Original Article

Sustained Release Bioadhesive Suppository Formulation for Systemic Delivery of Ornidazole: *In-silico* Docking Study

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ABSTRACT

Background and Objectives: Ornidazole is widely used as an antiprotozoal and anti-amoebic drug and its onset of action is within 2 h. The major extent of the drug is metabolized in the liver and excreted in the urine and faeces. Hence, the present study of suppository formulation for sustained systemic delivery of ornidazole is significant which could minimize abdominal disturbances and nausea and delayed onset of action particularly after oral administration. **Methods:** Bioadhesive suppository formulations were prepared for systemic delivery of ornidazole via rectal and vaginal route. **Results:** The physical drug-excipient-interaction was confirmed by *in-silico* docking study. The affinity between drug-HPMC and drug-PEG was found to be -2 and -0.9 k cal/mol respectively. *In vitro* drug release of the suppositories varied depending on the viscosity grade of HPMC used and all have followed mostly diffusion controlled mechanism. The formulation containing HPMC K100 showed the most sustained release of ornidazole in both the dissolution fluid of pH 7.4 and 4.5 (54.53 and 41.89 % respectively after 360 min). **Conclusion:** In conclusion, present bio adhesive suppositories could be utilized for sustained systemic delivery of ornidazole via rectal and vaginal route. The findings of this work will contribute to the current knowledge and encourage future pre-clinical research.

Key words: Ornidazole, Sustained release suppository, *In-silico* docking, Bioadhesive formulation, *in-vitro* dissolution.

INTRODUCTION

Ornidazole is widely used as an antiprotozoal and anti-amoebic drug and its onset of action is within 2 hr.[1] It prevents recurrence of peptic ulcer disease caused by *Helicobacter pylori*. It is also used effectively and safely for the treatment of inflammatory bowel disease.[2] Ornidazole is better tolerated than metronidazole; the major extent of the drug is metabolized in the liver and excreted in the urine and faeces.[3] The main adverse effects of ornidazole are headache, dizziness, anorexia, intestinal spasms, loose stools etc and the very common are significant abdominal disturbances, nausea etc.[4] Gastro retentive drug delivery systems were reported by increasing residence time to sustain the drug release in the g.i. tract for enhancing local action on *H. pylori*.[5,6] Other route of administration like rectal or vaginal could overcome these problems. The absorption of some drugs is notable from the vaginal wall.[7] Drugs used in the treatment of *Trichomonas* and *Candida* infections lead to systemic effect.[8] Prostaglandins, estrogens are hormones which are rapidly and extensively absorbed through the vaginal epithelium because of its large surface area. This route bypasses first pass metabolism so the rate of absorption is high.[9] Due to the mucoadhesive property it can attach to the mucus membrane of vagina and minimises the chance of detach and can sustain the drug release. Ornidazole suppository showed sustained release up to 90

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min only as reported by Ozyazici *et al.*[10] The very common adverse effects of ornidazole are the significant abdominal disturbances, nausea etc. Abdominal disturbances and nausea and delayed onset of action particularly after oral administration could be minimized by using sustained and controlled release delivery of non-invasive suppository formulation of ornidazole compared to transdermal delivery.[11] Hard surface of the tablets may cause irritation of the vaginal epithelium in case of intravaginal application. The slippery and smooth surface of suppositories may facilitate application and thus the irritation will be less.[12,13] Therefore suppository preparations are also suitable dosage forms for vaginal administration. The HPMC is an inert and semi-synthetic polymer used to control the release rate of drug and may be used as controlled delivery component in different medicaments.[14] PEG has the property of maximum water solubility.[15] In this study, the rectal and vaginal suppositories were prepared using PEG 400, PEG4000, HPMC K100, HPMC K15, HPMC E5 in different ratios to sustain the release of ornidazole and compare their *in vitro* drug release properties.

