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Research Article

DEVELOPMENT AND CHARACTERISATION OF ORAL SOFT GEL CONTAINING FLUCONAZOLE USP FOR THE TREATMENT OF ORAL CANDIDIASIS

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ABSTRACT

Fluconazole USP, a synthetic antifungal agent belonging to the group of triazole is one of the most commonly used antifungal agents for treating oral fungal infections, such as oral candidiasis. In order to bypass the disadvantages of solid dosage forms, the oral soft gel formulations may be proposed as better drug delivery tool for treating oral candidiasis. This study was conducted to develop a gel formulation of fluconazole using biodegradable polymers like chitosan and sodium alginate.The gels were evaluated for physical appearance, rheological behaviour, drug release and stability. Among all the gel formulations, chitosan showed superior drug release than sodium alginate. Drug release decreased with increase in polymer concentration. Stability studies showed that the physical appearance, pH and drug content remained unchanged upon storage for 45 days at ambient conditions.

Keywords: Fluconazole USP, Oral, Soft gel, Chitosan, Polymers.

INTRODUCTION

Oral delivery of antifungal drugs is mainly through conventional tablets, capsules, solutions, suspensions, and ointments etc. These existing dosage forms may result into poor bioavailability of drug in the buccal cavity or may cause degradation of drug in salivary fluid. These problems may be overcome by delivering the antifungal drugs into the oral cavity which may allow more of the drug to penetrate through the oral mucous layer. These properties may be fulfilled by incorporating the antifungal agents into oral soft gels. Gels are successfully used as drug delivery systems to protect the medicaments from a hostile environment. They often provide a faster release of drug substance without depending upon the water solubility of the drug, as compared to creams and ointments. Gels have several favourable properties such as being thixotropic, grease less, easily spreadable, emollient, non-staining, and compatible with several excipients and water soluble or miscible. Developments of gel formulations are very simple and cost effective[1].

Within the oral mucosal cavity, delivery of drugs is classified into three categories: (i)sublingual delivery, which is systemic deliver of drugs through the mucosal lining the floor of the mouth, (ii) buccal delivery, which is drug administration through the mucosal membranes lining the cheeks (buccal mucosa) and (iii) local delivery, which is drug delivery into the oral cavity. Among the three different categories of drug delivery within the oral cavity, the sublingual mucosa is relatively permeable, giving rapid absorption and acceptable bioavailability for many drugs, and is convenient, accessible, and generally well

accepted. Sublingual dosage forms are of two different designs, those composed of rapidly disintegrating tablets, and soft gelatin capsules filled with liquid drug. Such systems create a very high drug concentration in the sublingual region before they are systemically been absorbed across the mucosa. The buccal mucosa is considerably less permeable than the sublingual area and is generally not able to provide rapid absorption and good bioavailability. Local delivery to tissues of the oral cavity has a number of applications, including the treatment of toothaches, periodontal diseases, bacterial and fungal infections, aphthous and dental stomatitis[2].

Fluconazole USP (FLZ), a synthetic antifungal agent belonging to the group of triazole is one of the most commonly used antifungal agents for treating oral fungal infections, such as oral candidiasis. FLZ is mainly prescribed in tablet form. Apart from the draw backs of solid dosage forms, FLZ is reported to cause adverse effects such as nausea, vomiting, bloating and abdominal discomfort in patients. In order to bypass these disadvantages, the oral soft gel formulations may be proposed as better drug delivery tool for treating oral candidiasis[2,3]. The present study was conducted to develop a oral soft gel formulation of FLZ using biodegradable gelling agents like chitosan and sodium alginate. Effect of varying concentration of gelling agents on the physical appearance, rheological behavior, drug release and stability was investigated. The in vitro drug release profile was examined to ascertain the role of gelling agents and their concentration on the final formulation.

MATERIALS AND METHODS

Fluconazole USP was purchased from M/s Yarrow chemicals, Mumbai. chitosan and mannitol was procured from M/s Balaji chemicals, Gujarat,sodium alginate and PEG 400 were purchased from M/s Spectrum Reagents, Cochin. All the chemicals and solvents used were of analytical grade.

