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PREPARATION, IN-VITRO CHARACTERIZATION, AND CLINICAL STUDY OF PROPRANOLOL HCl VAGINAL CONTRACEPTIVE HOLLOW-TYPE SUPPOSITORIES

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ABSTRACT

Objective: Recently discovered that Propranolol Hydrochloride is a potent inhibitor of human sperm motility and can be used as a contraceptive drug through local anaesthetic or membrane stabilizing activity. The aim of this study is to prepare Propranolol Hydrochloride as vaginal hollow suppositories by using different bases. **Method:** Eight formulas of hollow suppositories were prepared and the influence of different hydrophilic (PEG) and lipophilic (Witepsol H 37) base on drug release and the physical properties were evaluated. On the other hand, the most appropriate hollow type formula was compared to the conventional prepared suppositories from the same base of. The selected formula was stored at 4°C and 28°C for 30 days to study the effect of storage time and storage temperature on the in vitro drug release and the physical properties. In clinical study the selected prepared Propranolol Hydrochloride vaginal hollow suppositories formula was given to ten healthy women (25-30 years) as contraceptive way for determination its clinical activity. **Results:** the results showed that F7 which contain PEG 400:4000(70:30) gave the best drug release about 99% with accepted physicochemical properties. In addition to that a significant increase ($P<0.05$) of drug release for the selected hollow type formula in the comparison with the conventional one (52% drug release). The obtained results demonstrated that the effect of storage on the suppositories prepared from PEG 400:4000(70:30) cause slight increase in melting time and hardness (physical properties) and slight increase in release rate of drug from suppository base. On the other hand, clinical study gave very effective and comfortable contraceptive properties of the

prepared propranolol HCl vaginal hollow suppositories. **Conclusion:** It can be concluded that propranolol HCl can be prepared as a suitable vaginal hollow type suppositories for contraceptive effect through rapid release process of drug and good clinical response.

KEYWORDS: propranolol HCl**,** hollow type suppositories**,** contraception, vaginal suppositories.

INTRODUCTION

Suppositories are solid dosage forms intended for insertion into body orifices (rectum, vagina or urethra) where they melt, soften, or dissolve and exert localized or systemic effects for severely debilitated patients or those who cannot take medicines orally,[1,2] Human vagina is often described as slightly S-shaped fibromuscular collapsible tubes between 6 and 10 cm long extending from cervix of the uterus.[3,4] The vaginal wall consists of three layers: the epithelial layer, the muscular coat and the tunica adventia.[5]

Vaginal route can be used for both local or systemic administration.[8] The factors that affect vagina absorption of drug may be divided into two main groups: physiological factors and physicochemical factors of the drug and base.[6]

A suppository base performs two important functions. First, it serves as a carrier for the active drug in an appropriate way considering both its physicochemical characteristics and its requirements during preparation. Second, it can be used to control delivery of the active drug at the site of absorption.[1] Ideal suppository base may be dissolved or melt at vaginal temperature 36°C, no metastable forms, non- toxic or non-irritating to sensitive and inflamed tissues, compatible with a broad variety of drugs and stable on storage.[7]

Hollow - Type Suppositories, which have a hollow cavity to accommodate drugs in various form as a powder and solution, have been given to a number of patients during the past ten years and their usefulness has been confirmed.[8]

Propranolol HCl[9] is 2-Propanol, 1-[(1-methylethyl)amino]-3-(1-naphthalenyloxy), hydrochloride as shown in Figure (1). propranolol HCl Readily soluble in water and ethanol. Its molecular weight is 295.80. the chemical formula of propranolol is $C_{16}H_{21}NO_2$.

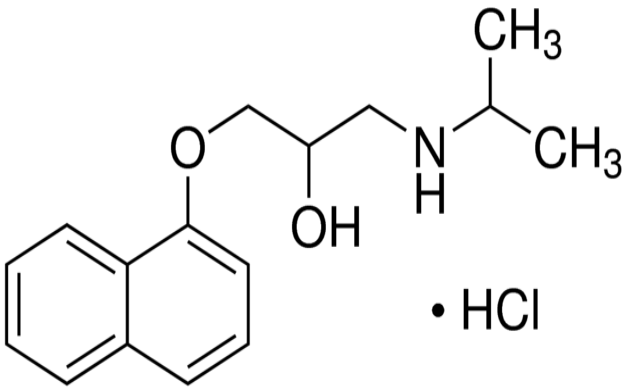

Figure (2): Structure of propranolol HCl.[9]

It is possesses local anesthetic or membrane stabilizing activity of short latency and fairly long duration and is a potent inhibitor of human sperm motility *in-vitro* and has a similar effect *in-vivo* in rats.[10]

The aim of this study is to prepare propranolol Hcl as contraceptive hollow-type suppositories using different suppositories bases. The physicochemical properties of the all prepared formulas were evaluated as melting time, hardness, softing time and drug release to select the most appropriated formula. The most appropriated formula was given to healthy women for clinical evaluations.

