

COMPARATIVE *IN VITRO* DRUG RELEASE EVALUATIONS OF NEW HYDROGELS CONTAINING OXICAMS

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Abstract

The paper presents the results of *in vitro* release and rheological evaluations performed on 12 experimental semisolid dosage forms containing oxicams as active pharmaceutical ingredient dispersed in hydrophilic matrix. Non-ionic tensioactive agents were added as both solubility-increasing excipients and absorption promoters. The *in vitro* release data obtained on a static vertical diffusion cell system was fitted with two kinetic models, square root law and first order kinetic models. The results were further analysed in relation with the rheological parameters and underline the critical role of correlated structural and release evaluations in the selection of optimal formulation candidate, during the research and development phase.

Rezumat

Lucrarea prezintă rezultatele evaluărilor cedării *in vitro* și a celor reologice pentru 12 forme farmaceutice experimentale semisolide conținând ca substanțe active oxiciami dispersați în matrici hidrofile. Au fost adăugați tensioactivi neionici ca excipienți cu rol solubilizant și promotori de absorbție. Datele cedării *in vitro* obținute într-un sistem de celule de difuzie, vertical statice, au fost analizate prin aplicarea a două modele cinetice, legea radicalului și cinetică de ordin I. Rezultatele au fost ulterior analizate în relație cu parametrii reologici și au subliniat rolul critic al evaluărilor corelate, structurale și difuzionale, în selecția formulării optime, pe parcursul etapei de cercetare-dezvoltare.

Keywords: meloxicam, piroxicam, tenoxicam, *in vitro* drug release, semisolids

Introduction

The *in vitro* drug release (IVR) methodologies for semisolid dosage forms have been adopted for the evaluation of possible changes in product performance after changes of the qualitative/quantitative composition, as well as of the variables of manufacturing process. The Scale Up Post Approval Changes guidance [1] issued by the US Food and Drug Administration specifically mentioned the instances where IVR can be used for regulatory purposes and clearly stated that they should not be considered as measures of bioequivalence. The vertical diffusion cell systems, also known as Franz cells, have been widely used as the main experimental device, with testing parameters adapted to the particularities of the pharmaceutical vehicle, as well as to the physico-chemical properties of the active pharmaceutical ingredient [2]. The overall methodological approach must provide an insight of the critical

interaction between the formulation and drug. For example, the membrane must be an inert support of the semisolid vehicle and should not display any significant diffusion resistance to the release of the active pharmaceutical ingredient (i.e. adsorption).

It was clear from the very first moment of the official adoption that the main intension was not to overrate the importance of IVR, but in the same time it was assumed that it also represented a first step in the regulatory recommendation of a tool for selection of the optimal formulation candidate, for research and development phase. Moreover, structural and release patterns, correlated or not, are critical for the *in vivo* performance of the semisolid drug products. Consequently, it is reasonable to assume that, if supplementary proofs of their relation with the *in vivo* pharmacokinetic data will be available, their use as surrogate procedures should be reconsidered.

Previous reports and current regulatory guidance [1] indicate that the release from semisolid dosage

forms can be adequately described by the Higuchi model. The active pharmaceutical ingredient is either dissolved or suspended in the vehicle. Accordingly, there is a linear relationship between the amount of active ingredient (Q , mg) released per unit area and the square root of time (t , min) [3]:

$$Q = (2C_i / A) \sqrt{\frac{D}{\pi}} \sqrt{t} \quad (1)$$

where C_i is the initial concentration in the donor compartment of the cell (mg/cm^3), A is the surface area available for diffusion (cm^2), D is the diffusion coefficient (cm^2/min).

By applying Fick's first law, one can estimate the permeability coefficient P (cm/sec) and, subsequently, the partition coefficient between the vehicle and the release media k_p [3], if the membrane doesn't display rate limiting properties:

$$Q = (P \cdot C_i \cdot A)t \quad (2)$$

$$k_p = P \cdot h / D \quad (3)$$

where h is the thickness of the interface. It is to be mentioned that first order kinetic model has also been used for the analysis of the release process (linear dependence of the logarithmic values of the unreleased fraction on time). Some reports suggest that the nature of the vehicle, mainly the hydrophilic character, determines the kinetic models that accurately fit the experimental data [4]. In fact, the experimental conditions, including a hydrophilic membrane and an aqueous receptor media, are responsible for the apparent underperformance of the lipid bases and the low values of the partition coefficient. The slow release profile can be adequately described by several models.

