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FORMULATION DEVELOPMENT AND EVALUATION OF METRONIDAZOLE CONTAINING THERMOSENSITIVE BIOADHESIVE GEL FOR VAGINAL DRUG DELIVERY

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ABSTRACT

The aim of the present study was to formulate and evaluate metronidazole containing thermosensitive bioadhesive gel for vaginal drug delivery to achieve a better therapeutic efficacy and patient compliance in the treatment for vaginosis. 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 whenstability testing was carried out at 25ºC/60% RH for 3 months.

KEYWORDS

Metronidazole, Thermosensitive, Bioadhesion, Gel and Bacterial vaginosis.

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INTRODUCTION

Traditionally, the vaginal cavity has been used for the delivery of locally acting drugs such as antibacterial, antifungal, antiprotozoal, antiviral, labor-inducing, spermicidal agents, prostaglandins and steroids[1]. Compared with other mucosal tissues, which are of interest for noninvasive drug administration, the vaginaoffers various advantages from a delivery point of view. The delivery system can be localized on the vaginal mucosa for many hours without causing a pronounced irritation unlike most other mucosal absorption membranes, such as the buccal or ocular mucosa. Intravaginal enzymatic activity is comparatively lower in the vagina than in the gastrointestinal tract[2]. Recently,

increased interest in the development of localized drug delivery systems within the vaginal cavity has been shown due to the advantage of localized drug levels, which reduces dosing frequency, drug administration, and side effects[3]. Topically administered agents are generally very well tolerated and systemic side effects can be overcome. Topical therapy is also safe for pregnant and nursing women under medical supervision, and there is no risk of damage to the unborn child. Moreover, a large number of interactions occur with many other drugs, and for this reason oral therapy may not be given to many patients, or it may only be given when absolutely vital, under close medical supervision.

The commercial preparations, such as creams, foams, gels, irrigations and tablets, are known to reside in the vaginal cavity for a relatively short period of time owing to the self- cleaning action of the vaginal tract, and often require multiple daily doses to ensure the desired therapeutic effect. Therefore, vaginal route appears to be highly appropriate for bioadhesive drug delivery systems[4].

By the use of bioadhesive polymers, the intravaginal retention time of drug delivery systems can be significantly prolonged. Consequently, the therapeutic efficacy of locally acting drugs can be improved by their increased availability at the target membrane[2]. Phase change polymers which exhibit sol–gel transition in response to body temperature and prolong the residence time of the dosage form in the vagina can be used along with bioadhesive polymers to improve the therapeutic efficacy and patient compliance[1].

The use of syringe applicators is probably the most efficient way for intravaginal administration of semisolid formulations; therefore, the formulation has to display a sufficiently low viscosity to be used in a syringe. Once administered, the viscosity of the gel or cream should be as high as possible in order to avoid a premature and inconvenient outflow of the formulation. As a result, *in situ* gelling polymers are in high demand for vaginal delivery systems. Overall, parameters needed for an *in situ* gelation are change in temperature, change in pH, increase in ionic strength, or access of oxygen. Poloxamers are polymers, which exhibit increase in viscosity at body temperature and have already been tested in vaginal delivery systems *in vivo*[2].

MATERIAL AND METHODS

Preparation of thermosensitive bioadhesive gel

Thermosensitive bioadhesive gel was prepared by cold method.

Metronidazole 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.

EVALUATION OF THERMOSENSITIVE BIOADHESIVE GEL

Evaluation of thermosensitivity

Gelation temperature

Gelation time

Solution was taken ina 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.

Determination of drug content

The prepared formulations were analyzed for drug content by taking 1 mL of gel in 10mL volumetric flask and the volume was adjusted with pH 4 citrate phosphate buffer. From the above solution 0.1mL was pipetted out in a 10mL volumetric flask and volume was adjusted with pH 4 citrate phosphate buffer. Absorbance was measured at 319.9nm.

Viscosity determination

The viscosity of all the formulation were measured by using Brookfield DVII+ viscometer using spindle no: 94 at 50rpm. Viscosity was measured at 37±1ºC using a thermo stated water jacket.

