

Comparison of static and dynamic sonication methods for particle size reduction, using a factorial design

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ABSTRACT

This article reports on particle engineering by a top-down method involving the organic solvent-free acoustic cavitation. The effects on particle size reduction of static and dynamic sonication methods were compared. The process parameters (volume, position and amplitude of sonotrode, concentration of drug, temperature, sonication time rpm of pump) were optimized by factorial design plan for particle size distribution of meloxicam (MEL) as response factor after sonication. It was found, that in case of the static sonication small sample volume, high amplitude and long sonication time influenced principally on the particle size reduction. Interactions were observed between amplitude of sonication, position of sonotrode and volume. By the dynamic sonication low rpm of pump, high amplitude and long sonication time were the dominant parameters. There was a correlation between the increased amplitude and temperature. Grinding with optimized process parameters resulted in 10.16 μm average particle size with static, and 14.60 μm with dynamic sonication. Samples sonicated with appropriate process parameters, were dried and characterized. During the solid state analysis

scanning electron microscopy (SEM) images showed, that the sonication resulted in a rounded habit of the particles. The thermoanalytical (DSC) and X-ray powder diffraction (XRPD) characterization displayed the crystalline structure of MEL in both cases. The FT-IR images demonstrated that no chemical degradation occurred.

The static sonication is recommended for development of preclinical samples, the dynamic sonication is suitable for scale-up of the static method.

Introduction

Particle design techniques have been developed to modify the physico-chemical and biopharmaceutical properties of the drug. The solubility, dissolution properties and permeability of drugs are of great importance determining their bioavailability. Number of possibilities are available in order to solve these problems, such as salt formation, complexation with cyclodextrins, crystallization, amorphization, milling etc. Particle engineering techniques can offer solutions for improvement of solubility, dissolution rate and permeability of poor water-soluble drugs [Rita GEM]. The qualified crystal-size, distribution and morphology can open a new, alternative administration route, e. g. intranasal and pulmonary route, where the particle size is determining factor [e, f, g,régi introd].

Acoustic cavitation is a novel possibility to modify the properties of particles, primarily to decrease particle size [IBU7-9]. It has the ability to erode and break down particles and increase the specific surface area [11]. Particle design uses ultrasound power in the frequency range 20-100 kHz to induce particle size reduction [12]. During sonication, the sound waves that form into the liquid media result in alternating high-pressure and low-pressure cycles, with rates depending on the frequency. During the low-pressure cycle, high-intensity ultrasonic waves evolve small vacuum bubbles or voids in the liquid. When the bubbles reach a volume at which they can no longer absorb energy, they collapse violently during a high-pressure cycle. This phenomenon is called ultrasonic cavitation [13].

Sonication is a mechanical process, which aims at achieve particle size reduction and uniform size distribution. There are two possibilities of wet grinding for particle size reduction using sonication. One of them is the static method, whereby the sample at rest is sonicated. Another possibility is the dynamic method which allows the continuous circulation of the sample using a peristaltic pump during the sonication. Ultrasound is applied in industry for preparation of metal nanoparticles [nanoparticles uh wave]. Sonochemistry involves the

use of ultrasound technique to promote chemical reactions [sonochem]. Ultrasound-assisted extraction is used as a strategy focused on sample preparation for metal determination in biological samples [biological sample]. The synergistic effect of ultrasound combined with UV is applied for secondary effluent disinfection [desinfition], and in combination with heat and low pressure ultrasound is used as an alternative pasteurization treatment [pasztöriz] etc.

On the field of pharmaceuticals, power ultrasound is applied for emulsification and for sedimentation of emulsions and suspensions [szakdoga 12-13]. Supercritical, solvent diffusion and melt emulsification (bottom-up) methods in the field of sonocrystallization are well known to solve solubility problems of drugs [Rita GEM]. Disintegration of drug particles (top-down approach) was not found in the professional literature.

Meloxicam (MEL) belongs to NSAIDs, and has anti-inflammatory, analgesic and antipyretic effect. We chose MEL as a model crystalline drug because of its poor (water-)solubility and high melting point (270 °C) [MEL melting point]. Co-grinding, as a dry milling procedure is well known to decrease particle size of MEL [Csaba. Levi].

Our aims were to compare the static and the dynamic sonication methods (as organic solvent-free, wet grinding techniques) and their effects on particle size reduction of MEL, using PVP K-25 as an agglomeration inhibitor. We optimized the process parameters using two-level fractional factorial design and studied the effects of power ultrasound on the physico-chemical properties of MEL.

