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FORMULATION AND EVALUATION OF METRONIDAZOLE MICROSPHERES FOR VAGINAL INFECTIONS

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ABSTRACT

Current research is about formulating microspheres containing metronidazole for vaginal infections for local action in bioadhesive polymers will improves patient compliance as well as disperses the drug throughout the vaginal cavity. The microspheres were prepared by solvent evaporation technique by using various proportions of eudragit RS and eudragit RL polymers. The microspheres were characterised for various parameters like FT-IR, DSC, % yield, entrapment efficiency, particle size determination, dissolution of microspheres and SEM, compatible. The % yield of microspheres ranged from 83.15 and 92.78. The encapsulation efficiency of metronidazole was in the range 63.82 to 72.26. The size of the microspheres ranged between 194 to 674µm. The microsphere formulation and microsphere loaded gel formulation follows Higuchi rule. The stability studies of microspheres was conducted at 4°C to 8°C and room temperature.

KEYWORDS

Bioadhesive, Microspheres, Metronidazole and Vagina infections.

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INTRODUCTON

Drug delivery by the conventional route was restricted to oral for systemic action and topical for local action. Self-administration of medicaments like inhalations, suppositories, injections took place in mid-1980s. The other non-oral routes like transdermal, intranasal, intra-vaginal routes were further developed provided self-administration. These non-oral administration which are advanced after the years gained lot of importance, since they avoided hepatic metabolism[1].

Presently available marketed vaginal formulations are having limitations which can be surpassed by inclusion of bioadhesive polymers. This improves patient compliance as well as disperses the drug throughout the vaginal cavity. Current research is about formulating a dosage form containing metronidazole for vaginal infections for local action. The drugs loaded microspheres was formulated and incorporated into the bio-adhesive carbopol gel which acts as a carrier.

Candidal infection is most commonly caused by *Candida albicans*, a fungal organism. This is a common of the regular flora of the vagina of reproductive-age women, but makes over 90% of cases of symptomatic vaginal candidiasis. When candidal infection is more by other *Candida* species, *Candida glabrata and Candida tropicalis,* it is mostly resistant to treatment. The use of a single drug would be effective in more than one type of infections caused by vaginitis. Most of these vaginal infections particularly respond to pathogen-specific treatments. Thus, it's very difficult for a one/single drug to treat the all sorts of frequent infections. In such case combination therapy may provide efficient treatment[2].

MATERIAL AND METHODS

Metronidazole was the gift sample obtained from KAPL (Bangalore). Eudragit RS and RL from Evonik Industries, Mumbai, Magnesium stearate and Carbopol 934P from lobachemie (Mumbai). All the other chemicals and solvents used in the experiments were of analytical grade.

Preparation of Microspheres

The microspheres were formulated by solvent-evaporation method. The various proportions of eudragit RS-100 polymer were used to prepare microspheres. Weighed amount of eudragit RS-100 polymer was dissolved in acetone. Metronidazole was added to the solution of polymer along with the magnesium stearate which acts as a dispersion agent. The resultant dispersion was transferred to a beaker comprised of mixture of liquid paraffin and n-hexane. The above suspension was stirred at 1200RPM for 5 hrs using a homogenizer. The stirring was continued for another 3 hrs at 1000rpm or until the complete evaporation of acetone. After the displacement of the acetone, the microspheres collected by vacuum filtration. Thus, obtained microspheres then washed several times by 50ml of n-hexane and dried at room temperature (25°C) for 24hrs. The Eudragit RL-100 microspheres were also prepared in the similar manner[3].

Evaluation of microspheres

Percentage yield

The percentage yield of prepared microspheres were calculated from the below equation.

Yield % = (M/Mo) x 100

Where: M is the weight of microspheres

Mo is called the total expected weight of drugs and polymer[4].

