Multivariate Statistical Analysis Regarding the Formulation of Oxicam-Based Pharmaceutical Hydrogels

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In this paper, it was analysed the influence of formulation factors over obtaining oxicam hydrogels, using the statistical analysis. Data analysis and predictive modeling by multivariate regression offers a large number of possible explanatory/predictive variables. Therefore, variable selection and dimension reduction is a major task for multivariate statistical analysis, especially for multivariate regressions. The statistical analysis and computational data processing of responses obtained from different pharmaceutical formulations, via different experimental protocols, lead to the optimization of the formulation process. It was found that the most suitable pharmaceutical formulations based on oxicams with the possibility of rapid release contained cyclodextrin, in particular 2-hydroxypropyl-β-cyclodextrin.

Keywords: oxicam, pharmaceutical formulation, hydrogel, cyclodextrin, multivariate statistical analysis

New trends in pharmaceutical research are aimed both towards the synthesis of new bioactive molecules, but as well on the design of improved dosage forms (capsules, tablets, gels, ointments, injections, patches etc.). The main objective is to obtain specific formulation that can deliver to the receptor the desired quantity of bioactive compound in a specific period of time. This objective is highly unrealizable for each available pharmaceutical formulation and individual patient.

Oxicam-type drugs are a class of structurally-closely related compounds belonging to nonsteroidal anti-inflammatory drugs (NSAIDs), used mainly due to their analgesic, antipyretic and anti-inflammatory properties. The most widely used oxicam-type drugs are piroxicam (PX), meloxicam (MX) and tenoxicam (TX), which are weak acids in aqueous solution and their mechanism of action is based on the bounding to plasma proteins [1,2]. Despite MX which shows slight preference for COX-2, PX and TX are unselective inhibitors of the cyclooxygenase (COX) enzymes [3, 4]. The structures of the analysed oxicams are presented in figure 1.

Following some previous studies [5-10] we studied the influence of the carbomer type (Carbopol Ultrez 10 – CU10 and hydroxypropylmethyl cellulose – HPMC, respectively) on some properties of the oxicam-containing hydrogels, as well as in clarifying the influence of cyclodextrin content and its solubility on the release of oxicams from these hydrogels.

Experimental part

As starting reagents, pharmaceutical grade PX, MX and TX (LaborMed Pharma, Romania) were used as received, without further purification. The alcohols were obtained as follows: polyethylene glycol 400 (PEG 400) and propylene glycol (PG) (BASF Chem Trade GmbH, Germany), absolute ethanol (Chimopar, România), glycerine (Gly) (Nordische, Germany). Other compounds, such as Solutol H15 (BASF, Germany), Tween 85 (Merck, Germany), β-cyclodextrin (BCD) (99%, Cyclolab, Hungary), 2-hydroxypropyl-β-cyclodextrin (2HPBCD) (>99%, Cyclolab), Carbopol Ultrez 10 (CU10) (Lubrizol, USA), hydroxypropylmethyl cellulose (HPMC) (Methocel E4 Premium, Colorcon Ltd., UK) triethanolamine (TEA) (Merck), monoethanolamine (MEA) (Stera Chemicals, România) were used. Preservative solution was prepared according to X-th Romanian Pharmacopoeia, and described elsewhere [11]. Also, we used phosphate buffer (*p*H = 7.4). The protocol for obtaining the hydrogels is described elsewhere [11].

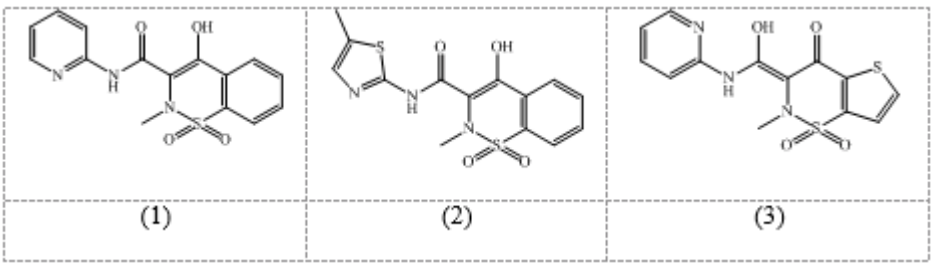

Fig. 1. Structures of oxicam-type active substances: 1 - piroxicam (PX), 2 -meloxicam (MX) and 3 - tenoxicam (TX).

