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Research Article

METRONIDAZOLE BIOADHESIVE VAGINAL SUPPOSITORIES: FORMULATION, IN VITRO AND IN VIVO EVALUATION

HAIDY ABASS*[1], RABAB KAMEL[2], AHMED ABDELBARY[3]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, German University in Cairo, El tagamoo El Khames Main Entrance Cairo, Egypt, [2]Department of Pharmaceutical Technology, National Research Center, Dokki, Cairo, Egypt, [3]Department of pharmaceutics and Industrial Pharmacy ,Faculty of Pharmacy ,Cairo University, Kasr El -Aini St., 11562, Cairo, Egypt. Email:haidy_miu2002@yahoo.com

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ABSTRACT

Drug administration via mucosal membranes, including the vaginal, has the advantage of by passing the hepatogastrointestinal first pass metabolism associated with oral administration. Metronidazole suppositories were prepared using different suppository bases viz., water soluble bases (PEGs and gelatin) emulsion and fatty bases. The physicochemical properties of most of the prepared MTZ suppositories comply with the pharmacopoeial limits and passed the quality control tests. In general, water soluble suppository bases gave higher release than did the emulsion in citrate buffer pH 4. PEG base (F14), gelatin base (Fl8) and emulsion base (F23) gave the highest drug release and selected for further investigation. The release of MTZ from polyethylene glycol bases followed, first and Higuchi order release model, while gelatin and emulsion obeyed first model. The tested suppository showed enhancement of drug absorption from tested suppository and the One way ANOVA analysis for AUC(0-∞) showed that the P value is 0.0502, considered not significant. The microbiological results showed that the bioadhesive formulae that released the concentration of 0.25 mg/ml of the drug and sustained this concentration for 120 min can be effective on *C. albicans* moreover bioavailability study was performed on flagyl ® vaginal suppository (market product) and the prepared pluronic127 –cp934 bioadhesive vaginal gel ,eight female rabbits were randomly divided into two groups, each containing four rabbits the results showed that the tested formulae did not exhibit enhancement in bioavailability in comparison to the market product which mean lower side effects and localized effect inside the vagina.

Keywords: Metronidazole bioadhesive, Vaginal suppositories

INTRODUCTION

In the recent years, research has focused on the vaginal placement of commercial tablets as a logic alternative for patients who cannot tolerate oral treatment. Many studies have demonstrated the superiority of the vaginal over the oral route in terms of dramatic minimization of general and gastrointestinal side effects.(1 and 2).The advent of biotechnology has renewed interest in using mucosal membranes as sites for non invasive drug delivery. Drug administration via mucosal membranes, including the vaginal and rectal membranes, has the advantage of by passing the hepatogastrointestinal first pass metabolism associated with oral administration. (3) Generally, suppositories are solid dosage forms intended for insertion into the body orifices where they melt, soften or dissolved and exert localized or systemic effects. Suppositories are commonly employed rectally or vaginally, occasionally urethrally and rarely aurally and nasally. They have various shapes and weights. The shape and size of a suppository must be capable of being easily inserted into the intended site.

The aim of study is to formulate Metronidazole suppositories using mainly water soluble and emulsion bases. The prepared suppositories were evaluated for their physical characteristics as well as the in-vitro drug release in pH 4 (simulating vaginal pH) using non membrane methods, kinetic analysis of the release data was also performed orifice without causing undue distension. (4)

MATERIALS AND METHODS

Materials

Metronidazole was purchased from El-Nasr chemical company,Egypt. Polyethylene glycol 600,Polyethylene glycol4000, polyethylene glycol 1500Polyethylene glycol 6000, polyethylene glycol200 (Sigma Chem.C0.,USA).Cocoa butter(B.P. grade). Witepso1 H15, Ovucire WL 3460 and SuppocireAM was kindly supplied from Gattefosse etäblissements, France.

Gelatin was purchased from The General Chemical and pharmaceutical C0., LTD, England. Propy1ene glycol was purchased from Evans Chem. C. Egypt. G1ycero1 (B.P. grade). Sodium alginate and Sodium carboxy methyl cellulose was purchased fromt he General Chemical and pharmaceutical CO; LTD, England). Cellophane ,mw-cutoff 10,000was purchased from Diachema, Germany. Secnidazole obtained from Sigma-Aldrich (USA) B No.20040626.

Flagy Vaginal Suppository®-containing 500 mg Metronidazole. Methanol and acetonitrile, HPLC grade, Sigma-Aldrich (USA). Sabouraud Dextrose Agar was purchased from Oxoid, England; its typical formula (g/l) mycological peptone 10.0; glucose 40.0; Agar *15.0,* pH 5.6 ± 02 Lot/CH, -B: 340 *53683*.Sabouraud liquid medium was purchased from Oxoid, England.*Candida. albicans* was isolated from the vaginal swab of a patient with vaginal cadidiasis who had received no antifungal therapy. Potassium dihydrogen phosphate and disodium monhydrogen phosphate, Sigma-Aldrich (USA)

Formulation of Metronidazole suppositories

MTZ suppositories, each containing *500* mg of the drug were formulated using water soluble, emulsion and fatty bases, Tables (I, II and III). The fusion method was adopted to prepare the different batches. For fatty and water soluble suppository, the base was melted first using water bath at a suitable temperature then MTZ powder was added to the melted base. Gentle stirring was continued to ensure complete mixing and to enhance cooling. The mass was poured into a metal mould just before congealing.

The emulsified suppositories were prepared by dissolving the surfactant in either the hydrophilic or lipophilic phase and polymer was dissolved in the water phase before starting emulsification. The bases used were melted then the aqueous phase was added, with continuous agitation (5). The drug was finally added and the mass was poured into a metal mould. After solidification at room temperature the prepared suppositories were packed in tightly closed containers and placed in a refrigerator. Before use the suppositories were left for 2 hours at room temperature.

