ORIGINAL ARTICLE

INFLUENCE OF FORMULATION VARIABLES ON KETOPROFEN DIFFUSION PROFILES FROM HYDROALCOHOLIC GELS

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Abstract

Ketoprofen is used to treat painful conditions such as arthritis, sprains and strains, gout, period (menstrual) pain, and pain after surgical operations. It eases pain and reduces inflammation. After oral administration it can have side effects especially on digestive system. That's why it is recommended with caution. In recent years we could note an orientation towards topical administration of NSAIDSs and hydrogels occupy one of the first places through the forms that were developed for topical use. Five different experimental formulations coded G1 to G5 containing 1% Carbomer 940 as gel forming agent were prepared in order to evaluate influence of both composition and preparation methodology on ketoprofen (KTP) diffusion kinetics. Release of KTP from the various gel formulations was studied using a modified Franz diffusion cell fitted with a standard cellophane membrane. The release profiles for all the experimental formulations as well for one commercial product (Fastum® gel) were compared in terms of diffusion coefficients (D). The hydrogel formulated with isopropyl alcohol, presented the slowest release of KTP, whereas the gels using ethanol and propylene glycol respectively were similar, yielding about 60% of the incorporated substance after 5 hours. Moreover, for the preparation of the KTP gels we recommend prior dissolution of the drug in the amount of the alcohol from the formulation. The release profile of the commercial formulation is intermediate between those of the experimental hydrogels. To establish the drug release mechanism from the tested hydrogels, several kinetic models were investigated: zero order, first order, Higuchi and Korsmeyer-Peppas models. For the tested hydrogel, the release mechanism is as follows: (i) *Fickian diffusional model* for the commercial product; (ii) zero order for the hydrogels G1-G4 (the release mechanism is represented by a *time-independent case-II, relaxational transport*); (iii) a complex mechanism for hydrogel G5, *super case-II transport*, where the diffusion is associated with hydrogel network swelling and destructuration, most probably due to its sensitivity by mechanical action during preparation. The results obtained confirmed that the *KTP in vitro* release kinetics, is influenced both by the type of alcohol from the gel basis and the preparation methodology, these variables being able to modify the bioactive compound release mechanism.

Rezumat

Pentru a evalua influenţa compoziţiei şi a metodei de preparare asupra cineticii proceselor de difuzie a ketoprofenului au fost preparate cinci formulări experimentale diferite, codificate de la G1 la G5, conţinând 1% carbomer 940 ca agent de formare a gelului. Eliberarea ketoprofenului (KTP) din formulările realizate a fost studiată cu ajutorul unei celule de difuzie Franz modificată, echipată cu o membrană de celofan. Au fost evaluate comparativ, prin intermediul coeficientului de difuziune (D), profilele de eliberare pentru toate formulările experimentale şi pentru produsul comercial (Fastum®). Hidrogelul formulat cu alcool izopropilic a prezentat cel mai lent ritm de eliberare a KTP, în timp ce gelurile folosind etanol, respectiv propilenglicol, au fost similare, cedând circa 60% din substanţa încorporată după 5 ore. În plus, pentru prepararea gelurilor cu KTP recomandăm dizolvarea prealabilă a acestuia în alcoolul din formulare. Profilul de eliberare al formulării comerciale este intermediar între profilurile hidrogelurile experimentale. Pentru a stabili mecanismul de eliberare a medicamentului din hidrogelurilor testate, au fost investigate mai multe modele cinetice: ordinul zero, ordinul întâi, modelele Higuchi şi Korsmeyer-Peppas. Pentru hidrogelurile testate, mecanismul de eliberare urmează: (i) modelul difuzional Fickian pentru produsul comercial; (ii) modelul de ordinul zero pentru hidrogelurile G1-G4 (transport relaxaţional independent de timp); (iii) un mecanism complex pentru hidrogelul G5, difuzia fiind asociată cu umflarea şi destructurarea reţelei hidrogelului, cel mai probabil datorită sensibilizării sale la acţiunea mecanică în timpul preparării. Rezultatele obţinute au confirmat faptul că cedarea *in vitro* a KTP este influenţată atât de tipul de alcool din baza de gel cât şi de metodologia de preparare, aceste variabile fiind în măsură să modifice mecanismul de eliberare a compusului bioactiv.