Preparation of oral soft gel

Dry gelling agents (0.2, 0.4 &0.8%) were dispersed into 50ml of distilled water maintained at 95°C. The dispersion was stirred at 95°C for 20min using a magnetic stirrer. The required quantities of co-solutes (sucrose & mannitol) were added to the gelling agent solution with continuous stirring & the temperature was maintained above 80°C. FLZ, PEG -400, citric acid and preservatives were added with stirring. Finally, required amount of sodium citrate was dissolved in distilled water and added to the mixture. The weight of the gel was monitored continuously during manufacturing and finally it was adjusted to 100gm with distilled water. The developed formulation was packed in suitable container with airtight seal and allowed to cool at room temperature to form gel[2,3].

Evaluation of physical characteristics

Developed formulations were visually inspected for clarity, texture and consistency[4].

Estimation of dug content

10gm of each gel formulations were transferred into a 250ml of volumetric flask containing 20ml of phosphate buffer solution of pH 6.8 and stirred for 30min. The volume was made up to 100ml and filtered. 1 ml of above solution was further diluted to 10ml using buffer solution.1ml of the above solution was further diluted to 10ml and the absorbance of the solution was measured at 266nm using UV-Visible spectrophotometer[4].

Determination of pH

The pH was measured in each gel, using a digital pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrodes were completely dipped in to the formulations and pH was noted[4,5].

Extrudability

The developed formulations were filled in to collapsible metal tubes and crimped at one end. After removing cap, the tubes were pressed to extrude the product/formulations. Extrudability of the formulation were checked and reported[4,5].

Viscosity

Viscosity of developed formulations were determined using Brook field viscometer LV DV-Prime I with a small volume adaptor. The viscosity was measured at varying the torque and rpm[5].

Spreadability measurement

Spreadability was measured using the self developed spreadability apparatus. The apparatus consists of two rectangular glass slides in which one slide is firmly fixed in a wooden frame while the other slide can easily slide over the surface of the fixed one. 1gm of sample was placed on one of the glass plate. Second plate was placed over the other one to sandwich sample between plates. A 20gm weight was placed on the top of upper plate to provide a uniform thin film of the sample between the plates. Weight was removed; excess of the gel sample was scrapped off from the

edges. The top plate was then subjected to pull by using string to which 20gm weight was applied. The time required by the upper plate to travel a distance of 6cm and separate from the lower plate was noted. A shorter interval indicates better spread ability[4,5].

In-vitro diffusion studies

The in-vitro diffusion studies of drug from developed formulations were studied using modified Franz diffusion cell and cellophane membrane. Weighed quantity of sample was placed on cellophane membrane which was then fixed on to diffusion cell. The cellophane membrane was made in contact with phosphate buffer solution of pH 6.8, which was used as receptor medium for this investigation. The temperature at receptor compartment was maintained at 37±2°C and it was stirred at 100rpm using a magnetic bead stirrer. The system was maintained for 1 hour. 5ml of sample was withdrawn from reservoir compartment at different time interval and absorbance was measured spectrophotometrically at 266nm[3,4,5].

In-vitro antifungal studies

Weighed quantity of sabouraud dextrose agar was transferred into a 500ml of conical flask. Sufficient quantity of purified water was added and heat is applied to dissolve it completely. Sterilize the medium for 15min at 121°C at 15lb pressure in autoclave. Cool the medium at room temperature and a loop full of fungal strain (Candida albicans) was transferred aseptically into the medium. Cultured medium with a concentration 1mg/ml was poured in to five petridishes and allowed it to cool at room temperature until it solidifies. Two cups were bored in each petridish with the help of sterile steel bore of 6mm. Calculated amount of pure Fluconazole solution (std), developed gel formulations were placed in each bores of selected petridishes and incubated for 72hrs at 37°C. The radius of the developed zone of inhibition was measured and reported[4,5,6].

Stability studies

Stability studies of developed formulations were carried out using ICH guideline for accelerated testing with required modifications. All the developed formulations were selected and filled in to the collapsible tubes and stored at temperature of 40±2°C/75±5%RH for a period of 45 days. After the period of 45 days, samples were tested for visual appearance, pH and drug content[4,5,6].