2. Experimental

2.1. Materials

MEL (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2Hbenzothiazine-3-carboxamide-1,1-dioxide) was obtained from EGIS Ltd. (Budapest, Hungary). The grinding additives, PVP K-25 (polyvinylpyrrolidone) was purchased from BASF (Ludwigshafen, Germany).

2.2. Methods

2.2.1 Preparation of sonicated formulations

A power ultrasound device (Hielscher UP 200S Ultrasonic processor with 200 W, Germany) was applied for energy input in the sample preparation. In each sample 0.5% of PVP K-25 was dissolved as a stabilizer, which formed hydrogen bond with MEL and reduce the agglomeration of the hydrophobe particles. Before sonication the suspensions were stirred with a magnetic stirrer for 5 minutes.

Samples at rest were treated in the case of static sonication. In the case of dynamic sonication, samples were circulated continuously during the sonication with a peristaltic pump (Heidolph PD 5006 Pump drive) in a double-walled flow cell (Flow Cell GD14K). The temperature was set with thermostat in both cases (Julabo, Germany).

The investigated parameters of sonications are given in Table 1 and Table 2.

Volume (ml)	25; 100
Position*	0.25; 0.75
Concentration of MEL (mg/10ml)	20; 180
Temperature (°C)	0; 36
Amplitude (%)	30; 70
Time (min.)	10; 30

*Position of sonotrode: 0.25: the sonotrode immersed to $\frac{3}{4}$ of the total height of the liquid

0.75: the sonotrode immersed to $\frac{1}{4}$ of the total height of the liquid

Pump (rpm)	50; 100
Concentration of MEL(mg/10ml)	20; 180
Temperature (°C)	0; 36
Amplitude (%)	30; 70
Time (min.)	10; 30

Preparation of solid products

Suspensions prepared with the optimized parameters were filtered using filter paper (MUNKTELL Filter Discs, Grade: 1290, Dia: 185 mm) and the filtrate was dried in a vacuum

dryer (Binder, Germany) at 40 °C in order to obtain solid products. After drying, the physico-chemical properties of the products were investigated.

2.2.2 Determination of particle size distribution by laser diffraction method

The volume particle size distribution of raw MEL was measured by laser diffraction (Mastersizer 2000, Malvern Instruments Ltd, Worcestershire, UK) with the following parameters: 300RF lens; small volume dispersion unit (2500 rpm); refractive index for dispersed particles 1.720; refractive index for dispersion medium 1.330. The MEL particle size was determined immediately on the initial water suspension, in which PVP was dissolved. After drying, the solid product was resuspended in water using an ultrasonic bath for 5 min. Due to the filtration, immediately after the sonication, PVP remains in the solution, and therefore only MEL particles were detected. Water was used as dispersant and the obscuration was in the range 11-16% for each measurement in both cases. In all cases the average size volume distribution, D 0.1, D 0.5, and D 0.9 were determined and evaluated.

2.2.3 Image analysis

The shape and surface characteristics of the samples were visualized by using a scanning electron microscope (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan). Briefly, the samples were sputter-coated with gold-palladium under an argon atmosphere, using a gold sputter module in a high-vacuum evaporator and the samples were examined at 15 kV and 10 μ A. The air pressure was 1.3-13.0 mPa.

2.2.4 Design of experiments

Six parameters were screened in the static sonication experiments using a two level fractional factorial design of resolution III. Here the main effects are not confounded with each other but they are confounded with two-factor interactions. This design is typically used to quickly identify the main effects governing the behavior of a multi-parameter system. High and low values for each parameter were set on the basis of our prior experience with similar tasks and are reported in Table 1. Dynamic sonication experiments were run by screening five parameters using the same experimental design type. High and low parameter values for the dynamic sonication experiments are presented in Table 2.

2.2.5 Further investigations of the optimized products

X-ray powder diffraction analysis (XRPD)

The physical state of IBU in the samples was evaluated by X-ray powder diffraction (XRPD). XRPD patterns were produced with an X-ray Diffractometer Miniflex II (Rigaku Co. Tokyo, Japan), where the tube anode was Cu with $K\alpha = 1.5405 \text{ \AA}$. The pattern was collected with a tube voltage of 30 kV and a tube current of 15 mA in step scan mode (4° min^{-1}). The instrument was calibrated by using Si.

