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FORMULATION AND EVALUATION OF BIOADHESIVE VAGINAL SUPPOSITORIES CONTAINING MICONAZOLE NITRATE

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ABSTRACT

Miconazole nitrate is an imidazole derivative antifungal agent, developed by Janssen Pharmaceutica, commonly applied topically to the skin or to mucous membranes to cure fungal infections. Over 90% of miconazole nitrate is reported to be bound to plasma proteins. In this study, miconazole nitrate was formulated into bioadhesive vaginal suppositories using different suppository bases such as fatty base, emulsion base and water soluble bases. Bioadhesive polymers (e.g Hydroxyethyl cellulose, sodium alginate and carbopol 934) are incorporated into the fatty and emulsion bases by the use of surfactant as tween 80. Studies are carried out to detect the effect of polymer addition at different concentration levels on the physicochemical properties and the in vitro release pattern. Analysis of the release data was carried out to determine the release kinetics of miconazole nitrate from all bases under investigation. Clinical evaluation of the efficacy of a single vaginal daily dose of miconazole nitrate formulated in different bioadhesive suppositories was carried out by selecting three formulations. They are evaluated for their clinical efficacy in treatment of vaginitis using 60 patients where they use 200 mg miconazole nitrate suppositories intra-vaginally once daily for 5 consecutive days and compared with conventional suppositories (Gynozol) which contain 200 mg drug, via trying on extra 20 patients. Bioadhesive vaginal suppositories showed 100% of the cured cases with the patient group received formula F 17, 90% of the cured cases with the patient group received formula F 9 and 85% of the cured cases with the patient group received formula F 4. On the other hand, Gynozol suppositories gave the lowest percentage of the cured cases, 70%.

Keywords: Miconazole nitrate, bioadhesive polymers, bioadhesive suppositories and clinical evaluation.

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INTRODUCTION

Vaginal delivery is an important route of drug administration for both local and systemic disease. The vaginal route has some advantages due to its large surface area, rich blood supply, avoidance of the first pass effect, relatively high permeability to many drugs and self-insertion [1&2]. The traditional 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[3]. The vaginal route appears to be highly appropriate for bioadhesive drug delivery systems in order to retain drugs for treating largely local conditions or for use in contraception. To prolong the residence time in the vaginal cavity, bioadhesive therapeutic systems have been developed in the form of semisolid and solid dosage forms. Bioadhesive systems have been widely investigated by Robinson et al.[4-6] and Nagai[7].

Miconazole nitrate is an antifungal agent that works by inhibiting the synthesis of ergosterol, a critical component of fungal cell membranes. It can also be used against certain species of leishmania protozoa which are a type of unicellular parasite that also contain ergosterol in their cell membranes. It has also some limited antibacterial properties [8].

MATERIALS AND METHODS

Materials

Miconazole nitrate was kindly supplied by PHARCO Pharmaceuticals Co., Alex, Egypt, Witepsol H-15 (Dynamit Nobel, Germany), Polyethylene glycols (PEG formula) 400 and 8000 (Union Carbdie, New York), Gelatin (BDH, UK), Carbopol 934 (B.F. Goodrich Co, USA), Hydroxyethyl cellulose (Kock-light Laboratories, Ltd., Golnbrook, bucks, England), Sodium alginate (BDH chemicals, Ltd., Poole, England), Potassium dihydrogen phosphate, glycerol, triethanolamine and tween 80 (Adwic, El Nasr Chem Co., Egypt), Methyl and propyl paraben (Nipa Lab., Hamburg, West Germany).All chemicals either of analytical or pharmaceutical grade were used without further purification.

Equipment

UV spectrophotometer (Jenway LTD, UK, Felsted, Dunow, Essex, CM6 3LB, Model 6105 UV/Vis, England), Thermostatically controlled shaking water bath (Grant instrument Cambridge Ltd., Barrington Cambridge, B2, 5002, England), pH meter (Beeckman Instr., Inc, USA), Magnetic stirrer (Heidolph, USA), Cellulose membrane, Spectrapor, M.W. cut-off 1200-1400 (Fisher Sci., Co., USA), Two-gram capacity suppository mould (ERBO), Prazisions Formenbeu GmH, Mod 128 BI Kauale Wester, Germany), Erweka hardness tester for suppositories (model SBT, Germany), Erweka deformation tester (model SSP, Germany).

Methodology

1.Spectrophotometric scanning of miconazole nitrate in presence of different suppository bases

A specified concentration of miconazole nitrate in the presence of all investigated suppository bases (each one individually) using phosphate buffer pH 4.75 were scanned spectrophotometrically at 200-400 nm to determine the wavelength of maximum absorption (λ_{max}).

2.Calibration of the mould

The mould was calibrated for suppository base used, by preparing suppositories using the base alone, weighing suppositories and taking the mean weight as the calibration factor[9].

3.Determination of the displacement value

It is necessary to determine the displacement value with respect to the suppository bases used for different formulations. The displacement value could be calculated using

formula: DV = xA / (B-C), where DV is the displacement value, x is the percentage of drug used, A is the weight of suppositories containing x% of the drug, B is the weight of un-medicated suppository, and C is the weight of the base in the medicated suppositories[10].the quantity of the base required is calculated using the following equation P = (NB) – F / DV where P is the amount of base required in grams, N is the number of suppositories to be prepared, B is the weight of non medicated suppositories, while F is the amount of drug in grams that is required.

4.Preparation of miconazole nitrate suppositories using plain bases

An oleaginous base and water soluble bases were used to formulate and prepare miconazole nitrate suppositories. Each vaginal suppository contains 200 mg of the drug. The fatty base employed was witepsol H-15 while water soluble bases were glycerinated gelatin and polyethylene glycols. For glycerinated gelatin base, 100 gm was prepared by the ratio 14:70:QS, for gelatin : glycerin : purified water, respectively[11] and PEG formula prepared in mixture ratio of 40:50:10 for PEG 8000 : PEG 400: distilled water[12].All batches of suppositories, except glycerinated gelatin batches, were prepared by fusion method[13], while glycerinated gelatin suppositories were prepared according to the method mentioned in The British Pharmacopoeia, 2011.

5.Preparation of miconazole nitrate suppositories using oleaginous base containing non ionic surfactant (tween 80)

Two gram witepsol H-15 suppositories containing 200 mg miconazole nitrate were prepared with different concentrations of non-ionic surfactant, tween 80 (T 80) which are 1, 3 and 5% w/w (Table 1). The drug was levigated with the specified amount of the surfactant and the molten base was then added with continuous stirring and just before congealing, the mass was poured into the moulds and allowed to solidify at room temperature. Not more than 5% of T 80 was used because higher surfactant concentrations produce retardant and/or irritative effects [14].

