

INVESTIGATIONS ON THE *IN VITRO* RELEASE MECHANISM OF PROPICONAZOLE NITRATE FROM HYDROPHILIC GEL FORMULATIONS

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Abstract

The aim of this study was the evaluation of the release kinetic of propiconazole nitrate (PN) from different topic formulations. There were prepared six types of suspension ointments with 1.5% PN, using 10% glycerol as wetting agent and enhancer, for formulas F1, F3, F5 and, respectively, 10 % propylene glycol for formulas F2, F4 and F6. The ointments' bases were as follows: two ointments based on 4% hydroxypropyl cellulose H (HPC-H), hydrogels (F1, F2); two ointments with polyethylene glycol modified base (F3, F4); and two ointments based on hydrated cetylic ointment (F5, F6). *In vitro* release of PN from gels was performed using the Enhancer cell of II dissolution apparatus with a semi-permeable membrane (cellophane). Quantitative determination of released PN was achieved by an UV spectrophotometric method at $\lambda = 270$ nm. In order to evaluate the kinetics of the *in vitro* PN release from the studied gels, the data were analyzed by the use of four mechanistic kinetic models, as follows: zero order kinetics, first order, Higuchi model and Peppas model. Data fitting was achieved through linear or non-linear regression with the Matlab 7.1 software. The results showed that F1-F4 formulas having as vehicle HPC-H and PEG 300-4000, are best fitted on the exponential model that defines the first order dissolution. The F5-F6 formulas, based on cetylic alcohol are best fitted on the Higuchi model. There are no significant differences between formulas containing the same polymer but a different type of wetting agent, leading to the conclusion that the latter does not influence the structural properties of the polymers.

Rezumat

Obiectivul acestui studiu a constat în evaluarea cineticii de cedare a nitratului de propiconazol (PN) din formulări topice cu baze diferite. Au fost preparate șase variante de unguente tip suspensie cu 1,5% PN, folosind ca agent de umectare glicerolul, în concentrație de 10% pentru formulările F1, F3 și F5, și respectiv 10% propilenglicol în formulările F2, F4, F6. Bazele de unguent au fost următoarele: 4% hidroxipropil celuloză-H (HPC-H) în formulările de hidrogeluri F1 și F2; formulele F3 și F4 au fost preparate pe bază de polietilen glicol modificat (PEG), iar formulele F5 și F6 au fost preparate cu baza de unguent cetilic hidratat. Cedarea *in vitro* a PN a fost realizată folosind celula Enhancer a

aparaturii II de dizolvare, cu membrană semipermeabilă (celofan). Determinarea cantitativă a PN cedat s-a realizat prin metoda spectrofotometrică la lungimea de undă $\lambda = 270\text{nm}$. Analiza cinetică de cedare *in vitro* a PN din gelurile studiate a fost realizată pe baza a patru modele cinetice: modelul de ordinul zero, modelul de ordinul unu, modelul Higuchi și modelul Peppas. Fitarea datelor s-a realizat prin regresie liniară sau neliniară utilizând software-ul Matlab 7.1. Rezultatele obținute au evidențiat faptul că formulele F1- F4, pe bază de HPC-H și PEG, se corelează cel mai bine pe modelul de cedare de ordinul unu. Formulările F5 și F6, pe bază de alcool cetilic se corelează cel mai bine pe modelul Higuchi. De asemenea, trebuie menționat că nu au fost identificate diferențe semnificative între formulele ce conțin același polimer, dar agent de umectare diferit, ceea ce denotă faptul că acesta nu modifică proprietățile structurale ale polimerului.

Keywords: propiconazole nitrate (PN), gel, wetting agent, release kinetics

Introduction

Drug release across the skin is an effective and targeted therapy for the infected area, used in local dermatological treatment. Generally, it is preferred to use antifungal agents in treating dermal infections because it allows direct access of high drug concentrations onto and inside the epidermis [3, 7, 8, 11]. Depending on the excipient used, the drug release occurs by diffusion through polymeric network and also by gel erosion [10, 12]. Propiconazole, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]-methyl]-1,2,4-triazole (Figure 1), is a triazolic-derived substance with antifungal action, synthesized in 1979, by Janssen Pharmaceuticals, Belgium.

