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Formulation and evaluation of liposomes in carbopol gels for mixed vaginal infections

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ABSTRACT

Objective: The aim of our study was to formulate and evaluate the liposomal carrier system for the local treatment for mixed vaginal infections. The combination of voriconazole and metronidazole were selected as model drugs for mixed vaginal infections.Methods:Multi lamellar liposomescomposed phosphatidylcholine and cholesterol along with combination of drugs was prepared by the thin film hydration method. The prepared liposome were characterized for fourier transform infrared, size distribution, entrapment efficiency, in vitrorelease study in simulated vaginal fluid and stability studies.The liposomes were loaded to carbopol gel. The liposomes loaded carbopol gels were evaluated for in vitrodrugs release study and compared with control gel. Results: FTIR study indicated that there is no significant chemical interaction between the components.The cumulative percent releasefrom liposomal gels FL1 was found to be 69.90% for metronidazole and 56.02 % for voriconazole.In vitro release studies of liposomes incorporated in the carbopol gel have shown a prolonged release of entrapped metronidazole and voriconazole compared to control gel. Stability studies showed that the vesicles were stable inrefrigerated temperature (4 °C) for 60 days without significant differences in drug entrapment.Conclusion:From the results it was evident that the liposomes are capable to efficiently deliver entrapped drug for the extended period of time within a bioadhesive gel. The bioadhesive gel in combination of these two drugs is effective in more than one type of vaginal infections.

Keywords: Bioadhesive gels,Metronidazole,Mixed vaginal infectionsVoriconazole

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INTRODUCTION

Vaginitis due to infection of two or more pathogens is termed as mixed vaginal infection[1].The three common infections associated with vaginal discharge in the adult woman are bacterial vaginosis, trichomoniasis and candidiasis. These infections are associated by vaginal discharge, irritation in vagina or foul vaginal odour and are diagnosed by vaginal pH measurement, vaginal discharge or microscopic examination[2,3]. In case of such mixed vaginal infections, it's difficult for a single drug to complete the treatment. The combination therapy may provide immediate and effective treatment for mixed vaginal infections[4]. Thus in the present study, combination of metronidazole and voriconazole is selected as the model drugs for the treatment of mixed vaginal infections. The voriconazole has a wide spectrum activity against yeast (including fluconazole susceptible and resistant candida species). voriconazole is indicated for vulvo vaginal candidiasis treatment[15]. Metronidazoleis classified therapeutically as an antibacterial, antiprotozoal drug[16]which is used in the treatment of trichomoniasis also.

The vagina provides a promising site for local infections[5].There are many vaginal dosage forms are availablewhich include tablets, films,ointments, pessaries, douches, suppositories and vaginal rings[6 7].The residence time of these dosage form is less to provide complete therapeutic effect.Thus, the bioadhesive polymer like carbopol is used in vaginal formulation that adheres to vaginal mucosa for long time. [8 9]

The incorporation of liposomes in Carbopol gels improves their stability and the applicability[10].Liposomal gels can act as a novel vaginal delivery system, able to provide controlled and sustained release of entrapped drug.Liposomes are studied extensively in topical applications for skin, oral and vaginal diseases.Liposomes act as "drug localizers", with low systemic absorption of the drugs. They also provide sustained drug release of drugs. [11-14]

The objective of our study was to prepare liposomesby thin film hydration method and to incorporate liposomes to carbopol 934 P gels.The liposomal suspension and liposomes loaded carbopol gels were characterized for different parameters.

MATERIALS AND METHODS

Metronidazole was the gift sample obtained from KAPL (Bangalore).Voriconazole was the gift sample from Ranbaxy. Soyalecithin(Phosphatidylcholine) was purchased from himedia,Cholesterol from SD fine chemicals,Carbopol 934P from loba chemie(Mumbai).All the other chemicals and solvents used in the experiments were of analytical grade.