Table I : Composition of polyethylene glycol suppository bases

Formula No.	Composition	%W/W
Fl	PEG20000:PG	80:20
F2	PEG 20000: PG	60:40
F3	PEG 20000: PG	50:50
F4	PEG 20000: PG	40:60
F5	PEG 20000: PG	20:80
F6	PEG 6000 : PG	60:40
F7	PEG 6000 : PG	50:50
F8	PEG 6000 : PG	40:60
F9	PEG 6000: PEG 600	60:40
Fl0	PEG6000:PEG600	50:50
F11	PEG6000:PEG600	40:60
F12	PEG 4000: PEG600	60:40
F13	PEG4000:PEG600	50:50
F14	PEG 4000: PEG 600	40:60

PG: propylene glycol; PEG: Polyethylene glycol

Table II: Composition of gelatin suppository bases:

Formula No.	Composition	%W/W
F15	Gelatin	14
	Glycerin	46
	Water	40
F16	Gelatin	14
	Glycerin	26
	Propylene glycol	20
	Water	40
F17	Gelatin	14
	Glycerin	16
	Propylene glycol	30
	Water	40
F18	Gelatin	14
	Glycerin	6
	Propylene glycol	40
	Water	40

Table III: Composition of emulsion suppository bases

Formula No.	Composition	%W/W
F19	Ovucire WL 3460	50
	Sodium CMC	1
	Tween 20	4
	Distilled water	45
F20	Ovucire WL 3460	50
	Sodium alginate— Tween 20	2
	Distilled water	4
		44
F21	Ovucire WL 3460	*34*
	Tween 20	5
	Span 60	1
	PEG 1500	40
	PEG 600	20
F22	Ovucire WL 3460	10
	Witepsol H15	24
	Tween 20	5
	Span 60	1
	PEG 1500	40
	Propylene glycol	20
F23	Cocoa butter	74
	Sodium alginate	2
	Tween 20	4
	Distilled water	20

Evaluation of Non-Medicated Suppositories

The prepared suppository bases were subjected to the following tests

Weight variation

Average weight was calculated by weighing twenty suppositories individually from each formulation and the percentage deviation from the mean was determined. All the prepared suppositories except F5 were within the pharmacopoeial limits for the uniformity of weight according to the B.P., 1998 method(6).

Disintegration / (dissolution) time

The test was performed in distilled water at37°C using the U.S.P. tablets disintegration apparatus. The disintegration time was recorded as soon as the suppositories placed in the basket either completely melted or dissolved. (6) The different formulations exhibited different Disintegration time they are either dissolved or softened and melted within the range of 12- 50 min for polyethylene glycol , (5-20) for gelatin ,(15-30) for emulsion and for fatty bases the melting time was (3-4)min. The (B.P., 1998)(6) states that the melting time, for a fat based suppositories should not exceed 30

minutes, while dissolution time of water soluble suppositories should not exceed 60 minutes which was coinciding with the above results.

Hardness Determination

This test was designed to measure the brittleness and fragility of the suppositories. The resistance of the prepared suppositories was evaluated using Erweka hardness tester (7) under the effect of increasing pressure at room temperature. The weight in Kg required for the deformation and breaking o the suppositories was calculated. The prepared suppository formulations exhibited hardness ranging from 0.8 to *4.2* kg .This is an important indication of the ability of different formulated suppositories to withstand pressure during handling, shipping and insertion.

Melting range determination

The test was carried out using the capillary method (7) in melting point apparatus. A straight capillary tube, 8 to 10 cm in length and 1 to 1.2 mm in internal diameter, opened at both ends was used. One end of the tube was dipped into the suppository bases and gently sufficient amount was packed to fill to a 1 cm column (no previous melting of the sample was done to avoid changing any stable modification to unstable one). The capillary tube was then placed in the apparatus attached to a thermometer. The melting range was recorded when the contents of the capillary tube started to melt. Melting point determination revealed considerable variability between the tested formulations. Water soluble bases showed higher melting ranges than emulsion and fatty bases, melting rang of gelatin suppositories can not be measured. Among emulsion bases witepsol based suppositories (F19-F22) showed higher melting range than cocoa better based suppositories (F23). Witepsol H15 (F20) has the lowest melting range as compared with the other fatty bases.

Uniformity of drug content

The B.P 1998 (6) method was adopted. Ten suppositories were randomly selected from each formula and assayed individually. a preweighted suppository was melted and dispersed in 25 ml of citrate buffer pH 4 then the volume was completed to 100 ml by the same buffer. The containers were allowed to rotate in a constant temperature water bath at 37 °C+ *0.5* for two hours. Aliquots were withdrawn from the aqueous phase, filtered, suitably diluted and assayed spectrophotometrically at 316 nm against blank. The drug content was found to comply with the requirements of the B.P. 1998, it ranged from 98-102% of the incorporated amount.

In -Vitro drug release in Citrate Buffer

U.S.P 25 dissolution method

The in—vitro release of Metronidazole from suppositories was performed using the U.S.P 25 dissolution apparatus II (Paddle type). The paddle was rotated at 50 rpm in 500 ml of citrate buffer pH 4, maintained at 37 ± 0.5°C. Metronidazle content was determined by measuring the absorbance at 316 nm against a blank. Each experiment was performed in triplicate.

Analysis of the drug release data

The release data were mathematically analyzed using linear regression method according to zero order, first order kinetics and Higuchi diffusion model (8 and 9).

Evaluation of Mucoadhesive Properties of Metronidazole Vaginal Suppositories

The mucoadhesive properties of the formulated vaginal suppositories were determined by measuring the work of adhesion between rabbit mucous vaginal membrane and the prepared suppository using-INSTRON, Model 2519-103 Serious 3340 Capacity 100 N USA.