Keywords: Carbopol 940, hydrogels, ketoprofen, diffusion profiles

Introduction

It is well known the importance of administering NSAIDs in the treatment of various aches, in osteo-articular therapy [1-3]. A member of this class is KTP. It is recommended in the symptomatic treatment of inflammatory diseases, metabolic or degenerative, in the treatment of rheumatoid arthritis, osteo-arthritis and ankylosing spondylitis. It shows potent inhibitory effects on prostaglandin synthesis by inhibition of both cyclooxygenase and lipoxygenase. It alleviates mild and moderate pain [4-6].

Ketoprofen is a 2-arylpropionic acid derivative non-steroidal anti-inflammatory drug (NSAID) and shows strong analgesic and anti-pyretic effects. It is one of the most interesting compounds and is widely used in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and abdominal cramps associated with menstruation. Recently, additional interest in KTP lies in their possible therapeutic benefits in the prevention of various cancers including colorectal and lung cancers and even in the treatment of neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease [7].

In powder form, KTP is stable at room temperature. The pK_a of ketoprofen in water is 4.45. The percentage of un-ionized form at pH 4.8 (acid mantle of skin) is 30.88. The pK_a affects skin permeability, as skin permeability of a drug increases with the increasing of the un-ionized fraction. The partition coefficient (log P) of ketoprofen in buffer pH 7.4 is 0.97 and the calculated log P is 2.81 [7, 8].

The pharmacokinetic data show that oral gastro-intestinal absorption is good. In oral dosage form, the absorption of ketoprofen is rapid and almost completed, with peak concentration occurring at 0.5 - 2 h [9]. The absolute bioavailability of ketoprofen is about 92% for oral administration. It diffuses into the synovial fluid, where it develops high concentrations. It is metabolized primarily in the liver and it is excreted through kidney. Percutaneous administration of 50 - 150 mg ketoprofen generates plasma concentrations of 0.08 to 0.15 μg/mL, 5-8 hours after application [7, 9-11].

Ketoprofen causes gastrointestinal disturbances, peptic ulceration with bleeding, if present in large doses in gastrointestinal tract upon oral administration [12, 13]. The topical application can assure higher local concentration of the drug at the site of initiation of the pain with lower or negligible adverse effects [7, 14]. Therefore, as an alternative to oral administration, the forms with topical administration are frequently recommended.

In the last twenty years, the gels and especially hydrogels have been extensively studied as semi-solid pharmaceutical formulations capable of ensuring efficient delivery of drug for oral, rectal, vaginal, ocular, cutaneous or subcutaneous administration. Thus, hydrogels have become widely used in biomedical and pharmaceutical fields as carriers system and biomedical devices because of their biocompatibility, the molecular network structure, the stability of bioactive compound built, the ease of application, the adverse reactions low incidence and their good tolerability [15]. More, using hydrogels as vehicles is justified by the fact that their high water content diminishes the irritability and markedly increases the patient compliance [16-17].

Carbomers are most suitable for topical formulation due to their numerous advantages that their usage brings: safety and efficiency – they are usable for long periods of time and they are considered to be safe and efficient in formulations for gels, lotions and creams; they are highly tolerated, without producing any local irritations and without causing sensitivity to repeated administration; they are an excellent vehicle for the drugs as they do not influence its biologic effects – due to their large molecular mass, they are unable to penetrate the skin and they can't affect the drug activity; they present excellent thickening, a good suspension and better emulsifying in topical formulations properties [18]. The adequate viscosity, for small values of the polymer concentration, the efficient suspension of the drug, the increased density and the low thixotropy, as well as the product clarity are the reasons for which, out of practical and esthetical considerations, the formulations which contain carbomers are preferred.

By considering the above factors, the present study intends to develop the ketoprofen topical formulations using Carbopol 940 as a gelling agent to overcome the gastric side effects, and also to assure an appropriate release rate and achieve the therapeutic benefit.