Table 1
Formulations of miconazole nitrate (200 mg) vaginal suppositories using different bases

formula	Suppository base Type		Conc (%w/w)	Tween 80(%w/w)	Distilled water(%w/w)	Bioadhesive polymer Type	conc (%w/w)	Drug content (mg) Theoretical	actual
F1	Fatty base	W H-15	100	--	--	--	--	200	199.1
F2		W H-15	99	1	--	--	--	200	198.9
F3		W H-15	97	3	--	--	--	200	200.1
F4		W H-15	95	5	--	--	--	200	199.9
F5	Emulsion base	W H-15	64	5	30	HEC	1	200	197.7
F6		W H-15	63	5	30	HEC	2	200	198.8
F7		W H-15	62	5	30	HEC	3	200	193.2
F8		W H-15	64	5	30	Sodalg	1	200	199.7
F9		W H-15	63	5	30	Sodalg	2	200	198.6
F10		W H-15	62	5	30	Sodalg	3	200	203.6
F11		W H-15	64	5	30	Carb934	1	200	197.9
F12		W H-15	63	5	30	Carb934	2	200	199.5
F13		W H-15	62	5	30	Carb934	3	200	193.1
F14		**Glycerinated Gelatin (B.P)**						200	198.7
F15	Water soluble bases	PEG8000/400	40:50	--	10	--	--	200	199.9
F16		PEG8000/400	40:50	--	9	HEC	1	200	201.7
F17		PEG8000/400	40:50	--	8	HEC	2	200	199.1
F18		PEG8000/400	40:50	--	7	HEC	3	200	198.9
F19		PEG8000/400	40:50	--	9	Sodalg	1	200	198.7
F20		PEG8000/400	40:50	--	8	Sodalg	2	200	199.1
F21		PEG8000/400	40:50	--	7	Sodalg	3	200	199.2
F22		PEG8000/400	40:50	--	9	Carb934	1	200	201.1
F23		PEG8000/400	40:50	--	8	Carb934	2	200	198.7
F24		PEG8000/400	40:50	--	7	Carb934	3	200	199.8

****W H-15:witepsol H-15 PEG: polyethylene glycol HEC: hydroxyethyl cellulose***
Sod alg: Sodium alginate Carb 934: carbopol 934

6.Preparation of miconazole nitrate suppositories containing different bioadhesive polymers

Miconazole nitrate suppositories were prepared using PEG formula and witepsol H-15 containing the selected bioadhesive polymers which are: Hydroxyethyl cellulose (HEC), Sodium alginate and Carbopol 934, each polymer used in three different concentrations (1, 2 and 3% w/w) as listed in table (1).

6.1.Preparation of miconazole nitrate suppository using PEG-formula containing different bioadhesive polymers

The specified amount of each polymer was added slowly to the calculated amount of distilled water with vigorous stirring. The pH of the dispersion of carbopol 934 was adjusted to 7.0 using triethanolamine. The polymeric phase was added to the melted base gradually with continuous stirring till a homogenous fluid is formed. Finally the drug was incorporated.

6.2.Preparation of miconazole nitrate suppository using witepsol H-15 containing different bioadhesive polymers

The fatty suppository bases containing bioadhesive polymer was prepared by the emulsification of the aqueous phase with the melted fatty base using tween 80 as an emulsifier. Several trials were carried out to prepare a stable emulsion that will be suitable physically as a suppository base and finally the best formula obtained was listed in table (1) and used for preparation of miconazole nitrate suppositories. When I tried to increase the concentration of bioadhesive polymer, it was difficult to incorporate the polymer in the mixture to ensure homogeneity. Before starting emulsification, the surfactant was mixed with the aqueous phase of the polymer. The melted fatty base was then added. The temperature was maintained at 40 ºC during the emulsification process and the impeller speed was kept at 200 rpm for the first five minutes then raised to 400 rpm for 25 minutes. The produced emulsion was kept for 12 hours at 40 ºC [(15)]. Then the drug was added to the prepared base while stirring and finally the product was molded and left to solidify overnight. All the prepared suppository batches were stored at 5 ºC for three days and then left at room temperature for 24 hours before testing.

7.Evaluation of miconazole nitrate suppositories

7.1.Weight variation

The weight variation was determined according to British Pharmacopoeia, 2011.

7.2.Uniformity of drug content

Uniformity of drug content was carried out according to the British Pharmacopoeia method, 2011. Ten suppositories were taken at random from each formula and assayed individually. A pre-weighed suppository was melted and dispersed in 50 mls of phosphate buffer pH 4.75. the solution was diluted with the same buffer to 100 mls and the containers were allowed to rotate in a constant temperature water bath at 37 ºC and at 120 rpm for two hours. The filtered samples were diluted and assayed spectrophotometrically at λ_{max} 316 nm. The concentrations were calculated against blank plain suppository bases with reference to the previously constructed calibration curve.

7.3.Measurement of pH value

The pH values of miconazole nitrate suppositories were determined by the method reported by Kassem et al., 1984[(16)].

7.4.Measurement of mechanical strength

The test is used to measure the mass (in kilograms) that a suppository can bear without breaking. The hardness of the suppository formulations was evaluated using Erweka Hardness Tester at room temperature. Suppository under test is positioned in an upright position and weights are added on it, starting with 600 gm weight and then add 200 gm weights every minute until the suppository broken. The weight under which a suppository collapsed was taken as the hardness of the suppository [(17&18)].

7.5.Measurement of melting zone

Melting zone or range is the term often preferred by some rather melting point because many suppository bases and medicated suppositories are mixtures and so do not have a precise melting point [(19)].the test was carried out according to Senior, 1974[(20)]. In this test a capillary tube, 8-10 cm in length and 1.0 - 1.2 mm in diameter, open at both ends was used. One end of the tube was dipped into the melted suppository to a depth of about 1 cm and then allowed to harden at room temperature. The capillary tube was then attached to a thermometer opposite to its bulb and placed in a thermostatically controlled water bath. The melting range was recorded when the contents of the capillary tube started to melt and rise under water pressure and when complete melting was observed.

8.Measurement of deformation time

The deformation time was determined according to The British Pharmacopoeia, 2011, using Erweka suppository deformation tester. Each suppository put in distilled water in the glass tube of the tester in a water bath equilibrated at 36.5 ±0.5 ºC and the tester rod was introduced until its end touches the flat end of the suppository. The time elapsed until the rod sinks down to the bottom of the glass tube was recorded as a deformation time.

9.In vitro release of miconazole nitrate from mucoadhesive vaginal suppositories

The dialysis method was used using modified Krowezinski method [21] in which one suppository was placed in a glass tube (10 cm^3) with cellophane membrane firmly tied at its end containing 5 ml of phosphate buffer (pH 4.75). The tube was vertically suspended in 250 ml beaker containing 100 ml of phosphate buffer (pH 4.75) and adjusted so that the membrane was just below the surface of the release medium. The temperature was maintained at 37±0.5 ºC using a thermostatically controlled shaking water-bath (at 25 rpm). One ml sample was withdrawn at predetermined time intervals and replaced by equal volume of fresh buffer solution at the same temperature. Samples were measured spectrophotometrically at λ_{max} 316 nm after appropriate dilutions against the same used phosphate buffer as a blank.

