

Formulation and Evaluation of Bioadhesive Gels Containing Miconazole Nitrate

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Abstract: Miconazole nitrate is an imidazole derivative characterized by longer half-life and higher efficacy in the treatment of the protozoal and anaerobic bacterial infection of the vagina. Over 90% is reported to be bound to plasma proteins. Since miconazole is not available as gel formulation, miconazole was formulated into buffered gels (pH 4.75) using different hydrophilic gel bases, including hydroxypropylmethyl cellulose (HPMC), carbopol 934 and sodium alginate. Determination of drug content, pH, viscosity, % adhesion and *in vitro* release through both artificial and natural diffusion barrier were investigated for all the prepared bioadhesive gels alone and incorporated with enhancers namely tween 80 (T80) and taurocholic acid. The results obtained revealed that a sodium alginate gel was found to have the highest viscosity and the highest bioadhesive strength by the *in vitro* evaluation. HPMC gel base showed the highest miconazole release through both cellulose membrane and rabbit skin at pH 4.75, while sodium alginate showed the lowest release rate. The total amounts of miconazole released through the cellulose barrier were higher than those released through the rabbit skin barrier. The amount of the drug released after 8 hours through cellulose barrier were 42.90%, 39.40% and 21.75% and through rabbit skin were 25.31%, 14.70% and 11.33% for HPMC, carbopol 934 and sodium alginate respectively. Tested enhancers were shown to increase the amount of miconazole released at an optimum concentration specific for each vehicle, as shown by 1% tween 80 which proved to be superior when incorporated with sodium alginate and carbopol 934 while 3% taurocholic acid was found also to be effective with sodium alginate. The best linear relations and the highest correlation coefficient was obtained with the non-Fickian diffusion equation for all gel bases. Two formulations were selected and evaluated for their clinical efficacy in the treatment of *Trichomonas vaginalis* using the chosen 40 patients where they use 200 mg miconazole nitrate intravaginally once daily at bed time for 5 consecutive days and compared with conventional treatment using miconazole nitrate (200mg) vaginal suppositories (Gynozol) tried on extra 20 patients. Bioadhesive vaginal gels showed 95% of the cured cases with the patient group received a gel of 15% sodium alginate and 1% tween 80. On the other hand, 80% of the patients received the gel formula containing 15% sodium alginate incorporated with 3% taurocholic acid were cured. While, Gynozol gave the lowest percentage of the cured cases (70%).

Key words: Miconazole nitrate, bioadhesive gels, cellulose membrane, rabbit skin and enhancers.

INTRODUCTION

Gels are used pharmaceutically as lubricants and as carriers for spermicidal agents^[5] and other drugs for their local effect and percutaneous absorption^[14]. The gel can be applied directly to the wounded area and once daily application of the gel had proved to be satisfactory in removing offensive odor emanating from such wound^[1]. A gel is a semisolid system of at least two interpenetrating phases: a gelling agent and a liquid. Gels that contain water are called hydrogels, while that contain an organic liquid are called organogels. Hydrogels in the broad sense include the

matrix of water - soluble materials such as cellulose derivatives and natural gums. These pseudohydrogels swell infinitely and the component molecules dissolve from the surface of the matrix. Drug molecules are released through the spaces in the network and also by the dissolution and/or disintegration of the matrix^[13]. Mucoadhesive polymers of natural, semisynthetic or synthetic origin are able to form hydrogels. In the simplest case the drug is dispersed in a mucoadhesive polymer which swells in the presence of water and exhibits bioadhesive properties^[6]. Vaginal gels are known to possess a higher biocompatibility and bioadhesivity and can be rapidly

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eliminated through normal catabolic pathways, decreasing the risk for irritative or allergic host reaction at the application site^[5]. It was reported that, 0.75% intravaginal metronidazole gel was proved to have a clinical cure rate similar to that for oral metronidazole for the treatment of bacterial vaginosis^[23]. Penetration enhancers were categorized according to their structural features^[2]. The effect of certain enhancers on the vaginal absorption of drugs and the acute histological effects of the enhancer formulations have been studied in ovariectomized rats^[17]. Transepithelial penetration enhancers were acting through two mechanisms of action. Firstly changes in the permeability of the membrane or/and secondly the physicochemical properties of the drug^[20].

The objective of the study is to formulate miconazole nitrate in the form of different gels using various bioadhesive polymers: cellulose derivative (hydroxypropylmethylcellulose); sodium alginate and polyacrylates (carbopol 934). The release of miconazole from the prepared gels alone and mixed with certain additives on the release of the drug was studied. Clinical evaluation of miconazole nitrate was also conducted.

MATERIALS AND METHODS

Materials:

- Miconazole nitrate was kindly supplied by PHARCO Pharmaceuticals Co., Alex., Egypt.
- Hydroxypropylmethylcellulose (HPMC), (Dow chemical company, USA).
- Carbopol 934 (B.F. Goodrich Co., USA).
- Sodium alginate and n-octanol (BDH chemicals, Ltd, Poole, England).
- Potassium dihydrogen phosphate triethanolamine and tween 80 (Adwic, El Nasr Chem. Co., Egypt).
- Taurocholic acid (Sigma chemical Co., USA).
- 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).
- Rotary viscometer (Haake inc., Germany).

- Cellulose membrane, Spectrapor, M.W. cutt-off 1200-1400 (Fisher Sci. Co., USA).
- MSE minor centrifuge (MSE Scientific instruments, Manor Royal, Grawley RH/0200 Susses, England).
- Magnetic stirrer (Heidolph, USA).
- Modified diffusion cell.

Methodology:

Spectrophotometric Scanning of Miconazole Nitrate in Presence of Different Gel Bases: A specified concentration of miconazole nitrate in phosphate buffer pH 4.75 and drug solution in all prepared gel bases in the same medium were scanned spectrophotometrically at 200 – 400 nm to determine the wavelength of maximum absorption (λ_{max}).

Preparation of Mucoadhesive Vaginal Gels:

Formulations of mucoadhesive vaginal gels containing miconazole nitrate alone and with certain enhancers are listed in Table (1). The formulated gels were prepared according to the following procedures:

Preparation of Hydroxypropylmethylcellulose Gel:

20% w/w of hydroxypropylmethylcellulose (HPMC) was dispersed in phosphate buffer adjusted at pH 4.75 in which methyl paraben 0.2% w/w and propyl paraben 0.02% w/w preservatives, were previously dissolved. The dispersion was mixed using a magnetic stirrer until a clear transparent gel free from air bubbles was obtained. The resultant gel mass was left overnight for complete swelling. Part of the plain gel was added to 1% w/w of the powdered drug with gentle stirring to produce a smooth layer of the gel. The rest of gel was added gradually portion by portion with continuous gentle stirring to avoid air entrapment till a homogenous dispersion was obtained.

