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IN VITRO RELEASE OF DIAZEPAM FROM DIFFERENT SUPPOSITORY FORMULATIONS.

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ABSTRACT

The effect of suppository vehicles, drug concentrations, liquid additives and some nonionic surfactants on the in vitro release of diazepam from different suppository formulation were investigated. The obtained data revealed that the drug release is significantly higher from suppository prepared using PEG than the other formulations. The drug release from oleaginous base was related inversely to its partition coefficient and the melting range of the tested oleaginous bases. The incorporation of some liquid additives (10%) was found to improve the release pattern of the drug from a PEG base. The degree of improvement is correlated well to the solubility of the drug in the utilized additives. The data, also, reveal that the release rate is directly proportional to the drug concentration. The incorporation of nonionic surfactants into Witepsol H_{15} in a 5% concentration was found to increase the release rate of diazepam. The enhancing effect was found to be dependent on the chemical structure of the surfactants. The release rate of medicament from different solid nonionic surfactants, as suppository bases, was also studied. The magnitude of drug release was markedly reduced as compared with PEG bases.

INTRODUCTION

The importance of drug release from a variety of suppository bases has been recognised by sevral authors[1-5] who attempted to identify the physicochemical properities of the drug and the bases that greatly influence the rectal absorption. Recently, the rectal therapy has be came more popular since the side effect of the I.M. injections can be circumvented, especialy in the pediatric population.

The rectal absorption of sodium ampicilline and other B-lactam antibiotics in rabbits and rats was enhanced by the addition of N-acyl collagen peptide into the suppository vehicle[6]. The rectal absorption of acetaminophen from an aqueous solution and PEG suppository bases was studied and a relationship was observed between acetaminophen bioavailability and the dielectric properties of the vehicles utilized[7]. The effect of particle size and drug concentration on the *in vitro* release of noramidopyrine methansulfonate sodium from witepsol H_{15} was studied and the data revealed that the finer drug particle size the less its release and the increase in medicament concentration the higher drug release[8].

The *in vitro* release of chlordiazepoxide hydrochloride from a variety of suppository bases was studied[9]. The authors found that the extent of drug release was dependent on the type of used bases.

Diazepam is a well accepted drug for the treatment of status epilepticus[10]. Status epilepticus is a serious and often a life-threating emergency, which requires prompt

treatment to minimize mortality as well as reducing neurological sequelae caused by prolonged seizure activity[11,12]. Current therapy by diazepam requires rectal or I.M. administration of the drug to achieve rapid onset of the required effect. The absorption characteristics of diazepam following intranasal administration was determined to assess the suitability of this approach for the treatment of status epilepticus in children[13]. The rectal therapy of benzodiazepines namely; diazepam was studied in humans and experimental animals.

The bioavailability of diazepam from rectal solution was studied by Moolenar et al[14]. They found that, the peak concentration was observed at 15 min. and the concentration was constant for about 3 hrs. The rectal absorption of lorazepam from fatty and water soluble suppository bases was studied and compared with the oral solution[15].

Diazepam is a benzodiazepine derivative, structurally and pharmacologically related to chlordiazepoxide. It is more potant than chlordiazepoxide. Due to its poor water solubility, several attempts have been done to improve its dissolution rate[16] and aqueous solubility[17].

A survey of the literature, revealed that there is no attempt was done to formulate diazepam suppositories. Therefore, the aim of the present article was to study the *in vitro* release of diazepam from different suppository formulations.

EXPERIMENTAL

Materials:

Diazepam (Hoffman La Roche Inc., Nutly, N.J.); Cacao butter (B.P. grade); Witepsols H_{12}, H_{15}, H_{37} and E_{75} (Nobel Dynmait, Germany); Massa estarinum B, C and BC (Nobel Dynmait, Germany); PEGs 300, 1000, 1540, 4000 and 6000 (Fluka AG, Switzerland); Glycerol (BDH, U.K.); Poloxamers 182, 402 and 407 (BASF, USA); Myrjs 53, 59 and Brijs 35,58 (ICI, USA); Standared cellulose dialysis membrane, spectrapor M.W. cutoff 12000-14000 (Fischer Sci. Co., U.K.) All other chemicals were of analytical or reagent grade and were used without further purification.