RESULTS AND DISCUSSION

Pure Fluconazole sample was scanned using phosphate buffer solution of pH 6.8 between 200nm to 400nm using UV visible spectrophotometer. The highest peak of fluconazole was obtained at 266nm and thus the λ_{max} of FLZ was fixed at 266nm and was used for further spectrophotometric evaluations during the study.FT-IR studies were carried out for pure drug, pure polymer and mixture of drug-polymer to confirm the interactions. The infrared spectrum of pure FLZ and physical mixture with excipients correspond to the similar wave numbers (Figure No.1,2 &3). Therefore, the study revealed that there may be no interaction between the drug and the excipients indicating that the excipients and the active pharmaceutical ingredient (API) may be compatible with each other[2,4,5].

In this investigation, the oral soft gels of FLZ were prepared using two different gelling agents with varying concentrations (Table No.1). The gelling agents were selected for this investigation based on their properties identified through literature review. The selected gelling agents were chitosan, and sodium alginate, which are biocompatible as well as biodegradable polymers of natural origin. Mainly three concentrations 0.2, 0.4 and 0.8% of polymers were selected. 10% PEG 400 is a non-ionic water soluble polymer which was used as solubilising agent in all the developed formulations. sucrose and mannitol were used as co-solute in this investigation[6,7].

The visual inspection confirmed the colour and texture of the developed formulations. The texture of soft gel in terms of stickiness and grittiness was evaluated by mildly rubbing the gel sample between two fingers. The formulations with polymer chitosan (SG1, SG2 and SG3) were transparent in appearance and formulations with Sodium alginate polymer (SG4, SG5 and SG6) were slightly yellowish-brown in appearance. The gels of batch SG1 and SG4 were smooth and non-sticky while gels of batch SG2, SG5 were smooth but slightly sticky. The gels of batch SG3, SG6 were hard and sticky in nature. As per the observations, with increase in concentration of polymer, final formulation showed increase in stickiness and gel was becoming harder in nature[6].

The highest drug content of 87.5% was recorded for SG1 with chitosan 0.2% as gelling agent. Formulation SG4 with sodium alginate 0.2% yielded 75.5% of drug content. But with increase in the concentration of gelling agent, the % drug content was reduced. The loading efficiency of drug mainly depends on the polymer-drug combination, polymer- drug

concentration and the method used. So the minimum of polymer- drug ratio resulted an increase in % drug content. Among the formulation SG1, SG4, the formulation SG1 with 0.2% chitosan showed maximum drug content[7,8]. The high percentage drug content assures the ability of the polymer to incorporate the maximum quantity of FLZ (Table No.2).

The pH of the prepared formulations was an important factor, which determines whether the formulation is free from producing any irritation to the oral mucosa. The pH for the developed formulations were between 6.2 to 6.8 (Table No.3) and within the acceptable range, which indicated the safety of the formulations in terms of its regular application in oral mucosa. The extrudability was assessed between satisfactory to excellent (Table No.3). Out of all the developed formulations, SG1 with chitosan (0.2%) showed excellent level of extrudability from the aluminium tube[4,6,7].

As per the collected data, the viscosity of developed formulations decreased with increase in shear rate which corresponds to a shear thinning system. Based on the rheological properties of the developed formulations, it may be categorised under pseudo-plastic system. For the topical semisolid formulations for better spreadability and extrudability, it may prefer to have shear thinning property. Hence in this investigation, developed formulations confirmed the same[2,4,5].

The spreadability was assessed on the basis of the time taken to move the slide at a distance of 6cm. The spreadability of developed formulations was ranging between 1.93 to 30.77g.cm/sec (Table No.4). The formulation SG1 showed good spreadability than other developed formulations. The reason for improved spreadability may be because of gel consistency attained by the gelling agent "chitosan" at its optimum concentration i.e. 0.2%. As per the previous investigations minimum polymer concentration resulted in good spreadability and as the concentration of polymer increases the final formulation resulted in poor spreading. So optimizing the polymer concentration may be a crucial factor for developing final formulation with good spreadability. The relationship between spreadability and viscosity was found to be linear. The rate of spreading depends on the viscosity of the formulations. Increasing the viscosity of polymer resulted in poor spreadability[4,9].