Preparation of Sodium Alginate Gel: 15% w/w of sodium alginate gel was prepared under the same conditions indicated in case of other formulation.

Preparation of Carbopol 934 Gel: For the preparation of 2% w/w carbopol gel, half the amount of phosphate buffer pH 4.75 was replaced with distilled water, in which the carbopol powder was dispersed by vigorous stirring taking care to avoid formation of indispersible lumps^[11]. A 0.1 ml of triethanolamine was added with stirring till a transparent clear gel was formed. The other half of the phosphate buffer pH 4.75 was added gradually with stirring till complete mixing. The incorporation of the drug into the prepared plain gel was followed the steps carried out in the preparation of the other gels.

Table 1: Formulations and drug content of the prepared miconazole gels.

Formula no.	Gel base	concentration	Claimed drug content	Actual drug content	Methyl Paraben	Propyl Paraben	Enhancer	concentration
1	HPMC	20	200	199.4	0.2	0.02	T80	1
2		20	200	199.0	0.2	0.02	T80	3
3		20	200	198.0	0.2	0.02	T80	5
4		20	200	197.0	0.2	0.02	TCA	1
5		20	200	201.0	0.2	0.02	TCA	3
6		20	200	198.0	0.2	0.02	TCA	5
7	Carbopol 934	2	200	202.0	0.2	0.02	T80	1
8		2	200	200.4	0.2	0.02	T80	3
9		2	200	199.5	0.2	0.02	T80	5
10		2	200	198.4	0.2	0.02	TCA	1
11		2	200	201.5	0.2	0.02	TCA	3
12		2	200	202.0	0.2	0.02	TCA	5
13	Sodium alginate	15	200	198.7	0.2	0.02	T80	1
14		15	200	199.2	0.2	0.02	T80	3
15		15	200	202.0	0.2	0.02	T80	5
16		15	200	201.0	0.2	0.02	TCA	1
17		15	200	200.5	0.2	0.02	TCA	3
18		15	200	199.6	0.2	0.02	TCA	5

Preparation of Vaginal Mucoadhesive Gels Containing Certain Enhancers: HPMC, Carbopol 934 and sodium alginate gels were prepared using certain enhancers according to the following method:

The drug was either levigated with tween 80 as liquid enhancer or levigated with taurocholic acid solid enhancer previously dissolved in a least amount of phosphate buffer pH 4.75. Each of the different gel bases pre-prepared was added to the drug treated with the enhancers under investigations.

Characterization of the Physicochemical Properties of the Drug and the Vaginal Gels:

Determination of Solubility of Miconazole Nitrate: In a stoppered glass bottle, an excess amount of miconazole nitrate was added to 10 ml of both n-octanol and phosphate buffer (pH 4.75). Agitation for 24 hour at 37°C ±1 was conducted. Aliquot of certain volume was centrifuged and the filtrate was diluted and measured spectrophotometrically at λ_{max} 316 nm.

Partition Coefficient Determination: An equal volume from n-octanol and phosphate buffer pH 4.75 were saturated with each other for 24 hours, the two phases were separated. Certain weight of either the drug alone

or an equivalent weight of the gel was dissolved in 10 ml of the aqueous phase to give the concentration of 0.5 mg/ml. The final solution transferred to a stoppered glass bottle containing 10 ml of n-octanol. The systems were agitated in a thermostated water bath at 37 ± 1°C for 24 hours, the phases were then separated, the aqueous phase was filtered and the concentration of the drug was determined spectrophotometrically at λ_{max} 316 nm against a blank solution prepared in an analogous manner. The concentration of the drug in octanol was calculated from the difference between the initial and final concentrations of the drug in the buffer phase. The partition coefficient was calculated according to Nernst equation^[4].

$$K = \frac{C_{org.}}{C_{aqu.}}$$

Where:

- K = partition coefficient
- C_{org.} = concentration of the drug in organic phase (octanol)
- C_{aqu.} = concentration of the drug in aqueous phase (buffer)

Determination of Actual Miconazole Nitrate Content in the Prepared Vaginal Gels:

One gram of the gel was accurately weighed and placed in tightly closed volumetric flask with 10 ml of methanol. The closed flasks were shaken for 10 minutes and then diluted to 100 ml with phosphate buffer (pH 4.75) and centrifuged. The supernatant was filtered and measured spectrophotometrically at λ_{\max} 316 nm for its drug content.

Determination of pH of Vaginal Gels: One gram of each gel was dispersed in 30 ml of distilled water and the pH was measured.

Rheological Measurement of the Gels: For the rheological measurements, entrapped air was removed by centrifugation of the prepared gel samples at 20,000 rpm for 10 minutes. Samples for examination were stored after centrifugation at room temperature for at least 24 hours before testing so that any stress in the material was able to relax^[18]. The viscosity of the samples were measured at $37 \pm 1^\circ\text{C}$ using a shear-rate-controlled rotary viscometer, in which sensor system consists of a cup and a bell-shaped rotor.

The viscosity was measured using the following equation.

$$\gamma = \frac{G.S}{n}$$

where:

- γ is the sample viscosity in mpa.s (mpa.s 1 Centipoise),
- G is the instrument factor depending on the type of the measuring head and sensor system used,
- S is the torque value and
- n is the present test speed (1 rpm).

In-vitro Determination of Bioadhesive Properties:

The vaginal gels were tested for bioadhesion properties using modified method described by Ranga Roa and Bari^[16].

Miconazole Nitrate In-vitro Release Studies:

Effect of the Type of the Gel Base:

Through Cellulose Membrane: The in-vitro release of miconazole nitrate from different hydrogel bases was performed using the dialysis method (Fig. 1). The semipermeable cellophane membrane which was previously soaked in phosphate buffer of pH 4.75 and dried, was stretched over the open end of a glass tube having a diameter of 3 cm, which was made

water tight by rubber band. Five grams of each formulation were accurately weighed and thoroughly spread on the membrane to occupy all 3 cm diameter circle. The tubes were then immersed upside – down in a 250 ml beaker containing 100 ml phosphate buffer pH 4.75 which is preheated and maintained at $37 \pm 1^\circ\text{C}$ in a constant temperature water bath. The tubes height was adjusted so that the membrane was just below the surface of the release medium.

The whole assembly was shaken at 25 strokes per minute during the entire time of diffusion. For each gel sample, 1ml was withdrawn at 15, 30, 60, 120, 180, 240, 300, 360, 420 and 480 minutes time intervals and replaced by equal volumes of fresh release medium maintained at the same temperature. Samples were measured spectrophotometrically at λ_{\max} 316nm after appropriate dilutions against the same used phosphate buffer as a blank. The amounts of drug released were calculated on the basis of the standard curve previously constructed. The percentage released was calculated on the basis of the actual miconazole nitrate content in 5g of the gel.