Procedure:

1.Preparation of diazepam suppositories.

The suppositories were prepared employing the fusion method. Each suppository was prepared in a manner to contain 20 mg of drug. Generally, the suppository ingredients were mixed and melted over a water bath. The drug was dispersed or dissolved in the molten base and then poured in a suppository mould (1 gm) and allowed to solidify at 20°C. The suppositories were removed from the mould and stored in a closed container at 10°C for 2-3 days prior to evaluation, the suppositories were allowed to stand for 4-6 hours at room temperature.

2.Release of diazepam from suppositories.

The procedure can be summarized as follows : the cellophane membrane was first soaked in distilled water for about 24 hours prior to use. The membrane was dried between two filter papers, streched over the lower end of an open ended glass cylinder, i.d.=22 mm, with the aid of a cotton thread and this acts as a donor. The donor was located vertically in a 50 ml beaker containing 20 ml of McIlvaine's phosphate buffer at pH 7.5, (acceptor). Two milliliters of the buffer were introduced into the donor compartment. It is quite worth to note that the donor was placed into the acceptor in a manner that the membrane was just below the

upper surface of the release medium. The whole dialyzing unit was placed into a mechanically shaken water bath, the rate of shaking was adjusted at 25 strakes/minute. The water bath was adjusted at 37°C. When a thermal equilibrium has been attained, one suppository of the tested suppository formulation, accurately weighed, was introduced into the donor compartment. At specified time intervals a of the release medium was withdrown and immediately replaced with an equal volume of the same buffer at the same temperature. The amount of drug release was estimated spectrophotometrically by measuring the absorbance at 231 nm after appropriate dilution with distilled water against a blank similarly treated. The cumulative amount of the drug dialysed into the acceptor was calculated. It should be noted that each experiment was done four times and the average amount released was calculated.

3. Determination of partition coefficient of diazepam.

Ten grams of the tested suppository base were accurately weighed and introduced into a glass stoppered tube, 50 ml, in capacity, followed by 10 ml of diazepam solution (50 ug/ml) in McIlvaine's phosphate buffer at pH 7.5. The tubes were tightly closed and allowed to rotate at 50 rpm in a thermostatically controlled water bath. The temperature was adjusted at 37°C. The tubes were allowed to rotate for 8 hrs. This time was found to be sufficient to attain equilibrium. The tubes were removed and allowed to stand vertically for one hour to allow complete separation of the two phases. The tubes were placed into the refrigerator and stand vertically for further one hour to be completely solidified. The aqueous phase was then filtered. The amount of the drug retained in the aqueous phase was determined by measuring the absorbance at 231 nm against a suitable blank. The amount of the drug partitioned into the oily phase was calculated by subtraction. The partition coefficient, K, could be calculated from the equation :

$$K = C_o / C_w$$

Where; C_o is the amount of the drug in the oily phase and C_w is that retained in the aqueous phase.

4. Solubility studies.

The solubility of drug was carried out according to Higuchi and Connors technique[18]. Excess amount of the medicament was accurately weighed and added to glass stoppered tubes, 50 ml in capacity, containing 20 ml of distilled water or water containing 10% of PEG 300, glycerol or propylene glycol. The tubes were rotated on a mechanical spindle at 37°C, at 50 rpm, for 48 hours. This time was sufficient to ensure solubility equilibrium for the tested solvents. After attainment of the equilibration, the contents of the tubes were filtered. Aliquot portions of the supernatant or the filterate were properly diluted with water and analysed for the drug content spectrophotometrically at 231 nm.