MATERIALS AND METHODS

Materials

Ornidazole was taken as a gift sample from Jagannath Pharmaceuticals (Jagatpur, Cuttack, India). HPMC E5, HPMC K15 and HPMC K100 were purchased locally from Burgoyne and Co., Mumbai, India). PEG400 and PEG 4000 were collected from Merck Specialities Private limited, India.

Preparation of suppository

Suppository formulations were prepared by fusion molding and congealing technique. PEG 4000, PEG 400, HPMC (K15, K100, E5), ornidazole and water were used in different ratio to constitute a one-gram suppository (Table 1).

PEG 4000 and PEG 400 were taken in a 25ml beaker and melted at 30°C for 4 to 5 min. The melted content was transferred to a china dish containing ornidazole, HPMC and water. Then the content was triturated for 4 to 5 min to get a uniform mixture. The china dish was kept in a hot air oven at 30°C for 2 to 3 min and finally the content was transferred to the suppository forming mould and preserved in refrigerator to settle down and solidification for overnight period. Next morning the solidified suppositories were removed by simply pressing in a forward direction and utilized for further testing.

Differential Scanning Calorimetry (DSC)

Thermal analysis of pure ornidazole and formulations were analysed by differential scanning calorimetry (DSC-1, Mettler Toledo software) in the range of 30 to 105°C at constant heating rate under liquid nitrogen gas purge.

Fourier-Transform Infrared Spectroscopy (FTIR)

Samples of pure ornidazole and prepared suppository formulations for FTIR study were placed over zinc selenide crystal and pressed on to the attenuated total reflectance crystal (ATR crystal) by using the integrated pressure application device by using Bruker infrared analyser (Bruker alpha; Ettlingen, Germany).

Scanning Electron Microscopy (SEM)

The surface morphology and crystalline nature of the samples were investigated by using Scanning Electron Microscope (ESEM-FEI Quanta-250). The drug and suppository formulations were placed over carbon tape and scanned at room temperature with voltage of 10 kV at low vacuum (100Pa).

In-silico docking study

Auto Dock Vina 1.1.2 program was used to calculate the binding between drug and polymer. The programme helps in pre-calculating the interaction between ornidazole-HPMC and Ornidazole-PEG also the binding interaction between them. Protein Data Bank (PDB)

Table 1: Sustained release bioadhesive ornidazole suppository formulation.

Formulation codes	Ornidazole (mg)	PEG 4000 (mg)	PEG 400 (mg)	Water (ml)	HPMC (mg)		
					E5	K15	K100
ORHL5	200	320	400	0.075	5	--	--
ORHL10	200	325	400	0.075	10	--	--
ORHM5	200	320	400	0.075	--	5	--
ORHM10	200	325	400	0.075	--	10	--
ORHH5	200	320	400	0.075	--	--	5
ORHH10	200	325	400	0.075	--	--	10

and its 3-D visualization were generated by using MGL Tools (an Auto dock tool). Marvin sketch helped in drawing the 3-D structure of (OR, HPMC, PEG). By using Auto Dock tools programme the PDBQT files of OR, HPMC, PEG were prepared. The OR was taken as ligand in opposition to the receptors like HPMC, PEG. The stability was deliberated on the basis of interaction energy between the ligand and receptor. The more negative score the better will be the binding.

In vitro drug release

The *in-vitro* dissolution tests of ornidazole suppositories were performed with USP dissolution apparatus (type-2, paddle type). Accurately weighed suppository samples were placed over the dialysis membrane (Dialysis Membrane- 150, LA401-30MT) (Av. Flat width-37.70 mm, Av. diameter- 25.4 mm, Capacity approx- 5.07 ml/cm, molecular weight cut-off 12000-14000, Himedia Laboratory Pvt. Ltd., Mumbai) in a both side open diffusion tube and tied with thread tightly. Diffusion tube was attached with the paddle and *in vitro* drug release was carried out in phosphate buffer (pH-7.4) and sodium acetate buffer (pH-4.5).[16] Drug release was continued in 200ml medium with a rotation speed of 50rpm at 37°C. Samples were withdrawn at regular time intervals and analysed in a UV spectrometer (Thermo scientific, Evolution 201-uv-visible spectrophotometer) at 319nm.