The present study followed on one hand the influence of the type of alcohol on physico-chemical properties of the experimental Carbopol based gels and on the other hand the influence of the KTP incorporation method on the same characteristics.

Materials and Methods

Materials

Carbomer 940 (Carbopol 940™) was purchased from Alpha-Pharma (Zwevegem, Belgium). Ketoprofen (KTP, purity ≥ 98%), glycerol and triethanolamine (TEA) were obtained from Sigma-Aldrich (St. Louis, MO, USA), whereas isopropyl alcohol, ethanol and propylene glycol (PG) were produced by Merck KGaA (Darmstadt, Germany). Double distilled water was used throughout the study. All the other reagents were of analytical grade, purchased from different commercial suppliers and used without further purification. Fastum® gel from Menarini Manufacturing Logistics and Services S.R.L (Italy) was the industrial hydrogel selected in the present study.

Preparation of hydrogels

Five different experimental formulations coded G1 to G5 containing 1% Carbomer 940 as gel forming agent (Table I) were prepared in order to evaluate influence of both composition and preparation methodology on ketoprofen diffusion kinetics.

Table I

Composition and codification of the experimental ketoprofen hydrogels

Component	*G1*	*G2*	*G3*	*G4*	*G5*
Ketoprofen	2.5	2.5	2.5	2.5	2.5
Carbomer 940	1	1	1	1	1
Glycerol	12	12	12	12	12
Ethanol	50	-	-	-	-
Isopropyl alcohol	-	50	-	50	50
Propylene glycol	-	-	50	-	-
Triethanolamine	0.2	0.2	0.2	0.2	0.2
Water *q.s. ad*	100	100	100	100	100

Preparation of the experimental hydrogels was based on a previously reported composition and method [13, 15]. For each formulation, 1 g of the gel forming agent (Carbomer 940) was slowly sprinkled into a beaker containing 30 mL distilled water under mild agitation. The obtained uniform dispersion was allowed to hydrate for 24 h. Then, 12 g of glycerol were added. Approximately 0.2 g triethanolamine were further used in order to neutralize the free carboxylic acid groups of Carbopol 940 to a pH value of 6 - 6.5.

In order to incorporate the active substance into the experimental formulations, 2.5 g ketoprofen were dissolved in 50 mL of different short chain alcohols, *e.g.* ethanol (G1), isopropyl alcohol (G2) and propylene glycol (G3) respectively. The obtained alcoholic solutions of were mixed with the previously prepared gel bases, under mild agitation at 25 rpm and the resulting hydrogels were adjusted to 100 g by further adding purified water.

Formulations G2, G4 and G5 were identical in terms of qualitative and quantitative composition, but different preparation methodology was employed, in order to induce different thermodynamic activity for the active compound: the macromolecule was allowed to soak and hydrate into the KTP hydro-alcoholic solution in case of G2 formulation, the KTP alcoholic solution was slowly incorporated into the hydrophilic gel base in case of G4, whereas G5 was obtained by simple dispersion of the active compound into the hydro-alcoholic matrix.

All samples were allowed to equilibrate for 48 hr at room temperature prior to further evaluation.

In vitro release kinetics study of ketoprofen from the experimental gels

Release of KTP from the various gel formulations was studied using a modified Franz diffusion cell with an effective diffusional area of 7.55 cm^2 in which a standard cellophane membrane was fitted.

1 g of gel was saline (pH 7.4) homogenously spread on the donor side of the cell, whereas freshly prepared phosphate buffer served as receptor medium in the experiments. The receptor medium was continuously stirred by a rotating Teflon coated magnet. The temperature was maintained at 37°C by using a circulating water jacket. All the membranes were pre-wetted in the receptor medium for 60 minutes before use.

Sample (5 mL) were withdrawn at predefined time intervals up to 420 minutes, and replaced with equal amounts of fresh dissolution media. The amount of ketoprofen released at each time interval was determined spectrophotometrically at λ_{max} = 254 nm against blank receptor medium. All analyses were performed in triplicate.