Preparation of liposomes:
The liposomes were prepared using the lipid film hydration technique using rotary flash evaporator[15]. In the appropriate volume of chloroform, the different ratio of the phospholipids, cholesterol and drugs namely, metronidazole and the voriconazolewere dissolved. The mixture was then transferred into a round bottom flask and the solvent was removed using a rotary flash evaporator at 40°C under vacuum at 20 RPM.The process was continued until the formation of a very thin lipids layer on the inner side of the round bottom flask. Then the flask was kept overnight under vacuum to ensure the complete removal of residual solvent. The dry lipid film was hydrated with phosphate buffersolution(PBS). The suspension was left undisturbed at room temperature for 2-3 hr to allow complete swelling of the lipid film.

Preparation of the Simulated Vaginal Fluid
Simulated vaginal fluid (SVF) was prepared from 3.51 g/l NaCl, 1.40 g/l KOH, and 0.222 g/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[13].

Simultaneous Estimation of metronidazole and voriconazole by UV Spectroscopy method:
For the simultaneous equation method, aliquots of voriconazoleand metronidazole (10 µg/ml) in SVF were scanned in the range of 200-400 nm. The 256 nm and 320 nm were selected as the two sampling wavelengths for voriconazoleand metronidazole respectively. The figure 1represents the overlain UV spectra of voriconazole and metronidazole.Voriconazole and metronidazole exhibited linearity in the range of 8-32 µg/ml and 2-14 µg/ml respectively at their respective selected wavelengths.

FT-IR Analysis
The FT-IR analysis was conducted to study the possible interaction between drugs and the other excipients.The FT-IR spectra of the samples were obtained using FT–Infrared Spectrophotometer by KBr pellet method.The samples were diluted with KBr pellet and then compressed into a tabletusing a manual tablet presser. The position of peak in FT-IR spectra of pure voriconazole and metronidazolewere compared with those in FT-IR spectra of drugs with the excipients.

Size distribution
Mean vesicle size and size distribution profile of liposome was determined by using Malvern particle size analyzer model SM 2000[16].The liposome suspension was added to the sample dispersion unit containing stirrer and stirred at 2000 rpm in order to reduce the interparticle aggregation. The average particle size was measured after performing the experiment.

Entrapment efficiency

The entrapment efficiency of liposomes were studied by centrifugationtechnique[17].The drug loaded liposomes were centrifuged at 20000 rpm for 1hr at the temperature of 4°C. Supernatant containing unentrapped drugs were withdrawn and analyzed by simultaneous estimation by UV spectrophotometrically at 320 nm for metronidazole and 256 nm for voriconazole against simulated vaginal fluid as blank. The entrapment efficiency was calculated separately for both the drugs. The experiment was carried out in triplicate and the values were expressed as mean standard deviation.

$$\textbf{\% Drug Entrapped (EE)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \times 100$$

