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Design, Formulation, and Evaluation of Piroxicam Niosomal Gel

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Abstract:
Objective: The objective of the present study was incorporation PIR niosomes in different gel bases to develop (PIR) gel for tansdermal delivery. **Method:** PIR niosomes were prepared by lipid evaporation method and all vesicles were evaluated for their entrapment efficiency (EE%), and in-vitro drug release. PIR gels were prepared using sodium alginate, methyl cellulose (MC), hydroxyl propyl methyl cellulose (HPMC), carboxy methyl cellulose (CMC), and xanthan gum. The effect of type and concentration of the employed gel bases on the permeation was tested. The viscosity of gel formulae and also the shelf life were evaluated. **Results:** The results showed that polymers type affect the drug permeation and rheological properties of PIR niosomal gel. F3 containing 4% MC showed the best permeation through rat skin (87.73 $\pm$ 2.06) and the lowest viscosity. **Conclusion:** All the studied gels are of acceptable physical properties and drug content. They exhibited pseudoplastic flow with thixotropic behavior. Considering in-vitro permeation, rheological properties, and shelf life, F3 (4% MC) formula was the best.
Key words: Piroxicam (PIR); entrapment efficiency (EE%); carboxy methyl cellulose (CMC); hydroxyl propyl methyl cellulose (HPMC); methyl cellulose (MC).

Introduction

PIR is a non steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, antirheumatoid arthritis, analgesic[1], and antipyretic activities in animal models. PIR like other non steroidal anti-inflammatory drugs causes side-effects on the gastro-intestinal system and other systems of the body. Because of these side effects the patient compliance may reduce. The best alternative route for administration of PIR is transdermal route. In recent years many researchers substantiating that niosomes are acting as best carriers for administration of drugs across the skin. These vesicular structures acts as carriers for drugs and helps to overcome the barrier properties of the skin. Niosomes or non-ionic surfactants vesicles are microscopic lamellar structures formed on the admixture of a non-ionic surfactant, cholesterol and phosphate with subsequent hydration in aqueous media[2].

The present study involves formulation of a transdermal PIR niosomal gel.

Material and Methods

Materials

PIR was provided by El-Mehan Drug Company, (Cairo,Egypt), Span 20 and Span 60 from Sigma Chemical Co., (Germany), Cholesterol from Sigma Chemical Co., (USA), Sodium hydroxide and Potassium dihydrogen phosphate, PureLab, Madison, USA, and Chloroform from Labscan Ltd, Dublin, (Ireland), HPMC, Alpha Chemica, Mumbai, India, MC, Oxford company, Hartlepool, United Kingdom,CMC, Oxford company, Hartlepool, United Kingdom, Sodium alginate, Xanthan gum. All other chemicals used were of analytical grade.

Equipment

An electric balance (SARTORIUS AG, Germany), Shimadzu UV spectrophotometer (2401/PC), Japan, Buchi rotavapor (R-3000, Switzerland), Digital Sonifier (Branson,Danbury, USA), Dissolution apparatus (Erweka GmbH, Germany), Shaker water bath (Julabo SW-20 C, Germany), pH meter, JENWAY (England), Centrifuge (Biofuge, primo Heraeus, Germany), and JEOL Transmission Electron Microscope (JTEM model 1010, Japan), Brookfield R/S+RHEOMETER, Rotary Viscometer, Brookfield Engineering Laboratories, Inc. (USA), Planimeter.

Methods

Preparation of PIR niosomes

Niosomes were prepared by lipid hydration method according to the composition in table (1).

Mixed surfactants and cholesterol were dissolved in 10 ml of chloroform. The solvent was evaporated using a rotary flash evaporator at speed 80 rpm, under low pressure at 60°C for preparing niosomes. Niosomes were formed by adding phosphate buffered saline, PBS (pH 7.4) containing 10mg PIR slowly to the dried thin film formed on the walls of the round-bottom flask, with gentle agitation. Dispersion of the mixture was carried using a sonicator for a period of 5 min.

