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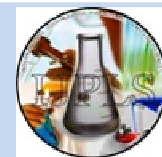

Formulation and Evolution of Ketoconazole Hydrogel Gel TopicalDelivery System against Fungal Infection

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Abstract

The aim of present study was to formulate and evaluate ketoconazole loaded hydrogel for effective topical delivery against fungal infections.

Hydrogels of KTZ were prepared by chemical polymerization method. Various concentrations of gelling agent and crosslinking agent were used in the preparation. All the 10 formulations HG1, HG2, HG3, HG4, HG5, HG6, HG7, HG8, HG9 and HG10 were optimized on basis of different optimization parameters such as structure, pH, spreadability, grittiness and homogeneity. Structure of hydrogels was determined by optical microscope. pH of the formulation was determined by digital pH meter and was found to be 6.81±0.5. It was observedon the basis of skin pH range 6.81 to 6.89. Formulation HG4 has optimum pH range. Spreadability of the formulation was determined by wooden block and glass slide apparatus. Formulation HG4 has good spreadability. Grittiness and homogeneity of the formulation was determined by visual inspection. HG4 has a transparent morphology and free from gritty particles. Viscosity of formulation was determined by Brookfield viscometer. The hydrogel were rotated at 0.3, 0.6 and 1.5 rotation per minute (RPM). The optimum range for hydrogel viscosities are 4000- 5000 cps. Formulation HG4 has optimum viscosity range. The optimized hydrogel HG4 was evaluated for drug entrapment and drug release studies. Drugentrapment was estimated spectrophotometrically. Formulation HG4 has higher drug entrapment. *In- vitro* drug release studies were performed using diffusion cell apparatus and samples were analyzed spectrophotometrically. Formulation HG4 shows better drug release than other formulations.The antifungal study was done using the agar well diffusion method and zone of inhibition was determined. In the present study zone of inhibition of the optimized formulation is equivalent to marketed formulation.

Keywords: Ketoconazole, Gel, Fungus

Introduction

Hydrogels are three-dimensional hydrophilic, polymeric networks that can swell in water and hold a large amount of water while maintaining the structure. Cross linking facilitates insolubility in water because of ionic interaction and hydrogen bonding. It also provides required mechanical strength and physical integrity to the hydrogels (Rowley *et al.,* 1999). Hydrogels are hydrophilic polymer networks that may retain a large amount of water and exhibit a semi-solid morphology. The hydrophilic three-dimension network formed by chemical or physical crosslinking can be considered as an ideal candidate for the drug delivery. Normally, the water content is more than 50% of the total weight when the term gel is used. Hydrogels are solids on the macroscopic scale. They have definite shapes and do not flow. Atthe same time, they behave like solutions on the molecular scale. Water-soluble molecules candiffuse in hydrogels with various diffusion constants reflecting the diffusant size and shape (Yoshimi *et al.,* 2005). Fungi are increasingly recognized as major pathogens in critically ill patients. *Candida spp.* and *Cryptococcus spp.* are the fungi most frequently isolated in clinical practice. Superficial and subcutaneous fungal infections affect the skin, keratinous tissues and mucous membranes. Systemic fungal infections may be caused by either an opportunistic organism that infects an at-risk host, or may be associated with a more invasive organism thatis endemic to a specific geographical area. There are various types of formulations like tablets, creams, shampoos and gels are available to treat the fungal infections but they have problems like first pass metabolism, skin rashes, allergic reactions, skin irritation etc. These problems necessitate the development of such system that can overcome problems associated with conventional system. Hydrogel could be a better option that avoids the complications of existing systems. These are hydrophilic polymer networks that may retain large amount of water and exhibit a semi-solid morphology. They preserve the active drug for a long time, biocompatible in nature and can be easily modified.

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Material and Method

Solubility Determination

A qualitative determination of the solubility was made by adding solvent in small incremental amount to a test tube containing fixed quantity of solute or vice versa. After each addition, thesystem was vigorously shaken and observed visually.

Table 1 Solubility determination in various solvents

S. no.	Components	Solubility
1	Methanol	Completely soluble
3	Methylene chloride	Sparingly soluble
4	Water	Completely insoluble

Partition coefficient or ΔLog P (BBB Permeability)

The partition coefficient of ketoconazole was determined using octanol-water system. It was found to be 4.33, was equivalent to reported value. This showed the lipophilic nature of drug.