10.Analysis of the release data

In order to determine the release model which describes the pattern of drug release, the in vitro release data were analyzed according to zero-order, first- order and according to Higuchi diffusion model [22].

11.Clinical evaluation of miconazole nitrate bioadhesive vaginal suppositories

Three formulations (F4, F9 and F17), which gave the highest in vitro release and good results for the physicochemical properties, were selected for clinical study. Each formulation was used by 20 female patients suffering from trichomonas vaginalis. On the other hand, extra 20 patients received conventional treatment (Gynozol suppository) for comparison. Local treatment was achieved by using one 200 mg miconazole vaginal suppository intravaginally once daily at bed time for 5 consecutive days. Results of clinical evaluation and direct microscopic examination were recorded on an evaluation sheet.

RESULTS AND DISCUSSION

Spectrophotometric scanning of miconazole nitrate in phosphate buffer (pH 4.75) alone and in presence of witepsol H-15, PEG formula or glycerinated gelatin was carried and it was found that the spectrum of miconazole nitrate is not affected by the presence of any of the investigated suppository bases and its maximum absorption was at λ_{max} 316 nm. By calibration of the mould, the average weight of different suppository bases used was 2.6, 2.5, 2.1 and 2.01 gm for glycerinated gelatin, polyethylene glycol formula, witepsol H-15 and emulsion bases, respectively.The displacement values of miconazole nitrate in the used bases were calculated and found to be 1.253, 1.120, 1.374 and 1.565 for glycerinated gelatin, polyethylene glycol formula, witepsol H-15 and emulsion bases, respectively. The weight variation and drug content uniformity of the prepared suppositories were found to comply with The British Pharmacopoeia requirements. Highest percent of deviation in weight from the average weight for all suppository batches prepared was 3.5%. Also, the drug content of the selected suppositories tested from each formulation did not deviate more than 5.6% from their labeled contents. The pH values of miconazole nitrate suppositories formulated in glycerinated gelatin, PEG formula, witepsol H-15 were found to be 5.57, 5.30 and 5.68, respectively (Table 2).Measurement of the mechanical strength of the prepared suppository revealed that witepsol H-15 had higher hardness value (2.2 kg) than PEG formula (1.9 kg), while the glycerinated gelatin base could not be evaluated for hardness due

to its elastic properties (Table 2). Concerning the melting range (zone), the prepared suppositories containing glycerinated gelatin showed the highest melting range (45-46 ºC) followed by PEG formula (42-43 ºC) and at last, witepsol H-15 (33-34 ºC) as shown in Table 2. Regarding the deformation time, results revealed that glycerinated gelatin suppository takes the highest time for deformation (45 min) followed by PEG formula (43 min), while witepsol H-15 had the lowest time for deformation (4 min) as shown in Table (2).These results comply with The British Pharmacopoeia, 2011 which allows 30 min for fatty base disintegration and 60 min for water soluble one. Also, results of deformation times of suppository under investigation correlated with their melting range values, i.e as the melting range increased, the deformation time increased. The pH values of miconazole nitrate suppositories formulated in all tested bases did not vary significantly after incorporation of tween 80. Also, the deformation time of suppositories formulated with witepsol H-15 and tween 80 in concentration 1,3 and 5% decreased up to 2 min for 5% tween 80 and 1 min for 1 and 3% tween 80 when compared with witepsol H-15 alone. The resultant decrease in deformation time upon incorporation of surfactant (tween 80) was in agreement with that reported by Ibric et al. [23], where the disintegration time of suppositories decreased by nonionic surfactant. From Table (2), it is obvious that there was no pronounced effect of surfactant on the melting range. From the results obtained, we can conclude that incorporation of tween 80 decreased the deformation time and the hardness of the investigated fatty base suppositories and the effect is dependent on the concentration of tween 80. The effect was more pronounced as the concentration of the added surfactant increased.

Table 2

Physical characterization of miconazole nitrate vaginal suppositories prepared using plain and fatty suppository bases containing different concentrations of tween 80

Formula no.	Type of base	Average weight (gm±SD)	pH	Mean hardness (kg±SD)	Melting range (ºC)	Mean deformation time (min±SD)
F14	Glycerinated-gelatin	2.6(±0.03)	5.57	--	45-46	45(±2.5)
F15	PEG	2.5(±0.01)	5.30	1.9(±0.1)	42-43	43(±2)
F1	W H-15	2.1(±0.01)	5.68	2.2(±0.5)	33-34	4(±0.5)
F2	WH15+1%T80	2.09(±0.03)	5.86	2.1(±0.3)	33-33.9	3(±0.21)
F3	WH15+3%T80	2.04(±0.01)	5.89	1.9(±0.25)	33-33.6	3(±0.2)
F4	WH15+5%T80	2.05(±0.02)	5.92	1.8(±0.31)	32.8-33.2	2.1(±0.3)

Addition of bioadhesive polymers, hydroxyethyl cellulose, sodium alginate and carbopol 934 into PEG formula and witepsol H-15 did not affect significantly pH values of the prepared suppositories (Table 3). Concerning the physical parameters of PEG suppositories after incorporation of bioadhesive polymers (Table 3), it was observed that increasing the polymer concentration was accompanied by an increase in hardness of PEG formula suppositories, the suppositories prepared with carbopol 934 were found to be the strongest against breaking forces by those prepared with HEC, while sodium alginate containing suppositories showed the lowest breaking values among the mucoadhesive suppositories prepared. It is observed also that increasing the polymer concentration resulted in an increase in the melting range and consequently in the time required for the deformation of the suppositories.

