American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

Development of Miconazole nitrate Thermosensitive Bioadhesive Vaignal Gel for Vaginal Candidiasis

Umme Hani* **and H.G Shivakumar**

Dept. of Pharmaceutics, JSS College of Pharmacy, JSS University, Sri Shivarathreeshwara Nagar, Mysore-570 015, Karnataka, India.

Address for Correspondence
Dept. of Pharmaceutics, JSS College of Pharmacy, JSS University, Sri Shivarathreeshwara Nagar, Mysore-570 015, Karnataka, India.

E-mail:
ummehaniahmed@gmail.com

ABSTRACT

Purpose: The aim of the present study was to formulate and evaluate miconazole nitrate containing thermosensitive bioadhesive gel for vaginal drug delivery to achieve a better therapeutic efficacy and patient compliance in the treatment for vaginal candidiasis.
Method: Here miconazole nitrate (1%) was formulated as a vaginal gel using thermosensitive polymer, pluronic F127 (20%) along with bioadhesive polymers such as carbopol 934, HPMC, SCMC and polycarbophil. The drug polymer compatibility was studied using FTIR. The prepared formulations were evaluated for parameters such as gelation temperature, gelation time, viscosity, bioadhesive strength, gel strength and drug release.
Results: Gelation temperatures for various formulations were found in the range of 30-38 ºC with gelation time varying from 1-5 min. The developed formulations had optimum viscosity, good bioadhesive strength and hence will have high retention property which is required for convenience at the site of application. Among the prepared formulations, one with the combination of pluronic F127, polycarbophil and carbopol 934 showed optimum gelation temperature, gelation time, viscosity, bioadhesive strength with sustained drug release for 12 hrs. The optimized formulation (F8) showed insignificant change in physical property and drug content when stability testing was carried out at 25ºC/60%RH for 3 months.
Conclusions: All the performed experiments confirm the applicability of bioadhesive In-situ gels as a novel delivery system for local therapy of vaginal candidiasis.

Keywords: Miconazole nitrate, Thermosensitive Bioadhesive gel, Vaginal candidiasis.

INTRODUCTION

In situ-forming systems are liquid aqueous solutions before administration, but gel under physiological conditions. There are several possible mechanisms that lead to in situ gel formation solvent exchange, UV-irradiation, ionic crosslinkage, pH change, and temperature modulation. These approaches, which do not require organic solvents, copolymerization agents, or an externally applied trigger for gelation, have gained increasing attention, such as a thermosensitive approach for in situ gel formation.[1] The development of in situ gel systems has recieved considerable attention over the past few years. This interest has been sparked by the advantage shown by these delivery systems such as ease of administration, reduced frequency of administration improved patient compliance and comfort.[2,3] In situ gel formulations are more likely to be accepted by patients because of the ease of administration. Several in situ gel formulations have been developed for the delivery of therapeutic agents [buprenorphine, Acyclovir, Fluconazole, Insulin etc].[4-7]

Vaginal candidiasis is a common condition and up to 75 % of all women suffer at least one episode of this infection during their lifetime. Candida albicans j1012 is the most important cause of vaginal candidiasis, accounting for over 80 % of the infection.[8] Antifungal imidazole drugs are a mainstay in the treatment of fungal infections. Imidazole drugs have low aqueous solubility because of their hydrophobic structures. This can have a negative impact on antifungal efficacy, side effects, pharmacokinetic variability and the development of drug resistance.[9]

The conventional dosage forms i.e preformed gel and solution have a number of lacunas, which has limited their use in vaginal drug delivery. Direct application of gels onto the infected sites of the vagina might be difficult, inconvenient as well as have frequent dosing because the conventional gels do not remain for long time at the site of application. A new and recent approach is to try to combine advantages of both gels and solution so that an accurate dose can be administered with ease of administration i.e in-situ gel system. These formulations remain to a solution state before administration but however transforms to gel after administration in to vaginal cavity. In the present study a thermosensitive bioadhesive vaginal gel was developed by incorporating the anti-fungal drug miconazole nitrate which is a most commonly employed drug in the treatment of vaginal infections.

