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Research Article

Formulation and evaluation of once daily mucoahdesive vaginal tablet of metronidazole

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ABSTRACT

The antitrichomonal imidazole derivative metronidazole is employed locally for the treatment of genitourinary tract bacterial infections and is formulated as creams, foams, tablets, or irrigations. In this study, a new dosage form was developed by using carbopol 934P (CP 934P) and hydroxypropylmethylcellulose (HPMC K4M) as bioadhesive polymers in different ratios. The mucoadhesive strength was evaluated by detachment force measurement from porcine vaginal mucosal membrane. The strong interaction between the polymer and the mucus lining of the tissue helps increase the contact time and permits the localization. CP 934P showed maximum bioadhesion and was directly proportional to its total content. The formulations were tested for their swelling behavior using agar gel plate. The swelling index increased with an increase in the content of HPMC. In vitro and in situ release studies were also carried out using porcine vaginal mucus membrane. The biopharmaceutical studies showed that the dosage form was able to release drug for 24 h. The similarity factor f_2 showed no significant difference between fresh and aged sample.

Keywords: Metronidazole; Bioadhesion; Controlled Release; Vaginal Tablet; HPMC K4M; Carbopol 934P.

INTRODUCTION

Improvements in the technology to develop the controlled drug delivery system (CDDS) have been ongoing for decades, with the formulation ranging from simple matrix, or reservoir type devices to transdermals, osmotic pumps, nanoparticles, liposomes, microspheres, bioadhesives, implants and more (Singla A.K. et al., 2000).

Prolonged contact time of a drug with a body tissue, through the use of bioadhesive polyermer, can significantly improve the performance of many drugs (Sakagami M et al., 2002). Pharmaceutical aspects of mucoadhesion have been the subject of great interest during recent years because mucoadhesion could be solution for bioavailability problems that result from a too short length of stay of the pharmaceutical dosage form at the site of application (Valenta C et al., 2001). It was hoped that bioadhesive vaginal dosage forms release the active ingredients slowly, so that the vagina would not be immediately exposed to entire dose of active ingredient thereby minimizing its possible toxic effects on the vaginal epithelium (Zaneveld L.J.D et al., 2001).

Obviously, the aim of present study was to develop once daily mucoadhesive vaginal delivery system for the drug metronidazole which is designed to improve the adhesion to the vagina in order to prolong the residence time and consequently to obtain a long therapeutic concentrations at the site of infection. Formulated vaginal tablets were evaluated for bioadhesive properties, in vitro and in situ release studies, in vitro swelling studies and stability studies.

EXPERIMENTAL

Materials

Metronidazole was a gift sample from JB Chemicals and Pharmaceuticals Ltd., Mumbai. CP 934P and HPMC K4M were obtained as gift samples from Ind—Swift Laboratories, Chandigarh, India and Colorcon Asia Pvt. Lt., Goa, India respectively. Potassium dihydrogen phosphate (E—Merk Ltd.), agar powder (Hi—Media Labs Pvt. Ltd.), magnesium stearate (SD Fine chemicals Ltd.) were obtained from commercial sources. All reagents and chemicals used were of analytical grade.

Preparation of Mucoadhesive Vaginal Tablets

The vaginal bioadhesive tablets of metronidazole with CP 934P and other excipients like HPMC K4M, magnesium stearate were prepared by direct compression method. The required weighed amount of drug , other materials were sieved through # 80 mesh and mixed for 5 min as per table 1 and compressed using double

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Table 1: Composition of different investigational mucoadhesive vaginal tablets

Investigational formulation	Drug (%)	Polymer ratio (%)	
		CP 934P	HPMC K4M
Batch A	10	80	0
Batch B	10	60	20
Batch C	10	40	40
Batch D	10	20	60
Batch E	10	0	80

CP 934P—Carbopol 934 P; HPMC K4M—Hydroxypropylmethylcellulose K4M

punch tablet machine (Cadmach Machines, Ahmedabad) fitted with flat—faced punches.

