International Journal of Advanced Pharmaceutical Sciences, Volume 1, Issue 04, Page 01-23
ISSN:2456-8147

Formulation And Evaluation of Mucoadhesive Thermosensitive Pluronic Lecithin Organogel of Miconazole Nitrate for Vaginal Candidasis

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An open access journal

Supporting Information:

Received: 07 March 2018
Accepted: 21 March 2018
Published: 05 April 2018

Competing Interests:
The authors have declared that no competing interests exist.

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ABSTRACT

Antifungal drugs are group of medications which inhibits or kills the growth of fungal species. The potential use for mucoadhesive systems as drug carriers lies in its prolongation of the residence time at the absorption site, allowing intensified contact with the epithelial barrier The present study give an insight on the formulation and evaluation of mucoadhesive mucoadhesive thermosensitive Pluronic Lecithin Organogel of Miconazole Nitrate for Vaginal Candidasis

Keywords : Candidasis, stability study ,Fickian diffusion

INTRODUCTION

In the 1980s, a new concept began to be applied to drug delivery systems called bioadhesion. It can be defined as the state in which two materials, at least one of which is biological in nature, are maintained together for a prolonged time period by means of interfacial forces.Vaginal candidasis is caused by the overgrowth of a fungal species .On the other hand, adhesion of preparations onto mucous membrane can be impaired by the mucociliary clearance system. This clearance, a natural defense mechanism of the body against the deposition of impurities onto the mucous membrane, can also remove the preparation. Thus, by using bioadhesive molecules, it is possible to retain the preparation at the action site and to direct the drug to a specific site or tissue. Other features associated with the development of controlled drug delivery systems using bioadhesive molecules include a decrease in drug administration frequency and an increase in patient compliance to the therapy (Woodley, 2001). Therefore, a bioadhesive system controlling drug release could improve the treatment of diseases, helping to maintain an effective concentration of the drug at the action site.

MECHANISM OF MUCOADHESION

The mechanism of mucoadhesion is generally divided into two steps; the contact stage and the consolidation stage. The first stage is characterized by the contact between the mucoadhesive and the mucus membrane with spreading and swelling of the formulation, initially its deep contact with the mucus layer.

In the consolidation step the mucoadhesive material are activated by the presence of moisture, Moisture plasticizer the system allowing the mucoadhesive molecules to break free and to link up by weak Vander Waals and hydrogen bonds. Essentially, there are two theories explaining the consolidation step; the diffusion theory and the dehydration theory. According to the diffusion theory; the mucoadhesive molecules and the glycoproteins of the mucus mutually interact by means of interpenetration of their chains and the building of secondary bonds. For this to take place, the mucoadhesive device has features favouring both chemical and mechanical interactions. For example, molecules with hydrogen bond building groups (-OH,-COOH), an anionic surface charge, high molecular weight, flexible chains and surface active properties, which help in spreading throughout the mucus layer can present mucoadhesive properties.

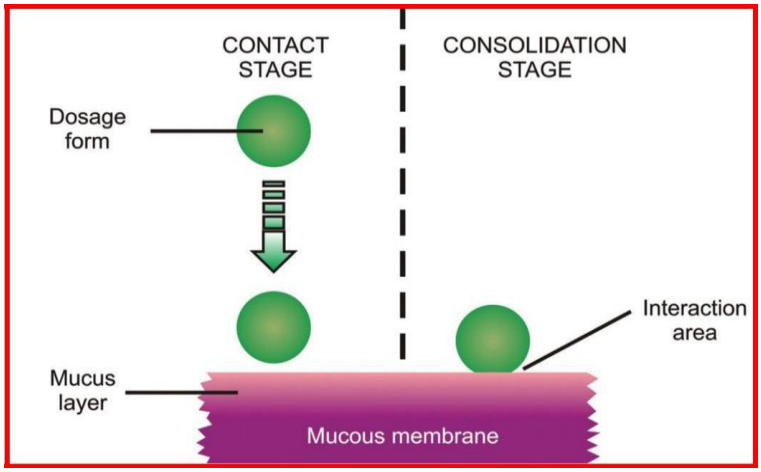

FIGURE 1: The two steps of the mucoadhesion process

However, the dehydration theory is not applicable for solid formulations or highly hydrated forms (Smart *et al*., 2005).