MATERIALS AND METHODS

Materials

Miconazole nitrate was a generous gift from Bhavani Pharmaceuticals Kanpur. Pluronic F127 was supplied by Sigma Aldrich. Hydroxy Propyl Methyl Cellulose E 15 LV, Sodium Carboxy Methyl Cellulose, Carbopol 934, Polycarbophil (Noveon AA-1), di-sodium hydrogen ortho phosphate, Citric acid was purchased from Loba Chemie, Mumbai. All other reagents were of analytical grade and used without further purification.

Methods

Preparation of the Simulated Vaginal Fluid

Simulated vaginal fluid (SVF) was prepared from 3.51 g/l NaCl, 1.40 g/l KOH, and 0.222g/l Ca (OH) 2, 0.018 g/l bovine serum albumin, 2 g/l lactic acid, 1 g/l acetic acid, 0.16 g/l glycerol, 0.4 g/l urea, 5 g/l glucose. The pH of the mixture was adjusted to 4.2 using 0.1M HCl.[8]

Preparation of Miconazole nitrate Thermosensitive bioadhesive gel

Thermosensitive bioadhesive gel was prepared by cold method (10). Miconazole nitrate and bioadhesive polymers except pluronic F127 were completely dispersed in pH 4 citrate phosphate buffer with continuous agitation at room temperature and cooled down to 4ºC. Pluronic F127 was then slowly added to the solution with continuous agitation. The resulting solution was then left at 4ºC until a clear solution was obtained.

Drug – Excipient Compatibility Studies

FT-IR Analysis

The FT-IR spectra of the samples were obtained using FT–Infrared Spectrophotometer (Shimadzu-8400 S, Japan) by KBr pellet method in the wave number range 600-4000 cm–1. The samples were diluted with KBr and then compressed into a tablet, 10mm in diameter and 3 mm in thickness, using a manual tablet presser (Techno search) at 300 kg/cm for 1 min. The position of peak in FT-IR spectra of pure miconazole nitrate is compared with those in FT-IR spectra of miconazole nitrate plus excipients.

Characterization of Thermosensitive Bioadhesive Gel

Gelation temperature

The gelation temperature of the formulations was determined as follows: a 20-mL transparent vial containing a magnetic bar in 5 mL of Pluronic F127 gel was placed in a water bath. A thermometer connected to a thermistor was immersed in the gel, which was heated at a rate of 2ºC/min with constant (150 rpm) stirring. When the magnetic bar stopped moving because of gelation, the temperature displayed on the thermistor was recorded as the gelation temperature.[11,12.]

Gelation time

The gelation time was determined by test tube inverting method. Solution was taken in a thin walled tube and kept at the respective gelation temperature on a water bath. The test tube was taken out every 1 min and inverted to observe the state of the sample. The gelation time was determined by flow or no-flow criterion with the test tube inverted.

Mucoadhesive force determination

Mucoadhesive forces of all the prepared formulations were determined using a mucoadhesive force measuring device, which is a modified balance. By measuring the force required to detach the formulation from a mucin disc using the measuring device. At the right arm of the balance, a mucin disc was horizontally glued to the lower surface of the right pan of the modified balance. The mucin disc was hydrated with distilled water prior to the mucoadhesion test. Three drops of each formula were placed on the upper surface of an inverted beaker, which was placed directly below the right pan. The *in situ* forming liquid sample was exposed to a source of heat to allow gelation. The upper stage of the modified balance containing the hydrated mucin disc was adjusted to be in contact with the preparation. A preload of 10 grams was immediately applied for 1 minute to ensure intimate contact between the mucin disc and the sample and to allow formation of an adhesive bond. The preload time and the force were kept constant for all the tested formulations. After completion of the preload time, water was allowed to drip from the infusion set into a preweighed plastic jar placed on the left pan of the balance at a constant rate of 5 mL per minute. The dripping of water was stopped when the mucin disc was detached from the tested sample, the filled plastic jar was reweighed and the mass of water required to detach the tested sample from the mucin disc was

calculated from the difference. [13] Measurements were repeated three times for each of the gel preparations, but a fresh smooth gel surface was created before each measurement.

