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FORMULATION AND EVALUATION OF MIDAZOLAM SUPPOSITORIES: 1-FORMULATION OF MIDAZOLAM SUPPOSITORIES ADOPTING HPLC TECHNIQUE FOR ANALYSIS

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ABSTRACT

Infantile suppositories each containing 10 mg midazolam maleate were prepared using polyethylene glycol(PEG) bases, cacao butter, witter H15 and witepsol E75. Some physico-chemical tests were carried out on the prepared suppositories with or without the medicament. High performance liquid chromatographic assay has been described for successful quantitative determination of the drug using B-CyD-bonded stationary phase. The proposed method is accurate and was established from the standard solution. The permeation and dissolution patterns of the drug from the tested bases were investigated and were found to be base dependent. Carbowax base consisting of a mixture of PEG 1000, 4000 (97:3) gave the best permeation of the drug and the fastest dissolution rate.

INTRODUCTION

Midazolam, 8-chloro-6-(fluorophenyl-1-methyl-4H-imidazo[1,5-a][1,4] benzodiazepine (midazolam Ro 21-3981,Dormicum^{R}) is a new benzodiazepine derivative with unique properties. It is water soluble and also stable in its acid formulation, but is highly lipid soluble in-vitro[1-3]. It has rapid onset and short duration of action. Other benzodiazepines which forms water soluble salts like chlordiazepoxide and oxazepam have a slow onset of action. There are many uses for midazolam in pre-operative period including premedication, anesthesia induction and maintenance, and sedation for diagnostic and therapeutic procedures[1-3].

The product is not yet in the Egyptian market. It has been investigated clinically in the form of i.m. or i.v. injectable solution (maleate in an early phase, thereafter hydrochloride) and in the form of tablets containing the maleate salt. No information about the

rectal use of this new promising drug has been reported.

However, the rectal administration of some benzodiazepines have been studied[4-9]. Diazepam rectal solution gave faster and regular absorption than the oral or the i.m. administration with no observed differences in the bioavailability[5-8]. On the other hand, lorazepam suppositories formulated in water soluble bases gave the same extent of absorption as that of an oral solution of the drug in mongrel dogs[9]. Suppositories may be convenient for pre-operative medication, especially for children to avoid painful injection.

Titrimety[10], complexiметry[10], colorimetry[11] and spectrophotometry[12] have been used for quantitative determination of benzodiazepines. In addition GLC and HPLC have been developed for the analysis of benzodiazepines in drug formulations[13-14] and in biological fluids[15].

Recently, there is considerable interest in the utilization of cyclodextrins as stationary or mobile phase in chromatographic analysis because of their ability to form inclusion complexes with a variety of organic molecules[16,17].

However, the literature revealed that midazolam determination have not received much study. Hence, the aim of the present work is to formulate suppositories containing midazolam maleate using different suppository-bases and to study the physico-chemical properties, in-vitro permeation and the dissolution patterns of the drug from the prepared suppositories. Also, this study concerned with the construction of an HPLC technique for successful quantitative determination of the drug.

EXPERIMENTAL

Materials :

Midazolam maleate (La Roche and Co. LTD., Basel, Switzerland), Carbowaxes 400, 1000, 1500 and 4000 (Fluka AG, Switzerland), cacao butter (B.P grade), witepsol H15, E75 (Nobel Dynamit, GFR), and standard cellophane membrane (30/32 Fisher Sci., Co. England). All other chemicals were of analytical reagent.

Preparation of Suppositories:

Five different suppository bases were selected (Table 1). Suppositories of infantile size (1 gm) each containing 10 mg of midazolam were prepared adopting the fusion method. The prepared suppositories were placed in a refrigerator at 4°C for 3 days and another 2 days at room temperature before testing. Suppositories without the drug were prepared under the same condition to be used as reference control.

Table 1 : Composition of Suppository bases used.