Differential scanning calorimetry (DSC)

DSC measurements were carried out with a Mettler Toledo DSC 821^e thermal analysis system with the STAR^e thermal analysis program V9.0 (Mettler Inc., Schwerzenbach, Switzerland). Approximately 2-5 mg of pure drug or product was examined in the temperature range between 25 °C and 300 °C. The heating rate was 5 °C min⁻¹. Argon was used as carrier gas at a flow rate of 10 l h⁻¹ during the DSC investigations.

Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra were recorded with a Bio-Rad Digilab Division FTS-65A/896 FTIR spectrometer (Bio-Rad Digilab Division FTS-65A/869, Philadelphia, USA) between 4000 and 400 cm⁻¹, at an optical resolution of 4 cm⁻¹ operating conditions: Harrick's Meridian SplitPea single reflection, diamond, ATR accessory. Thermo Scientific GRAMS/AI Suite software (Thermo Fisher Scientific Inc., Waltham, USA) was used for the spectral analysis.

3. Results and discussion

3.1. Effects of process parameters on particle size distribution

3.1.1 Static sonication

An analysis of the results measured by laser diffraction revealed that the static sonication resulted in roughly 25-70% decrease in the average MEL particle size, regarding to the sonication parameters. The D 0.1, D 0.5 and D 0.9 values are reported in Table 3. Their relationship with the sonication variables is analyzed quantitatively on the basis of D 0.5 figures in the main effects plots (Fig 1) and interaction plots (Fig 2). The figures of D 0.1 and D 0.9 would provide similar results. A main effect plot for a given parameter is obtained by

[AK1] megjegyzést írt: Nekem kicsit soknak tűnik 4 mélységig tagolni egy ekkora cikket.

[AK2] megjegyzést írt: Egészen biztos, hogy nem kellene mindkét módszernek mindegyik D szinten mindkét ábráját berakni. Ez összesen 12 ábra lenne, aminek szerintem nem sok értelme van. Én azt javasolnám, hogy válasszunk ki EGY D szintet (Én D0.5-öt választanám, de Önök tudják hogy az jó-e tényleg vagy D0.9 kell inkább?), és azon az egy szinten mutassuk meg az ábrákat, összesen 4 darabot a 12 helyett. Az ábrákat mindenképpen újra kell csinálnom (barna háttér eltüntetés, felirat pontosítás, méretezés stb), kérem írják meg hogy melyikeket választják, mert azokat csinálom meg rendesen.

averaging the results of each run where this parameter was set to low or to high and connecting these averages with a line. If the studied parameters are independent, the plot gives a clear indication of response of the system to the selected variable. The main effects plots for the MEL particle size distribution indicate that small sample volume, high amplitude and long sonication time were preferred for efficient particle size reduction, whereas the position of the sonotrode, the concentration of the solution and the temperature influenced the particle size less.

Interaction plots shows the effects between variables, which are not necessarily independent by showing the means of the responses for each level of a factor for each level of a second factor pairwise for all factors involved in the study. The interaction plots presented in Fig 2 for the D 0.5 furthermore can be used to gain insight into the complex interactions between the sonication parameters. Increased sonication time resulted in a particle size reduction, independently of the other process parameters. The context between the physical parameters of sonication (amplitude, position, volume) was unequivocal. High amplitude resulted in a particle size decrease independently of the sonotrode-position and sample-volume. The context between the chemical parameters (concentration, temperature) and amplitude shows that the particle size reduction effect of the increased amplitude occurred at low concentration and high temperature. The relationship between temperature and concentration, and temperature and volume was unidirectional, in contrast with temperature-position relationship: increase of the temperature was useful in the upper position. At high concentration the upper, at low, the under position resulted in smaller particle size.

Summarizing the results, the appropriate parameters for static sonication were long sonication time (30 min), high amplitude (70%), small sample-volume (25ml), high temperature (36 °C), upper position of sonotrode (0.25) and low concentration of MEL (20mg/10ml). In sample at rest the distribution of sonication effect was inhomogeneous and near the sonotrode (therefore in small volume) was the most effective. The increased amplitude—due to the large energy investment and long sonication time—because of the sustained energy exposure—was required to achieve the small particle size. With the raising of the temperature, the kinetic energy of the particles increased, which affected adversely to cohesive forces. In case of using low concentration of MEL, the amount of energy per particle was greater.