Estimation of Entrapment Efficiency

Encapsulation Efficiency = (Actual drug content/Theoretical drug content) x 100

Particle Size Determination

The size was established by using a stage micrometer and eyepiece 86 (Magnus MLX-DX, Olympus, India). The mean particle size was ascertained by randomly selecting around three hundred particles per batch.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was used to study surface characteristics, and investigate the morphology of surface. The sample was dried and coated with a gold ion for 5 to 6 min[5].

In vitro Release Studies of Microspheres

The USP 'basket type' dissolution test apparatus was used to investigate release of drugs. Microspheres were placed into the basket. The dissolution chamber was filled with 900mL of SVF of pH 394.7 at 100rpm and with temperature of 37 ± 1°C. At one hour of the time intervals, 5mL of the sample were withdrawn and replaced by fresh pre-warmed SVF as dissolution medium. Samples were analyzed spectrophotometrically at 320nm for metronidazole.

Kinetics of drug release

To study the mechanism and kinetics of drugs release the formulations were analyzed by using PCP-Disso-V2 software.

Differential Scanning Calorimetry (DSC)

The DSC of pure drug metronidazole and along with drug-loaded microspheres were conducted by heating at a rate 10°C/min. The samples were hermetically sealed in an aluminium crucible. Nitrogen gases were purged at a rate of 10ml/min. The DSC of metronidazole, and the formulation F1 and F3 were shown in Figure No: 7-10.

EVALUATION OF MICROSPHERES LOADED GELS

Screening of anti-bacterial activity

Determination of the Minimum Inhibitory Concentration (MIC) and zone of inhibition was carried out for formulation FM1. For *in vitro* antibacterial activity against gram positive bacterial strains *Staphylococcus aureus, Bacillus subtilis;* and two gram negative bacterial strains *Escherichia coli, Klebsiella pneumonia* were used. For *in vitro* antifungal activity against *A. niger, Candida tropicalis, Candida albicans and Penicilliumnotatum* were used[6,7].

Screening of Antifungal Activity

Fluconazole was used as standard drug. The sterile Saboutaud's nutrient Agar Media was cooled to 45°C with stirring Fungal culture was inoculated to the media under aseptic condition. Media was poured in to the Petri plates under aseptic condition and kept the plates for solidification 1 h. In every plate cups of 6mm diameter bores were done and microspheres loaded gels was incorporated. All the plates were kept in incubator at 27° C for 48 h. After 48 h incubation, zone of inhibition was measured.

Determination of pH

The pH of the bioadhesive gel FMI was analyzed by a digital pH meter. The electrode was immersed in to liposomal gel and microsphere loaded gel formulation and constant reading was noted.

Bioadhesive strength measurement of gel

Detachment stress (dyne/cm²) = m.g /A

Where:

M: the weight added to the balance in gram

G: acceleration due to gravity

A: area of tissue exposed[8].

Viscosity determination of gel

The T-bar spindle (T95) was utilized to determine the viscosity of the FM1. The average of three readings taken in one minute was noted as the viscosity of gels[9].

Spread ability

The spread ability of the FM1 gel formulation was studied by measuring diameter of 1gm gel between horizontal plates after 1 minute[10].

Rabbit vaginal irritation test

Eighteen female rabbits were allocated into three category containing six rabbits in one category. Animals in category II and category III received 1ml of liposomal gel and microsphere loaded gel respectively via intra-vaginal route for ten continuous days. The rabbits were held in a supine position. The intra-vaginal administration of 1ml of liposomal gel and microsphere loaded gel was done by incorporated tuberculin syringes at a depth of 8cm in to vaginal cavity. The gel was displaced at depth differing 8-10cm. Rabbits were individually observed daily for clinical signs (redness, genital swelling, vaginal bleeding including discharge from vagina). Rabbits were sacrificed and were inspected macroscopically for all clinical signs. The vaginal tissues were removed and were fixed in 10% neutral-buffered formalin for microscopic examination. The vaginal tissues were implanted in paraffin, sectioned at a thickness of 4-6μm and stained with hematoxylin and eosin (H and E) and investigated by light microscopy.