Base type	Composition	% w/w
Water soluble		
Polyethylene glycol (PEG)		
A	PEG 400	30
	PEG 1500	30
	PEG 4000	40
B	PEG 1000	97
	PEG 4000	3
Fatty		
Cacao butter	Cacao butter	100
Witepsol H_{15}	Witepsol H_{15}	100
Witepsol E_{75}	Witepsol E_{75}	100

Physicochemical tests :

Hardness : This was designed to measure the brittleness and fragility of the suppositories. It was determined using Erweka hardness tester(Type SBT, Erweka, Heusenstamm. Germany).

Melting point : Was measured by the U-tube method. Weight variation and disintegration time were varied according to the B.P. 1980[18].

HPLC Assay :

Apparatus :

The apparatus used for HPLC was a Spectra Physics pump module, equipped with a stainless steel column (100 X 4.6 mm) packed with B-CYD chemically bonded to high-purity silica gel (Cyclobond I, Advanced Separation Technologies, USA). Detection was effected spectrophotometrically at 215 nm using a Du Pont variable-Wavelength UV spectrophotometer. SP 4100 computing integrator was used to monitor the chromatographic characteristics. pH-meter (HANNA instruments) was used to adjust the pH.

Solutions :

Sodium dihydrogen phosphate (0.05M) solution was prepared using distilled water and adjusted to pH 7 with 0.1M NaOH.

For construction of calibration graphs stock solution (100 mg ml^{-1}) of midazolam was prepared in distilled water and serially diluted with distilled water to give final concentrations of 6-60 ug ml^{-1}.

Chromatographic conditions :

The mobile phase was consisted of phosphate buffer (pH 7.0) : methanol in the ratios of 50:50, 60:40, 65:35, 70:30, and 80:20. The mobile phase was degassed directly before use. 50 u l sample of the prepared solution was injected. Flow-rate was 1 ml min^{-1}, chart speed was 1 cm min^{-1} and attenution unit for full-scale deflection was sit at 1 mV.

Assay of midazolam suppository (drug content) :

Ten suppositories from each base were assayed individually. A preweighed suppository was melted and dissolved in 20 ml distilled water. Take 0.2 ml of clear solution of the prepared sample under test in 10-ml calibrated flask and complete with distilled water. The final concentration was equivalent to 10 $ugml^{-1}$. The drug was determined by the HPLC method, the peak area measured for the drug was directly related to the concentration.

Permeation Study :

The permeation study was carried out through cellophane membrane as follows[19]: The membrane was soaked in distilled water overnight, withdrawn, rinsed with distilled water. The membrane was then stretched firmly over one end of a glass tube (28 mm internal diameter) and tied with a cotton thread. A volume of 5 ml phosphate buffer pH 7.4 at 37°C ± 0.5 was poured into the tube. The prepared tube was suspended in 250 ml beaker containing 100 ml phosphate buffer at 37°C ± 0.5°. The system was placed into a constant temperature water bath and kept to attain a temperature of 37°C ± 0.5°. After the temperature is

equilibrated, one suppository was introduced into the tube. The buffer inside the beaker was stirred by slowing rotating magnetic stirrer.

At intervals, an aliquot of 5 ml was withdrawn from the beaker and replaced with the same volume of fresh phosphate buffer at 37°C ± 0.5°. The amount of the drug permeated was determined by the HPLC method. The peak area measured for the drug was directly related to the concentration.

Dissolution Study :

The USP rotating basket apparatus was used for the test. A suppository was positioned upright in the wire basket, which was then suspended in a USP vessel containing 250 ml phosphate buffer pH 7.4 dissolution medium kept at ± 0.1°C. The basket was rotated at 50 rpm. Samples of 5 ml were withdrawn at specified time intervals and assayed for their drug content using the HPLC analysis by measuring the peak area which directly related to the concentration. An equivalent amounts of fresh buffer was added to the dissolution medium to compensate for sampling,

RESULTS AND DISCUSSION

PHYSICO-CHEMICAL PROPERTIES :

The determined hardness values (1.4-4.6 kg) Table 2 are in agreement with Erweka requirements. The test was carried out on medicated and control suppositories. The results revealed that no difference in hardness was found between the medicated suppository and its control except for cacao butter, where as, incorporation of the drug resulted in an increase of its hardness value (4.6 kg) as compared to its control (2.00 kg). A result which is required for good manipulation.