MATERIALS AND METHOD

Materials: Propranolol HCl (awa medica Drug Industry), Witepsol H37(Samara Drug Industry, Iraq**)**, Polyethylene glycols1000 and 6000 (BDH Chemicals Ltd, England), Di-sodium hydrogen phosphate (Seelze – Hoover, Germany), liquid paraffin (Fluka AG, Switzerland) and Lactose(Hazard Ltd, England).

Determination of Melting Point

The melting point of propranolol HCl was measured by the electrical melting point apparatus using capillary method.[11]

Determination of λ_{max}

Propranolol HCl solution of (30 µg/ml) in citric phosphate buffer pH 4.2 was prepared, then the solution was scanned by spectrophotometer from 200-400 nm and λ max of the drug was determined.[12]

Calibration Curve of Propranolol HCl

Calibration curve of propranolol HCl in citric phosphate pH 4.2 were obtained by preparing serial dilutions from stock solution (30µg /ml) and the prepared samples were analyzed spectrophotometrically at λ max that determined from above. The measured absorbance of each sample was plotted versus concentrations.

Preparation of Hollow-type Suppositories of Propranolol HCl

Different Eight formulas of Hollow-type suppositories as shown in table 1 were prepared by fusion method. various suppository bases were melt by using gentle heat (45°C) in water bath, then the melted bases were poured into 2 gm suppository molds equipped by cylindrical tube in the center (figure 2) and allowed to stand for 2 hours at room temperature to solidify. After construction of a hollow cavity was filled with 80 mg propranolol HCl and 20 mg of lactose powder.[12] The openings at the back part of the suppositories were sealed with the melted bases.

Figure (2): A modified mold (2gm) for preparation of hollow-type suppositories.

Table (1): Composition of Propranolol Hcl Hollow-Type Suppositories.

Formula No.	Quantity of propranolol Hcl (mg)	Type of the Base
F1	80	PEG_{1000}
F2	80	PEG_{4000}
F3	80	$PEG_{1000:4000}$(50:50)
F4	80	$PEG_{1000:4000}$(70:30)
F5	80	$PEG_{1000:6000}$(70:30)
F6	80	$PEG_{400:6000}$(70:30)
F7	80	$PEG_{400:4000}$ (70:30)
F8	80	Witepsol H 37

Effects of the Type and Ratio of Polyethylene Glycols

Formulas 1 (PEG_{1000}) and 2(PEG_{4000}) were prepared to study effect of PEG types on the physical properties and drug release from the prepared hollow type suppositories (table 1). Formulas 3-7 were prepared to investigate the effect of mixture of different PEG types with different ratio as demonstrated in table 1 on the physical properties and drug release from all the prepared hollow type suppositories.

Effect of Types of Suppositories Base

Formulas 1-7 which contain hydrophilic bases and formula 8 which contain lipophilic base were prepared to study the effect of suppository base type on the physical properties and drug release from the prepared hollow type suppositories.

Preparation of Propranolol HCl Conventional Suppositories

Propranolol HCl conventional suppositories were prepared by fusion method using PEG 400-4000 with ratio 70:30 as a suppositories base.

In general, the fusion method involved melting of the base by gentle heating (45°C) on water bath, followed by addition of the drug (80 mg for each suppository) with continuous and gentle stirring until a homogenous preparation was achieved. The mixture was poured into a 2 gram suppository mold and the suppositories were allowed to solidify over a night in a refrigerator.[14]

When the suppositories are hard, the mold is removed from the refrigerator and allowed to come to room temperature.

Effect of Suppositories Types on Drug Release

Conventional suppositories of propranolol HCl and formula 7 of hollow type suppositories were used to study the effect of suppositories type on the release of propranolol HCl from prepared suppositories.