Two processes are frequently observed to interfere during the in vitro release testing. The first one is triggered by the concomitant diffusion of several components of the pharmaceutical formulation. A previous report suggested that high amounts of tensioactive agents could generate deviation from Higuchi model, based on their accumulation in the receptor component and correlated changes of the solubility and thermodynamic activity of the analyte on both sides of the membrane [5, 6]. Secondly, there is a clear difference between the natural and artificial membranes. The later ones are mainly used for quality control purposes and provide important information on the interaction between a drug and the semisolid matrix.

The criteria for the evaluation of structural equivalence between the bio-batch and the new or optimized formula are not well defined. The spreading phenomenon at the site of absorption altered the results obtained by skin stripping technique, but quantitative evaluations were not available nor suggested [7]. According to an available technical note, distinct rheological behaviours are not correlated with the bioequivalence conclusion [8]. Nevertheless, the complexity of the interaction pattern within the semisolid matrix makes difficult the attempts to directly correlate the structure with the release characteristics [9].

The aim of the current paper was to evaluate the relationship between IVR data and rheological assessment of four types of formulations with meloxicam, piroxicam and tenoxicam as single active ingredients with low aqueous solubility, dispersed or dissolved in a hydrophilic semisolid matrix.

Materials and Methods

The hydrophilic formulations were prepared by separate hydration (with 50% of the water quantity available in the formula) of two types of macromolecular agents, carbomer 940 (1%) and a cellulose derivative, methylcellulose or hydroxypropyl-methylcellulose (3%).

Table I

The composition of topical semisolid dosage forms containing oxicams (g/100g)

Component	Formula			
	F1	F2	F3	F4
Oxicam (meloxicam, piroxicam, tenoxicam)	0.5	0.5	0.5	0.5
Carbomer 940	1.5	1.5	1.5	1.5
Hydroxypropyl-methylcellulose	3.0	3.0	-	-
Methylcellulose	-	-	3.0	3.0
Triethanol amine	0.5	0.5	0.5	0.5
Macrogol glycerolhydroxystearate (Cremophor® RH 40)	1.0	-	1.0	-
Macrogol 15 hydroxystearate (Solutol HS® 15)	-	1.0	-	1.0
Isopropyl-myristate	1.0	1.0	1.0	1.0
Ethanol absolute	2.5	2.5	2.5	2.5
Purified water	90.0	90.0	90.0	90.0

The oxicam was dispersed in the mixture of ethanol, isopropyl myristate and a non-ionic tensioactive agent (macrogol glycerolhydroxystearate, and, respectively, macrogol 15 hydroxystearate). Each product was mixed at 2000 rpm for 10 minutes, using a Heidolph RZR 2020 mechanical stirrer. The viscosity and IVR assessment were performed after at least 24 hours of storage in ambient conditions.

The structural characteristics were assessed using a rotational viscosimeter Thermo Haake VT550 (ViscoTester VT550), Thermo Electron (Karlsruhe) GmbH, Germany, with a SV-DIN coaxial cylinder assembly (interval of shear rates: 0-100 s⁻¹; sample volume: 10 ml; six replicates per sample, recorded at ambient temperature). The recorded flow patterns were characterized by three parameters: hysteresis area (difference in area under the share stress vs. share rate plot, for the ramp up/ramp down curves, Pa s⁻¹); flow index - n and consistency index - K, calculated according to Ostwald de Waele model:

$$\tau = K(\dot{\gamma})^n$$

where:

τ is the share stress (Pa),

and $\dot{\gamma}$ is the share rate (s⁻¹).

The IVR procedures were performed on a Hanson Microette system (Hanson Research Inc., USA) with 12 mL vertical diffusion cells and 50% ethanol absolute in purified water as receptor media. Approximately 300 mg of each formulation was applied on polysulfone membranes (Tuffryn[®], PALL Life Sciences HT-450, 0.45 μ m average pore size, batch no. T72556), with 0.5 ml samples collected at 30, 60, 90, 120, 150, 180, 210 and 240 minutes after application. The tests were performed in triplicate, at 32 \pm 0.5°C and at 400 rpm stirring rate. The samples were analysed for oxicam content using a spectrophotometric method, at 223.4 nm, on a

Jasco UV-Vis V-530 spectrophotometer (equipped with Spectra Manager software for Windows 95/NT, version 1.54.03). Higuchi and first order kinetics models, in the forms described in the introduction chapter, were applied for the analysis of release data.

