OPTIMIZATION OF THE SONICATION PROCESS FOR MELOXICAM NANOCRYSTALS PREPARATION

SONIA IURIAN[1], IOAN TOMUȚA[1], LUCIA RUS[2], MARCELA ACHIM[1], SORIN E. LEUCUTA[1]

[1]**Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania**
[2]**Department of Drug Analysis, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania**

Abstract

Background and aims. Meloxicam, a widely recommended AINS, presents poor water solubility, which limits its bioavailability and effect onset. The objective of this study is the investigation of the most important factors that influence the efficiency of sonication in the preparation of meloxicam nanocrystals.

Methods. The effects of crucial technological sonication parameters (amplitude, time and applied cycle) on the crystal sizes and dissolution were investigated using a central composite experimental design with three factors and three levels. Different mathematical models were applied for the evaluation of the influence of each factor on the measured responses.

Results. The amplitude and the time were found as the most important variables. Their increase determined significant size reduction and homogeneity due to cavitation phenomenon, while the applied cycle was less important. The crystal size greatly influenced dissolution; a strong correlation was noted between small crystals and fast dissolution after freeze-drying the nanosuspensions. The optimal formulation was obtained by sonication at 100% amplitude, for 45 minutes and cycle 1, conditions which led to 600 nm crystals with 0.521 polydispersion index. The morphological analysis revealed small, round-shaped crystals with narrow size distribution.

Conclusions. The results provided the optimal sonication conditions needed to obtain meloxicam nanosuspensions with high drug dissolution capacity.

Keywords: nanocrystals, nanosuspensions, meloxicam, high dissolution, design of experiments

Background and aims

In the last years, fast dissolving dosage forms gained attention from the research and industrial media due to their ease of administration and wide acceptance of different groups of patients [1]. One of the challenges in the preparation of these dosage forms, especially where fast absorption is desired, is fast active pharmaceutical ingredient dissolution [2]. For this reason, nanosizing has become an important research topic and several methods to reach the nano range were developed.

Based on the Noyes-Whitney equation, dissolution depends, among other factors, on the contact surface between the drug and the liquid, so the preparation of nanocrystals increases the surface area and thus it improves absorption, when it is dissolution rate limited [3]. Several techniques were used in the preparation of nanocrystals, like milling, solvent evaporation, high pressure homogenization and acoustic cavitation [4,5]. The green methods, without the use of organic solvents, are usually preferred due to their

lack of toxicity [6]. Among them, the sonication method showed good results at the meloxicam size reduction in previously reported studies [7].

Meloxicam is a non-steroidal anti-inflammatory drug with selectivity on the COX-2 enzyme, recommended in rheumatoid arthritis, osteoarthritis and postoperative pain [8]. Its plasma half life is of 20 hours and the peak concentration is reached after 5-6 hours [9]. Meloxicam was chosen as a model drug because of its low water solubility that can limit its oral bioavailability and its high melting point, 254°C [10].

Ultrasound mediated size reducing and synthesis of nanomaterials was reported to yield particles with uniform size distribution, high surface area and high stability. Passing high frequency sound waves through a course suspension generates the cavitation phenomenon - the sequential formation, growth and collapse of voids in the liquid, that lead to particle erosion and breakage [11]. Thus, the sonication process efficiency depends on the input power into the liquid medium, meaning that as the power increases, the efficiency increases until an optimal value beyond which no change occurs [12].

The objective of this study was to develop and optimize an ultrasound method for meloxicam crystals size reduction, which could be further used for the preparation of orodispersible tablets via freeze-drying. Three factors that determine the sonication efficiency were studied – the amplitude, the time and the pulse control.

Materials and methods
Materials

Meloxicam (Mel) (UNICHEM Laboratories Ltd., India) was used as a model drug, in micronized form. PVP K25 (Kollidon, BASF, Germany) was used as suspension stabilizer.

Experimental Design

A central composite experimental design with three factors and three levels was used. The independent and dependent variables are listed in Table I. Fourteen experimental runs and three center points were generated, thus a total of 17 experimental determinations were performed according to the design matrix presented in Table II.