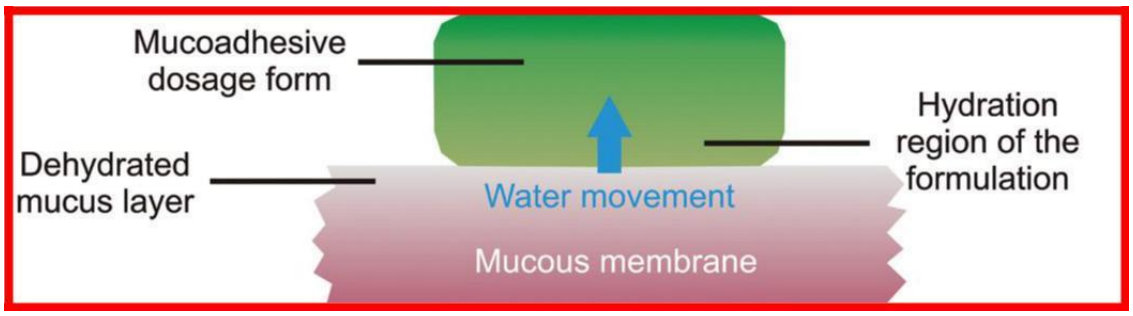

FIGURE 3: Dehydration theory of mucoadhesion

1.3 MUCOADHISIVE THEORIES

Mucoadhesive is a complex process and numerous theories have been proposed to explain the mechanism involved. These theories include mechanical interlocking,

- Wetting theory
- Diffusion theory

- Fracture theory
- Electrostatic theory
- Absorption theory

1.3.1 Wetting theory

The wetting theory applies to liquids systems which present affinity to thesurface in order to spread over it. This affinity can be found by using measuring techniques such as the contact angel, the greater is the affinity (figure 3). The contact angle should be equal or close to zero to provide adequate Spreadability. The Spreadability coefficient can be calculated from the difference between the surface energies γB and γA and the interfacial energy γAB as indicated in an equation given below. This theory explains the importance of contact angle and reduction of surface and interfacial energies to achieve good amount of mucoadhesion. (Smart *et al.,)*

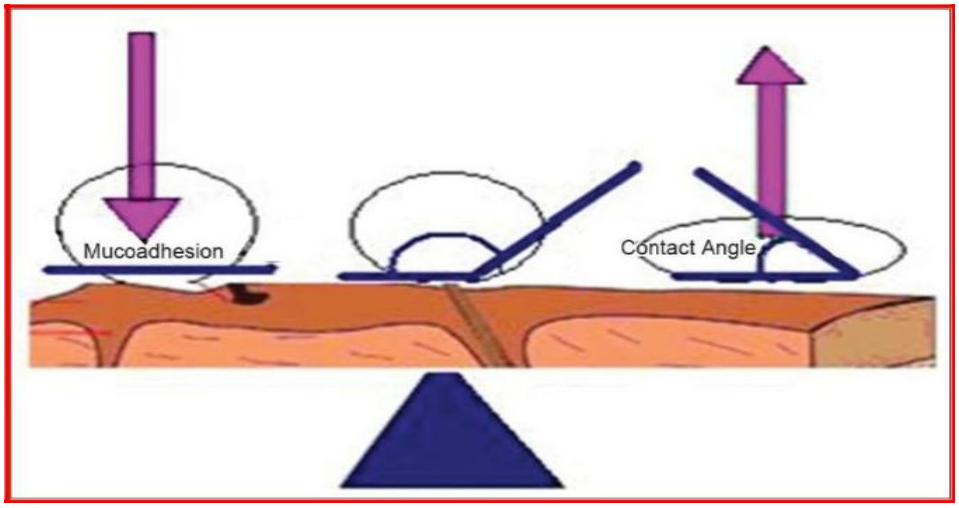

FIGURE 4: Influence of contact angle on mucoadhesion

1.3.2 Diffusion theory

Diffusion theory describes the interpenetration of both polymer and mucin chains to a sufficient depth to create a semi-permanent adhesive bond (figure 4). It is believed that the adhesion force increases with the degree of penetration of the polymer chains. This penetration rate depends on the diffusion coefficient, flexibility and nature of the mucoadhesive chains, mobility and contact time. According to the literature, the depth of interpenetration required to

produce an efficient bioadhesive bond lies to the range 0.2-0.5 . This interpenetration depth of polymer and mucin chains can be estimated by the following equation.

$= (\quad)^{1/2}$

Where t is the contact time and D is the diffusion coefficient of the mucoadhesion material in the mucus. The adhesion strength for a polymer is reached when the depth of penetration is approximately equivalent to the polymer chain size, In order for diffusion to occur, it is important that the components involved have good mutual solubility, that is both the bioadhesive and the mucus have similar chemical structures. The greater the structural similarity, the better is the mucoadhesive bond.

