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Formulation of Tinidazole Rectal Suppositories

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الخلاصة:

تم تحضير التنيدازول كلبوسات شرجية لغرض جعل الدواء متوفر داخل الجسم عن طريق اخر غير الفم، وبذلك يمكن تجاوز الاثار الجانبية للدواء على القناة الهضمية، وكذلك من الممكن استعمال هذا الشكل الدوائي عند صعوبة اعطاء الدواء عن طريق الوريد.

لقد تم دراسة تاثير نوع القاعدة اللبوسية وتاثير الموادالمخفضة للشد السطحي المضافة على الخواص الفيزيائية و على قابلية تحرر التنيدازول من اللبوسات الشرجية باستخدام القواعد الشحمية (الوايتبسول ھ 15 والوايتبسول ھ 35) والقواعد الذائبة في الماء [خليط من البولي اثيلين كلايكول 6000 :400 :200 (30 :35 :35) و 4000 :1000 (30 :70)].

لقد اوضحت النتائج ان قابلية تحرر التنيدازول اكبر من القواعد الذائبة في الماء مقارنة بالقواعد الشحمية والسبب في ذلك يعود ربما الى وجود اتحاد بين الدواء والترايكليسرايدات الموجودة في تلك القواعد الشحمية. كذلك وجد ان استخدام المواد المخفضة للشد السطحي قد زاد من تحرر التنيدازول من اللبوسات الحاوية على الوايتبسول ھ 15، وكانت الزيادة اكبر في حالة استخدام السبان 80 مقارنة بالتوين 80. كذلك تم دراسة تاثير درجة الحرارة وفترة الخزن على الصفات الفيزيائية و قابلية تحرر الدواء بواسطة خزن اللبوسات الشرجية لمدة 1 ,15 ,30 و 45 يوم بدرجتي حرارة 25 °م و 4 °م.

لقد اوضحت النتائج ان لدرجة حرارة الخزن تاثير على قابلية تحرر التنيدازول من اللبوسات الحاوية على الوايتبسول ھ 15 دون ان تؤثر غلى الخواص الفيزيائية لهذه اللبوسات، اما فترة الخزن فقد كان لها تاثير على كل من الخواص الفيزيائية وقابلية تحرر التنيدازول من هذه البوسات.

من ناحية اخرى، فقد وجد ان لدرجة حرارة الخزن تاثير على كل من قابلية تحرر التنيدازول والخواص الفيزيائية للبوسات الحاوية على البولي اثيلين كلايكول بينما لم تكن لفترة الخزن اي تاثير على هذه اللبوسات.

واخيرا وجد ان مدة صلاحية التنيدازول في اللبوسات الحاوية على البولي اثيلين كلايكول 4000 :1000 (30 :70) تبلغ حوالي 3,3 سنة.

Abstract:

Tinidazole was prepared as a suppository dosage form that could be used rectally in an attempt to render the drug available systemically by a route other than oral route, thus overcoming the side effects of the drug on the gastrointestinal tract. Also such dosage form could replace the intravenous administration whenever it is inconvenient.

The influence of the type of suppository base and added surfactants on the physical properties and drug release was studied using lipophilic bases (witepsole H-15, witepsole H-35) and water soluble bases [mixtures of polyethylene glycols PEG 6000:400:200 (30:35:35) and PEG 4000:1000 (30:70)].

The results showed a good release of tinidazole from PEG bases compared to lipophilic bases because of the interaction between the drug and triglycerides of the lipophilic bases. Also the non ionic surfactants increase the release of tinidazole from witepsole H-15 base containing suppository and the increase was greater for span 80 than tween 80.

Also the influence of temperature and storage period on the physical properties and release was investigated by storing the prepared suppositories at 4˚C and 25˚C for 1, 15, 30 and 45 days. The results appeared that the temperature of storage affect the release of tinidazole from witepsole H-15, but had no effect on physical properties of the suppositories. While the storage period at both temperatures had some effects on the physical properties. On the other hand, temperature of storage affects the physical properties of polyethylene glycol suppositories and release of the drug, in contrast to the period of storage, which had no effect.