RESULTS AND DISCUSSION

Suppositories were solidified and found smoothed surface (Figure 1) and studied characterizations are described below:

Differential Scanning Calorimetry (DSC)

A sharp endothermic peak of pure ornidazole was observed at 91.26°C indicating pure crystalline form (Figure 2). Formulationsshowed endothermicappearance in the range of 50 - 59°C due to presence of PEG.[17] Ornidazole melting peak has been disappeared in all the formulations indicating amorphous nature of the drug in the suppositories.

Fourier-Transform Infrared Spectroscopy (FTIR)

The peaks at 3172 and 3314 cm^{-1} in the FTIR spectrum of ornidazole are due to the C-H stretching and –O-H stretching mode respectively. The peaks at 1532 cm^{-1} and 1365-1264 cm^{-1} are due to asymmetric and symmetric stretching of NO_2 respectively. The C-O stretching vibration is confirmed by the presence of peak at 1186 cm^{-1}. The carbon connected with NO_2(C-N) has been credited by the presence of peaks at 824 and 732 cm^{-1} is due to the stretching frequency of C–Cl bond vibration. Peaks at 3500 and 3000 are due to O-H stretching of water and C-H stretching. Another peak at 1646 is due to N-H bending which signifies the interaction between polymer and drug through H-bonding (Figure 3).

Scanning Electron Microscopy (SEM)

The surface morphology of the pure drug and the suppositories are shown in Figure 4. Distinct brick shaped crystals were seen in the SEM image of ornidazole. The disappearance of definite crystal geometry is seen in the SEM images of all the suppository formulations. The disappearance of crystal geometry conforms the uniform distribution of drug throughout the formulations.

Analysis of molecular interaction (Docking)

The docking scores have been cited in Table 2. *In-silico* study gave an idea about the interaction between drug and carrier molecule (Figure 5). The physical interaction and possible conformation were well predicted by docking study. The binding interaction in between the Ornidazole and PEG showed lowest energy as compared to OR and HPMC. The negative energy in between drug and carrier indicates stable interaction. The more

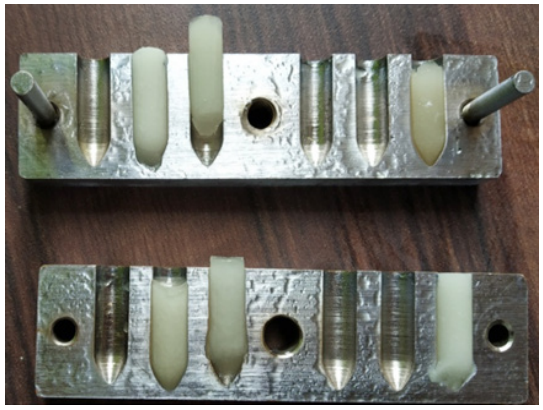

Figure 1: Prepared suppositories.

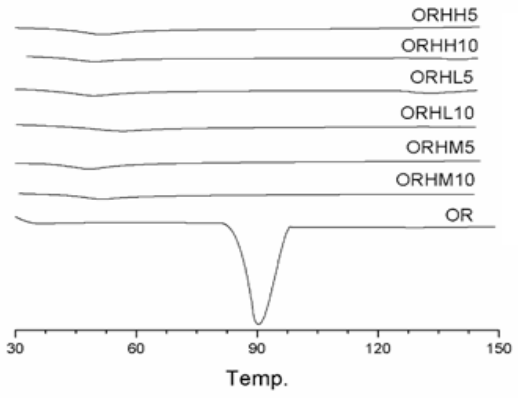

Figure 2: DSC thermo gram of ornidazole and the prepared suppositories.