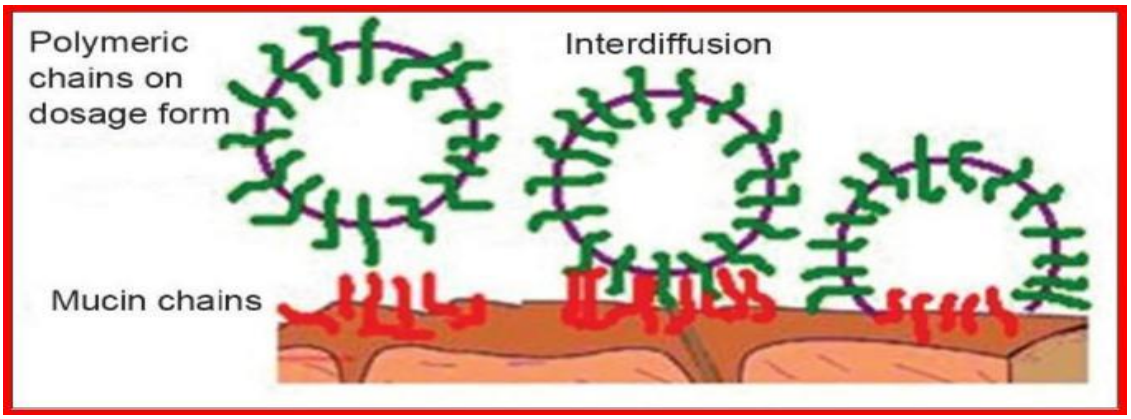

FIGURE 5: Secondary interaction between mucoadhesive device and of mucus

1.3.3 Fracture theory

This is perhaps the most used theory in studies on the mechanical measurement of mucoadhesion. It analyzes the force required to separate two surfaces after adhesion is established. This force is frequently calculated in tests of resistance to rupture by the ratio of maximal detachment force and the total surface area involved in the adhesive interaction. Since the fracture theory is concerned only with the force required to separate the parts, it does not take into account the interpenetration or diffusion of polymer chains. Consequently, it is appropriate for use in the calculations for rigid or semi-rigid bioadhesive materials, in which the polymer chain do not penetrate into the mucus layer. (Hegerstron *et al*.,)

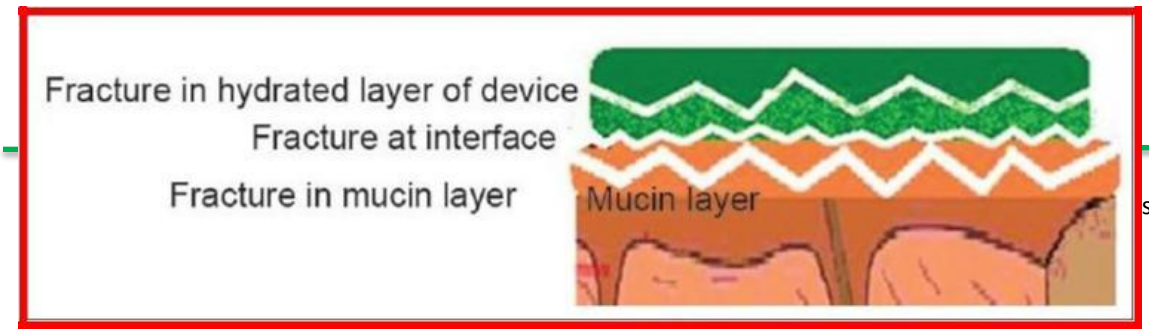

FIGURE 6: **Fractures occurring for mucoadhesion**

1.3.4 The electronic theory

This theory describes adhesion occurring by means of electron transfer between the mucus and the mucoadhesive system, arising through differences in their electronic structures. The electron transfer between the mucus and the mucoadhesive results in the formation of double layer of electrical charges at the mucus and mucoadhesive interface. The net result of such process is the formation of attractive forces within the double layer. (Dodou *et al*.,)

1.3.5 The adsorption theory

In this instance, adhesion is the result of various surface interactions (primary and secondary bonding) between the adhesive polymer and mucus substrate. Primary bonds due to chemisorptions result in adhesion due to ionic, covalent and metallic bonding, which is generally undesirable due to their permanency. Secondary bonds arise mainly due to vander walls forces, hydrophobic interactions and hydrogen bonding. Whilst these interactions require less energy to "break", they are the most prominent from of surface interaction in mucoadhesion process as they have the advantages of being semi-permanent bonds. (Kinloch *et al*.,)

All these numerous theories should be considered as supplementary process involved in the different stages of the mucus/substrate interaction, rather than individual and alternative theories. Each and every theory is equally important to describe the mucoadhesion process. There is a possibility that there will be initial wetting of the mucin and then diffusion of the polymer into mucin layer, thus causing the fracture in the layers to effect the adhesion or electronic transfer or simple adsorption phenomenon that finally leads to the perfect mucoadhesion. The mechanism by which mucoadhesive bond is formed will depend on the

nature of the mucus membrane and mucoadhesive material the type of formation, the attachment process and the subsequent environment of the bond. It is apparent that a single mechanism for mucoadhesion proposed in many texts is unlikely for all the different occasions when adhesion occurs. (Jemenez *et al*.,)