Table 3
Physical characterization of miconazole nirate' vaginal suppositories prepared by PEG containing different bioadhesive polymers

Formula no.	Bioadhesive polymer Type	conc(%w/w)	Average weight (gm±SD)	pH	Mean hardne-ss (kg±SD)	Melting range (ºC)	Mean deformation time (min±SD)
F15		-	2.5±0.01	5.30	1.9±0.1	42-43	43±2
F16	HEC	1	2.4±0.02	5.25	2.1±0.2	43-44	49±2
F17		2	2.4±0.01	5.15	2.2±0.3	44-45	54±3
F18		3	2.5±0.03	5.0	2.3±0.1	44.5-45	58±0.1
F19	Sod alginate	1	2.5±0.01	5.3	1.9±0.4	43-43.5	45±2.5
F20		2	2.5±0.03	5.2	1.9±0.3	43-44	47±2
F21		3	2.5±0.02	5.1	2.0±0.2	43.5-44.5	50±1.5
F22	Carbopol 934	1	2.4±0.01	5.4	2.1±0.1	44-45	51±0.9
F23		2	2.5±0.03	5.2	2.2±0.3	45-46	55±0.8
F24		3	2.4±0.02	5.0	2.6±0.4	45-47	60±1.8

From table (4), it is obvious that changes in physical parameters were found to be in direct correlation to polymer concentrations. As the polymer concentration increased, the suppository melting range and deformation time increased. Hardness values showed nearly no marked change. The deformation time is most of the formulae containing witepsol H-15 and mucoadhesive polymers were increased by about two times the suppositories with no mucoadhesive polymers. The unexpected features concerning both deformation time and melting ranges compared to hardness values could be explained by Yahagi et al., 1999 [24] , where he explained that the emulsion produced from the emulsification process of witepsol H-15 and tween 80 as emulsifier, lead to unclear measurements for deformation test and melting range. Thus, it is possible to conclude that, as the polymer concentration increases in the suppository, the melting range and the deformation time increases for all the investigated polymers. The highest effect was observed with carbopol 934.

Table 4
Physical characterization of miconazole nirate' vaginal suppositories prepared using witepsol H-15 containing different bioadhesive polymers

Formula no.	Bioadhesive polymer Type	conc(%w/w)	Average weight (gm±SD)	pH	Mean hardness (kg±SD)	Melting range (ºC)	Mean deformation time (min±SD)
F1		--	2.1±0.01	5.68	2.2±0.5	33-34	4±0.5
F5	HEC	1	2.2±0.02	5.9	0.8±0.1	36.5-37	8±0.05
F6		2	2.1±0.03	5.8	0.7±0.2	36.5-37.5	8±0.01
F7		3	2.0±0.01	5.9	0.8±0.1	36.5-37.5	8.5±0.01
F8	Sod alginate	1	2.1±0.13	5.7	0.7±0.2	36-37	7.5±0.2
F9		2	2.2±0.13	5.9	0.8±0.3	36-37	7.5±0.05
F10		3	1.9±0.12	5.8	0.8±0.1	36-37.5	8±0.05
F11	Carbopol 934	1	2.2±0.03	5.7	0.7±0.3	36-37.5	9±0.03
F12		2	2.0±0.05	5.8	0.7±0.3	36-37.5	9±0.01
F13		3	2.1±0.09	5.8	0.8±0.1	36-38	9.5±0.05

Figure (1) showed the release of miconazole nitrate from different suppository bases in phosphate buffer pH 4.75 and at 37 ±1 ºC. witepsol H-15 showed higher release than glycerinated gelatin and PEG bases. The difference in the release from suppository bases seemed to be highly affected by variation in melting range and deformation time. The highest drug release from W H-15 may be attributed to the shorter deformation time (4 min). This is due to the fact that, the melting point as well as the softening time are the rate limiting factors in the release of drugs from fatty bases at 37 ºC [25] . Results are in agreement with that reported by Calis et al. [26]. Glycerinated gelatin suppository showed higher release rate when compared with PEG suppositories, this may be attributed to the higher aqueous solubility of glycerol-gelatin base. Figure (2) reveals the effect of addition of tween 80, as a non-ionic surfactant, to the fatty base W H-15. It is clear that increasing concentration of tween 80 resulted in increase in percent of miconazole nitrate released from the fatty base W H-15. Results were in agreement with Ibric et al. [23].

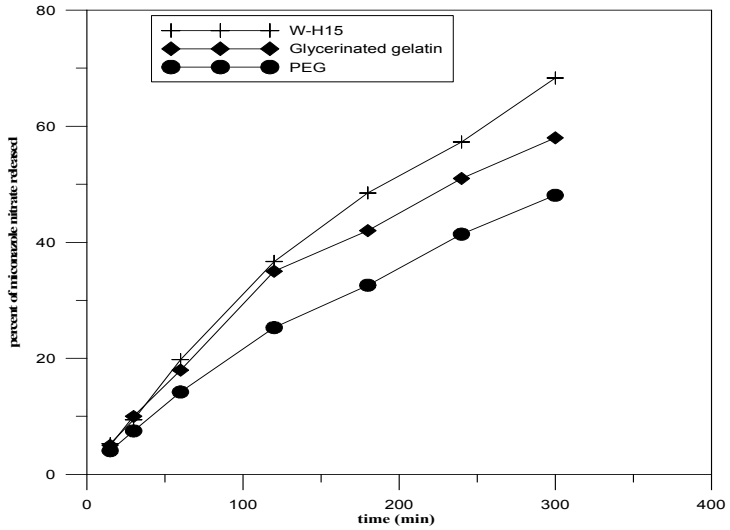


Fig. (1): Effect of different suppository bases on the in vitro release of miconazole nitrate in phosphate buffer pH 4.75 at 37±1 ºC

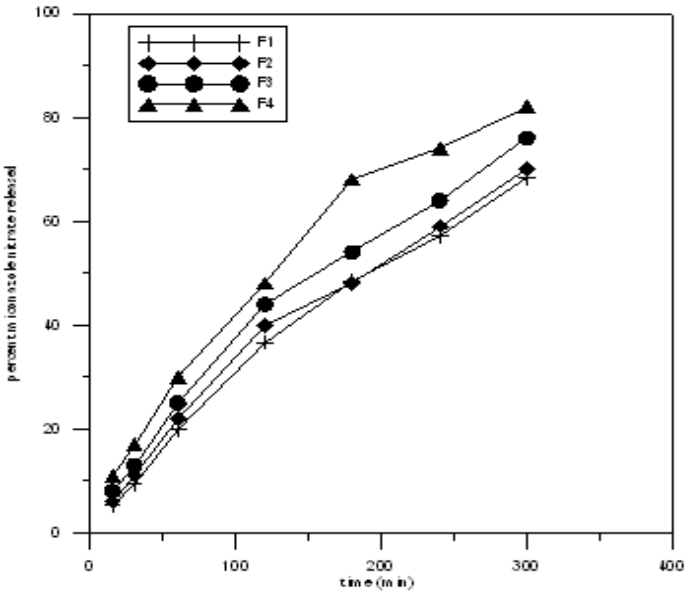


Fig. (2): Effect of different concentrations of tween 80 on in vitro release of miconazole nitrate from witepsol H-15 suppositories in pH suppositories in phosphate buffer pH 4.75 at 37±1 ºC

Figures (3-5) illustrate the effect of incorporation of different mucoadhesive polymers on release of miconazole nitrate from suppositories containing witepsol H-15. The amount of drug released was found to be in indirect correlation with polymer concentrations.

