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IN-VITRO RELEASE CHARACTERISTICS OF PAPAVERINE HYDROCHLORIDE FROM MULTIPLE EMULSION SYSTEMS

H.Abdel-Monem Sayed, E.Hafez and S.M.Safwat

Dept. of Pharmaceutics, Faculty of Pharmacy,

Assiut University, Assiut, Egypt

ABSTRACT

A two-step emulsification procedure was adopted to prepare stable multiple emulsion of the W/O/W Type. An aqueous solution of the drug and paraffin oil at ratio of 1:2 were used to prepare a W/O type primary emulsion (1st step) using blend of surfactants (HLB 3.8) at 10% concentration. While a blend of surfactants (HLB 12.3) was used as the second emulsifying agent for incorporating the prepared primary emulsion into the outer aqueous phase (1:2) to produce the final emulsion. Drug release from the prepared system was evaluated 24 hours after preparation and periodically at weekly intervals thereafter for four weeks. Multiple emulsion systems, each containing either sodium chloride, sorbitol or methylcellulose were similarly prepared and evaluated for drug release thereof. Drug release from the prepared systems was found to be greatly retarded in comparison with that from the corresponding aqueous solutions. The drug release rate from the prepared systems, with the exception of those containing methylcellulose was slightly changed to lower values. The effect was greater at the second week testing while, a constant release pattern was found thereafter. The presence of methylcellulose lead to a greater lowering in the release rate.

INTRODUCTION

Papaverine is a smooth muscle relaxant alkaloid obtained from opium or synthetically prepared. The drug has the analgesic and narcotic properties of morphine[1,2]. It is used as an antispasmodic in a variety of conditions affecting the vascular system and the gastro-intestinal and genito-urinary tracts[3]. It is given by mouth and intravenous or intramuscular injections for providing postoperative pain relief[4-6]. The drug was also used in the management of chronic brain syndrome secondary to cerebral arteriosclerosis[7]. It is well absorbed after oral administration[8]. It is a weak base with a pka of 6.4[9]. The hydrochloride salt is soluble in water and an aqueous solution of this salt yields peak blood levels within 1 to 2 hours after oral administration[10]. The drug biological half-life in humans is 60-120 minutes[11,12].

Sustained-release papaverine hydrochloride products are used widely to treat conditions that may be improved by relief of spasm in certain blood vessels. These products usually contain 150 or 300 mg of the drug, and the recommended dosing interval is 8 to 12 hours[3]. Timko and Lordi[13] evaluated three commercial sustained-release products of the drug by the in-vitro release testing. Papaverine hydrochloride was tested as a model drug in the preparation of sustained-release forms using new carriers[14]. A reasonable correlation between the in-vivo bioavailability in humans and the in-vitro dissolution data was reported with some papaverine dosage forms [15,16].

Interest has recently increased in the pharmaceutical applications of multiple emulsions. It is possible that such emulsions may be used for oral prolonged action products or intramuscular depot therapy[17-23]. The inherent physical instability is regarded as one of the major problems in the application of such systems. Therefore, the objective of this work is the development of a sustained release liquid form of papaverine. More specifically, is to formulate the drug in a multiple phase emulsion and, to follow the physical stability of the prepared system by determining the time dependence of the drug release profile.

EXPERIMENTAL

Materials :

Papaverine hydrochloride (pharmaceutical grade obtained from the Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt). Light paraffin oil (Pharmaceutical grade). Non ionic surfactants; Span 80, Span 85 and Polysorbate 80 (Atlas Chemical Industries, Wilmington. U.S.A). Methylcellulose and Sorbitol (ROTH. FDR). All other chemicals were of analytical reagent grade, and were used without further purification.

Procedure :

Assay Method : Beer's law curves were tested for standard solution of papaverine hydrochloride. The maximum wavelength for the drug in the tested isotonic sodium chloride solution was 310 nm. The same maximum wavelength was reported for In-vitro evaluation of commercial sustained-release products of the drug[13]. This wavelength allowed direct absorbance

readings under all the tested experimental conditions. linearity was followed in the concentration ranges used. The linear regression analysis of absorbance data revealed the following linear equation :

$$Y = 0.182\ X - 0.014 \quad \text{.....eq. 1}$$

$$X = \frac{Y + 0.014}{0.182} \quad \text{.....eq.2}$$

where Y : Absorbance & X : Concentration in mg/100 ml.

Preparation of W/O/W Type Multiple Emulsions :

A two step emulsification procedure was adopted[24] (Chart I).