Melting point determination

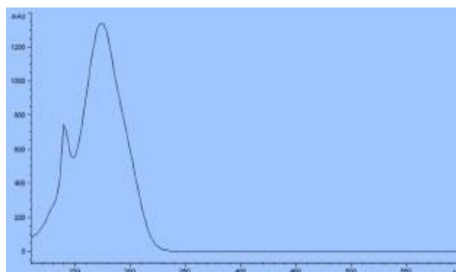

Figure 1: Determination of λmax by UV spectroscopyCalibration curves in different solvents

Melting point of ketoconazole was determined using open capillary method. Melting point of drug sample was found to be **144±2ºC.**

Determination of λmax by UV spectroscopy

For determination of λmax, stock solution of drug (concentration 1000µg/ml) in methanol wasprepared by dissolving 10 mg of ketoconazole in 10 ml methanol. The working solutions in the concentration range of 5-50 µg/ml were prepared. Drug sample was scanned in the range of 200- 400 nm and absorption maximum (λ_{max}) was found to be 231 nm as per specification.

- Calibration curve of ketoconazole was prepared with the help of UV spectroscopy.

Calibration curves were prepared in methanol, phosphate buffer 6.8 and phosphate buffersaline 7.4.

- Preparation of stock solution:

The stock solution of ketoconazole was prepared to obtain final strength of 1000 µg/ml inmethanol, phosphate buffer 6.8 and phosphate buffer saline 7.4.

- Preparation of standard solution:

Standard solution was prepared in the concentration range 5-50 µg/ml from stock solution by suitable dilutions. Absorbance of standard solutions was measured at 231nm by UV spectrophotometry.

Table 2 Calibration curve in phosphate buffer 6.8 at 231 nm

S. no	Concentration	Absorbance
1	5	0.125
2	10	0.242
3	15	0.331
4	20	0.462
5	25	0.65
6	30	0.728
7	35	0.841
8	40	0.98
9	45	1.011
10	50	1.124

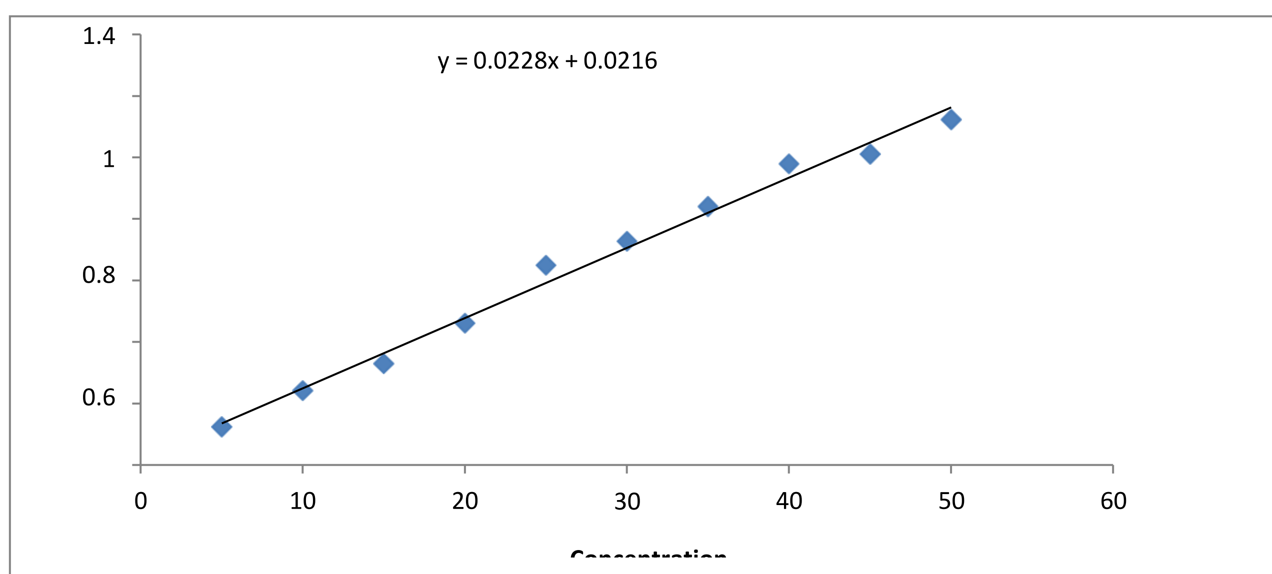

Figure 2 Calibration curve in phosphate buffer 6.8 at 231 nm

Table 3 Calibration curve in phosphate buffer saline 7.4 at 231 nm

S. no	Concentration	Absorbance
1	0	0.00

2	10	0.241
3	20	0.456
4	30	0.628
5	40	0.816
6	50	0.989

y = 0.022x + 0.021

Figure: 3 Calibration curve in phosphate buffer saline 7.4 at 231 nm

Powder Flow Properties

Angle of repose

The flow characteristic is measured by angle of repose. Improper flow of powder is due to frictional forces between the particles. Angle of repose of drug was determined by funnel method and it was found to be 39.19^0 which indicated that the drug had fair flow.