RESULTS AND DISCUSSION

The *in vitro* release pattern of diazepam from different suppository bases was investigated at 37°C. Figure 1 shows the obtained data for the drug release from witepsol bases. From this figure, it could be observed that witepsol H_{15} exhibited the higher release rate. The tested bases could be ranked in the following order according to their ability to release the drug : witepsol H_{15} > witepsol H_{12} > witepsol H_{37} > witepsol E_{75}. This finding is attributed to both chemical composition and the melting range of the tested bases (Table 1).

Table 1. Melting Range of the Tested Oleaginous Suppository Bases.

Suppository base	Melting range (C°)
Cacao Butter	30.0 - 35.0
Witepsol H_{12}	32.0 - 33.5
Witepsol H_{15}	33.5 - 35.5
Witepsol H_{37}	36.0 - 38.0
Witepsol E_{75}	37.0 - 39.0
Massa Estarinum B	33.5 - 35.5
Massa Estarinum C	36.0 - 38.0
Massa Estarinum BC	33.5 - 35.5

The affinity of diazepam towards the tested oleaginous bases followed through determination of the partition coefficient between these bases and McIlvaine,s buffer at pH 7.5. The values of partition coefficient, K, are presented in Table 2. From this table, a slight difference between the K values could be observed. Generally, the partition coefficient values could be arranged as witepsol H_{37} < witepsol E_{75} < witepsol H_{15} < witepsol H_{12}. The data reveal that the release rate of diazepam from witepsol bases having lower melting range (e.g. witepsol H_{12} and witepsol H_{15}) was higher than that from the other candidate having higher melting range; witepsol H_{37} and witepsol H_{75}.

Table 2. Partition Coefficient of Diazepam, K, Between Oleaginous Suppository Bases and Phosphate Buffer pH. 7.5

Suppository base	K	±S.D.	C.V. (5)
Cacao butter	2.041	0.06	1.96
Witepsol H_{12}	2.246	0.07	3.12
Witepsol H_{15}	1.795	0.03	1.67
Witepsol H_{37}	1.572	0.05	3.18
Witepsol E_{75}	1.748	0.06	3.43
Massa estarinum B	1.948	0.04	2.05
Massa estarinum C	1.931	0.05	2.59
Massa estarinum BC	1.896	0.07	3.69

On comparing the release pattern of drug from witepsol H_{12} and witepsol H_{15}, it was explored that the release rate is inversely proportional to the partition coefficient. Also, the release rate profile from witepsol H_{37} and witepsol E_{75} could be explained on the same assumption.

Figure 2 depicts the release pattern of diazepam from massa estarinum B (MS B), massa estarinum C (MS C), massa estarinum BC (MS BC) and from cacao butter suppository bases. It should be noted that all of these bases melted completely during initial time of the release study. Those bases could be ranked according to their ability to release the medicament as : MS BC > MS C > MS B. This finding is attributed to base type affinity of drug to tested bases as well as the partition coefficient of the drug, Table 2. Although cacao butter exhibits a higher partition coefficient value, the release rate of diazepam was faster. This reveals that the chemical composition of the base affects, to some extent, the release pattern of the drug.

The *in vitro* release of the medicament from different PEG suppository bases was also studied. The composition of these vehicles is surveyed in Table 3. Figures 3 and 4 reveal that the composition of the PEG base affects, markedly, the release rate. The appreciable differences between the release rates from the tested PEG bases may be predicted from a knowledge of the physico-chemical properties the PEG bases. The data, also, show that when mixtures of PEGs were used, the rate of release increase as the percentage of PEG 1540 was increased, Table 3. The same finding was attained when mixtures of PEG 1000 and PEG 4000 were used as suppository vehicles. The results obtained indicate that the character of polyethylene glycol base is the most influential.

Table 3. Composition of PEG Suppository Bases.