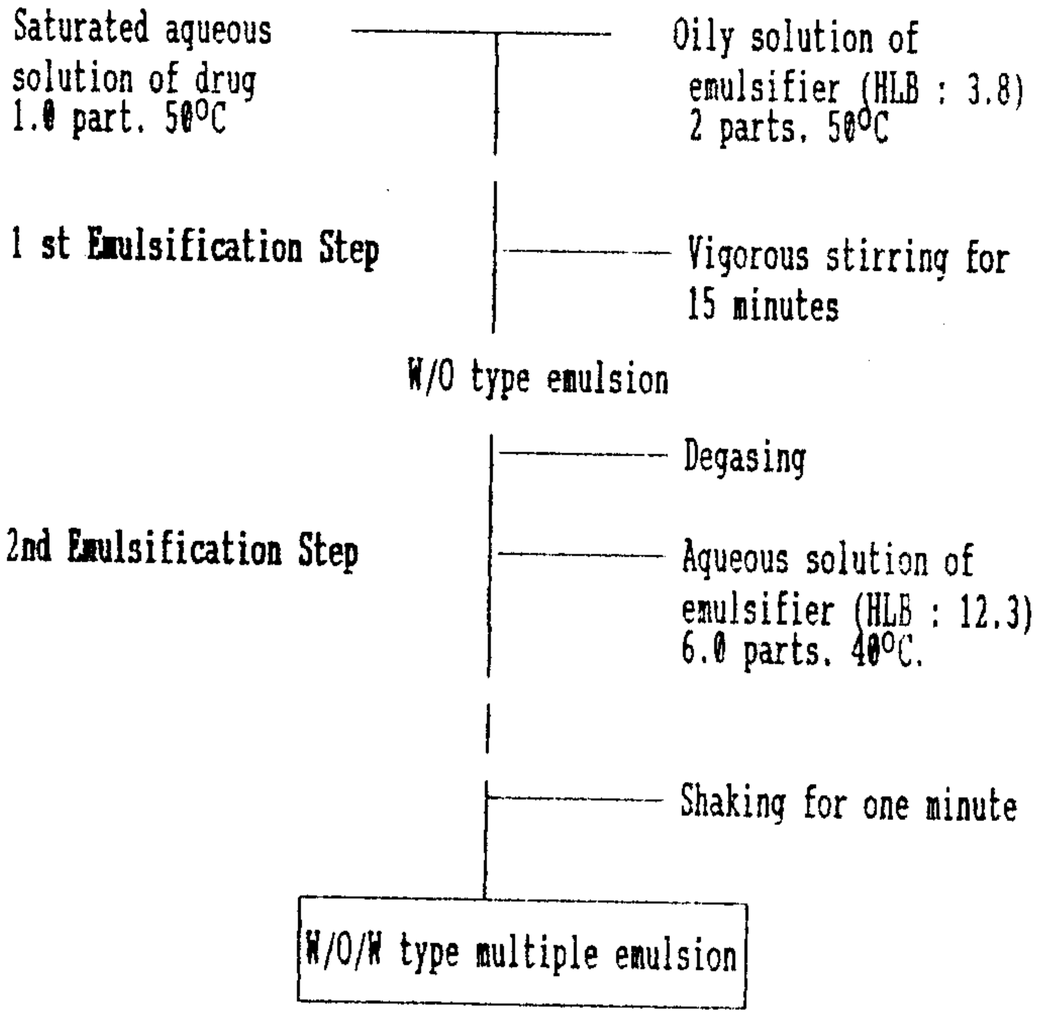


Chart I : Preparation of W/O/W type Multiple Emulsion by A Two-Step Procedures of Emulsifications.

An aqueous solution of papaverine hydrochloride and paraffin oil at ratio of 1:2 were used to prepare a W/O type emulsion (first emulsification step). Surfactant blend (HLB 3.8) of both span 80 and span 85 was used at 10% concentration. In the second emulsification step. the prepared primary emulsion was incorporated into the outer aqueous phase (1:2) to produce the final W/O/W multiple emulsion. Surfactant blend (HLB 12.3) was used at 5% concentration. Polysorbate 80 constitutes the major proportion of the second emulsifying agent. The prepared emulsion was denoted formulation P. Three other systems were similarly prepared but one of these materials; sodium chloride (4.5%), sorbitol (4.5%), and methylcellulose (0.9%) was added to their outer aqueous phases. These prepared systems were denoted as formulations N.S and M respectively. The prepared emulsions were kept at room temperature for 24 hours before testing.

Partition Coefficient Determination :

The partition coefficient of papaverine hydrochloride between paraffin oil and isotonic sodium chloride solution was determined as follows : Different concentrations of the drug namely 100.200.300.400 and 500 mg/100 ml were prepared in the saline solution. 50 ml of each of the mentioned concentrations was added to a separating funnel. An additional 50 ml of paraffin oil was added to the funnel content and shaken gently until equilibrium. The aqueous phase was separated and passed through filter paper. The ultraviolet absorbance at 310 nm was then measured against a blank

prepared in an analogous manner. Drug Concentration in the oil phase (C_o) was determined using equation (3).

$$C_o = C_t - C_w \quad \text{.....eq.3}$$

where C_o : Total drug concnetration.

C_w : Drug concentration in aqueous phase.

Release Studies :

Release of papaverine hydrochloride from W/O/W multiple emulsion systems was investigated by a dialysis method employing cellophane membrane (2.5 cm in diameter). Emulsion sample (10 ml) was introduced into the dialysis tube and dialysed in a 50 ml of isotonic sodium chloride solution at 37°C. Stirring was effected mechanically using mechanical shaker at 50 rpm to ensure proper agitation of both the emulsion sample in the dialysis tube and the large volume sink medium. At intervals, 1 ml of the saline medium was withdrawn and 1 ml of fresh one was added instead. The drug concentration was measured spectrophotometrically at 310 nm using fresh saline solution as a blank. The in-vitro release test was done on the emulsion systems 24 hours after preparation and then periodically at weekly intervals for 4 weeks.

Analysis of Data :

The in-vitro release data were kinetically analysed according to zero order and first order kinetics as well as according to the diffusion controlled release mechanism (Higuchi's diffusion model)[25]. Also, the data of in-vitro release from fresh systems were statistically treated through the analysis of variance[26].

RESULTS AND DISCUSSION

Papaverine hydrochloride was formulated in multiple phase emulsion with the aim of the development of a sustained-release liquid dosage form. W/O/W multiple type emulsions were tried because of their convenience for both preparation and oral administration. Paraffin oil was selected as the oily phase in this study. It is colourless, tasteless, odourless and not absorbed in the gastrointestinal tract[27]. Moreover, as a mineral oil, it is unlike edible fats and oils, does not have to be protected against microorganisms and oxidation. The physical stability of the W/O type primary emulsion was tested in a previous work[24].