Drug Profile and Description

Sl. no.	Drug profile name	Drug profile properties
1.	**Drug name**	Miconazole nitrate
2.	**IUPAC NAME**	1-(2-(2,4-dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl)imidazolium nitrate.
3.	**Molecular weight**	479.15
4.	**Half life**	2.1 hour
5.	**Dose**	5 to 10gm of a 2% gel daily for 7-14days
6.	**Solubility**	Ether, isopropyl alcohol, methyl alcohol, propylene glycol
7.	**Melting point**	179-180^{0}C
8.	**Therapeutic use**	Antifungal agent

POLYMER PROFILE

Sl.no	Name	Functional category	Solubility
1.	**Carbopol 934**	Mucoadhesive polymer and release modifying agent.	Soluble in water after neutralization in ethanol and glycerin.
2.	**Pluronic f 127**	Thermosensitive polymer, solubilizing agent and wetting agent.	Water.
3.	**PEG 400**	To adjust viscosity and for soft gelation purpose.	Soluble in water, acetone, benzene
4.	**Soya lecithin**	Dispersing, emulsifying and helps in drug permeation.	Water, chloroform.
5.	**Isopropyl myristate**	Semisolid bases and in topical application.	Acetone, chloroform, ethanol

1. DIFFERENT APPROACHES

the mucoadhesion drug delivery system may include the following:

1. Buccal drug delivery system
2. Sublingual drug delivery system
3. Vagina drug delivery system
4. Rectal drug delivery system
5. Nasal drug delivery system
6. Ocular drug delivery system

FACTORS AFFECTING MUCOADHESION :

Mucoadhesion may be affected by a number of factors, including

1. hydrophilicity,
2. molecular weight,
3. cross-linking,
4. swelling,
5. pH

6. the concentration of the active polymer

METHODOLOGY

METHOD OF PREPARATION (COLD METHOD)

PLOs were prepared by using the cold method. Aqueous phase were prepared by dispersing carbopol 934 in citrate-phosphate buffer (0.1 M, pH 4.0) at 4ºc with gentle mixing. Pluronic F 127was then added to carbopol 934 solutions and allowed to dissolve over night at 4ºc. Oil phase was prepared by dissolving soya lecithin and sorbic acid in appropriate quantity of isopropyl myristate. The mixture was kept overnight at 4ºc in refrigerator for complete dissolution of its constituents. MZN was initially dissolved in the mixture of methanol and polyethylene glycol (PEG) 400 (3:5) and mixed with lecithin isopropyl myristate solution., Finally aqueous phase (70%) was slowly added in oil phase (30%) in with stirring at 400 rpm using mechanical stirrer.(Amit jain *et al*.,)

FORMULATION OF PLURONIC LECITHIN ORGANOGEL

- Pluronic lecithin organogel were prepared by cold method technique which requires aqueous phase and oil phase both are mixed by mechanical stirrer with slowly.

FORMULATION DESIGN OF PLURONIC LECITHIN ORGANOGEL

Formulations	Miconazole (%)	Soya Lecithin (%)	Carbopol 934(%)	Pluronic F127 (%)	Isopropyl Myristate Upto(ml)	Sorbic Acid (%)	Water Upto (ml)
F1	1	4	0.2	15	100	0.2	100
F2	1	4	0.2	25	100	0.2	100
F3	1	4	0.2	35	100	0.2	100
F4	1	4	0.2	50	100	0.2	100
F5	1	4	0.8	15	100	0.2	100
F6	1	4	0.8	25	100	0.2	100
F7	1	4	0.8	35	100	0.2	100
F8	1	4	0.8	50	100	0.2	100
F9	1	4	1.4	15	100	0.2	100
F10	1	4	1.4	25	100	0.2	100
F11	1	4	1.4	35	100	0.2	100
F12	1	4	1.4	50	100	0.2	100

NEED FOR THE STUDY

Currently vaginal delivery systems include creams, foams, gels, tablets, pessaries and irrigations, which are limited use because of less residence time at the genito urinary tract: they are removed rather rapidly by the self-cleansing action of the vaginal tract. Moreover, the physiological conditions imposed by the protective mechanism of the genital tract, limiting the residence time and thus impairing the therapeutic efficacy of the drug, make multiple and frequent administration necessary for treatment. Patient compliance when administering the dosage forms and following the repeated-dose therapeutic regimen is an important challenge in vaginal drug delivery. Patients are generally reported to tolerate organogel better than other dosage forms

EVALUATION OF PLURONIC LECITHIN ORGANOGEL AND GEL FORMULATION

Determination of mucoadhesive force

- The mucoadhesive force of organogel on vaginal mucosal tissues was determined by means of mucoadhesive force measuring apparatus, fabricated in our laboratory.
- At the time of testing, a section of tissue was secured (keeping the mucosal side out) to the upper side of a glass vial using a cyanoacrylate adhesive.