Table 3. Results of the static sonication

Volume (ml)	Position of sonotrode	Conc (mg/10ml)	Temp (°C)	Amplitude (%)	Time (min.)	D0.1 (µm)	D0.5 (µm)	D0.9 (µm)
25	0.25	20	36	70	30	1.51	10.16	19.53
100	0.25	20	0	30	30	4.81	23.07	46.88
25	0.75	20	0	70	10	2.75	18.45	42.87
100	0.75	20	36	30	10	5.92	26.52	53.39
25	0.25	180	36	30	10	3.95	19.62	41.51
100	0.25	180	0	70	10	5.19	24.16	46.98
25	0.75	180	0	30	30	3.53	17.12	29.22
100	0.75	180	36	70	30	7.19	20.83	36.62

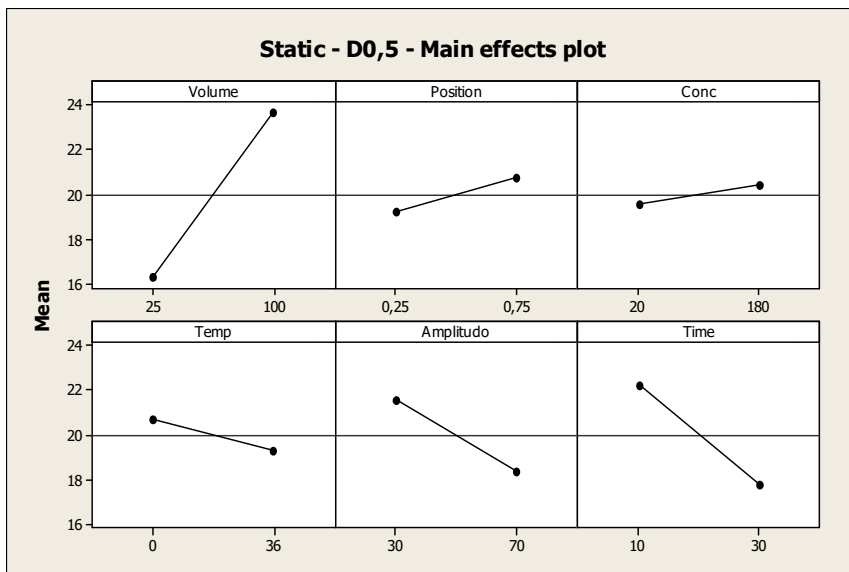


Fig.1 The main effect plots of the static sonication

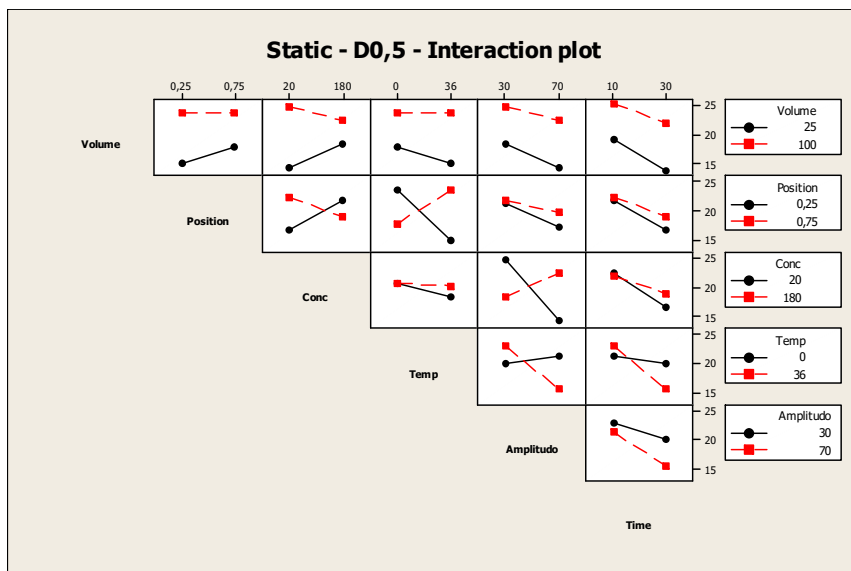


Fig. 2 Interaction plots of the static sonication

3.1.2 Dynamic sonication

An analysis of the results measured by laser diffraction revealed that the dynamic sonication resulted in roughly 15-60% decrease in the average MEL particle size, regarding to the sonication parameters. The size distribution function is reported in Table 4 and their relationship with the sonication variables were analyzed quantitatively on the basis of D 0.5 figures in the main effects plots (Fig 3) and interaction plots (Fig 4). The figures of D 0.1 and D 0.9 would provide similar results. The main effects plots for the MEL particle size distribution indicate that from the investigated parameters, circulation of the sample at low rpm, high amplitude and long sonication time resulted in the most significant particle size reduction, whereas the concentration of the suspension influenced the particle size less. High temperature had more significant effect on particle size under dynamic sonication than when using static sonication.