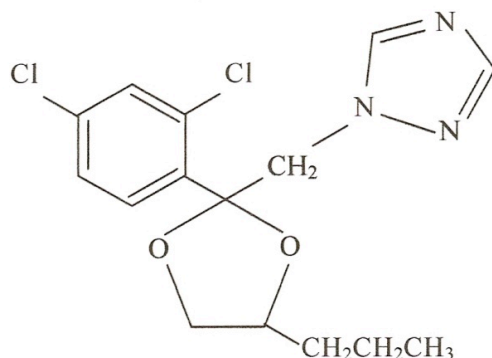


Figure 1
Propiconazole – chemical structure

At first, this substance has been used in agriculture as systemic foliar fungicide, nowadays being used for the fungistatic action when treating mycoses with different localisations. Propiconazole's fungistatic mechanism is based on C-14 demethylation during ergosterol biosynthesis, and leading

to the accumulation of C-14 methylsterols. The biosynthesis of these ergosterols is critical to the formation of cell walls in fungi. This lack of normal sterol production diminishes or stops the fungus growth, thus preventing further infection. Therefore, propiconazole is considered to be fungistatic, rather than fungicidal [4]. Also, it was observed that propiconazole molecule binds to the fatty acids and to the phospholipids which are present at the fungus membrane level, specifically inhibiting certain enzymes as: cytochrome C, peroxydase and catalase. All of these mechanisms determine a potent fungistatic activity of propiconazole on a wide spectrum of fungi including *Candida species* that resist to other antifungal agents [2, 6, 13, 15]. Drug released quantity depends both on its solubility into the dissolution medium and on the gel base properties [5, 14]. In order to evaluate the *in vitro* release of propiconazole nitrate (PN) from the studied formulations we used the Enhancer cell with semi-permeable membrane (cellophane) [1, 9].

The aim of the present study was the evaluation of the dissolution profile of PN from formulas of gels with different ointment bases, and two wetting agents: glycerol and propylene glycol, by fitting with four mathematical models.

Materials and Methods

Materials

Propiconazole (CHWAY Chemicals & Pharmaceuticals LTD., China) (it was transformed into nitrate in our laboratory), *hydroxypropyl cellulose H* (HPC-H, Nisso, Japan, high viscosity grade 1.000–4.000 mPa·s), *polyethylene glycol* 300 and 4000 (PEG 300 and 4000, Labo-Chemie Wien, Fischamed, Österreich), *glycerol* (Sigma Aldrich, Germany), *propylene glycol* (Merck, Schuchard, München, Germany), *triethanolamine* (Alpha Aesar GmbH & CoKG, Germany), *cetylic alcohol*, *lanoline* and *vaseline* (Sigma Aldrich, Germany). All the chemicals used comply with the 10th Romanian Pharmacopoeia requirements.

Methods

Gels preparation

There were prepared six types of suspension ointments with 1.5 % propiconazole nitrate, using as wetting agent 10 % glycerol, for formulas F1, F3, F5 and, respectively, 10 % propylene glycol for formulas F2, F4, F6:

- two ointments based on 4% HPC-H, hydrogels;
- two ointments with polyethylene glycol (PEG) modified base;
- two ointments based on hydrated cetylic ointment (Table I).