Differential scanning calorimetry (DSC)

DSC curves for the individual substances (Mel and PVP), their binary physical mixtures (1:1=m:m) and the lyophilized nanosuspensions were obtained in a Mettler Toledo DSC 822 cell. 40 µL aluminum crucibles were used, with about 2mg of samples, under dynamic N_2 atmosphere (flow rate: 80 mL/min) and at a heating rate of 10°C/min in the temperature range from 25 to 400°C.

Suspension preparation

For the preparation of the stock suspension PVP K25 was dissolved in distilled water at a concentration of 0.5% (w/v). Micronized Mel was added under stirring at 1000 rpm at a concentration of 2 mg/ml. The suspension was kept under stirring for 10 minutes.

Suspension sonication

A power ultrasound device (Hielscher UP100H Ultrasonic Processor with 100 W, Germany) was used to apply energy input to 40 ml of the previously prepared suspensions. The process parameters – amplitude, time and cycle – were set according to the experimental design matrix (Table II). During sonication the samples were kept into an ice bath. to prevent excessive heating.

Nanosuspension lyophilization

After sonication, 0.5 ml samples from the suspensions were poured into blister sockets and submitted to lyophilization using Virtis Advantage Plus (SP Scientific, Gardiner, USA) freeze dryer, according to a previously optimized freeze-drying cycle (results not shown). The lyophilization process consisted in freezing for 10 hours at -55°C, then primary drying at -25°C and 150 Torr for 12 hours, then secondary drying for 5 hours at +5°C and 300 Torr. PVP K 25 acted like a suspension stabilizer, but during the freeze-drying process – also like matrix forming agent.

Table I. The independent variables with their variation levels and the dependent variables.

Type of variable	Formulation variable	Symbol	Level		
			-1	0	+1
Independent	Amplitude (%)	X_1	40	70	100
	Time (min)	X_2	15	30	45
	Cycle	X_3	0.5	0.75	1
Dependent	Crystal size (nm)	Y_1			
	Polydispersity Index	Y_2			
	The % of meloxicam dissolved after 2 minutes	Y_3			
	The % of meloxicam dissolved after 4 minutes	Y_4			
	The % of meloxicam dissolved after 6 minutes	Y_5			
	The % of meloxicam dissolved after 12 minutes	Y_6			
	The % of meloxicam dissolved after 18 minutes	Y_7			
	The % of meloxicam dissolved after 30 minutes	Y_8			

Table II. Experimental design matrix.

Exp Name	Run Order	X_1	X_2	X_3
N1	11	40	15	0.5
N2	6	100	15	0.5
N3	7	40	45	0.5
N4	17	100	45	0.5
N5	12	40	15	1
N6	10	100	15	1
N7	14	40	45	1
N8	3	100	45	1
N9	2	40	30	0.75
N10	4	100	30	0.75
N11	5	70	15	0.75
N12	15	70	45	0.75
N13	8	70	30	0.5
N14	13	70	30	1
N15	1	70	30	0.75
N16	9	70	30	0.75
N17	16	70	30	0.75

X_1 - amplitude (%); X_2 – time (min); X_3 – cycle.

The particle size distribution

The average size measurement of Mel nanocrystals was performed by dynamic light scattering at a backscattering angle of 90° using a Malvern Zetasizer Nano ZS. The size measurements were done on dilute suspensions before and after sonication and for each of them at least 3 measurements at 25°C were performed. The average size and polydispersity index (PdI) were calculated.

Meloxicam *in vitro* dissolution

In vitro dissolution studies were performed on the content of one blister alveole containing 1mg Mel, according to the Eur.Ph.8.0, using the paddle method. The dissolution media was 500 ml phosphate buffer pH 7.4. kept at 37°C and at a rotation speed of 100 rpm. At certain time intervals: 2 minutes, 4 minutes, 6 minutes, 12 minutes, 18 minutes and 30 minutes, 5 ml samples were withdrawn and replaced with the same volume of fresh media. The samples were filtered and the amount of dissolved drug was determined spectrophotometrically at 360 nm, by a previously validated method. The dissolution test was performed in duplicate.

