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Original Research Article

Detail work on formulation development and evaluation of micro sponges gel of clotrimazole for treatment of vaginal fungal infection

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ABSTRACT

Background: Vaginal infection is so widespread that women have to seek medical counseling. In fact, almost 70% of women experience vaginal infections in their life. Vulvovaginal candidiasis is responsible for vaginal infections and Candida albicans is the major agent. Vaginal infections are caused by hormonal changes, negative sexual effects, irrelevant quality of life, high mortality rate, depressive mood and various kind of anxiety.

Aim & Objective : Detail Work on Formulation Development and Evaluation of Micro sponges gel of Clotrimazole for Treatment of Vaginal fungal Infection. The microsponge-based novel delivery system has been developed for vaginal delivery of Clotrimazole. The method adopted was quasi-emulsion solvent diffusion. Formed microsponges were a spherical in shape Different drug–polymer ratio reflected good particle size, drug content and entrapment efficiency. Microsponge-based gel showed in vitro drug release reflected highest regression value for Koshmeyer-Peppas and in vitro antifungal activity of CLZ microsponges gel was higher than the market formulation.

Methods: Drug, chemical and solvent to be used are listed as Clotrimazole, Eudragit RL-100, Polyvinyl alcohol, Carbopol 934, Sodium Citrate dihydrate, Citric Acid, Dichloromethane, Triethanolamine, Agar 1. Preparation of citrate buffer pH 4.5 solution 2. Preparation of standard curve of citrate buffer pH 4.5 solution: methanol 3. FTIR spectroscopy 4. Selection of method for the preparation of microsponges 5. Quasi emulsion solvent diffusion (two step method) is selected for the preparation of microsponges 6. Selection of amount of drug (clotrimazole): 7. Selection of optimum volume of solvent (dichloromethane): 8. Selection of amount of emulsifier (polyvinyl alcohol): 9. Preparation of microsponges.

Result : The f23 formulation, which had a manufacturing yield of 66.58%, an entrapment efficiency of 91.26%, and an actual drug content of 67.28 percent, was determined to be more trustworthy than the other formulations. The CDR was 66.18%, and the Flux value was 76.17(g/cm2/h).With (r2)0.9738 and a n value of 0.3981, Koshmeyer Peppas was judged to be the model that suited the data the best.A measurement of the viscosity revealed non-Newtonian flow. The ZOI, which exceeded the advertised preparation, was discovered to be 2.2 cm.

Conclusions : For vaginal delivery of Clotrimazole, a new delivery mechanism based on microsponges has been created. The technique used was solvent diffusion from a quasi-emulsion. Microsponges that had been formed had a spherical shape. Different drug-polymer ratios were indicative of good drug content, entrapment effectiveness, and particle size. Microsponge-based gel demonstrated in vitro drug release that had the greatest regression value for Koshmeyer-Peppas, and CLZ microsponges gel had stronger in vitro antifungal activity than the commercial formulation.

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1. Introduction

1.1. Vaginal infection

Vaginal infection is so widespread that women have to seek medical counseling. In fact, almost 70% of women experience vaginal infections in their life. Vulvovaginal candidiasis is responsible for vaginal infections and Candida albicans is the major agent. Vaginal infections are caused by hormonal changes, negative sexual effects, irrelevant quality of life, high mortality rate, depressive mood and various kind of anxiety.

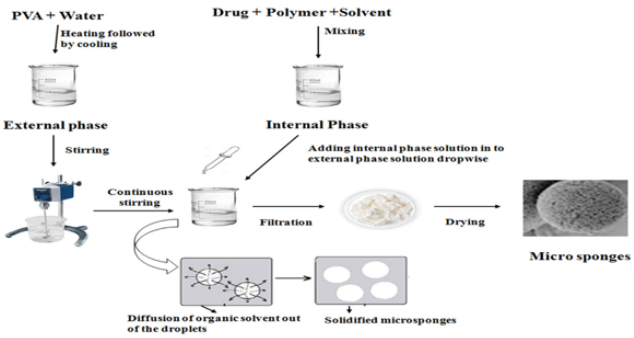

Fig. 1: Quasi-emulsion solvent diffusion for preparation ofmicriosponges.

2. Materials and Methods

2.1. Materials

Drug, chemical and solvent to be used are listed as Clotrimazole, Eudragit RL-100, Polyvinyl alcohol, Carbopol 934, Sodium Citrate dihydrate, Citric Acid, Dichloromethane, Triethanolamine, Agar

2.2. Equipments

Equipment to be used in project work are listed as UV-visible double beam

Spectrophotometer, Hot air oven, Sonicator, Magnetic stirrer, Digital melting point apparatus, Franz diffusion cell, pH meter, Digital melting point, Disintegration apparatus, Optical microscope, Scanning electron microscopy (SEM), Fourier transforms infrared spectroscopy (FTIR), Digital weighing balance.