Bulk density and tapped density of drug sample

Bulk density and tapped density were determined as per procedure and were found to be 0.171g/ml and 0.316 g/ml respectively. Measurement of compressibility was determined by bulk density.

Formulation and optimization of hydrogel

The hydrogels were prepared by chemical polymerization method (Sanap *et al.,* 2013). In thismethod carbopol 934 was dissolved in distilled water and allowed to swell overnight. Drug sample was first dispersed in small quantity of tween 80 then added to carbopol solution and stirred by automatic magnetic stirrer. The remaining ingredients were added to it and the mixture was neutralized (to pH 6.0) by drop wise addition of 20% solution of sodium hydroxideto allow gel formation. Mixing was continued until a transparent gel was obtained.

Various concentrations of the polymer and cross linking agents were taken to get best possiblehydrogel with good consistency and structure. These formulae were altered on the basis of consistency of hydrogel. The drug was added accordingly and then prepared hydrogel were optimized via various parameters (Table 4). Similarly plain hydrogel (without drug) was also prepared by taking different concentration of Carbopol.

Table: 4 Composition for different hydrogel formulation

S. no	**Ingredients**	**HG1**	**HG2**	**HG3**	**HG4**	**HG5**	**HG6**	**HG7**	**HG8**	**HG9**	**HG10**
1	Carbopol 934 (%)	0.25	0.5	0.75	**1**	1.5	0.25	0.5	0.75	1	1.5
2	Ketoconazole (%)	2	2	2	**2**	2	2	2	2	2	2
3	Tween 80 (ml)	2.5	2.5	2.5	**2.5**	2.5	2.5	2.5	2.5	2.5	2.5
4	Glycerol (ml)	5	5	5	**5**	5	5	5	5	5	5

5	Glutaraldehyde (ml)	0.5	0.5	0.5	**0.5**	0.5	1	1	1	1	1
6	20% NaOH (ml)	q.s	q.s	q.s	**q.s**	q.s	q.s	q.s	q.s	q.s	q.s
7	Distilled water(up to ml)	100	100	100	**100**	100	100	100	100	100	100

Optimization Parameters

The prepared hydrogels were optimized on the basis of different parameters such as structure,pH, spreadability, grittiness and homogeneity. The values are shown in Table 5.

Table: 5 Optimization of different formulations

Formulation code	pH	Spreadability	Grittiness	Homogeneity
HG1	6.91±0.5	9.37	Not found	Opaque
HG2	6.78±0.5	8.33	Found	Transparent
HG3	6.29±0.5	10	Not found	Opaque
HG4	**6.81±0.5**	**18.7**	**Not found**	**Transparent**
HG5	6.72±0.5	15	Not found	Transparent
HG6	6.40±0.5	12.5	Found	Slight transparent
HG7	7.21±0.5	7.5	Not found	Opaque
HG8	7.14±0.5	15.6	Not found	Opaque
HG9	6.89±0.5	12.8	Found	Slight transparent
HG10	7.28±0.5	6.25	Not found	Transparent