Spreadability

- For the determination of Spreadability, excess of sample was applied between the two glass slides
- and compressed to uniform thickness by placing 1000 gm weight for 5 mins.

Gelling capacity

- The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of simulated vaginal fluid (pH 4.0) freshly prepared.
- equilibrated at 37°C and visually assessing the organogel formation and noting the time for gelation and the time taken for the organogel formed to dissolve.

Viscosity

- The viscosity of the different gel formulations was determined using a Brookfield viscometer with spindle no 7 at 50 rpm.

Drug content

- 1gm of gel was accurately weighed dissolved using 10 ml of 0.1m HCl and 50 ml of isopropyl alcohol, sonicated for a period of 10-15 min and made up to the mark in 100 ml volumetric flask with isopropyl alcohol.
- The absorbance was measured spectrophotometrically at 272 nm against blank gel treated in the same manner as sample.

***In vitro* release studies**

- *In vitro* studies of the gel were carried out across the egg membrane extracted by using the concentrated HCL.
- The receptor compartments were filled with 0.1M citrate-phosphate buffered saline (PBS) pH 5.5

and the temperature was maintained at 37^{o}c throughout the study.

kinetics of drug release

- The drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q_0-Q) v/s t].

Antifungal efficacy studies

- The antifungal efficacy study against *Candida albicans* was determined by agar diffusion method, employing 'cup plate technique'.
- The zone of inhibition (ZOI) was measured and compared with that of the pure drug and entire operation was carried out in aseptic condition.

Stability studies

- The prepared final formulation F11 was transferred to collapsible aluminum tube which was charged for accelerated stability studies in ICH certified stability chamber maintained at 40°c and 75% RH.

pH , viscosity *in vitro* release and drug content were tested accordingly

Characteristics of various miconazole nitrate organogel formulations

FORMULATIONS	pH*	VISCOSITY*	SPREADABILITY*
		(cps)	(g.cm/s)
F1	6.3	3185±15	11.90±0.5
F2	5.9	3195±60	10.12±0.3
F3	6.4	3395±45	12.90±0.4
F4	6.6	3495±35	12.80±0.4
F5	6.5	3105±25	10.20±0.4
F6	6.2	3290±20	12.10±0.1
F7	6	3370±25	11.80±0.6
F8	6.2	3415±45	10.30±.07
F9	5.6	3145±55	12.80±0.1
F10	5.9	3330±30	13.10±0.6
F11	6.3	3355±20	12.70±0.4
F12	6.3	3405±30	10.12±0.2

FORMULATIONS	pH*	VISCOSITY* (cps)	SPREADABILITY* (g.cm/s)
F1	6.3	3185±15	11.90±0.5
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F4	6.6	3495±35	12.80±0.4
F5	6.5	3105±25	10.20±0.4
F6	6.2	3290±20	12.10±0.1
F7	6.0	3370±25	11.80±0.6
F8	6.2	3415±45	10.30±.07
F9	5.6	3145±55	12.80±0.1
F10	5.9	3330±30	13.10±0.6
F11	6.3	3355±20	12.70±0.4
F12	6.3	3405±30	10.12±0.2

*Average of three determination.

: Characteristics of various miconazole nitrate organogel

FORMULATIONS	DRUG CONTENT(%W/W)	*MUCOADHESIVE FORCE(dynes/ cm^2)	GELLING CAPACITY
F1	98.8±0.49	30.9±2.8	+
F2	97.3±0.68	30.6±0.7	+
F3	93.5±.044	56.3±5.3	+++
F4	99.4±0.75	55.6±4.2	+++
F5	98.7±0.55	45.5±1.2	++
F6	99.1±0.47	47.7±1.5	++
F7	95.5±0.33	46.2±2.4	++
F8	93.6±1.44	29.6±1.0	+
F9	97.5±0.99	54.6±5.6	+++
F10	98.4±0.55	55.5±7.5	+++
F11	99.7±0.65	51.1±2.8	+
F12	98.7±0.65	31.1±2.8	++

***Average of three reading; + gel after few minutes, dissolved rapidly; ++gelation immediately, remains for few hours; +++ gelation immediately, remains for extended period.**