Bioavailability and Microbiological Study

Microbiological Study

A turbid 48-h culture of *C. albica.s* was prepared in 200 ml liquid Sabouraud medium, the turbidity is adjusted to contain approximately $l0^5$ cells/ml (10)

Then sets of MTZ solutions at increasing concentrations (0.25, 0.5, 0.75 and 1 mg/mL) were prepared. These concentrations correspond to the 25%, 50%, 75% and 100% of MTZ released from bioadhesive MTZ suppositories, respectively. From each concentration of MTZ, a mixture of the yeast and MTZ solution was made in a sterile tube in ratio of 1:10, respectively. After 15 and 120 mm 100 µl of the yeast suspension containing 0.25 mg/ml MTZ was inoculated into tubes containing 900 µl liquid Sabouraud medium. Similar transfers were made, from 0.5 mg/ml suspension after 30 and 120 min, from 0.75mg/ml suspension after 90 and 120 min, and from 0.l mg/ml suspension after 120 and 180 min.(11) and(12)

The Bioavailability Study

Study design

The study was performed for two formulae, namely; flagyl ® vaginal suppository (market product), F23 Vaginal suppository. Eight female rabbits were randomly divided into four groups, each containing two rabbits. A cross over design was applied on two phases, so that each group received a single vaginal dose of one of the tested formulae in each phase. A washout period of seven days was left between phases. The tested formulae were vaginally administered to rabbits. The rabbits were fasted for 24 hours before drug administration and continued fasting until 4 hours post dose, with water allowed.

Blood sampling

Blood samples were withdrawn from the marginal ear vein of the rabbits just prior to drug administration and at time intervals of 0.5, 1, 1.5,2, 2.5, 3, 4, 5, 7, 10 and 24 hours after drug administration. The blood samples were withdrawn into tubes washed with diluted heparin to guard against coagulation of blood. The blood samples were then centrifuged at 3000 rpm for 10 minutes and the clear plasma was then collected in polyethylene capped tubes and deep frozen at -20°C till required for analysis.

HPLC assay method

A modified method of Nasir M et al, (13)M.J. Jessaa et al,(14) and J.I.D.Wibawa et al.(15).was adopted with some modifications. Analysis of samples was performed using a Shimadzu HPLC system equipped with spectrofluorimetric detector. The mobile phase was a mixture of water and methanol (70:30 v/v). The flow rate was 1 ml per minute. The detection was carried on at 317 nm .As excitation and emission wavelengths, respectively. A calibration curve was plotted for Metronidazole the range of 0.01 —20 ug/mL. secnidazole was used as internal standard.

Plasma samples preparation for Metronidazole determination

To 1 ml of plasma in a glass centrifuge tube, 1ml of secnidazole were added (as internal standard) and 1 ml of acetonitrile. After mixing (30 s), the mixture was centrifuged for 10 mm at 300 rpm. Then 20 uml of supernatant were injected into liquid chromatograph. Concentrations of Metronidazole in unknown samples were calculated with reference to the prepared calibration curve.

For calibration curve, plasma standards were prepared by spiking one mL of drug-free rabbit plasma with the internal standard working solution (to prepare 20ug/mL) containing appropriate amounts of Metronidazole to produce concentrations of 10, 20, 30, 50, 100, 200, 300 , 500,1000,2000,3000,5000,10000,20000 ng/mL. The spiked plasma standards were processed as described above. The calibration curve was obtained by plotting the chromatographic peak area ratios (drug/internal standard) against the corresponding nominal Metronidazole concentration added. Samples were prepared and injected on the same day.

Assay validation

The assay procedures were validated in terms of linearity and extraction recovery.

1- Linearity: The linearity was checked by determining the best fitting line equation and the correlation between the added concentrations and the measured peak area ratios.

2- Extraction recovery: Relative recoveries of 10, 20, 30, 50, 100, 200, 300 , 500,1000,2000,3000,5000,10000,20000 ng/mL were

evaluated by assaying the plasma standards as described above and comparing the peak area ratios with those obtained from direct injection of unprocessed reference solutions of the same concentrations.

Determination of the pharmacokinetic parameters

To assess the bioavailability of Metronidazole, the plasma concentration- time data were evaluated, and the pharmacokinetic parameters were calculated.

Statistical Analysis

Two-way Analysis of variance (ANOVA) was applied to assess the significance of formulation and period effects on the pharmacokinetic parameters of the tested formulae and Flagyl ® vaginal suppository LSD test for multiple comparisons was then performed in order to determine the source of difference using SPSS® software, version 16.0 (SPSS Inc., Chicago, IL). Differences are considered to be significant at $p = 0.05$.

RESULTS AND DISCUSSION

The prepared suppositories are well formed with a smooth shinning surface except F5 which did not congeal and was too soft so it was rejected from the PEGs and no further experiment was done on it on the other hand the rest suppositories are well formed, white or creamy white in color for PEGs, fatty and emulsion bases and yellow for gelatin bases. After slicing the suppositories longitudinally, they did not show any fissures, cracks or contraction holes.

In-vitro release of Metronidazole from different suppository formulations in citrate buffer PH 4

No standard laboratory method or apparatus design for the release of drugs from suppositories(16) . The following methods were attempted to investigate the in-vitro release of MTZ in a sequence of efficacy.