All the developed formulations were subjected for in-vitro diffusion study for 1 hour duration with fixed sampling intervals. The release of FLZ from the gel was varied according to concentration of polymer. The drug release of FLZ after the completion of 60 min from formulation SG1 was 95.71% and from formulation SG4 it was found to be 90.47%. The progressive increase in the amount of drug diffusion through the cellophane membrane was from formulation SG1 and SG4 attributed to gradual decrease in the concentration of polymer[9]. Viscosity of the formulation was increased with the increase of polymer concentration. Viscosity is negatively related to the release of active substance from formulations and its penetration through the diffusion barriers. The decrease in the release could be attributed to increased viscosity of the gel by increasing polymer concentration. Thus, both high concentration of polymer and high viscosity caused the reduced release of FLZ from the developed formulations. The study suggested that, if with increased concentration of polymer, the diffusion of drug through the barrier may forced to reduce. The amount of drug diffused from formulation SG1 was 95.71 in 60 min which was higher among all the gel formulations. Hence it may be important to optimize the concentration of polymer for a maximum release of drug from the final formulation[3,4,10].

Diameter for zone of inhibition was taken as the parameter to assess the in-vitro antifungal activity of all the developed formulations (Table No.6). As per the findings,chitosan based formulations, SG1 was measured with zone of inhibition of 14mm, which was reduced to 10mm and 08mm respectively with SG2 and SG3. Among the formulations containing sodium alginate, SG4 was reported with highest zone of inhibition of 11mm which was reduced to 8mm and 7mm respectively for SG5 and SG6. The data recorded for pure FLZ was 12mm. When all the formulations were cross checked for their diameter for zone of inhibition, formulation SG1 with chitosan was showing superior activity (Figure No.5). The improved antifungal activity performed by chitosan may be due to its inherent antifungal property. Since other polymers or excipients were not reported with any inherent antifungal property, this may be an added advantage for the developed soft oral gel formulation of FLZ with chitosan. As per the in-vitro antifungal study, increase in concentration of polymer, the formulations were showing reduced activity, it may be because of the release retardation of drug from the polymer base[10,11,12].

Accelerated stability studies were performed in accordance with ICH guidelines with required modifications for a period of 45 days. The studies were carried out to verify the changes in

physical characteristics, pH along with changes in % drug content at selected condition of 40±2°C/75±5%RH. Among all the developed gel formulations, formulation SG1 showed no change in physical appearance , while all the other formulations showed considerable changes.. Formulations SG1, SG4 were not reported with any significant change in % drug content even after 45 days. There was change is pH reported for all the formulations except SG1.The data obtained from stability studies did prove the ability of chitosan based soft gels to remain stable throughout in comparison with sodium alginate[8,9,10].

CONCLUSION

Fluconazole oral soft gel formulations showed acceptable levels of physical properties and drug release study. All prepared gel showed acceptable physical properties concerning color, consistency, pH value, extrudability, spreadability and viscosity. The polymers chitosan and sodium alginate may be suitable for developing soft gels. The concentration of polymer may be an important factor which may affect the properties as well as the release profile of FLZ from the developed formulations. Among all the developed soft gel formulations, chitosan with 0.2% concentration showed superior drug release. In all the developed formulations, the drug release was decreased with increase in polymer concentration. Hence, the concentration of polymers may be optimized for the successful development of oral soft gel containing Fluconazole USP. As per the investigations, it may be suggested that chitosan at 0.2% may be the optimum concentration to develop a stable soft oral gel for delivery of fluconazole USP for the effective treatment of Oral candidiasis. Further investigations may be necessary to prove its efficacy at clinical level.