Table 2 : Physico-chemical Properties of Midazolam Maleate Suppositories Prepared in Different Suppository Bases.

Suppository base	Hardness (Kg)	Melting range (C°)	Average weight (gm/± S.D)	Disintegration time (min)
PEG				
A	2.9	46-47	0.987±0.0117	25
B	1.4	36-37	0.978±0.0329	12
Cacao butter	4.6	33-34	0.789±0.0139	6
Witepsol H_{15}	4.4	33-35	0.780±0.011	9
Witepsol E_{75}	4.8	37-39	0.834±0.009	29

The weight variation results showed that all the prepared suppositories were found to meet the acceptable limits of weight variation according to B.P. 1980 (± 0.5%).

The disintegration time was determined according to the B.P. 1980. All results were fit with the pharmacopeias requirements.

Assay :

The inclusion complexes of some other benzodiazepines with α-, β- and ɣ-cyclodextrins (CYDs) in aqueous solution and in solid phase was studied[20]. The magnitude of stability constant values increase in the order of B-› ɣ -› α-CyD, suggesting that in aqueous media the cavity size of B-CyD most favorably accommodates the benzodiazepine molecules. When using aqueous-organic mobile phases for HPLC with B-CyD stationary phase, retention is predominantly due to inclusion

complex formation. The organic mobile phase modifier tends to compete with solute for preferred location in the hydrophobic cavity of CyD. Hence, increasing the concentration of the organic modifier will decrease solute's interaction with the CyD, resulting in decreased retention. The finding is in accordance with the fact that water is indispensable for the formation of inclusion complex between cyclodextrin unit and guest molecule[16].

The strength of interaction of B-CyD with midazolam was observed from the elution order. The elution order was dependent on the amount of phosphate buffer (pH 7.0) in the mobile phase (Table 3). Using 80:20 phosphate buffer (pH 7.0) : methanol gave long retention time with broadening of the peak. Good chromatographic separation of midazolam was achieved using phosphate buffer : methanol (65 : 35).

Table 3 : Effect of Mobile Phase Constituent on Retention Time.

Phosphate buffer (pH 7): methanol v/v	Retention time (min)
50 : 50	2.11
60 : 40	2.96
65 : 35	3.33
70 : 30	4.16
80 : 20	6.09

Under the specified HPLC experimental conditions, the peak area measured for midazolam was directly related to the concentration used in the range 6-60 ug ml^{-1} (standard solution). This dependence is found strictly linear as depicted from the regression analysis data given in Table 4.

Table 4 : Calibration Data for Standard Solution.

Compound	Concentration $ugml^{-1}$	Correlation coefficient r(SD)*	Slope	Intercept
Midazolam	6-60	0.999(0.011)	0.151	0.05

* Average of six determinations.

As far as the efficiency of the developed HPLC procedure is concerned, no separation trouble would be expected on account of the existence of the investigated midazolam in suppository formulation.

As revealed from (Fig. 1), midazolam could be analysed with good separation. There was no indication that the suppository bases interfered with the separated peak.

The HPLC method was simple, rapid, precise and accurate (Table 5).

Table 5 : Drug Content Analysis of the Prepared Suppositories.

Base type		Added mg/g	Found mg/g	Recovery** %	SD	CV,*%
PEG	A	10	10.09	100.9	0.18	1.70
PEG	B	10	10.19	101.9	0.11	1.07
C.B		10	11.48	114.8	0.07	0.61
W.	H_{15}	10	3.00	30	0.02	0.66
W.	H_{75}	10	6.1	61	0.01	2.4

* Standard deviation of the mean of five determinations divided by the mean expressed as percentage.

** The drug content uniformity of PEG base A,B and cacao butter are of the B.P.C. 1973[21].

Consideration of these results can afford a basis for application of the developed HPLC procedure for the analysis of midazolam.