Permeability Through Rabbit Skin: The permeability of miconazole nitrate from different mucoadhesive vaginal gel through rabbit skin was studied using abdominal skin of old female rabbits. The skin was excised just prior to the experiments, removing of the hair and cleaning with saline to remove all visceral debris. When the skin is not used immediately, it was stored at -20°C and used in less than three months^[18]. The skin barrier was mounted on the open end of the diffusion cell with the inside surface facing the donor chamber. All the experimental conditions were adjusted as mentioned in the in-vitro release studies.

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 and diffusion controlled release mechanism according to the Higuchi model^[9].

Effect of Certain Enhancers on the Release of Miconazole Nitrate from Different Gel Bases:

The effect of different types of enhancers on the release of the drug from the mucoadhesive gel formulations in phosphate buffer pH 4.75 was studied. Various enhancers used were tween 80 (1,3 and 5% w/w) and taurocholic acid (1, 3 and 5% w/w).

Determination of the Permeability Parameters: The amount of the drug permeated per unit surface area ($\mu\text{g}/\text{cm}^2$) was plotted versus time (minutes) and the flux ($\mu\text{g}/\text{cm}^2 \text{min}^{-1}$) was calculated from the slope of

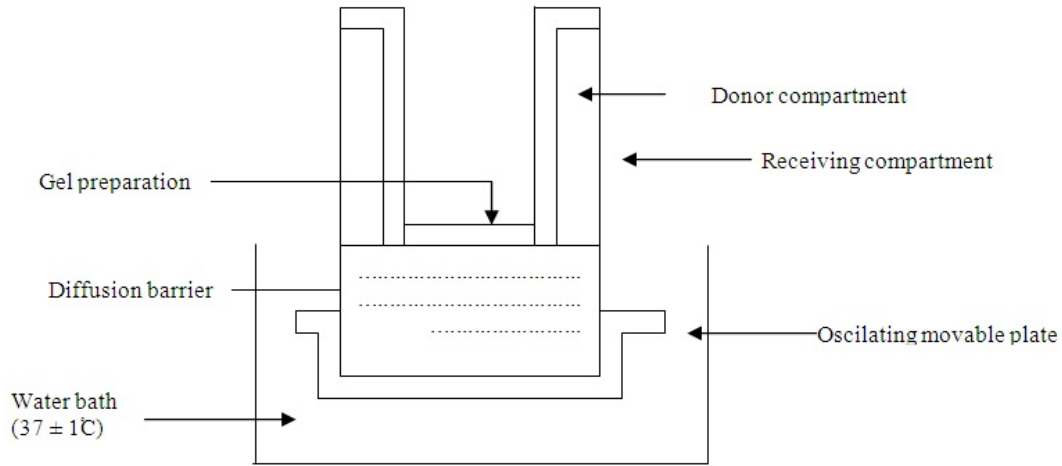


Fig. 1: Schematic diagram of the diffusion apparatus.

the line^[22]. The method reported by Yoneto *et al.*,^[25] was used for the analysis of the permeation data. The permeability coefficient was then calculated according to the following equation^[19].

$$P_m = J_{ss} / C_d$$

Where:

- P_m = permeability coefficient
- J_{ss} = flux
- C_d = concentration of the drug in the donor side.

The partition coefficient, which is an indication of the distribution of miconazole nitrate between the gel bases and the receiving medium was calculated by using the following equation:

$$P_m = K_p D / h$$

Where:

- P_m = permeability coefficient
- K_p = partition coefficient
- D = Diffusion coefficient
- h = thickness of the membrane (cm)

In order to normalize the permeability data for each enhancer treated formulations in respect to its own control, enhancement factors as percent were calculated as reported by Shojaei *et al.*,^[21].

$$\text{Enhancement factor(\%)} = \frac{P_{enh}}{P_{control}} \times 100$$

- P_{enh} = permeability coefficient obtained for gels containing enhancer.

- $P_{control}$ = permeability coefficient obtained for gels without enhancer.

Clinical Evaluation of Miconazole Nitrate Bioadhesive Vaginal Gel:

Each formula under investigation was used by 20 patients. On the other hand, 20 patients received conventional treatment (Gynozol suppository) for the comparison. Local treatment was achieved by using 200 mg miconazole nitrate intravaginally once daily at bed time for 5 consecutive days. For the accuracy, we use a plastic applicator to the required dose (200 mg). The patient's complaints, the results of the clinical evaluation and the results of the direct microscopic examination were recorded on an evaluation sheet.

RESULTS AND DISCUSSION

The solubility of the drug in phosphate buffer of pH 4.75 and n-octanol was found to be 10.88 mg/ml and 3.77 mg/ml respectively. pH 4.75 of the phosphate buffer was chosen to simulate the pH of the vagina, the site at which the drug will be released. Kistner, 1978 has claimed that the normal pH of the vagina ranges from 4.0 – 5.0.

The optimum partition coefficient of either miconazole nitrate alone or formulated into vaginal gel between n-octanol and phosphate buffer pH 4.75 was determined and found to be 0.40 at 37°C. The low partition coefficient of miconazole nitrate (0.4) was referred to the higher solubility of the drug in the phosphate buffer compared to that in n-octanol. This value auger well for effective vaginal treatment because the first layer of the vaginal mucosa is epithelial layer which is constructed with an elaborate system of channels. These channels are considered to

Table 2: Physical evaluation of different mucoadhesive vaginal gels containing miconazole nitrate (1% w/w).

Gel base	pH	Viscosity (cp) at 1 rpm	Bioadhesion (%)
20% HPMC	4.81	23358	46.53
2% carbopol 934	5.71	41220	54.03
15% sod. Alginate	4.87	126408	66.31

be an important pathway of watery secretions from the blood network to the tissue. This may discuss the expected features for the drug towards the epithelial layer.

The actual miconazole nitrate was determined for each vaginal gel formulation. It is obvious that the percentage of miconazole nitrate content in all prepared vaginal gel formulations was in the range from 96.5% to 101.6% of the claimed content.

pH, viscosity and bioadhesion properties of the tested gels were determined and listed in Table (2). The prepared vaginal gels were prepared to be in acidic side as they are prepared in acidic phosphate buffer solution, to resemble the vaginal tract to avoid any irritation may occur due to the change in the pH. The pH of vaginal gels for each polymer gel base was measured directly after preparation.

The viscosity of semisolid preparations plays an important role in drug release from the vehicles and greatly affects drug bioavailability. In addition, it is reported that, the mucoadhesive properties of a range of well defined polymers are greatly influenced by their viscosities and molecular weights^[24].