Partition Coefficient Determination :

The knowledge of partitioning behaviour of the drug is helpful in understanding the drug release tendency from the prepared multiple emulsion systems. The drug must partition itself through the oil phase before being available for release from the innermost aqueous phase. Moreover, the drug must cross a variety of membranes to gain access to the target tissues.

The partition behaviour of papaverine hydrochloride between paraffin oil and isotonic sodium chloride solution was studied. Different concentrations of the drug were tested. The partition ratio or apparent partition coefficient ($P_{app.}$) was given by the equation :

$$P_{app.} = C_o/_w \quad \text{.....eq. 4}$$

where C_o and C_w: the total concentration of drug in paraffin oil and aqueous phase respectively.

To investigate the possible association of the drug in paraffin oil and to determine the true partition coefficient (K), the data of drug distribution between the two phases, were treated according to the equation[28]:

$$K = (C_o)^{1/n}/C_w \quad \text{.....eq. 5}$$

Where K : the true partition coefficient of drug with the assumption that the drug is totally undissociated in water. and n : degree of drug association in paraffin oil.

Thus :

$$\text{Log } K = 1/n \log C_o - \log C_w \quad \text{.....eq. 6}$$

or $$\log C_w = 1/n \log C_o - \log K \quad \text{.....eq. 7}$$

Equation 7 is one of linear relation (Y = a x + b). When this equation is plotted with log C_w on the vertical axis and log C_o on the horizontal one, a linear plot must be found with a slope equals to 1/n and an intercept equals to - log K.

Figure 1 shows a linear plot for the distribution data between paraffin oil and the aqueous phase. From this plot, the degree of association of drug in paraffin oil was found to be 1.02 (about 1). A result which indicates that papaverine hydrochloride exists predominantly in the form of single molecules (unassociated) in paraffin oil. The calculated partition coefficient was found to be 1.738×10^{-1}. These results give an evidence on the suitability of paraffine oil as an oily phase for the preparation of W/O/W emulsion containing papaverine hydrochloride. The limited affinity of drug towards the oily phase makes it available to the oil barrier. At the same time this lower affinity in addition to its unassociation tendency makes it easy to cross that barrier to the outermost aqueous phase.

Release Studies :

Papaverine hydrochloride release from the prepared multiple emulsions was conducted in isotonic sodium chloride solution. Mechanical shaker was used to ensure proper agitation of both the emulsion sample in the dialysis tube and the large volume dissolution medium. The agitation in the dialysis tube was regarded as essential requirement to avoid creaming of the oily phase. An effect which may lead to reduction in the release rates specially in delayed stages[23].

The release data were analysed according to zero, first and diffusion controlled mechanisms[25]. The results of data analysis appear in Table 1. The high correlation coefficient values, obtained by the analysis of the percentage amount released versus the square root of time give an evidence that the release pattern follows the diffusion controlled release mechanism (Higuchi's model). Similar findings for release from emulsions were reported[23,24,29-31]. The fact that both fresh and stored emulsions as well as those containing additives give the same release mechanism is an evidence on the stability and similarity of the prepared systems. In another meaning, we can say that the prepared systems remain as multiple phase emulsions in spite of the change in the release rate of drug thereof.

Analysis of variance[26] of the in-vitro release data from the freshly prepared systems (Table 2) re-

vealed significant differences between some formulations. The systems containing either methylcellulose or sodium chloride were of insignificant difference in the release rates. Also, the addition of sorbitol lead to insignificant difference in the release rate. On the other hand, significant differences were found to be existed between emulsions containing either methylcellulose or sodium chloride and those containing either sorbitol or no additives. The added substances were described to get emulsions with different release patterns by adjusting the osmotic gradient between the aqueous phases[17,20,32]. Figure 2 shows the release patterns of papaverine hydrochloride from the different freshly prepared systems. It is clearly obvious that emulsion containing sodium chloride exhibited the greatest prolongation of drug release. An effect which may be probably due to salting out of the surfactant at the interfaces and competing for water molecules. When surfactant molecules loose water, the structure of the interfacial layer (liquid crystalline phase) becomes more rigid and thus more effective as a mechanical barrier for the transfer of drug. Sorbitol prolonged the drug release but to a less extent. Methylcellulose effect was greater specially in the early stage of release.

The release of papaverine hydrochloride from the prepared W/O/W emulsions was slower than that from drug solutions in the corresponding outer aqueous phases (Figures 3-6). These results suggest that the rate limiting step is not transport through the dialysis tube but transport through the oily phase. Thus, the result also assure the preparation of multiple phase emulsion of the drug. An exception was found with emulsion containing methylcellulose (Figure 6) where the release in the early stage (up to 5 hours) from the emulsion was closely related to that from the solution. An effect which can be attributed to the high viscosity of the outer aqueous phase.

The time dependence of drug release profile was investigated. The release patterns of drug from fresh as well as stored emulsions were determined (Figures 3-6 and Table 2). It is evident that although sorbitol has added a limited stability to the prepared emulsions, its system exhibited the lower stability properties after 4 weeks period of storage. In this respect, more than three-folds enhancing of the release rate was found after that period. Emulsion formulations containing no additives exhibited more than twofolds increase in the release. Formulations containing either sodium chloride or methylcellulose exhibited more constant release pattern during storage. Less than 10% enhancing in the release was found during the first three weeks. After the fourth week the change was increased to about 22 and 25% in case of methylcellulose and sodium chloride respectively . These results confirm the formulation of the rigid mechanical barrier. Formulations containing methylcellulose is regarded as more preferable since they are of more stable release pattern and as a more convenient oral preparations. Finally, further studies on the preparation of more stable emulsion systems have to be done.

Table 1 : Release Characteristics of Papaverine Hydrochloride from The Prepared Multiple Emulsion Systems.