The expiration date of tinidazole in formula containing polyethylene glycol 4000:1000 (30:70) was found to be about 3.3 years.

Keyword: Tinidazole, suppositories, rectal preparations, suppository base.

Introduction:

Rectal preparations are intended for rectal use in order to obtain a systemic or local effect or may be intended for diagnostic purposes [1].The suppository is a medicated solid dosage form generally intended for use in the rectum, vagina and to a lesser extent, the urethra[2]. They disintegrate in the body cavity either by melting or by dissolution [3]. The rectal route has many advantages such as it can avoid the hepatic first pass effect, and avoid the undesirable effect of meals on drug absorption[4, 5]. In addition the rectal route is useful in decreasing gastrointestinal side effects as found for Aspirin [6]. It is also preferred in the treatment of patients who are unable to make use of oral route. This may be the case when the patient has a problem in the gastrointestinal

tract like nausea and vomiting episode or case of unconsciousness [4,7]. Further more, several categories of the patients, i.e. the very young, the very old or the mentally disturbed, may more easily use the rectal than the oral route [8]. The drug may be insufficiently stable at the pH of the stomach or susceptible to enzymatic attack in the gastrointestinal tract[9]. The suppository dosage form used to avoid unpleasant tasting or smelling drugs whose oral use is limited as metronidazole [10].

Tinidazole, like other structurally related drug metronidazole, demonstrate activity against the following protozoa: *Trichomonas vaginalis, Giardia lamblia and Entamoeba histolytica*. It also has a prophylactic use to prevent post operative anaerobic infections and used for the eradication of Helicobacter in peptic ulcer diseases [11].

The aim of this study is to formulate tinidazole as suppository dosage form, which suggested to be used rectally to obtain systemic effect in order to replace the intravenous route and to avoid the side effects of tinidazole on the gastrointestinal tract when used orally.

Materials and Methods:

Materials:

Tinidazole (Sigma chemical Co.), witepsole H-35 (supplied by Sammara Drug Industry SDI), witepsole H-15 (supplied by Al Shahbaa Pharma, Syria), PEG 4000 and PEG 200 (Hopkin &Williams, England), PEG 6000, PEG 1000, PEG 400, Potassium dihydrogen ortho-phosphate (BDH chemicals, Ltd. Pool, England), tween 80 (Merck schuchardt, Germany), Disodium hydrogen phosphate (E.Merck Darmstadt, West Germany), Span 80 (Atlas-chemie, Germany).

Instruments:

Balance (Sartorius AG, Gottingen, Germany), Dissolution apparatus (Copley, type FH 16-D, Nottingham, England), pH meter (pH 211, microprocessor, Italy), Hardness tester, Softening time tester (Erweka, Apparatteban GMBH, SBT, West Germany), UV visible spectrophotometer (Carrywin UV, Varian, Australia), suppository moulds 2 gm (stainless steel, ERBO Prazision Formenbau, GMPHD-7470 Albstadt 3).

Methods:

Preparation of tinidazole suppositories:

Tinidazole suppositories were prepared by fusion method in which 500 mg tinidazole and the surfactant (when used) were incorporated into the suppository base after it was melted by gentle heating on a water bath. The amount of the base used after calculating the displacement value. The melted mass was stirred constantly but slowly to avoid air entrapment, then the mixture poured into a 2 gm suppository

mold and then cooled in a refrigerator maintained at 5 ̊C. After that, any excess suppository mass was removed from the mold by scraping and then the mold was opened and the suppositories were removed [12].

For suppository containing mixture of polyethylene glycols, the higher molecular weight polyethylene glycol was first melted, then the lower molecular weight polyethylene glycol were added and mixed well[2].