The viscosity of sodium alginate gel was proved to be the highest viscosity (126408 cp).

The bioadhesive properties of the different bioadhesive systems used were 66.31%, 54.03% and 46.53% for sodium alginate 15%, carbopol 934 2% and HPMC 20% respectively. This result can be explained by the low degree of swelling of the polymer in the more viscous gels as it has been reported that, excessive swelling results in abrupt drop in the adhesive strength^[7].

Fig. (2) shows the release of miconazole nitrate in phosphate buffer pH 4.75 from different gel formulations. It is clear that the release of the drug varies from 45.93% to 22.95% after 8 hours depending on the type of gel bases. The obtained results revealed that the amount of the drug released from cellulose derivative gel base was significantly higher than that released from carbopol and sodium alginate. This may be attributed to the higher viscosity of sodium alginate gel than that of the other tested gels which is responsible for hindering the release from it. It was demonstrated that under acidic conditions (pH 4.75) swelling of carbopol 934 increase slightly and consequently the viscosity. In contrary, HPMC gel

show the highest drug release since it is non-ionic polymer and so pH has no effect on their swelling behavior and consequently low viscosities and bioadhesion percent will be obtained. Also, it was noticed that during the first 60 minutes, all the prepared gels except sodium alginate gel show similar release pattern. These systems were swellable in water and a minimum of one hour was needed for their complete swelling. The swelling and viscosity of sodium alginate gel seems to be effective before the first hour of release is elapsed.

The results of the *in vitro* release study through cellulose membrane showed that miconazole nitrate transport through this artificial membrane was dependent on the nature and the viscosity of the gel in which the drug is incorporated. In order to validate these findings, it was found appropriate to study the drug transport through a real skin barrier. The results of the percentages drug transport from the different formulations up to 8 hours are shown in fig. (3). It is noticed that smaller amounts of miconazole nitrate were able to penetrate through the rabbit skin in comparison to the amounts of the drug penetrated through the cellulose barrier. The same release behavior concerning the different gel bases was the same as in case of cellulose membrane. The release pattern follows the following descending order: HPMC > carbopol 934 > sodium alginate. The first hour release was found to be affected by the nature of the rabbit skin. These bioadhesive gels are characterized by a very slow release rate of miconazole nitrate. Only 10.21% of miconazole was released after 8 hours from 15% sodium alginate gel, which has shown the highest viscosity (126408 cp), while gel of HPMC show the same dissolution rate through the first hour of release. These lower values of the percentage drug transported through the rabbit skin for all gel formulations could be related to the increased thickness and the rather complex structure of the rabbit skin barrier compared with cellulose membrane. Cellulose membrane is easily to be hydrated by the dissolution medium, the case which causes the pores of cellulose film wider and consequently the drug release would be faster. Miconazole nitrate transport through rabbit skin from different gel bases was ranked descendingly as follows: HPMC > carbopol > sodium alginate that conforms to that observed in the transport study through cellulose barrier. Since there is little information available on

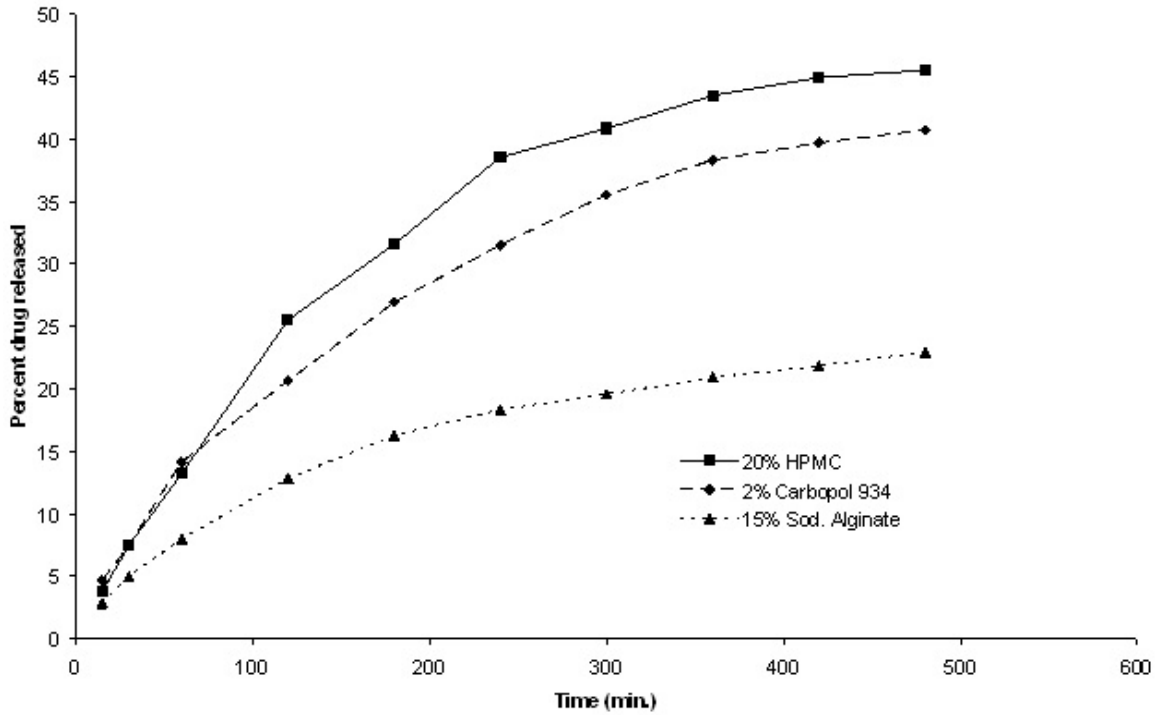


Fig. 2: Effect of different gel bases on the release of miconazole through cellulose membrane in pH 4.75 phosphate buffer at $37 \pm 1^\circ\text{C}$