### Meloxicam crystal morphology

The crystal morphology was studied by optical microscopy (Optika. Italy) on the raw suspensions, with 500x degree of magnification for the initial crystals and 1000x - for the nanocrystals. The images were captured with an Optikam camera.

Results
Experimental design analysis. Quality of fit

The data fitting was done using Modde 10.0 (Umetrics. Sweden) statistical software by the Partial Least Squares (PLS) method. Figure 1 illustrates the statistical parameters with values between 0 and 1 as histograms for each of the measured responses: R^2 which represents the regression coefficient, Q^2 which indicates the predictive power of the model, the model validity and the reproducibility. The results for the ANOVA test showed that p for the model was less than 0.05 for all responses, while p for the error was higher than 0.05 for all the responses.
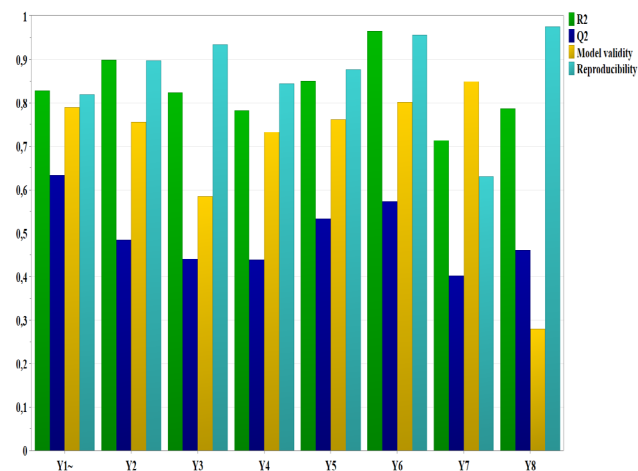
Figure 1. The fitting of the experimental data to the chosen model Y_1 – average size, Y_2 – polydispersion index, Y_3 – % of meloxicam released after 2 minutes, Y_4 - % of meloxicam released after 4 minutes, Y_5 - % of meloxicam released after 6 minutes, Y_6 - % of meloxicam released after 12 minutes, Y_7 - % of meloxicam released after 18 minutes, Y_8 - % of meloxicam released after 30 minutes.

Nanocrystal size and polydispersity index. The influence of process factors on the nanocrystal size and polydispersity index

The result for the initial size of raw meloxicam was 4.51±0.57 μm and the PdI was 1. After the sonication treatment, the average size for the 17 formulations strongly decreased to values comprised between 638.73±52.73 nm and 1187±215.00 nm. The polydispersity index ranged between 0.521 and 0.897.

Crystal morphology

The captured images (Figure 3) indicate the appearance of meloxicam crystals before and after the ultrasound treatment.

DSC studies

The DSC curves of the physical mixture of Mel with PVP and the lyophilized Mel:PVP optimal formulation are illustrated in Figure 4.

The DSC curve of Mel showed an endothermic event between 258.90 and 264.37°C with a temperature of T_{onset} =262.80°C corresponding to its melting, followed immediately by a decomposition exothermic event. The value of the enthalpy corresponding to the melting event is ΔH_{fusion} =1835.27 mJ/g.