Permeation :

The release of midazolam from different suppository bases and its diffusion through cellulosic membrane in phosphate buffer pH 7.4 was investigated. Generally, the results revealed that the rate of drug release seemed to be dependent mainly on chemical composition of the tested bases.

With respect to carbowax bases A and B (Fig. 2), the permeation is fast during the first hour, followed by relatively a slaw permeation pattern. This may be due to dissolution of PEG bases in the aqueous phase of the donor compartment creating an increase in the osmotic pressure, which lead to an influx of water molecule from the acceptor into donner compartment. Consequently a decrease in the concentration gradient was attained resulting in slowing of the release rate. Also, the increase in viscosity due to the dissolution of carbowaxes, hence a decrease in the diffusion rate of the drug, may explain the slow release phase. A result in agreement with Habib et al[4] and Ibrahim et al[22].

However, PEG base B was found to provide higher release rate compared to PEG base A (Fig. 2 and Table 6). The difference obtained in drug release is mainly related to both chemical composition and the hardness values (Table 2) of the two bases which will affect the rate of solubility and hence drug release.

Table 6 : Analysis of Permeation Data of Midazolam from Suppository Bases According to Different Release Kinetics.

Base Type	Model	Correlation coefficient	K	Mechanism
PEG				
A	Zero	0.943	8.172	
	First	0.941	0.088	Diffusion
	Diffusion	0.971	12.621	
B	Zero	0.960	13.63	
	First	0.977	0.151	Diffusion
	Diffusion	0.001	20.74	
Cacao Butter	Zero	0.986	1.86	
	First	0.976	0.0184	Diffusion
	Diffusion	0.996	2.86	

For fatty bases, both cacao butter and witepsol H_{15} are characterized by having the same melting range (33-35°C) but vary mainly in their chemical composition. Unexpected results were obtained. Witepsol H_{15} which contains self emulsifier, monoglyceride[23] permeates indetectable amounts of the drug over 2 hrs as compared with cacao butter. At the same time, witepsol E_{75} which possess the same chenical composition as witepsol H_{15} but vary in melting range, was found to permeate negligible amounts of the drug.

Fig. 3 illustrates the permeation patterns of midazolam from PEG base B (The best release rate) in phosphate buffer pH 7.4 and in distilled water.

The relatively slow release of the drug in phosphate buffer pH 7.4 may be due to the basic nature of

the drug, which reduces its solubility in the alkaline medium observed through out the experiment.

To know the mechanism of midazolam release from suppositories and its diffusion through the cellulosic membrane, the data obtained (over on hour) were analysed using the linear regression according to zero order and first order and also according to simplified Higuchi model[24]. The results of data analysis are presented in Table 6. It revealed that the release of the drug from the carbowax bases and cacao butter followed the diffusion controlled mechanism, as indicated by the higher correlation coefficient.

Dissolution :

Fig. 4 shows the dissolution profiles of midazolam from carbowax and cacao butter bases. As expected, the PEG base B shows the highest dissolution rate (100% after ½ hr), followed by about 76% for PEG base A. Whereas cacao butter provided lower dissolution of the drug.

On the other hand, the amount of the drug dissoluted from witepsol H_{15} and witepsol E_{75} was not detected. This finding was in agreement with the drug permeation data and also the determined drug content (Table 5). The retarding effect of these bases on the release and dissolution of the medicament may be explained as a sort of interaction between the base and drug. An effect which is mainly attributed to their chemical structure. Baichwal yelvigi[25] reported that, polyglycerols have been used as dissolution retarding materials for some medicaments like aspirin, sulphanilamide and ferrous sulphate.

Hence, the witepsol H_{15} and witepsol E_{75} bases must not be recommended as suppository bases for midazolam.