In vitro bioadhesion studies of thermosensitive bioadhesive gel[3]

The bioadhesion strength of the formulations was evaluated by an *in vitro* method reported by Varsha[23]. Sheep vaginal mucosa was used as model membrane. The mucoadhesive potential of each formulation was determined by measuring a force

required to detach the formulation from vaginal mucosal tissue. A section of sheep vaginal mucosa was fixed on each of two glass slides using cyanoacrylate adhesive. 0.1mL of formulation was placed on first slide. While another slide with mucosal section attached to a pan was placed in inverted position above the first slide. Both the slides with gel formulation between them held in contact with each other, for 2 mins and 5mins using preload of 50g to ensure intimate contact between them. Then weight was kept rising in pan until th mucosa on each slides get detached from each other. The mucoadhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

Mucoadhesive Strength (dynes/cm^2) = mg/A, Where, m = weight required for detachment in gram,

g= Acceleration due to gravity (980cm/s^2), A = Area of mucosa exposed.

The vaginal mucosa was changed for each measurement.

***In vitro* drug release study**

The *in vitro* release study of metronidazole 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 pH 5.5 citrate phosphate buffer 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 metronidazole was analyzed at 319.9nm by UV spectrophotometer.

Mathematical model fitting of obtained drug release data

The release data were fitted into various mathematical models using PCP.Disso-V2.08 software to know which mathematical model will best fit the obtained release profile. The parameters like 'n' the time exponent 'k' the release rate constant and 'R' the regression co-efficient were determined to know the release mechanisms. The various models studied were

First order

Zero order

Matrix model

Peppas model

Hixon- crowell model

Peppas model fitting

The data obtained from *in vitro* release studies best fitted fit into Peppas model.

Koresmeyer-Peppas equation

$M_t / M_\infty = 1 - A (exp-kt)$ ———— (1)

$\text{Log} (1 - M_t / M_\infty) = \log A - kt/2.303$ ———— (2)

Where, M_t = Amount of drugs released at time t

M_∞= Total amount of drug loaded

K = Diffusion constant/Release rate constant A = intercepts

The value of 'n' determined from Korsmeyer-Peppas equation if found to be below 0.5, it indicates that the drug release from the formulation follows Fickian diffusion, if 'n' value is between 0.5-0.85, indicates Non-Fickian diffusion or anomalous mechanism (relaxation controlled) and if 'n'value is above 0.89, indicates Super case II transport.

Stability studies

Drug decomposition or degradation occurs during storage, because of chemical alteration of the active ingredients or due to product instability, leading to lower concentration of the drug in the dosage form, hence the stability of pharmaceutical preparation need to be evaluated. The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and relative humidity (RH) conditions.

A drug formulation is said to be stable if it fulfills the following requirements:

It contains at least 90% of the stated active ingredient

It contains effective concentration of the added preservatives, if any

It does not exhibit discoloration or precipitation, nor develops foul odour

It does not develop irritation or toxicity

Optimized formulation was selected for stability studies. Formulations were packed in screw capped bottles and studies were carried out for 90 days by keeping at 25 ± 2°C and 60 ± 5% RH.

Samples were withdrawn on 15th, 45th, 60th and 90th day and checked for changes in physical appearance and drug content spectrophotometrically at 319.9 nm.

***In vitro* bioadhesion studies**

Bioadhesive strength was determined in terms of detachment stress. Coadhesive 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. 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.

***IN VITRO* DRUG RELEASE STUDY**

The *in vitro* drug release of metronidazole form the formulations are reported in Table No.3 and their profile in Figure No.3.

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 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[28]. The increase in gel strength and/ or molecular interaction between the drug and polymers could also retard release of the drug.

Release kinetics

The release pattern of the drug from the formulations was obtained by plotting Log Mt/M∞ versus Log t. The data obtained is given in Table No.4 to Table No.6 and the respective kinetic plots are shown in Figures No.4 to Figure No.6.