Table I
Formulations of 1.5 g % propiconazole nitrate ointments

Ingredients	Formulations (g)					
	F1	F2	F3	F4	F5	F6
Propiconazole nitrate	1.5	1.5	1.5	1.5	1.5	1.5
HPC-H	4	4	—	—	—	—
Glycerol	10	—	10	—	10	—
Propylene glycol	—	10	—	10	—	10
Polyethylene glycol 300	—	—	23.5	23.5	—	—
Polyethylene glycol 4000	—	—	40	40	—	—
Cetylic alcohol	—	—	5	5	2.5	2.5
Lanoline	—	—	—	—	6	6
White petrolatum	—	—	—	—	50	50
Triethanolamine	1	1	1	1	1	1
Distilled water	ad 100					

In vitro release of PN from gels

In vitro release of PN from gels was performed using the Enhancer cell with a semi-permeable membrane (cellophane). In order to increase its permeability, the membrane was maintained in distilled water for 24 hours. As medium receptor we used distilled water : ethanol (90:10 w/w), at a temperature of $32\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, 50 rpm. We used a 0.5 g sample for each gel, placing it smoothly on the Enhancer cell membrane. When applying the sample, the membrane was checked to verify the absence of air bubbles underneath the membrane. The Enhancer cell including the sample was placed into the dissolution flask, which contained 100 mL of receptor medium. Aliquots of 3 mL were withdrawn from the dissolution flask at 15, 30, 60, 120, 180, 240, 300, 360 minutes, being replaced with fresh medium every time.

Quantitative determination of PN released

The collected samples were analyzed using a HITACHI 2000 UV/VIS spectrophotometer at $\lambda = 270\text{ nm}$. The experiments were repeated three times for each gel taking into consideration the average values of the determinations.

Kinetic analysis of PN in vitro release profiles

In order to accomplish a kinetic evaluation of *in vitro* release of PN from studied gels, the release data were analyzed by four mechanistic kinetic models (Equations 1-4, Table II) [16 - 17].

Zero order kinetics

$$R = \alpha_1 + \alpha_2 \cdot t \quad \text{Equation 1}$$

where: R is the amount of drug substance released at time " t "; α_1 is the initial amount of drug in the solution; α_2 represents the release rate constant for zero order kinetics; t is the time;

First order

$$R = 1 - e^{-k \cdot t} \quad \text{Equation 2}$$

where: R is the amount of drug substance released at time " t "; k is the release rate constant for exponential kinetics, t is the time;

Higuchi model

$$R = k_1 + k_2 \cdot t^{0.5} \quad \text{Equation 3}$$

where: R is the amount of drug substance released at time " t "; k_1 is the initial amount of drug in the solution for the Higuchi model; k_2 is the release rate constant (the Higuchi dissolution constant); t is the time;

Peppas model

$$R = k_1 - k_2 \cdot t^n \quad \text{Equation 4}$$

where: R is the amount of drug substance released at time " t "; k_1 is the initial amount of drug in the solution; k_2 is the release rate constant for the Peppas model; n is the release exponent; t is the time.

Table II

Kinetic parameters used to analyze the release profile of PN from gels

Equation	Kinetic model	Parameters
1	Zero order	α_1, α_2
2	First order (exponential)	k
3	Higuchi	k_1, k_2
4	Peppas	k_1, k_2, n

Data fitting was achieved through linear or non-linear regression with the Matlab 7.1 software. The criterion for the best choice in matters of models was R^2 , the correlation coefficient.

Results and Discussion

The experimental results obtained from the dissolution test are shown in Figure 2.