1- U.S.P 25 dissolution method

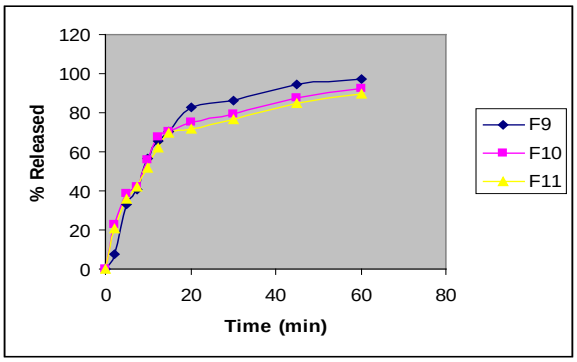
This method was adopted by many investigators (17-20). However the results obtained from water soluble polyethylene glycol bases showed that, the release of MTZ from those formulations was identical. This may be attributed to the large volume of dissolution medium and that (500 mg) of the drug in the suppository which completely released after 15 min of the release time. Accordingly the test was not continued for other bases.

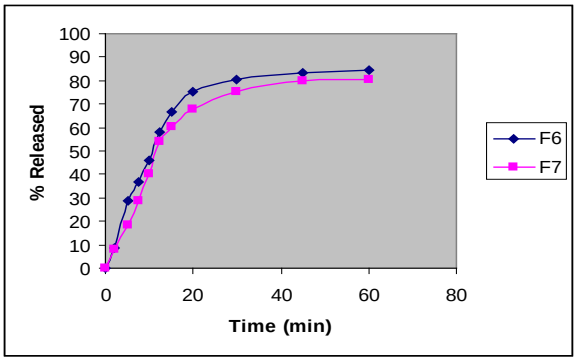
2-Dialysis method

The second technique tried was the dialysis membrane method(21 and 22) . The amount released of MTZ from the different polyethylene glycol bases was very low after 8 hrs. This distinct decrease in drug release behavior by this method may be attributed to the molecular weight (Mwt: of the drug which can not be delivered through the cellophane membrane this was coinciding with Ozyazici M**,** et al (23). they used Three different dissolution methods to evaluate the in vitro drug release from Vaginal suppository formulations of metronidazole which were prepared using six different bases as Witepsol H15, Cremao, Ovucire WL2944, Ovucire WL3264, PEG 1500, PEG 6000 but they found that The diffusion studies which were performed through synthetic (cellophane) and natural membrane (rabbit vagina), but the drug did not showed no good permeation for the drug from natural membrane although its cut off is 10.000. In-vitro release of Metronidazole from polyethylene glycol bases

The in-vitro release of MTZ from polyethylene glycol bases (Fl to F14) is presented in Fig. 1. It is obvious that the release is affected in one hand by the solubilizing effect of propylene glycol and liquid PEG 600 on the drug, on the other hand, by the content of solid PEGs (20000,6000 or 4000) in the suppository which contributed to increased solubility and dissolution in the aqueous medium (24).

By increasing solid PEGs concentration and decreasing PEG 600 or propylene glycol concentration in the base resulted in raising the melting point and increasing the hardness of the base, consequently retarding the in-vitro release of the drug and vice versa. Relatively, suppository base (F4) which contained 80% of propylene glycol gave the highest drug release while (F5) excluded as it was too soft and did not congeal. this is also coinciding with Vromans H et al., (25) whom studied the rectal absorption of metronidazole from an aqueous suspension by preparing fatty suppository and three different polyethylene glycol suppositories and studied them in healthy volunteers and then compared them with absorption from an oral solution. They found that the polyethylene glycol suppositories gave the highest peak plasma levels.





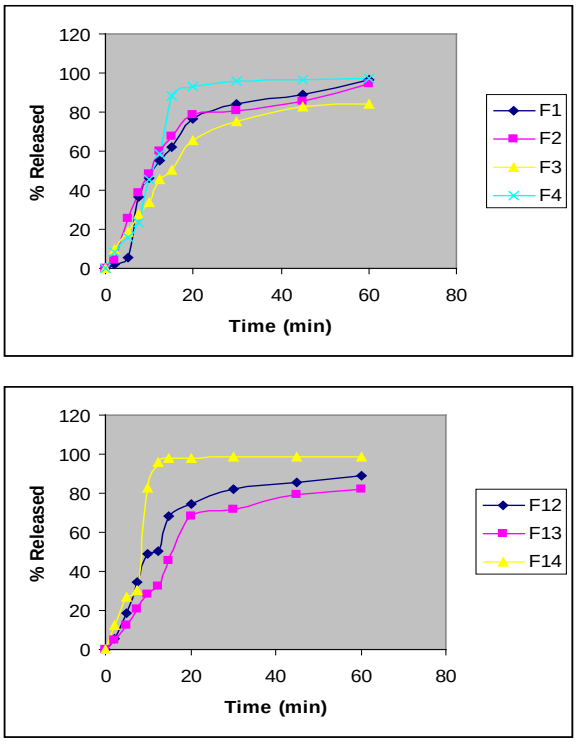


Fig. 1: In-Vitro Release of Metronidazole from Different Poly Ethylene Glycol Suppository Bases in Citrate Buffer PH 4 at 37° C

In-vitro release of Metronidazole from glycerogelatin bases

Fig. (2) represent drug release from different gelatin bases .The tested bases could be arranged according to their release rate as follows F18>F15>F17>F16

The percentage of MTZ released from them was 95%,94.2%.,90 %and 89.6% respectively after one hour this is coinciding with Ofoefule SI et al.,(26)who found high in vitro dissolution profile of metronidazole glycerogelatin suppositories .These observation may also be explained on the bases that increasing the propylene glycol concentration lead to decrease the dissolution time ,therefore increasing the release rate .In addition to the enhancing effect of propylene glycol on the solubility of the drug as reported(27).

In-vitro release of Metronidazole from fatty suppository bases

The results of drug release from fatty suppository bases (cocoa butter, witepsol H15 and suppocire Am) are cited in Fig. 3 and 4.