Evaluation of the Hollow Suppositories

Hardness of suppositories

Hardness of the hollow type suppositories bases was evaluated to determine the mechanical strength of the prepared suppositories.[11]

The breaking strength test was carried out using the Erweka hardness tester. This test determines, under defined conditions, the resistance of suppositories to rupture, and it is measured by the mass needed to rupture them by crushing.

The temperature inside the testing chamber was controlled at 25°C by means of circulating water from a thermostat connected to the tester. The suppository was placed into the holding device with the tip upwards and the test chamber was then closed with glass plate. At this point, the initial load, which was given by the weight of the entire suspended block, was 600 g. After one minute a disc of 200 g weight, was added and this weight addition was continued every minute until the suppository collapsed under the load of the weight.

If the suppository collapsed within 20 seconds of placing the last disc, then this mass was not taken into account. If the suppository collapsed between 20 and 40 seconds of placing the last disc, then half of this mass was used in calculation, i.e. 100 g.

If the suppository remained uncrushed for more than 40 seconds after last disc was placed, then all the mass was used in calculation. Ten suppositories were used in each measurement.[15]

Determination of the Melting Time

The release of the active ingredient from the vehicle is related to the melting point of the vehicle and the solubility of the drug in the vehicle. The suppositories were placed into a glass tube (2.5 cm diameter); 2 ml of citric phosphate buffer solution of pH 4.2was then added. The tube was placed in a water bath at 37°C$\pm$0.5°C. The time required for each suppository to melt completely or to disintegrate was determined.[16]

Softening Time Determination for lipophilic suppositories

The softening time test indicates how long certain preparation takes to lose its physical structure. The suppository was inserted in the spiral shaped glass basket of the test tube with the tip pointed upwards and the tube was then closed. A thermostat connected to the tester provided circulating distilled water inside the test tube at the constant temperature 37°C and constant flow rate. The time required for the first drop of the suppository base to

appear floating on the surface of the water inside the testing tube was considered softening time.[17]

In Vitro Drug Release

The release of propranolol HCl from prepared hollow type suppositories was done by placing the suppositories in ajar containing 900ml citric phosphate buffer Ph 4.2, with a paddle rotating at 100 r.p.m[12] at a constant temperature of (37°C ± 0.5°C). determination of release rates of propranlol HCL from the various suppository bases. At appropriate time intervals (5, 10, 15, 20, 25,30,35 and 40 minutes), 5ml samples were withdrawn and fillter through the volume of the dissolution medium was kept constant by replacing the withdrawn volume of the sample with equal volume of fresh dissolution medium maintained at the same temperature. A minimum of triplicate drug release determinations were made for each suppository preparation. propranlol HCl samples were analyzed using an ultraviolet spectrophotometric method.

Effect of Storage Time and Temperature

Formula (7) was wrapped with aluminum foil and stored at 4°C and 28°C for 30 day to study the effect of storage and temp. on the physical property and the drug release from the prepared hollow type suppositories.[18,19]

Clinical study

Formula (7) was prescribed to ten healthy women (25-30 years) at dose of one vaginal suppositories inserted one half hour before intercourse for two months and its contraceptive ability was evaluated to determine its clinical activity.

Statistical Analysis

The results obtained were statistically analyzed by using one way analysis of variance (ANOVA). Differences of ($P< 0.05$) were considered to be significant.[20]

RESULT AND DISCUSSION

Determination of propranlol HCl melting point

The measured melting point of propranlol HCl was found to be 165°C with decomposition. This result was the same as reported in references, which indicates the purity of the supplied drug powder.

Determination of λ max of propranlol HCl

The UV scan of the solution which contains (30 µg/ml) of propranlol HCl in citric phosphate buffer ph 4.2 by UV spectrophotometer at 200-400 nm gave the spectrum with a λ_{max}. at 289 nm.