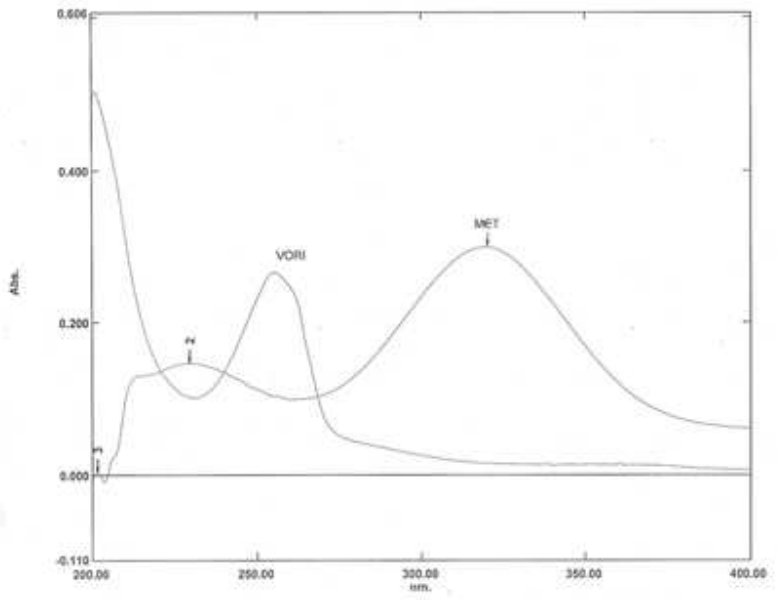


Figure No.1:Overlain Spectra of voriconazole and metronidazole

***In vitro* release of liposomes:**

The prepared liposomes were separated from unentrapped drug by centrifugation method andresuspended in PBS pH (7.4), filled in a dialysis tube to which a sigma dialysis sac was attached to one end[18].The dialysis tube was suspended in Simulated Vaginal Fluid (SVF) pH (4.2), stirred with a magnetic stirrer and samples were withdrawn at specific time intervals and analyzed using UV spectrophotometer. To maintain a constant volume, an amount of medium equivalent to the volume of sample withdrawn was added immediately.

Stability studies

The drug retentive behaviour was assessed by storing the formulationF6 liposomal suspensions and liposomal loaded gels at two different temperature conditions, i.e., 4-8 °C (Refrigerator; RF), 25±2 °C (Room temperature; RT), for a period of 60 days[19]. Samples were withdrawn periodically and analyzed simultaneously for the entrapment efficiency studies.

Gel preparation

The gels were prepared by dispersing the Carbopol 934P (1 g) in distilled water by continuous stirring with the help of glass rod, in which glycerol (10g) was previously added allowed to soak for 2 hr. The Mixture was then and neutralized by drop wise addition triethanolamine. Mixing was continued until the formation of a transparent gel[20]. Control gel (gel containing pure drugs) was also prepared in the similar manner.Liposomes containing drugs was mixed into the 1 % (w/w) Carbopol 934P gel by an electrical mixer (25 rpm, 2 min) to gel.

In vitro release from gel:

The *in vitro*reslease from the gel was studied from modified open diffusion cell[21]. The semi permeable membrane was used to study. The donor compartment contained the gel and receptor compartment was filled with SVF .The temperature was maintained at 37°C and stirred at 25 rpm. The samples were withdrawn at one hour time interval and analyzed for drugs using UV spectrophotometer aganist SVF as blank.

RESULTS

The FTIR studies:

The FTIR study report is presented in figure 2-4. The IR stretching of puremetronidazoleshowed 3364,3024,2855,1517 and 720 cm^{-1}wave number as major peaks.Thevoriconazole showed 3209,2829,1181 and 1534 cm^{-1} wave number as major peaks. the results revealed no considerable changes in their peaks of metronidazole and voriconazole when mixed with excipients compared to pure metronidazole and voriconazole.