2.3. Methodology

2.3.1. Selection of drug

Vulvovaginal candidiasis infection is often disease especially in adults and the cause due to the fungal infections. Clotrimazole drug is seems to be effective and less toxicity [1]

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2.4. Identification of drug

2.4.1. Determination of melting point

Drug was filled in capillary tube which was closed one side and heat was increased by the rise of temperature the drug starts melting this temperature. [2]

2.5. FTIR spectroscopy

FTIR analysis for was done by FTIR NICOLET 6700. Each sample was mixed with potassium bromide in 1:100 and compressed into potassium bromide (KBr) pellets later observed at the range from 3500cm-1 to 1500cm-1. [3]

2.6. UV spectroscopy

10mg of clotrimazole was weighed and transferred to 100ml volumetric flask and diluted up to the mark with methanol (1000μg/ml). 10ml from the prepared solution was pipetted out in a 10ml volumetric flask and dilute up to the mark. From this 1.5ml of the solution was pipetted into a 10ml volumetric flask and diluted up to the mark with methanol to form 27μg/ml that was scanned in the range of 200-400nm. [3]

3. Analytical Methods

3.1. Preparation of citrate buffer pH 4.5 solution

4.8 g of citric acid dissolved 500ml of water then add 9 g of sodium citrate to the first solution after that solution is placed in Sonicator. Adjust final pH of solution by HCl. [4]

3.2. Preparation of standard curve of citrate buffer pH 4.5 solution: methanol

2 to 27μg/ml solution were prepared and scanned in the range of 200-400nm using UV visible spectrophotometer [4]

3.3. Preparation of standard plot in methanol

2 to 27 μg/ml solution were prepared and scanned in the range of 200-400nm using UV visible spectrophotometer. [5]

3.4. Preformulation studies

3.4.1. Aqueous solubility

To determine aqueous solubility the drug, saturation shake-flask method was used which involve agitation of excess amount of sample in distilled water at 50rpm and 37°C for 24-72 h followed by phase separation of saturated solution from undissolved solute‘. [6]

3.5. FTIR spectroscopy

The identification of pure drug Clotrimazole was done evaluated by recording spectrum by using FTIR. KBr pellet was used for the determination of functional group of drug by FTIR. FTIR spectrum was recorded between scanning

ranges of 1500-3500 cm-1. Finally obtained spectrum was compared with reference spectrum.[3]

3.6. Formulation development

3.6.1. Selection of dosage form

Microsponges loaded with drug clotrimazole was prepared in vaginal gel and the application of the gel over the vaginal infections is easy and convenient and it increases the retention time of drug. Gel or jellified emulsion is stable one and better vehicle for hydrophobic or water insoluble drugs as clotrimazole.

3.7. Selection of method for the preparation of microsponges

Quasi emulsion solvent diffusion (two step method) is selected for the preparation of microsponges.[2]

3.8. Selection of amount of drug (clotrimazole)

Drug and other excipient ratio were varied in different formulation but the concentration of polymer remain same in each and every formulation. Formulation f1 to f27 drug concentration were changed (100 to 400 mg of clotrimazole).Stirring rate and stirring time were also kept constant at 1000 rpm and an agitated up to 30 min for the formation of microspheres and after 8 h of stirring, the experiment was stopped as the dichloromethane has been removed from the reaction medium. The aforementioned f1-f27 formulations with varying drug (CLZ) concentration were evaluated for % yield, drug content, % drug entrapment efficiency and particle size. The best obtained formulation was used for further formulation development.

3.9. Selection of optimum volume of solvent (dichloromethane)

Formulation F1 to F27 were prepared by altering the volume of dichloromethane. The volume was increased from 5 to 20ml in each formulation. Production yield, actual drug content, theoretical drug content, particle size and shape of the microsponges were studied and the formulation with best result was selected for the selection of optimum volume of dichloromethane.

3.10. Selection of amount of emulsifier (polyvinyl alcohol)

Formulation F1 to F27 were prepared by altering the volume of emulsifier. The volume was increased from 20 to 60ml in each formulation. Production yield, actual drug content, theoretical drug content, particle size and shape of the microsponges were studied and the formulation with best result was selected for the selection of optimum volume of emulsifier.

Table 1: Formulation table

Formulation code	Drug (clotrimazole)	Polymer (mg)	PVA (mg)	Solvent (ml)
f1	100	100	20	5
f2	100	100	40	15
f3	100	100	60	10
f4	100	100	20	5
f5	100	100	40	10
f6	100	100	60	15
f7	100	100	20	5
f8	100	100	40	10
f9	100	100	60	15
f10	200	100	20	5
f11	200	100	40	10
f12	200	100	60	15
f13	200	100	20	5
f14	200	100	40	10
f15	200	100	60	15
f16	200	100	20	5
f17	200	100	40	10
f18	200	100	60	15
f19	400	100	20	5
f20	400	100	40	10
f21	400	100	60	15
f22	400	100	20	5
f23	400	100	40	10
f24	400	100	60	15
f25	400	100	20	5
f26	400	100	40	10
f27	400	100	60	15

3.11. Preparation of microsponges

Clotrimazole microsponges was prepared by an emulsion solvent diffusion method. In this method, the organic internal phase containing clotrimazole and Eduragit RL100 in dichloromethane was gradually added into distilled water which contained different concentrations of polyvinyl alcohol (PVA) as emulsifying agent. The mixture was stirred for 8 h, at 25ºC to remove dichloromethane from the reaction flask. The formed microsponges were filtered and washed with distilled water before being tray-dried at room temperature for 24hrs.[2]

4. Evaluation of Microsponges

4.1. Physical appearance

Prepared microsponges were checked for appearance, texture and uniformity.