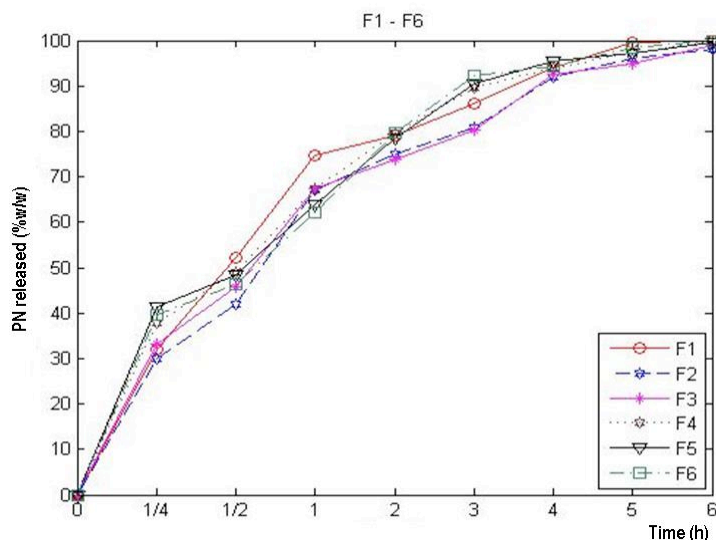


Figure 2

PN dissolution release profile from F1 – F6 gel formulas

The results of data fitting to the zero order kinetic model are summarized in Table III and Figure 3.

Table III

The parameters values of the kinetic model release

Kinetic model		Formula					
		F1	F2	F3	F4	F5	F6
Zero order	α_1	11.6293	11.7225	11.4700	11.2657	11.4462	11.7070
	α_2	22.0893	17.6978	19.3233	22.7307	22.5376	21.2431
	r^2	0.8672	0.9054	0.9025	0.8772	0.8769	0.8844
First order	k	2.5641	3.1250	3.0303	2.7778	2.6316	2.7027
	r^2	0.9949	0.9911	0.9912	0.9925	0.9800	0.9770
Higuchi	k_1	0.3194	-2.8423	-1.1307	0.9058	1.3928	0.0459
	k_2	37.6907	37.2176	36.6127	37.2248	36.9413	37.5461
	r^2	0.9803	0.9822	0.9896	0.9864	0.9830	0.9789
Peppas	n						
	0.2	r^2	0.8709	0.8351	0.8588	0.8796	0.8799
	0.4	r^2	0.9762	0.9664	0.9784	0.9824	0.9794
	0.7	r^2	0.9471	0.9676	0.9688	0.9544	0.9516
	0.9	r^2	0.8947	0.9281	0.9260	0.9037	0.9026