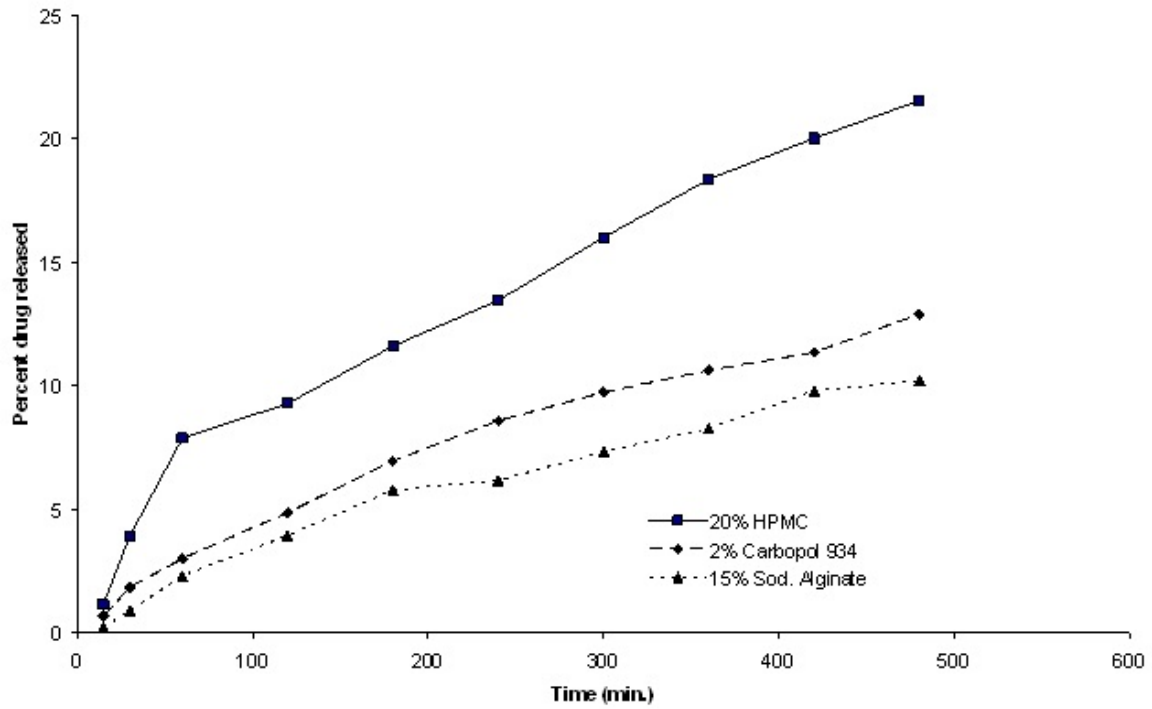


Fig. 3: *In-vitro* Miconazole transport from different gel bases through rabbit skin in pH 4.75 phosphate buffer at $37 \pm 1^\circ\text{C}$

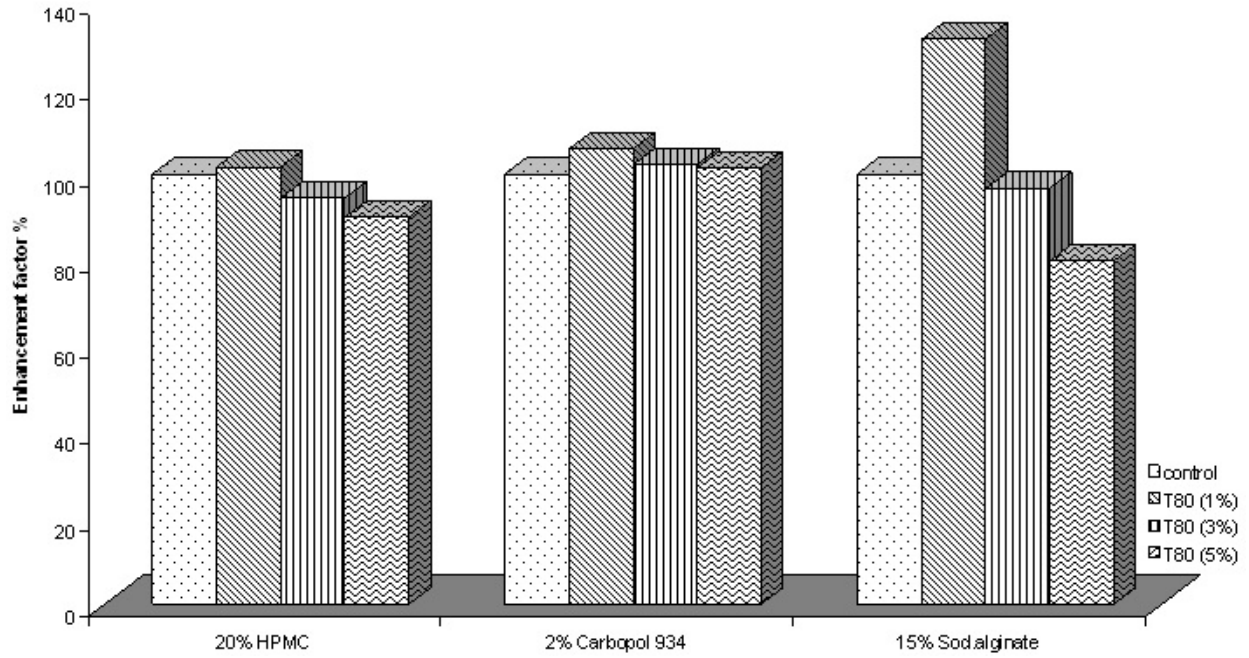


Fig. 4: Enhancement factors for the penetration of miconazole through cellulose barrier from different bioadhesive gels containing different concentrations of tween 80.