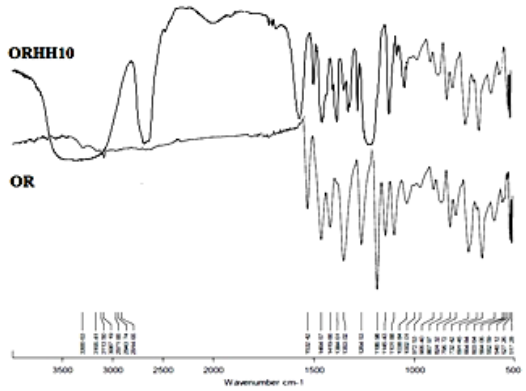

Figure 3: FTIR spectrum of OR and ORHH10.

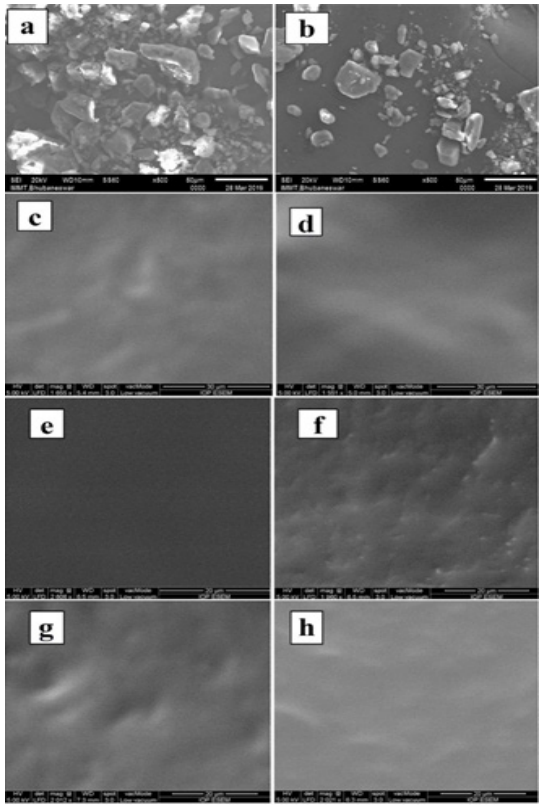

Figure 4: SEM image of pure crystalline drug ornidazole: (a) (magnification 500X), (b) (magnification 500); and suppository formulations: (c) ORHL5 (magnification 1655), (d) ORHL10 (magnification 1551); (e) ORHM5 (magnification 1551), (f) ORHM10 (magnification 1960); (g) ORHH5 (magnification 2012), (h) ORHH10 (magnification 2021).

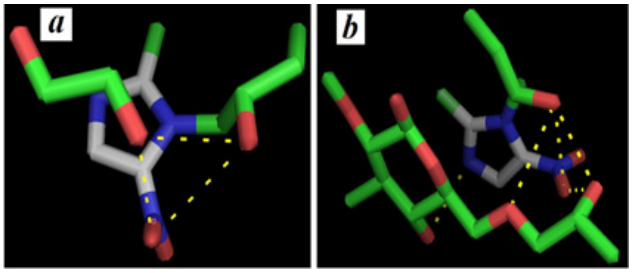

Figure 5: Docking interaction of (a) ornidazole-PEG and (b) ornidazole-HPMC.

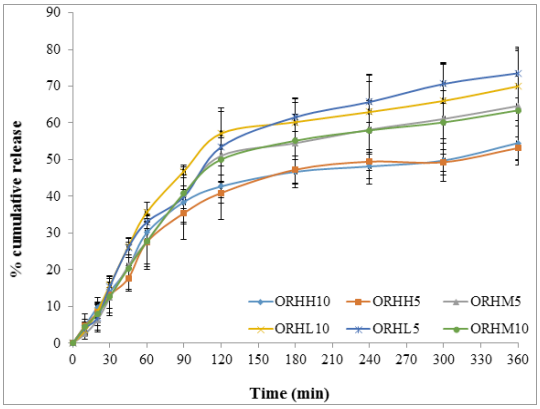

Figure 6: Ornidazole release from suppositories in phosphate buffer of pH 7.4(Results are mean ± SD of three independent experiments, *n*=3).