In the mixtures with PVP before and after lyophilization, the melting domain of meloxicam was between 211.82°C and 239.74°C in the first case, respectively between 227.01°C and 248.31°C in the second
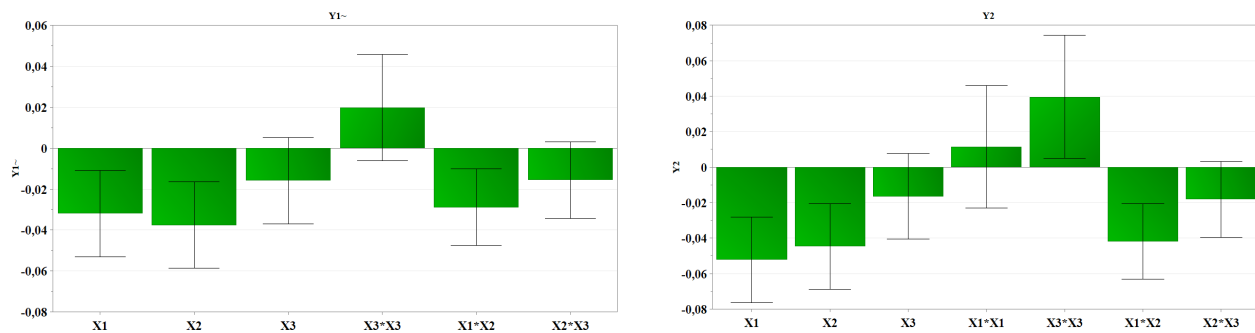
Figure 2. The influence of the technological factors on the nanocrystal size (Y$_1$) and polydispersity index (Y$_2$).
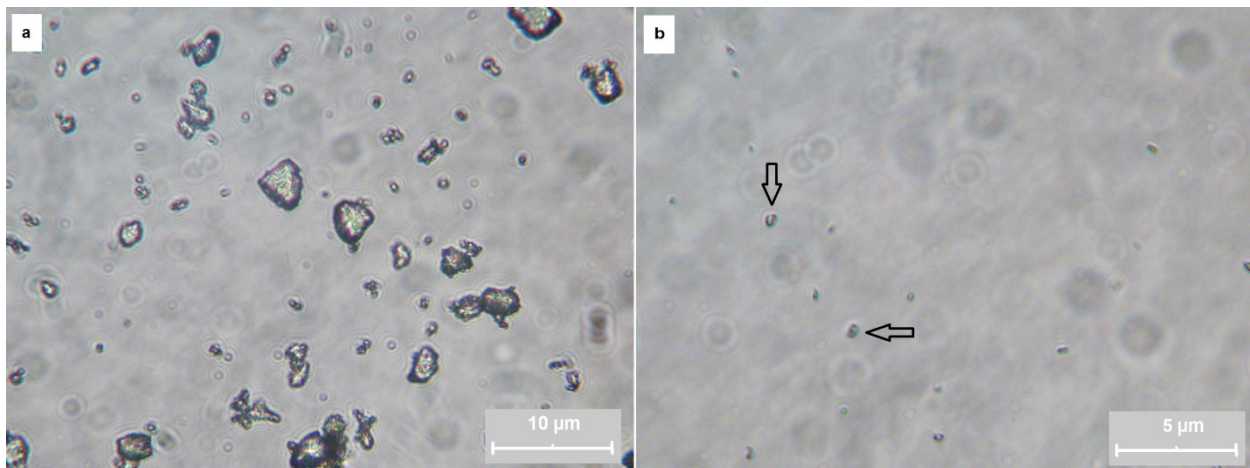
Figure 3. The aspect of the initial meloxicam suspension (a) compared to the nanosuspension obtained by ultrasound treatment for 45 minutes at 100% amplitude and cycle 1 (formulation N8) (b).
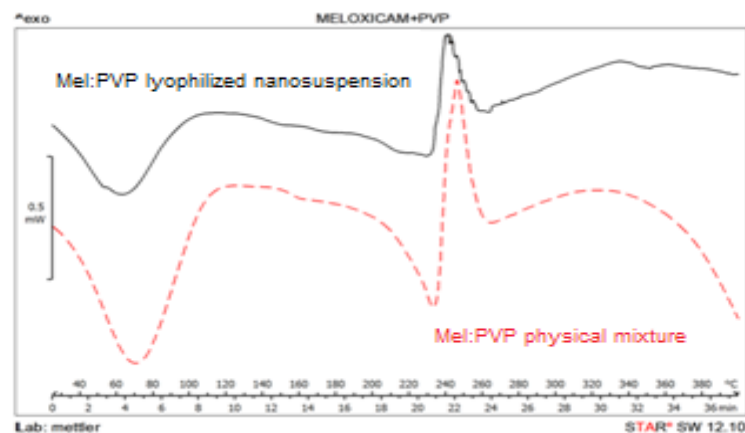
Figure 4. The thermal behavior of Mel:PVP (1:1=m:m) physical mixture and lyophilized nanosuspensions.

one. The melting temperatures and enthalpies were 233.14 ºC and 229.19ºC respectively, and 66.66 mJ/g and 0.51 mJ/g respectively.