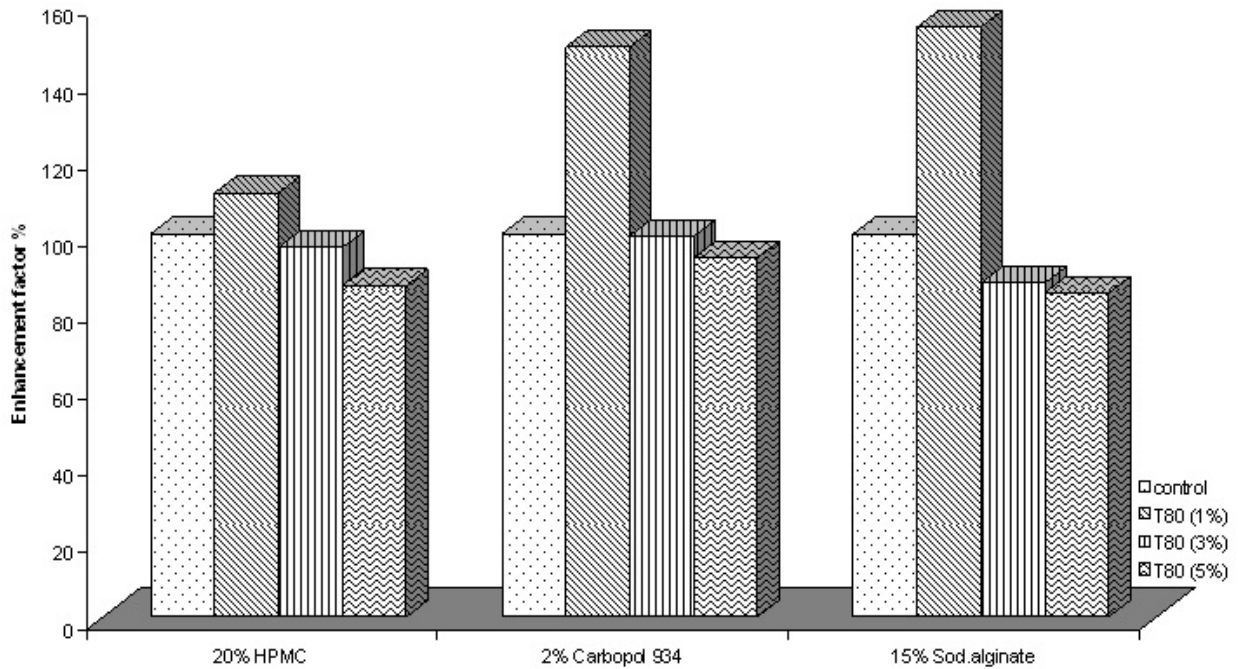


Fig. 5: Enhancement factors for the penetration of miconazole through rabbit skin from different bioadhesive gels containing different concentrations of tween 80.

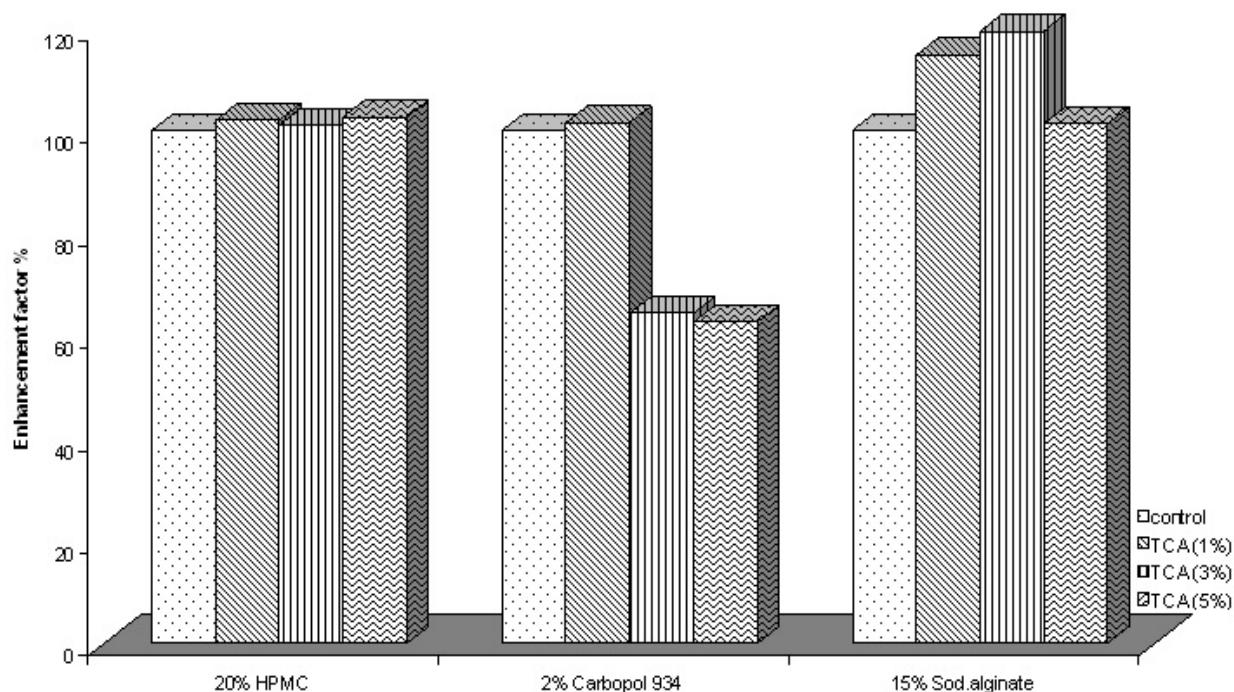


Fig. 6: Enhancement factors for the penetration of miconazole through cellulose barrier from different bioadhesive gels containing different concentrations of taurocholic acid.

vaginal mucosal enhancement and none of miconazole nitrate vaginal gel products are available in the market, it was found appropriate to study the effect of permeation of the drug delivered from the different gel bases under investigation.

Tween 80 (T80 at 1, 3 and 5% w/w) and taurocholic acid (TCA at 1, 3 and 5% w/w) were used as enhancers. Each enhancer has been incorporated separately in the selected gel bases. The effect of enhancers at different concentrations on the release of miconazole from gels through the cellulose membrane and rabbit skin is shown in Figs. (4 - 7). From the results, it is clear that, all enhancers used were able to increase the release rate of the drug from gels through the cellulose barrier and rabbit skin to a variable extent depending on the type of enhancer. The enhancer effect was more pronounced with transport of the drug through the rabbit skin for all gel formulation subjected for investigation than that through the cellulose barrier. These findings could be attributed to the effect of enhancers on the physical properties of rabbit skin. Permeation enhancers which reversibly reduces the barrier properties of the skin may allow more drug to permeate into the viable tissues^[3,8]. Enhancers would emulsify the lipid layer of the rabbit skin and cause an erosion for the surface layer, this would increase the pores volume of the membrane. This would

consequently lead to higher release rate. The effect of enhancers on drug solubility and partitioning is also another factor could help in producing the fast release of drug in presence of emulsifying types of enhancers. 1% concentration of enhancers used seems to be the optimum concentration at which the maximum release is obtained except with formula 14 containing 3% taurocholic acid using cellulose membrane. 1% tween 80 show the highest enhancement factor with formula 13 (containing sodium alginate 15%) when we use cellulose membrane, and also when we use rabbit skin. Also 1% taurocholic acid show the highest enhancement factor with formula 13 (containing sodium alginate 15%) when we use rabbit skin.

Concentration of enhancers beyond the maximum concentration would be responsible for permeability coefficient declined and reducing of enhancement effect. Enhancer at high level showed a lower tendency to solubilize the drug which may be attributed to complex formation.

The greatest enhancement factor % (154.05) was obtained with sodium alginate gel (Tables 3 - 6).