Formulation	Storage period (Week)	Zero order		First order		Higuchi's diffusion	
		Q r	t k	log Q r	t $Kx10^{-1}$	Q r	t B
P	F	0.954	4.05	0.875	2.70	0.979	12.45
	1	0.948	7.80	0.936	4.53	0.992	23.20
	2	0.986	10.54	0.891	4.52	0.998	31.93
	3	0.973	9.42	0.905	3.74	0.992	28.72
	4	0.987	9.63	0.922	3.97	0.985	28.74
S	F	0.949	4.35	0.880	1.51	0.978	13.55
	1	0.994	6.34	0.949	3.83	0.998	18.88
	2	0.986	8.73	0.952	3.88	0.989	25.98
	3	0.980	8.73	0.933	4.11	0.984	26.31
	4	0.997	14.74	0.953	4.64	0.980	41.21
N	F	0.961	3.98	0.923	4.93	0.969	11.98
	1	0.972	3.95	0.898	2.87	0.983	11.82
	2	0.994	4.05	0.971	2.94	0.991	12.01
	3	0.976	4.39	0.943	2.97	0.986	13.13
	4	0.996	5.04	0.961	3.31	0.996	15.06
	F	0.926	5.71	0.854	4.74	0.962	17.87
	1	0.978	4.87	0.914	3.87	0.993	18.80
	2	0.991	6.47	0.909	4.08	0.998	19.48
	3	0.987	5.86	0.912	3.72	0.991	17.59
	4	0.987	7.37	0.951	3.84	0.990	21.64

Q: Amount released (%), t: time of release (hours). r: Correlation coefficient
K: Specific rate constant, B : Slope of linear plot.
F: Fresh samples (24 hours after preparation).

Table 2 : Effect of Formulation on The In-Vitro Release Profile of Papaverine Hydrochloride from The Prepared Multiple Emulsion Systems.

Formulation	Amount of medicament released (%) after the following time intervals in hours							
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0
P	5.9	12.8	13.3	16.6	17.2	22.2	22.5	26.0
S	4.2	7.8	10.1	16.9	17.2	19.3	21.7	23.8
N	2.3	3.0	4.3	7.6	12.5	14.7	16.2	18.1
N	2.9	4.6	11.8	16.2	20.1	25.3	25.9	26.4

Analysis of Variance[26]

Source of variation	S.S	d.f	S.S/d.f.	F Calculated	F Tabulated p=0.05
Time	1450.56	7	207.22	16.81*	2.49
Formulation	258.98	3	86.33	7.00*	3.07
Error	94.78	21	12.33	--	--
Total	1804.32	31	--	--	--

$$L.S.D. = t_{0.05} \times \frac{2\ SS/df = 3.663}{r}$$

Treatment	Mean % Released X_i	Difference between means		
		$X_i - X_D$	$X_i - X_C$	$X_i - X_B$
P	$X_A = 17.063$	7.225*	1.938	0.438
M	$X_B = 16.625$	6.787*	1.500	--
S	$X_C = 15.125$	5 287*	--	--
N	$X_D = 9.838$	--	--	--

* : Significant differences.

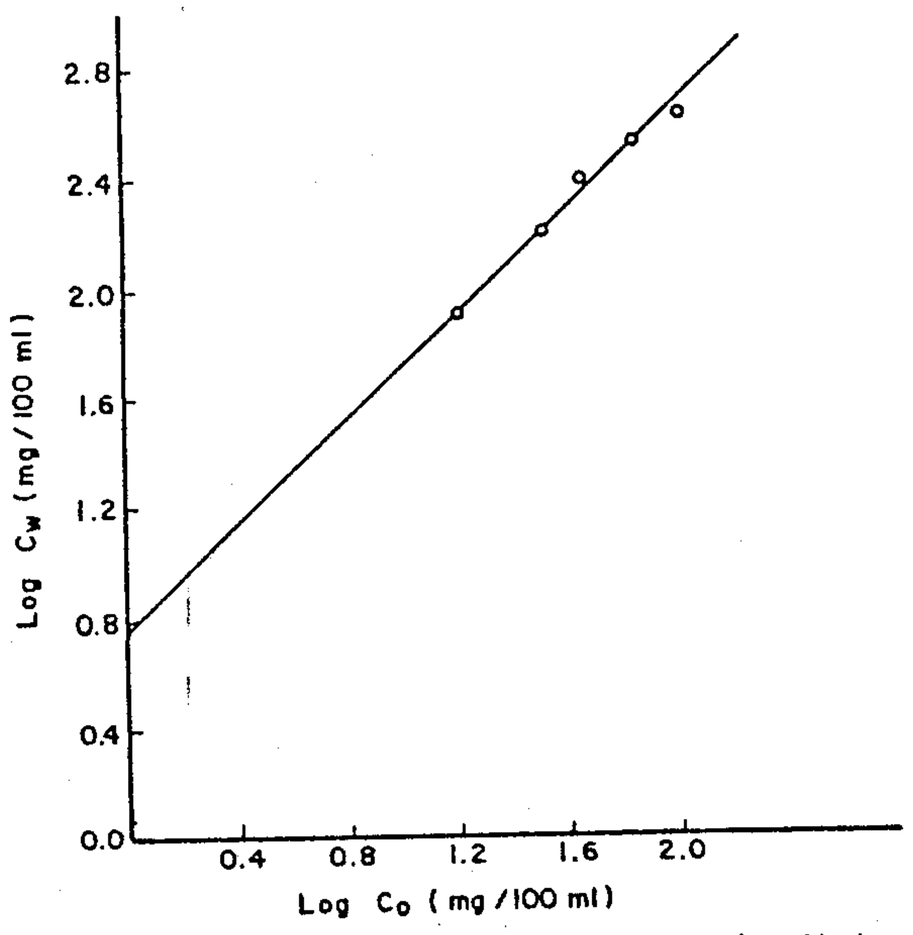
S.S : Sum of squares.

d.f : Degree of freedom.