FigureNo.2: FT-IR Spectra of Metronidazole pure drug

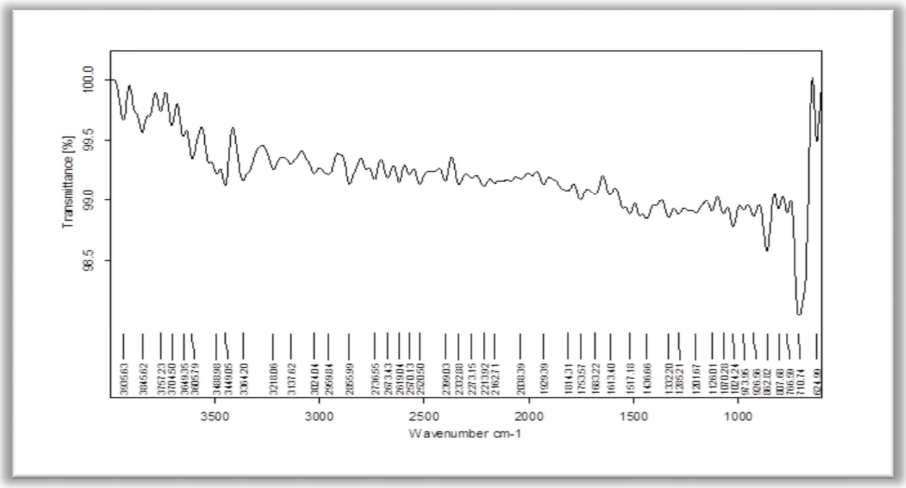


Figure No.3:FTIR Spectrum of Voriconazolepure drug

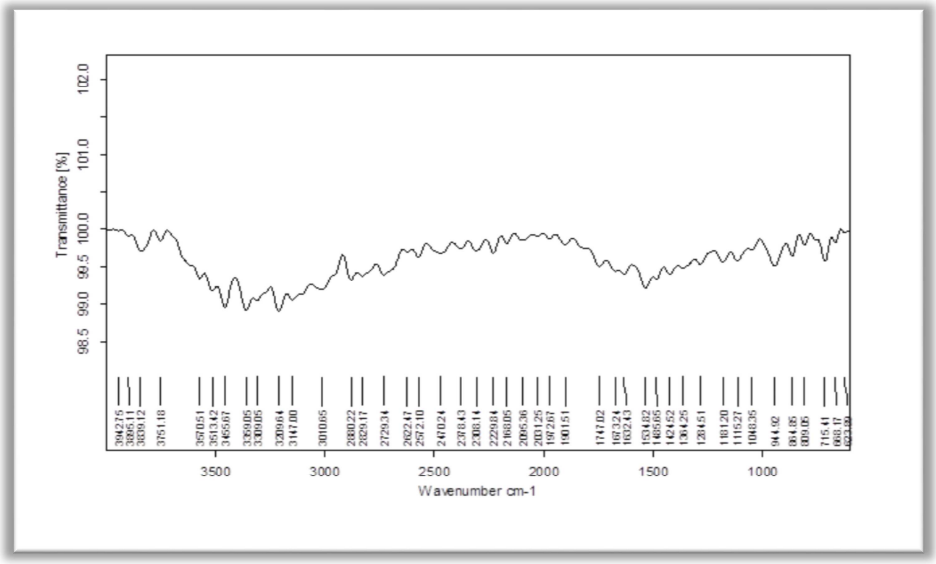
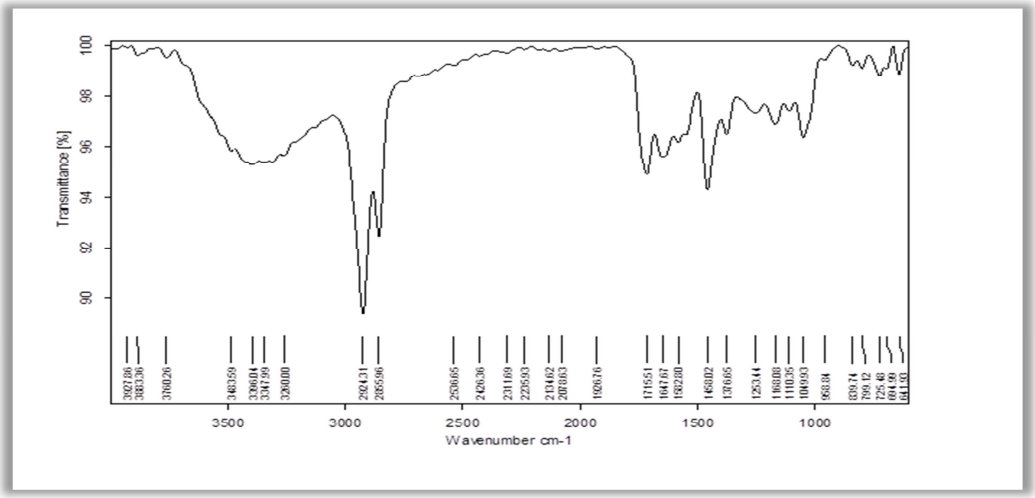


Figure No.4:FTIR Spectrum of Combination of drugs and excipients



Discussion: It was found that the drugs in the mixture was found be intact. The results indicates that there was no interaction between the pure drug and the polymer mixture. It was observed that there was no major shift in the wave length of functional groups of metronidazole and voriconazole.