Meloxicam dissolution profile

The dissolution profiles of the 17 formulations are presented in Figure 5 as the percentage of dissolved Mel as a function of time during the first 30 minutes of dissolution.

The influences of technological factors on the percentages of dissolved Mel at certain times are shown in Figure 6 as histograms.

Optimization of the sonication process

The optimization software generated the conditions for the obtention of the smallest crystals with the lowest polydispersity index: 100% amplitude, sonication for 45 minutes and cycle 1.
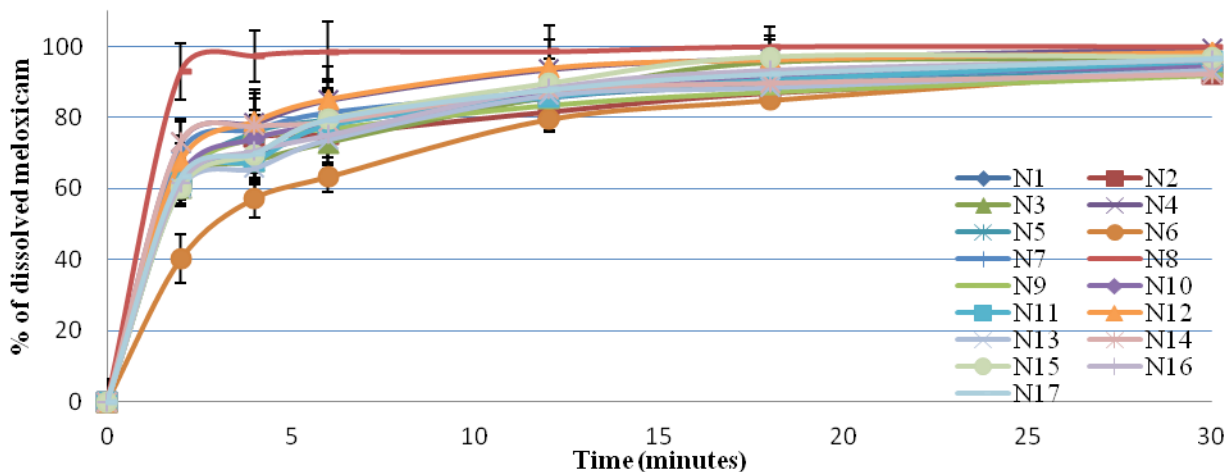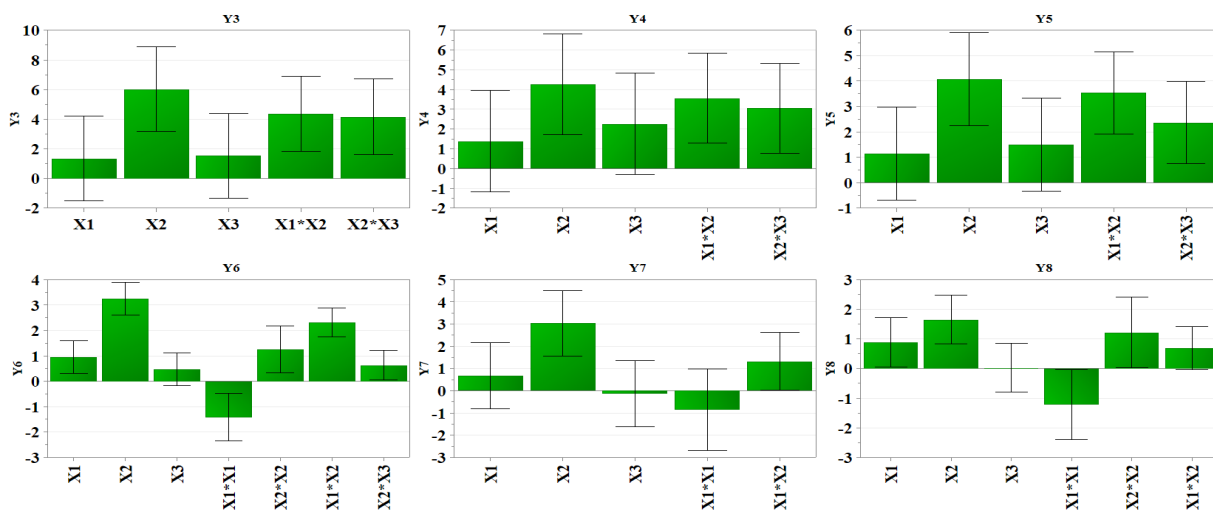
Figure 5. The percentages of dissolved meloxicam.