The evaluation plan was also applied to available data, partially presented in a previous paper [9]. The aim was to extent the analysis to more lipophilic formulations (two topical creams containing 3% piroxicam, available on the local market; C1 and C2)), as well as to include the profile of the reference dosage form (Feldene 1% gel, R). The distribution coefficient in binary biorelevant systems were obtained by shake-flask method and experimental protocol will be published in a separate report.

All the other reagents and the analytical standard of meloxicam, piroxicam and tenoxicam were purchased from Sigma Aldrich. The purified water was generated by a SGW Ultraclear UV Plus[™] system. Cremophor[®] RH 40 and Solutol HS[®] 15 were kindly donated by BASF Ludwigshafen, Germany.

Results and Discussion

The results obtained by fitting the experimental data with the previously described models for structural and diffusional assessments are presented in Table II.

Table II

The results of structural analysis and modelling of *in vitro* release profiles from semisolid dosage forms

Parameter	LogD _{5,4}	LogD _{7,4}	Formula	Model						D cm ² /s (x10 ⁸)	P cm/s (x10 ⁶)	k _p		
				Ostwald de Waele			Higuchi						First order kinetics	
				m (Pa/s)	n	R ²	K μg/cm ² /min ^{1/2}	lag min ^{1/2}	R ²				k ₁ min ⁻¹ (x10 ⁴)	R ²
Oxicam	0.589	0.800	F1	12.32	0.44	0.9999	38.58	0.76	0.9974	42.90	0.9909	17.94	7.72	0.448
			F2	17.53	0.42	0.9999	40.85	1.01	0.9992	46.80	0.9956	19.57	8.11	0.420
			F3	18.87	0.41	0.9999	27.88	2.16	0.9990	23.23	0.9936	9.71	5.18	0.576
			F4	31.97	0.37	1.0000	30.14	2.88	0.9985	24.53	0.9977	10.26	5.32	0.506
Piroxicam	0.548	0.661	F1	8.18	0.52	0.9998	49.87	1.01	0.9988	75.26	0.9962	40.52	9.99	0.347
			F2	11.20	0.48	0.9999	41.42	0.61	0.9986	50.43	0.9948	27.15	8.44	0.425
			F3	14.86	0.44	0.9999	36.62	1.76	0.9956	36.54	0.9954	19.67	7.13	0.460
			F4	20.55	0.42	0.9999	42.97	3.01	0.9952	42.11	0.9987	22.67	7.53	0.353
Tenoxicam	0.636	0.749	F1	3.95	0.59	0.9993	54.58	0.93	0.9972	100.14	0.9946	58.30	10.85	0.315
			F2	7.96	0.53	0.9996	51.87	0.94	0.9979	83.36	0.9973	48.53	10.21	0.328
			F3	11.61	0.45	0.9999	53.75	3.00	0.9993	63.76	0.9983	37.12	9.28	0.278
			F4	17.82	0.41	1.0000	45.22	3.19	0.9981	44.37	0.9996	25.83	7.74	0.327

LogD_{5,4} - distribution coefficient determined in triplicate, using binary systems composed of isopropyl-myristate - phosphate buffer pH=5.4 10 mM; LogD_{7,4} - distribution coefficient determined in triplicate, using binary systems composed of n-octanol - phosphate buffer pH=7.4 10 mM; m - flow consistency index; n - flow behavior index; K - release rate according to Higuchi model for solutions; k₁ - first order rate constant; D - diffusion coefficient; P - penetration coefficient; k_p - partition coefficient; R² - correlation coefficient.

Noteworthy, the *in vitro* drug release evaluations were more accurately described by the square root law, compared to the first order kinetics, although both models generated correlation coefficients above 0.99 thresholds. The amounts of oxicams

recovered in the receptor compartment indicated that selection of the receptor media composition was adequate, providing sink conditions for the entire duration of the test (Figure 1). All the parameters calculated as estimators of the diffusion

pattern from the semisolid matrix (K , P , D , k_1 and k_p) were clearly influenced by the nature of cellulose derivative.