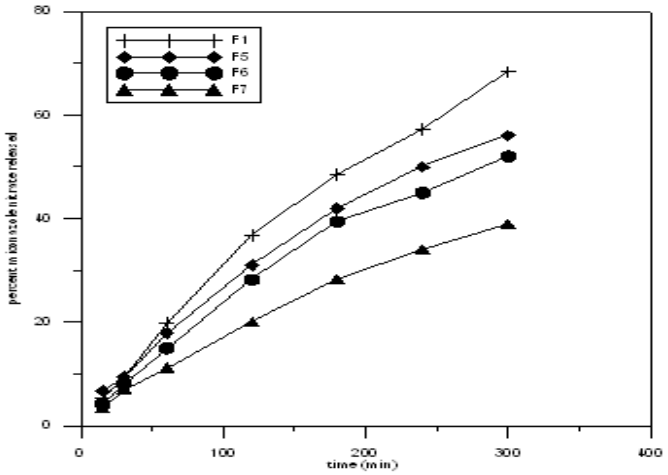


Fig. (3) In vitro release of miconazole nitrate from witepsol H-15 mucoadhesive vaginal suppositories containing different concentrations of HEC in phosphate buffer pH 4.75 at 37±1°C

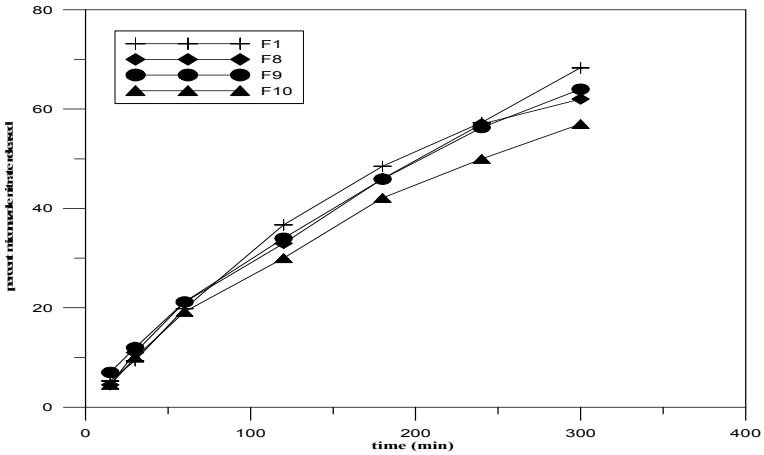


Fig. (4): In vitro release of miconazole nitrate from witepsol H-15 mucoadhesive vaginal suppositories containing different concentrations of sodium alginate in phosphate buffer pH 4.75 at 37±1 °C

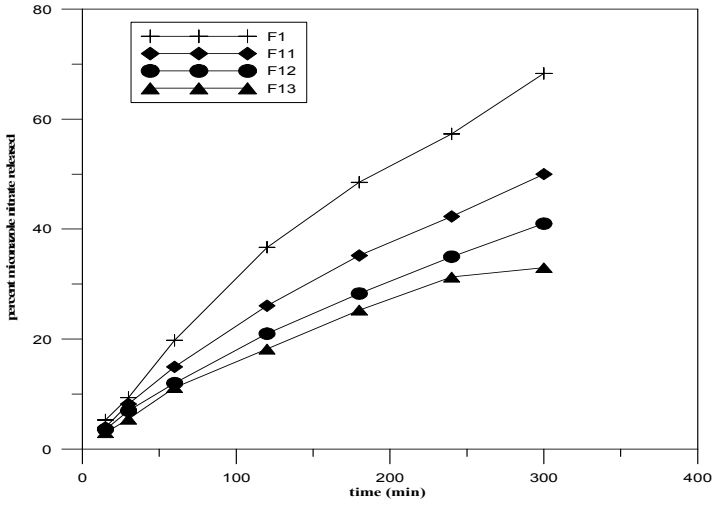


Fig. (5): In vitro release of miconazole nitrate from witepsol H-15 mucoadhesive vaginal suppositories containing different concentrations of carbopol 934 in phosphate buffer pH 4.75 at 37±1 °C

Figures (6-8) show the effect of different mucoadhesive polymers on release of miconazole nitrate from suppositories containing polyethylene glycols 8000 / 400, 40:50. The amount of drug released was found to be the highest with formula F 17 which contains HEC 2% followed by F 23 which contains carbopol 934, 2% followed by formula F 16 which contains HEC 1%.

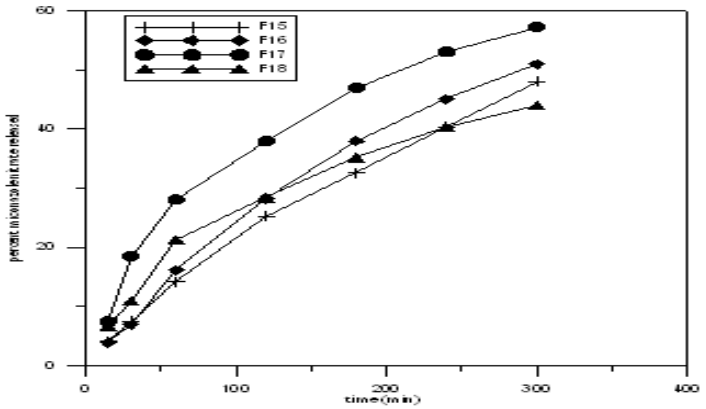


Fig. (6): In vitro release of miconazole nitrate from PEG-mucoadhesive vaginal suppositories containing different concentrations of HEC in phosphate buffer pH 4.75 at 37±1 °C

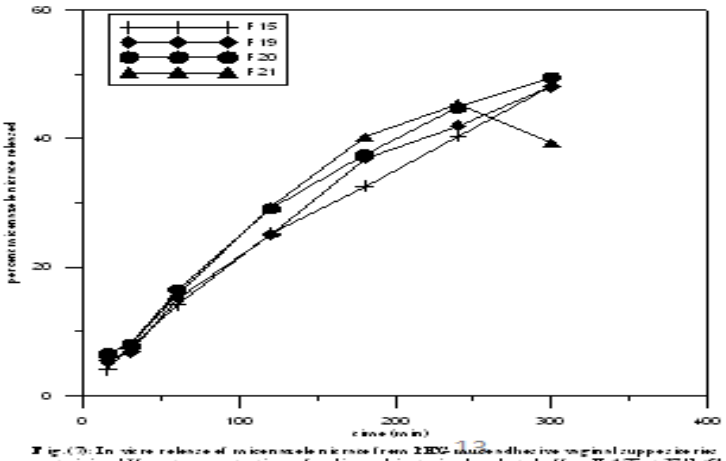


Fig. (7): In vitro release of miconazole nitrate from PEG-mucoadhesive vaginal suppositories containing different concentrations of sodium alginate in phosphate buffer pH 4.75 at 37±1 °C