The interaction plots presented in Fig 4 for the D 0.5, furthermore can be used to gain insight into the complex interactions between the sonication parameters. Increased amplitude and temperature resulted in significant particle size reduction. The long sonication time had

no high effect in case of the low temperature and low amplitude. The increase of the concentration had adverse effect on the particle size at low circulation rate.

In conclusion we can say, that the optimized process parameters for dynamic sonication were long sonication time (30 min.), high amplitude (70%) and temperature (36 °C), low circulation rate (50 rpm) and low concentration of the samples (20 mg/10ml). Due to the continuous circulation of the samples, the distribution of sonication effect was homogeneous. The cavitation reduced efficiently the particle size at low circulation rate. The sample was resident longer in the cavitation space during one sonitacion cycle. The explanation of the other parameters is the same as for the static method.

Table 4. Results of the dynamic sonication

Pump (rpm)	Conc (mg/10ml)	Temp (°C)	Amplitude (%)	Time (min.)	D0.1(μm)	D0.5(μm)	D0.9(μm)
50	20	36	70	30	2.20	14.60	35.02
50	20	0	30	30	4.56	24.22	47.05
100	20	0	70	10	5.70	26.90	51.92
100	20	36	30	10	5.90	26.15	52.20
50	180	36	30	10	4.40	22.69	53.54
50	180	0	70	10	6.27	23.54	46.77
100	180	0	30	30	9.06	29.31	45.58
100	180	36	70	30	2.87	16.73	38.03