Data analysis

All experiments were run in triplicate. The release profiles for all the experimental formulations were compared in terms of diffusion coefficients (D). The results were expressed as mean ± standard deviation. The data were compared by one way analysis of variance (ANOVA) followed by Turkey's HSD multiple comparison test. A level of significance of $p < 0.05$ was considered.

In order to study the transport mechanism from the experimental hydrogels, different diffusion models were considered to fit the experimental data up to 60% KTP released [19, 20] *i.e.* zero order kinetics, first order kinetics, Higuchi and Korsmeyer-Peppas [21] models (Tabel II).

Table II

Mathematical equations of the evaluated kinetic models

Kinetic model	*Ecuation**
Zero order kinetics	$F = k_0 * (t - T_{lag})$
First order kinetics	$F = 100 * \{1 - Exp[-k_1 * (t - T_{lag})]\}$
Higuchi	$F = k_H * (t - T_{lag})^{0.5}$
Korsmeyer-Peppas	$F = k_{KP} * (t - T_{lag})^n$

– k_0, k_1, k_H, k_{KP}, represents constant values specific to each kinetic model, T_{lag} – latency time of the release, n – diffusional exponent, dependent on the geometry of the release system and of the release mechanism.

Experimental data were analysed by a nonlinear least-squares regression, using the value of the coefficient of determination as model selection criteria.

However, since a larger number of model parameters could lead to a higher probability of obtaining a better R-squared value, the use of a discriminatory criterion to effectively compare models with a

different number of parameters was also considered. For this reason, the Akaike Information Criterion (AIC) was applied, since effectively penalizes the model for having too many variables and offers the best trade-off between maximizing the R-squared value, and minimizing the number of predictors in the model.

The AIC can be defined as $AIC = N \ln(WSS) + 2p$ [22] where N is number of experimental data points, WSS is the weighed sum of squared residuals and p is the number of parameters. The model with the minimum value for the AIC is the one which, statistically, describes the best the drug release mechanism.

Results and Discussion

All the prepared gel formulations were transparent viscous preparation with a smooth and homogeneous appearance, and very good skin spreadability and pH ranging between 6 and 6.2.

Influence of the nature of the alcoholic component on the diffusion profiles and kinetic parameters of KTP release from the experimental hydrogels

The *in vitro* release of KTP expressed as cumulative percent of drug released in time from the G1-G3 formulations is presented in Figure 1.

The release profiles of KTP from the selected formulations were compared in terms of diffusion coefficients (D) by one way analysis of variance (ANOVA) followed by Turkey's HSD multiple comparison test. A level of significance of $p < 0.05$ was considered.

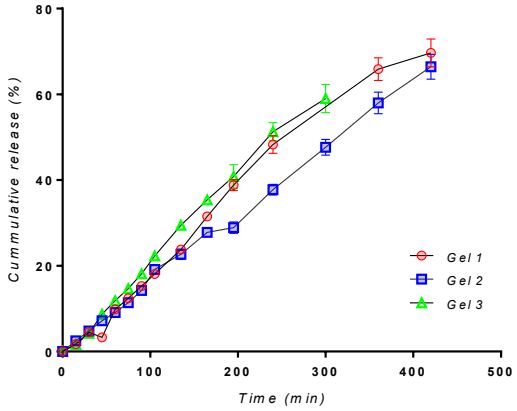

Figure 1.
Cumulative release of KTP from the G1-G3 experimental hydrogels (n = 3). Error bars represent the standard deviation (SD)

Plotting of the amount diffused per unit area (mg/cm^2) as a function of the square root of time allowed drug diffusion coefficient in the semisolid matrix (D) to be obtained from the straight line slope (Figure 2), using the Higuchi adaptation of the Fick's first law of diffusion, considering that KTP is totally in solution in the gel matrix:

$$q = 2C_0\sqrt{\frac{D \cdot t}{\pi}}$$

where q represents the amount of drug released into a sink medium per surface unit, C_0 is the drug concentration into the matrix and D represents diffusion coefficient of the drug through the matrix [23].