In vitro drug release study

The *in vitro* release study of miconazole nitrate from thermosensitive bioadhesive gels was carried out at 37ºC and with the stirring rate of 100rpm using an orbital shaking incubator. Formulation equivalent to 50mg of drug was placed into a 150mL beaker and incubated at 37ºC to form gel. Then 100mL of SVF was added to the beaker and the medium was stirred at 100rpm. At predetermined time interval, 1mL of the medium was collected and replenished by 1mL of fresh medium. The amount of released miconazole nitrate was analyzed at 272nm by UV spectrophotometer.

Measurement of Gel Strength

A sample of 50 gm of gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37^0C. The apparatus for measuring gel strength (weigh or apparatus as shown in Figure 2, weighing 27 gm) was allowed to penetrate in Pluronic F127 gel. The gels strength, which means the viscosity of the gels at physiological temperature, was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel.

Content uniformity and Rheological studies

All prepared gel formulations were tested for content uniformity. The rheological studies were conducted using (Brookfield Model DV-II Viscometer (Essex, UK). The viscosity of the formulated solutions was measured using spindle no: 94 at a shear rate of 50 rpm at 37°C. The temperature was maintained by a thermally controlled water bath. The samples were equilibrated for 10 minutes to reach the running temperature prior to each measurement.

Stability study

The formulation F8 showing optimum gelation, viscosity and drug release was selected for the stability study which was conducted according to the International Conference on Harmonization guidelines, 2003. A sufficient quantity of gel solution in glass vials was stored in desiccator's containing saturated solution of sodium chloride to maintain an approximate relative humidity of 60 ± 5%. The desiccator was kept at room temperature (30 ± 2°C) and samples were withdrawn at 0, 30, 60, 90 days. The physical stability of the gel was inspected periodically by checking clarity, gel temperature, viscosity, *in-vitro* drug release profile.

RESULTS AND DISCUSSION

Drug – Excipient Compatibility Studies

To find out the compatibility between the drug and polymer the IR spectra of pure miconazole nitrate and Formulation F8 was compared. The IR spectra did not show any significant difference in peak position of miconazole nitrate even after formulating a gel (F8).

These obtained results prove that there was no positive evidence for the interaction between miconazole nitrate and excipients hence they are compatible with each other. The spectra is reported in Figures 3.

Selection of polymers

Thermosensitive bioadhesive gel formulations containing miconazole nitrate were prepared by using pluronic F127 and bioadhesive polymers in a mixture which gels at the body temperature at a specific concentration. Carbopol, SCMC, HPMC, polycarbophil in the concentrations 0.2 and 0.4 % w/v were used as mucoadhesive polymers required to modulate the gel

strength and increase the residence time of the gel by adhering to vaginal mucosa. Pluronic F127 is of particular interest since concentrated solutions (≥20% w/w) of the copolymer are transformed from low viscosity transparent solutions to solid gels on heating to body temperature. At low temperatures in aqueous solutions, a hydration layer surrounds pluronic F127 molecules. However, when the temperature is raised, the hydrophilic chains of the copolymer become desolvated as a result of the breakage of the hydrogen bonds that had been established between the solvent and these chains. This phenomenon favors hydrophobic interactions among the polyoxypropylene domains, and leads to gel formation. Because of the dehydration process, the hydroxyl groups become more accessible.[14]

Gelation temperature (Tgel) and gelation time

Tgel is the temperature at which the liquid phase makes a transition to gel. An ideal in situ gel should be a free flowing liquid at room temperature so as to allow reproducible administration into the site of application where it undergoes in situ phase transition to form a strong gel. The human vaginal temperature is 37.2°C, so Tgel of vaginal thermoreversible gels were considered to be suitable if they were in the range of 25–37°C. If the Tgel is lower than 25°C, a gel might be formed at room temperature, leading to difficulties in manufacturing, handling, and administering. If Tgel is higher than 37°C, a liquid dosage form still exists at vaginal temperature, resulting in drainage of the formula from the vagina at an early stage. PF-127 alone at various concentrations results in gelation a temperature which is not within the suitable range.[13] An increase in PF-127 concentration resulted in a decrease in Tgel; this finding was in agreement with the data of Edsman et al.[15] Gelation temperature is dependent largely on polymer content, together with the high percentage of Pluronic (20%), known to lower gelation temperature.[16] Increase in the concentration of mucoadhesive polymers from 0.2% to 0.4% decreased the gelation temperature.