The release rate of the drug from these bases is less than the release rate from the water soluble or emulsion bases. This release pattern was expected due to the higher affinity of the hydrophobic MTZ to the lipophilic bases. The lipophilic bases can be arranged according to drug release as follows: F26 > F25 > F24. These results can be attributed to dependency of release pattern on both melting behavior and the chemical composition of the bases. Witepsol H15 (F25) which has the same melting range (34-35 °C) as cocoa butter (F24), but different in chemical composition, gave greater release than cocoa butter, a result may be attributed to the presence of the self emulsifying agents in the former base, which may facilitate the dispersion of the medicament into the surrounding medium (26). The release rate from cocoa butter (F24) was relatively lower than from suppocire AM (F26) and witepsol H15 (F25), Similar results were obtained by Nair and Bhargava(17) who reported that, the release rate of fluconazole was greater from witepsol W45 than from cocoa butter.

The collected results. are in good agreement with that obtained by Hosny et al.(19)working on mebeverine suppositories. They found that, the hydrophilic bases released the drug more rapidly than lipophilic bases. Also, higher release of propranolol hydrochloride (5), fenbufen(22) and zonisamid (31) were reported with hydrophilic bases compared to lipophilic bases.

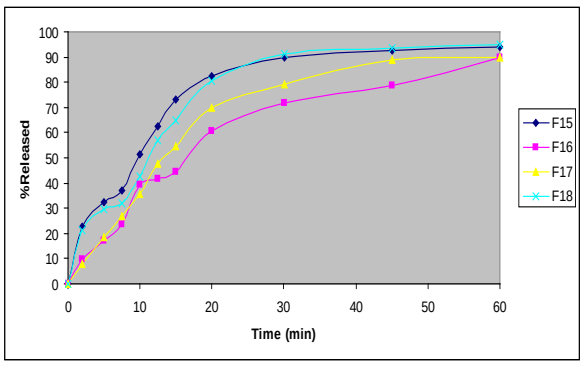


Fig. 2: In-Vitro Release of Metronidazole from Different Gelatin Suppository Bases in Citrate Buffer PH 4 at 37° C

In-vitro release of Metronidazole from emulsion suppository bases

The in-vitro release pattern of MTZ from the tested emulsion suppositories is presented in and Fig. 3 and 4, the tested bases could be arranged according to their release rate as follows F23> F22 > F21 > F19. The obtained results revealed an inverse relationship between the amount of drug released and the melting point and dissolution time of the suppositories. A result indicated that the lower the melting point and shorter dissolution time of the suppository, the higher the release rate.

The base components were found to determine the melting range as well as the dissolution time of the suppository. In this respect, witepsol H15 and cocoa butter when used as the oily phase lead to the formation of an emulsion bases with lower melting range and shorter dissolution time compared to Ovucire WL 3460. The bases contained witepsol H15 or Ovucire WL 3460 gave higher release rate than the base contained cocoa butter as the former bases contain self emulsifying agents. This is coinciding with Ozyazici M et al,(28) who found high dissolution and vaginal absorption characteristics of metronidazole prepared by a simple fusion method using Witepsol H15 further more E. Bergogne et al, (29)also found that suppositories containing Witepsol H-15 only, or with the addition of Tween 80 (1%), with or without diclofenac sodium, a non-steroidal anti-inflammatory drug, were significantly increased the rectal absorption of latamoxef, with bioavailabilityas high as 72%.

Suppositories contained sodium CMC (F 19) showed lower release this may be due to more gelling behavior exhibited by sodium CMC (30). When PEG 1500 with PEG 600 or propylene glycol is used as the aqueous phase in F2l and F22 suppositories instead of water, a higher release rate was observed compared to other emulsion suppositories. The high release rate of these bases may be attributed to the concomitant rapid dissolution of the suppository and the effect of PEGs and propylene glycol on the solubility of the drug.

Kinetic analysis of release data

Tables: (IV) summarize the kinetic analysis of MTZ release data (using the beaker method) by linear regression according to zero, first order kinetics and simplified Higuchi model, and the release constant, K was calculated. The release of MTZ from polyethylene glycol bases followed, first and Higuchi order release model, while gelatin and emulsion obeyed first model. The release of the drug from fatty bases followed either first order or Higuchi model. Table (IV).

Evaluation of Mucoadhesive Properties of Metronidazole Vaginal Suppositories

Vaginal suppositories formulated using PEG, emulsifying base with out additives and with cocoa base with out additives showed no work of bioadhesion while those prepared with gelatin bases, emulsifying base with additives and cocoa butter base with additives namely F15,F16,F17,F18 ,F19 ,F20 and F26 showed bioadhesion .

Table (V) and Fig. (5a) and Fig. (5b) indicate that the higher bioadhesion was for gelatin suppositories F15>F16>F17>F18 as the propylene glycol increase the bioadheion strength decrease then F20>F19>F26

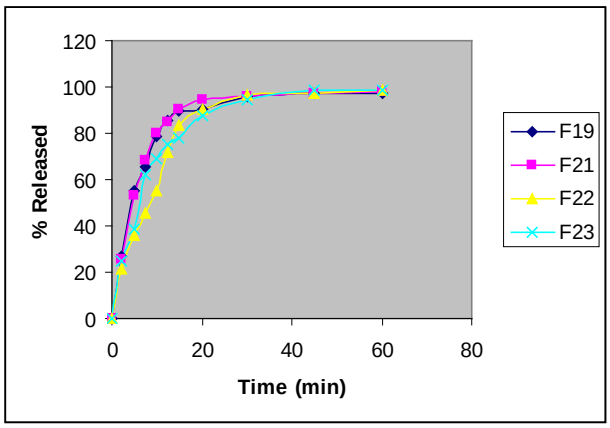


Fig. 3: In-Vitro Release of Metronidazole from Different Emulsion Suppository Bases in Citrate Buffer PH 4 at 37° C