Formulations:

Different formulas were prepared using different types of bases and surfactants as shown in (Table-1) the displacement value of tinidazole in these bases was first determined and the amount of the base needed was calculated.

Physical properties of the prepared suppositories:

Melting time determination:

The suppositories were placed in a glass tube (2.5 cm diameter); 2 ml of Sorensen's phosphate buffer of pH 7.4 was then added. The tube was placed in water bath at 37˚C. The time required for each suppository to melt completely or to disintegrate was determined [13].

Hardness test (Resistance to rupture):

This test determines, under defined conditions, the resistance to rupture of suppositories measured by the mass needed to rupture them by crushing. This test carried out using the Erweka hardness tester. The temperature inside the testing chamber was controlled at 25˚C by means of circulating water from 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 entire suspended block, was 600 gm. After one minute a disk of 200 gm was added and this weight addition was continued every minute until the suppository crush under the load of the weight.

The mass required to crush the suppository was calculated by the sum of the masses weighing on the suppository when it was collapsed (including the initial mass of the device i.e. 600 gm) [14].

Softening time test (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 as softening time [15].

In vitro dissolution test:

The dissolution rate of tinidazole from suppository was determined using a rotating basket dissolution apparatus at 50 rpm and at a constant temperature of 37˚C. The medium was 900 ml of Sorensen's phosphate buffer solution of pH 7.4. At appropriate time intervals (0, 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes), 5 ml samples were withdrawn through syringe Millipore filter and the amount of tinidazole was determined by ultraviolet spectrophotometer at 310 nm.

Factors affecting the formulation:

Effect of type of suppository base:

Lipophilic bases [witepsole H-35 (formula 3) and witepsole H-15 (formula 4)] and hydrophilic bases [PEG 6000:400:200 (30:35:35) (formula 1) and PEG 4000:1000 (30:70) (formula 2)] were used to investigate the influence of the type of suppository base on physical properties and dissolution rate of tinidazole from the prepared suppositories.

Effect of type of surfactant:

The effect of type of surfactant on the physical properties and the dissolution rate of tinidazole from the prepared suppositories were studied by incorporating 5% span 80 and 5% tween 80 with witepsole H-15 containing suppository [formula (5) and formula (6), respectively].

Effect of storage time and temperature on tinidazole release and physical properties of the selected suppositories:

Experiments were conducted for studying the effect of storage time and temperature on the release of tinidazole and physical properties of different suppository formulas. The study was carried out using suppositories stored for 1, 15, 30 and 45 days at 4˚C and 25˚C. Two formulas were selected for this study [formula (2) and (5)]. The suppositories were wrapped with aluminum foil, placed in tightly closed containers and stored at the mentioned temperatures for the periods indicated [2].

Finally, formula (2) was stored for 30, 60, 120 and 180 days at 25˚C to determine the shelf life.

Results and Discussion:

Factors affecting the formulation of tinidazole suppositories:

Effect of suppository base:

The effect of the type of the base on the physical properties of the prepared suppositories was illustrated in (Table -2). It appears that all the suppositories were within the limits recommended by the British Pharmacopoeia (disintegration occurs within 30 minutes for fat based suppository and less than 60 minutes for water soluble suppository). Also it was found that hydrophilic bases (polyethylene glycol) have long

melting time than oleaginous bases (witepsole). Beside that the hardness of the suppositories prepared from polyethylene glycol was found to be less than that observed for oleaginous bases [14].

Figure (1) illustrates the effect of changing the suppository base type on the in vitro release of tinidazole from the prepared suppositories. It appears that the amount released was higher from hydrophilic bases [formula (1) and (2)] when compared with the oleaginous bases [formula (3) and (4)]. This is due to the lower water solubility of tinidazole, so the affinity of the drug to lipophilic bases is higher than the hydrophilic bases which make the entrapment of the drug within these bases easy. On the other hand, the high release percentage of tinidazole from hydrophilic bases may be related both to the affinity of the drug for these bases, and the water solubility of the base in the aqueous medium (i.e. the solubility of the drug in the base greatly influence the amount of the drug released from those suppositories). The results are in consistence with the results obtained in the formulation of flurbiprofen [16] as suppository dosage form.