Figure 6. The influence of technological factors on the % of meloxicam dissolved after 2 minutes (Y_3), 4 minutes (Y_4), 6 minutes (Y_5), 12 minutes (Y_6) 18 minutes (Y_7) and 30 minutes (Y_8).
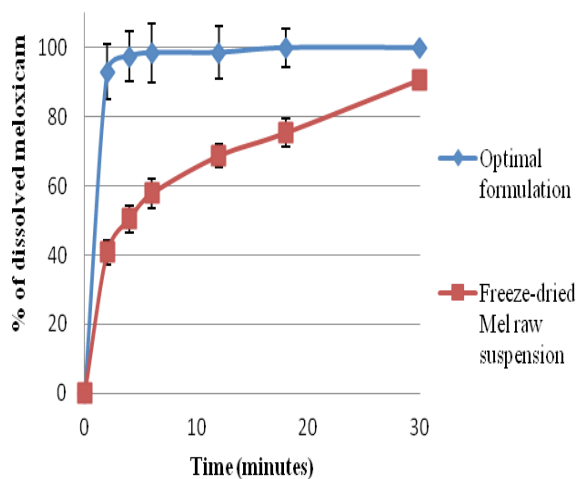

Figure 7. The dissolution of the freeze-dried optimal formulation compared to the freeze-dried meloxicam raw suspension

After freeze-drying the optimal formulation, it presented an average size of 645.7±29.66 nm and a polydispersion index of 0.543±0.07. Its dissolution profile compared to the one corresponding to the Mel freeze-dried raw suspension is presented in Figure 7.

Discussion
Experimental design analysis. Quality of fit

The reliability of the experimental design was checked through the determination of the following statistical parameters: R^2, Q^2 and the ANOVA test. R^2 indicates the variation fraction of the response explained by the model and Q^2 the variation fraction of the response that can be predicted by the model; their values are between 0 and 1, but higher ones indicate a good model with good predictive power [13]. In this particular case, the regression coefficient R^2 ranged between 0.714 and 0.964, meaning that the model explained at least 71% of the response variance. The Q^2 ranged between 0.317 and 0.634, which

showed acceptable predictive power for the model. The chosen confidence level was 95% and the models for all responses showed high significance with *p*-values below 0.05.

Nanocrystal size. The influence of process factors on the nanocrystal size

The ultrasonic processor generates longitudinal mechanical vibrations by means of reversed piezoelectric effect with a frequency of 30 kHz. The sound waves that propagate into the suspension result in alternating high-pressure and low-pressure cycles, with rates depending on the frequency. During the low pressure cycles, the waves create small voids into the liquid which collapse when they attain a high volume, during the high pressure cycle. The cavitation phenomenon produces intense heating, high pressures and high particle mobility which further make possible large particles colliding and surface erosion that result into particle size reduction [14].

The amplitude is the rate at which the pressure lowers and increases at each stroke. Higher amplitudes mean higher cavitation effects, thus a more intense destructive effect on suspended solid particles [14]. The cycle or pulse mode indicates the power discharge: continuously for 1 or interrupted for 0.5 and 0.75 (power discharge for 0.75 seconds, followed by 0.25 seconds pause). The interrupted application of ultrasounds proved to be effective in emulsification processes, so the same technique was tested with the nanocrystal formation [15].

The size of raw Mel without previous treatment was measured on the stock suspension. The size was as declared in the producer's quality certificate and the PdI indicated a highly polydisperse sample. Thus, the process resulted in a decrease from 26.31% to 14.14% of the initial size.

The effect of the studied factors and their interactions on the responses are reflected by the coefficients of the equations used to fit the experimental data. They are represented graphically as histograms. The most influential factor on the crystal size was the time, as seen in Figure 2; the increased time of sonication determined the size reduction. The amplitude increase from 40% to 100% induced more pronounced nanonization, so the amplitude was the second influencing factor. The applied pulse mode had no significant influence on the size.