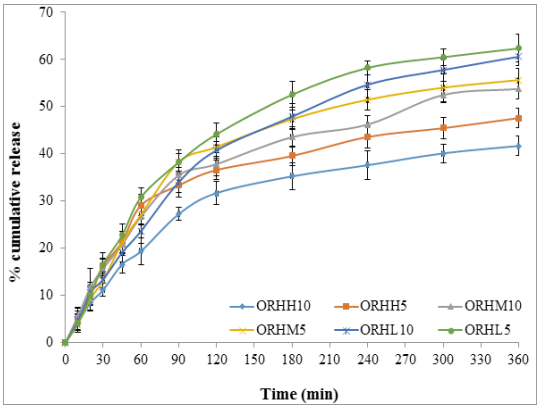

Figure 7: Ornidazole release from suppositories in phosphate buffer of pH 4.5(Results are mean ± SD of three independent experiments, *n*=3).

Table 2: Docking score of drug and polymer interaction.

Formulation	Docking score (Affinity)
OR-HPMC	-2.0
OR-PEG	-0.9

negative value indicates more stable binding than that of less negative value. The binding energy values for OR-HPMC and OR-PEG were -2.0 and -0.9 k cal/mol respectively. Figure 5 shows the docking interaction of ornidazole and the polymer.

In vitro drug release

The *in-vitro* release of ornidazole from suppositories containing bases of different composition were analysed in different time intervals. As shown in the Figure 6 and Figure 7 the diffusion of ornidazole from suppositories were in a sustained manner and continued to 6 h to release about 50-70%. Ornidazole release pattern from the suppositories were in the order of ORHH10>ORHH5>ORHM10>ORHM5>ORHL10>ORHL5.

Table 3: Drug release kinetics of ornidazole suppository formulations at pH 7.4.

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	K_0 (%.min^{-1})	r^2	K_F (min^{-1})	r^2	K_H (%. min$^{-1/2}$)	r^2	n	Kp	r^2
ORHL5	0.207	0.859	1.955	0.946	4.739	0.959	0.787	0.012	0.950
ORHL10	0.191	0.791	1.935	0.881	2.205	0.926	0.757	0.002	0.973
ORHM5	0.182	0.825	1.953	0.896	4.337	0.938	0.797	0.099	0.946
ORHM10	0.178	0.824	1.949	0.890	0.343	0.939	0.790	0.024	0.965
ORHH5	0.143	0.806	1.948	0.859	1.073	0.937	0.711	0.039	0.972
ORHH10	0.140	0.782	1.940	0.844	0.342	0.927	0.705	0.072	0.964

Table 4: Drug release kinetics of ornidazole suppository formulations at pH 4.5.

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	K_0 (%.min^{-1})	r^2	K_F (min^{-1})	r^2	K_H (%. min$^{-1/2}$)	r^2	n	Kp	r^2
ORHL5	0.171	0.848	1.947	0.920	1.932	0.964	0.741	0.038	0.969
ORHL10	0.161	0.893	1.960	0.950	3.352	0.979	0.723	0.037	0.986
ORHM5	0.151	0.822	1.947	0.885	1.316	0.949	0.726	0.027	0.968
ORHM10	0.139	0.845	1.946	0.908	0.351	0.969	0.681	0.116	0.970
ORHH5	0.119	0.778	1.940	0.839	1.927	0.934	0.672	0.105	0.954
ORHH10	0.110	0.832	1.958	0.874	0.167	0.959	0.659	0.049	0.977

The *in-vitro* release of ornidazole from suppository containing 10mg of HPMC K100 (ORHH10) showed the most sustained release as compare to all the formulation.[18] The drug release up to 360 min was considered as the main criterion for understanding the most sustaining effect. Drug release kinetics was not considered as the criterion for understanding the most sustaining effect. Suppository ORHH10 exhibited 41.68 % drug release whereas; ORHM10 has shown 53.75% up to 360 min.