The dependence on the type of nonionic tensioactive agent was less evident. It is to be mentioned that their presence in the composition was imposed by previous reports suggesting the net improvement of the absorption profile through the skin [10]. Moreover, since it was assumed that their surfactant properties generated emulsion-type hydrogels, a supplementary limiting step must be considered, i.e. the partition between the internal

lipidic phase and the hydrophilic matrix. The latter process was dependent upon the affinity of the active pharmaceutical phase, controlled by its physico-chemical properties.

On the other hand, their interposition between the hydrate macromolecular chains triggered distinct, structure dependent flow behaviours. The *in vitro* release profiles of the hydrophobic active entities seems to be controlled by both diffusional resistance, correlated with the viscosity, and the specific values of the distribution coefficient.

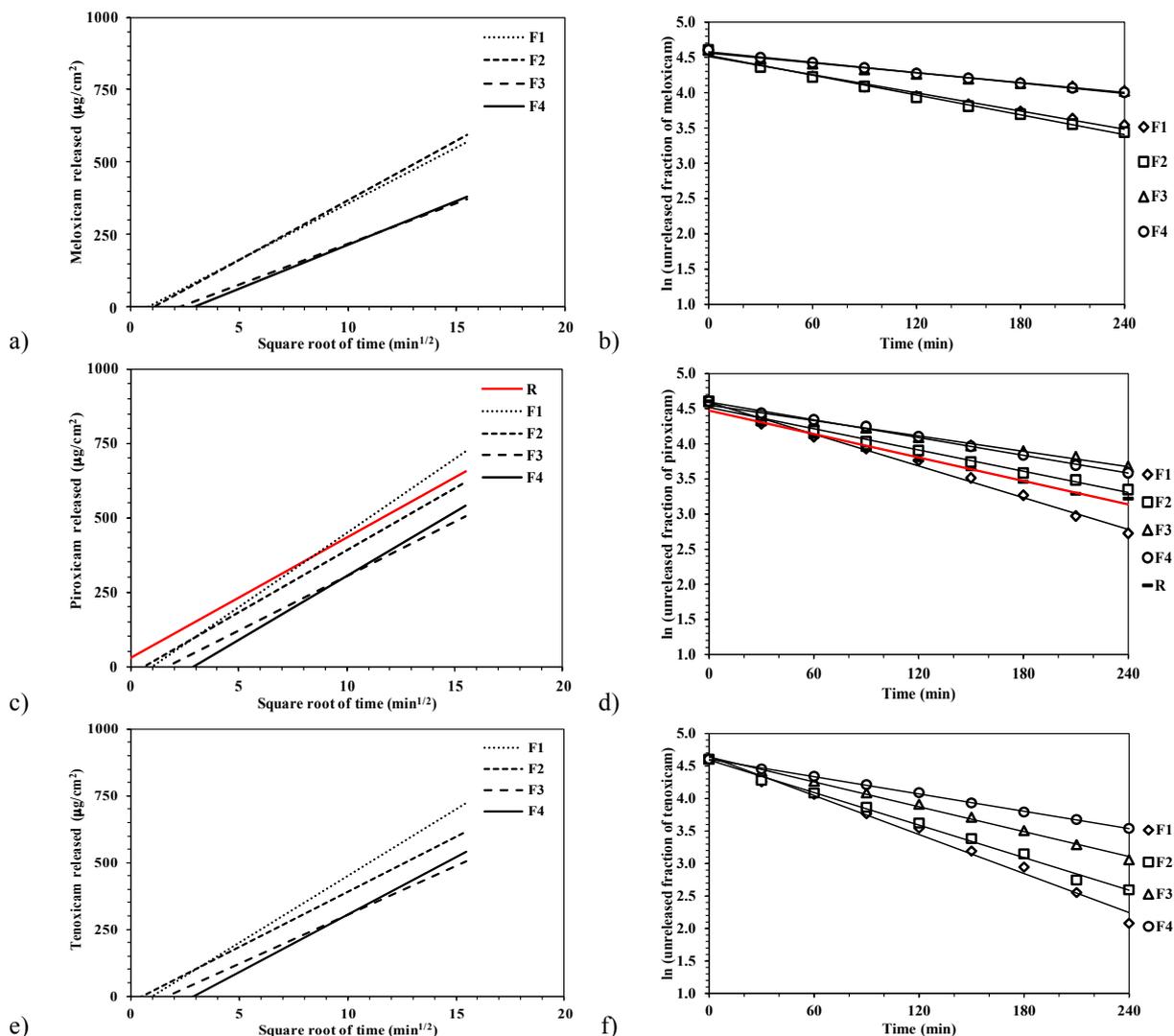


Figure 1.