S.S./d.f : Variance estimate = S^2.

L.S.D. : The least significant difference.

In-vitro Release Characteristics of Papaverine Hydrochloride From Multiple Emulsion Systems



Figure(1): Distribution Pattern of Papaverine Hydrochloride between Paraffin Oil and Saline Solution at 25°C.

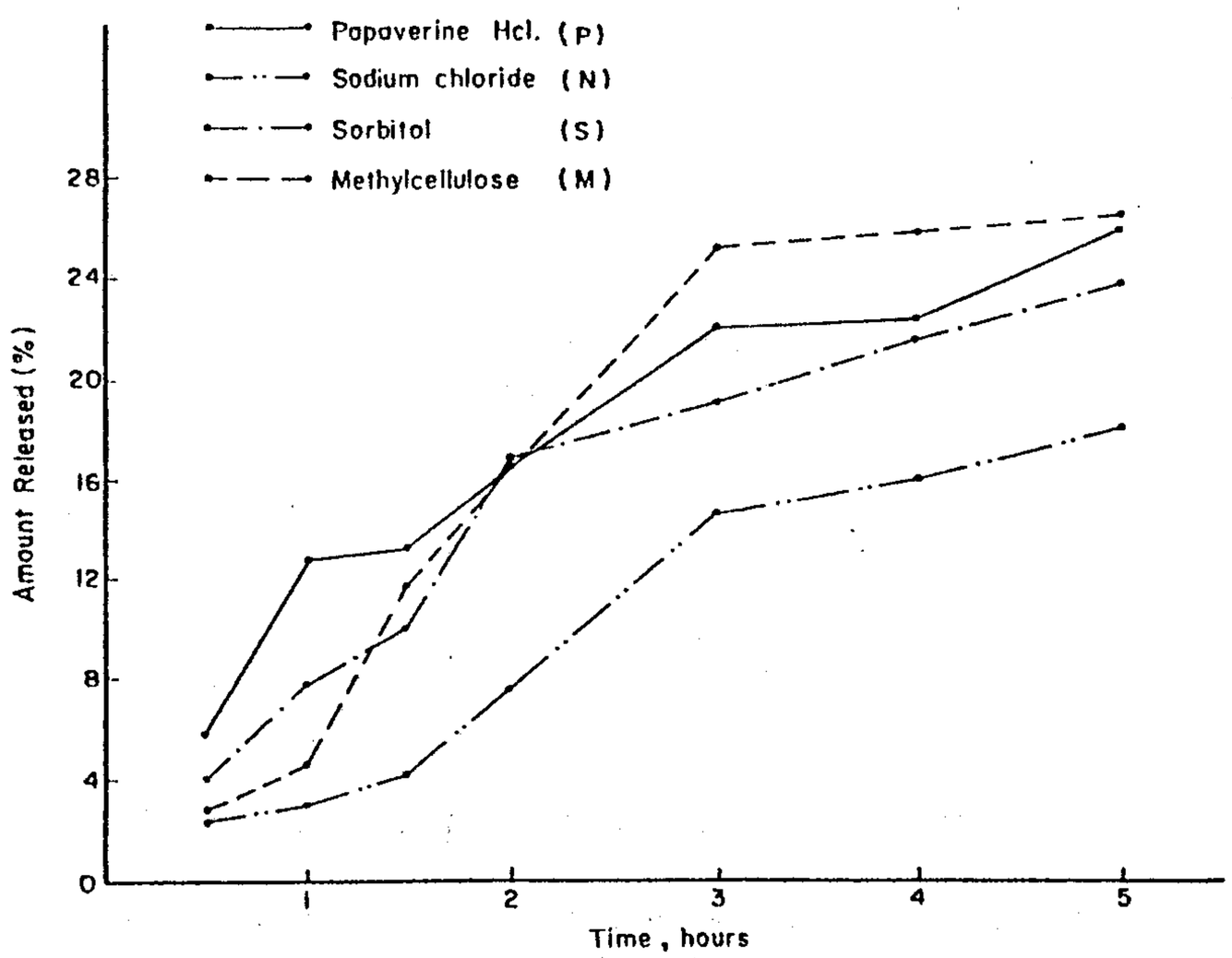


Figure (2): In – Vitro Release of Papaverine Hydrochloride from the Fresh Prepared Emulsion Systems .