Other researchers reported sonication at 30 to 70% amplitude, which resulted in the decrease of crystal size and increase in temperature [7,15]. They had to limit the amplitude values so that the produced heat would not determine the melting of the active principles. In our case the temperature was maintained at 25-30°C with the ice bath, so meloxicam could not be damaged.

Polydispersity index. The influence of process factors on the polydispersity index

The PdI indicates the width of the sizes distribution. When compared to the initial suspension, the dispersion degree of the crystal size diminished significantly. It was strongly influenced by the process parameters, as seen in Figure 2, first by the amplitude and then by time. The amplitude increase determined higher homogeneity with lower PdI. A longer processing time produced the crystal erosion with size decrease, the detachment of aggregates and thus the decrease of polydispersity [16].

A double effect was seen from the variation of the cycle value. Its increase determined higher PdI, so lower cycle values could be correlated to higher homogeneity.

Nanocrystal morphology and thermal behavior

Raw Mel consisted in individual or agglomerated crystals with angular or prismatic shapes and wide size distribution. The crystals obtained after sonication were regular sized with more roundish shapes and polished surfaces, probably due to the erosive phenomenon that took place during the process [17]. The size distribution also significantly narrowed.

The small value of the melting enthalpy of size-reduced meloxicam in the mixture with PVP after lyophilization (0.51 mJ/g) as compared to the physical mixture (66.66 mJ/g) demonstrates that after the ultrasound mediated size reduction and lyophilization process of Mel with PVP, the active ingredient converts in a form that needs a smaller quantity of energy for melting.

Meloxicam dissolution profile

The size reduction of Mel crystals was done to improve its dissolution profile at the preparation of freeze-dried orodispersible tablets. Usually the preparation of such dosage forms requires the addition of matrix forming polymers and cryoprotectants, which have been reported to alter the dissolution profiles of the active principles. For that reason, in this case PVP that acted as stabilizer for the suspension was the polymer that also formed the freeze-dried matrix.

During the dissolution study, samples were redrawn every 2 minutes and then more rarely until the end – at 30 minutes when almost all the active principle – between 91.78% and 99.50% was dissolved. The highest dissolution rate was obtained for the formulation N8 prepared by sonication for 45 minutes, at 100% amplitude and cycle 1; after 2 minutes 92.08% of the Mel was already dissolved. The same formulation N8 displayed the smallest average size, which confirmed the strong correlation between the dissolution behavior and the crystal sizes.

The dissolution percentage was mostly influenced by the sonication time, as presented in Figure 5. The longer time determined smaller crystals and higher dissolved Mel percentage. A significant interaction was found between the amplitude (X_1) and time (X_2), meaning that the sonication at a high amplitude for a long time produces crystals that dissolve faster. The second factor (X_2) – the time and the third factor (X_3) – the ultrasound pulse interacted with the increase in the dissolved percentage. Apparently, the continuous sonication for a long time – 45 minutes - leads to higher Mel dissolution.

Optimization of the sonication process

The statistical analysis led to the correlation between factors and responses as polynomial equations. The size of the crystals and their polydispersity index were highly influenced by the process parameters, therefore the optimal sonication parameters were generated in order to minimize the average size and polydispersity index.

The generated conditions were identical to the ones applied for the N8 formulation. The suspension was sonicated and freeze-dried according to the same lyophilization cycle. The average size was, as predicted, in the range between 626.099 nm and 674.205 nm. The PdI also was in the estimated interval, between 0.515 and 0.548. The model was validated by these results.

Figure 6 shows the difference between the dissolution profile of the optimal freeze-dried formulation - N8 compared to the freeze-dried raw suspension. After 2 minutes, the dissolution of Mel from the optimal sonicated formulation was almost complete – 92.92%, while from the stock suspension it was 40.73%. After 30 minutes, the drug from the optimal formulation was completely dissolved – 99.95%, while from the stock suspension the percentage was 90.74%. These results show that Mel crystals processed by ultrasound method have significantly improved dissolution with high impact on the drug release from the final dosage form.