Also there was a slight increase in the release of tinidazole from formula (2) as compared with formula (1). This may be due to the fact that the release from polyethylene glycol base was found to be increased as molecular weight decreased [17].

Changing the type or ratio of polyethylene glycol mixture from formula (1) to (2) affect the physical properties of the suppositories due to the fact that the melting point and hardness of polyethylene glycols increase as a function of polymerization of the polymer used, that increase with the molecular weight used [1].

Effect of addition of surfactants to the suppository base:

Non ionic surfactants were used in an attempt to improve the release of tinidazole from the suppository [18]. This was achieved by incorporating 5% (w/w) span 80 or tween 80 in witepsole H-15 [formula (5) and (6) respectively.

Witepsole H-15 was chosen because it was found to be the base of choice for use in countries of continental climate [19].

The addition of these non ionic surfactants resulted in an increase in the amount released of tinidazole from witepsole H-15 suppository base as shown in figure (2). This could be due to an increase in the wetting and spreading properties of the base and subsequent increase in the dissolution rate of the drug [20] also may be due to the micellar solubilization of the drug by surfactants [19]. Moreover, surfactants decrease the disintegration time and thus increase the release of active ingredients from suppository base [21].

It was found that span 80 (formula 5) produced the highest release compared with tween 80 (formula 6). This could be due to the HLB

(hydrophilic-lipophilic balance) value of span 80 which is equal to 4 which is considered to be the optimal HLB value for surfactant incorporated in suppository base [22].

Non ionic surfactants also affect the physical properties of the prepared suppository. It was found that the addition of these surfactants resulted in a decrease in the melting time, softening time and the hardness as shown in (Table -3). This could be due to the fact that these adjuvants are miscible with lipophilic excepients, so homogenous mixture of heterogeneous composition was obtained. Consequently, the melting temperature of the base-excepients was decreased [23].

Effect of storage time and temperature on tinidazole release and physical properties of the selected suppository:

Formula (2) and (5) were used in this study because of their higher release behaviors. Samples were selected from these formulas and stored for 1, 15, 30 and 45 days at 4°C and 25°C.

(Table -4) showed that there was a reduction in the melting time and hardness of formula (2) tinidazole suppository [PEG 4000:1000 (30:70)] stored at 4°C, especially after 30 and 45 days of storage, with slight changes in these parameters on storage at 25°C. This observation can be explained by the fact that storing polyethylene glycols based suppositories at low temperatures cause an increase in their brittleness with subsequent decrease in melting time and hardness [24].

The effect of the storage conditions at different temperatures on the release behavior of tinidazole from the selected formulas was studied. For formula (2) which was stored at 4°C, showed an increase in the release rate for samples stored at 45 days compared to those tested after one day of preparation, as presented at figure (3). This may be due to an increase in their brittleness which makes the drug rapidly released from the suppository [24].

While figure (4) represent the effect of storage time and temperature on the release of tinidazole from formula (2) suppositories stored at 25 °C. It shows no significant changes in the release rate for samples stored at different time intervals since the base is stable at 25 °C and undergo no brittleness [24].

On the other hand, formula (5) shows an increase in the melting time and hardness on storing at different time intervals at 25 °C with no significant changes at 4 °C as shown in (Table -5). This may be due to the crystallization of witepsole base at room temperature when stored for prolong time [25].

For the same reason, there was a decrease in the release rate of tinidazole from formula (5) stored at 25 °C for samples tested after 45 days compared to ones tested soon after preparation due to the crystallization of the witepsole bases when stored at room temperature for

prolong time [25] as shown in figure (6). While figure (5) illustrates that there was no significant change in the release rate of tinidazole from formula (5) at 4 °C.