Determination of Calibration Curve

Figure (3) shows the calibration curve of propranlol HCl in citric phosphate buffer ph 4.2. A straight line was obtained by plotting the absorbance versus concentration with high regression coefficient (R^2 = 0.9997). This indicates that calibration curve obeys Beer-lambert's law at λ max 289 nm within the range of concentration used.[21]

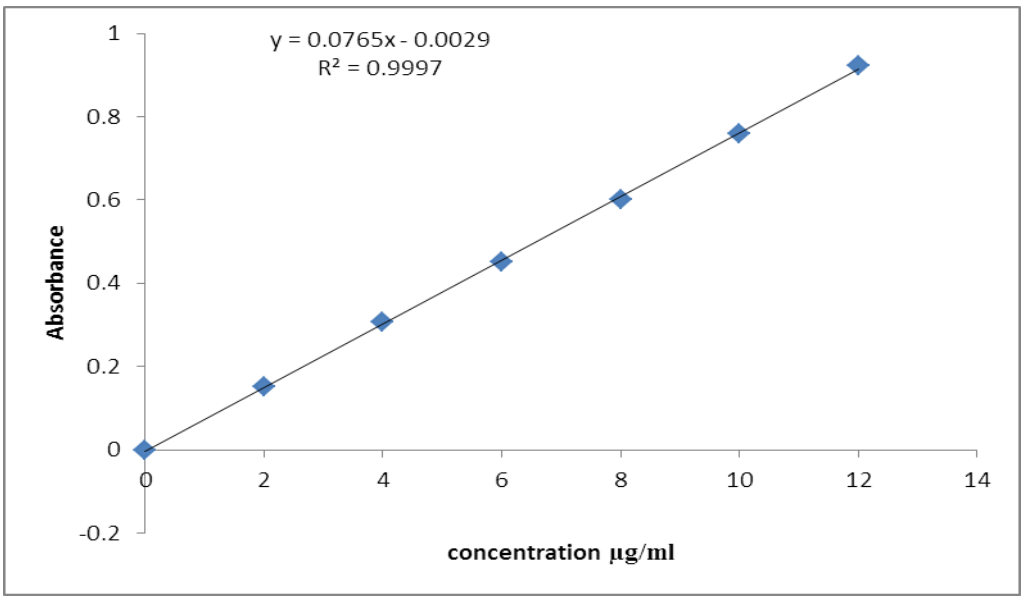

Figure (3): Calibration curve of propranlol HCl in citric phosphate buffer pH 4.2.

Evaluation of the Prepared Suppositories

Effects of the Type and Ratio of Polyethylene Glycols

Table (2) shows the effects of changing the type and ratio of polyethylene glycols on the physical properties of hollow-type suppositories. The results indicated that formula 2 (which contain PEG 4000) showed higher melting time compare to other formulas. In addition to that melting time for lipophilic bases (witepsol H 37) was to be longer than to hydrophilic bases (polyethylene glycols). Formulase 2,5 and 6 were no significant differences ($P<0.05$) in hardness between water-soluble base and oleaginous base.

Figure 4 demonstrated that the % drug release show no significantly differences ($P>0.05$) in the dissolution rate of propranlol HCl from formulas 2, 3 and 4 but there was slightly

decrease in release rate of drug of formula 5 as show in figure (4). This may be due to that the water solubility and hygroscopicity of polyethylene glycols (PEG) decrease with increase average molecular weights. This result was in agreement when these bases used in the formulation of ibuprofen suppositories.[22]