4.2. Microscopy

The morphology and size of microsponge were observed by scanning electron microscopy. Prepared microsponges were analyzed for surface morphology using SEM (Carl Zeiss) as it provides topographical information up to 10X to

50.00 KX equipped with EDS detector (Oxford instruments) in terms of percentage concentration. This is useful for material verification and contaminant identification. Samples were fixed on a double-faced adhesive tape operated at a 20- kV and 5- kV acceleration voltage respectively.[2]

4.3. Particle size

Particle size of prepared microsponges was measured by using optical microscopy. The eye piece micrometer was calibrated with the help of a stage micrometer. More than 300 microsponges were measured randomly for their diameter. The average particle size was determined by using Edmondson's equation.[7]

$$Dmean = \Sigma n.d / \Sigma n$$

Where, n = Number of microsponges checked; d = Mean size range

4.4. Determination of production yield

Percentage yield was determined by calculating the initial weight of raw materials and the weight of microsponge. Percentage yield was calculated by using the following formula.[7]

$$\text{Percentage yield} = \text{Practical yield} \times 100$$

4.5. Theoretical yield

4.5.1. Determination of entrapment efficiency

Microsponge equivalent to 20 mg of the clotrimazole was taken for determination of loading efficiency. The amount of drug entrapped was estimated by dissolving the microsponges in DCM and measuring the absorbance in UV at 297.5 nm after necessary dilutions. The amount of drug entrapped in the microsponges were calculated by the following formula

$$\text{Entrapment efficiency} = \text{actual weight of drug in microsponges} \times 100$$

4.6. Theoretical weight of drug

4.6.1. Preparation of microsponges based gel

Weighed amount of carbopol 934 was slowly added to purified water with continuous stirring and dispersion was then allowed to hydrate and swell for 4 h at room temperature. The pH of the formed gel was checked and neutralized to pH 4.5 until the desired pH value obtained. During neutralization process, the mixture was stirred until a homogenous clear gel was formed. Finally, the prepared carbopol gel was stirred by using a mechanical stirrer at 1000 rpm with a slow addition of prepared microsponge's powder to form microsponges based gel formulation.[8]

4.7. Evaluation of microsponges based gel formulation

4.7.1. Visual appearance

The gel was subjected to visual inspection by preparing smears of formulation on glass slide and to be examined under a microscope to check homogeneity and uniformity.

4.7.2. Determination of pH

The pH of gel formulations was measured using the pH meter. 5g gel was dispersed in 45 ml distilled water at 27 ºC and solution pH was measured. The pH measurements was recorded in triplicate to generate an average pH value for each formulation.[9]

4.7.3. In vitro release studies

In vitro release studies was carried out using Franz diffusion cells with a receptor compartment volume of 20 mL and an effective diffusion area of 3.14 cm2. Cellulose dialysis membrane was soaked in receptor media (citrate buffer pH 4.5) for 8 h before experiment. 1 gram of gel-containing microsponges was placed on the donor side. The receptor medium was continuously stirred at 600 rpm and thermostated at 32±0.5°C with a circulating jacket. At predetermined time intervals, 2 mL samples was withdrawn from the receiver compartment and replaced with an equal volume of fresh buffer. The collected samples was analyzed by UV.[10]

4.7.4. Mechanism of drug release

The mechanism of drug release was determined by fitting the release data into various kinetic models such as zero-order (% drug release vs time), first-order (log % drug retained vs time), Higuchi (% drug release vs square root of time) and Koshmeyer Peppas.[11–13]

Zero-order model: Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$$Qt = Q0 + K0t$$

Where, Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0 = 0) and K0 is the zero-order release constant expressed in units of concentration/time.

To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

4.8. Application

This relationship can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs in coated forms, osmotic systems, etc.

4.9. First order model

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order kinetics can be expressed by the equation:

$Qt = Qoe^{-kt}$ or $\ln Qo + kt$

Where, Qt is the amount of drug released in time t, Q0 is the initial concentration of drug in the solution and k is the first order rate constant. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of -K/2.303.

4.10. Application

This relationship can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices.