Shelf life study:

To evaluate most promised formula for tinidazole rectal suppository, formula (2) was introduced to study the shelf life. The suppositories were stored at 25°C and samples analyzed for drug content at 30, 60, 120 and 180 days.

The degradation of tinidazole followed first order kinetics since the plot of the logarithm of percent remaining of tinidazole versus time gave straight line [26] as shown in figure (7). The degradation rate constant of tinidazole at 25° C ($K_{25\,^{o}C}$) was determined and found to be (0.4×10^{-4}) day^{-1}. The shelf life then was calculated from the following equation and found to be 3.3 years.

$t_{10\,\%} = 0.105 / K_{25\,^{o}C}$

Conclusion:

The release of tinidazole from water soluble bases was better than lipophilic bases. The non ionic surfactants improve the release from witepsole H-15 base especially for span 80 and to a lesser extent tween 80.

The storage time and temperature affect both the physical properties of the suppositories and the release of tinidazole. The expiration date of tinidazole in suppository dosage form was about 3.3 years.

Formula no.	Base	Tinidazole 500 mg	Span 80 (w/w)	Tween 80 (w/w)	Displacement value
1	PEG 6000:400:200 (30:35:35)	500	-	-	1.19
2	PEG 4000:1000 (30:70)	500	-	-	1.3
3	Witepsole H-35	500	-	-	1.45
4	Witepsole H-15	500	-	-	1.5
5	Witepsole H-15	500	5%	-	1.5
6	Witepsole H-15	500	-	5%	1.5

Table-1: Composition of tinidazole suppositories using different bases and surfactants and the calculated displacement values

Formula no.	Base type	parameters		
		Melting time (min.)	Softening time (min.)	Hardness (Kg)
1	PEG 6000:400:200 (30:35:35)	33	_	3.0
2	PEG 4000:1000 (30:70)	31	_	2.8
3	Witepsole H-35	13	6	3.5
4	Witepsole H-15	11	5	3.3

Table-2: Effect of changing the type of base on the physical properties of tinidazole suppositories.

Formula no.	Base type	parameters		
		Melting time (min.)	Softening time (min.)	Hardness (Kg)
4	Witepsole H-15	11	5	3.3
5	Witepsole H-15 + 5% span 80	9	4	2.6
6	Witepsole H-15 + 5% tween 80	10	5	2.9

Table-3: Effect of addition of surfactant on the physical properties of tinidazole suppositories.

Storage time (days)	parameters			
	4°C		25°C	
	Melting time (min.)	Hardness (Kg)	Melting time (min.)	Hardness (Kg)
1	31	2.8	31	2.8
15	30	2.6	32	2.6
30	28	2.2	29	2.7
45	24	2.0	30	2.8

Table-4: Effect of storage time and temperature on the physical properties of tinidazole suppository formula (2) at 4°C and 25°C.

Storage time (days)	parameters			
	4°C		25°C	
	Melting time (min.)	Hardness (Kg)	Melting time (min.)	Hardness (Kg)
1	9	2.6	9	2.6
15	8	2.5	9	2.6
30	9	2.5	10	2.7
45	10	2.6	12	2.8

Table-5: Effect of storage time and temperature on the physical properties of tinidazole suppository formula (5) at 4°C and 25°C.