Entrapment efficiency of niosomes (EE%)

The unentraped drug was separated from the niosomal dispersions by centrifugation at 15,000 rpm for 45 min. The supernatant was separated, diluted to 100 ml with PBS Ph 7.4, filtered using a membrane filter (0.45µm pore size), and measured using a spectrophotometer at 354 nm. EE% was calculated by the following equation[3].

EE%=[(Ct-Cr\Ct)] ×100%

Ct is the concentration of total PIR.

Cr is the concentration of free PIR.

In-vitro release of PIR

This study was carried out using a USP dissolution tester (Apparatus I). Niosomal suspension (5ml) was placed in cylindrical tubes (2.5cm in diameter and 6cm in length). Each tube is tightly covered with a molecular porous membrane from one end and attached to the shafts of the USP Dissolution apparatus, instead of the baskets, from the other end. The shafts were then lowered to the vessels containing 250 ml of PBS (Ph 7.4) at 37±0.5 ºC, and 50 rpm. 5ml samples were withdrawn at time intervals of 1, 2, 3, 4, 6, 8, 10, and 12 hr. followed by replacement with fresh medium. The samples were analyzed spectrophotometrically at 354 nm. The obtained data were subjected to kinetic treatment according to zero, first, and Higuchi diffusion models[4]. The correlation coefficient (r) was determined in each case.

Transmission electron microscopy (TEM)

A sample drop was diluted 10-fold using de-ionized water and a drop of this diluted dispersion was applied to a collodion-coated 300 mesh copper grid and left for 5 min to allow some of the niosomes to adhere to collodion. A drop of 2% aqueous solution of uranyl acetate was applied for 1 min. The sample was air dried and examined with TEM.

Preparation of PIR niosomal gel

0.5% PIR niosomal gel was formulated using, sodium alginate (4 and 6%), MC (4 and 6 %), CMC (2 and 4 %), HPMC (2 and 4 %) and xanthan gum (0.5 and 1%). The weighed amount of polymer powder was sprinkled gently in beaker, containing 70 ml boiling distilled water (hot water was used in case of HPMC) and stirred magnetically at a high speed. Stirring was continued until a thin hazy dispersion, without lumps, was formed. 10 gm glycerin and 10 gm propylene glycol were added as permeation enhancers with continuous stirring followed by 0.2 gm methyl Paraben and 0.02 gm propyle Paraben as preservatives then niosomal suspension containing 0.5% PIR was added with stirring to get a homogeneous dispersion of noisome in the gel. Finally total weight adjusted to 100 gm by distilled water, Table (I).

Table (1): Composition of the PIR noisome

Independent variables	**Levels used**
X_1 = Speed (rpm)	80
X_2 = Amount of chloroform (ml)	10
X_3 = HLB	6.036
X_5 = Amount of drug (mg)	10
X_7 =Sonication time (min)	5
X_6 = Amount of total lipid (mg)	50
X_4 = Surfactant -Cholesterol ratio	1:1

Evaluation of Gels

Clarity

It was determined by visual inspection under black and white background and it was graded as follows: turbid: +, clear: ++, very clear (glassy): +++[5].

Homogeneity

It was determined by visual inspection for the appearance of gel and presence of any aggregate[6].

Spreadability

A spreadability test was conducted by pressing 0.5 g of gel between two glass slides and leaving it for about 5 min. until no more spreading was expected. The diameter of the formed circle was measured and used as

comparative values for spreadability[7].

Extrudability

1 gm of gel was filled in clean collapsible tube**;** 0.25 gm weight was placed on the free end of the tube and was just touched for 30 second. Amount of gel extruded was noted[5].

pH

Two grams of gel was dispersed uniformly in 20 ml of distilled water using magnetic stirrer for 2 hrs. The pH of dispersion was measured by using digital pH meter[6].