The gelation temperature lowering effect might be caused by increased viscosity after dissolution of mucoadhesive polymer[17] or Due to their ability to bind to the polyoxyethylene chains present in the pluronic molecules. This will promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding which will lead to gelation at lower temperature[18,19]. Gelation temperatures of the prepared formulations are tabulated in Table 2. Formulations F1–F9 were found to gel between 25 and 37°C, so they were considered to be suitable for vaginal application.

Gelation time was defined as the time when the elasticity modulus became higher than the viscosity modulus. When the solutions were heated at 37ºC, they transformed into non flowing gels within less than 10 minutes. It can be seen that increase in concentration of mucoadhesive polymers decreased gelation time. Data is shown in table 2. Figure 6 is a Photography showing the appearance of Miconazole nitrate solution & In situ gel formed in SVF of pH 4.5 at 37^0C.

Mucoadhesive force determination

Mucoadhesive force is an important and crucial physicochemical parameter for *in situ* forming vaginal gels since it prevents the formulation from rapid drainage and hence prolongs its residence time [13]. Data in Table 2 indicated that the increase in the concentration of bioadhesive polymers increased the bioadhesive strength. The mucoadhesive polymers could be arranged according to their mucoadhesive force

enhancing effect at 0.2% concentration of vaginal gel as, CP> Polycarbophil >SCMC > HPMC. Increasing the polymer amount may provide more adhesive sites and polymer chains for interpenetration with mucin, resulting consequently in aggrandization of mucoadhesive strength. The mechanism of the mucoadhesion enhancing effect of different polymers might be related to hydrogen bonding between the polymers and the mucosal membrane (glycoprotein) via carboxyl groups in the mucoadhesive polymers[20]. The mucoadhesive effect of HPMC could be due to the cellulose derivatives having many hydroxyl groups promote dehydration of poloxamers and consequently the hydrophobic interactions between the poly(oxypropylene)blocks [21].

In vitro release drug release study

Figure 5 shows the cumulative amount of miconazole nitrate released *vs.* time profiles for various formulations. From the *in vitro* drug release studies it was observed that the concentration of mucoadhesive polymers affected the drug release from the formulations. The addition of mucoadhesive polymers like SCMC, carbopol and polycarbophil retarded the drug release from the formulations, whereas HPMC exhibited burst release. The retardation of drug release increased with increase in the concentration of mucoadhesive polymers. Increase in the overall product viscosity might contribute to the retarding effect of these mucoadhesive polymers[22] as well as their ability to distort or squeeze the extra-micellar aqueous channels of poloxamer micelles through which the drug diffuses thereby delaying the release process [23]. The increase in gel strength and/ or molecular interaction between the drug and polymers could also retard release of the drug.

Measurement of Gel Strength

At 37^0 C, the gel strength of formulation F3, F5, F7, F8 and F9 was found to be more as the concentration of polymer is more as compared to formulation F1, F2, F4, and F6. And among all formulations gel strength of formulation F8 was found to be good. Data is reported in table 2.

Rheological studies

Rheological behavior is the key part in the formulation of PF-127 preparations. Viscosity studies showed a marked increase in viscosity of the gels at 37ºC due to sol-gel conversion (Table 2). Concentration of bioadhesive polymers also had significant effect on the viscosity of the gels. Increase in the concentrations of the bioadhesive polymers increased the viscosity of the gels. The order for increase in viscosity observed can be given as follows: Polycarbophil > Carbopol > SCMC > HPMC.

Stability studies

Depending on gelation temperature, bioadhesive strength, viscosity, drug content and *in vitro* drug release, formulation F8 was selected as optimized formulation. The stability studies for F8 were carried out at 25ºC/ 60%RH for 90 days. There was no marked change in the physical property and the drug content during the study period.