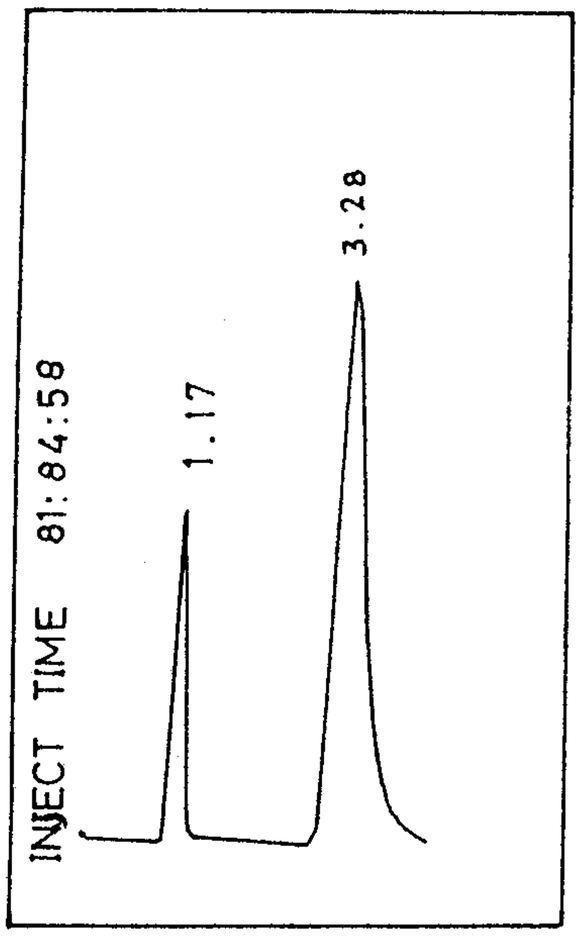

Fig. 1: Chromatogram of midazalam. Chromatographic condition:
mobile-phas:phosphate buffer (PH_7): methanol(65:35) flow-rate 1m min^{-1}, max^{215}.

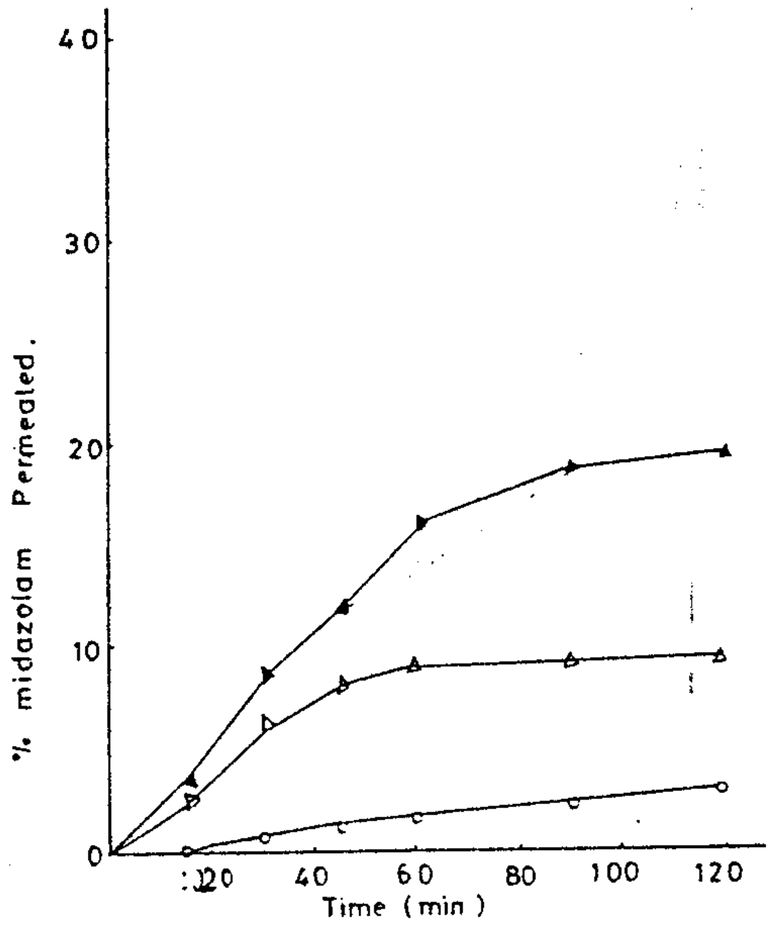

Fig.2. Permeation Profiles of midazolam from suppository bases, (Δ—Δ) PEG A, (▲—▲) PEG B and (o—o) cacao butter in phasphate buffer PH 7.4