Table No.1: Formulation chart of thermosensitive bioadhesive gel

S.No	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Pluronic- F127 (%w/v)	20	20	20	20	20	20	20	20	20
2	HPMC (%w/v)	0.2	-	0.2	-	-	-	-	-	-
3	SCMC (%w/v)	-	0.2	0.2	-	-	-	-	-	-
4	Carbopol (%w/v)			-	0.2	0.4			0.2	0.4
5	Polycarbophil(%w/v)	-	-	-	-	-	0.2	0.4	0.2	0.4
6	Metronidazole(%w/v)	1	1	1	1	1	1	1	1	1
7	pH 4 citrate phosphatebuffer	q.s to 20mL	q.s to 20mL	q.s to 20mL	q.s to 20mL	q.s to 20mL	q.s to 20mL	q.s to 20mL	q.s to 20mL	q.s to 20mL

Table No.2: Bioadhesive strength of Formulations F1-F9

S.No	Formulation code	Bioadhesive strength (dynes/cm^2) Mean ± SD	
		2mins	5mins
1	F1	1068.1 ± 3.78	1470.8 ± 2.36
2	F2	1905.3 ± 4.01	2177.1 ± 3.78
3	F3	2364.2 ± 2.65	2940.1 ± 4.9
4	F4	3277.0 ± 2.89	3920.2 ± 3.97
5	F5	3800.0 ± 4.12	4028.3 ± 2.54
6	F6	3214.2 ± 3.96	3484.5 ± 4.35
7	F7	3270.5 ± 2.87	3593.2 ± 2.86
8	F8	4120.7 ± 3.61	4464.5 ± 4.20
9	F9	4693.1 ± 4.25	4900.5 ± 3.91

*Standard deviation, mean n=3

Table No.3: Cumulative % drug release from formulations F1-F9

S.No	Time in hrs	Cumulative % drug release								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	0.5	28.1 ± 4.62	22.6 ± 4.13	22.6 ± 3.87	25.2 ± 5.71	23.5 ± 3.67	24.8 ± 3.72	22.7 ± 5.1	18.8 ± 4.69	18.0 ± 3.9
2	1	54.6 ± 5.01	35.3 ± 4.01	35.3 ± 4.26	38.6 ± 5.64	35.3 ± 3.04	37.3 ± 4.11	35.0 ± 5.23	27.3 ± 3.67	22.7 ± 4.81
3	2	78.5 ± 4.1	46.7 ± 4.68	45.6 ± 3.98	52.9 ± 4.1	49.8 ± 4.25	56.1 ± 4.26	45.0 ± 4.36	36.5 ± 4.16	34.9 ± 4.56
4	3	89.0 ± 3.83	56.9 ± 3.71	58.8 ± 4.11	64.6 ± 5.02	58.8 ± 2.69	67.5 ± 2.89	57.4 ± 4.05	46.8 ± 5.03	44.6 ± 5.13
5	4	91.2 ± 3.04	69.8 ± 2.14	68.1 ± 3.58	73.8 ± 4.23	71.4 ± 5.06	78.6 ± 3.67	65.2 ± 3.89	55.4 ± 2.79	55.3 ± 4.03
6	5	90.4 ± 2.15	79.4 ± 2.01	79.4 ± 2.79	78.7 ± 3.97	76.4 ± 4.15	80.7 ± 5.04	74.2 ± 4.72	67.5 ± 3.58	63.8 ± 2.67
7	6	90.1 ± 2.36	85.1 ± 2.67	87.0 ± 2.87	81.7 ± 2.79	79.6 ± 3.76	83.2 ± 2.97	81.0 ± 2.96	79.2 ± 4.24	75.1 ± 3.09
8	8	--	84.2 ± 2.39	89.0 ± 3.45	87.1 ± 3.41	81.9 ± 4.03	89.1 ± 3.5	84.1 ± 5.02	84.0 ± 3.25	81.2 ± 3.96
9	10	--	--	--	87.0 ± 2.59	83.5 ± 2.97	89.6 ± 4.24	83.7 ± 4.33	88.8 ± 2.16	84.8 ± 2.74
10	12	--	--	--	--	--	--	--	93.6 ± 3.26	87.1 ± 2.69