The increase in the penetration rate by the use of lower concentrations of taurocholic acid could be attributed to the fact that, the fraction of the free drug in solution considered as the only driving force for penetration. At higher concentrations of these

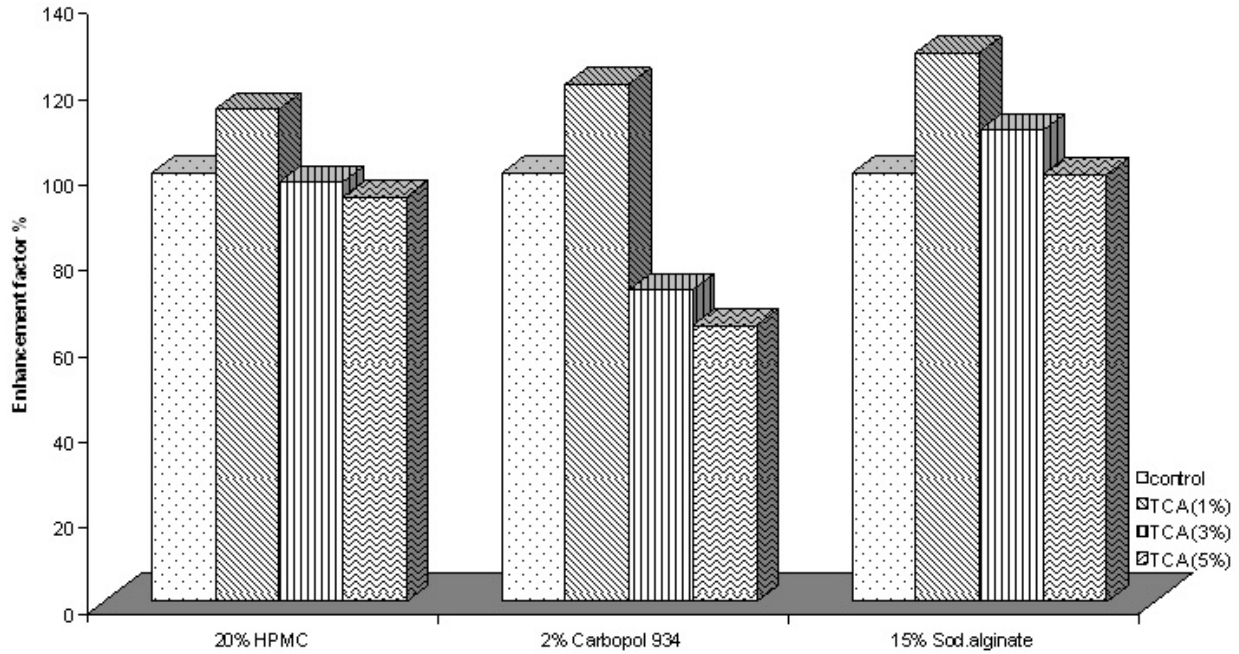


Fig. 7: Enhancement factors for the penetration of miconazole through rabbit skin from different bioadhesive gels containing different concentrations of taurocholic acid.

Table 3: Transport data of miconazole from gels containing Tween 80 as a penetration enhancer in pH 4.75 phosphate buffer at $37 \pm 1^\circ\text{C}$ using cellulose membrane as a barrier.

Type of barrier	Cellulose membrane											
Formula no.	1				7				13			
Enhancer conc. /time (min)	0	1	3	5	0	1	3	5	0	1	3	5
	control				control				control			
15	265	325.1	2990	278	327.92	327.9	278.4	180	200	247.36	167.48	146.28
30	560.4	546.3	525.8	495	524.4	573.8	519.4	440	394.84	463.6	362.56	322.28
60	933.6	932.9	883.4	775	1000	102	918	821	563.96	721.56	509.56	447.36
120	1804	1903	1314	1271	1458.6	1656	1413	1426	906	1154.1	785.16	686.2
180	2231	2377	2086	1802	1903.2	1996	1811	1732	1150.52	1438.2	1000	830.4
240	2726	2809	2543	2136	2229	2264	2100	1972	1296.12	1658.6	1199.3	943.48
300	2888	2900	2739	2514	2513.1	2707	2495	2385	1383.04	1806.4	1342.8	1124.4
360	3074	3180	2850	2707	2710.2	2827	2682	2580	1479.16	1895.4	1412.7	1155.48
420	3177	3259	2938	2756	2806.3	2963	2755	2714	1542.76	2044.5	1466.4	1201.4
480	3218	3322	2968	2852	2879.8	3084	2933	2827	1621.92	2102.5	1521.6	1316.6
Flux $\mu\text{g. cm}^{-2}.\text{min}^{-1}$	6.4	6.56	6.04	5.76	5.52	5.84	5.64	5.6	2.94	3.86	2.84	2.34
Permeability coeff * $10^2 (\text{cm}^{-2}.\text{min}^{-1})$	0.06	0.065	0.06	0.06	0.055	0.058	0.056	0.06	0.029	0.038	0.028	0.023
Enhancement factor (%)	100	101.6	94.37	90	100	105.8	102.2	101	100	131.29	96.59	79.72

Table 4: Transport data of miconazole from gels containing tween 80 as a penetration enhancer in pH 4.75 phosphate buffer at $37 \pm 1^\circ\text{C}$ using rabbit skin as a barrier.

Type of barrier	Rabbit skin											
Formula no.	1				3				5			
Enhancer conc. /time (min)	0	1	3	5	0	1	3	5	0	1	3	5
	control				control				control			
15	81.69	81.96	77.72	65.72	48.76	65.72	65.72	48.8	16.24	58.64	14.84	7.76
30	278.44	280.56	262.2	255.12	130.76	137.8	98.24	106	65	114.48	63.6	53
60	556.88	563.96	494.68	447.36	212.72	212.72	164	154	163.96	305.32	141.4	135
120	655.84	704.6	632.52	578.08	344.16	426.16	311.7	283	278.44	509.56	221.9	202.12
180	819.8	868.56	779.52	730.04	491.88	638.88	426.2	394	409.2	643.8	352	308.84
240	950.52	1016.2	908.12	847.36	606.36	894.68	590.1	541	436.76	777.4	424	361.12
300	1130.76	1229	1086.2	989.4	688.32	1000	655.8	623	518.72	885.52	488.3	474.2
360	1296.8	1383	1226.8	1107.4	751.24	1101.8	759.7	642	585.16	989.4	508.8	494.68
420	1414.12	1542.8	1342.8	1222.6	802.12	1185.2	800.7	731	693.28	1062.2	567.5	559
480	1521.56	1619.1	1463.6	1310.2	911.68	1224.7	869.2	845	721.56	1134.3	636	588.68
Flux $\mu\text{g.}$ ($\text{cm}^{-2}.\text{min}^{-1}$)	2.84	3.1	2.73	2.45	1.78	2.65	1.77	1.66	1.48	2.28	1.29	1.25
Permeability coeff * 10^2 ($\text{cm}^{-2}.\text{min}^{-1}$)	0.028	0.031	0.027	0.024	0.017	0.026	0.017	0.02	0.014	0.022	0.012	0.012
Enhancement factor %	100	110.71	96.42	86.47	100	148.87	99.43	93.7	100	154.05	87.16	84.59

Table 5: Transport data of miconazole from gels containing taurocholic acid As a penetration enhancer in pH 4.75 phosphate buffer at $37 \pm 1^\circ\text{C}$ using cellulose membrane as a barrier.