Drug content

The gel of 250mg was dissolved in 100 ml of phosphate buffer pH 7.4 of three samples of formulation from top, middle and bottom of gel were transferred into three different 100 ml volumetric flask. The volumetric flask containing gel solution was shaken for 2 hr on mechanical shaker in order to get complete solubility of drug[8]. Then, samples were analyzed spectrophoto metrically

In-vitro permeation of PIR gels

In vitro permeation was determined by a modified USP XXVII dissolution apparatus I using a cylindrical tube (2.5 cm in diameter and 6 cm in length). Accurately weighed 1gm gel was spread uniformly on the epidermal surface of excised rat abdominal skin which was stretched over the lower open end of the tube with SC side facing upwards and the dermal side facing downwards into the receptor compartment[9]. The dissolution medium was 250 ml of phosphate buffer pH 7.4 .The stirring speed was 100 rpm, and the temperature was maintained at 37°C ± 0.5°C for 12 hr (13). Samples of 5 ml were withdrawn at predetermined time intervals 1, 2, 3, 4, 6, 8, 10, and 12hr and replaced with fresh medium at appropriate time intervals to maintain a constant volume. The samples were filtered through 0.45µ nylon disc filter, and analyzed by UV spectrophotometer at the previously determined λ_{max} after suitable dilutions using phosphate buffer (pH 7.4) as a blank. The release experiments were repeated in triplicates

The average cumulative amount of PIR permeated per unit surface area (µg/cm^2) was plotted as a function of time. The drug flux at steady state (JSS) was calculated from the slope of the straight line. Permeability coefficient (KP) was calculated using the following equations: KP = JSS/Co (where Co is the initial concentration of the drug). D (diffusion coefficient, cm^2/min) was calculated as follows: D = h^2/6 Lt Where h is the thickness of the skin in cm and Lt the Lag time in minutes[7].

<u>Kinetic treatment and parameters for the permeation of PIR niosomal gels</u>

The obtained data were subjected to kinetic treatment according to zero, first, and Higuchi diffusion models[4]. The correlation coefficient (r) was determined in each case.

Rheological properties determination

The viscosity was determined using Brookfield R/S+RHEOMETER using spindle CC 14. The measurement was started at 1 rpm; the speed was gradually increased till reached 200 rpm, the speed was then reduced gradually until reaching the starting rpm. Measurement of thixotropic behavior of was determined using the planimeter in order to calculate the hysteresis loop between the upward curve and downward

Stability studies of PIR niosomal gels

The prepared plain and medicated gel bases were stored in well stoppered polyvinyl chloride (PVC) plastic containers in the dark for 12 months at room temperature. They were checked for drug content.

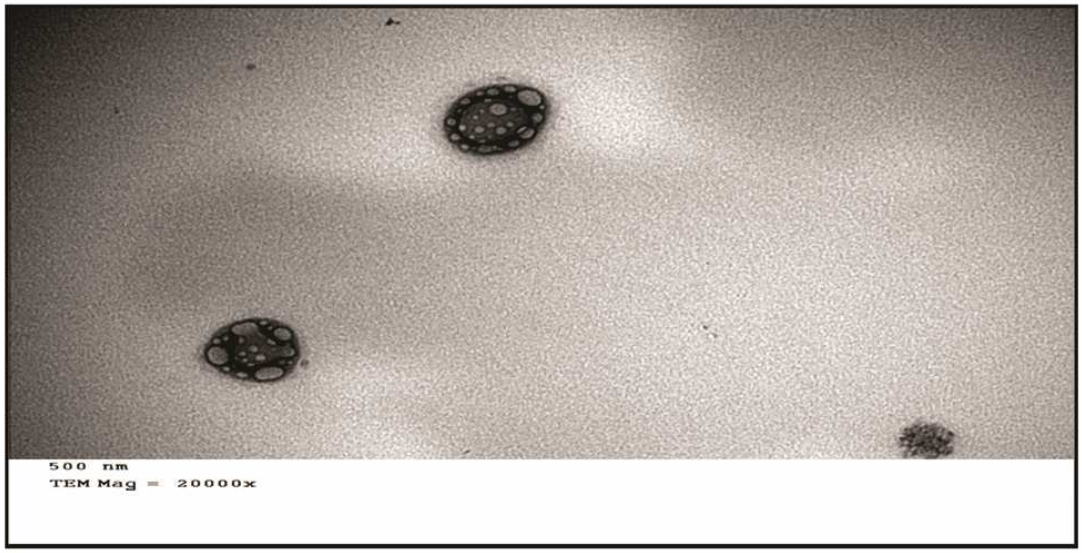

Figure (1): TEM micrograph of the PIR niosome

Results and Discussion

Formulation of piroxicam niosome

The piroxicam niosome prepared by lipid hydration method. EE% was found to be 41.52 ± 0.42%. the in-vitro release was 91.68% after 12 hr.