Formulae	% PEG			
	PEG 1000	PEG 1540	PEG 4000	PEG 6000
1	--	100	--	--
2	--	80	--	20
3	--	50	--	50
4	75	--	25	--
5	25	--	75	--
6	10	--	90	--

The significant increase in the release rate of drug from PEG bases, as compared with oleaginous bases, is attributable to an increase in the diazepam solubility in presence of the tested PEG bases. However, PEG 4000 was found to increase the aqueous solubility as well as the dissolution rate of diazepam[19]. The data can also, be treated according to the fact that the lower molecular weight PEG is more water soluble than that of higher molecular weight. Accordingly, as the percentage of either PEG 1000 or 1540 was increased, Table 3, the dissolution rate of drug from the tested base will be improved. Generally, the release of drug from water soluble bases is superior than from fatty type. Among the tested formulations, PEG 4 exhibited the higher release rate.

The influence of liquid additives on the release behaviour of diazepam from PEG 4000 and PEG 6000 (1:1) base was studied Figure 5. The appreciable differences between the release rates of diazepam could be attributed to the variable effects of those liquid additives on the aqueous solubility of drug.

Table 4 shows the solubility of diazepam in water containing 10 % of each liquid additive. From this table, the tested additives could be ranked in the following decreasing order according to their ability to solubilize diazepam : Propylene glycol > PEG 300 > glycerol. This improving effect in the drug solubility could be taken as a parameter to explain the release rate pattern. Inspection of Figure 5 reveals that the increase in the amount of drug released from this PEG base is correlated with the solubilizing power of those additives.

Table 4. Solubility of Diazepam in the Tested Liquid Additives at 37°C.

Solvent	Solubility (mcg/ ml)	± S.D.	C.V. (%)
Water	72.04	0.73	0.51
Water + PEG 300	177.10	0.31	0.17
Water + Propylene glycol	287.60	0.81	0.28
Water + Glycerol	101.10	0.15	0.15

Figure 6 reveals the release pattern of diazepam from Brij 35 an Brij 58 suppository bases. From the data obtained, it could be observed that the release of diazepam from Brij 35 is faster than that from Brij 58. Figure 7 illustrate that the suppository bases prepared from Myrj 59 exhibited a higher release rate than that prepared with Myrj 53. A comparisone between these bases indicates that Brij 35 is far supperior to the other tested surfactant bases.

Inspection of the results for the effect of drug concentration on the amount of drug released reveals that a higher release was attained as the drug concentration was increased, Figure 8. The drug was used in a concentration range of 10-30 mg/g suppository base. This finding could be explained on the basis of Fick's low; i.e as the concentration gradient increases, the release rate will be consequently increased. This study was carried out employing PEG_4 as a suppository base. On comparing the release pattern of diazepam from two different classes of water soluble suppository bases i.e. PEG and nonionic surfactant bases, it should be pointed out that the amount of the drug released in case of PEG bases is much greater than that from the surfactant bases. This could be explained on the assumption that when these surfactant completely dissolved in water present in the donor, they will dis

solve excess amount of the drug through micellar solubilization. The solubilized drug molecules will be partitioned between that micellar and intermicllar phases[20]. Diazepam, a poorly water soluble drug, will be partitioned in a favour of the micellar phase. Accordingly, the concentration of the free drug in the intermicllar phase, which is available for dialysis will be reduced. Another explanation for such observation is that diazepam will react with the surfactant monomers resulting in the formation of drug surfactant complex which is not ready to diffuse through the cellophane membrane[21].

The incorporation of 5% w/w of some selected nonionic surfactants into witepsol H15 was found to modify the release rate of diazepam. The surfactants tested were Poloxamer 182, Poloxamer 402 and Poloxamer 407. The data obtained revealed that all the tested poloxamer improve the release rate, (Figure 9). This improving effect may be due to an increase in the dissolution rate of the drug due to the presence of such surfactants. The higher enhancing effect was attained in the presence of poloxamer 407. The lower effect was produced by poloxamer 402 while an intermediate effect was obtained with poloxamer 182.

In conclusion, the combined factors that affect the *in vitro* availability of diazepam from suppositories are: a)The type and chemical composition of the tested suppository base, b) the partition coefficient of the drug, C) the solubilizing effect of the surfactant and d) formation of a complex between the drug molecule and the surfactant monomers.