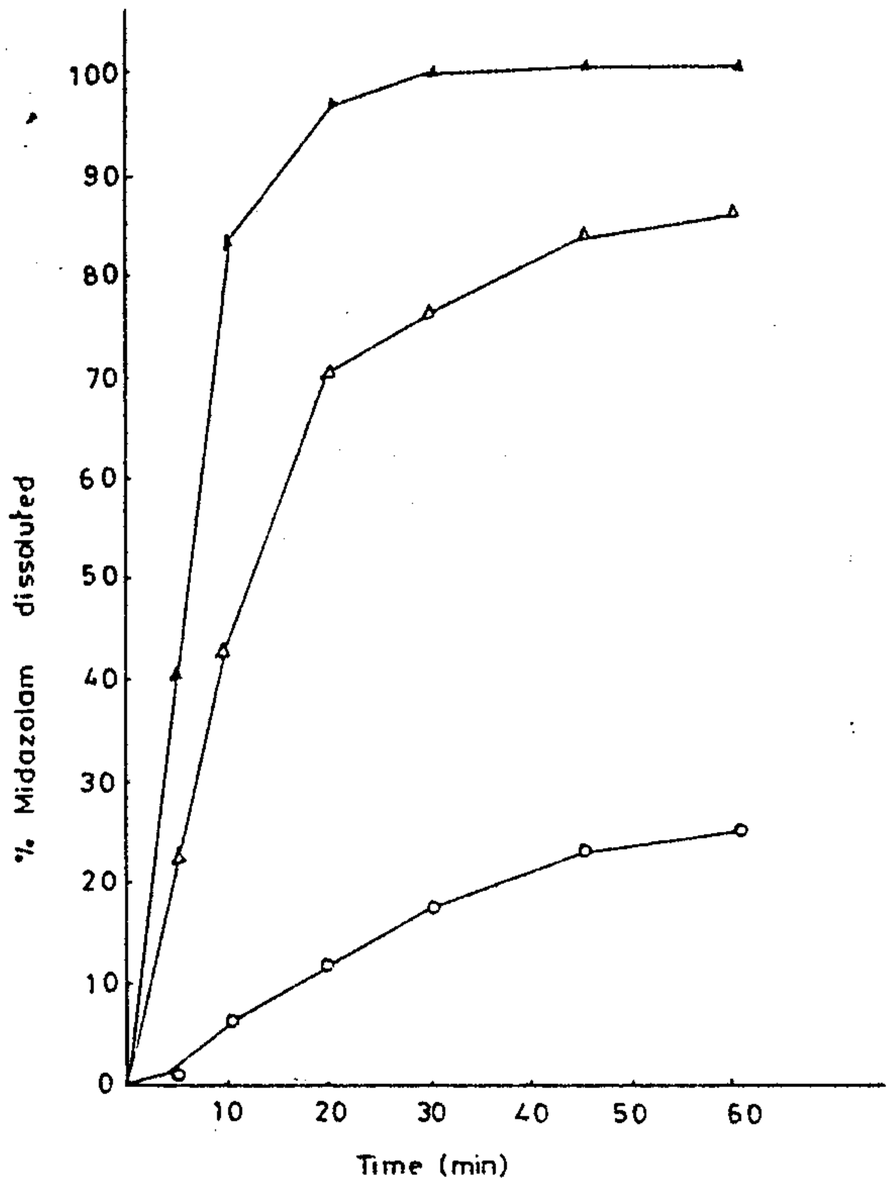

Fig.4. Dissolution Profiles of midazolam From Suppository bases: (Δ—Δ) PEGA, (▲—▲) PEGB, (o—o) Cacao butter.