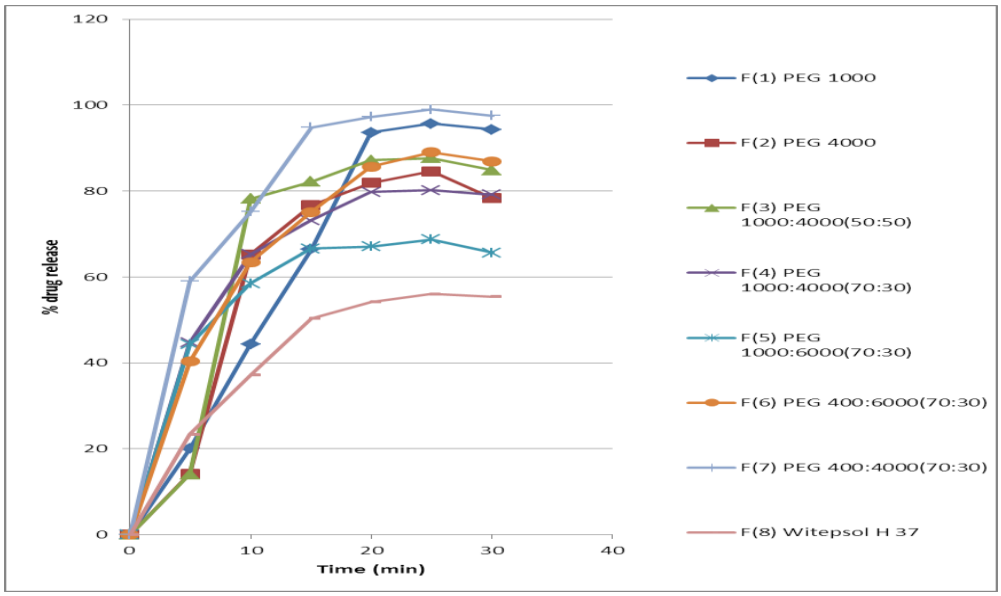

Figure (4): Effect of Type and ratio of Suppository Base Used on the in vitro release of propranolol HCl in citric phosphate buffer pH 4.2 at 37°C.

Table (2): Physical Properties of Hollow-Type Suppositories of propranlol HCl.

Formula No.	Base Type	Parameters		
		Softening Time (minutes)	Melting Time (minutes)	Hardness (Kg)
F1	PEG_{1000}	-	15	1.2
F2	PEG_{4000}	-	34	3.3
F3	$PEG_{1000:4000}$ (50:50)	-	27	2.5
F4	$PEG_{1000:4000}$ (70:30)	-	30	2.5
F5	$PEG_{1000:6000}$ (70:30)	-	28	3.1
F6	$PEG_{400:6000}$ (70:30)	-	26	3
F7	$PEG_{400:4000}$ (70:30)	-	23	2.3
F8	Witepsol H 37	10	43	3.2

Effect of Suppository Types

Table (3) represent the physical properties (melting times and hardness of hollow suppositories formula 7 and conventional suppository. It appeared that conventional type

have a longer melting point and higher hardness compare to hollow suppositories formula 7. This may be due to the presence of cavities fill of drug which might affect the skeleton structure of the hollow-type suppositories.[42]

Figure (5) shows the dissolution behavior of propranolol HCl from hollow suppositories formula 7 and conventional type, it indicated that there was a significant increase ($P<0.05$) of drug release for hollow type formula7 when compared with convential.Watanabe.et.al demonstrated that drug was released more rapidly and absorbed more efficiently from a hollow-type suppository than from a conventional suppository.

This result was in agreement with the results obtained from the formulation of morphine as conventional and hollow-type suppositories.[23]

Table (3): Physical Properties of propranolol HCl Hallow and Conventional Suppositories.

Suppositories Type	Melting Time (minutes)	Hardness (Kg)
Hollow type	23	2.3
Conventional type	29	3.2

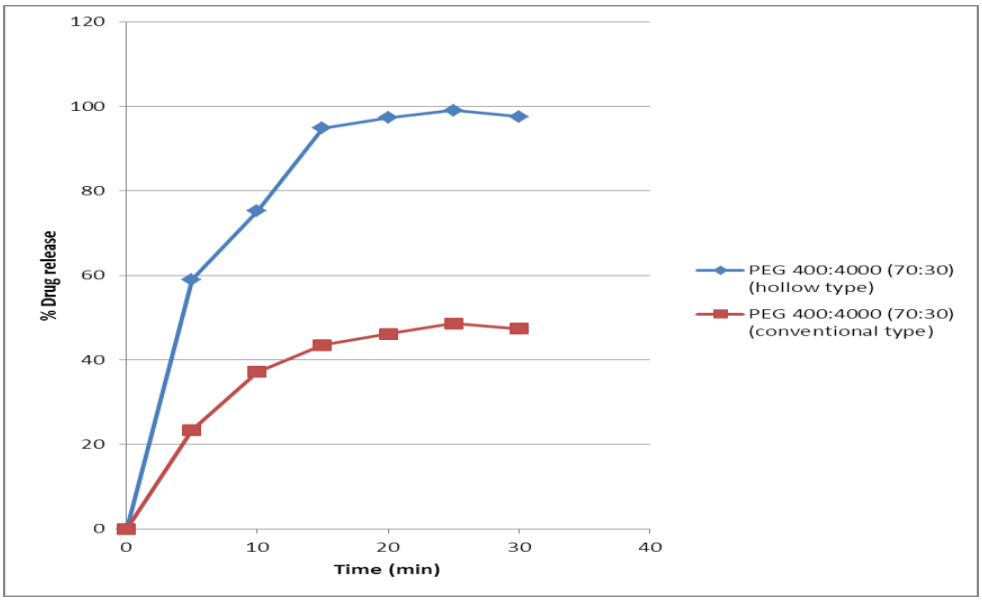

Figure (5): Effect of suppository type on the in vitro release of propranolol HCl from PEG 400:4000(70:30) as hallow and convential suppositories in citric phosphate buffer pH 4.2 at 37°C.