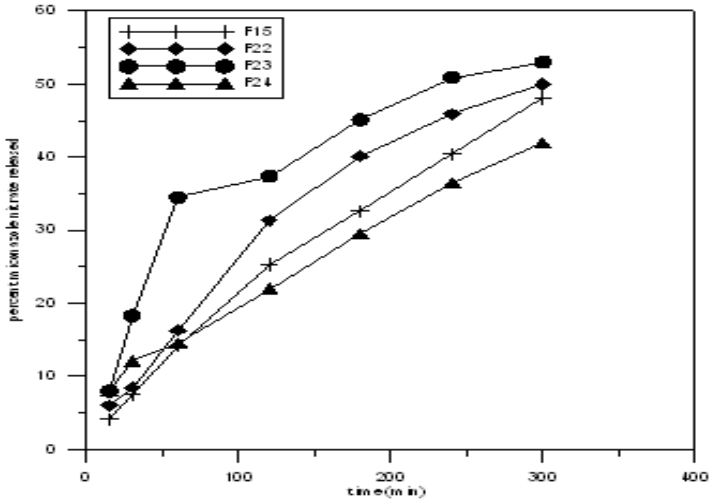


Fig. (8): In vitro release of miconazole nitrate from PEG-mucoadhesive vaginal suppositories containing different concentrations of carbopol 934 in phosphate buffer pH 4.75 at 37±1 °C

From the data obtained, it is clear that Witepsol H-15 gave the highest drug release after 30 min when incorporate tween 80 as a surfactant, as it released about 82% for F 4 which contain 5% T80 and released about 76% from F3 which contain 3% T80. This was explained by Desai et al. [27], who stated that, surfactants make more channels for drug release, thus increasing the effective porosity of the matrix. In addition, being an emulsifying agent, surfactant acts on the base-water interface reducing the surface tension, which can help the flow of medicament from the interface to the dissolution medium [28].The release data was analyzed mathematically according to zero-, first-order and Higuchi diffusion model. The exponential equation by Peppas [29] was used. The mathematical treatment of the release data was presented in Tables (5-8). Regarding the kinetic evaluation of the in vitro release data, it was evident that no one model was able adequately to describe the drug release profiles. Also, it was found that the water-soluble PEG-formula suppositories showed biphasic release profiles with two slopes, while witepsol H-15 mucoadhesive suppositories showed single-phase release profiles with one slope [30].

Concerning PEG formula suppositories, results (tables 5-7) indicated that, HEC containing suppositories showed the best fitting linear relations with the highest correlation coefficients were found with the Higuchi diffusion equation. PEG formula suppositories containing sodium alginate or carbopol 934 showed a combination of first-order and Higuchi release mechanisms, while zero-order kinetics showed the lowest correlation coefficients. For further confirmation, logarithm of released amount (log Q) was plotted against logarithm of time (log t) as reported by Peppas. By reviewing the data, it could be deduced that, the majority of the formulations exhibited values of n= 0.5 to 1 indicating an anomalous or non-Fickian diffusion release.

Table 5

kinetic modeling of miconazole nitrate (200 mg) released from PEG mucoadhesive vaginal suppositories containing different concentrations of hydroxyethyl cellulose

Polymer conc%w/w			1		2		3	
parameter			First phase	second phase	First phase	second phase	First phase	second phase
Zero-order	r_o		0.996	0.986	0.953	0.960	0.966	0.967
	K_o ($mg.min^{-1}$)		0.235	0.168	0.268	0.162	0.207	0.128
First-order	r_1		0.9980	0.997	0.969	0.984	0.973	0.982
	K_1x10^{-3} (min^{-1})		2.8	2.4	3.5	2.6	2.5	1.8
	$t_{1/2}$(min)		245		207		273	
Higuchi diffusion model	Q/A Vs $t^{1/2}$	r_h	0.997	0.999	0.989	0.991	0.991	0.999
		$Dx10^5$ ($mg.cm^{-2}.min^{-1}$)	1.826	2.091	2.580	1.433	1.495	1.135
	logQ Vs log t	r_h	0.9982	0.998	0.980	0.992	0.991	0.999
		slope	0.956	0.669	0.731	0.458	0.714	0.520

K = rate constant r = correlation coefficient D = diffusion coefficient

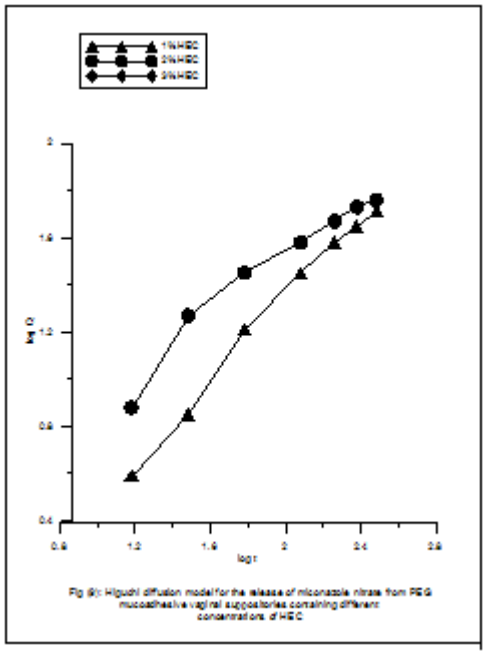


Fig (6): Higuchi diffusion model for the release of miconazole nitrate from PEG mucoadhesive vaginal suppositories containing different concentrations of HEC

Table 6

Kinetic modeling of miconazole nitrate (200 mg) released from PEG mucoadhesive vaginal suppositories containing different concentrations of sodium alginate

Polymer conc%w/w			1		2		3	
			First phase	second phase	First phase	second phase	First phase	second phase
parameter								
Zero-order	r_o		0.997	0.984	0.996	0.984	0.995	0.982
	K_o ($mg.min^{-1}$)		0.197	0.117	0.221	0.116	0.224	0.111
First-order	r_1		0.997	0.992	0.997	0.991	0.996	0.989
	K_1x10^{-3} (min^{-1})		2.35	1.87	2.687	1.932	2.705	1.817
	$t_{1/2}$(min)		327		304		314	
Higuchi diffusion model	Q/A Vs $t^{1/2}$	r_h	0.988	0.993	0.999	0.994	0.986	0.991
		$Dx10^5$ ($mg.cm^{-2}.min^{-1}$)	1.390	1.791	2.280	1.740	1.721	1.545
	logQ Vs log t	r_h	0.986	0.990	0.971	0.991	0.977	0.990
		slope	0.801	0.672	0.760	0.604	0.754	0.571

K = rate constant ***r = correlation coefficient*** ***D = diffusion coefficient***

Table7

Kinetic modeling of miconazole nitrate (200 mg) released from PEG mucoadhesive vaginal suppositories containing different concentrations of carbopol 934