CONCLUSION

The *in situ* gelling liquids are considered a more convenient formulation for topical application into the vagina. The insitu formulation will have better patient acceptability since formulation will be applied in the form of sols which upon contact will form the corresponding gels causing less irritation or pain. From the study, it can be concluded that the temperature sensitive bioadhesive gel can be used to achieve sustained drug release. All the gels formulated had gelation temperature well below body

temperature thus they readily became gels, making them ideally suited to function as drug depot. Thus the developed dosage form was found easy to administer, simple, comfortable, with increased patient compliance.

ACKNOWLEDGEMENTS

The authors would like to thank Bhavani Pharmaceuticals Kanpur for the gift sample of Miconazole. And a special thank for Inspire Fellowship for providing fund. We also thank the JSS University and JSS Mahavidyapeetha, Mysore for their valuable support to carry out this research.

REFERENCES

1. Varshosaz J, Tabbakhian M and Salmani Z. Designing of a thermosensitive chitosan/poloxamer *in situ* gel for ocular delivery of ciprofloxacin. *The Open Drug Delivery Journal* 2008; 2: 61-70.
2. Zhidong L, Jiawei L, Shufang N, Hui L and Weisan P. Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin. *Int J Pharm* 2006; 315:12–16.
3. Peppas NA and Langer A. New challenges in biomaterials. Science. 1994263,1715.
4. Richard A. Graves, Thomas Freeman and Tarun K. In vitro dissolution method for evaluation of buprenorphine in situ gel formulation: A technical note. *AAPS PharmSciTech* 2007; 8 (3):1-4.
5. Yu Liu, Yi-ying Zhu and GangWei,Wei-yue Lu. Effect of carrageenan on poloxamer-based *in situ* gel for vaginal use: Improved in vitro and in vivo sustained-release properties. *Eur J Pharma Sci* 2009; 37:306–31.
6. Indrajeet D. Gonjari, Avinash H. Hosmani, Amrit B. Karmarkar, Appasaheb S. Godage, , Sharad B. Kadam and Pandurang N. Dhabale. Formulation and evaluation of *in situ* gelling thermoreversible mucoadhesive gel of fluconazole. *Drug Discov Ther* 2009; 3(1):6-9.
7. Feirong Kang and Jagdish Singh. In vitro release of insulin and biocompatibility of in situ forming gel systems. *Inter J Pharma* 2005; 304: 83–90.
8. Hani U, Bhat RS, Shivakumar HG. Formulation design and evaluation of metronidazole microspheres in a bioadhesive gel for local therapy of vaginal candidiasis. *Lat Am J Pharm* 2011; 30 (1): 161-7.
9. Dina W. Ameen. development and in vitro evaluation of bioadhesive vaginal tablet using econazole nitrate as a model drug. *Iraqi J Pharm Sci* 2011; 20(1): 57-65.
10. Erem Bilensoy, Abdur Rouf M, Imran Vural, Murat Sen, and Atilla Hıncal A. Mucoadhesive, thermosensitive, prolonged-release vaginal gel for clotrimazole:*β*-cyclodextrin complex. *AAPS Pharmscitech* 2006; 7 (2) Article 38.
11. Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK and Kim CK. Development of in situ gelling and mucoadhesive acetaminophen liquid suppository. *Int J Pharm* 1998; 165: 33- 44.
12. Miyazaki S, Nakamura T, Takada M. Thermo-sensitive sol-gel transition of Pluronic F-127. *Yakuzaigaku* 1991; 51:36-43.
13. El-Sayed Ali Ibrahim, Sayed Ismail, Gihan Fetih, Omar Shaaban, Khaled Hassanein and Noura Hassan Abdellah. Development and characterization of thermosensitive pluronic-based metronidazole *in situ* gelling formulations for vaginal application. *Acta Pharm* 2012; 62: 59–70.
14. Escobar JJ, Lopez CM, Naik A, Kalia YN, Quintanar GD and Ganem AQ. Applications of thermoreversible pluronic F127 gels in pharmaceutical formulations. *J pharm Pharmaceut Sci* 2006; 9(3): 339-358.
15. K. Edsman J. Carlfors and R. Petersson. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. *Eur J Pharm Sci* 1998; **6:**105–112.
16. Kim EY, Gao ZG, Park JS, Li H and Han K. rhEGF/HP-β-CD complex in poloxamer gel for ophthalmic delivery. *Int J Pharm* 2002; 233:159-167.