Comparative presentation of the *in vitro* release patterns fitted with square root and first order kinetic models for: a, b - meloxicam; c, d - piroxicam; e, f - tenoxicam

The topical semisolid dosage forms containing piroxicam represent a special case, various types of vehicles being used for conditioning different strengths. Moreover, there are no particular indications for administration, despite claimed content varies by 6 orders of magnitude. The reference drug product is a simple hydrophilic gel,

containing two macromolecular agents without the addition of a tensioactive agent. The cream formulations had a remarkably high consistency, with an increased structural resistance opposed to the release of the low solubility, high permeability drug (Figure 2).

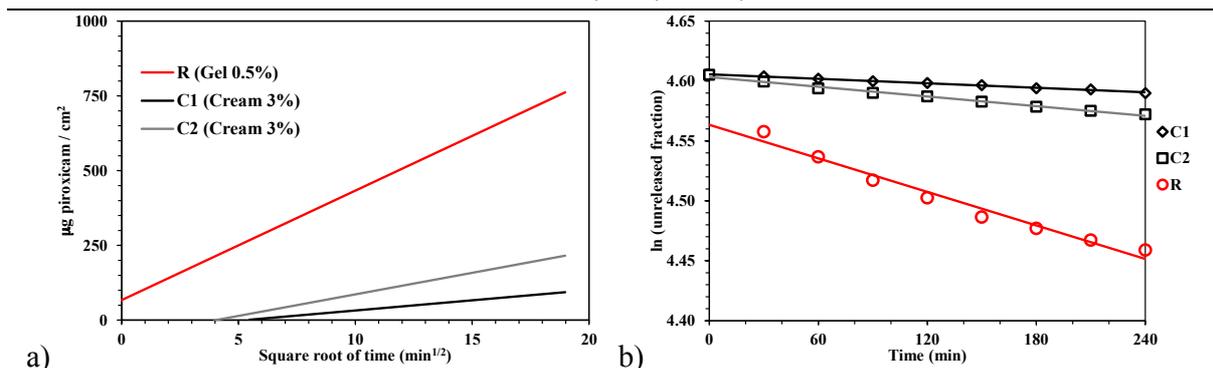


Figure 2.
Comparison of IVR data for registered drug products containing piroxicam
a) square root law; b) first order kinetics

The main problem in the phase of research and development of new, innovative generic drug products is how to choose the target profile, when multiple options are available with presumably the same therapeutic outcome. Furthermore, it is not clear how adequate is to extrapolate the data generate on dosage forms containing drugs from the same therapeutic class, with a considerable degree of similarity in terms of structure and correlated biopharmaceutical properties. Considering the qualitative and quantitative composition, as well as the assumed mechanism of release, the experimental formulations should have displayed an intermediate behaviour.

The rheological profiles indicated that all the formulations presented a pseudoplastic behaviour (Figure 3). The Ostwald de Waele model fitted

adequately the recorded experimental data. The values of the flow behaviour index were subunitary. Noteworthy, a rank order relationship rather than a direct proportionality was observed between the parameters characterizing the stress-induced deformation pattern and the in vitro diffusion. Both procedures showed an adequate discriminatory character for the variable of composition. For the experimental formulations, the supplementary partition of the active ingredient is compensated by a decreased diffusional resistance, based on the lower consistency and, most probably, higher mobility of oxicams within the swollen hydrophilic matrix. This is one of the multiple consequences of the specific interactions induced by surfactants [11], within an intermeshing network [12].