In Situ Bioadhesive Strength Studies

According to previously published method (Gupta A et al., 1992) investigation of the bioadhesive strength of tablests was done with a modified physical balance (Precision Balance Works, Varanasi). The vaginas of freshly sacrified porcine were removed and the mucus membrane (2 mm thickness) was excised by removing the underlying connective tissue and thoroughly washed with phosphate buffer (pH 4.0 ± 0.1) and glued over the protrusion of Teflon block by using cynoacrylate adhesive and placed in to the jacketed glass beaker filled with phosphate buffer and maintained at 37°C. Teflon cylinder along with tablet to be tested was lowered thereby subjected to contact with mucus membrane. After 3 min the weights were slowly increased by adding water drop by drop to the PVC beaker until the tablet just detached from membrane surface. The excess weight on the left—hand side of balance was taken as force required to detach the tablet from the membrane.

In Vitro Swelling Studies

The swelling rate of vaginal bioadhesive tablets were evaluated by using 1% w/v agar gel plate method (Machida H et al., 1979). Tablets formulations were placed on gel surface in petridish , which were placed in an incubator and allowed to swell at 37°C for at least 30 h. The tablets were periodically removed and their weight was measured before and during the swelling by using electronic balance (Afcoset ER—182 A, Mumbai). Finally swelling index of all investigational formulations were calculated.

In Vitro Release Studies

In order to determine in vitro release of metronidazole from different vaginal tablets dissolution test apparatus USP type 2 paddle (Tab Machines, Mumbai) method was used. The 1 M phosphate buffer (pH 4.0±0.1) at 37°C was used as the dissolution medium with constant stirring (50rpm) for up to 100% release of drug from the dosage form. Samples of 3 ml were withdrawn at predetermined intervals in triplicate and replaced by phosphate buffer maintained at 37°C. The amount of metronidazole was analyzed spectrophotometrically at λ max 319 nm (Beckman USA DU®—640 B). Dissolution tests were performed in triplicate and standard deviation was applied.

In Situ Release Studies

In situ release was studied using the vagina of freshly sacrified porcine. Tablets to be tested were placed within the mucus membrane of porcine vagina and kept in a 100 ml beaker which contains 10 ml phosphate buffer (pH 4.0 ± 0.1) and properly covered. For maintaining the simulated condition of vagina this set up was placed in an orbital shaking incubator (Remi Instruments, Mumbai) with 50 rpm at 37ºC. Samples of 0.5 ml were withdrawn at predetermined intervals and replaced with buffer solution and amount of metronidazole was analyzed spectrophotometrically (Gursoy A et al., 1989).

Stability Studies

The best batch was subjected to stability studies at elevated temperature (i.e. 60°C / ambient humidity) for six weeks. The samples of the dosage form were withdrawn at end of sixth week and evaluated for the changes in physical characters and drug release pattern. Similarity factor f_2 test was performed on the in vitro dissolution data of fresh and aged samples of selected batch (Shah V.P et al., 1998).

RESULTS AND DISCUSSION

In Situ Bioadhesive Strength Studies

Since vaginal mucus membrane consists of oligosaccharide chains with sialic acid, polymers (CP 934P) with carboxyl and hydroxyl groups can bind strongly to these chains, resulting in strong bioadhesive force than HPMC K4M (with neutral groups). However, the bioadhsive force of a vaginal tablet should be controlled since excess bioadhesive force can damage the vaginal mucus membrane (Yong C.S. et al., 2001). HPMC K4M could be used to control the strong bioadhesive force of CP 934P. Thus a mixture of CP 934P and HPMC K4M was selected as the bioadhesive additive for the metronidazole vaginal tablet. As shown in table 3, the bioadhesive force of HPMC K4M was less than with CP 934P and depended upon its concentration. The reason for this dependence is of course that the carboxylic groups of the CP 934P create ionic interactions with the glycoprotein network of the porcine vagina, thus promoting the firm establishment of the bioadhesive bond.

Table 2: Observed swelling index of investigational formulations using agar gel plate method