Polymer conc%w/w			1		2		3	
parameter			First phase	second phase	First phase	second phase	First phase	second phase
Zero-order	r_o		0.997	0.982	0.887	0.960	0.990	0.991
	K_o ($mg.min^{-1}$)		0.244	0.106	0.256	0.090	0.118	0.116
First-order	r_1		0.998	0.989	0.901	0.970	0.991	0.999
	K_1x10^{-3} (min^{-1})		3.03	1.77	3.39	1.607	1.38	1.71
	$t_{1/2}$(min)		300		320		441	
Higuchi diffusion model	Q/A Vs $t^{1/2}$	r_h	0.989	0.992	0.940	0.975	0.990	0.999
		$Dx10^5$ ($mg.cm^{-2}.min^{-1}$)	2.12	1.41	2.616	1.019	0.488	1.667
	logQ Vs log t	r_h	0.988	0.993	0.957	0.981	0.989	0.999
		slope	0.830	0.526	0.944	0.410	0.441	0.751

K = rate constant r = correlation coefficient D = diffusion coefficient

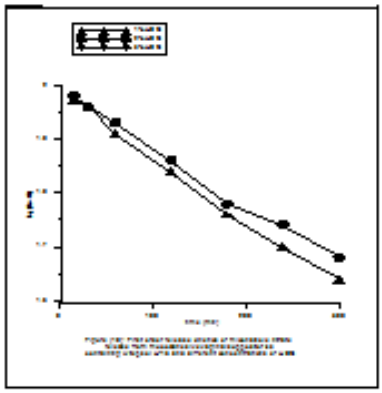
Table 8

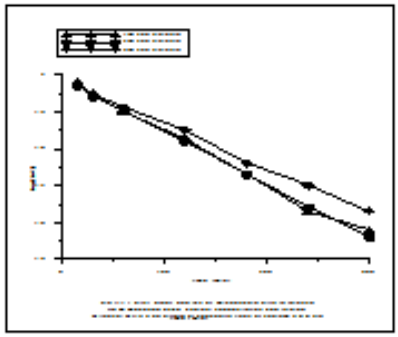
kinetic modeling of miconazole nitrate (200 mg) released from witepsol H-15 mucoadhesive vaginal suppositories containing different concentrations of different polymers

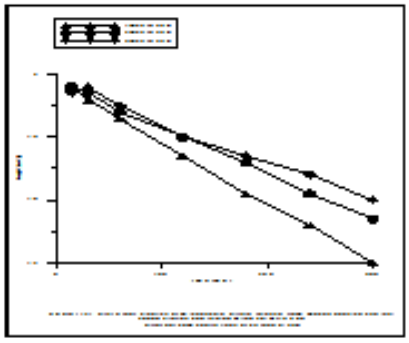
			HEC			Sod alginate			Carbopol 934		
			1	2	3	1	2	3	1	2	3
Zero-order	r_o		0.989	0.982	0.989	0.981	0.983	0.986	0.998	0.989	0.993
	K_o ($mg.min^{-1}$)		0.181	0.174	0.126	0.208	0.200	0.188	0.164	0.130	0.114
First-order	r_1		0.999	0.996	0.997	0.998	0.999	0.998	0.999	0.998	0.997
	K_1x10^{-3} (min^{-1})		2.765	2.534	1.613	3.450	3.220	2.760	2.305	1.614	1.420
	$t_{1/2}$(min)		252	274	431	201	216	251	302.1	431.3	490.4
Higuchi diffusion model	Q/A Vs $t^{1/2}$	r_h	0.995	0.996	0.992	0.997	0.997	0.996	0.996	0.996	0.997
		$Dx10^5$ ($mg.cm^{-2}.min^{-1}$)	1.752	2.182	1.130	3.120	2.928	2.519	1.883	1.176	0.929
	logQ Vs log t	r_h	0.997	0.998	0.929	0.987	0.998	0.991	0.996	0.997	0.998
		slope	0.745	0.813	0.794	0.861	0.762	0.834	0.873	0.817	0.845

K = rate constant r = correlation coefficient D = diffusion coefficient

Concerning the order of release, it was evident from the results that the best fitting with the highest correlation coefficient was found for Zero-order kinetics with the glycerinated gelatin suppositories. On the other hand, kinetic assessment of the release data of miconazole nitrate from emulsion bases prepared with witepsol H-15 and different mucoadhesive polymers (Table 8) indicated that, the highest correlation coefficient (0.996 - 0.999) were exhibited with the first order release mechanism. Log-Log plot of the release data showed values of n= 0.5 to 1 which indicate that non-Fickian diffusion release mechanism could be applicable for drug release from these suppositories.







Clinical evaluation of miconazole nitrate bioadhesive vaginal suppositories was carried out by using the three selected formulae: F4, F9 and F17. These formulae were selected according to the best physicochemical characters and in vitro drug release. Formula F4 contains 95% witepsol H-15 and 5% tween 80, formula F9 contains 63% witepsol H-15, 5% tween 80, 30% distilled water and 2% sodium alginate. Formula F17 contains PEG 8000 / 400 (40% : 50%), 8% distilled water and 2% HEC. The number of cured and non - cured patients after 5 days of treatment using different formulations is listed in Table (9).

Table 9
Clinical evaluation of different bioadhesive vaginal formulations compared with conventional treatment using commercial gynozol suppositories

formula	No. of cured cases	No. of not cured cases	Statistic X^2-value*
F4	17 (85%)	3	2.143
F9	18 (90%)	2	3.810[b]
F17	20 (100%)	0	8.571[a]
Gynozol-suppositories	14 (70%)	6	--

**Critical X^2 at P (0.05) = 3.841 and at P (0.10) = 2.706*
a: significant difference at 0.10 > P > 0.05
b: significant difference at P ≥ 0.10

Otherwise, no significant difference in the clinical efficacy between the formula under investigation and the conventional miconazole nitrate therapy. The higher clinical efficacy of formula F17 (100%) could be attributed to strong bioadhesive properties of HEC as a polymer. These bioadhesive properties increase the contact time of the drug with the absorbing vaginal membrane. Regarding witepsol H-15 suppositories, the higher clinical efficacy of the formula containing the bioadhesive sodium alginate, F9 (90%) when compared with the formula which does not contain bioadhesive polymer, F4 (85%) could also be attributed to the increased residence time in the vagina. While the statistical analysis of the results using X^2 - test was done to compare between the clinical efficacy of each formula under investigation and the conventional treatment with the intravaginal miconazole nitrate (Gynozol suppositories), the results are shown in Table (9). The statistical results proved that only formula F17 showed significant higher efficacy.