Entrapment efficiency:

The entarapment efficiency was shown in table No.1.The entrapment efficiency of voriconazole was found to be from the range of 48.4± 0.19 to 63.0 ±0.18 and for metronidazoleit was found to be in the range of 38.4 ±0.10 to 49.0 ± 0.11.

Discussion: A positive correlation was observed with the concentration of phosphatidylcholine cholesterol, and entrapment efficiency. Thus, as the concentration of lipids increases, the entrapment efficiency was found to be increased. This may be due to the cholesterol which is known to impact rigidity to the bilayer membrane. This prevents the drug leakage from bilayer membrane so that the retention of the drug is enhanced. The entrapment efficiency of voriconazole is slightly greater than the metronidazole. This may be due to more lipophilic nature of voriconazole.

The entrapment efficiency of liposomes was found to be increased with increase in lipidsconcentration,but the effective drug-lipid ratio decreased.This may be due to increase in total amount of lipids in the formulation.Thedrug:lipid ratio for F1 was 42.6:1 for metronidazole and 53.7:1 for voriconazole and for F6 it was 37:1 for metronidazole and 48.4:1 for voriconazole.

Mean Particle size:

The mean particle size was shown in table No.1.The mean particle size liposomes was found to be from the range of 755 nm to 1104 nm forformulation F1 to F6.It was observed that as the concentration of soyalecithin and cholesterol increases the mean particle size was also found to be increased.

Figure No.5:Size distribution curve of F1

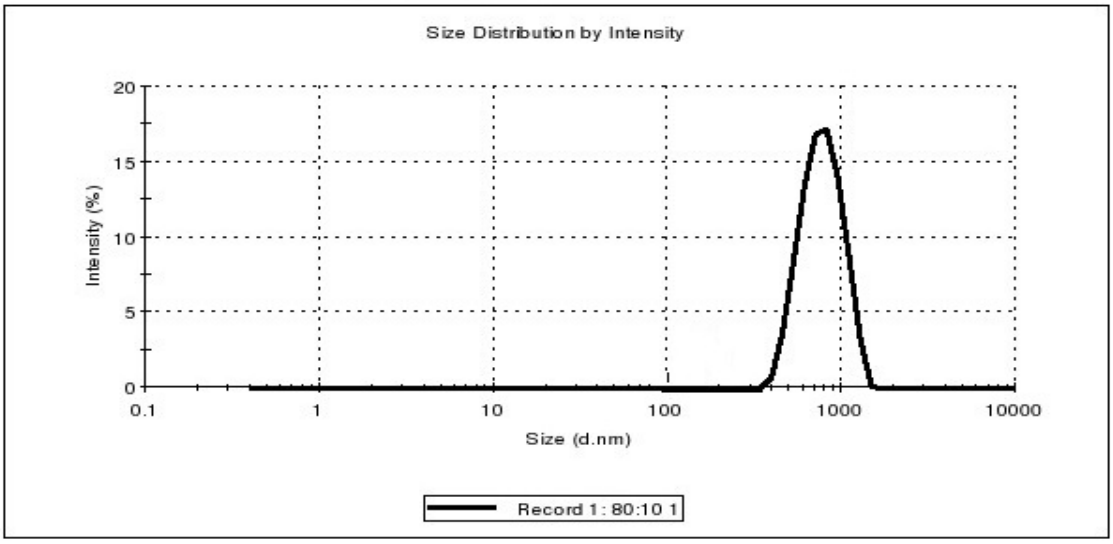


Table No.1:Table showing entrapment efficiency, Drug-lipid ratio and Particle size in nm