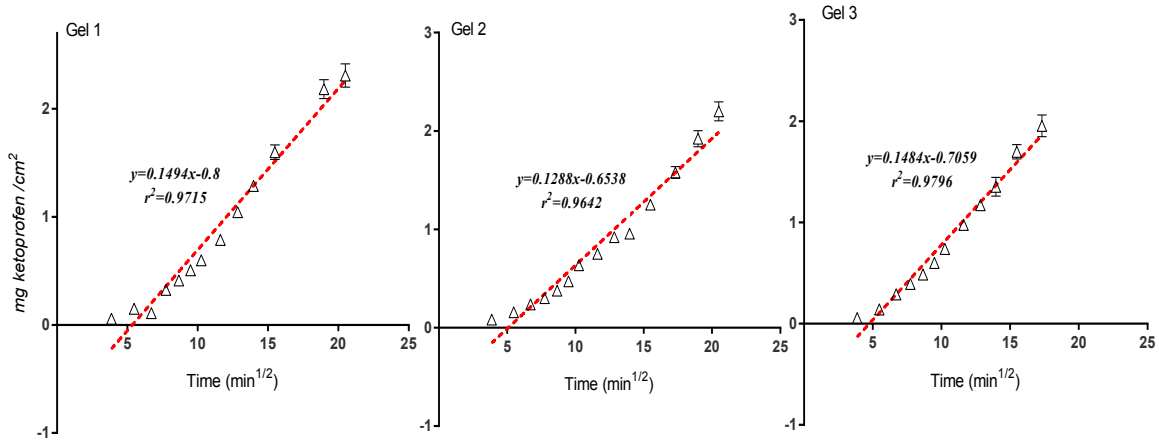

Figure 2.
In vitro KTP cumulative diffusion profiles in pH 7.4 PBS, using a cellophane membrane

Table III

Value of the KTP diffusion coefficients for the G1 - G3 experimental hydrogels

Code of experimental formulation	*Diffusion coefficients – D ± SD * 10^5 (cm^2/min)*	*Time lag - T_{lag} (min)*
Gel 1	2.81 ± 0.29	28.65
Gel 2	2.09 ± 0.23	25.76
Gel 3	2.77 ± 0.25	22.56

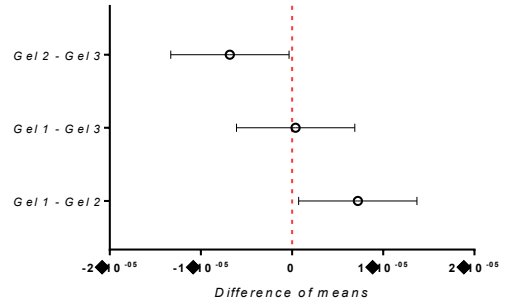

Figure 3.

95% confidence intervals for the difference of means of the diffusion coefficients for the G1 - G3 hydrogels

Based on experimental data we found that the value of D increases in the order G2 < G3 < G1.

Also, the diffusion from the G2 formulation (D = 2.09 ± 0.23 cm^2/min) is significantly slower from both G1 (D = 2.81 ± 0.29 cm^2/min) and G3 (D = 2.77 ± 0.25 cm^2/min), while G1 and G3 formulations are similar in terms of diffusion coefficients.

As a conclusion, the hydrogel formulated with isopropyl alcohol, presented the slowest release of KTP, whereas the gels using ethanol and propylene glycol respectively were similar, yielding about 60% of the incorporated substance after 5 hours.

Influence of incorporation technique on diffusion profiles and kinetic parameters of KTP release from the experimental hydrogels

The *in vitro* release of KTP expressed as cumulative percent of drug released in time from the G2, G4 and G5 formulations is presented in Figure 4.

The release profiles of KTP from the selected formulations were compared in terms of diffusion coefficients (D) by one way analysis of variance (ANOVA) followed by Turkey's HSD multiple comparison test. A level of significance of $p < 0.05$ was considered.

It was noticed that the value of D increased in the following order G5 < G4 < G2, but statistically significant differences are recorded only between G5 and G2.