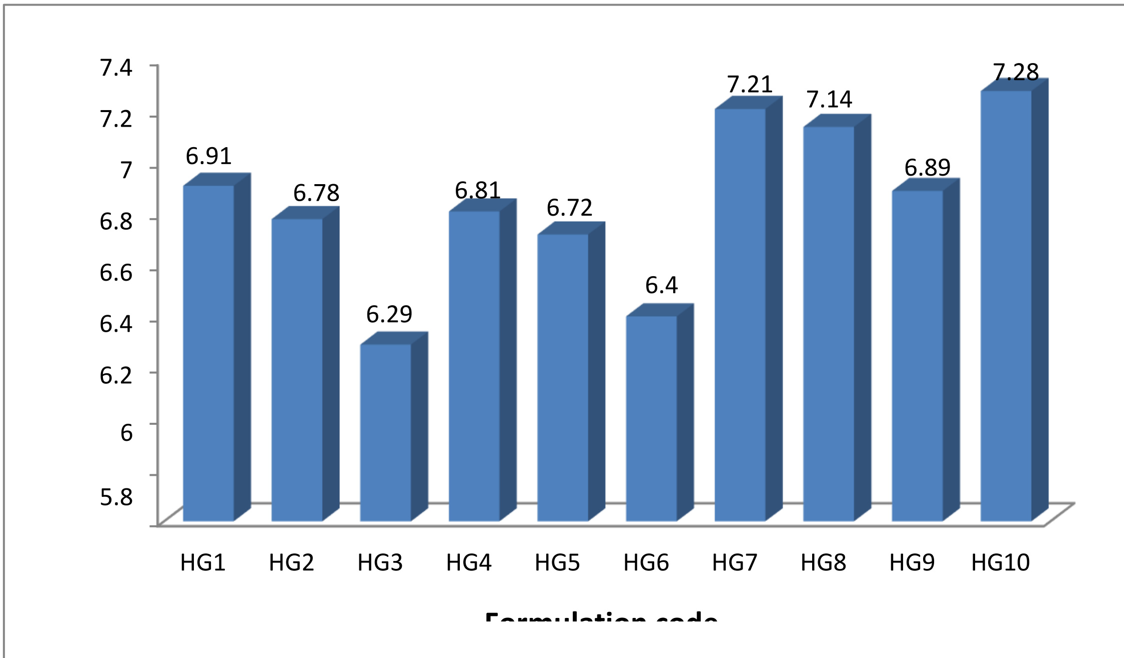

Figure: 4 Determination of pH

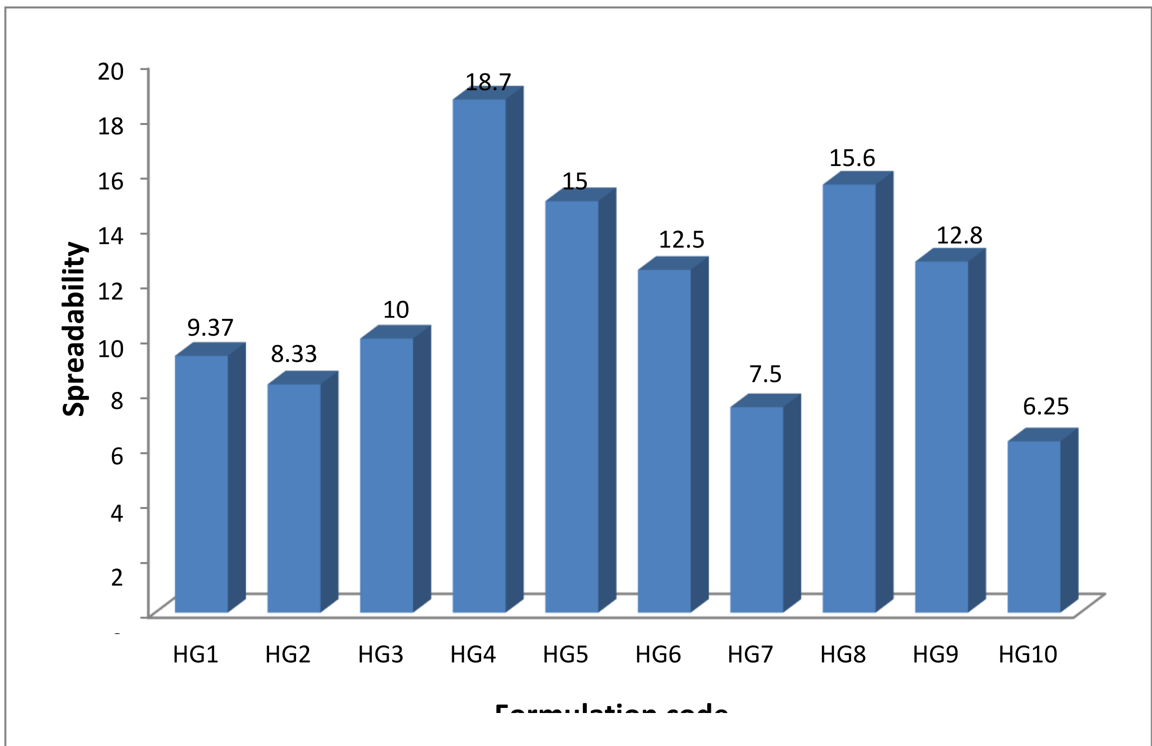

Figure: 5 Determination of spreadability

Viscosity:

Brookfield digital viscometer was used to measure the viscosity of prepared gel. The T shapedspindle was selected (T3) and rotated at different rpm. The reading, near to 100% torque was noted down. A sample was measured at 30±1°C.