Table No.4: Release kinetic data of formulations F1-F3

Time in hrs	Log t	Mt/M∞			Log Mt/M∞		
		F1	F2	F3	F1	F2	F3
1	0.000	0.546	0.353	0.353	0.263	0.452	0.452
2	0.301	0.785	0.467	0.456	0.105	0.331	0.341
3	0.477	0.890	0.569	0.588	0.051	0.245	0.231
4	0.602	0.912	0.698	0.681	0.040	0.156	0.167
5	0.699	0.904	0.794	0.794	0.044	0.100	0.100
6	0.778	0.901	0.851	0.870	0.045	0.070	0.060
8	0.903	--	0.842	0.890	--	0.075	0.051

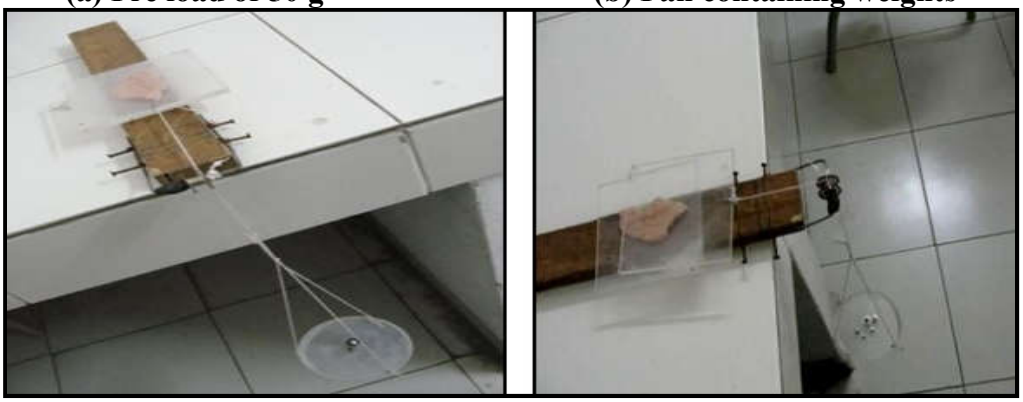
Table No.5: Release kinetic data of formulations F4-F6

Time in hrs	Log t	Mt/M∞			Log Mt/M∞		
		F4	F5	F6	F4	F5	F6
1	0.000	0.386	0.353	0.373	0.413	0.452	0.428
2	0.301	0.529	0.498	0.561	0.277	0.303	0.251
3	0.477	0.646	0.588	0.675	0.190	0.231	0.171
4	0.602	0.738	0.714	0.786	0.132	0.146	0.105
5	0.699	0.787	0.764	0.807	0.104	0.117	0.093
6	0.778	0.817	0.796	0.832	0.088	0.099	0.080
8	0.903	0.871	0.819	0.891	0.060	0.087	0.050
10	1.000	0.870	0.835	0.896	0.060	0.078	0.048

Table No.6: Release kinetic data of formulations F7-F9

Time in hrs	Log t	Mt/M∞			Log Mt/M∞		
		F7	F8	F9	F7	F8	F9
1	0.0	0.350	0.273	0.227	0.456	0.564	0.644
2	0.3	0.450	0.365	0.349	0.347	0.438	0.457
3	0.5	0.574	0.468	0.446	0.241	0.330	0.351
4	0.6	0.652	0.554	0.553	0.186	0.256	0.257
5	0.7	0.742	0.675	0.638	0.130	0.171	0.195
6	0.8	0.810	0.792	0.751	0.092	0.101	0.124
8	0.9	0.841	0.84	0.812	0.075	0.076	0.090
10	1.0	0.837	0.888	0.848	0.077	0.052	0.072
12	1.1	--	0.936	0.871	--	0.029	0.060