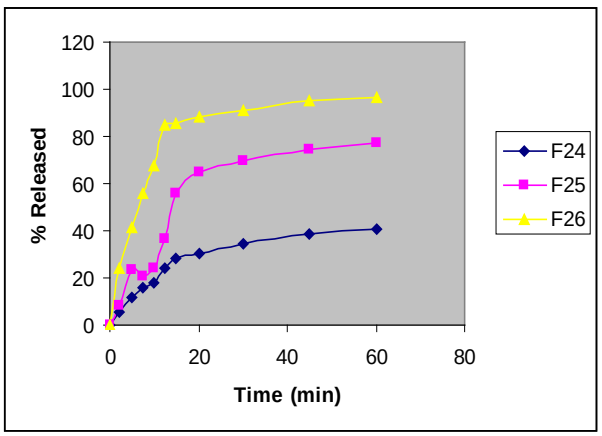


Fig. 4: In-Vitro Release of Metronidazole from Different Emulsion Suppository Bases in Citrate Buffer PH 4 at 37° C

Table IVV: The Kinetic Analysis of the Release of Metronidazole from Different Metronidazole Vaginal Suppository

Suppository Formulae		R^2			Release Order
Type	**Formula**	**Zero**	**First**	**Diffusion**	**Mechanism**
PEG	F1	0.742	0.933	0.87	First
PEG	F2	0.7022	0.8677	0.8638	First
PEG	F3	0.8708	0.965	0.9590	Firs
PEG	F4	0.676	0.8298	0.808	Diffusion
PEG	F6	0.728	0.860	0.880	Diffusion
PEG	F7	0.765	0.894	0.899	Diffusion
PEG	F9	0.734	0.967	0.887	First
PEG	F10	0.740	0.9125	0.883	First
PEG	F11	0.750	0.903	0.893	First
PEG	F12	0.738	0.8901	0.8839	First
PEG	F13	0.852	0.921	0.9299	Diffusion
PEG	F14	0.477	0.63	0.6411	Diffusion
Gelatin	F15	0.779	0.9318	0.9009	First
Gelatin	F16	0.8800	0.9670	0.96223	First
Gelatin	F17	0.8692	0.9847	0.959	First
Gelatin	F18	0.834	0.9475	0.922	First
Emulsion	F19	0.559	0.888	0.7477	First
Emulsion	F21	0.540	0.8585	0.7322	First
Emulsion	F22	0.714	0.9183	0.86211	First
Emulsion	F23	0.7010	0.9943	0.8600	First
Fatty	F24	0.827	0.866	0.9415	Diffusion
Fatty	F25	0.7982	0.8737	0.8854	Diffusion
Fatty	F26	0.604	0.855	0.7803	First

Table V: The Peak Detachment Force and Work of Bioadhesion of Different Metronidazole Vaginal suppositories

Type	Peak detachment Force(N) ±S.D	Work of Bioadhesion (mJ)±S.D
F15	1.423±0.12	2.364±0.12
F16	1.235±0.14	1.895±0.16
F17	0.987±0.15	1.254±0.11
F18	0.895±0.14	1.201±.021
F19	1.069±0.05	1.58±.05
F20	1.021±0.04	1.68±0.012
F26	1.258±0.041	1.532±0.035

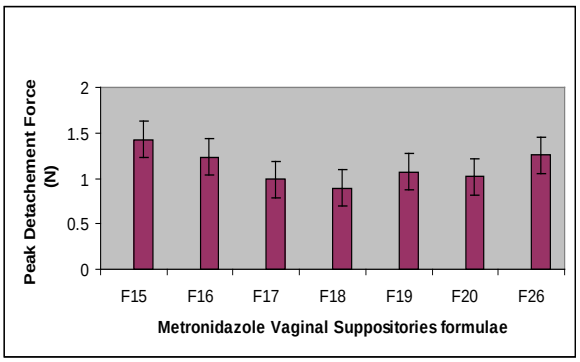


Fig. 5a: The Peak Detachment Force of Different Metronidazole Vaginal suppositories formulae

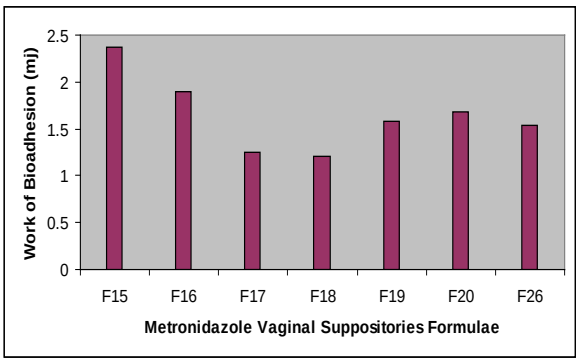


Fig. 5b: The Work of Bioadhesion of Different Metronidazole Vaginal suppositories formulae

(A) Microbial study

In the microbiological part of this study

- The 24-h incubation of the 1:10 suspension of *C. albicans* that had contacted the 0.25 mg/ml solution of MTZ for 15 min yielded the growth of the yeast on SDA. Plates.

- The 24-h incubation of the 1:10 suspension of *C. albicans* that had contacted the 0.50 mg/mI solution of MTZ for 120 mm yielded no growth of the yeast on SDA for 90 and 120 mm yielded no growth of the yeast on SDA plates.

-The 24-h incubation of the 1:10 suspension of *C. albicans* that had contacted the 0.75 mg/ml solution of MTZ for 120 and 180 mm yielded no growth of the yeast on SDA plates.

In other words

- The 0.25 mg/ml concentration of MTZ was not effective on *C. albicans* in 15 min, but showed fungistatic effect in 120 min.

- The 0.50 mg/mI concentration of MTZ was not effective on *C. albicans* in 30 min, but showed fungistatic effect in 120 min.

- The 0.5 mg/mI concentration of MTZ showed fungistatic effect on *C. albi cans* in 90 min as well as in 120 min.