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In order to obtain hydrogels with/without oxicam complexes (PX, MX, TX) with cyclodextrin (BCD or 2HPBCD), CU-10 or HPMC were used as gel-forming polymers. Also, as solubilizers and/or absorption enhancers of the active substances, respectively as rheological modifiers were used as cosolvents (EtOH, PG, Gly and PEG400) and surfactants (Tween 85 or Solutol H15). The used vehicle was preservative solution, and as neutralizing agents (for the adjustment of pH) organic bases (MEA or TEA) or inorganic base ($NaOH_{aq}$ 10%) were used.

Principal Component Analysis (PCA) is the basis of the multivariate analysis of the data. Theoretical bases regarding the use of multivariate analysis in domains such as pharmacy and pharmacognosy are revealed by numerous studies [12-18].

In order to obtain valid results, an experimental plan was developed, by using an optimization software. For multivariate statistical analysis and modeling, the method of cluster analysis - HCA - implemented in software package Statistica 6.0 - StatSoft, Inc., USA, was used. Data fitting was realized by using the design to latent structures (PLS), using the software package Unscrambler 6 (Camo AS, Trondheim, Norway) by *cross validation* method.

Results and discussions

The choose of independent variables

Besides the presence of cyclodextrins in the final formulations, it was considered important the concentration of rheology modifiers (glycerol and polyethylene glycol), ethanol or surfactant. For the oxicams/cyclodextrin complexes used in hydrogel formulations, it was considered important the presence (concentration) of cyclodextrin in the formulation and its water solubility (BCD has a solubility in water of ~2%, while 2HPBCD has a solubility in water of ~40 %). The independent variables that were taken into consideration and the levels are presented in table 1.

The choice of dependent variables

The following variables were measured as dependent variables: the final pH of the formulation, the penetration, the spreadability surface for a maximum applied mass of 750 g, oxicam concentrations achieved after 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min (expressed as a percentage or in ìg cm^{-2}), respectively, as specific diffusion parameters: flow through the membrane, permeability coefficient, transfer rate, diffusion coefficient and time lag (table 2).

Table 1

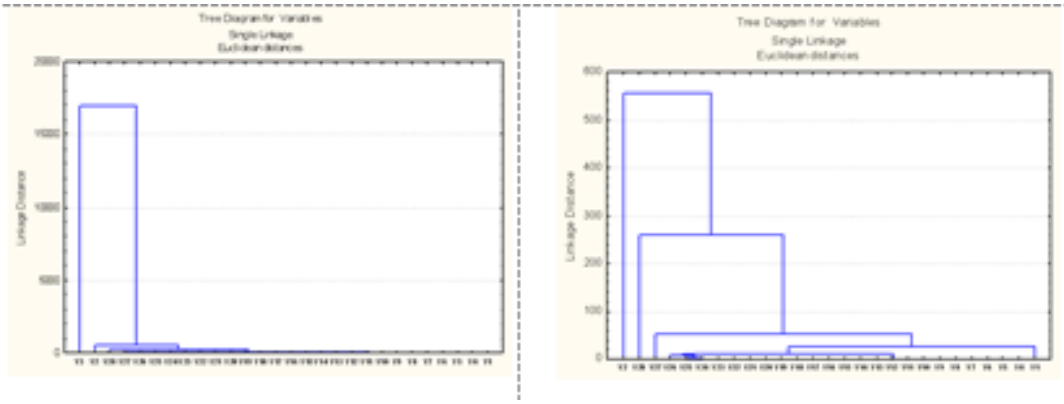
INDEPENDENT VARIABLES USED IN THE DESIGN AND OPTIMIZATION OF PHARMACEUTICAL FORMULATION WITH CU10 AND HPMC

Carbomer type	Factor	Level		
		(-)	(+/-)	(+)
CU10	**X1**: Concentration ratio surfactant/rheological modifier	0		0.5
	X2: Concentration of rheological modifier (%)	0	10	20
	X3: Product between the presence (incidence) of cyclodextrin and solubility	0	2	40
HPMC	**X1**: Concentration of ethanol (%)	0	25	50
	X2: Concentration of rheological modifier (%)	0	10	20
	X3: Product between the presence (incidence) of cyclodextrin and solubility	0	2	40