17. Varsha G. Formulation and evaluation of *in situ* gel of metoprolol tartrate for nasal delivery. *J Pharm Res* 2010; 3(4): 788-793.
18. Gilbert JC, Richardson JL, Davies MC, Palin KJ and Hadgraft J. The effect of solutes and polymers on the gelation properties of pluronic F127 solutions for controlled drug delivery. *J Control Rel* 1987; 5: 113-118.
19. Ryu JM and Chung SJ. Increased bioavailability of propranolol in rats by retaining thermally gelling liquid suppositories in the rectum. *J Control Rel* 1999; 59(2):163-72.
20. Lehr CM, Bouwatra JA, Bodde HE and Junginger HE. A surface energy analysis of mucoadhesion: contact angle measurement on polycarbophil and pig intestinal mucosa in physiologically relevant fluids. *Pharm Res* 1992; 9: 70-75.
21. Liu T and Chu B. Formulation of homogenous gel-like phases by phase transition. *J. Appl. Cryst* 2000; 33: 727-730.
22. Desai SD and Blanchard J. Evaluation of pluronic F127 based sustained-release ocular delivery systems for pilocarpine using albino rabbit eye model. *J Pharm Sci* 1998; 87: 1190-1195.
23. Vermani K, Garg S and Zaneveld JD. Assemblies for *in vitro* measurement of bioadhesive strength and retention characteristics in simulated vaginal environment. *Drug Dev Ind Pharm* 2002; 28: 1133–46.

Table 1. Formulation chart of Thermosensitive Bioadhesive Gel

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Pluronic-F127 (%w/v)	20	20	20	20	20	20	20	20	20
HPMC (%w/v)	0.2	-	0.2	-	-	-	-	-	-
SCMC (%w/v)	-	0.2	0.2	-	-	-	-	-	-
Carbopol (%w/v)			-	0.2	0.4			0.2	0.4
Polycarbophil (%w/v)	-	-	-	-	-	0.2	0.4	0.2	0.4
Miconazole Nitrate (%w/v)	1	1	1	1	1	1	1	1	1
pH 4 citrate phosphate buffer	q.s to 20 mL	q.s to 20 mL	q.s to 20 mL	q.s to 20 mL	q.s to 20 mL	q.s to 20 mL	q.s to 20 mL	q.s to 20 mL	q.s to 20 mL

Table 2. Results of characterization of thermosensitive bioadhesive gel. Each value represents mean ± SD: * n = 3

Formulation	Gelation temperature (°C)*	Gelation time (mins)*	Viscosity (cps) at 37°C	Mean mucoadhesive Force x 102 (N cm2)*	Gel strength T (sec)*	Drug content %W/W*
F1	35.8 ± 1.2	6.0 ± 0.8	16050	13.1±2.7	113	98.79±0.63
fiF2	35.0 ±1.3	4.2 ± 0.7	20130	16.9±1.4	116	96.90±0.70
F3	37.0 ± 1.5	4.0 ± 0.89	23685	17.9±2.5	142	96.95±0.43
F4	36.0 ± 0.9	3.5 ± 0.4	29040	19.6±3.1	118	96.95±1.36
F5	35.3 ± 1.3	3.0 ± 0.2	29880	20.8±2.2	151	98.33±0.52
F6	36.5 ± 1.2	4.5 ± 0.3	30975	22.3±2.3	119	97.47±1.03
F7	35.0 ± 0.8	4.0 ± 0.9	31628	19.6±1.5	148	98.16±0.94
F8	34.2 ± 0.6	3.7 ± 0.3	33930	21.4±1.8	146	97.87±0.87
F9	33.0 ± 1.4	3.0 ± 0.6	35104	23.9±1.6	172	98.56±1.34