- The 1 mg/ml concentration of MTZ showed fungi- static effect on *C. albicans* in 120 min as well as in 180 min Fig.s .

The quantity of MTZ in mg/ml released from the prepared bioadhesive gel, tablets and suppository were given in Chapter 1, 2 and 3. In the microbiological part of this study, 0.25 mg concentration of the drug totally inhibited the growth of *C. albicans* in 120 min coinciding with.Yesmin et al,who studied Efficacy of a new ketoconazole bioadhesive vaginal tablet on Candida albicans (10) . In the light of this finding, it can be stated that bioadhesive formulae that released the concentration of 0.25 mg/ml of the drug and sustained this concentration for 120 mm can be effective on *C. albicans.* For this theory, when bioadhesive tablets prepared were examined according to the dissolution results, all the bioadhesive tablet formulations were fitting to the criteria stated above (in Chapter 1, 2 and 3). However, among the formulae prepared the best, almost ideal, sustained release was obtained with pluronic –cp bioadhesive vaginal gel , F23 Vaginal suppository and C3 bioadhesive vaginal tablet.

(B) The Bioavailability Study

I. Assay validation of Metronidazole in rabbit plasma

Fig. 7 shows representative chromatograms of; (a) Blank rabbit plasma, (b) Rabbit plasma spiked with metronidazole and scinidazole (internal standard). Metronidazole and scinidazole were eluted after 5.3 and 3.38minutes, respectively. As can be seen from Fig. 7, a good separation of the drug and the internal standard was achieved under the specified chromatographic conditions. No interfering endogenous peaks were observed at the retention times of either metronidazole or the internal standard when blank rabbit plasma was analyzed.

The peak area ratio of metronidazole to the internal standard in rabbit plasma was linear with respect to metronidazole concentration over the range .01-20µg/mL. The linear regression equation of the calibration curve was: Y = -0004X - 0.0292, where *Y* and X are metronidazole / scinidazole peak area ratio and metronidazole concentration in ng/mL, respectively. The determination coefficient (r^2) between peak area ratio and metronidazole concentration was 0.9993 over the concentration range used.

Data for the extraction recovery of metronidazole from plasma samples fortified with the drug in concentrations of 10, 20, 30, 50, 100, 200,500 and 1000 ng/mL . The mean extraction recovery was 96.1±6.228965

II. Bioavailability of Metronidazole from the tested formulae

Plasma metronidazole concentrations obtained following a single vaginal dose administration of 500mg of the market product (Flagyl®) suppository, and the three selected formulae; F23 Vaginal suppository , C3 bioadhesive vaginal tablet and pluronic –cp bioadhesive vaginal gel, to four rabbits are compiled in tables (VI-XI). The mean plasma metronidazole concentrations versus time are graphically illustrated in Fig. 78. The individual and the mean pharmacokinetic parameters calculated from metronidazole plasma concentration-time data of the rabbits following the administration of each of the tested formulae are shown in tables (VI-XI) and Fig. (6). The mean values for the maximum plasma concentration (Cpmax) were 13133.1 ± 4846.719634, 12618.025 ± 7444.32,7501±1204.61 and 1114.86 ± 186.36 ng/mL after vaginal administration of the market formula, tested suppository, tested bioadhesive tablets and tested gel to four rabbits, respectively. One way ANOVA analysis showed a significant change between treatments (p=0.016). In addition the mean values for the area under plasma concentration-time curve AUC(0-24) were 51009.038 ± 25145.2, 235543.53 ± 329870.28,3147.003±4981.19 and 6163.32 ± 1078.8 ng.hr/mL, for the four previously mentioned formulations, respectively. One way ANOVA analysis for AUC(0-24) showed that the P value is 0.3512, considered not significant variation among the treatments means is not significantly greater than expected by chance. Also the mean values for the area under plasma concentration-time curve AUC(0-∞) were 43755.443 ± 19224.998, 43285.487 ± 26154.975,33006.392±4981.19 and 6714.3229 ± 1258.56 ng.hr/mL, for the four previously mentioned formulations, respectively. One way ANOVA analysis for AUC(0-∞) showed that the P value is 0.0502, considered not quite significant. Variation among treatment means is not significantly greater than expected by chance.

Table V: Plasma Concentration of Metronidazole Following Vaginal Adminisatration of the Commercial Available Product to four Rabbits (500mg Flagyl Suppository)

Time	Plasma Concentration (ng/ml) of four Rabbits					
	1	2	3	4	Mean	± S.D
0	0	0	0	0	0±	0
0.5	5402	3167.5	10752	13834.5	8289±	4877.96
1	14302	5267	14445.2	18518.2	13133.1±	5596.51
1.5	8627	4327	12405.2	13190.2	9637.35±	4062.198
2	3849.5	2719.5	9567.7	11727	6965.925±	4365.826
2.5	1927	1876.5	10452	10387	6160.625±	4917.84
3	1127	1129	6152	11387	4948.75±	4902.215
4	386.25	1013	7094.5	7539.5	4008.3125±	3833.409
6	295.25	950	4077	4884	2551.5625±	2267.39
8	213.75	920	1889.5	4059.5	1770.6875±	1673.37
12	735.75	901.75	924	498.175	764.91875±	196.6661
24	69.5	558	890.2	254	442.925±	359.8315

Table VI : Pharmacokinetic Parameters of Metronidazole Following Vaginal Adminisatration of the Commercial Available Product to four Rabbits (500mg Flagyl Suppository)

Parameter	Plasma Concentration (ng/ml) of four Rabbits					
	1	2	3	4	Mean	± S.D
Cp_{max}(ng/ml)	14302	5267	14445.2	18518.2	3933.05±	6357.58407
T_{max}(hour)	1	1	1	1	1±	0
$AUC_{(0-24)}$ (ng.hr/ml)	26013.3	26267	14445.2	70622	51009.038±	25145.218
$AUC_{(0\infty)}$(ng.hr/ml)	26312	28682.584	74475.31	82233.417	43755.443±	19224.998
$t_{1/2}$ (hour)*	2	2.5	2.6	3	2.525±	0.356195
Kel($(hour^{-1})$**	0.3465	0.2772	0.2665	0.231	0.2803±	0.041872

* Elemination half life; ** Elimination rate constant.