As shown in figure (1). The examined niosomes appeared as spherical unilamellar nano vesicles with size ranged between 85.79 and 176.84 nm (mean 118.43 nm).

Formulation of PIR niosomal gel

The prepared formulae are described in Table (2). Concentraion of PIR in marketed gel is 0.5% w/w, so we choose this concentration in our formulation.

Evaluation of Gels

All the prepared gel formulae are of smooth and homogenous appearance. spreadability values for all prepared formulae ranged between 6.2 –10.3 cm which indicates that the gels can be spared easily on skin surface with a little stress. The percent of gel extruded ranged between 75.85- 98.36%. The pH values were found to be in the range of (5.7-6.6) which is within the required physiological range, i.e., pH 4-7 units and was considered to be safe and non-irritant for transdermal application. Drug content of PIR gel formulae was found to be in the range of 88.94-98.44% which shows a good content uniformit**y,** Table (3).

In vitro skin permeation of PIR gels

The permeation profile of PIR gels are illustrated figures (3-7). It was found that drug permeation is highly dependent on polymer type and polymer concentration.

1- Effect of polymer type on the diffusion profile of PIR

The in vitro skin permeation of PIR gel formulations was investigated through rat skin. Drug permeation is highly dependent on polymer type. As shown in Figure (2), it was clear that the permeation of PIR from 4% MC gel bases was higher than that from other tested gel bases.

The permeation of PIR niosomal gels formulae from different gel bases could be arranged in a descending manner as follows: F3 (4% MC) > F7 (2% CMC) > F1 (4% Na alginate) > F9 (1% xanthan) > F5 (2% HPMC) > F4 (6% MC) > F2 (6% Na alginate) > F8 (4% CMC) > F10 (2% xanthan) > F6 (4% HPMC). These

differences may be attributed to the variation in shape and dimension of the crystallites of the solid fraction and their ordering in the 3-dimensional structure within the resulting network[10].

Table (2): Suggested formulae of PIR niosomal transdermal gel

Composition	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Na alginate (gm)	4	6								
MC (gm)			4	6						
HPMC (gm)					2	4				
CMC (gm)							2	4		
Xanthan (gm)									0.5	1
Methyl Paraben (gm)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (gm)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Glycerin (gm)	10	10	10	10	10	10	10	10	10	10
Propylene Glycol (gm)	10	10	10	10	10	10	10	10	10	10
Niosomal suspension (ml)	10	10	10	10	10	10	10	10	10	10
Water to (gm)	100	100	100	100	100	100	100	100	100	100

N.B. niosomal suspension equivelant to 0.5 gm PIR

Table (3): Evaluation of PIR gel formulae

Formula	Clarity	Homogeneity	spreadability	Extrudability	pH	Drug content
F1	+	good	9.9±0.21	92.18±2.09	6.60±0.19	93.43±2.5
F2	+	good	7.5±0.26	84.72±1.02	6.56±0.14	89.28±1.5
F3	+	good	10.3±0.31	98.36±1.26	5.70±0.12	95.08±1.5
F4	+	good	7.3±0.25	86.85±1.31	5.77±0.16	88.94±3.8
F5	+	good	7.7±0.26	87.65±0.51	5.86±0.11	90.9±1.9
F6	+	good	6.2±0.20	75.85±0.80	5.90±0.17	91.86±3.6
F7	+	good	9.4±0.25	95.66±0.66	6.28±0.11	98.44±3.1
F8	+	good	6.7±0.15	83.52±1.46	6.31±0.19	95.25±2.5
F9	+	good	9.7±0.17	90.03±0.95	6.25±0.15	92.28±3.1
F10	+	good	7.1±0.23	80.32±0.62	6.27±0.17	90.05±3.8