The drug content in organogel was found in range of 93.5% to 99.7%. The higher drug content found in F11 i.e. 99.7% due to the optimum concentration of pluronic F127 and soya lecithin. The *in vitro* release profiles of miconazole from its various organogel formulations are represented in Table 11 and 12. Higher drug release was observed with formulations F11. This finding may be due to presence of optimum level of carbopol 934 (1%) and soya lecithin (4%). **Percentage Drug Release from Organogel Formulations (F1-F6)**

Time(hrs)	F1	F2	F3	F4	F5	F6
1	9.76±0.37	10.83±0.29	10±0.57	16.5±0.73	11.3±0.31	10.5±0.3
2	11.88±0.3	11.71±0.45	11.5±0.45	20±0.38	18.3±0.39	17±0.51
3	13.21±0.4	14.1±0.31	13.2±0.58	25.8±0.34	21±0.18	18±0.59
4	16.09±0.3	15.1±0.40	17.2±0.69	30.7±0.25	26.4±0.89	24±0.73
5	21±0.35	16.4±0.46	19±0.54	33.2±0.12	29.4±0.82	27±0.83
6	23.74±0.5	17.4±0.29	22.3±0.48	35.3±0.67	32±0.64	30±0.61
7	26.9±0.9	18.72±0.29	22.9±0.55	38.9±0.64	36±0.53	33±0.90
8	32.5±0.5	20.03±0.38	24.3±0.72	41.3±0.59	39±0.42	36±0.41

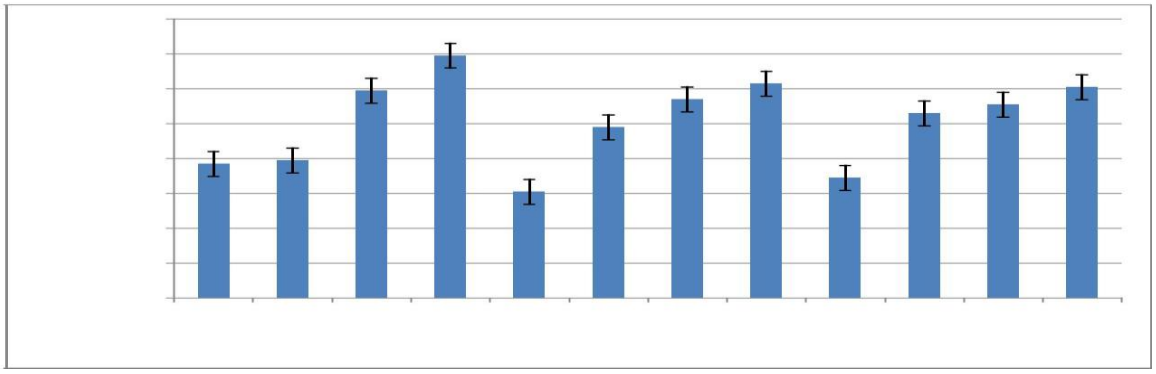
9	35.22±0.3	22.52±0.47	25.12±0.70	47.6±0.49	45±0.17	44±0.71
10	37.48±0.3	24.65±0.39	26.13±0.89	50.6±0.43	48±0.83	46±0.42
24	61.7±0.41	42.06±0.36	51.06±0.35	87±0.11	82±0.19	78±0.49

Percentage Drug Release from Organogel Formulations (F7-F12)

Time (hrs)	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
1	11±0.66	10±0.54	10.7±0.37	10.6±0.41	26.6±0.42	12±0.54
2	15±0.76	14±0.35	17.5±0.45	16.6±0.53	32.4±0.49	18±0.12
3	17±0.44	16±0.54	21±0.38	20.6±0.37	37.4±0.51	21±0.32
4	22±0.62	20±0.84	21±0.38	22.4±0.48	38.1±0.59	23±0.43
5	27±0.41	25±0.94	23.7±0.35	25±0.42	39.2±0.29	26±0.52
6	29.4±0.64	28±0.15	26.3±0.33	26.2±0.39	41.07±0.74	27±0.74
7	35.5±0.58	34±0.19	32.4±0.37	29.3±0.41	42.7±0.78	30±0.79
8	39±0.64	38±0.51	33.5±0.39	31.1±0.50	44.5±0.81	32±0.54
9	41±0.76	40±0.23	39.4±0.43	32.5±0.34	52.68±0.89	33±0.83
10	44±0.85	42±0.11	41.4±0.39	34.3±0.43	55.51±0.39	36±0.19
24	75±0.69	72±0.78	62.14±0.46	68.9±0.43	94.8±0.29	70±0.45