Variable	Description
Y1	pH
Y2	Penetration (mm)
Y3	Spreadability surface for a maximum applied mass of 750 g (mm^2)
Y4-Y13	Concentration of yielded oxicam (%) after: 15', 30', 45', 60', 90', 120', 150', 180', 210', 240'
Y14-Y23	Concentration of yielded oxicam (µg/mm^2) after: 15', 30', 45', 60', 90', 120', 150', 180', 210', 240'
Y24	Flow through the membrane, J (mg cm^{-2} min^{-1})
Y25	Permeability coefficient, Kp (cmx10^7 min^{-1})
Y26	Transfer rate, k (µg/cm^2/$min^{1/2}$·10^{-2})
Y27	Diffusion coefficient, D (cm^2/min·10^{-8})
Y28	Time lag, t_L (min)

Table 2

DEPENDENT VARIABLES USED IN THE DESIGN AND OPTIMIZATION OF FORMULATION CONTAINING CU10 AND HPMC



a b

Fig. 2. Dendrogram from HCA analysis of dependent variables (Y) for pharmaceutical formulations based on CU10: a) all variables, b) all variables except Y3

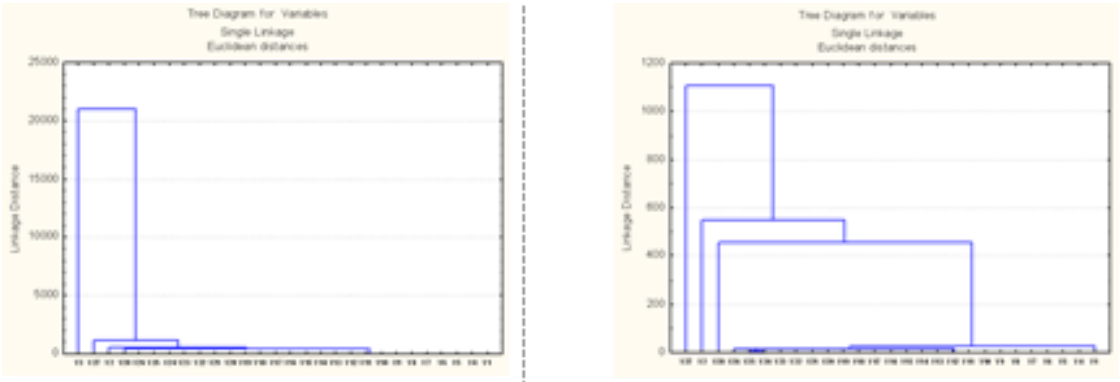


Fig. 3. Dendrogram from HCA analysis of dependent variables (Y) for pharmaceutical formulations based on HPMC: a) all variables, b) all variables except Y3

Similarity evaluation and selection of dependent variables

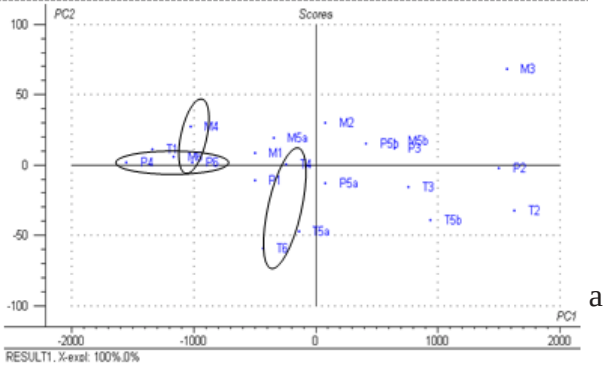
To assess the similarity of the dependent variables and selection of the most important ones in multivariate statistical analysis and modeling, we used the cluster analysis (HCA - Hierarchial Cluster Analysis). Similarity/ dissimilarity of variables can be observed in the dendrograms presented in figure2 (for the formulations based on CU10) and in figure 3 (for the formulations based on HPMC).