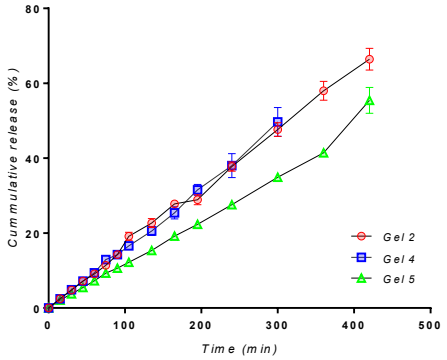

Figure 4.

Cumulative release of KTP from the isopropyl alcohol experimental hydrogels (n = 3). Error bars represent the standard deviation (SD)

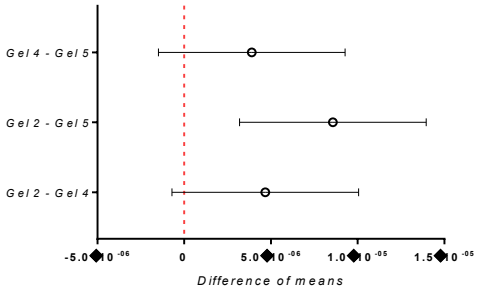

Figure 5.

95% confidence intervals for the difference of means of the diffusion coefficients for the G2, G4 and G5 hydrogels

This behaviour could be due to a possible precipitation of KTP when the alcoholic solution is added over the gel basis (case of the G4 gel) or to an incomplete solubilisation and to an eventually mechanical destructuration of the gel basis during the preparation (case of the G5 gel). Based on the experimental data, for the preparation of the KTP gels we recommend prior dissolution of the drug in the formulation alcohol.

Table IV

Value of the KTP diffusion coefficients for the G2, G4 and G5 experimental hydrogels

Code of experimental formulation	*Diffusion coefficients – D ± SD * 10^5 (cm^2/min)*	*Time lag – T_{lag} (min)*
Gel 2	2.09 ± 0.23	25.76
Gel 4	1.62 ± 0.22	21.37
Gel 5	1.23 ± 0.19	27.50

The in vitro performance comparison of the experimental and commercial hydrogels

The release profile of the commercial formulation is intermediate between those of the experimental hydrogels. The value of D is practically identical to the one obtained for the hydrogel G2 containing isopropyl alcohol in formulation. The kinetic profiles are almost superposable up to 5 hours of experiment, followed by a slower release from the commercial formulation, while the release from the gel G2 remains constant and follows an apparent zero order kinetics. The gels containing ethanol and propylene glycol present values of D significantly higher compared to commercial formulation, leading to a faster release, which could recommend them for the treatment of acute pain. The diffused amount of KTP is significantly higher for G1 and G3 gels in comparison with commercial formulation Fastum® gel (about 70% *versus* 50% after 7 hours of experiment).

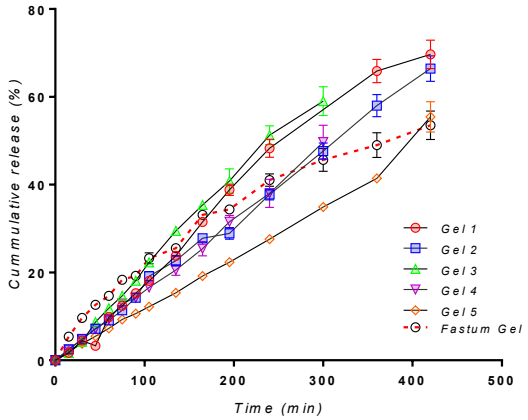

Figure 6.
Comparative evaluation of KTP diffusion profiles from the tested hydrogels

Table V
Value of the KTP diffusion coefficients for the all the tested hydrogels

Code of experimental formulation	*Diffusion coefficients – D ± SD * 10^5 (cm^2/min)*	*Time lag – T_{lag} (min)*
Gel 1	2.81 ± 0.29	28.65
Gel 2	2.09 ± 0.23	25.76
Gel 3	2.77 ± 0.25	22.56
Gel 4	1.62 ± 0.22	21.37
Gel 5	1.23 ± 0.19	27.50
Fastum® Gel	2.08 ± 0.26	25.75