Table 6 Determination of viscosity

S. no.	Formulation code	RPM	Viscosity (cps)
1	HG1	0.3	4056
		0.6	4078
		1.5	3809
2	HG2	0.3	4589
		0.6	4490
		1.5	4215
3	HG3	0.3	4950
		0.6	4870
		1.5	4724
4	**HG4**	**0.3**	**5148**
		0.6	**4965**
		1.5	**4398**
5	HG5	0.3	5000
		0.6	4980
		1.5	4867
6	HG6	0.3	5009
		0.6	4598
		1.5	4500
7	HG7	0.3	6061
		0.6	5874
		1.5	5523
8	HG8	0.3	6276
		0.6	6012
		1.5	5870
9	HG9	0.3	6500
		0.6	6435
		1.5	6209
10	HG10	0.3	6081
		0.6	5845
		1.5	5611

Table: 7 Optimized formula of hydrogel

S. no.	Ingredients	Quantity
1	Carbopole 934	1%
2	Ketoconazole	2%
3	Tween 80	2.5 ml
4	Glycerol	5 ml
5	Glutaraldehyde	0.5 ml
6	Sodium hydroxide (20%)	q.s
7	Distilled water	upto 100 ml

Evaluations of Optimized Hydrogel

Structure of hydrogel

The structure of prepared hydrogel was determined by the optical microscope. A drop ofprepared hydrogel was put into glass slide and observed under the microscope.

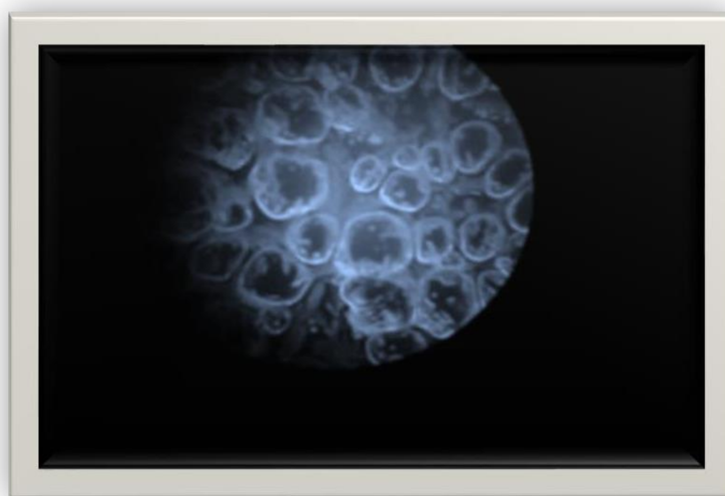

Figure: 6 Optical images of hydrogels, before dry

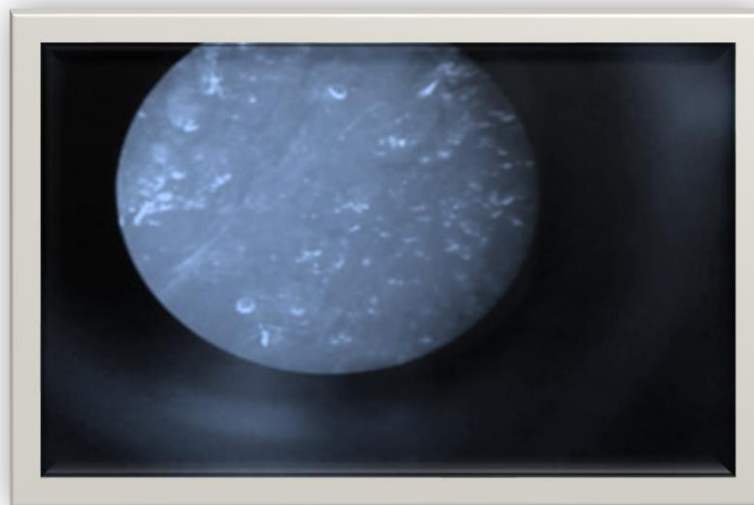

Figure: 7 Optical images of hydrogels, after dry

Drug entrapment

A specific quantity of optimized formulation (1 g) was taken and dissolved completely in 100ml of phosphate buffer (6.8). The volumetric flask containing gel was shaked for 2 h on a mechanical shaker in order to get uniform solution. Solution was filtered by 0.45µm membrane filter and estimated spectrophotometrically at 231 nm using phosphate buffer 6.8 as a blank solution (Table 8).