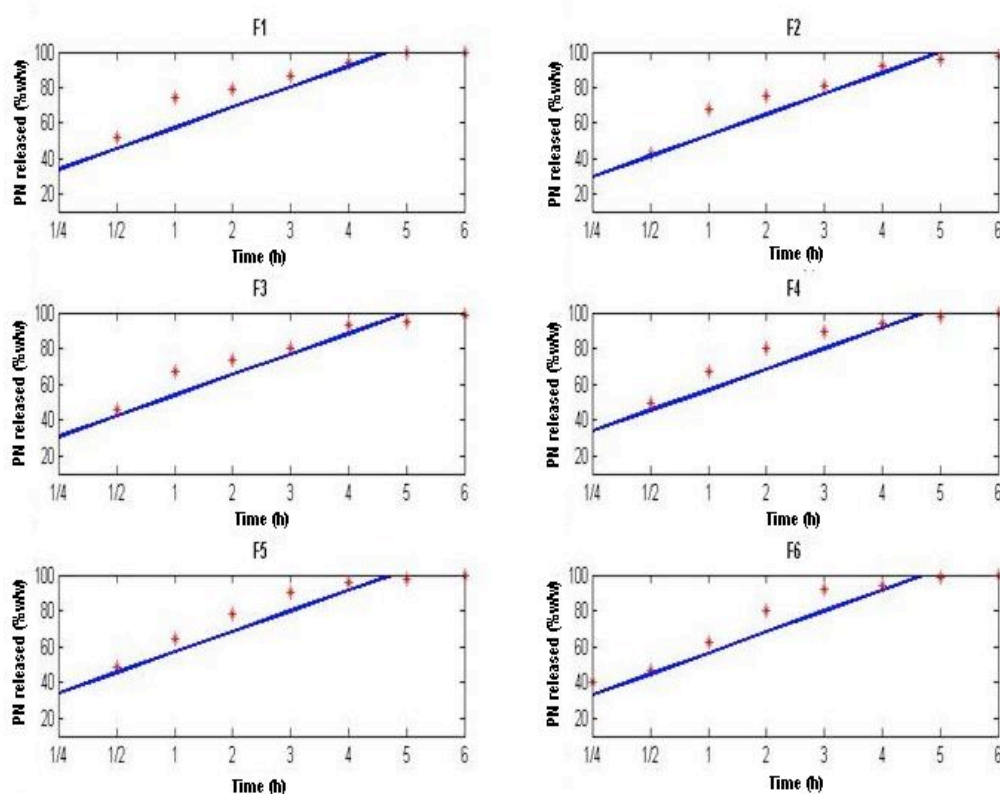


Figure 3

The fitting plot of the PN dissolution profiles from F1 – F6 with the zero order kinetic release model

The above presented data show that, in case of zero order kinetics model fitting, none of the formulas of the studied gels is characterized by a linear profile of release.

The exponential model fitting of data, highlighted that all the studied gel formulations exhibit a first order kinetic, showing that the release rate depends on the concentration (Figure 4). For all formulations based on HPC-H and PEG, $R^2 > 0.99$ (Table III). Therefore these two polymers have gel structuring properties and, used in the selected concentrations, ensure the PN release from gels, following a first order kinetics.

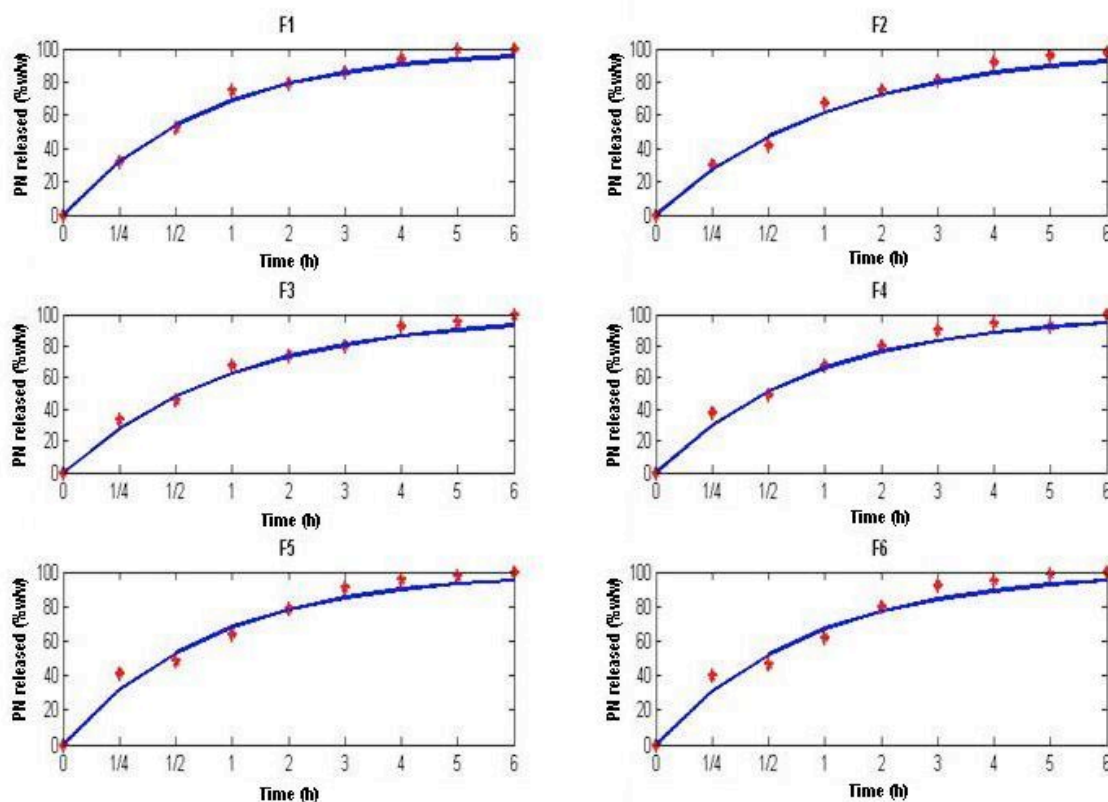


Figure 4

The fitting plot of the PN dissolution profiles from F1 – F6 with the first order kinetic model