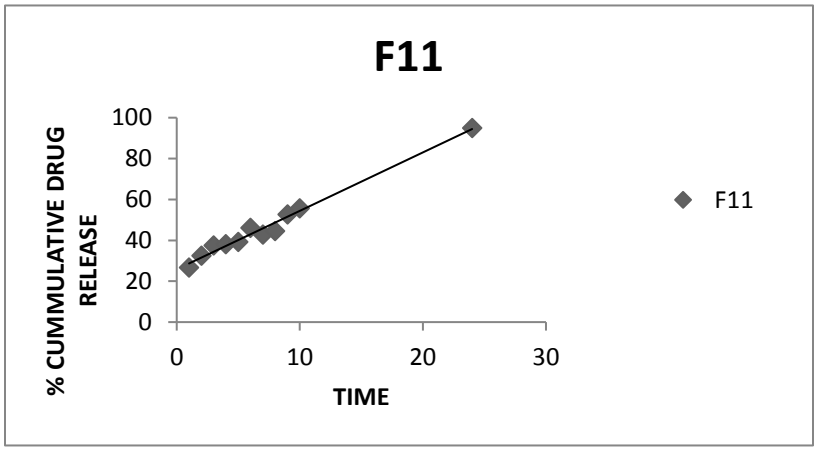
Average of three determination.