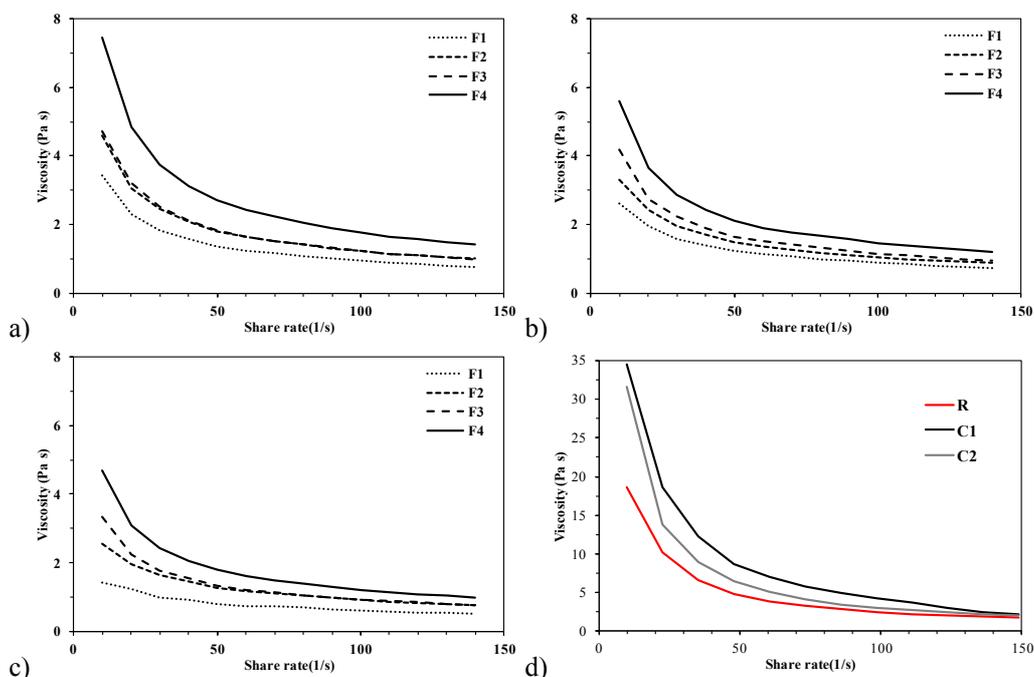


Figure 3.
Deformation curves for the experimental formulations
a) meloxicam; b) piroxicam; c) tenoxicam; d) registered products

Although the square root law fitted better the mean release profiles and there is a consensus regarding the regulatory acceptance, the first order kinetic models could be relevant in case of atypical (non-Higuchian) patterns. Preliminary data generated in case of SUPAC comparison suggested that, when the first order rate constants were used for calculation of 90% confidence intervals, with a 75-133.33% acceptance interval, the procedure can underline more clearly the pharmaceutical non-equivalence. Of particular importance is the

apparent relation between the flow behaviour index and those constants (Figure 4). Despite the non-linearity, it reveals that, in case of adequate selection of the experimental parameters (membrane type, porosity, tortuosity, sink conditions, sampling schedule etc.), the methodological approach could be sensitive for changes in structure and/or composition. Therefore, it can be considered as total quality control test, similar to current view on the dissolution tests for oral solid dosage forms.

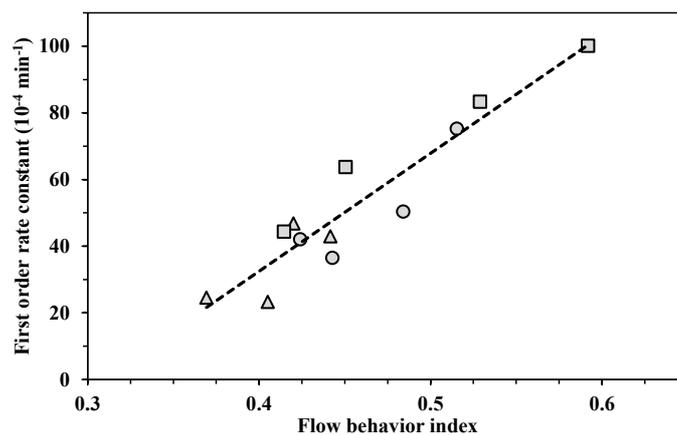


Figure 4.

Correlation between the first order rate constant and flow behaviour index

(Δ - meloxicam; \circ - piroxicam; \square - tenoxicam)

Conclusions

Twelve experimental semisolid dosage forms containing oxicams as active pharmaceutical ingredient were prepared by dispersion of the hydro-alcoholic solutions in hydrophilic matrix.

The results were further correlated with the rheological parameters and underline the critical role of correlated structural and diffusional evaluation in the selection of optimal formulation candidate, during the research and development phase.

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