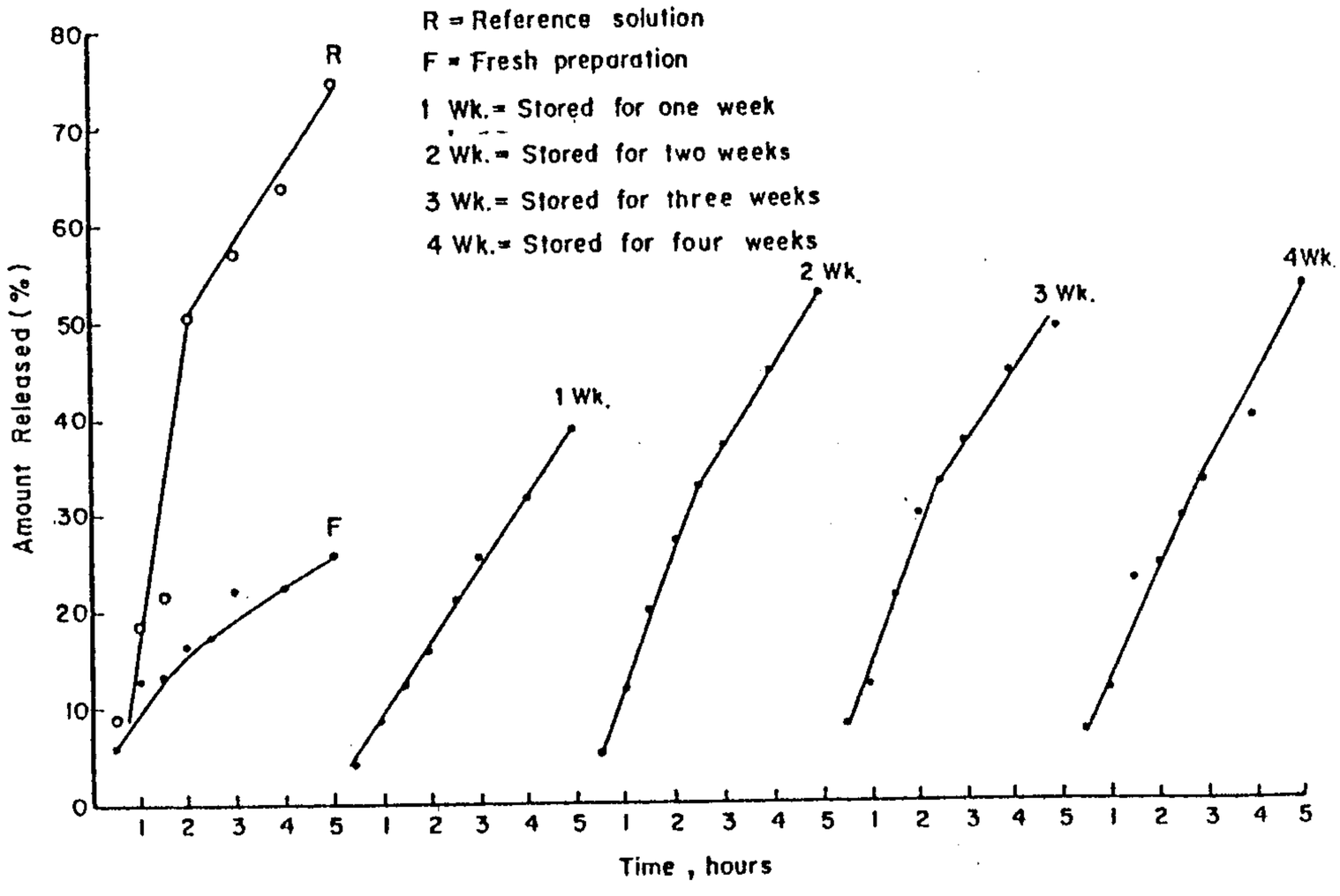
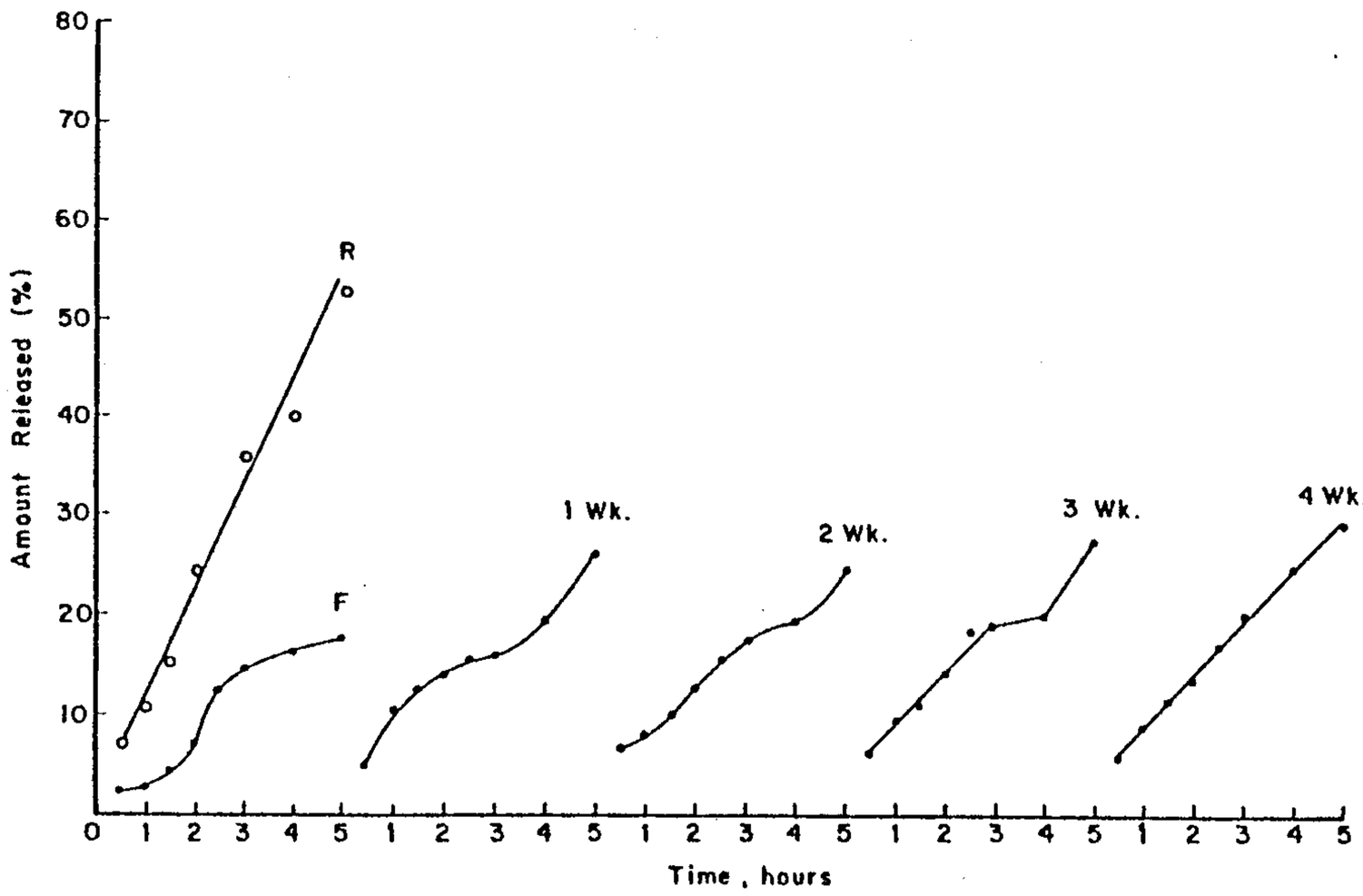