It can be seen that in both cases, Y3 (which is spreadability surface for a maximum applied mass of 750 g) is totally dissimilar to other variables. Following this, HCA analysis is carried out without this variable, when there was observed an obvious similarity between the variables that describe the concentrations of yielded oxicams (Y4-Y13 and Y14-Y23, respectively). After this, only one remaining variable was used in statistical analyze, namely Y23. Also, other variables like Y28, Y27, Y26, Y25, Y24 and Y23 were dissimilar, with the degree of dissimilarity decreasing in this order. Therefore, for multivariate statistical analyses, dissimilar variables Y28 and Y27 were selected. Y24-Y26 variables have a lower degree of dissimilarity, and therefore only variable Y25 was selected for further analysis. Although it was dissimilar to the other variables, *p*H value, not dependent on the considered independent variables, but only on the presence of the agent for adjusting the *p*H, value of which was not used in the statistical analysis.

PCA analysis

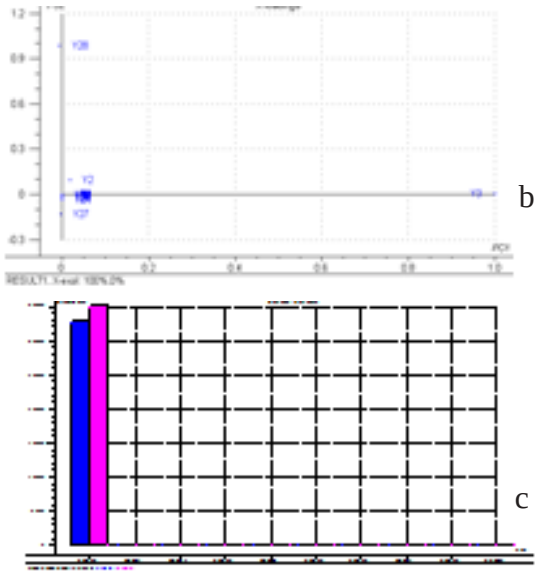
Principal component analysis (PCA) was realised for all oxicams, in order to obtain a classification of samples regarding both independent variables (X) and the dependent ones (Y).

The PCA analysis of all samples based on CU10 revealed a very good group of the cyclodextrin samples to the left of the graph of the scores (fig. 4), when using all dependent variables in the analysis. Important variable in the analysis is especially Y3 for principal component 1 (PC1) and Y28 for PC2, respectively, the variance of data are almost entirely explained by PC1 (~100%) (fig. 4).



a

Fig. 4. Graph of: a) scores)



b

c

Fig. 4. Graph of: b) X-loadings and c) residual variance for PCA for samples based on CU10

If the analysis is performed for each group of oxicam formulations, it can be noticed that there is a grouping of the cyclodextrin samples within the same area and the same influence of variables, however, more important for Y2 and Y27 samples containing PX, respectively for Y2, Y27 and Y28 to the MX and TX formulations.

Regarding the samples based on HPMC, the grouping was more evident when using the dependent variables, PC1 explaining 96 % of the data variance, and PC2 4 %. The samples containing cyclodextrins are grouped (with one exception, P4b) on the left graph of the scores of PCA analysis of all data (fig. 5). In this case too, the important variables for classification are Y3 for PC1 and Y27 for PC2. By grouping the samples that contain cyclodextrins, it can be noticed that the PCA analysis performed for each type of oxicam, reveals that the best grouping occurs for the samples containing MX and TX.