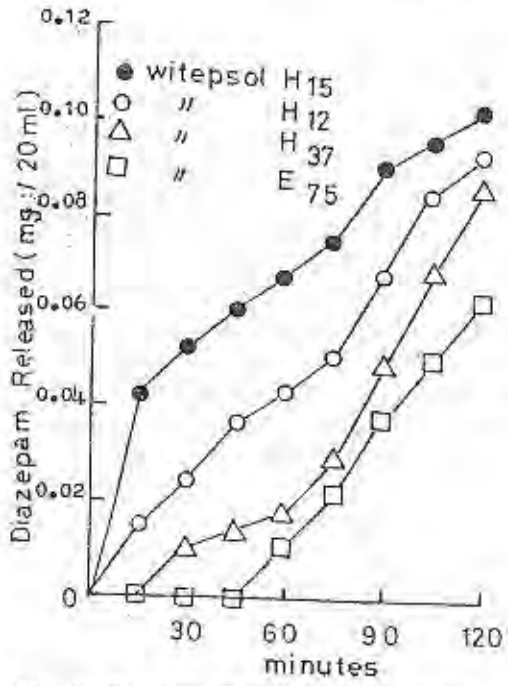

Fig. 1. In-vitro Release of Diazepam from Different Witepsol Suppository Bases.

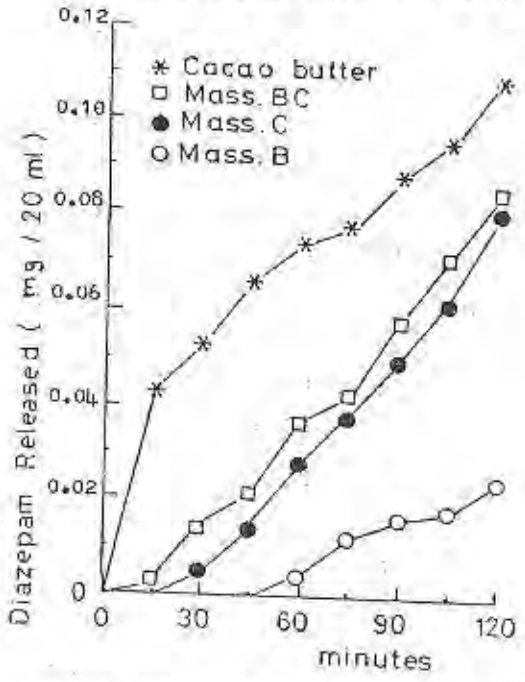

Fig. 2. In-vitro Release of Diazepam From Cacao Butter and Different Massa Estarinum Suppository Bases.

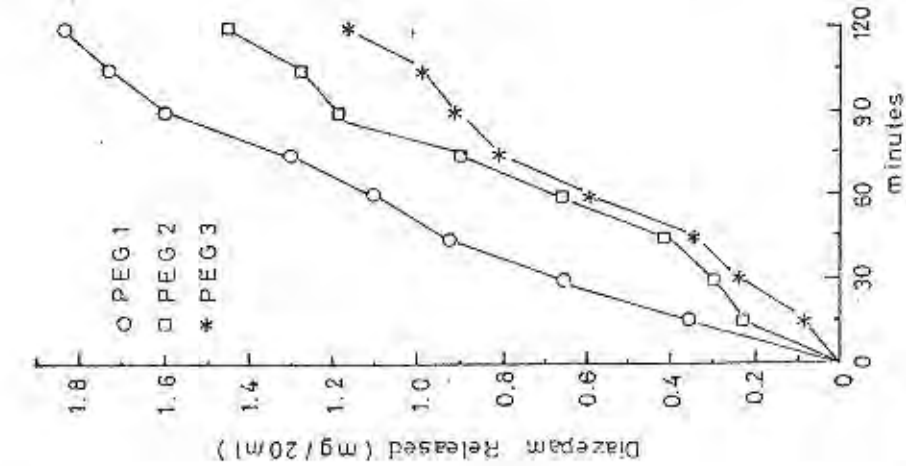

Fig. 3. In-vitro Release of Diazepam from PEG Suppository Bases (1,2,3).