+ Satisfactory, ++ Good

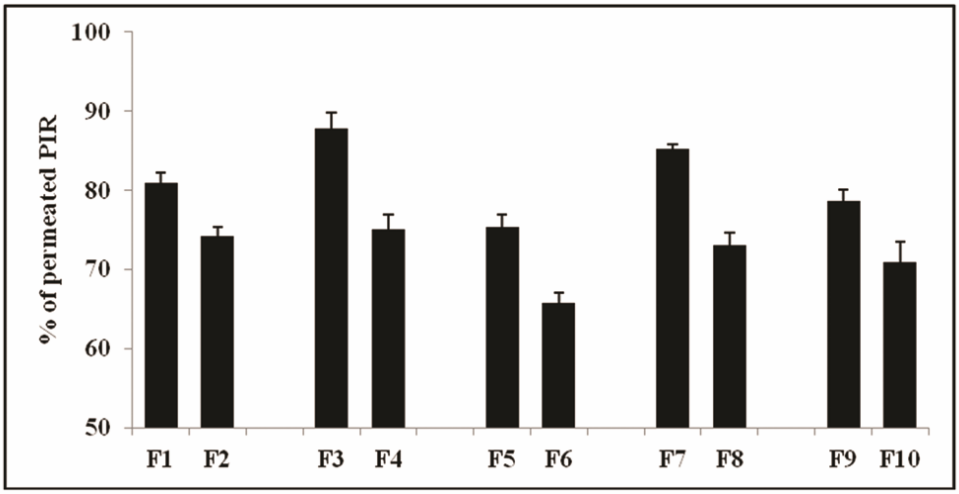

Figure (2): The effect of polymer type and polymer concentration on the in-vitro drug permeation of PIR

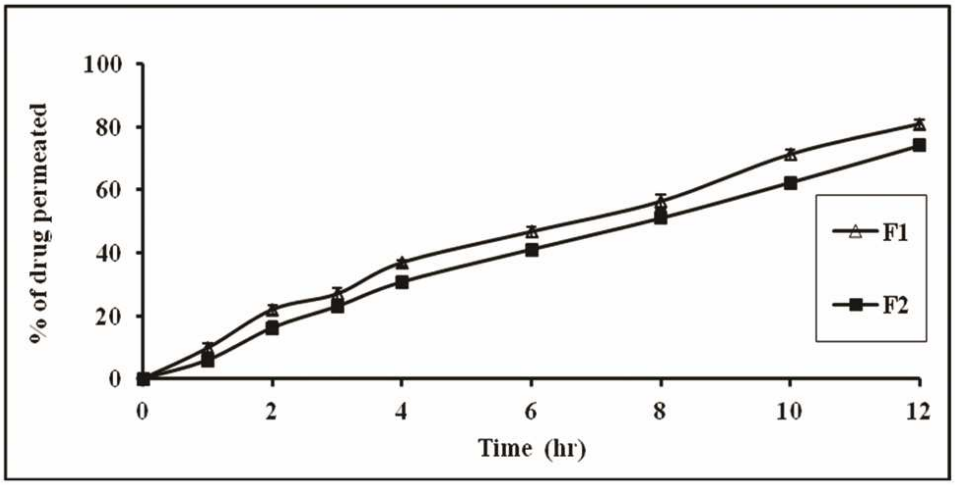

Figure (3): The effect of sodium alginate concentration on the in-vitro drug permeation of PIR