(a) Pre load of 50 g **(b) Pan containing weights**



(c) Pan containing weights (another view) **(d) Detachment of mucosa**

Figure No.1: Assembly used for the mucoadhesive strength measurement

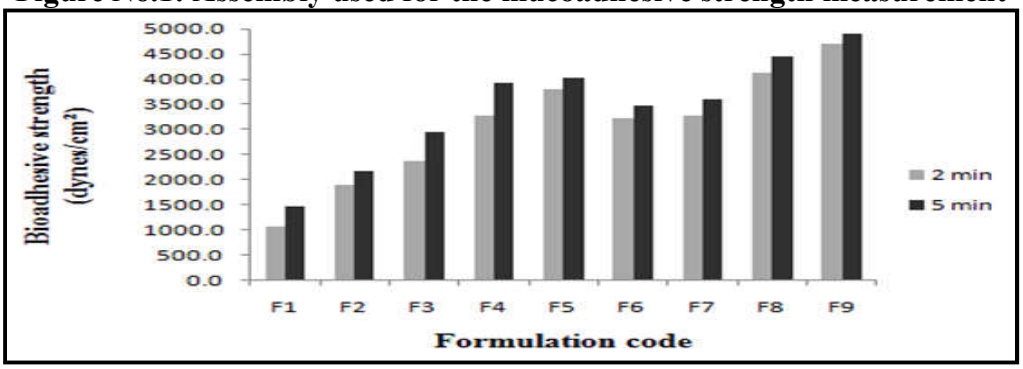


Figure No.2: Bioadhesive strength of formulations F1-F9

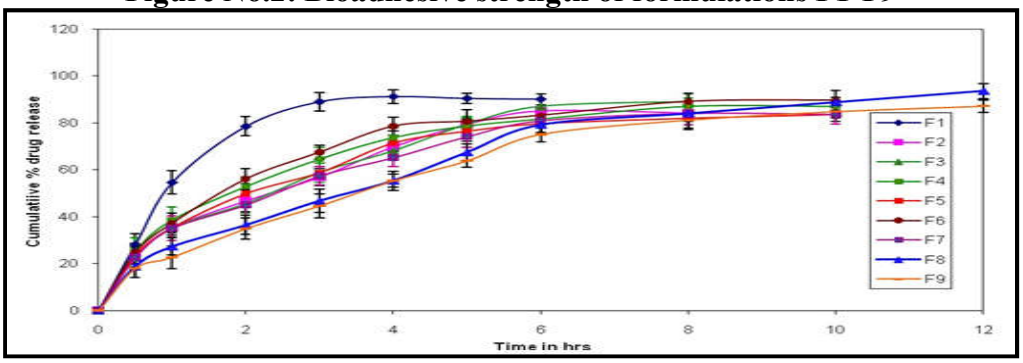


Figure No.3: *In vitro* drug release of formulations

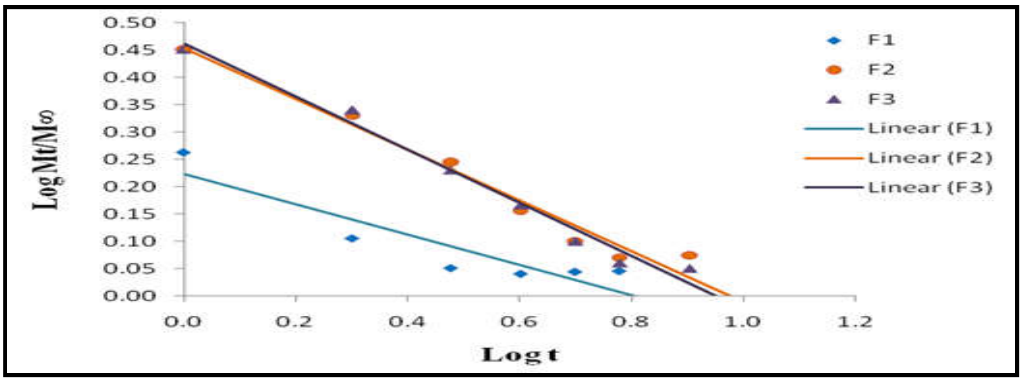


Figure No.4: Release kinetics of Formulations F1, F2, F3

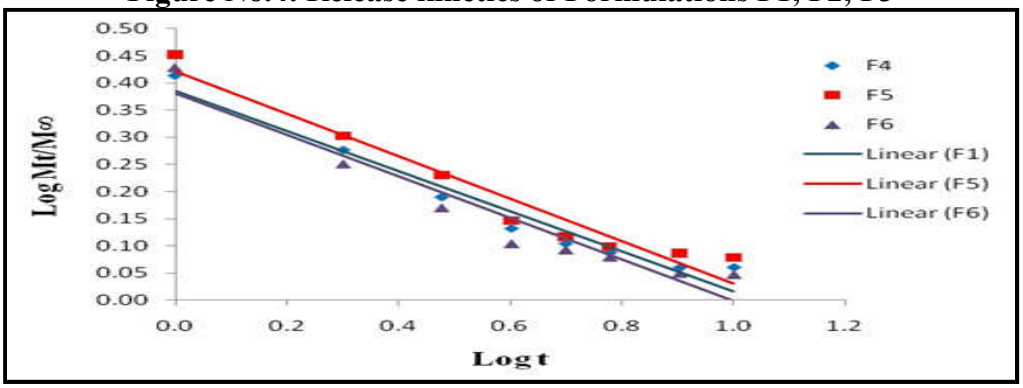


Figure No.5: Release kinetics of formulations F4, F5, F6

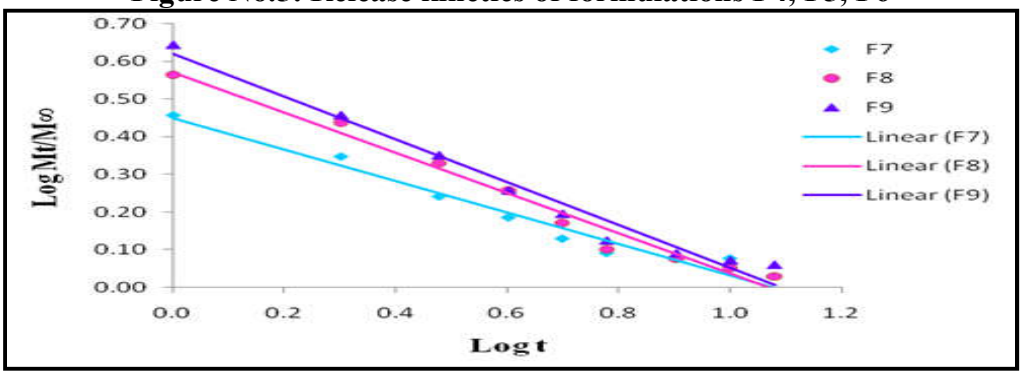


Figure No.6: Release kinetics of formulations F7, F8, F9

SUMMARY AND CONCLUSION

The objective of this study was to prepare a thermosensitive bioadhesive gel containing metronidazole for vaginal drug delivery for the treatment of bacterial vaginosis. Pluronic F127 was used as temperature sensitive polymer along with bioadhesive polymersto increase the residence time of drug at the site of action. Also the bioadhesive polymers used could sustain the release of the drug from the formulation. Prepared formulations were characterized by FTIR to study drug polymer compatibility. The formulations were evaluated for various parameters like gelation temperature, gelation time, drug content, viscosity, bioadhesive strength and *in vitro* drug release. Stability studies were carried at 25ºC/60% RH out for optimized formulation. The prepared formulations were stable and in solution state at or below room temperature. While it transformed into gel at body temperature (37ºC) and released drugfor prolonged time.

The following conclusions were obtained drawn from the results obtained.