4.11. Higuchi model

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then extended to different geometrics and porous systems. Accordingly, model expression is given by the equation:

$ft = Q = A\sqrt{D(2C-Cs)\,Cs\,t}$

Where, Q is the amount of drug released in time t per unit area A, C is the drug initial concentration, Cs is the drug solubility in the matrix media and D is the diffusivity of the drug molecules (diffusion coefficient) in the matrix substance. This relation is valid during all the time, except when the total depletion of the drug in the therapeutic system is achieved. To study the dissolution from a planar heterogeneous matrix system, where the drug concentration in the matrix is lower than its solubility and the release occurs through pores in the matrix, the expression is given by equation:

$ft = Q = \sqrt{D\delta\,(2C - \delta Cs)\,Cs\,t\,\tau}$

Where, D is the diffusion coefficient of the drug molecule in the solvent, δ is the porosity of the matrix, τ is the tortuosity of the matrix and Q, A, Cs and t have the meaning assigned above. Tortuosity is defined as the dimensions of radius and branching of the pores and canals in the matrix. In a general way it is possible simplify the Higuchi model as (generally known as the simplified Higuchi model):

$ft = Qt = KH\,t1/2$

Where, KH is the Higuchi dissolution constant. The data obtained were plotted as cumulative percentage drug release versus square root of time

4.12. Application

This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs.

4.13. Koshmeyer-Peppas model

Koshmeyer et al.1983 derived a simple relationship which described drug release from a polymeric system. To find out the mechanism of drug release, first 60% drug release data were fitted in Koshmeyer-Peppas model.

$Mt / M\infty = Ktn$

Where, $Mt/M\infty$ are a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices. In this model, the value of n characterizes the release mechanism of drug as described in table.

To find out the exponent of n the portion of the release curve, where $Mt / M\infty < 0.6$ should only be used. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time.

4.14. Viscosity measurement of CLZ microsponges gel

Brookfield Viscometer was used for rheological studies. The sample (30 g) was placed in a beaker and was allowed to for 2 min before measuring the reading using a spindle at 10, 20, 50, and 100 rpm. At each speed, reading on the viscometer was noted. [10]

4.15. In vitro bioadhesion study

Comparing the CMZ-MSG to the commercial clotrimazole gel (CandidV® gel), the bioadhesive capability of the latter was assessed.A pH 4.5 citrate buffer was used to make an agar plate (1% w/w). The centre of the plate was filled with the test sample. The agar plate was pushed up and down in a pH 4.5 citrate buffer at 37°C after being attached to a US Pharmacopoeia disintegration test instrument for 5 minutes. At its lowest point, the sample on the plate was submerged in the solution; at its highest point, it was not. Visual observations were made of the test samples' time spent on the plate. [14]

4.16. Antifungal activity

Using Candida albicans, an in-vitro inhibition zone test was carried out. After autoclaving, freshly produced agar medium was added to the Petri dishes to create Sabouraud Dextrose Agar (SDA) plates. Using sterile cotton swabs, the fungal suspension of Candida albicans was applied to the solidified agar and left to dry for 10 minutes. Using a

cork borer and prepared wells on agar plates, a produced gel with drug-loaded microsponges was aseptically deposited onto the wells and incubated for two days. For any sign of antifungal activity, the test sample's distinct zones of inhibition were displayed.[15]

5. Result and Discussion

5.1. Identification of drug

5.1.1. Determination of absorption maxima (λmax) in methanol

The UV-visible spectrophotometer was used to conduct the clotrimazole identification test. The measured and reported absorbance maximum values for methanol solution were compared. The typical value for that compound is the wavelength at which the absorption maxima (max) occur. Clotrimazole had an observed value of 264 nm, which was determined to match its claimed value exactly. Comparing the two results proved that the sample was accurate. clotrimazole.[3]

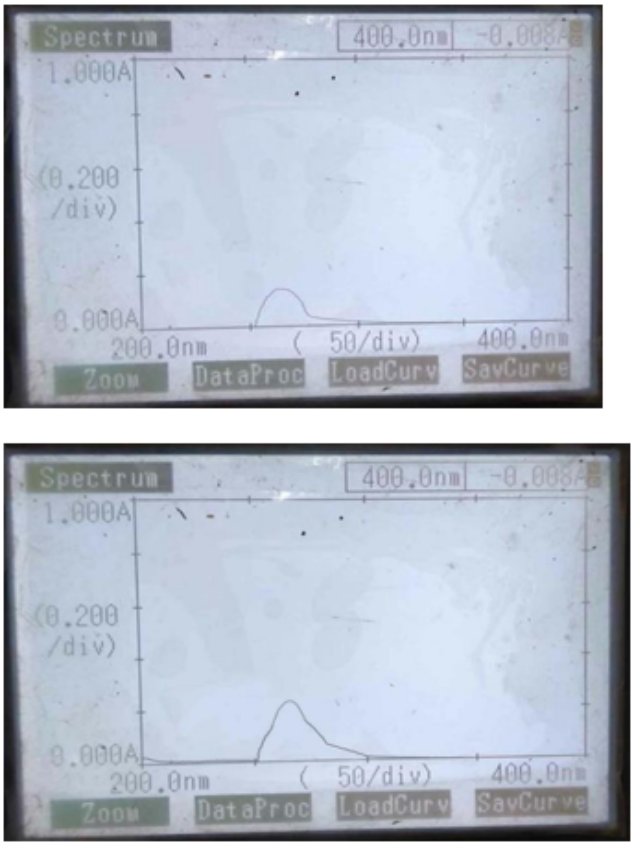

Fig. 2: Wavelength of maximum absorbance in methanol & buffer observed by UV spectroscopy