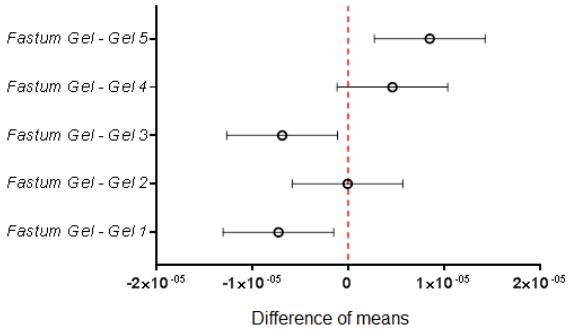

Figure 7.
95% confidence intervals for the difference of means of the diffusion coefficients for the tested hydrogels

Evaluation of the release mechanism through mathematical modelling

Mathematical modelling increases understanding of the drug release mechanism and contributes to reduce the number of experiments for the optimization of the formulation. For this purpose, several kinetic models were investigated [24]: zero order, first order, Higuchi and Korsmeyer-Peppas models.
In all cases, the fitting of the *in vitro* experimental kinetic profiles was performed using mathematical expression of the drug released fraction as a function of time, described by each model (non-linear fitting). The values of the rate constant and dissolution time-lag (T_{lag}) were determined from the mathematical equation corresponding to the specific kinetic model.
In most cases Korsmeyer-Peppas model generated data that best approximated experimental parameters, as expected taking into account that this model involves a high number of parameters in comparison with classical models (zero order, first order, Higuchi). On the other hand one can mention that Korsmeyer-Peppas is an empiric model, able to describe different release models. The selection of a certain mechanism is based on the release exponent (n) values. Thus, for the tested hydrogel, the release mechanism is as follows: (i) *Fickian diffusional model* for the commercial product; (ii) zero order for the hydrogels G1 - G4 (the release mechanism is represented by a *time-independent case-II, relaxational transport*); (iii) a complex mechanism for hydrogel G5, *super case-II transport*, where the diffusion is associated with hydrogel network swelling and destructuration, most probably due to its sensitivity by mechanical action during preparation.

Table VI

The fitting parameters for the experimental data related to KTP release from the tested formulations using different diffusion kinetic models

Parameter	***Gel 1***	***Gel 2***	***Gel 3***	***Gel 4***	***Gel 5***	***Fastum® Gel***
Zero order						
k0	0.182	0.158	0.209	0.162	0.123	0.116
Tlag	3.493	-1.612	2.854	1.673	4.428	-76.664
Rsqr	***0.9908***	***0.9971***	***0.9941***	***0.9974***	***0.9902***	0.9517
First order						
k1	0.003	0.002	0.003	0.002	0.002	0.002
Tlag	24.986	17.095	17.005	11.812	16.321	-31.844
Rsqr	*0.9814*	0.9825	0.9946	0.9845	0.9704	0.9830
Higuchi						
kH	3.461	3.055	3.544	2.592	2.302	2.628
Tlag	57.542	57.045	54.177	42.410	57.283	21.491
Rsqr	0.9715	0.9440	0.9579	0.9247	0.9077	0.9792
Korsmeyer-Peppas						
kKP	0.554	0.180	0.412	0.088	0.020	1.637
Tlag	26.264	1.184	13.166	-9.034	-36.142	8.997
N	***0.894***	***0.979***	***0.883***	***1.103***	***1.290***	***0.580***
Rsqr	***0.9925***	***0.9971***	***0.9970***	***0.9983***	***0.9945***	***0.9900***

Conclusions

The results obtained confirmed that the *in vitro* KTP release kinetics is influenced both by the type of alcohol from the gel basis and the preparation methodology, these variables being able to modify the bioactive compound release mechanism. The values of ketoprofen diffusion coefficients determined experimentally recommend the formulation with ethanol and glycerol as optimal for KTP topical conditioning.

Acknowledgement

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