Kinetics of drug release

Drug release mechanism has been predicted to develop a rational formulation utilizing mathematical models. Different kinetic models like Zero order, First order, Higuchi and Korsmeyer-Peppas model[19-31] were used to describe the kinetics of ornidazole release of suppository. The model that uses highest level of correlation (r^2) was used as the model-fitting kinetics. These models are represented as follows:

Zero order model: $Q=Q_0+K_0t$---------- (i)

First order model: $Log\ (100-Q)= \frac{K_F.t}{2.303}$---------- (ii)

Higuchi model: $Q=K_H\times\sqrt{t}$ ---------- (iii)

Korsmeyer-Peppas model: $Q=K_p\times t^n$

$LogQ=LogK_p+n.Logt$ ------ (iv)

Q = Cumulative percent drug release at time t

Q_0 = Cumulative percent drug release at time t = 0

K_0 = Zero order release rate constant

K_F = First order release rate constant

K_H = Higuchi release rate constant,

K_p = Peppas release rate constant or, Parameter reflecting the structural and geometric characteristics of the delivery device,

n = Power law exponent, or release exponent.

The kinetic parameters as per model are presented in the Table 3 and Table 4. Result indicates that the release kinetics of all the suppository formulations (pH 7.4 and pH 4.5) is mostly following the Korsmeyer-Peppas model because of higher r^2 value (0.946-0.986) compare to other models. The release exponent values (n= 0.659-0.797) are a sign of partially diffusion controlled and partially erosion controlled release. Fitting of release data with Higuchi model has shown r^2 values (0.926-0.929) closer to Korsmeyer-Peppas model indicating also diffusion controlled release to some extent.

CONCLUSION

The drug and polymer showed a significant interaction between them which was conformed from FTIR study and was supported by molecular level study (*in-silico* docking). The *in-vitro* release of ornidazole from suppository containing 10mg of HPMC K100 (ORHH10) showed the most sustained release as compare to all the formulation. Due to the presence of HPMC, it works as mucoadhesive so that it can attach to the mucus membrane of vagina and rectus for longer period of time. So this can be an approach in sustaining the release of ornidazole up to 6 hr and minimises the repeating of dose.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

HPMC: Hydroxy Propyl Methyl Cellulose; **PEG:** Poly Ethylene Glycol; **SD:** Standard Deviation.

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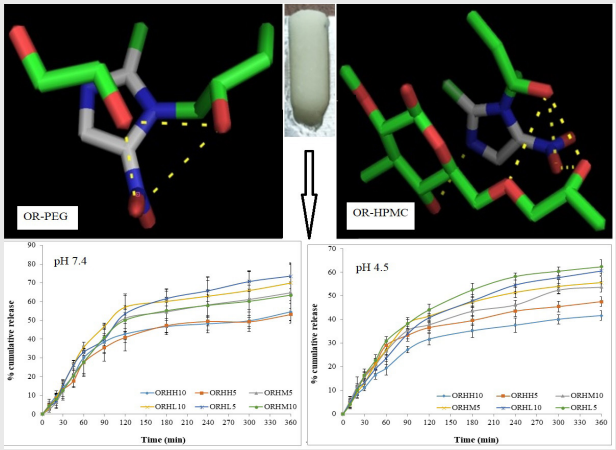
PICTORIAL ABSTRACT



SUMMARY

- Sustained release ornidazole suppositories were prepared for systemic delivery to avoid abdominal disturbances and delayed onset of action after oral administration.
- *In silico* docking study revealed the affinity between ornidazole-HPMC and ornidazole-PEG of -2 and -0.9 k cal/mol respectively.
- Suppository (HPMC K100) showed the most sustained diffusion controlled release of ornidazole at pH 7.4 and 4.5.
- Bioadhesive suppositories could be conveniently utilized for sustained systemic delivery via rectal and vaginal route for more than 6 hr.

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