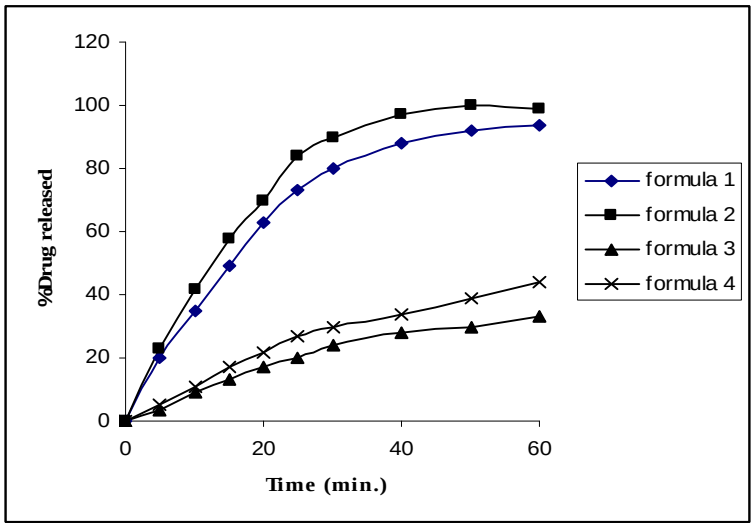

Figure-1: Effect of suppository base type on the in vitro release of tinidazole from the prepared suppository in Sorensen's phosphate buffer of pH 7.4 at 37 °C. (formula 1: PEG 6000:400:200 (30:35:35), formula 2: PEG 4000:1000 (30:70), formula 3: Witepsole H-35, formula 4: Witepsole H-15).

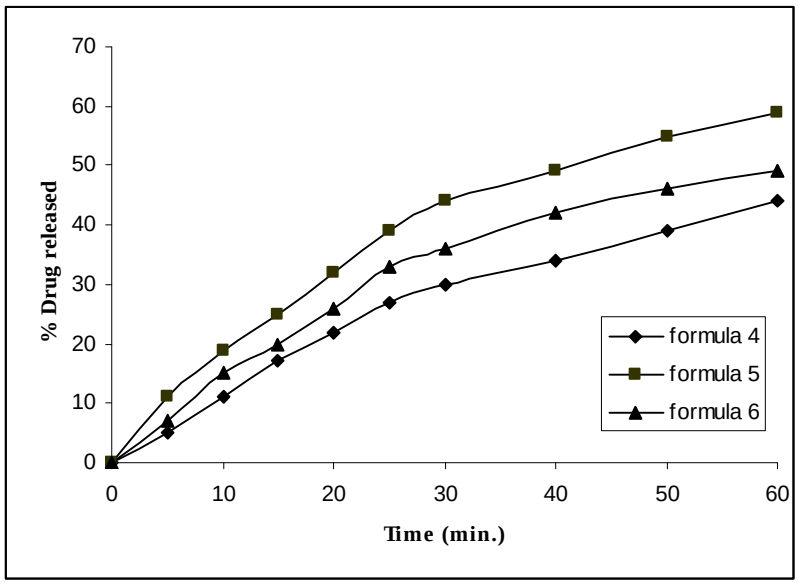

Figure-2: Effect of type of surfactant on the in vitro release of tinidazole from the prepared suppositories in Sorensen's phosphate buffer of pH 7.4 at 37 °C. (formula 4: Witepsole H-15, formula 5: Witepsole H-15 + span 80 (5% w/w), formula 6: Witepsole H-15 + tween 80 (5% w/w)).

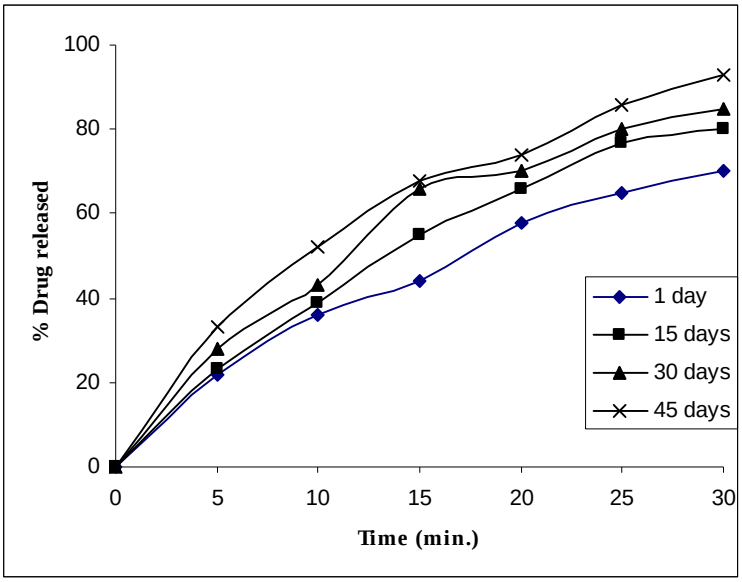

Figure-3: Effect of storage time and temperature on the release of tinidazole from formula (2) suppositories stored at 4◦C using Sorensen's phosphate buffer of pH 7.4 at 37 ◦C.