Type of barrier	Cellulose membrane											
Formula no.	4				6				12			
Enhancer conc. /time (min)	0	1	3	5	0	1	3	5	0	1	3	5
	control				control				control			
15	265	314.48	248.76	380.2	327.92	354	241.6	219.8	200	205.64	208	205.64
30	560.44	626.16	527.92	691.9	524.4	601.4	480	450.16	394.84	350.52	350.52	375.28
60	933.56	966.08	819.08	1016	1000	1178	861.2	821.92	563.96	574.56	571.6	567.48
120	1804.2	1902.5	1591.5	1984	1458.6	1595	1161	1113.08	906	983.04	980	961.96
180	2231.1	2313.1	1982.2	2411	1903.2	2048	1020	1360.44	1150.52	1258.6	1261.4	1237.44
240	2725.8	2791.5	2463.6	2873	2229	2380	1592	1537.12	1296.12	1431.1	1440.9	1392.92
300	2887.6	2969.6	2559.7	3035	2513.1	2654	1720	1668.56	1383.04	1607.8	1462.3	1435.32
360	3074.2	3139.9	2746.4	3211	2710.2	2830	1864	1816.96	1479.16	1643.1	1682	1484.08
420	3176.7	3267.1	2830.6	3348	2806.3	2912	1945	1901.08	1542.76	1746.3	1780.4	1554.08
480	3217.7	3356.9	2855.5	3446	2879.8	3018	1957	1910.24	1621.92	1766.8	1842	1678.44

Table 5: Continued

Flux mcg. (cm ⁻² .min ⁻¹)	6.4	6.52	6.46	6.56	5.52	5.6	3.55	3.45	2.94	3.37	3.5	2.97
Permeability coeff * 10 ² (cm ⁻² . min ⁻¹)	0.064	0.065	0.064	0.065	0.055	0.056	0.035	0.034	0.029	0.033	0.035	0.029
Enhancement factor %	100	102	101.06	102.6	100	101.4	64.34	62.6	100	114.69	119.31	101.22

Table 6: Transport data of miconazole from gels containing taurocholic acid as a penetration enhancer in pH 4.75 phosphate buffer at 37 ± 1°C using rabbit skin as a barrier.

Type of barrier	Rabbit skin																										
Formula no.	4			5			6			10			11			12			16			17			18		
Enhancer conc. /time(min)	0			1			3			5			0			1			3			5					
	control						control						control														
15	81.69	147.72	78.24	78.44	48.76	81.96	72	48.76	16.24	40.28	36.04	16															
30	278.44	319.44	194.28	184.2	130.76	163.96	135.64	114.48	65	111.68	84.8	61.08															
60	556.88	633.2	335.64	321.6	212.72	245.92	208.44	180.2	163.96	265	248.04	152.44															
120	655.84	763.96	628.88	615.6	344.16	449.48	330.12	294.68	278.44	293.28	283.4	268.4															
180	819.8	868.56	761.24	746.3	491.88	698.24	486.92	436.04	409.2	494	474.2	399.96															
240	950.52	1082	923.32	899.6	606.36	736.4	560.44	507.44	436.76	566.08	512.36	430.12															
300	1130.8	1327.2	1088.7	1052	688.32	879.16	588	524.36	518.72	727.92	578.8	503.88															
360	1296.8	1499.6	1120	1153	751.24	932.16	615.6	540.64	585.16	777.4	657.24	575															
420	1414.1	1641	1320.7	1260	802.12	1046.6	654	573.84	693.28	848.76	760.44	680															
480	1521.6	1756.2	1383.3	1357	911.68	1062.9	678.92	590.12	721.56	945.76	841.28	720.03															
Flux mcg. cm ⁻² .min ⁻¹	2.84	3.26	2.78	2.68	1.78	2.15	1.29	1.14	1.48	1.89	1.62	1.47															
Permeability coeff * 10 ² (cm ⁻² . min ⁻¹)	0.028	0.032	0.027	0.026	0.017	0.021	0.012	0.011	0.014	0.018	0.016	0.014															
Enhancement factor %	100	114.92	97.88	94.36	100	120.78	72.8	64.49	100	128.1	110	99.72															

Table 7: Clinical evaluation of bioadhesive vaginal gel formulations compared with conventional treatment using commercial gynozol vaginal suppositories.

Formula No.	No. of cured cases	No. of not - cured cases
13	19	1
17	16	4
Gynozol supp.	14	6

enhancers, the reduced concentration of the free drug due to micellar encapsulation will result in a proportional of the penetration of the drug^[15]. Similar explanation was discussed for tween 80 by Muller and Keruter,^[12] who reported that surfactants can cause considerable interfacial barriers, retarding the transport of solutes solubilized in the micelles by the orders of magnitude. Clinical evaluation of miconazole nitrate

bioadhesive vaginal gel was carried and the numbers of the cured and non-cured patients after 5 days of treatment using the different formulations are listed in Table (7). From the results, it is clear that the highest percentage of the cured cases, 95%, (19 cases) was obtained with formula No. 13 (15% sodium alginate + T80). On the other hand, 80% (16 cases) of the patients receiving formula No. 17 (15% sodium

alginate + 3% TCA) were cured. While, 70% (14 cases) only were cured for patients receiving the commercial gynozol suppositories. These results are not in complete accordance with the invitro release results as the in-vitro release of miconazole nitrate from 15% sodium alginate gel showed the lower release rate than the other gel bases. The higher clinical efficacy of formula No. 13 could be attributed to the strong bioadhesive properties of sodium alginate polymer. These bioadhesive properties of the polymer, increasing the contact time and intimate contact of the drug with the absorbing vaginal membrane.

Conclusion:

- Sodium alginate hydrogels was found to have the highest bioadhesive percent by the invitro evaluation.
- Hydroxypropylmethyl cellulose gel base proved to be superior vehicle which give the highest miconazole release through both cellulose membrane and rabbit skin at pH 4.75.
- Kinetic analysis of the release data indicated that the release of miconazole from all the prepared hydrogels followed non – Fickian diffusion pattern.
- The inclusion of enhancers: tween 80 and taurocholic acid caused an increase in the rate and the amount of the drug released.
- Of the tested enhancers, 1% tween 80 proved to be superior when incorporated with sodium alginate (using both cellulose membrane and rabbit skin) while 3% taurocholic acid ranked as number two when used with sodium alginate (using cellulose membrane barrier).
- The selected formulae for bioavailability study are formula No. 13 &17. Clinical evaluation proved that the highest percentage of the cured cases, 95%, was obtained with formula no. 13.

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