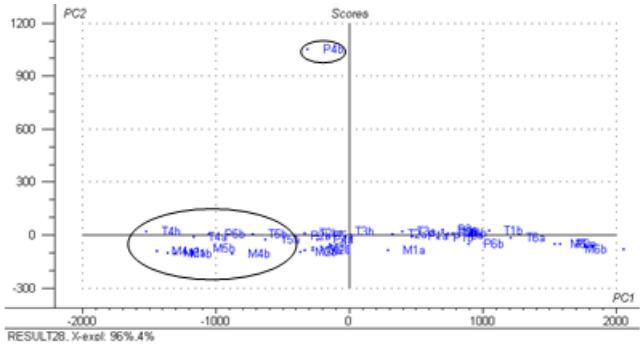


Fig. 5. Graph of: a) scores,

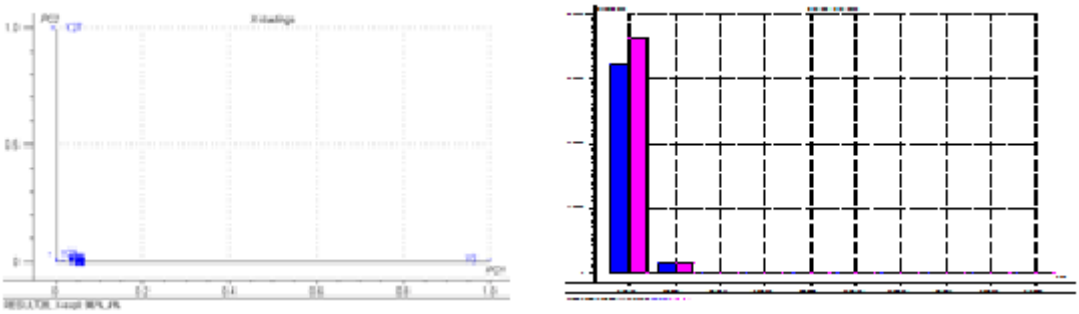


Fig. 5. Graph of: b) X-loadings and c) esidual variance for PCA for samples based on HPMC.

Mathematical models obtained by multivariate statistical analysis (PLS method)

For obtaining mathematical models that can fit and describe the experimental data variance and their selection for biological activity, the PLS (Partial Least Squares) method was used in both PLS1 and PLS2 variants (using a single dependent variable or more dependent variables, respectively) [19-27]. The currently used model was a second-degree polynomial, as seen in equation 1:

$$Y_n = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 + b_3 \cdot X_3 + b_{11} \cdot X_1^2 + b_{22} \cdot X_2^2 + \\ + b_{33} \cdot X_3^2 + b_{12} \cdot X_1 \cdot X_2 + b_{13} \cdot X_1 \cdot X_3 + b_{23} \cdot X_2 \cdot X_3 \quad (1)$$

where $X_{1,2,3}$ are independent variables, Y_n are dependent variables, b_0 is the medium value of Y_i, b_i is the medium value of X_i and b_{ij} represent the interaction between X_{ij} factors.

The selected independent variables are presented in tables 1 and table 2, while dependent variables were selected based on both cluster analysis and PCA analysis. They are presented in table 3, namely variables Y2, Y3, Y23, Y25, Y27 and Y28. All the results obtained by applying the PLS methods are presented in table 3.

In the case of applying PLS1 method for the samples containing CU10, it was not possible to obtain the mathematical model with appropriate prediction. This fact could be associated with the clear difference in the influence of cyclodextrin in the formulation. The use of variable Y2 model is closer to the statistically significant (r^2=0.39), but the predictive capacity is reduced (q^2=0.07). The important independent parameters for the model are X3 and X2, namely the concentration of cyclodextrin and the concentration of rheology modifier (model 1).

Much better results were obtained from PLS1 analysis using Y3 as dependent variable. The correlation in the case was 0.48, and the predictive correlation coefficient about 0.4, with the same significance for X variables as above (model 2). For the other studied dependent variables, the results were weaker. For instance, in the case of variable Y28 (lag time), correlation was below 0.51 (model 7).

For HPMC-based formulations, both PLS1 and PLS2 analyses led to good results. The PLS1 analysis for the dependent variables Y2 and Y3 led to statistically significant results for both mathematical models (coefficient of determination >0.83) and the prediction capability (coefficient of determination > 0.73). In these cases (models 4 and 5), the most important variables for modeling were X2 and X3 (the concentration of the rheology modifier and the incidence of the cyclodextrin). If the dependent variable used in the PLS1 analysis was Y25 (model 6), the results were poor, so that the resulting model had a correlation coefficient of 0.48, while the prediction was not significant (q^2=0.24).

Instead, the dependent variable Y28 led to better results compared with the previous situation, the model with a correlation coefficient of 0.7 was obtained, and the prediction was statistically significant (q^2=0.5). In this case (model 7), important variables are the ones that affect the viscosity (concentration of the ethanol and rheology modifier).

Better results were obtained at PLS2 analysis when using two or more dependent variables of the previously selected.