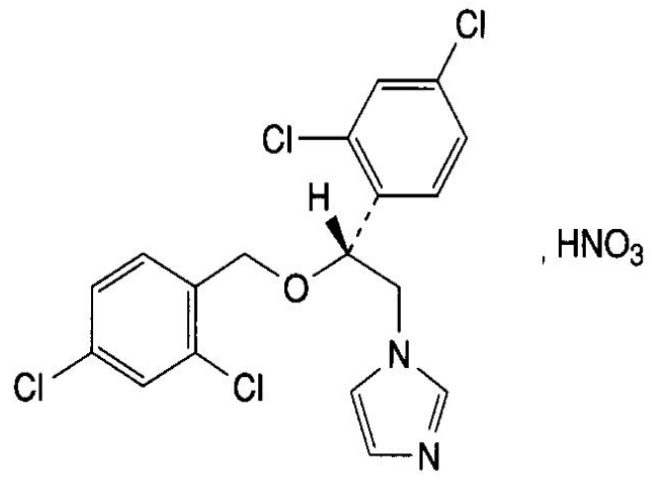

Figure.1. Structure of antifungal drug Miconazole nitrate

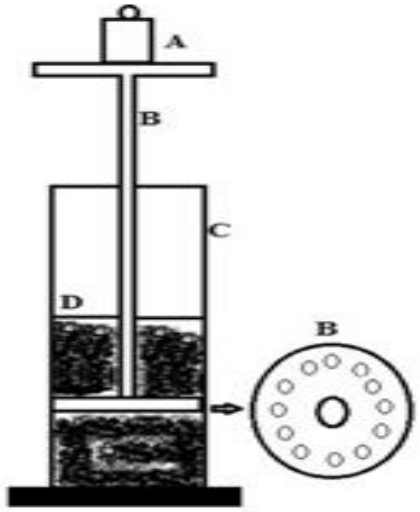

Figure.2. Gel strength-measuring device. A) Weight, B) Device, C) Cylinder, D) Gel