5.1.2. Determination of absorption maxima (λmax) in citrate buffer pH 4.5 solution and methanol

UV Visible Spectrophotometer was used to identify CLZ. The medium employed was a methanolic solution, and the observed and reported values of the absorption maxima were compared. The maximum absorbance wavelength (max) serves as a defining parameter for a substance. The obtained CLZ's observed value of 260 nm was discovered to be the same as its reported value, confirming the sample's identity as a CLZ. compared to the given value indicating that the sample is CLZ.[3]

5.2. Melting point

The capillary method was used to detect the melting point, an easy test to identify the substance acquired. The observed melting point verified that the substance was CLZ because it was found to match the claimed value exactly i.e.…148±1.35.[2]

5.3. FTIR spectroscopy

The CLZ FTIR study revealed absorption for aromatic C-H stretching at 3000- 3100 cm1, 3010-3100 cm1 for C=C stretching, 1400-1600 cm1 for C=O vibration, and 1670-1820 cm1 for C-H stretching.[3]

5.4. FTIR spectroscopy of pure CLZ

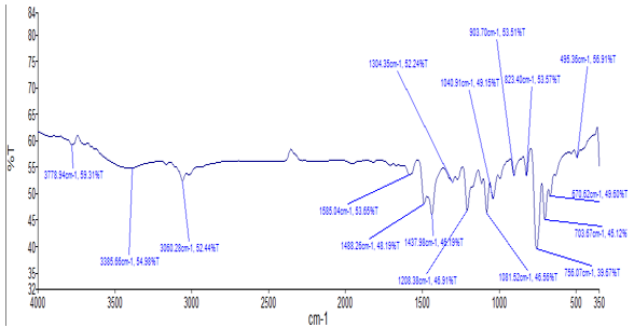

Fig. 3: FTIR spectrum of pure powder of Clotrimazole

5.5. pH determination

The organised vaginal gel was created, and the pH of the gel was determined using a pH metre. The pH was measured to be 4.5-5.5, which is close to the pH of the typical vaginal area.[9]

6. Analytical Methods

6.1. Preparation of standard plot in citrate buffer 4.5 and methanol

In the citrate buffer 4.7 and methanol, the standard plot for CLZ was created. Max 261 nm, which was used for subsequent study of absorption for concentrations ranging from 2 to 27 g/ml, was the observed absorption maxima. The plot and observations were both shown in Table 5. After obtaining the linear plot, the correlation coefficient (r2) value was determined to be 0.9911.[4]

Table 2: Standard Plot data for CLZ in citrate buffer 4.5 and methanol

Conc (μ/ml)	Abs1	Abs2	Abs3	Avg	Std
2	0.073	0.098	0.115	0.095333	0.02112
5	0.165	0.182	0.184	0.177	0.01044
7	0.234	0.236	0.229	0.233	0.003606
9	0.276	0.279	0.263	0.272667	0.00850
12	0.374	0.379	0.37	0.374333	0.00450
15	0.468	0.463	0.484	0.471667	0.01097
17	0.536	0.548	0.534	0.539333	0.0075
20	0.639	0.645	0.643	0.642333	0.00305
22	0.699	0.714	0.721	0.711333	0.01124
25	0.807	0.801	0.822	0.81	0.010817
27	0.954	0.943	0.961	0.952667	0.009074

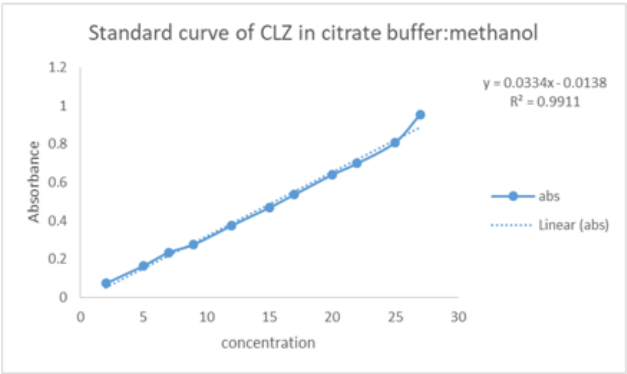

Fig. 4: Standard plot for CLZ citrate buffer 4.5 pH and methanol

The linear equation calculated from the above analysis was further used for determination of drug concentration.

6.2. Preformulation study

In order to identify the physicochemical characteristics of the CLZ that might influence the drug's performance, preformulation tests were carried out. It assisted in gathering crucial information needed for the creation of an effective dosage form and formulation design. In this study, permeability and solubility were assessed using a reliable approach.