Table: 8 Drug entrapment

S. no.	Formulation code	Drug entrapment
1	HG4	95±0.5

***In-vitro* release profile**

In- vitro release studies was performed by using a diffusion cell with a receptor compartment capacity of about 20 ml. The egg membrane was mounted between the donor and receptor compartment of the assembly.

The formulated preparation (HG4) was weight up to 1g was placed over the membrane and thereceptor compartment of the diffusion cell was filled with phosphate buffer 6.8. The whole assembly was fixed on magnetic stirrer, and the solution in receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm with 37±0.5°C temperature. 5 ml sample was withdrawn at time interval of 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390 min, and after 24 h. Samples were analyzed for drug concentration spectrophotometrically at 231 nm against blank. The receptor compartment was replaced withan equal volume of phosphate buffer at each time of the sample withdrawn (Table 9).

Table 9 Drug release studies of optimized hydrogel (HG4)

S. no.	Time	Cumulative drug release (%)
1	10 min.	0.305±0.5
2	20 min.	0.82±0.5
3	30 min.	1.35±0.5
4	40 min.	1.96±0.5
5	50 min.	2.6±0.5
6	60 min.	3.3±0.5
7	90 min.	4.1±0.5
8	120 min.	5.1±0.5
9	150 min.	6.5±0.5
10	180 min.	8.3±0.5
11	210 min.	10.3±0.5
12	240 min.	12.7±0.5
13	270 min.	16±0.5
14	300 min.	19.4±0.5
15	330 min.	23.2±0.5
16	360 min.	35.6±0.5
17	390 min.	58.0±0.5
18	after 24 h	91.2±0.5

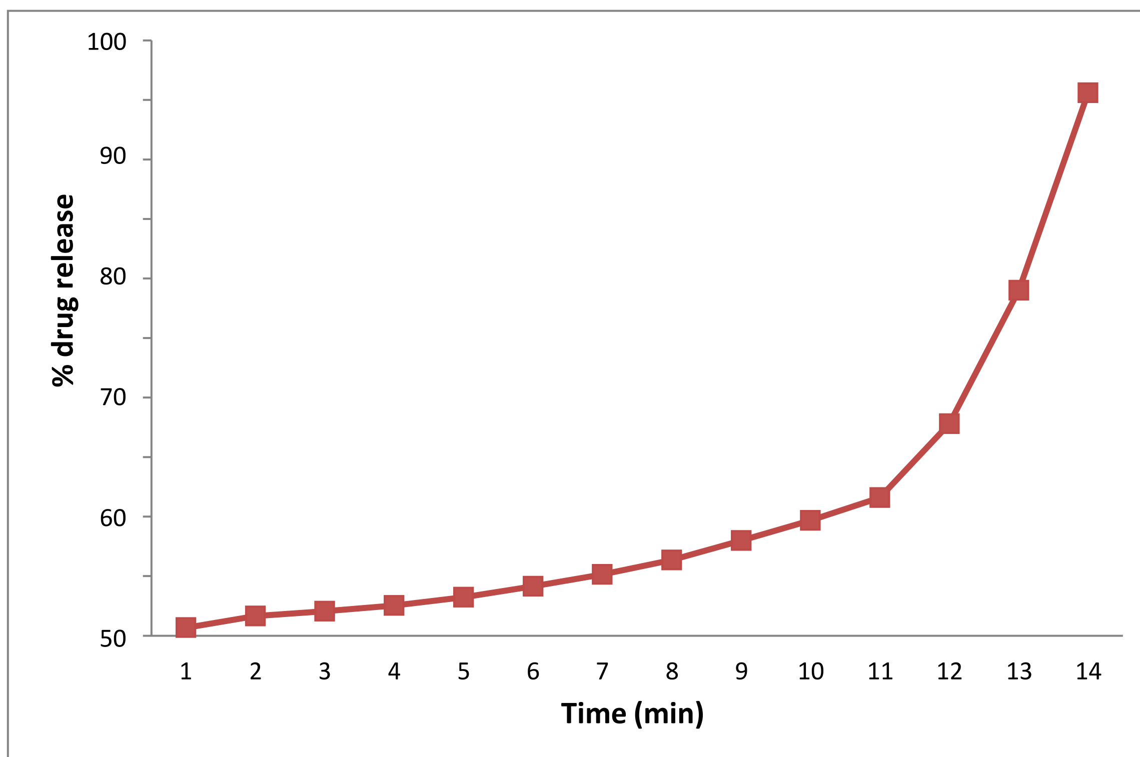

Figure: 6 % drug release of formulationStability studies

The stability studies were carried out for all the hydrogel formulation by freez- thaw cycling. Here, by subjecting the product to a temperature of 4°C for 1 month, then at 25°C for 1 monthand then at 40°C for 1 month. After this, the hydrogel is exposed to ambient room temperature.