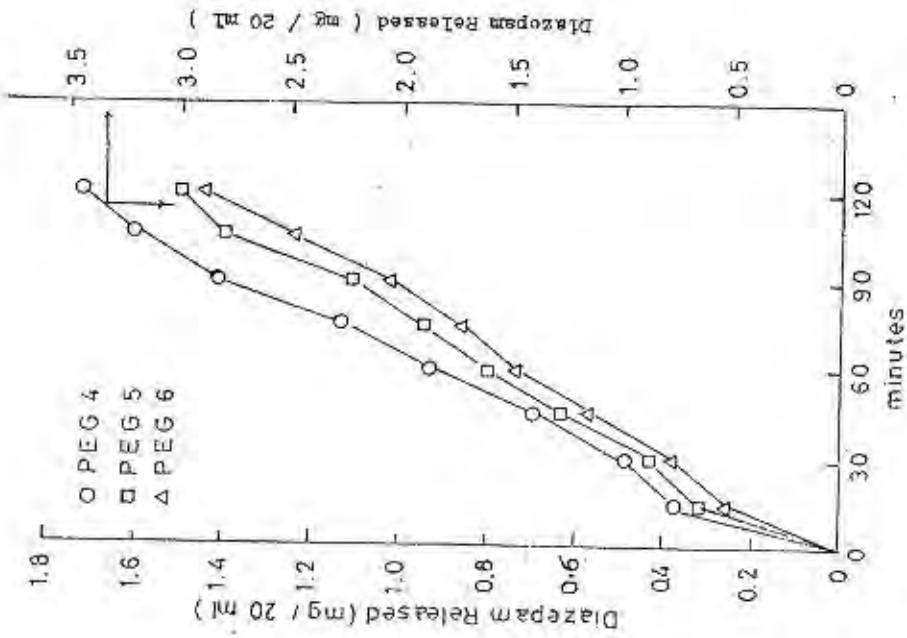

Fig. 4. In-vitro Release of Diazepam from PEG Suppository Bases; (4,5,6).

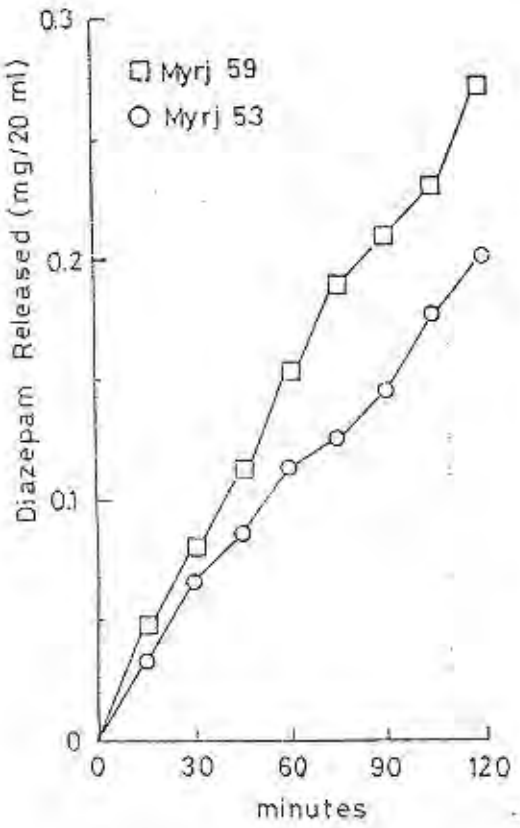

Fig. 7. In-vitro Release of Diazepam from Myrjs 53 and 59 as Suppository Bases.