RESULTS AND DISCUSSION

The microspheres were formulated by solvent-evaporation method. Metronidazole showed maximum absorption at 320nm by UV simultaneous estimation method with good reproducibility. The linearity for metronidazole was found in the range 2-14μg/ml with the regression coefficient (r^2) 0.998. The interaction study in between drug and polymer are evaluated by FT-IR Spectrometer. It was evident that drug and excipient were compatible. The melting thermo grams of F1 and F6 showed that there was no drug to polymer interactions. Shown in Figures No.4-7. Entrapment efficiency microspheres ranged from 83.15 and 92.78. The

size of the microspheres ranged between 194 to 674µm. SEM photographs of eudragit polymers microspheres were spherical with rough surface. The release studies of the microsphere (F1-F6) showed that with increased concentration in the polymer concentration the drugs release was found to be decreased. (Figure No.12-14). It was noticed that more than 68% of metronidazole was released over the period of 10 h from microsphere loaded gel. (Figure No.14-15). All the microsphere formulation and microsphere loaded gel formulation follows higuchi rule. (Table No.6). The stability studies indicated that the all the selected formulations F1 was stable during the period. The selected formulation did not show more deviation in the entrapment efficiency and in vitro release after 60 days when stored at RT.

Table No.1: Melting point of metronidazole

S.No	Drug Name	Description	Melting Point		Solubility
1	Metronidazole	white to pale-yellow crystals	161ºC	7.2mg/ml	6.3mg/ml

Table No.2: Entrapment efficiency and % yield of metronidazole

S.No	Formulation code	Drug: polymer ratio	Entrapment efficiency* Metronidazole	% yield
1	ERL1	1:1	63.82±1.10	91.15
2	ERL2	1:2	66.90±1.34	83.15
3	ERL3	1:4	69.82±1.20	89.09
4	ERSI	1:1	64.28±1.08	86.27
5	ERS2	1:2	67.02±1.21	87.38
6	ERS3	1:4	72.26±1.64	92.78

Table No.3: Kinetic data of Metronidazole microspheres

S.No	Formulation code	KINETIC MODELS							Mechanism of release
		Zero-order Plot		First-order plot		Higuchi plot	Peppas		
		K0	R^2	K	R^2	R^2	n	R^2	
1	F1	7.25	0.8392	-0.098	0.9583	0.9716	0.332	0.9593	fickian diffusion
2	F2	7.24	0.8933	-0.079	0.9827	0.9901	0.412	0.9747	fickian diffusion
3	F3	3.91	0.9202	-0.024	0.9560	0.9749	0.4187	0.9208	fickian diffusion
4	F4	7.37	0.889	-0.087	0.9753	0.9813	0.385	0.9753	fickian diffusion
5	F5	6.44	0.9325	-0.053	0.9856	0.9899	0.4839	0.9657	fickian diffusion
6	F6	3.75	0.9471	-0.022	0.968	0.9687	0.4811	0.9238	fickian diffusion