Analysis type	Sample	Selected variable Y	R^2	q^2	X variable
PLS1	CU10	Y2	0.39	0.07	X3; X2
PLS1	CU10	Y3	0.48	0.39	X3; X2
PLS2	CU10	Y2; Y28	0.48	0.40	X3; X2
PLS1	HPMC	Y2	0.87	0.80	X2; X3
PLS1	HPMC	Y3	0.83	0.74	X2; X3
PLS1	HPMC	Y25	0.48	0.24	X3; X1; X2
PLS1	HPMC	Y28	0.70	0.51	X2; X1
PLS2	HPMC	Y2; Y3	0.87	0.82	X3; X2
PLS2	HPMC	Y2; Y23	0.87	0.81	X3; X2
PLS2	HPMC	Y2; Y25	0.87	0.80	X3; X2
PLS2	HPMC	Y3; Y23	0.83	0.75	X3; X2
PLS2	HPMC	Y3; Y27	0.84	0.76	X3
PLS2	HPMC	Y2; Y3; Y25	0.87	0.82	X3; X2
PLS2	HPMC	Y3; Y23; Y25	0.83	0.73	X3
PLS2	HPMC	Y3; Y23; Y28	0.83	0.77	X3
PLS2	HPMC	Y2; Y3; Y23; Y25	0.87	0.81	X3; X2
PLS2	HPMC	Y2; Y3; Y23; Y27	0.87	0.80	X3; X2
PLS2	HPMC	Y2; Y3; Y23; Y28	0.87	0.81	X3; X2
PLS2	HPMC	Y3; Y23; Y25; Y27	0.83	0.75	X3
PLS2	HPMC	Y3; Y25; Y27; Y28	0.83	0.76	X3

Table 3
THE RESULTS OBTAINED BY MULTIVARIATE STATISTICAL ANALYSIS

For example, for the Y2 variable, PLS2 analysis led to the mathematical models statistically significant for both the initial model and the predictive power of the model ($R^2 \cong 0.87$, $q^2 \cong 0.81$). Best results have been obtained with the use of variable Y2 with Y3 (model 8), Y23 (model 9), Y25 (model 10).

By considering the pairs of dependent variables Y3 to Y23 (model 11), Y27 (model 12) and Y28, respectively, the results of PLS2 indicated that correlation coefficients of the models are very close ($R^2 = 0.834$), and the prediction coefficients ranging between 0.74 and 0.76. For all models, the most important independent parameter was X3.

PLS2 analysis for HPMC-based pharmaceutical formulations using three statistically significant dependent variables improved the quality and strength of predictive models, and in particular to the use of combinations of the variables Y2, Y3 with Y23, Y25, Y27 and Y28, in these cases so correlation coefficients > 0.8 were observed, with a significant prediction (model 13). In all other cases (models 14 and 15), PLS2 analysis revealed predictive correlation coefficient under 0.77, although the mathematical models have correlation coefficients higher than 0.83.

If in PLS2 modelling, four dependent variables are used, the accuracy of correlation is slightly improved. Thus, the combinations of Y2 and Y3 with other significant variables yield the best results (correlation coefficients for modeling over 0.86, and for the prediction over 0.80) (models 16-18). In all other cases, the results were slightly weaker, so that combinations Y-2/3-23-25-27/28 have correlation coefficients over 0.83 and 0.75, respectively (models 19-20). In almost all presented cases, X3 was the most important independent parameter for modeling/prediction.

However, the results of this study will be completed by an in-depth instrumental analysis regarding the stability of the pharmaceutical formulations by means thermal stability and spectroscopic investigations [28-46], by already established experimental protocols.

Conclusions

This paper was focused on analyzing the bahviour of oxicam-based hydrogels with Carbopol Ultrez 10 (CU10) and hydroxypropylmethylcellulose (HPMC) by statistics and regression model. Dependent variables with importance in conditioning and controlled release show high similarities and dissimilarities. There can be distinguished six dissimilar types of variables: penetration degree, applying surface, concentration of yielded oxicam, permeability, diffusion coefficients and time lag (latency), respectively, using hierarchical clustering procedures (HCA) and principal component analysis (PCA).

Regression models of PLS1 type have led to significant results when using variables regarding the penetration and lag (latency) time, for both type of formulations (with CU10 or HPMC). PLS2 type regression indicated a high importance in modeling for the same dependent variables, but the quality of models is considerably improved, as well as the predictive power. Most regression analyses showed significance for the independent variables which are the concentration of the rheology modifier and the concentration of cyclodextrins (adjusted for water solubility).

It was observed that the most suitable pharmaceutical formulations based on oxicams with the possibility of rapid release of bioactive compounds are cyclodextrin formulations, in particular the ones containing 2-hydroxypropyl-β-cyclodextrin.

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