The effect of temperature and time on the physical as well as chemical characteristics was evaluated for assessing the stability of prepared formulation. The different parameters such as physical properties, pH, grittiness, and drug entrapment were observed.

Table 9 Stability testing parameters of optimised formulation HG4 at temperature 4^{0}C

S. no.	Parameters	Initial	After 7 days	After 14 days	After 21 days	After 1 month
1	Morphology	Transparent	Transparent	Transparent	Transparent	Transparent
2	Odour	Moderate	Moderate	Moderate	Moderate	Moderate
3	pH	6.81	6.82	6.83	6.85	6.83
4	Grittiness	Not found	Not found	Not found	Not found	Not found
5	Drug entapment	95.0±0.5	95.1±0.3	94.8±0.3	95.3±0.4	95.4±0.2

Table 10 Stability testing parameters of optimised formulation HG4 at temperature25^{0}C

S. no.	Parameters	Initial	After 7 days	After 14 days	After 21 days	After 1 month
1	Morphology	Transparent	Transparent	Transparent	Transparent	Transparent
2	Odour	Moderate	Moderate	Moderate	Moderate	Moderate
3	pH	6.81	6.84	6.86	6.82	6.81
4	Grittiness	Not found	Not found	Not found	Not found	Not found
5	Drug entapment	95.0±0.5	95.1±0.5	94.8±0.6	95.3±0.4	95.4±0.3

Table 11 Stability testing parameters of optimised formulation HG4 at temperature40^{0}C

S. no.	Parameters	Initial	After 7 days	After 14 days	After 21 days	After 1 month
1	Morphology	Transparent	Transparent	Transparent	Transparent	Transparent
2	Odour	Moderate	Moderate	Moderate	Moderate	Moderate
3	pH	6.81	6.82	6.83	6.81	6.81
4	Grittiness	Not found	Not found	Not found	Not found	Not found
5	Drug entapment	95.0±0.2	95.5±0.5	95.4±0.5	95.7±0.4	95.3±0.3

Antifungal activity

Preparation of Inoculums

For evaluation of antifungal activity, culture of fungi was grown. Fresh culture of fungi was suspended in sterile water to obtain a uniform suspension of microorganism.

Preparation of Sabouraud's Dextrose Agar Plates

Suspended 16.25 gm sabouraud's dextrose agar in 250 ml distilled water and boiled the suspension to uniform mixing until the clear solution appeared. Solution was autoclave at 15 ibs pressure (121^{0}C) for 15 min. After that the solution was poured into glass plates andkept at 37±0.5^{0}C temperature for overnight.

Determination of Zone of Inhibition

Antifungal activity was checked by agar well diffusion method. In this method a previously liquefied medium was inoculated with 0.2 ml of fungal suspension having a uniform turbidity at 40±0.5°C temperature. 20 ml of Sabouraud's dextrose agar culture medium were poured into the sterile glass plates having an internal diameter of 8.5 cm. Care were taken for the uniform thickness of the layer of medium in different plates.

After complete solidification of liquefied inoculated medium, the wells were made aseptically with cork borer having 6 mm diameter. In each of the plates hydrogel solution was placed carefully. Plates were kept for pre-diffusion for 30 min, after that plates were incubated at 37±0.5°C for 24 h. After incubation period was over, the zone of inhibition was measured.

Table 12 Antifungal activity (Zone of inhibition) for HG4

Fungal species	Zone of inhibition (mm)			
Candida albicans	Formulation	Standard		Control
	HG4	KET (S1)	Marketed preparation(S2)	
	20.2	25.6	20.5	No activity