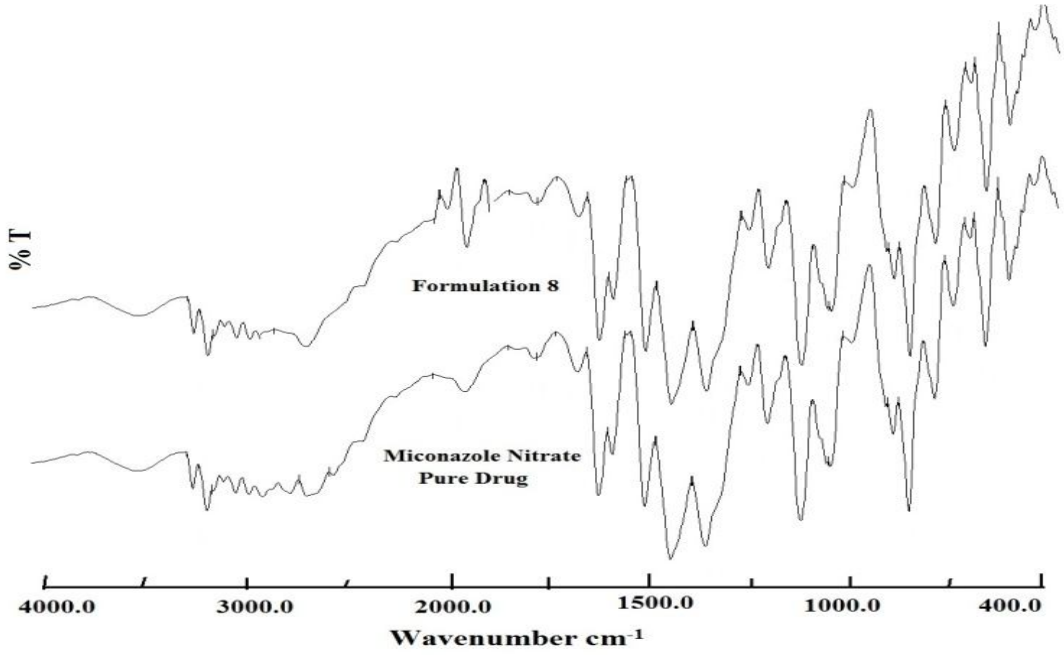

Figure.3. FTIR spectra of pure drug and formulation

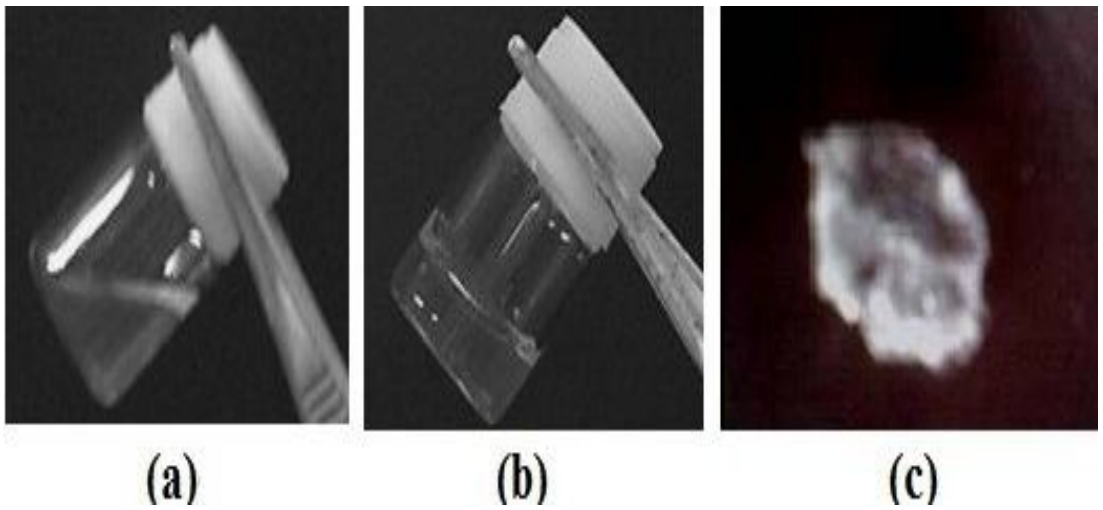

Figure.4. (a)Miconazole Nitrate In situ gel at room temperature (b) & (c) In situ gel at body temperature

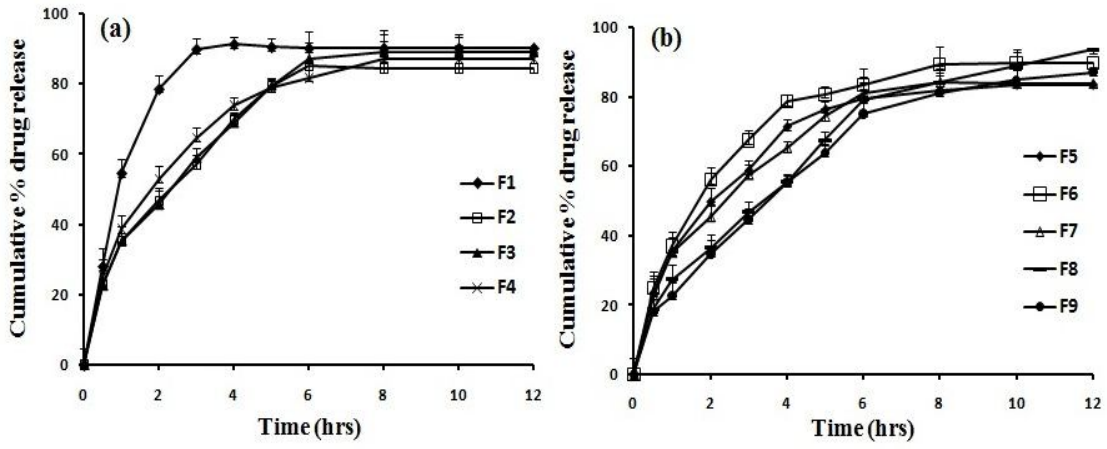

Figure.5. *In vitro* release profiles of formulations in SVF (**a**) F1 to F4 and (**b**) F5 to F9. (n = 3, mean ± standard deviation)