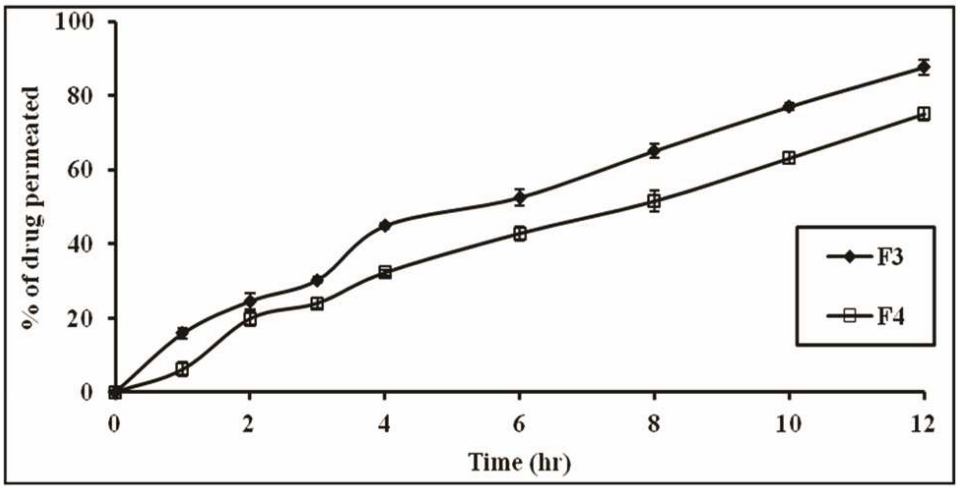

Figure (4): The effect of MC concentration on the in-vitro drug permeation of PIR

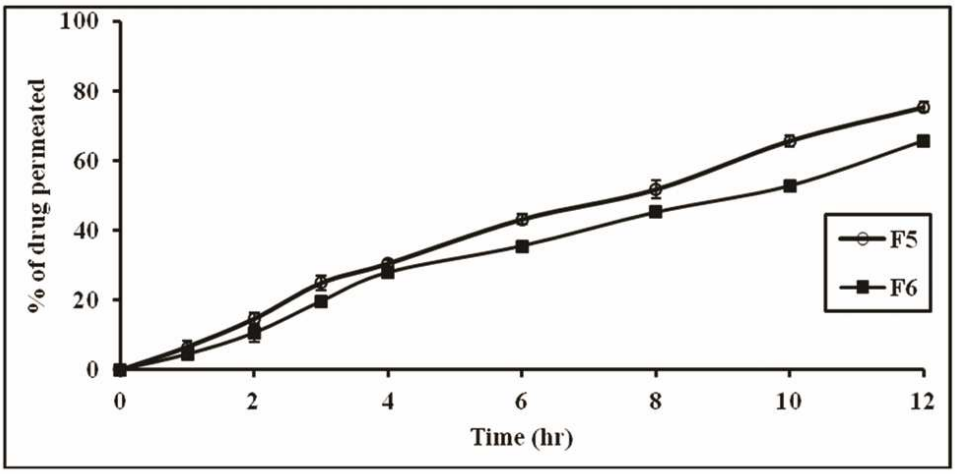

Figure (5): The effect of HPMC concentration on the in-vitro drug permeation of PIR

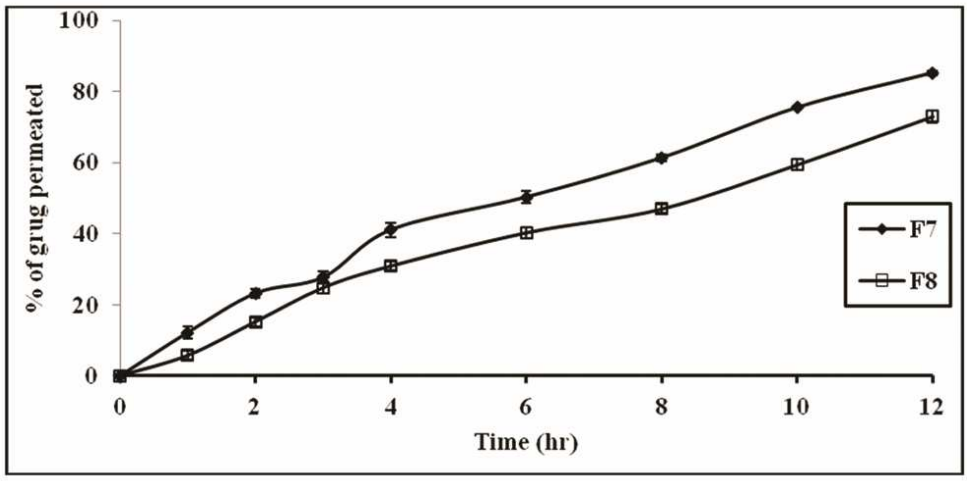

Figure (6): The effect of CMC concentration on the in-vitro drug permeation of PIR