6.3. Determination of solubility

The saturation shake-flask method was used to calculate the solubility of pure CLZ. The drug's poor aqueous solubility behaviour was demonstrated by the low solubility of CLZ in aqueous media, which was observed to be 0.35 mg/ml. Due to CLZ's poor aqueous solubility, which was identical to the findings of the study conducted by, it is necessary to increase the drug's dermal bioavailability using appropriate techniques. This necessity had made it necessary for us to do research on improving the solubility of the CLZ.[6]

7. Preparation of Drug Loaded Microsponges

An emulsion solvent diffusion approach was used to create clotrimazole microsponges. In this procedure, distilled water with various concentrations of polyvinyl alcohol (PVA) as an emulsifying agent was mixed with an organic internal phase comprising clotrimazole and Eudragit RL 100 in dichloromethane. To remove dichloromethane from the reaction flask, the liquid was agitated for 4 hours at a temperature of 25°C. The created microsponges were filtered, cleaned with distilled water, and tray-dried for 24 hours at room temperature.[2]

Table 3: Formulation table

Formulation code	Drug (mg)	Polymer(mg)	PVA(mg)	Solvent(ml)
f1	100	100	20	5
f2	100	100	40	10
f3	100	100	60	15
f4	100	100	20	5
f5	100	100	40	10
f6	100	100	60	15
f7	100	100	20	5
f8	100	100	40	10
f9	100	100	60	15
f10	200	100	20	20
f11	200	100	40	5
f12	200	100	60	10
f13	200	100	20	15
f14	200	100	40	20
f15	200	100	60	5
f16	200	100	20	10
f17	200	100	40	15
f18	200	100	60	20
f19	400	100	20	5
f20	400	100	40	10
f21	400	100	60	15
f22	400	100	20	5
f23	400	100	40	10
f24	400	100	60	15
f25	400	100	20	5
f26	400	100	40	10
f27	400	100	60	15

7.1. Effect of Drug: polymer ratio on production yield, drug content and entrapment efficiency

Drug and excipient ratios were adjusted to create different formulations. In every formulation, the polymer content is constant. The best dose of the medicine to be used was chosen after studying its impact on several criteria.

Drug content, entrapment effectiveness, and production yield are all impacted by the drug to polymer ratio.

1. When drug: polymer ratio was 1:1, the production yield was very low (less than 30%) formulation f1 to f9 the production yield were 27.85 to 28.87(%) shown in table 5.7.
2. Increasing the drug: polymer ratio increased the production yield, it was seen that there was a significant difference in production yield of f10 to f27, production yield were 49.28 to 69.68 (%) shown in table 5.7.
3. Actual drug content was lower than the theoretical values in all formulations knowing that the drug is insoluble in water.
4. Higher drug content was obtained at higher drug: polymer ratio. drug content for formulations f1 to f9 were 35.75 to 38.56 (%) as shown in table 5.7.
5. Drug content of formulation f10 to f27 were obtained 53.99 to 71.24(%)as shown in table 5.7
6. Entrapment efficiency was obtained for formulation f1 to f9 (78.7 to 92.9(%)) as shown in table 5.7 and for formulation f10 to f27 the entrapment efficiency was 85.25 To 92.29(%).
7. This conclude that higher the drug and polymer ratio higher the production yield, entrapment efficiency and drug content.

7.2. Effect of emulsifier concentration on production yield, drug content and entrapment efficiency

1. The type and concentration of emulsifier has an important role to play in the preparation of microsponges.
2. The effect of change in concentration of PVA on production yield, drug content and entrapment efficiency was noticed. Shown in fig 5.7.
3. An increase in the production yield was observed on decreasing the amount of the PVA

7.3. Effect of solvent (internal phase) volume on production yield, drug content and entrapment efficiency

1. As dichloromethane concentration was increased from 5 to 15 ml, the microsponges' manufacturing yield and drug content declined. depicted in table 5.7.
2. As indicated in table 5.7, this is caused by the drug's lower concentration in the dichloromethane's higher volume.
3. The data shown in table 5.7 indicate that the production yield increased, but the amount of actual drug content reduced as the volume of dichloromethane increased.
4. Due to the slower droplet formation time and increased drug precipitation in the microsponge due to the decreased dichloromethane diffusion rate from the concentrated solution. This is what leads to increased entrapment effectiveness. The effect of dichloromethane volume on the morphology of microsponges was also investigated.