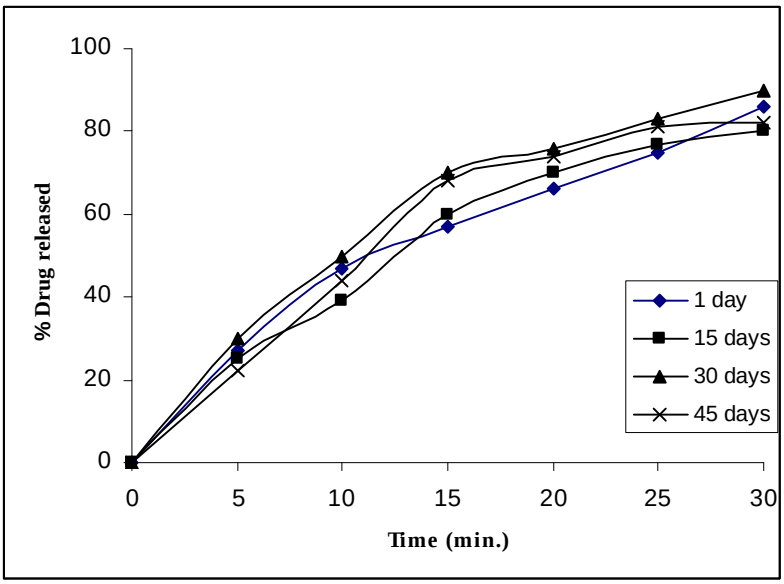

Figure-4: Effect of storage time and temperature on the release of tinidazole from formula (2) suppositories stored at 25°C using Sorensen's phosphate buffer of pH 7.4 at 37 °C.

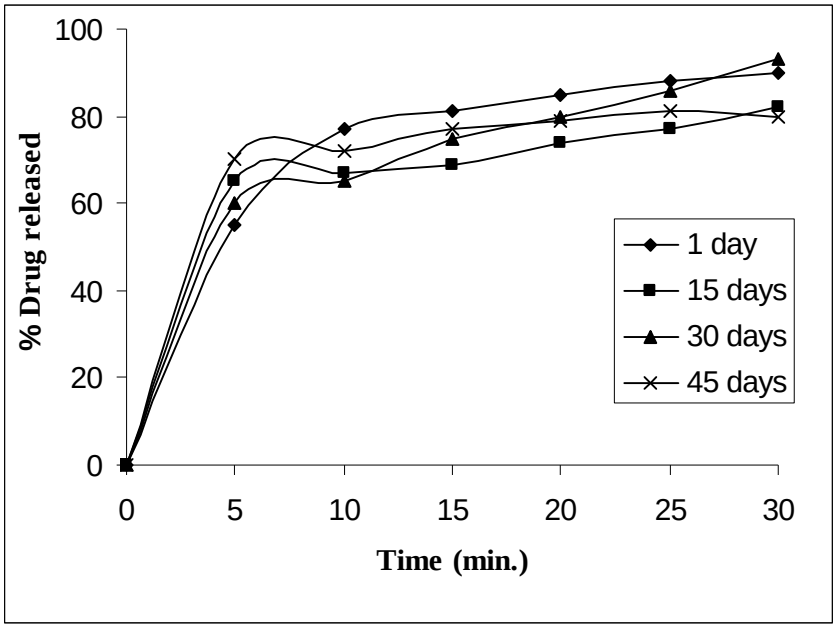

Figure-5: Effect of storage time and temperature on the release of tinidazole from formula (5) suppositories stored at 4◦C using Sorensen's phosphate buffer of pH 7.4 at 37 ◦C.