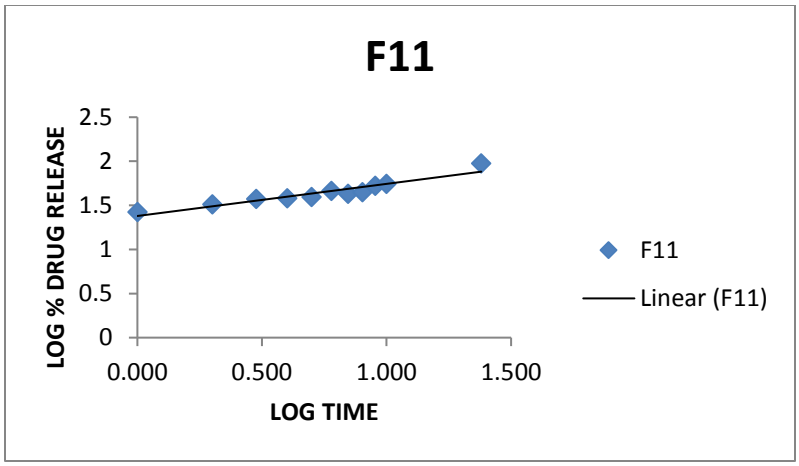
Viscosity of organogel formulations

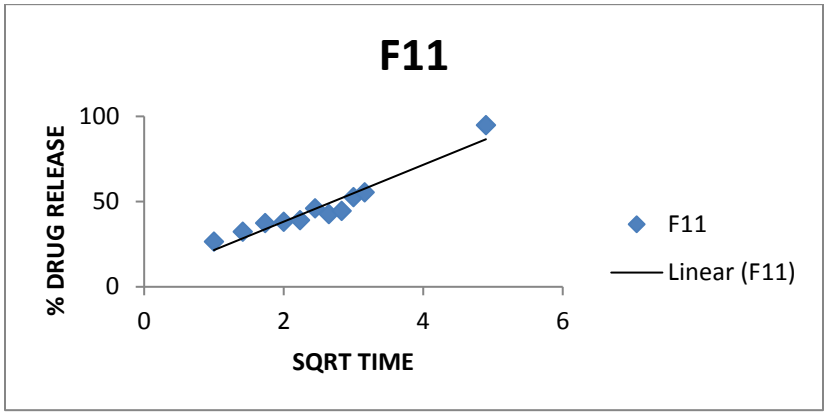


F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11

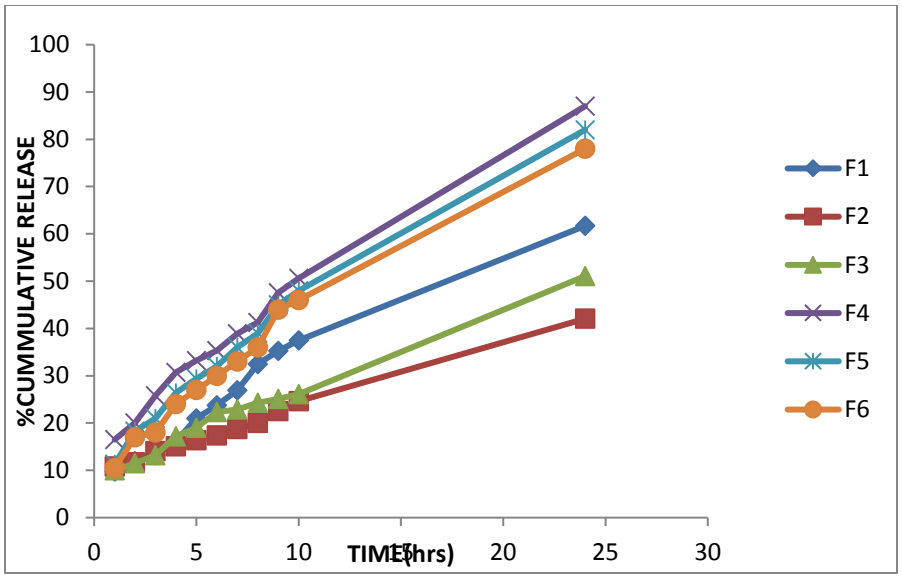
FORMULATIONS KINETICS OF DRUG RELEASE



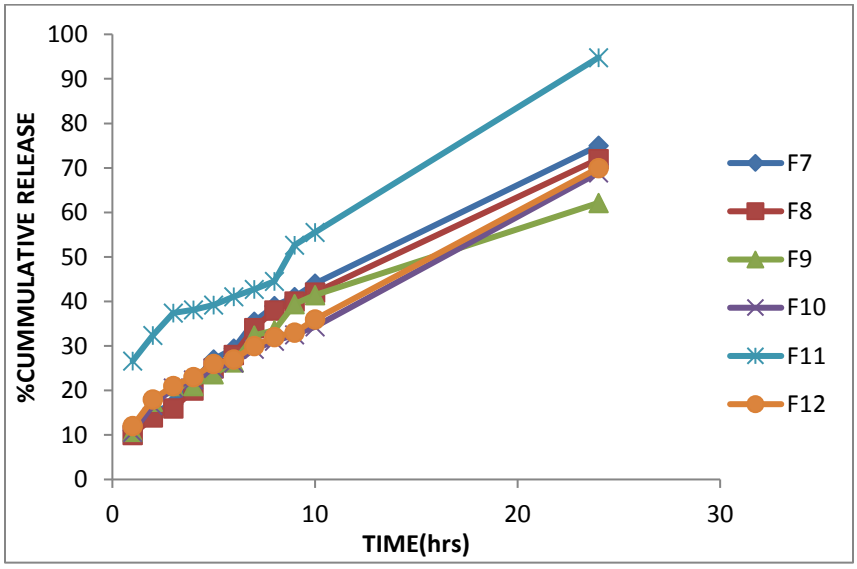




Graphical Representation of Drug Release



. EVALUATION OF PLURONIC LECITHIN ORGANOGEL



CONCLUSION

The present study was to design, develop and evaluate the pluronic lecithin organogel incorporated gel for topical controlled drug delivery of miconazole nitrate for extended release. Miconazole nitrate is easily inactivated by the gastric environment and produce gastric disturbances such as diarrhoea, nausea, abdominal pain and vomiting. The study design focused

on standardization of process parameters and formulation parameters involved in the preparation of miconazole pluronic lecithin organogel by cold method. Carbopol 934 was used as a polymer to exhibit mucoadhesive property and pluronic F 127 used as a thermosensitive polymer, soya lecithin as a wetting agent, methanol and polyethylene glycol-400 as solvents in the oil phase, water as aqueous phase. By compatibility studies it was found there was no interaction between the drug and excipients. The best standardized F11 formulation showed good mucoadhesive force, gelling capacity, pH, drug content and viscosity**.**

The best formulation F11 was incorporated into gels and gels were evaluated for physical parameters and showed controlled release upto 24 hrs. Analysis of drug release mechanism showed that the drug release followed fickian diffusion and the best fitted model were found to be Higuchi.

Stability studies at room temperature revealed that there was no noticeable change in the homogeneity, pH, spreadability, mucoadhesive force, viscosity, drug content and *in vitro* release at the end of three months.

Thus it was concluded that the selected antifungal drug can be developed into organogel. The data in this study support the potential effectiveness of a vaginal gel with mucoadhesive properties to ensure longer residence time in the application site because of prolonged release properties controlled release of the incorporated drug is achieved, suggesting better patient compliance and higher therapeutic efficacy.

CONCLUSION

Based on mathematical data revealed from models, it was concluded that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism, the diffusion exponent ‘n’ values were found to be in the range of 0.36 for the MNZ organogel indicating Fickian diffusion. Thus it was concluded that the selected antifungal drug can be developed into organogel. The data in this study support the potential effectiveness of a vaginal gel with mucoadhesive properties to ensure longer residence time in the application site because of prolonged release properties controlled release of the incorporated drug is achieved, suggesting better patient compliance and higher therapeutic efficacy.

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