The Higuchi model, a representative model for the release of the soluble substances from the pharmaceutical formulas based on hydrophilic polymers, stands on the principle that the drug substance release profile decreases in time, due to the increase in the length of the diffusion pathway followed by the drug substance. The experimental results obtained after the data fitting to this model revealed satisfactory values of the equation parameters, summarized in Figure 5 and Table III; it is noteworthy to mention that R^2 values obtained for F3 and F4 formulas are higher than the values obtained when data fitting to the exponential model was performed.

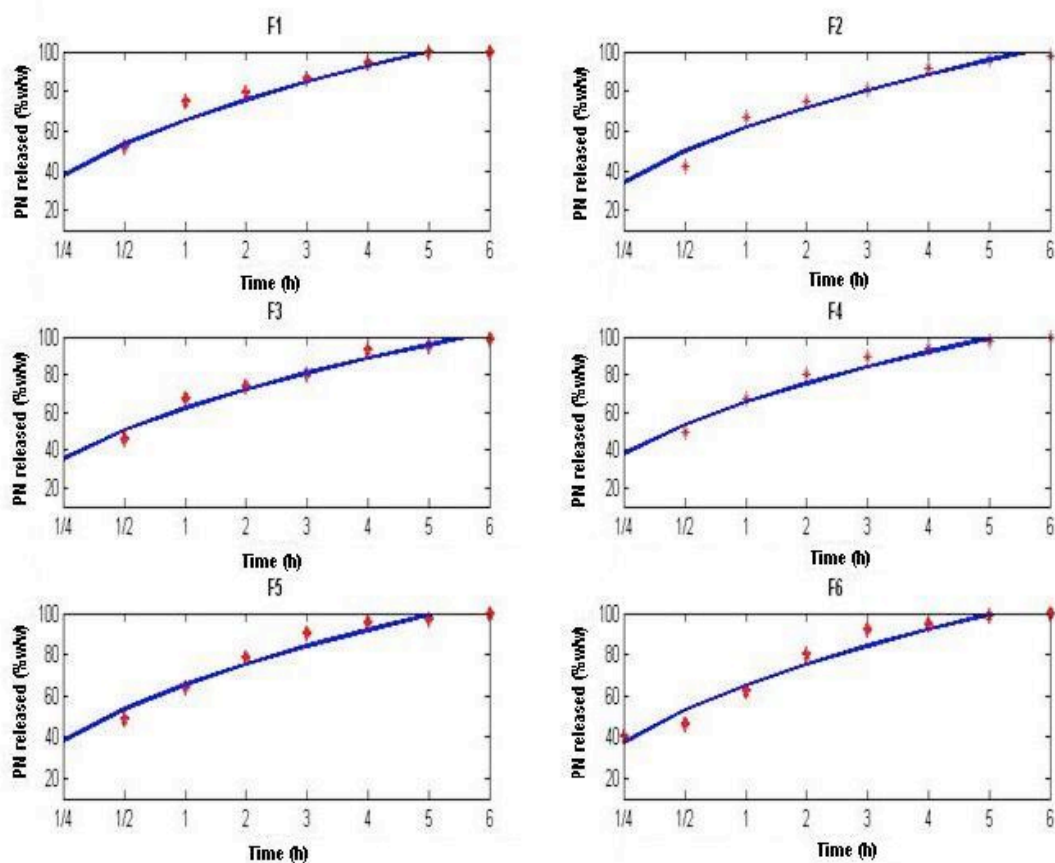


Figure 5
The fitting plot of the PN dissolution profiles from F1 – F6
with Higuchi model

The *Peppas model* is a decision model regarding the drug substance release mechanism, situated between the linear model and the Higuchi model. The results of data fitting to this model revealed, as expected, the best match for the n values closer to 0.5 (Figure 6 and Table III).