Table 4: Data of production yield, drug content and entrapment efficiency of f1 to f27

Formulations Code	Production yield (%)	Entrapment efficiency (%)	Actual drug content (%)
f1	27.85	84.933	38.56
f2	28.87	81.894	37.18
f3	25.49	78.744	35.75
f4	27.35	88.048	36.1
f5	29.3	86.829	35.6
f6	28.45	89.024	36.5
f7	25.98	92.924	35.59
f8	21.86	92.202	35.59
f9	27.98	91.879	35.19
f10	49.28	88.567	55.39
f11	54.92	87.927	54.99
f12	47.35	89.382	55.9
f13	48.92	90.615	53.59
f14	54.92	91.156	53.91
f15	47.35	90.277	53.39
f16	48.92	87.973	46.89
f17	45.16	86.660	46.19
f18	49.49	87.711	46.75
f19	68.78	87.705	67.7
f20	69.68	85.257	65.81
f21	61.45	92.291	71.24
f22	63.58	88.578	65.3
f23	66.58	91.268	67.28
f24	55.89	90.002	66.35
f25	61.45	91.241	64.38
f26	62.49	89.965	63.48
f27	67.89	91.638	64.66

7.4. Morphology and surface topography by scanning electron microscopy

The microparticles were evidently porous on SEM pictures. The solvent's migration from the microparticles' surface caused the pores to form. The particles were known as microsponges because of their distinctive look.

In SEM analysis, the surface properties of the medication and microsponges were clearly distinguished. The SEM images of clotrimazole microsponges at various

resolutions have provided examples. The virtually spherical, highly porous structures seen in the microphotographs of clotrimazole microsponges show how microsponges are formed. The result of the solvent (DCM) evaporating off the surface of the microsponges is the creation of pores. This demonstrated altered crystal geometry and verified the development of microsponges containing clotrimazole.[2]

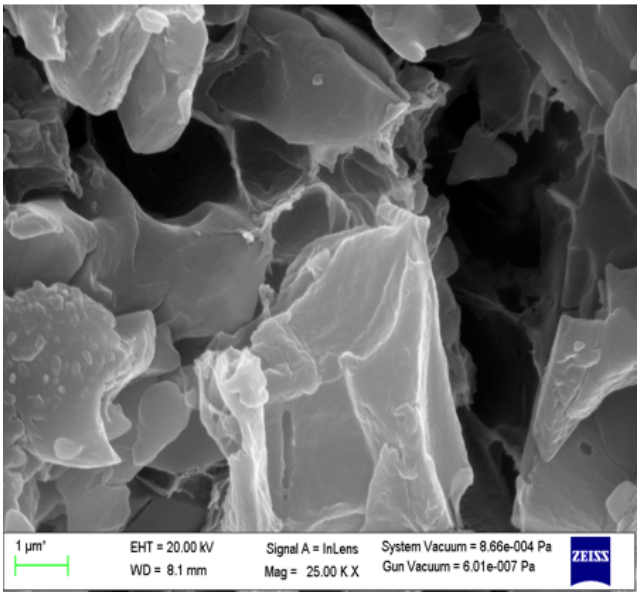

Fig. 5: SEM image of formulation (f23)

7.5. *Microscopy*

7.5.1. *Microscopic images of prepared microsponges*

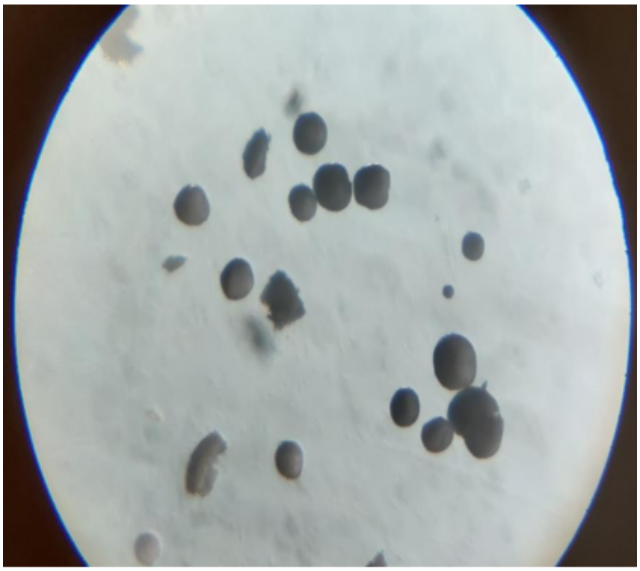

Fig. 6: Microscopic image of formulation f23

7.5.2. *Particle size determination*

When medication concentration was increased for formulations f21–f23 and f27, particle size decreased. The internal phase's viscosity has a direct impact on the microsponges' particle size. smaller particle size results from a viscosity differential between the internal and external phases that is smaller the bigger the volume of the internal phase. Emulsion globules are produced as a result of a less viscous dispersed phase being obtained with a higher volume of solvent. There is no doubt that these emulsion globules split into smaller droplets, resulting in microscopic particle size.[7]

The average particle size for all formulations f21, f23 and f27 (μm) are 52.3±1.23, 55.4±1.36, 49.2±0.35.