Table 1: Composition of developed formulations

Ingredients (%w/w)	SG1	SG2	SG3	SG4	SG5	SG6
Fluconazole	2	2	2	2	2	2
Chitosan	0.2	0.4	0.8	_	_	_
Sodium alginate	_	_	_	0.2	0.4	0.8
PEG 400	10	10	10	10	10	10
Citric acid	0.05	0.05	0.05	0.05	0.05	0.05
Mannitol	0.3	0.3	0.3	0.3	0.3	0.3
Sucrose	66	66	66	66	66	66
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3
Methyl paraben	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water up to	100	100	100	100	100	100

Table 2: Drug content estimation of developed formulations

Formulation Code	% Drug content
SG1	87.5%
SG2	67.5%
SG3	55.0%
SG4	75.5%
SG5	60.0%
SG6	52.5%

Table 3: The pH and Extrudability of developed formulations

Formulation Code	pH	Extrudability
SG1	6.8	+++
SG2	6.4	++
SG3	6.7	+
SG4	6.2	++
SG5	6.7	+
SG6	6.5	+

+++ -Excellent, ++ -Good, + - Satisfactory

Table 4: Spreadability of developed formulations

Formulation code	Time taken to slide (sec)	Spreadability (g.cm/sec)
SG1	3.9	30.77
SG2	8	15.0
SG3	60	2.0
SG4	7.8	15.38
SG5	18.8	6.38
SG6	62	1.93

Table 5: In-vitro diffusion data for developed formulations

Time (min)	% Drug diffused					
	SG1	SG2	SG3	SG4	SG5	SG6
0	0	0	0	0	0	0
5	13.09	39.76	24.64	28.93	11.66	7.62
10	27.97	44.16	37.26	36.54	20.95	13.81
15	35.83	50.95	57.27	44.64	33.09	30.95
20	56.07	68.93	66.78	53.57	41.31	39.04
30	75.59	73.21	74.52	62.26	55.24	43.09
45	85.95	89.04	84.76	76.19	61.66	57.73
60	95.71	94.76	88.93	90.47	80.35	72.85

Table No.6: Antifungal study of developed Formulations

Formulation code	Zone in mm
SG1	14
SG2	10
SG3	08
SG4	11
SG5	08
SG6	07
Pure FLZ	12