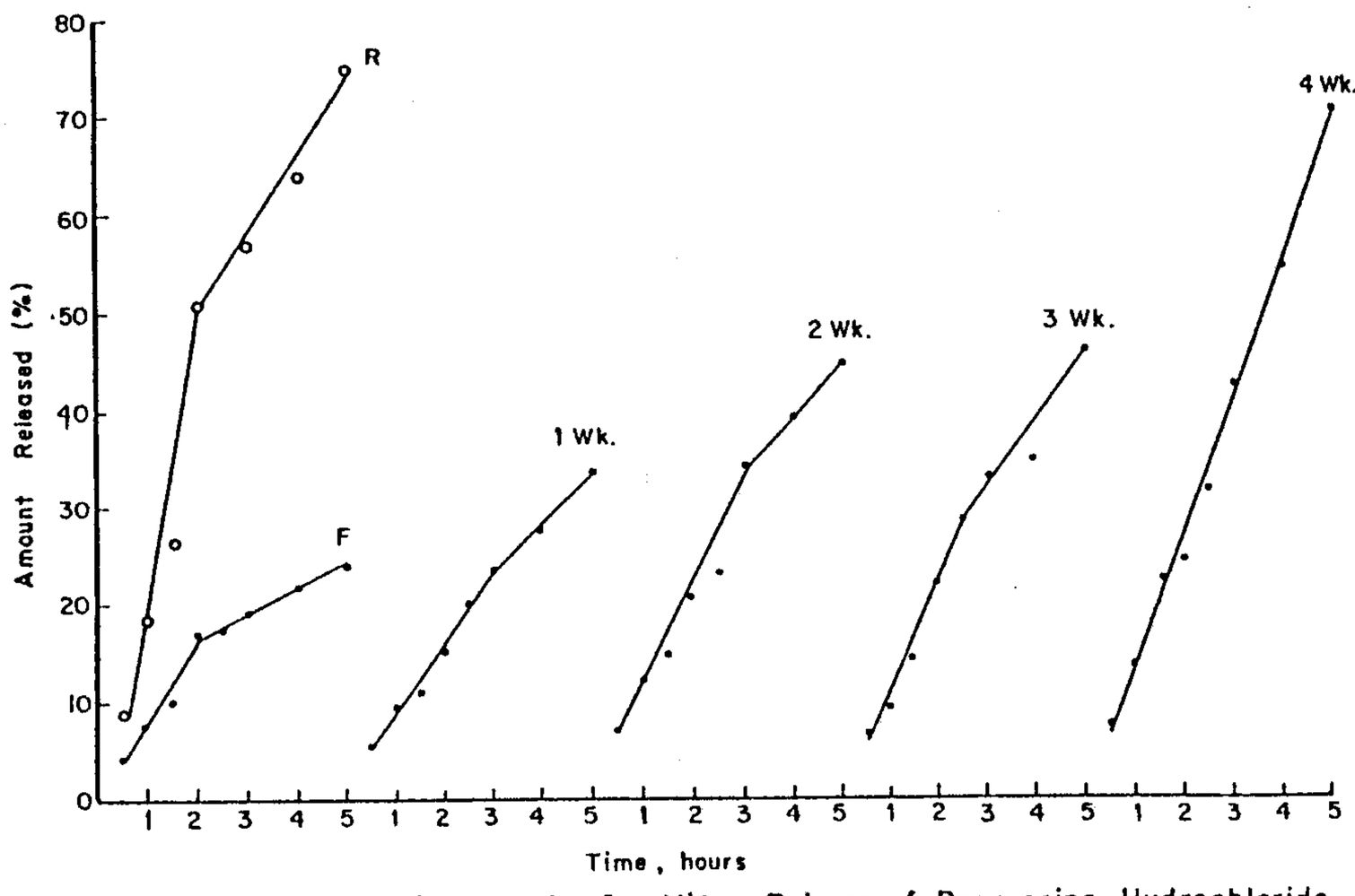