S. No	Time (h)	Swelling index of investigational formulation				
		Batch A	Batch B	Batch C	Batch D	Batch E
1	0.5	0.0700 ±0.0039	0.0678 ±0.0120	0.0866 ±0.0038	0.0934 ±0.0034	0.0945 ±0.0034
2	1.0	0.1024 ±0.0120	0.0974 ±0.0018	0.1391 ±0.0093	0.1450 ±0.0208	0.1948 ±0.0096
3	2.0	0.02507 ±0.0271	0.2738 ±0.0111	0.2872 ±0.0082	0.2962 ±0.0077	0.3497 ±0.0305
4	4.0	0.3122 ±0.0101	0.3667 ±0.0114	0.3990 ±0.0061	0.4889 ±0.0151	0.5829 ±0.0198
5	6.0	0.5975 ±0.0062	0.6449 ±0.0519	0.7003 ±0.0145	0.8891 ±0.0287	1.1282 ±0.1368
6	8.0	1.2211 ±0.2666	1.7642 ±0.2007	1.8342 ±0.2126	2.1553 ±0.3166	2.2963 ±0.4096
7	10.0	2.1205 ±0.5462	2.1359 ±0.1323	1.9814 ±0.0440	2.2933 ±0.1736	2.7629 ±0.1955
8	12.0	2.1623 ±0.1607	2.2589 ±0.4361	2.0108 ±0.0566	2.3922 ±0.3351	2.9515 ±0.1420
9	24.0	2.4541 ±0.3258	2.6838 ±0.2284	2.9113 ±0.0296	2.9493 ±0.1373	3.1331 ±0.0869
10	30.0	2.6447 ±0.4642	2.7098 ±0.1880	3.0281 ±0.0271	3.0317 ±0.0681	3.4234 ±0.3970

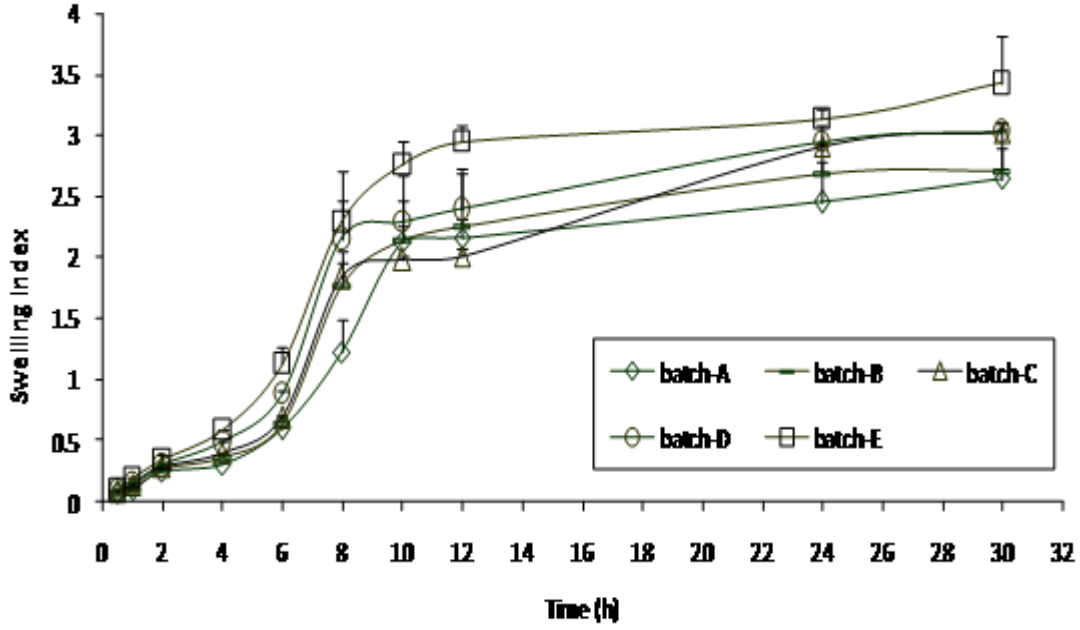

Figure 1: In vitro swelling index versus time of investigational formulations. Each data point represents mean ± S.D. (n = 3)

In Vitro Swelling Studies

The swelling index of formulations containing only CP 934P was less than the formulations with CP 934P and HPMC K4M. As shown in figure 1 & table 2, swelling of tablets with HPMC K4M was higher and increased with increase in HPMC K4M content. These results are in agreement with those obtained by Garcia—Gonzalez (Garcia-Gonazlez et al., 1993). In tablets formulated with a higher concentration of cross—linking polymer (CP 934P), cross—link density will be higher and inter-link length will be correspondingly lower, resulting in less swelling index. On other hand, formulations with higher concentration of hydrophilic polymer (HPMC K4M), due to its lower cross—link density, has in-creased chain extensibility thereby resulting in more swelling.