Table VII : Plasma Concentration of Metronidazole Following Vaginal Adminisatration of the 500 mg Metronidazole tested vaginal suppository to four Rabbits

Time	Plasma Concentration (ng/ml) of four Rabbits					
	1	2	3	4	Mean	± S.D
0	0	0	0	0	0±	0
0.5	3131.1	17889.5	16832	3084.5	12921.78±	8240.284
1	4754.25	21477	16054.5	9809.5	15200.99±	7288.322
1.5	1144.5	17072	6151.75	9027	9389.613±	6665.412
2	328.75	16289.5	5677	6652	8505.563±	6644.484
2.5	876.75	13027	3354.5	6259.5	6911.313±	5248.31
3	1171.25	13417	1379.5	4787	6838.688±	5730.509
4	230	3694.5	1127	2599.5	3147.75±	1537.889
6	454.25	4435.25	938.75	2016	2678.063±	1773.906
8	113.75	1053.1	651	1263.5	1469.338±	506.1332
12	136.15	965.5	354	781	488.4563±	380.9432
24	98.3	412	188	636.6175	238.075±	241.1995

Table VIII: Pharmacokinetic Parameters of Metronidazole Following Vaginal Adminisatration of the 500 mg Metronidazole tested vaginal suppository to four Rabbits.

Parameter	Plasma Concentration (ng/ml) of four Rabbits					
	1	2	3	4	Mean	± S.D
Cp_{max}(ng/ml)	3131.1	21477	16054.5	9809.5	12618.025±	6858.942286
T_{max}(hour)	1	1	1	1	1	0
$AUC_{(0-24)}$ (ng.hr/ml)	58119.86	806707.8	34550.5	70622	235543.53±	329870.28
$AUC_{(0\infty)}$(ng.hr/ml)	9695.404	82530.311	35364.353	45551.879	43285.487±	26154.975
$t_{1/2}$ (hour)*	2.3	2.2	3	2.5	2.5±	0.308221
Kel($(hour^{-1})$**	0.301	0.315	0.231	0.2772	0.28105±	0.0319

* Elemination half life; ** Elimination rate constant.

From the previous results, it is evident that in comparison with the market product, the tested suppository showed enhancement of drug absorption from tested suppository but regarding the bioadhesive tablet and gel, it was clear that it did not exhibit enhancement in bioavailability in comparison to the market product which mean lower side effects and localized effect inside the vagina.

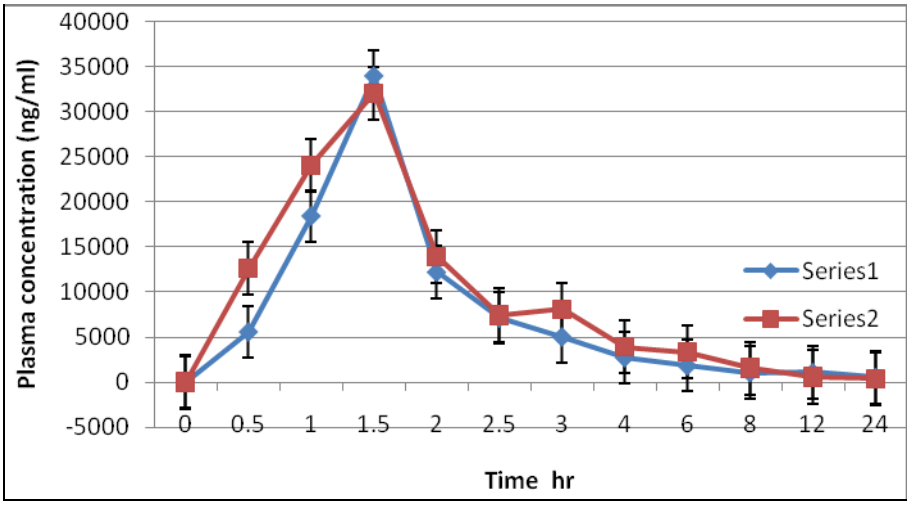


Fig. 6: Mean Plasma Metronidazole Concentration Following single Vaginal Dose of F23 Vaginal suppository and the market product

CONCLUSION

On the basis of the previous findings, the following could be concluded, Water soluble suppository bases gave higher release than did the emulsion in citrate buffer pH 4. Among the different bases used. PEG base (F14), gelatin base (Fl8) and cocoa base with additives (F26) gave the highest drug release and selected for further investigation. The release of MTZ from polyethylene glycol bases followed, first and Higuchi order release model, while gelatin and emulsifying base with or with out additives obeyed first model except f(23) which obeyed Higuchi model. The release of the drug from cocoa butter base with or with out additives followed either first order or Higuchi model. Water soluble bases showed no bioadhesion while gelatine bases showed the highest work of adhesion.The cocoa butter base and the emulsifying base showed no bioadhesion but the addition of bioadhesion polymers lead to increase their work of bioadhesion. The tested suppository showed enhancement of drug absorption from tested suppository and the One way ANOVA analysis for AUC(0-∞) showed that the P value is 0.0502, considered not quite significant. Variation among treatment means is not significantly greater than expected by chance. tested suppository did not exhibit enhancement in bioavailability in comparison to the market product which mean lower side effects and localized effect in side the vagina also it can be stated that bioadhesive formulae that released the concentration of 0.25 mg/ml of the drug and sustained this concentration for 120 min can be effective on *C. albicans.*

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