Figure (3): In-Vitro Release Profile of Papaverine Hydrochloride from its Prepared Emulsion System .

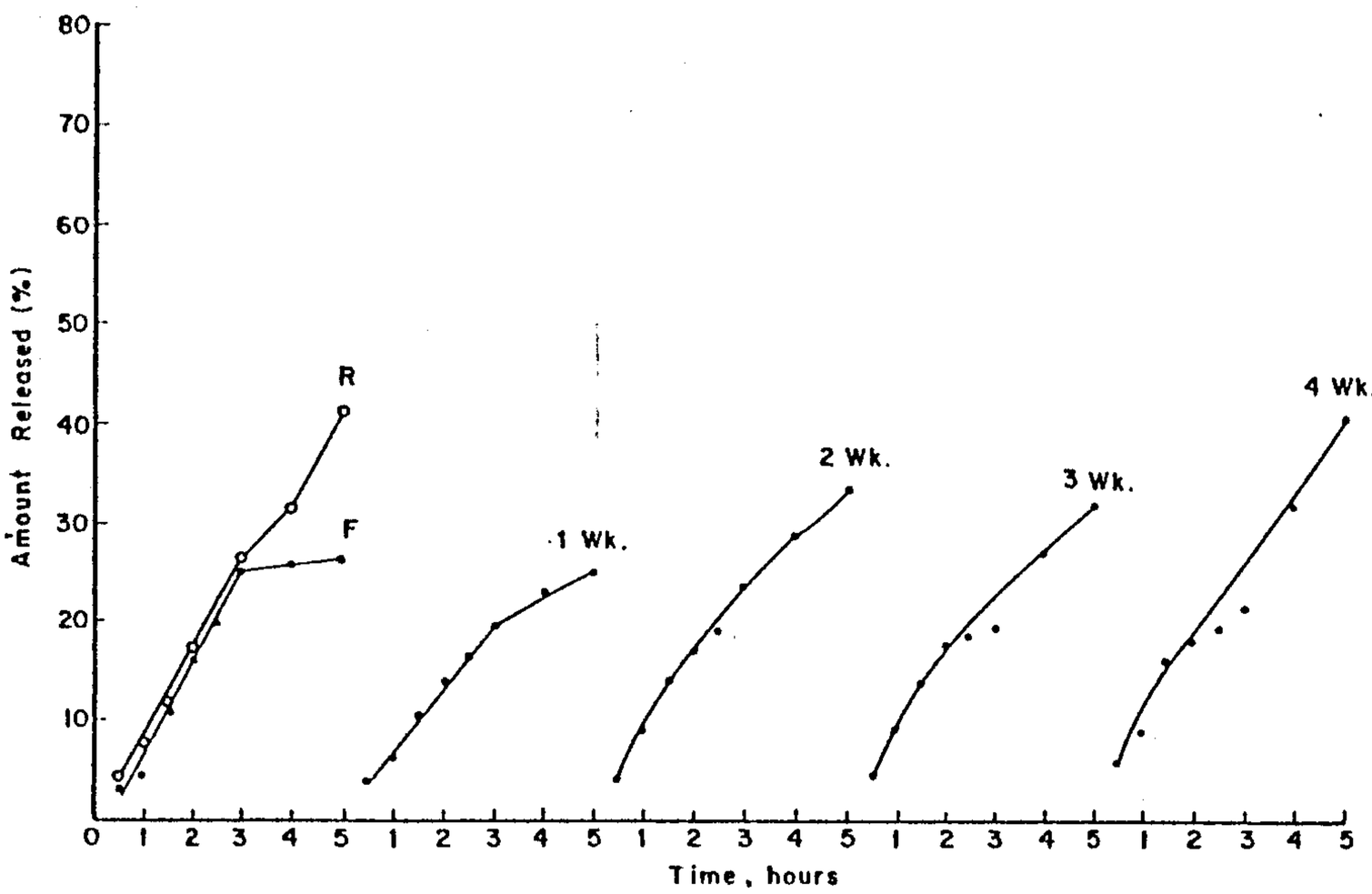


Figure(4): Effect of Sodium Chloride on the In-Vitro Release Profile of Papaverine Hydrochloride from its Prepared Emulsion System.

In-vitro Release Characteristics of Papaverine Hydrochloride From Multiple Emulsion Systems



Figure(5): Effect of Sorbitol on the In-Vitro Release of Papaverine Hydrochloride from its Prepared Emulsion System.



Figure(6): Effect of Methylcellulose on the In-Vitro Release Profile of Papaverine Hydrochloride from its Prepared Emulsion System.

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In-vitro Release Characteristics of Papaverine Hydrochloride From Multiple Emulsion Systems.

خصائص الانطلاق المعملى لهيدروكلوريد البابافرين من المستحلبات المتعددة

حسين عبد المنعم ـ احسان حافظ ابراهيم ـ سلوى محمود محمد صفوت

قسم الصيدلانيات ـ كلية الصيدلة ـ جامعة اسيوط

فى هذا البحث امكن تحضير مستحلبات متعددة من نوع ماء / زيت / ماء تحتوى على عقار هيدروكلوريد البابافرين وقد استخدمت صياغات مختلفة لهذه المستحلبات .

وتم تحضير هذه المستحلبات على خطوتين منفصلتين . تمثلت الخطوة الاولى فى تحضير مستحلب اولى من نوع ماء / زيت وذلك باستخدام محلول مائى من العقار مع زيت البرافين بنسبة ١ الى ٢ وفى الخطوة الثانية تم ادماج المستحلب الاولى فى الوسط الماء الخارجى بنسبة ١ الى ٢ . وقد استخدمت خلطات مناسبة من المواد ذات النشاط السطحى فى كل خطوة على حدة .

وقد تم تقييم انطلاق العقار من المستحلبات المحضرة بعد ٢٤ ساعة من تحضيرها وكذلك بعد فترات اسبوعية لمدة اربعة اسابيع بعد التخزين . كما تم تقييم العقار من المستحلبات التى يحتوى كل منها على احدى هذه المواد: كلوريد الصوديوم، أو السوربيتول آو ميثيل السيليلوز. واظهرت النتائج ان معدلات الانطلاق من الانظمة المحضرة اقل كثيرا مقارنة بذلك من محاليل العقار فى الاوساط المائية . هذا وقد اظهرت المستحلبات المحتوية على ميثيل السليلوز احسن النتائج حيث ثبات معدلات الانطلاق .

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