From the FTIR spectra, it was observed that similar characteristic peaks appear with minor differences for the drug and their formulations. Hence, it appeared that therewas no interaction between drug and the polymer used.

From the results of drug content determination, it can be inferred that drug content was satisfactory and the drug was uniformly distributed in all the formulations.

Bioadhesive strength of the formulations increased with increase in polymer concentration. Carbopol showed maximum bioadhesive strength followed by polycarbophil and SCMC, whereas HPMC had least bioadhesive property.

From the *in vitro* drug release data, it was observed that the concentration of bioadhesive polymers affected the drug release from the formulations. The release rate of drug was found to decrease with increase in the concentration of the bioadhesive polymers. Formulation F1 containing HPMC showed burst release of about 91% in 4^{th} hour, whereas F2 and F3 showed maximum release of about 84% and 89% respectively at 6^{th} hour. Formulations F4, F5, F6 and F7 showed maximum release of about 87%, 83%, 89% and 83% respectively at 10^{th} hour. Formulations containing combination of polycarbophil and carbopol F8 and F9 showed maximum release of 93% and 87% respectively at 12^{th} hour.

The result of stability studies carried out on the optimized formulation, F8 indicated that after 90 days there was no significant change in the drug content when stored at recommended accelerated storage conditions i.e., 25ºC/60% RH.

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.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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