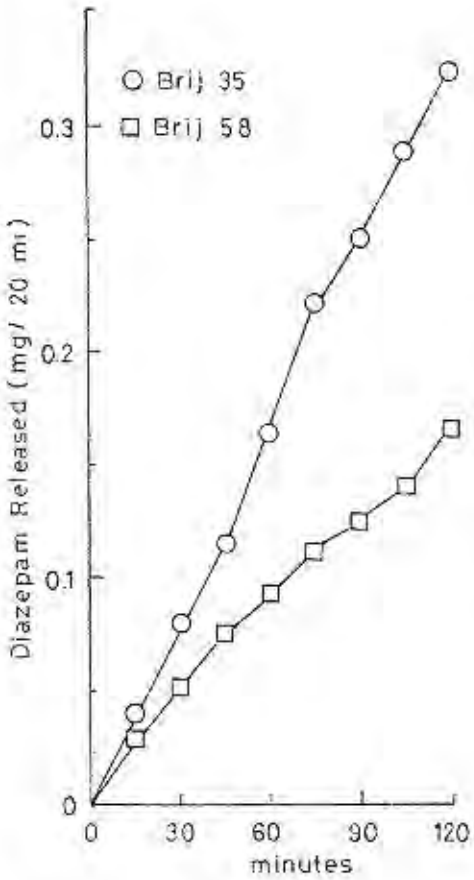

Fig. 6. In-Vitro Release of Diazepam from Brijs 35 and 58 as Suppository Bases.

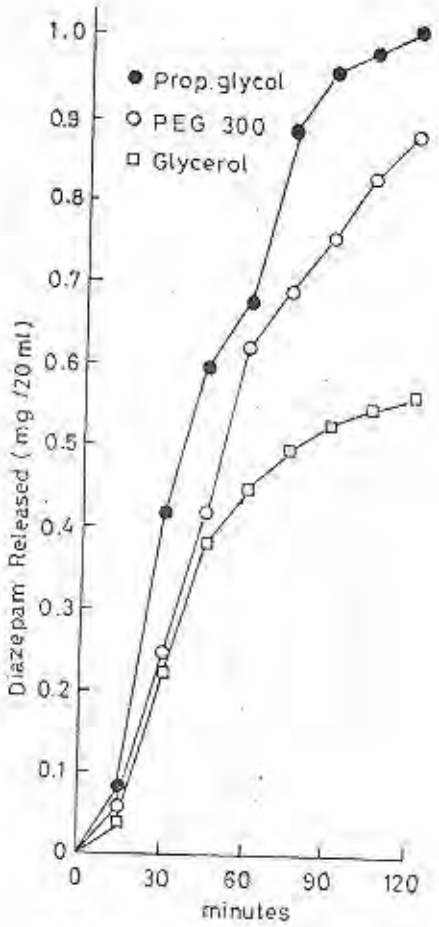

Fig. 5. Effect of Liquid Additives on the In-Vitro Release of Diazepam from PEG Base.

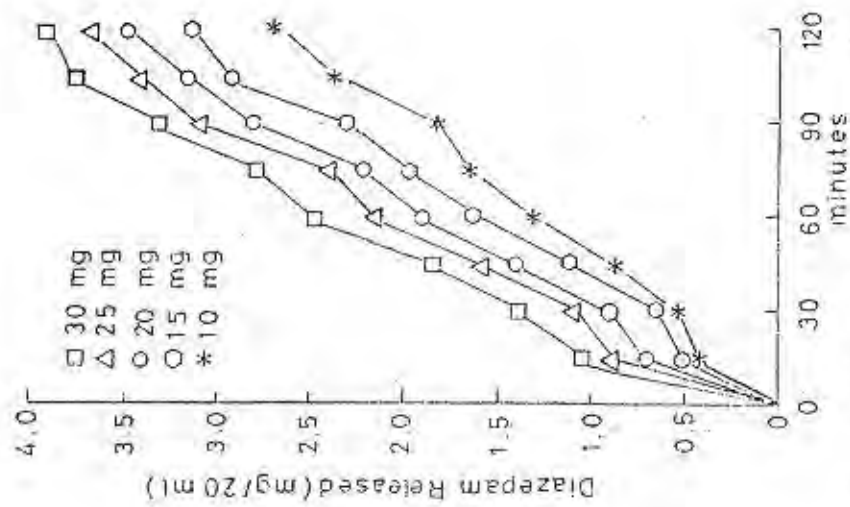

Fig. 8. Effect of Diazepam Concentration on in-vitro Release of Drug from Suppositories.