CONCLUSION

The release of miconazole nitrate frm different bases followed the order of witepsol H-15 > glycerinated gelatin (B.P) > polyethylene glycol. The incorporation of tween 80 to witepsol H-15 (oleaginous base) was found to decrease both mechanical strength and deformation time and slightly decrease the melting range. Miconazole nitrate was significantly increased by tween 80 from this base in a concentration dependent manner. While, the release mechanisms were not affected by surfactant incorporation into this base.The addition of different mucoadhesive polymers; HEC, sodium alginate and carbopol 934 was found to affect the physical properties of the prepared suppositories. For PEG formulae, the direct polymer addition increased the mechanical strength, the melting range and the deformation time to an extent depending on the polymer concentration. While, the incorporation of the polymers into oleaginous base by emulsification was found to decrease the mechanical strength but lightly increase the deformation time. Analysis of the release data by the linear regression of miconazole nitrate from glycerinated gelatin base proved that the release followed Zero- order kinetics. While, for PEG formulae, the release followed combined first- order and non-Fickian diffusion kinetics through a biphasic release pattern. But in case of witepsol H-15, the release followed non-Fickian diffusion mechanism. The selected formulae for clinical evaluation are formulae no. F4, F9 and F17. Clinical evaluation proved that the highest percentage of cured cases, 100%; was obtained with formula no. F17, which contains PEG and 2% HEC.

REFERENCES

1) Valenta, C., 2005. The use of mucoadhesive polymers in vaginal delivery, Adv Drug Deliv Rev, 57: 1692 – 1712.
2) Knuth, K, Amiji, M., Robinson, J.R., 1993. Hydrogel delivery systems for vaginal and oral applications, Formulation and biological considerations, Adv Drug Deliv Rev, 11: 137-167.
3) Robinson, JR and Bologna, W.J., 1994. Vaginal and reproductive system treatments using a bioadhesive polymer, J Control Release, 28: 87-94.
4) Park, K and Robinson, J.R., 1984. Bioadhesive polymers for oral controlled drug delivery :Method to study bioadhesion, Int J Pharm, 19: 101-127.
5) Park, K. and Robinson, J.R., 1987. Mechanism of mucoadhesion of poly (acrylic acid) hydrogels, Pharm Res, 4: 457-464.
6) Leung, S. and Robinson, J.R., 1988. The contribution of anionic polymer structural features to mucoadhesion, J Control Release, 5: 223-231.
7) Nagai, T., 1985. Adhesive topical drug delivery systems, J Control Release, 2: 121-134.
8) Russel. E.L., Pharm D, 2010. ‘’Antifungal Drug Interactions’’ in ‘’Doctorfungus’’, 23 (1).
9) Carter, S., 1981. ‘’Cooper and Guns, Dispensing for pharmaceutical students’’ 12th Ed., Pitman Medical Publishing Col., Ltd., Lord P. 238.
10) Virdas, N.J, Ried, V.E., Bohidar, N.K. and Plakogiannis, F.M., 1982. ‘’ Medicament release from suppository bases. Part I,’’ Physicochemical characteristics and bioavailability of indomethacin in rabbits’’ J.Pharm. Sci., 71, 945.
11) ‘’British Pharmacopoeia’’, 2011. The stationary office, London, UK.
12) Ansel, H.C, Popovich, N.G. and Allen, L.V., 2011. ‘’Suppositories and Inserts’’ , Chapter 12 in: ‘’Pharmaceutical dosage forms and drug delivery systems’’, 9th Ed., Williams and Wilkins, eds., Lea and Febiger, USA, p. 312-330.
13) Anschel, J. and Lieberman, H.A., 1976. In ‘’The theory and practice of Industrial Pharmacy’’, Lea and Febiger, Philadelphia, p. 245.
14) Dan, Y., Shaofeng, Z., Yumei, H., Ming, Y., Yun, Q and Xiaohe, X., 2011. ‘’Microcalorimetric Investigation of toxic effects of three injectable solubilizing excipients on tetrahymena thermophila BF_5 growth’’, in Chin. J. Chem., 29, 871 – 876.
15) Takamura, A., Minow, I., Noro, S. and Kubo, T., (1979). Effects of tween and span emulsifiers on the stability of o/w emulsions, Chem. Pharm. Bull., 27, 2921.
16) Kassem, A.A., Elmiligi, M. F. and Ali, S. A., 1984. Formulation and stability of heptaminol supp. Part II: formulation and characterization of heptaminol supp., Bull. Pharm. Sci, Assiut University, 7 (2), 276.
17) Erweka Co., 1964. ‘’The pamphlet of testing apparatus for the pharmaceutical industry’’, Erweka Apparatus, GMBH., Frankfurt, West Germany.
18) Coben, L. J. and Lieberman, H. A., 1986. ‘’Suppositories’’ in ‘’The theory and practice of Industrial pharmacy’’, Lachman, L., Lieberman, H. A. and Kang, J. L., eds, Lea and Febiger, Philadelphia, p.564 – 588.
19) Coben, L.J and Lordi, N.J., 1980. Physical stability of semisynthetic suppository base, J Pharm Sci, 69: 955 – 960.
20) Senior, N., 1974, in ‘’Advanced Pharmaceutical Sciences’’ vol. 4, Bean, H.S, Beckett, A.H. and Carless, J.E., eds., Academic Press, London, p. 366 – 433.
21) Krowczinski, L., 1970. ‘’Suppository in New Medicinal’’, Poland, p.131.
22) Higuchi, W.I. and Suzuki, A, 1970. ‘’Theoritical model studies of drug absorption and transport in the gastrointestinal tract’’, J. Pharm. Sci., 59, 651.

23) Ibric, S., Duric, Z., Jovanovic, M., Parojcic, J. and Jovicic, I., 2002. ‘’The influence of drug particle size, surfactant addition and temperature of cooling on disintegration and drug release from suppositories’’, FIP-World Congress, 62, 125.
24) Yahagi, R., Onishi, H. and Machida, Y., 1999. ‘’Preparation and evaluation of double phased mucoadhesive suppositories of lidocaine utilizing carbopol and white bees wax’’, J Contr Release, 61 (27), 1.
25) Thomas, W.H. and McCormack, R., 1971. ‘’The drug release characteristics of various rectal suppositories as determined by specific ion electrodes’’, J Pharm Pharmacol, 23, 490.
26) Calis, S., Suman, M and Hincal, A.A., 1994. ‘’Effect of suppository bases on the release properties of a potent antimicrobial agent (31G)’’, Pharmazie, 49, 336.
27) Desai, S.J., Simonelli, A.P. and Higuchi, W.I., 1965. "Investigation of factors influencing release of solid drug dispersed in inert matrices, J Pharm. Sci., 54, 1459.
28) Farid, S.F., 1996. "A Pharmaceutical Study on tiaprofenic acid in different dosage forms", "Master thesis", Cairo University, Egypt.
29) Peppas, N. A. and Sahlin, J. J., 1989: "A simple equation of description of solute release III coupling of diffusion and relaxation, Int. J. Pharm. 57: 169-172.
30) Asikoglu, M., Ertan, G. and Cosar, G., 1995. "The release of isoconazole nitrate from different suppository bases: in-vitro dissolution, physicochemical and microbiological studies", J Pharm Pharmacol, 47 (9): 713-6.