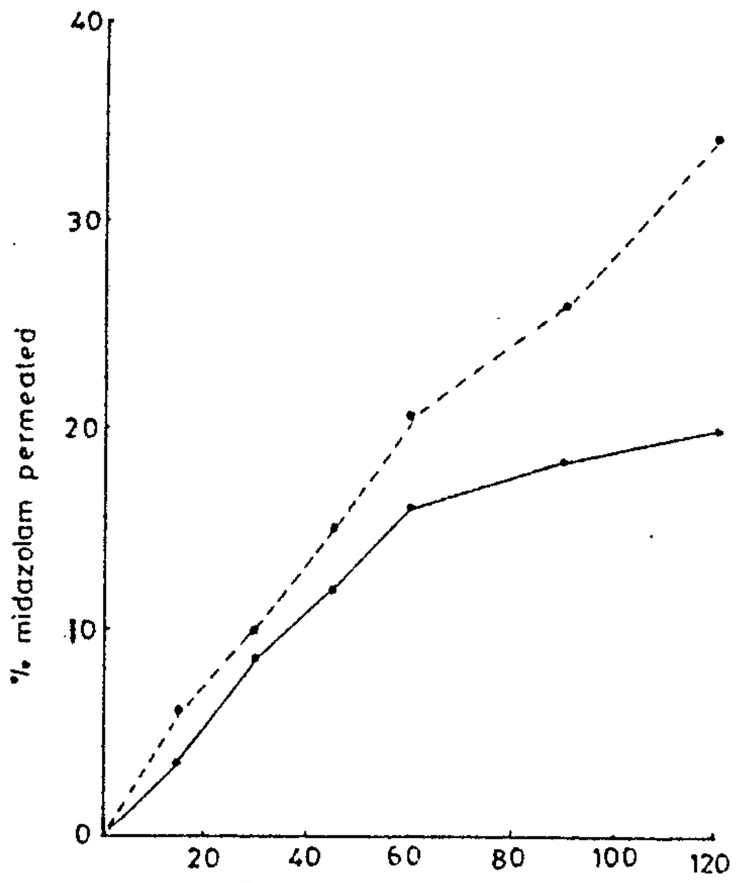

Fig .3. Permeation Profiles of midazolam From PEG base B, (——) in phosphate buffer PH 7.4, (----) in distilled water.

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صياغة وتقييم اقماع الميدازولام

١ ـ صياغة الميدازولام فى اقماع باستخدام كروماتوجرافيا السوائل ذات الضغط العالى فى تعينه

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تضمن هذا البحث تحضير اقماع الميدازولام باستخدام نوعيات مختلفة من قواعد الاقماع وقد تم دراسة تأثير نوع القاعدة ومكوناتها على معدلات انطلاق العقار . وقد اثبتت النتائج ان القاعدة المكونة من خليط ايثيلين الجليكول ١٠٠٠ ، ٤٠٠٠ (٩٧ : ٣) تعطى افضل النتائج . فى حين ان قاعدة الويتيسول و٧٥ منعت انطلاق العقار مما دعى النصح بعدم استخدامهما لصياغة اقماع الميدازولام . وتضمن البحث ايضا استخدام طريقة كروماتوجرافيا السوائل ذات الضغط العالى للتعين الكمى وتقنية العقار فى الاقماع المحضرة . وقد استخدم البيتاسيكلوديكسترين المعالج كميائيا بحزيئات السيليكا كوسط ثابت والذى يعتبر اضافة جيدة فى مجال التحليل الكمى الصيدلى .

وتم فصل مركب الميدازولام بكفاءة عالية وذلك بتخفيض نسبة الكحول المستخدم والذى كان افضلهم ٣٥ / كحول : ٦٥ / ماء .

واثبتت نتائج التحليل انه قد تم استرجاع المادة الفعالة بدقة عالية دون تدخل من القاعدة المستخدمة من الاقماع .

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