Table 3: Selection of best batch based on its bioadhesive strength and in vitro, in situ release profiles

S.No	Investigational formulation	Detachment force (gm)	In vitro Release Profile		In situ Release Profile	
			Time (h)	%	Time (h)	%
1	Batch A	26.34±3.73	21.0	98.90±0.52	22.0	95.86±1.24
2	Batch B	21.26±1.37	24.0	98.50±0.14	24.0	96.34±1.0
3	Batch C	15.39±2.04	25.5	98.81±0.73	27.0	94.98±2.12
4	Batch D	10.32±1.06	27.0	98.03±1.68	28.5	96.99±1.59
5	Batch E	4.83±1.48	28.0	98.93±0.76	30.0	97.25±1.08

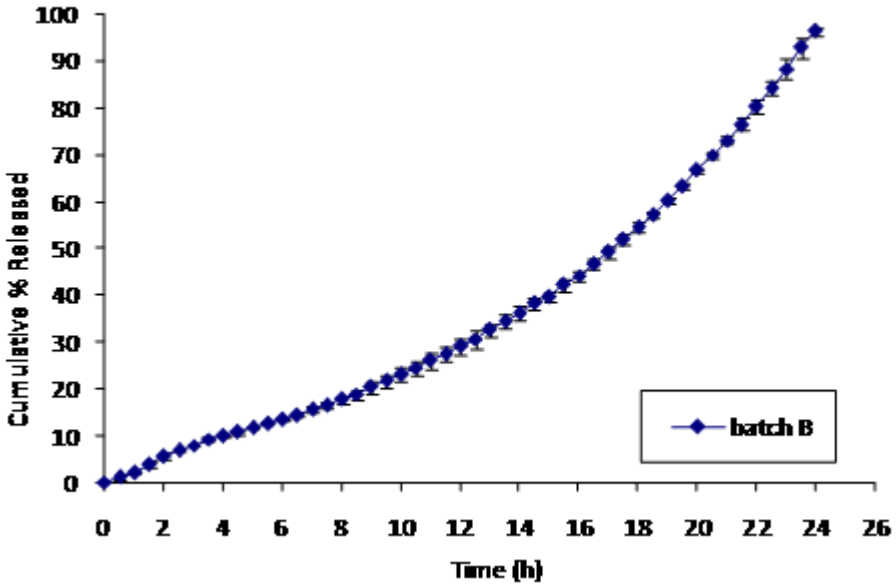

Figure 2: In situ release profile of metronidazole from the best batch of investigational formulation. Each data point represents mean ± S.D. (n =3)

In Vitro and In Situ Release Studies

The results indicated that as the CP 934P content increased there was an increase in the percent of drug release and it can be explained by the swelling behavior of HPMC/CP matrix system. Since CP 934P is a more hydrophilic than HPMC, tablets containing high amount of CP 934P swell at higher rate with lesser extent, which may be responsible to a high rate of drug diffusion.

In all formulations there was good correlation between in vitro and in situ drug release profile. Obviously, in the absence of or at low levels of HPMC (especially batch A&B), the cellulose ether could not maintain the integrity of the matrix in the presence of higher levels of CP 934P resulted in burst release as compared to the formulations containing higher HPMC content.

An inverse relationship was found between swelling index and percent release of metronidazole in all formulations. When the amount of HPMC in the matrix is high, it causes a greater degree of swelling. This in turn reduces the drug release, as the diffusion path length of drug is now longer, resulting in controlled release. The percent release of metronidazole per time was slow in situ as compared to in vitro release in all investigational formulations (Table 3).

Stability Studies

The batch B has been selected as best batch among all investigational formulations based on different studied parameters (as per table 3), which was able to release the metronidazole more than 95% in a optimum time period with sufficient adhesive strength. Accelerated stability study testing (AST) on selected batch was carried out to determine the rate of degradation of the formulation. There was no physical change in the tablets after 6 weeks of AST. Since the resulting similarity factor f_2 was 74.07 and it lies between 50 and 100, both fresh and aged samples of selected batch were not significantly different as respect of their drug release profiles.

CONCLUSION

Metronidazole is the drug of choice in the treatment of bacterial vaginosis but dose and duration of therapy are still controversial. The current recommendation is a treatment with metronidazole orally with 500 mg twice daily for 7 days (Centers for Disease Control and Prevention 1998). Since batch B was released 98.56% (in situ) of metronidazole within 24 h and had sufficient

bioadhesion strength. Thus, improved bioadhesive properties of batch B tablets comprising 3:1 of CP 934P:HPMC K4M in combination with the controlled release of metronidazole seems to be a matrix for vaginal delivery system for the treatment of genitourinary infections.

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