Effect of Storage Time and Temperature

Hallow type suppositoties formula 7 was chosen as the most appropriated formula since it showd a faster drug release and acceptable physicochemical properties as compared with

other formulas. Therefore the effect of storage time and temperature was done on it. Table (4) demonstrated the effect of storage period and temperature on the physical properties for the hallow type suppositoties formula 7.

Samples were selected from this formula and stored for 30 days at 4°C and 28°C.

The result indicated that on storage of these suppositories samples of formula 7 at 4°C and 28°C there was no significant change in the physical properties, but the slightly increase in the softening time and melting time and hardness for samples of formula 7. Beside the dissolution behavior of propranlol HCl from these samples of this formula during the storage at a various temperatures was found to be no significant ($p>0.05$) change in release of drug from the formula tested as show in figures (6).

This result was in agreement with the results obtained from suppositories containing montelukast sodium.

Table (4): Effect of Storage Time and Temperature on the Physical Properties Formula 7.

Storage temperature	Melting Time (minutes)	Hardness (Kg)
4°C	29	3.3
28°C	24	2.8

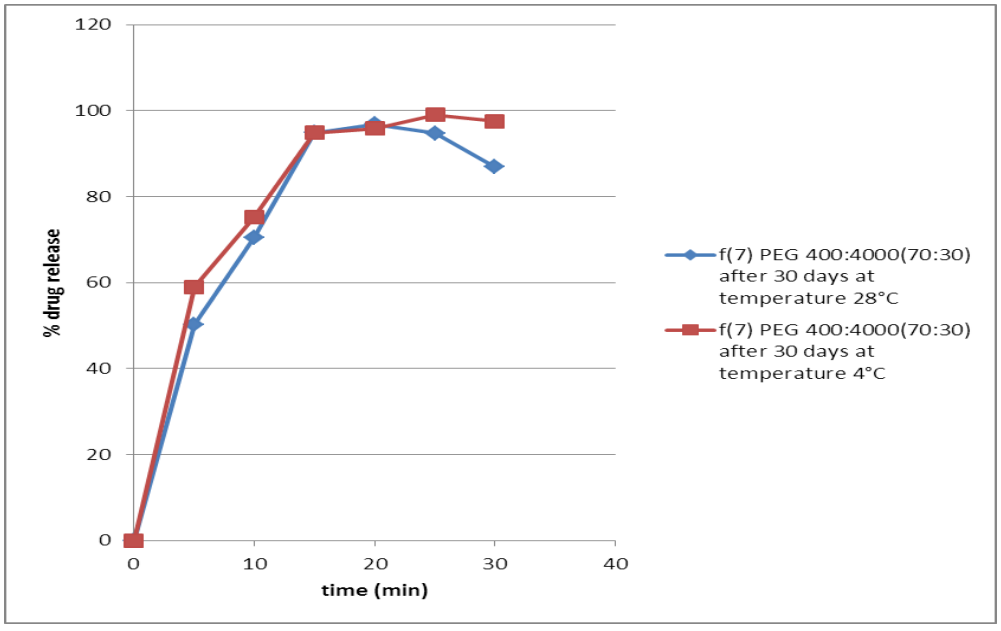

Figure (6): Effect of storage period and temperature on the release of propranolol HCl from hollow-type suppositories using PEG 400:4000(70:30) base in citric phosphate buffer pH 4.2 at 37°C.

The result of clinical study of the prepared formula indicated highly efficient and comfortable dosage form.

CONCLUSION

From the results obtained, we can conclude that the maximum release rate of the drug was achieved from hollow-type vaginal suppositories formulated by using [PEG $_{400:4000}$ (70:30)] as a suppository base with better drug release from hollow type suppositories compared with conventional suppositories formulated from the same base. Hollow-type suppositories are useful as a promising approach for enhancing the release of drugs administered vaginally to get efficient, rapid, comfortable pharmacological effect.

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