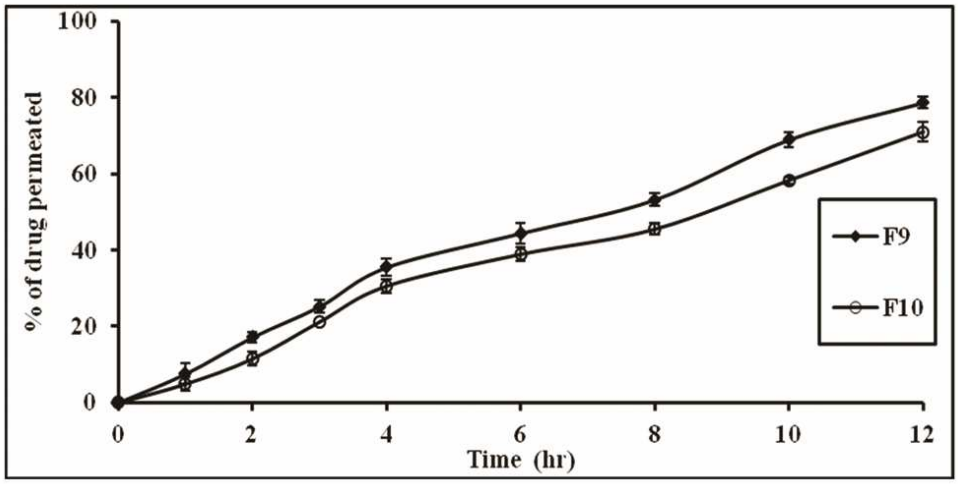

Figure (7): The effect of Xanthan gum concentration on the in-vitro drug permeation of PIR

2- Effect of polymer concentration on the diffusion profile of PIR

As shown in figure (2) the permeation of PIR from the prepared gels is highly dependent on polymer concentration. It was found to be decreased as the concentration of the gel increased. The release of PIR from 4% Na alginate (F1), 4% MC (F3), 2% HPMC (F5), 2% CMC (F7) and 1% Xanthan (F9) gel bases were higher than that from 6% Na alginate (F2), 6% MC (F4), 4% HPMC (F6), 4% CMC (F8) and 2% Xanthan (F10) gel bases.

It was found that the drug permeation is inversely proportional to polymer concentration. This may be attributed to at the higher polymer concentrations, the active substance is trapped in polymer chains and it is structured by its close proximity to those polymer molecules thus increasing the diffusional resistance. The mechanism for such enhanced resistance may be due to reduction in the number and dimension of water channels within the gel structure. Also, the density of chain structure which has been observed in gels microstructure increases at the higher polymer concentration and this limits the active substance's movement area[11].

In addition, viscosity increased as polymer concentration increased. Viscosity is negatively related to the release of active substance from formulations and its penetration through the diffusion barriers. The decrease in the release could be attributed to increased micro viscosity of the gel by increasing polymer concentration. Thus, both high concentration of polymer and high viscosity compete each other in decreasing the release of active substance from the formulation[12]. In our study, the finding that higher polymer concentration resulted in lower drug release from the vehicles is in agreement with Lauffer.s molecular diffusion theory of polymer gels[13],

which states that the diffusion coefficient of a solute is inversely proportional to the volume fraction occupied by the gel-forming agent.

Permeation data analysis

As shown in table (4), the diffusion flux increased with increasing of gel concentration. There was very good correlations were obtained between steady state flux and permeability coefficients. The diffusion coefficient of the prepared gels was in the range of $4.33E^{-08}$ Cm^2 hr^{-1} for F7 to $1.15E^{-07}$ Cm^2 hr^{-1} for F6. The partition coefficient of the prepared gels was in the range of 47.402 for F6 to 149.343for F3.

No direct correlation was observed between the lag time and the apparent flux released.

Kinetic treatment and parameters for the in-vitro permeation of PIR gels

As shown in table (5), it is clear that the higher correlation coefficient values for Higuchi diffusion model suggesting that the permeation release of PIR from all prepared niosomal gel preparations can be best described by Higuchi's diffusion model.