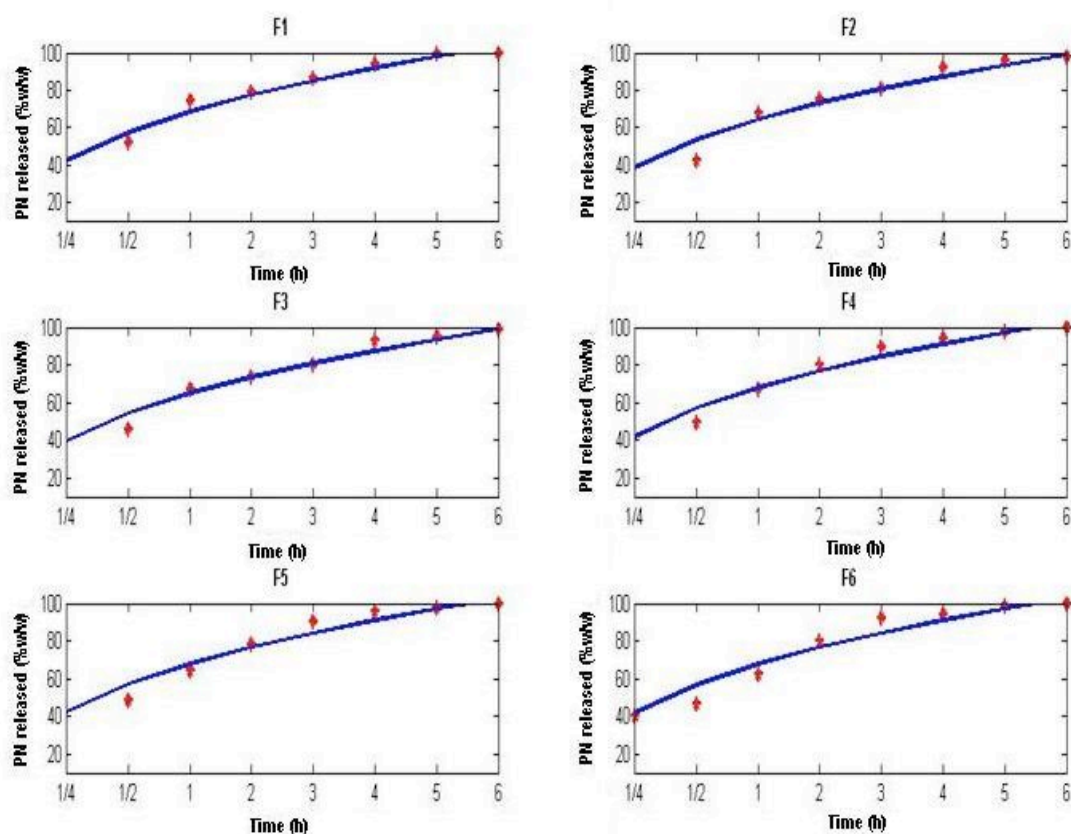


Figure 6
*The fitting plot of the PN dissolution profiles from F1 – F6 with Peppas model
 ($n = 0.4$)*

Conclusions

The dissolution profile of PN from three ointment bases prepared in two variants using glycerol and propylene glycol as wetting agents was evaluated based on four kinetic models. The obtained results showed that the F1-F4 formulas based in HPC-H and PEG 300-4000 are best fitted on the exponential model that defines the first order dissolution.

The F5-F6 formulas, based on cetylic alcohol are best fitted on the Higuchi model. There are no significant differences between the formulas containing the same polymer, but a different type of wetting agent, which means that it does not modify the structural properties of the polymers.

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