Conclusions

This study showed that the application of ultrasounds on suspended drug particles can lead to the obtention of nanosized crystals in accurately controlled conditions. The chosen technological variables were of great importance to the process efficiency. High amplitudes applied continuously for a long time gave satisfying results. The most efficient combination of variables that led to the optimal formulation was the sonication for 45 minutes at 100% amplitude and cycle 1, which resulted in a suspension containing 645.7±29.66 nm Mel crystals with 0.543±0.07 polydispersity index and fast drug dissolution.

These results represent the basis of the development of novel oral lyophilisates formulations with improved Mel dissolution.

References

1. Iurian S, Tomuta I, Leucuta SE. Formulation of orodispersible tablets containing meloxicam and their *in vitro* and *in vivo* characterization. Farmacia. 2014;62(6):1097-1108.
2. Lai F, Pini E, Angioni G, Manca ML, Perricci J, Sinico C, et al. Nanocrystals as tool to improve piroxicam dissolution rate in novel orally disintegrating tablets. Eur J Pharm Biopharm. 2011;79:552-558.
3. Gadadare R, Mandpe L, Pokharkar V. Ultra Rapidly Dissolving Repaglinide Nanosized Crystals Prepared via Bottom-Up and Top-Down Approach: Influence of Food on Pharmacokinetics Behavior. AAPS PharmSciTech. 2014:1-13 DOI: 10.1208/s12249-014-0267-8.
4. Müller RH, Gohla S, Keck CM. State of the art of nanocrystals–special features, production, nanotoxicology aspects and intracellular delivery. Eur J Pharm Biopharm. 2011;78:1-9.
5. Keck CM, Muller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur J Pharm Biopharm. 2006;62:3-16.
6. Kurti L, Kukovecz A, Kozma G, Ambrus R, Deli MA, Szabo-Revesz P. Study of the parameters influencing the co-grinding process for the production of meloxicam nanoparticles. Powder Technol. 2011;212:210-217.
7. Bartos C, Szabo-Revesz P, Ambrus R. Optimization of technological parameters by acoustic cavitation to achieve particle size reduction. Farmacia. 2014;62(1):34-47.
8. Loza E. Systematic review: is the use of NSAIDs effective and safe in the elderly? Reumatol Clin. 2008;4:172-182.
9. Turck D, Roth W, Busch U. A review of the clinical pharmacokinetics of meloxicam. Br J Rheumatol. 1996;35:13-16.
10. Cristancho DM, Martinez F. Solubility and preferential solvation of meloxicam in ethyl acetate + ethanol mixtures at several temperatures. J Molecular Liquids. 2014;200:122-128.
11. Patil MN, Pandit AB. Cavitation – a novel technique for making stable nano-suspensions. Ultrason Sonochem. 2007;14:519-530.
12. Pinjari DV, Prasad K, Gogate PR, Mhaske ST, Pandit AB. Intensification of synthesis of zirconium dioxide using ultrasound: Effect of amplitude variation. Chem Eng Process Process Intensif. 2013;74:178-186.
13. Eriksson L, Johansson E, Kettaneh-Wold N, Wikstrom C, Wold S. Design of Experiments: Principles and Applications. Umea: Umetrics Academy; 2000.
14. *** Technical Note Hielscher T. Ultrasonic production of nano-size dispersions and emulsions. Available from: http://pdf.directindustry.com/pdf/hielscher/ultrasonic-production-nano-size-emulsions-dispersions-hielscher/24814-379929.html
15. Ramisetty KA. Pandit AB. Gogate PR. Ultrasound assisted preparation of emultion of coconut oil in water: Understanding the effect of operating parameters and comparison of reactor designs. Chem Eng Process Process Intensif. 2015;88:70-77.
16. Bartos C. Kukovecz A. Ambrus R. Farkas G. Radacsi N. Szabo-Revesz P. Comparison of static and dynamic sonication as process intensification for particle size reduction using a factorial design. Chem Eng Process Process Intensif. 2015;87:26-34.
17. Jordens J, De Coker N, Gielen B, Van Gerven T, Braeken L. Ultrasound precipitation of manganese carbonate: The effect of power and frequency on particle properties. Ultrason Sonochem. 2015;26:64-72.