Formulation Code	Phosphatidyl choline : Cholesterol in mg	Drug entrapped (mg± SD)		Entrapment efficiency		Drug-lipid ratio (µg: mg)		Particle size in nm
		VORICONAZOLE	METRONIDAZOLE	VORICONAZOLE	METRONIDAZOLE	VORICONAZOLE	METRONIDAZOLE	
F1	80 : 10	4.84± 0.19	3.84 ±0.10	48.4	38.4	53.7:1	42.6:1	791.6
F2	80 : 20	4.99± 0.11	3.83± 0.17	49.9	38.3	49.9:1	38.3:1	946.9
F3	80 : 30	5.37±0.13	4.19± 0.18	52.2	41.9	48.8:1	38.0:1	1036
F4	100 : 10	5.71± 0.18	4.52±0.12	57.1	45.2	51.0:1	41.0:1	802.0
F5	100 : 20	6.12±0.14	4.75 ±0.14	60.2	47.5	50.1:1	39.5:1	971.1
F6	100 : 30	6.30±0.14	4.90±0.11	63.0	49.0	48.4:1	37.6:1	1104

***In vitro* drug release studies and kinetic studies:**

The *in vitro* release studies from liposome suspension for all the formulations F1 to F6were shown in figure 6 and 7. The *in vitro* drug release from formulation F1 was found to be 84.9% for metronidazole and 71.0% for voriconazoleand for formulation F6 itwas found to be 69.90% for metronidazole and 61.7% for voriconazole respectivelyat the end of 10^{th} hour.The liposomalgel formulation FL1 showed releaseof69.90% for metronidazole and 60.02% for voriconazole and forformulation FL6, the 61.70% for metronidazole and 47.90% for voriconazolerespectively at the end of 10^{th} hours. The control gel(containing drugs) showed the release of 60.4% for metronidazole and 46.8% for voriconazole respectively and didn't show much further release at the end of 10^{th} hour. It was found that in all the formulation the R value of model was close to zero order release. The diffusion co-efficient values indicated that the release from all the formulation follows diffusion mechanism.

Discussion: The liposomal gel showed good sustain release compared to liposomal suspension. *In vitro* drug release of liposomes loaded gel was found to be much slower compared with liposomal suspension. Slow release from the gel may be attributed due to diffusion of drugs from the gel matrix.

The release of the drugs from the control gel(plain gel with drugs) was found to be much greater than the liposomes suspension and liposomes loaded carbopol gel.The control gel released upto 60 % of metronidazole and 46 % of voriconazoleat the end of 10^{th} hour and further there was no much release took place.The liposomes loaded gels provided controlled release of drugs for more than 10 hours.(Figure 8 and 9)

The *in vitro* studies revealed that as the concentration of lipid increases, the release of the drug was found to be decreased. This may be due to the increased rigidity of cholesterol so that, the release of the drug from the bilayer membrane decreases.

Stability studies:

The results of stability studies are shown in table 2.Stability studies were conducted for the formulation F6. The stability studies were performed at room temperature i.e 25°C and 60%RH and 4°C to 8°C for period up to 60 days. The liposomal gels were analyzed for % entrapment efficiency of voriconazole and metronidazoleat a time interval of 30 days till a period of 60 days.

Discussion:The stability studies revealed that there was slight decrease in the entrapment efficiency when stored in room temperature. Formulations showed no much variation in % entrapment efficiency when stored at 4-8°C.Therefore, the formulations were supposed to be stored at 4-8°C to retain its efficacy. These results indicated that all the formulations were stable for a period of 60 days when stored in refrigerated condition.