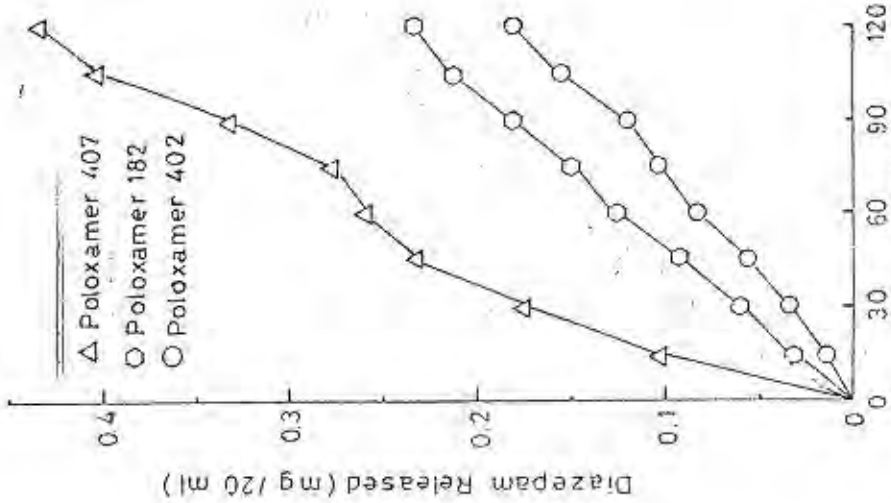

Fig. 9. Effect of Different Surfactants on the In-vitro Release of Diazepam from Witepsol H_{15}.

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دراسة معملية لانطلاق عقار الديازيبام من أقماع شرجية فى صياغات مختلفة

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استهدفت الدراسة صياغة عقار الديازيبام فى صورة أقماع شرجية وقد استخدمت فى هذه الصياغة العديد من القواعد الدهنية والذائبة فى الماء وكذلك مخاليط من قاعدة الويتبسول هـ ١٥ وبعض منشطات السطح الغير متأينة وتم تقييم الأقماع المحضرة معمليا بدراسة تأثير نوع القاعدة المستخدمة ومكوناتها ، تركيز العقار ، اضافة بعض السوائل مثل الجلسرين - وعديد اثيلين الجليكول ٣٠٠٠ والبروبيلين الجليكول وكذلك اضافة بعض منشطات السطح الغير متأينة على معدلات انطلاق العقار من هذه القواعد المحضرة.

وقد أوضحت النتائج ما يلى:

١- القواعد المحضرة من عديد اثيلين الجليكول تعطى معدلات عالية لانطلاق العقار معمليا.
٢- أن معدل انطلاق العقار يتناسب طرديا مع تركيزه فى القواعد المستخدمة.
٣- ان اضافة الجلسرين وعديد اثيلين الجليكول ٣٠٠ والبروبيلين جليكول بنسبة ١٠٪ الى القاعدة المكونة من خليط عديد اثيلين الجليكول ٤٠٠٠ ، ٦٠٠٠ (١: ٪) يزيد من معدل انطلاق العقار.
٤- ان اضافة منشطات السطح الغير متأينة بنسبة ٥٪ الى قاعدة الويتبسول هـ ١٥ يزيد من معدل انطلاق العقار.
٥- استخدام بعض منشطات السطح الغير متأينة كقاعدة يقلل من انطلاق العقار اذا ما قورن بانطلاق من قاعدة عديد اثيلين الجليكول.

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