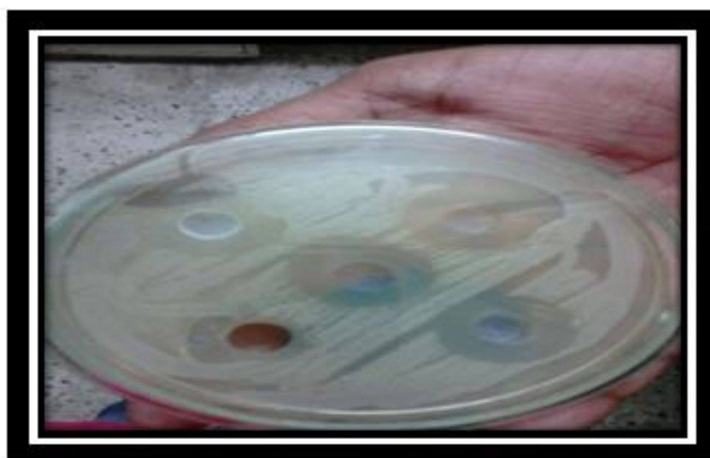

Figure: 7 Antifungal activity of formulation HG4 in Comparison with marketed for

Figure: 8 Antifungal activity of formulation HG4 in Comparison with pure KTZ

Results and Discussions

Hydrogels of KTZ were prepared by chemical polymerization method. Various concentrations of gelling agent and crosslinking agent were used in the preparation. All the 10 formulations HG1, HG2, HG3, HG4, HG5, HG6, HG7, HG8, HG9 and HG10 were optimized on basis of different optimization parameters such as structure, pH, spreadability, grittiness and homogeneity.

Structure of hydrogels was determined by optical microscope and results are shown in figure7 and 8 .

pH of the formulation was determined by digital pH meter and was found to be 6.81±0.5. It was observed on the basis of skin pH range 6.81 to 6.89. Formulation HG4 has optimum pH range. The results are shown in table 5 and figure 4.

Spreadability of the formulation was determined by wooden block and glass slide apparatus. Formulation HG4 has good spreadability and results for the spreadability are shown in table 5and figure 5.

Grittiness and homogeneity of the formulation was determined by visual inspection. HG4 has a

transparent morphology and free from gritty particles. Results are shown in table 5.

Viscosity of formulation was determined by Brookfield viscometer. The hydrogel were rotated at 0.3, 0.6 and 1.5 rotation per minute (RPM). The optimum range for hydrogel viscosities are 4000- 5000. Formulation HG4 has optimum viscosity range. The results for viscosity are shownin table 6 .

The optimized hydrogel HG4 was evaluated for drug entrapment and drug release studies. Drug entrapment was estimated spectrophotometrically. Formulation HG4 has higher drug entrapment. The result for drug entrapment is shown in table 8.

In- vitro drug release studies were performed using diffusion cell apparatus and samples were analyzed spectrophotometrically. Formulation HG4 shows better drug release than otherformulations. The results are shown in table 9 and figure 6.

The stability studies were carried out at 4°C, 25°C, 40°C and room temperature. The physical stability including morphology, odour, and grittiness of optimized formulation were shows no changes. pH, and drug entrapment were shows slight acceptable changes during under all storage conditions for 1 month. Formulation HG4 was stable under all storage conditions

The antifungal study was done using the agar well diffusion method and zone of inhibition was determined. In the present study zone of inhibition of the optimized formulation is equivalent to marketed formulation. The results are reported in table 12 and figure 7, 8.

Conclusion

Fungi are increasingly recognized as major pathogens in critically ill patients. *Candida spp.* and *Cryptococcus spp.* are the fungi most frequently isolated in clinical practice. Superficial and subcutaneous fungal infections affect the skin, keratinous tissues and mucous membranes. Systemic fungal infections may be caused by either an opportunistic organism that infects an at-risk host, or may be associated with a more invasive organism that is endemic to a specific geographical area.

There are various types of formulations like tablets, creams, shampoos and gels are available to treat the fungal infections but they have problems like first pass metabolism, skin rashes, allergic reactions, skin irritation etc. These problems necessitate the development of such system that can overcome problems associated with conventional system. Hydrogel could be a better option that avoids the complications of existing systems. These are hydrophilic polymer networks that may retain large amount of water and exhibit a semi-solid morphology. They preserve the active drug for a long time, biocompatible in nature and can be easily modified. KTZ is effective antifungal drug available in cream formulation, from which the drug is in direct contact with skin and hence prone to local reaction such as skin irritation, while hydrogel based formulations will effectively preserve the drug and efficiently surpass the epidermal layer and shows the high therapeutic benefit. The conventional cream of ketoconazole has the low penetration, while the hydrogel based formulations can improve the high penetration to the skin.The aim of present study was to formulate and evaluate ketoconazole loaded hydrogel for effective topical delivery against fungal infections. The antifungal study was done using the agar well diffusion method and zone of inhibition was determined. In the present study zone of inhibition of the optimized formulation is equivalent to marketed formulation

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