7.6. *Determination of pH*

The vaginal gel with microsponges was made, and its pH was measured using a pH metre. It was discovered to be 4.5 to 5.5, which is close to the pH typically observed in the vagina during a fungus infection.[9]

7.7. *In vitro permeability study and flux*

The best microsponges gel was discovered to have a maximum drug release% (% permeability) of 66.183 of%, which was higher than the formulation used in marketing.[10]

Table 5: Permeability studies of clotrimazole, market preparation and developed vaginal gel formulation

Time (hr)	%CDR(%Permeabiliy)			
	Marketed formulation	f23	f21	f27
1	17.071	31.21	29.64	32.85
2	19.59	36.59	34.45	36.62
3	23.01	41.45	38.73	40.81
4	26.68	46.45	43.07	46.23
5	31.37	51.39	47.38	50.77
6	34.55	56.28	51.82	52.43
7	41.45	61.48	56.12	60.04
8	48.15	66.18	60.61	64.85

7.8. *Drug release kinetic data analysis*

To better understand the process of drug release, the cumulative percentage drug release data from clotrimazole-loaded microsponges gel was fitted into the Zero order, First order, Higuchi model, and Peppa's model. The r2 determination coefficients of regression's slopes were listed.

The findings revealed that the r2 values for zero order kinetics ranged from 0.213 to 0.304, first order kinetics from 0.372 to 0.386, the Higuchi model from 0.896 to 0.928, and the Peppas model from 0.973 to 0.971.

In order to determine whether the release mechanism is fickian diffusion or non-fickian diffusion, Peppas' model is well known to be frequently utilised. Different release mechanisms could be described using the value of the n (release exponent of the Koshmeyer Peppas model).

The Koshmeyer Peppas model was present in all created formulations, and the r2 is indicated in table 5.12. Exponents (n) value was discovered and is displayed in table 5.12.N values between 0.45 and 0.89 suggest non-fickian transport. The release curve for an exponential release vs. time function in non-fickian transport is linear. The correlation coefficient was determined to be optimal for the Koshmeyer Peppas model. The best r2 score among the others was for f23, at 0.9716. [11–13]

Release kinetics of microsponges based vaginal gel

Table 6: Drug release kinetics data derived from various mathematical models

Zero order (R^2)	f21	0.213
	f23	0.304
	f27	0.294
First Order (R2)	f21	0.372
	f23	0.549
	f27	0.433
Higuchi (R2)	f21	0.896
	f23	0.928
	f27	0.906
Koshmeyer Peppa's (R2)	f21	0.971
	f23	0.973
	f27	0.966

7.9. Viscosity measurement

Using a Brookfield viscometer, the viscosity of the gel formulation (f23) was calculated. The viscosity was measured at rpms of 10, 20, 50, and 100, and the results were 12,435, 6,874, and 4,127. 2,016(cp)

Each of the three formulations exhibited non-Newtonian behaviour, with viscosity decreasing with increasing shear rate (rpm). The flow curves demonstrate that the viscosity of the microsponges gel was reduced at the same shear rate values. The patient benefits from this since it makes it easier to apply the medication to the vagina and boosts patient compliance. While allowing for full medication release, the mucoadhesion qualities of carbopol gel allow for longer retention of the gel in the vagina.

7.10. In vitro bioadhesion study

In comparison to the commercial formulation, the bioadhesive ability of the produced microsponges loaded with clotrimazole vaginal gel formulation was assessed.

At the lowest point, the prepared formulation on the plate was submerged in the solution; at the highest point, it was not. Table 5.13's visual representation of the residence time of the formulation on the plate was taken.

Results of In vitro bioadhesion Studies of f23,f21,f27 and marketed formulation were 51.2 min,48.6 min,43.1 min and 29.3 min.

7.11. Antifungal study

For the purpose of determining the antifungal activity of the marketed formulation and created microsponges based gel, the average zone of inhibition (ZOI) for Candida albicans was taken into consideration. The investigation was conducted using commercially available formulation and prepared microsponges; the commercially available formulation's vaginal ZOI was determined to be 1.3(marketed formulation) cm while that of the prepared microsponges was found to be 2.2 cm of f21,1.8cm of f27 and 1.5 cm of f23. [15]

8. Conclusions and Future Directions

For vaginal delivery of Clotrimazole, a new delivery mechanism based on microsponges has been created. The technique used was solvent diffusion from a quasi-emulsion. Microsponges that had been formed had a spherical shape. Different drug-polymer ratios were indicative of good drug content, entrapment effectiveness, and particle size. Microsponge-based gel demonstrated in vitro drug release that had the greatest regression value for Koshmeyer-Peppas, and CLZ microsponges gel had stronger in vitro antifungal activity than the commercial formulation. Carbopol gel may extend the amount of time that the medicine is in touch with the vaginal mucosa and lengthen the period that the dose form is retained in the vaginal mucosa. This study's formulation of a gel containing microsponges showed promise as a novel delivery mechanism for the treatment of vaginal fungal infectionsOverall, the f23 formulation outperformed the other formulations in terms of production yield, entrapment effectiveness, real drug content, % CDR, and flux (g/cm2/h). The best-fitting model was discovered to be Koshmeyer Peppas. A measurement of the viscosity revealed non-Newtonian flow. The ZOI was a preparation that went beyond marketing.

9. Source of Funding

None.

10. Conflict of Interest

None.

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