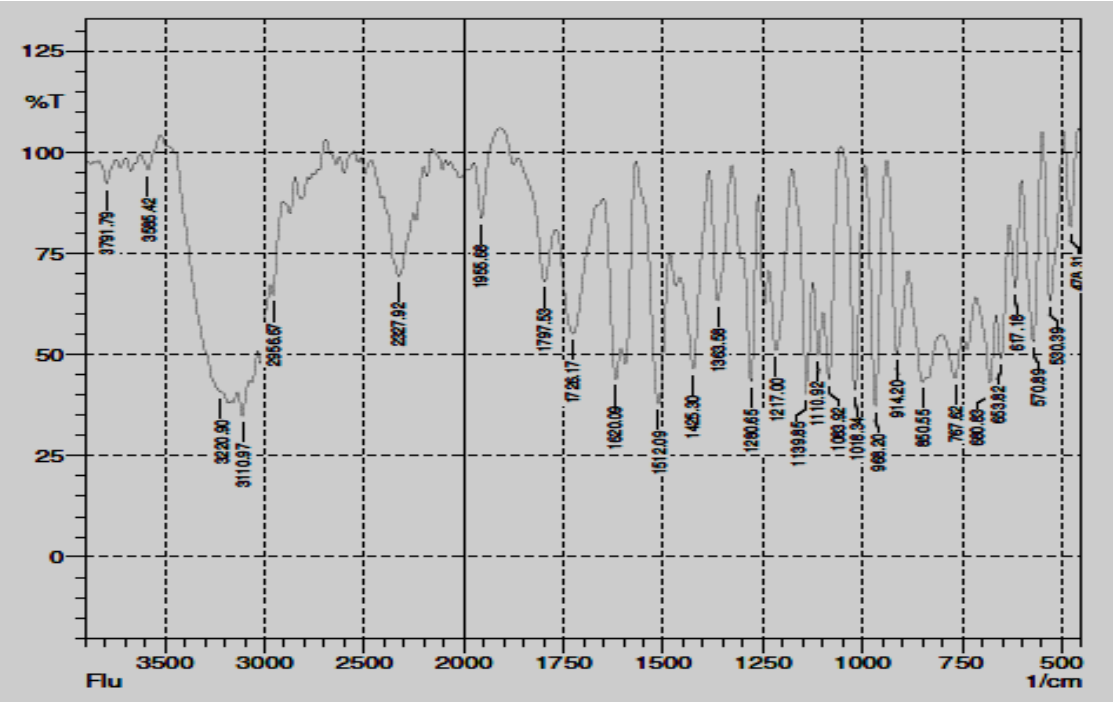

Fig. 1: FT-IR spectrum of pure FLZ

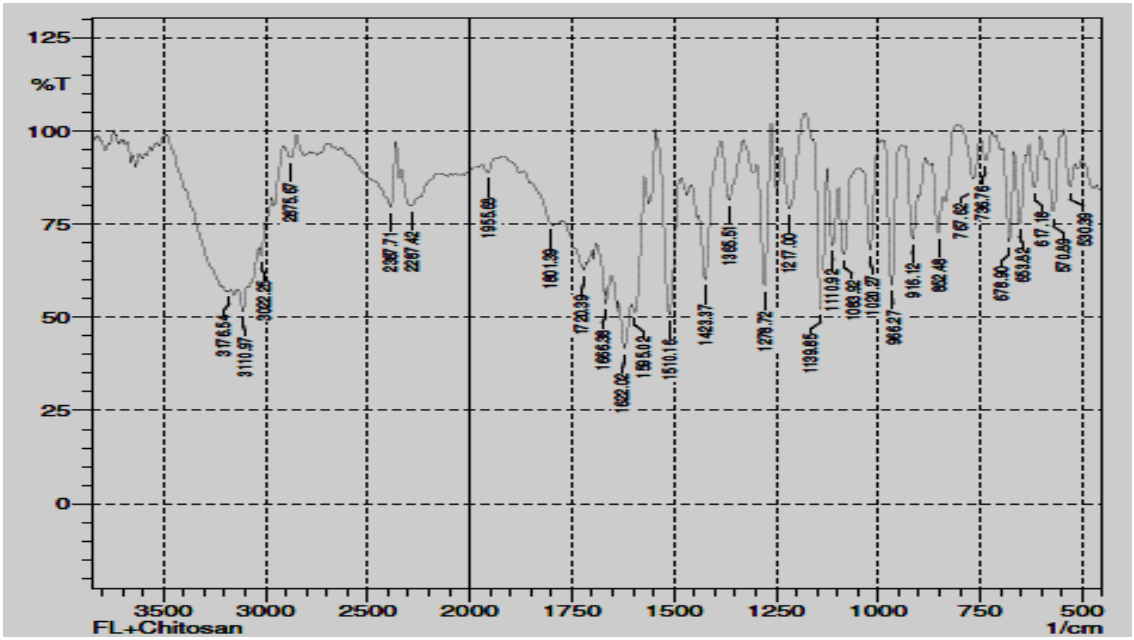

Fig. 2: FT-IR spectrum of FLZ+ Chitosan

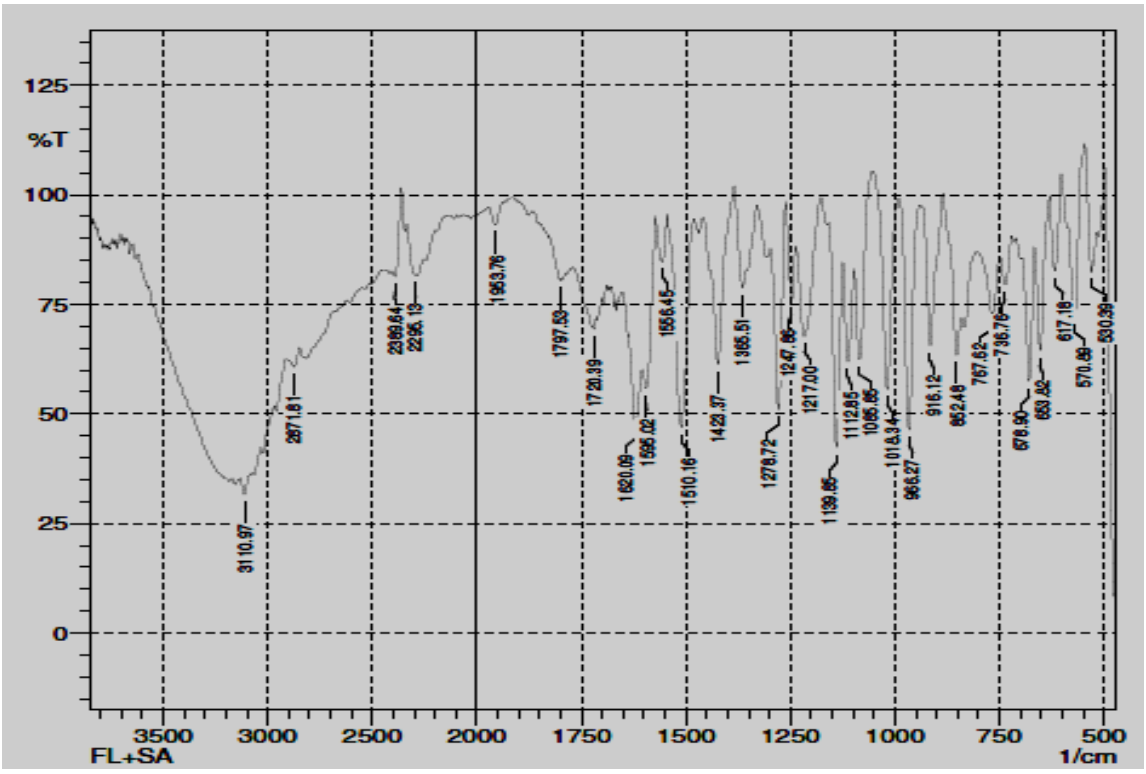

Fig. 3: FT-IR spectrum of FLZ+ Sodium alginate