Figure No.6. *In vitro* release of Metronidazole from F1 to F6

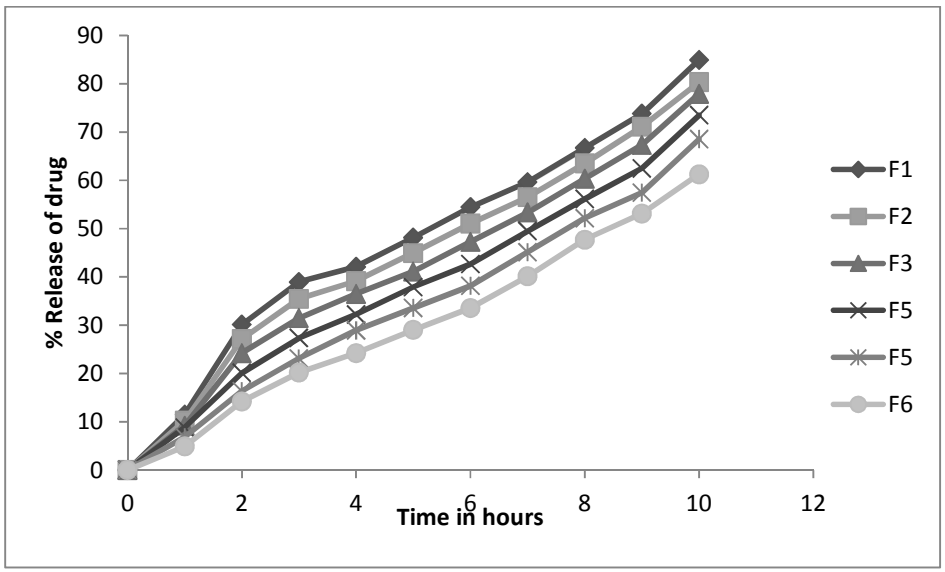


Figure No.7.*In vitro* release Voriconazole from F1 to F6

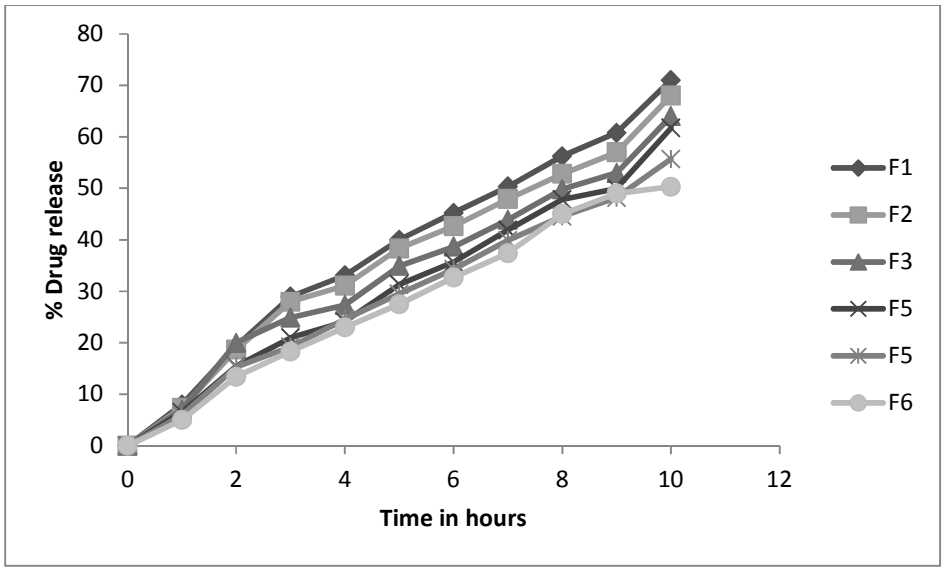


Figure No.8.*In vitro* release of Metronidazole from FL1 and Control gel

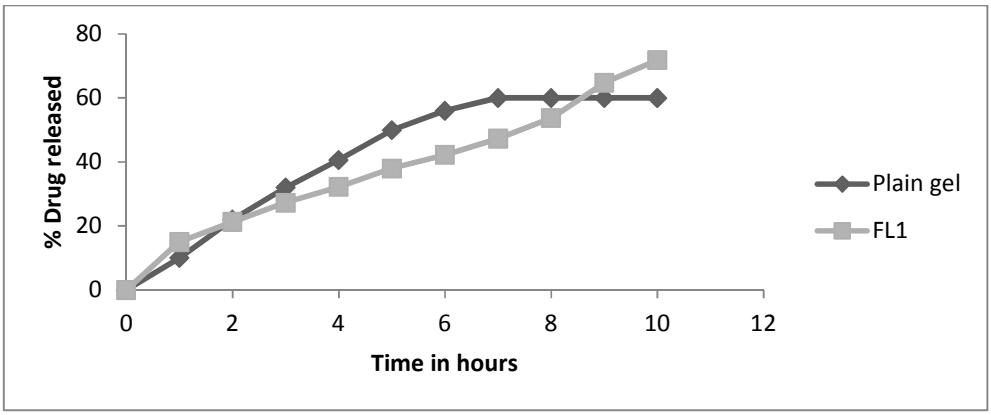


Figure No.9.*In vitro* release of Voriconazole from FL1 and Control gel

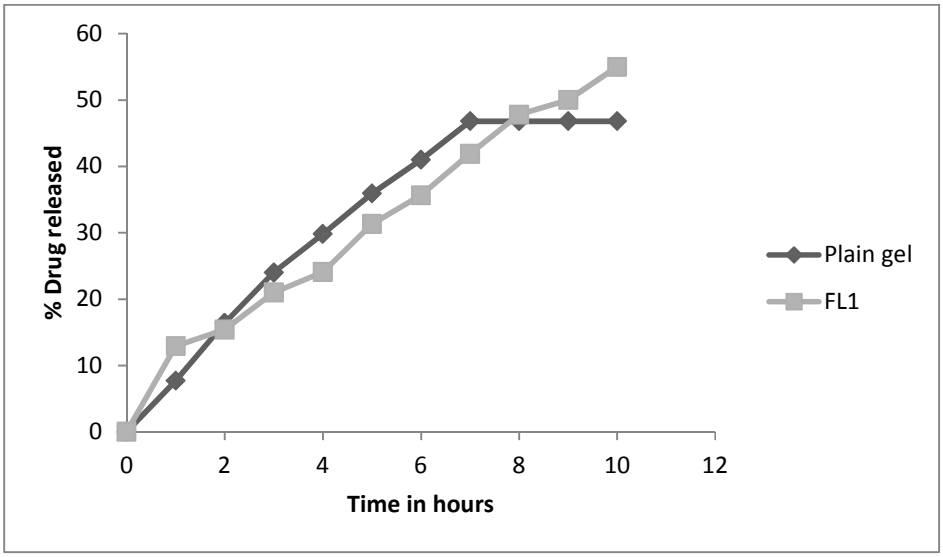


Table No.2.:Stability studies of F6

Sl. No.	Number of days	Entrapment efficiency			
		Metronidazole		Voriconazole	
		4-8°C	Room Temp.	4-8°C	Room Temp.
1.	0	49.0	48.3	63.0	62.2
2.	30	48.2	46.9	62.3	60.3
3.	60	46.8	45.5	61.9	59.2

CONCLUSION

The liposomes are prepared by thin film lipid evaporation method successfully.The liposomes provided sustain drug release, which is one of the desired characteristics for local vaginal infection. Liposomes loaded bioadhesive gel provided an extended period of drug delivery for more than 10 hours and was also found to be stableupto 60 days. These gels are safer over the vaginal cavity and also has the desired viscosity and bioadhesive propertiesThus, liposomes loaded with drugs in a carbopol gel is the well suited approach for mixed vaginal infections improving the patient compliance.

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