Drug polymer compatibility studies by *FT-IR spectrum*

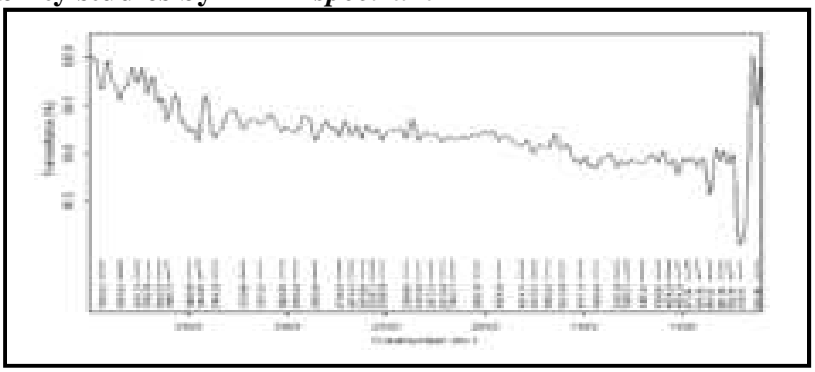

Figure No.1: Metronidazole pure drug

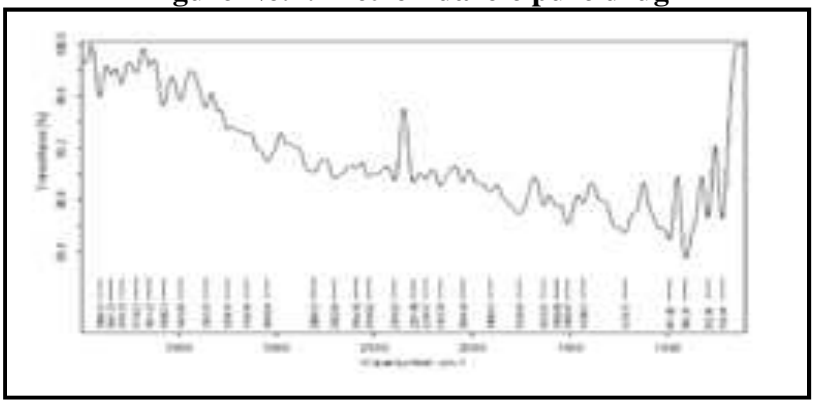

Figure No.2: Metronidazole and Eudragit RS 100

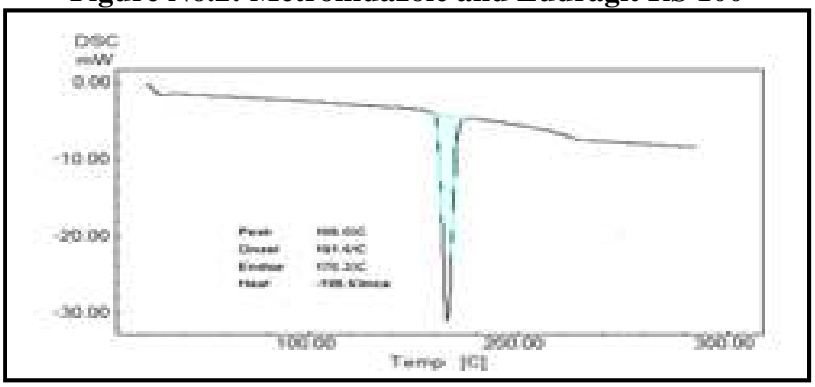

Figure No.3: DSC of pure drug Metronidazole

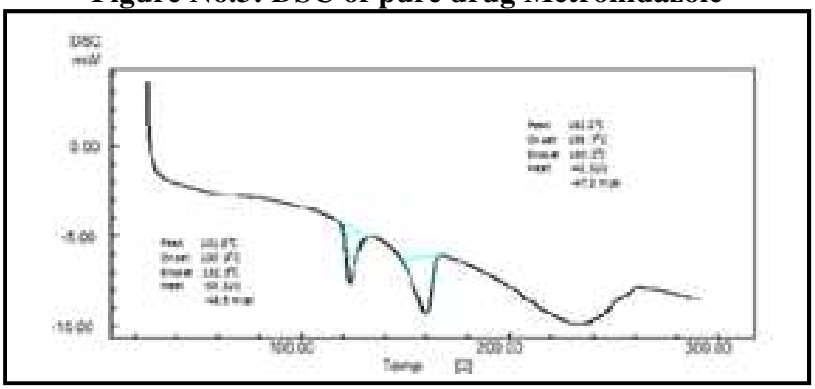

Figure No.4: DSC of Formulation F1

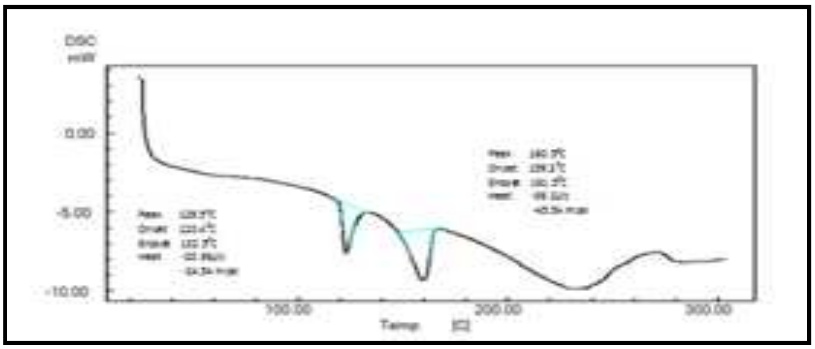

Figure No.5: DSC of Formulation F6

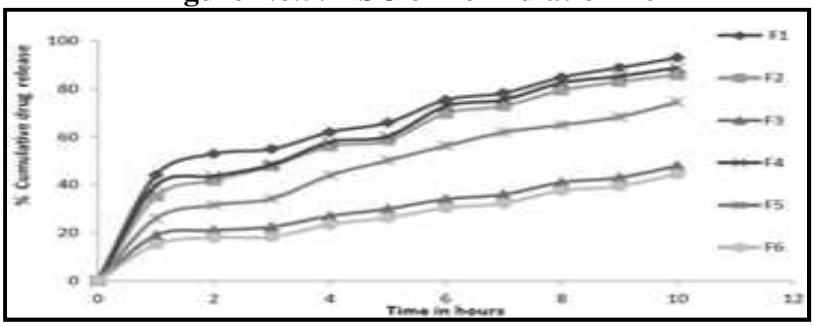

Figure No.6: *In vitro* release of metronidazole from F1-F6

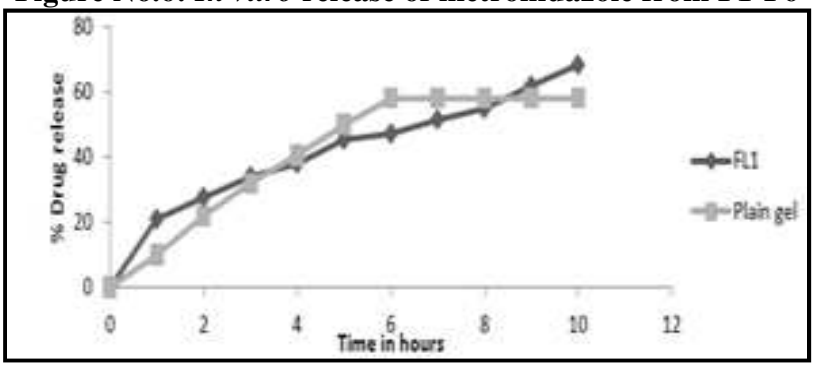

Figure No.7: *In vitro* release of metronidazole FM1 with control gel

CONCLUSION

In the present research work, metronidazole drug is used to treat vaginal infections. The gel acted as a carrier of these liposomes and microspheres and also helped in the adhesion of gel onto the vaginal mucosa for prolonged period which improved in better therapeutic effect. *In vitro* anti-microbial activity, pH, Spread ability of gels, and *in vitro* drug diffusion studies showed that these bioadhesive vaginal gels of metronidazole will deliver the drugs for extended time. The new formulations were therapeutically more efficacious, as it shows extended release of drugs. Therefore, it can be concluded that the some of the chosen formulation complies the mandatory essentials for a stable vaginal formulation. The developed formulation contains both antibacterial and antifungal drugs like metronidazole and liposomes in muco-adhesive gels. Thus, developed method is the substitute to traditional vaginal gels as it reducing application of several formulations vaginal infections.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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