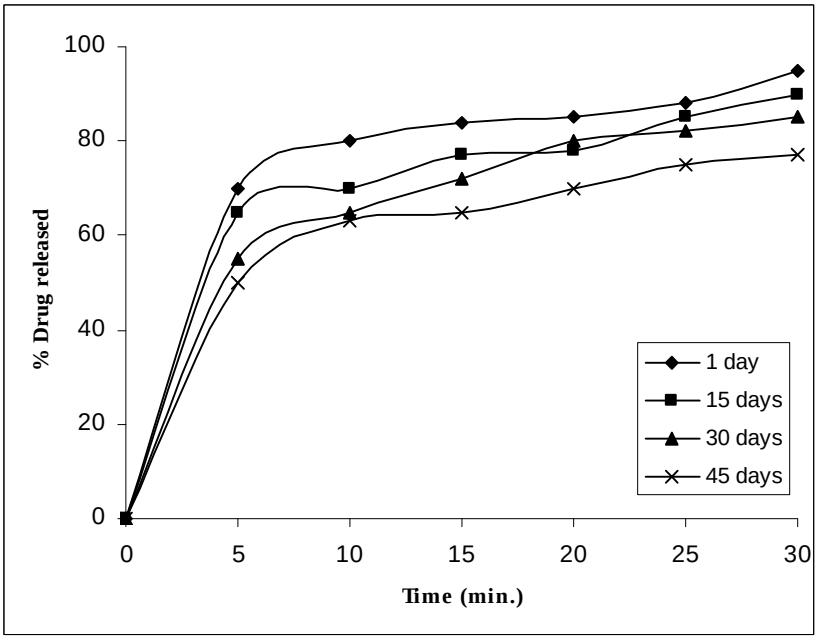

Figure-6: Effect of storage time and temperature on the release of tinidazole from formula (5) suppositories stored at 25°C using Sorensen's phosphate buffer of pH 7.4 at 37 ◦C.

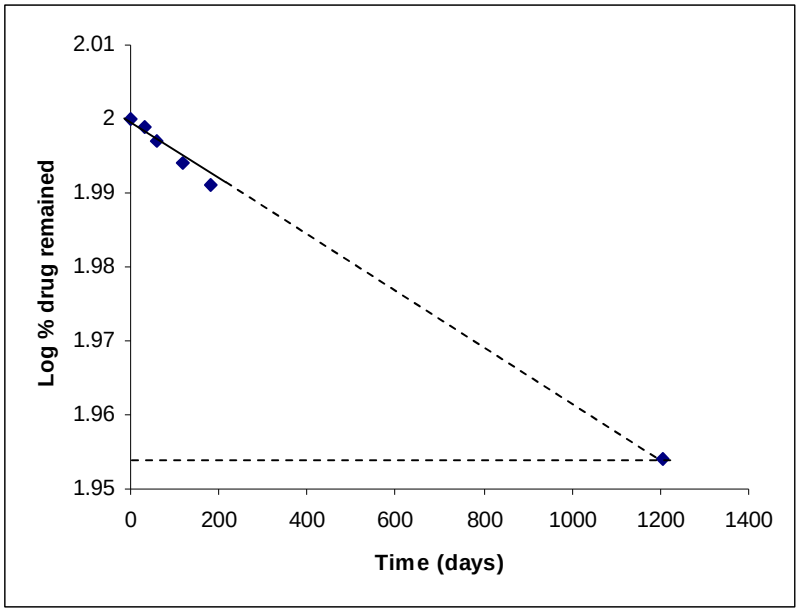

Figure-7: Determination of the expiration date of tinidazole in formula (2) suppositories stored at 25°C.

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