12. Unde, S., & Kurup, N. (2021). Development and validation of ultraviolet spectroscopic method for estimation of methoxsalen in bulk using methanol and phosphate buffer (pH 7.4). *Indian Journal of Pharmaceutical Education and Research*, *55*(2), S572.
13. Siddique, I. M. (2024). Exploring functional groups and molecular structures: a comprehensive analysis using FTIR spectroscopy. *development*, *1*(2).
14. Verma, N. K., Alam, G., Mishra, J. N., Vishwakarma, D. K., & Singh, V. K. (2015). Formulation development and characterization of ethyl cellulose microspheres of ibuprofen. *Pharm Lett*, *7*, 280-4.
15. Singh, R., Goel, S., Sharma, P. K., & Agarwal, A. (2021). Hydrogel as a novel drug delivery system: recent advancements and patents. *Current Nanoscience*, *17*(1), 14-25.
16. Jiang, N. (2015). Electron beam damage in oxides: a review. *Reports on Progress in Physics*, *79*(1), 016501.
17. De Leo, V., Milano, F., Mancini, E., Comparelli, R., Giotta, L., Nacci, A., ... & Catucci, L. (2018). Encapsulation of curcumin-loaded liposomes for colonic drug delivery in a pH-responsive polymer cluster using a pH-driven and organic solvent-free process. *Molecules*, *23*(4), 739.
18. Badwaik, C. B., Lade, U. B., Agarwal, T., Barsagade, P., Nandgave, M., &Gaddamwar, N. (2022). Formulation and evaluation of herbal face cream. International Journal of Pharmaceutical Research and Applications, 7(1), 955-960.
19. Yasir Siddique, M., Nazar, M. F., Mahmood, M., Saleem, M. A., Alwadai, N., Almuslem, A. S., ... & Akhlaq, M. (2021). Microemulsified gel formulations for topical delivery of clotrimazole: structural and in vitro evaluation. *Langmuir*, *37*(46), 13767-13777.
20. Greive, K. A., & Barnes, T. M. (2016). Bioequivalence of 0.1% mometasone furoate lotion to 0.1% mometasone furoate hydrogel. *Australasian Journal of Dermatology*, *57*(2), e39-e45.
21. Rai, S. Y., & Ravikumar, P. (2016). Development and evaluation of microsphere based topical formulation using design of experiments. *Indian Journal of Pharmaceutical Sciences*, *78*(2), 182-192.
22. Khanum, M. (2017). *Development and Evaluation of Nanoparticle Based Topical Gel Containing Antifungal Drug Fluconazole* (Master's thesis, Rajiv Gandhi University of Health Sciences (India)).
23. Adadey, S. M. (2014). *Isolation and Characterization of Antifungal Agents Produced by Wood Decaying Fungi From Ghana* (Doctoral dissertation, University of Ghana).
24. El Khetabi, A., Ezrari, S., El Ghadraoui, L., Tahiri, A., Ait Haddou, L., Belabess, Z., ... & Lahlali, R. (2021). In vitro and in vivo antifungal activities of nine commercial essential oils against brown rot in apples. *Horticulturae*, *7*(12), 545.
25. González-González, O., Ramirez, I. O., Ramirez, B. I., O'Connell, P., Ballesteros, M. P., Torrado, J. J., & Serrano, D. R. (2022). Drug stability: ICH versus accelerated predictive stability studies. *Pharmaceutics*, *14*(11), 2324.