Rheological properties of gel formulae

All the rheological data of the different gels were fitting to the power's law with (R^2) values ranged between (0.923- 0.996).The minimum viscosities were in the range (97.3– 209.1) cPs, while the maximum viscosities were in the range (4123– 12055.3) cPs, Table (6). The maximum viscosities of MC gel bases were lower than that of other tested cellulose derivatives. This may be attributed to variation in shape and dimensions of crystallites of different polymers[14]. F3 was the lowest formula in viscosity. Thixotropic behavior ranged between (5.6 Cm^2 – 12.2 Cm^2). The pseudoplastic behavior is evidenced by that the flow curves approach the origin with no yield values and N value is higher than1, it ranged between (2.58- 5.27).

Stability study of PIR niosomal gels

The percent of drug degraded after 12 months were: 6.31, 3.99, 3.85, 5.16 and 3.69 for sodium alginate, MC, HPMC, CMC and xanthan gum respectively.

According to the results obtained from the kinetic analysis of the stability test, it was obvious that the degradation of PIR was found to obey zero order reaction for all the tested gel bases, based on the values of the correlation coefficient (r), table (7). The shelf life of PIR formulae can be calculated according to zero order by the following equation: $t_{90} = a / 10 K$ Where (a) is the initial drug concentration. As shown in table (8) the shelf life of prepared PIR niosomal gels ranged between 1.619 year for F1 and 2.516 year for F9.

Table (4): Permeation parameters of PIR from prepared gels

F. NO.	Steady state flux Jss (µg cm^{-2} hr^{-1})	Permeability coefficient (Cm hr^{-1})	Lag time (hr)	Diffusion coefficient (Cm^2 hr^{-1})	Partition coefficient
F1	70.419	0.00704	2.360	5.59E-08	112.028
F2	67.469	0.00675	1.613	8.19E-08	73.348
F3	73.463	0.00735	3.015	4.38E-08	149.343
F4	67.688	0.00677	1.738	7.60E-08	79.298
F5	69.081	0.00691	1.682	7.85E-08	78.320
F6	61.375	0.00614	1.146	1.15E-07	47.402
F7	70.900	0.00709	3.049	4.33E-08	145.736
F8	64.613	0.00646	1.701	7.76E-08	74.073
F9	69.244	0.00692	2.131	6.20E-08	99.472
F10	62.563	0.00626	1.752	7.53E-08	73.904

Table (5): The Calculated Correlation Coefficients for The In-Vitro permeation of PIR gels Employing Different Kinetic Orders or Systems

Formula No.	Correlation coefficient (r)		
	Zero	**First**	**Diffusion**
F1	0.9945	0.9853	**0.9950**
F2	0.9959	0.9900	**0.9964**
F3	0.9931	0.9805	**0.9945**
F4	0.9930	0.9879	**0.9952**
F5	0.9954	0.9912	**0.9963**
F6	0.9927	0.9912	**0.9947**
F7	0.9939	0.9836	**0.9951**
F8	0.9915	0.9817	**0.9924**
F9	0.9935	0.9863	**0.9948**
F10	0.9916	0.9854	**0.9923**

Table (6): Data of viscosity, thixtropic behavior and Farrow's constant of PIR formulae

Formula	Max. viscosity (CP)	Min. viscosity (CP)	Thixtropic behavior (Cm^2)	Farrow's constant
F1	6596.7	209.1	10.2	2.817
F3	4123	149.8	11	2.665
F5	12055.3	196.6	12.2	4.340
F7	5307	188.3	5.6	2.580
F9	8428	97.3	11.2	5.275

Table (7): The calculated correlation coefficients for the degradation of PIR formulae at room temperature employing different kinetic orders

Formula	correlation coefficients (r)		
	Zero order	**First order**	**Second order**
F1	**0.98608**	0.98525	0.98435
F3	**0.99914**	0.99898	0.99879
F5	**0.99950**	0.99944	0.99935
F7	**0.99726**	0.99703	0.99676
F9	**0.99939**	0.99924	0.99905

Table (8): Rank order for the stability study for PIR formulae according to the chosen order

Formula	t_{90} (year)	RO
F1	1.619	5
F3	2.485	3
F5	2.498	2
F7	2.310	4
F9	2.516	1

Conclusion

All the studied gels are of acceptable physical properties and drug content. They exhibited pseudoplastic flow with thixotropic behavior. Considering in-vitro permeation, rheological properties and shelf life, F3 (4% MC) formula was the best among the studied formulations.

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