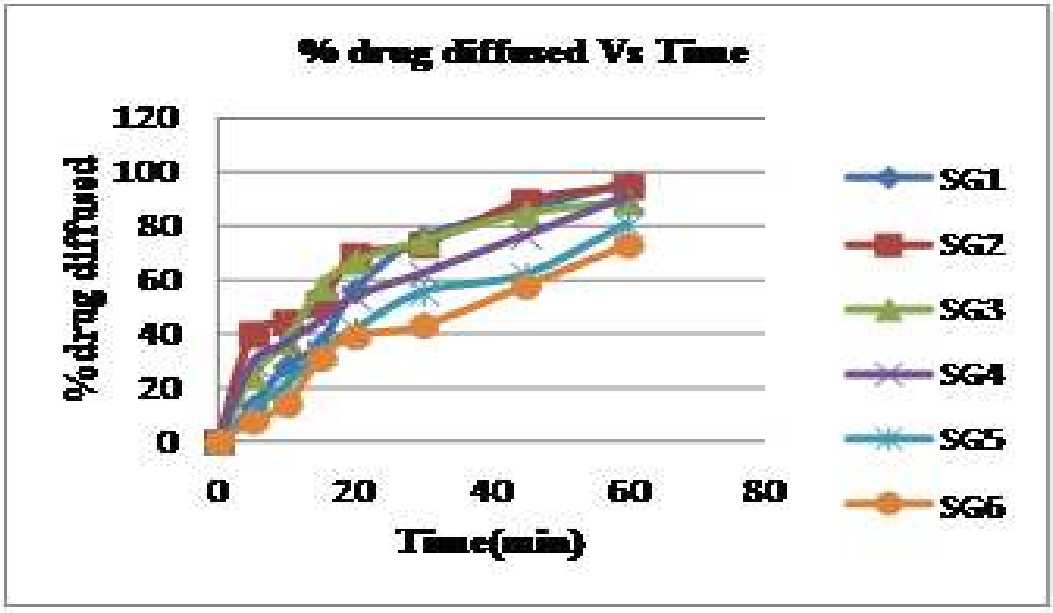

Fig. 4: Percentage drug diffused for developed Formulations

Fig. 5: Antifungal study of developed Formulations

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