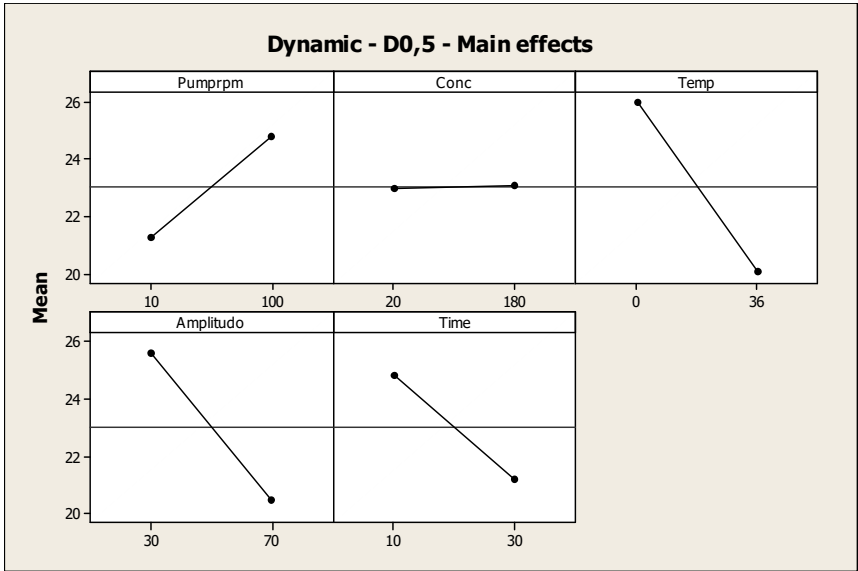


Fig. 3 The main effect plots of the dynamic sonication

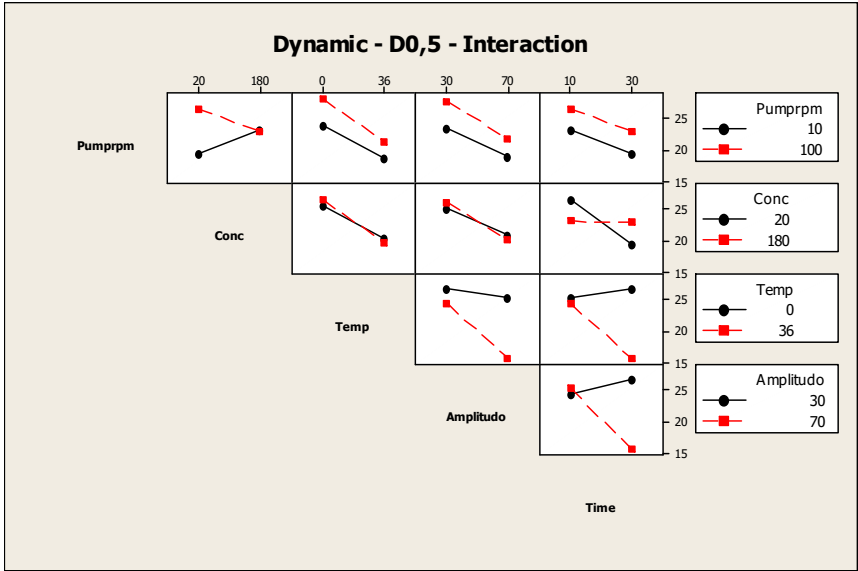


Fig. 4 Fig. 2 Interaction plots of the dynamic sonication

3.2 Characterization of dried product

3.2.1 Particle size distribution

The change in average particle size on drying was not significant as compared with the suspension of samples: aggregation did not occur. (MALVERN, fajlagos felület mindkét módszernél)

3.2.2 SEM

The SEM pictures (Fig. 5) provided an indication of the morphology of the modified particles. The crystal habit of pure MEL changed significantly. The raw MEL consisted mainly of angled crystals with a broad focal size distribution. The dried product comprised irregular-shaped, roundish crystals with an average size of 10 μm in case of the static sonication and 15 μm in case of the dynamic sonication. The crystal lattice of MEL demonstrated defects and cracks, along which the crystals were disintegrated due to acoustic cavitation. This factor accounted for the relatively rough surface. The cracking (due to the energy investment) and the surface dissolution? were responsible for the roundness of the particles. The SEM images show the small broken pieces of the crystals on the surface of the bigger particles (Oldékonyság!!!!!!).

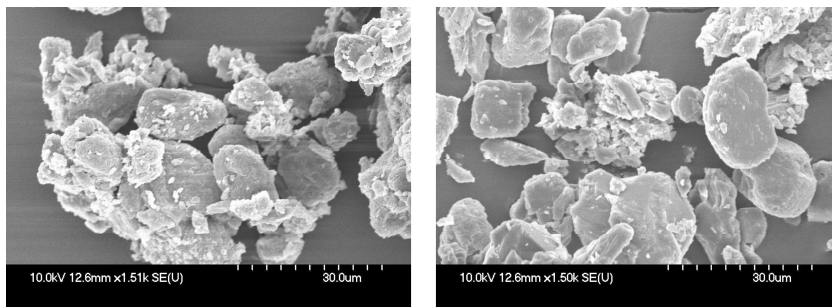


Fig. 5 SEM picture of raw MEL(HIÁNYZIK) (A), and dried products (B, C)

3.2.3 X-ray diffraction

The XRPD pattern of pure MEL demonstrated its crystalline structure, as expected. The characteristic 2θ data were following: 13.22, 15.06, 26.26 and 26.67. The raw MEL and the

sonicated dried MEL composite in both cases displayed same X-ray diffraction patterns (Fig. 6). This means that the crystalline form of the micronized MEL was not changed by the sonication and drying procedure.

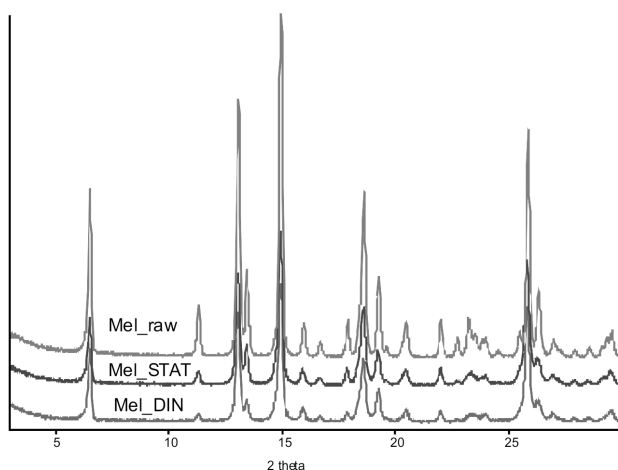


Fig. 6 XRPD examination of raw MEL and dried products

3.2.4 DSC

DSC was employed to investigate the crystallinity and the melting of MEL in the pure form and in the both sonicated dried products. The DSC curve (Fig. 7) of pure MEL revealed a sharp endothermic peak at xxx °C, which is its melting point, confirming its crystalline structure. The DSC curves exhibited sharp endothermic peak of the MEL after drying in the static at 258.62 and in the dynamic at 259.81 °C, indicating that the crystallinity of the drug was retained.

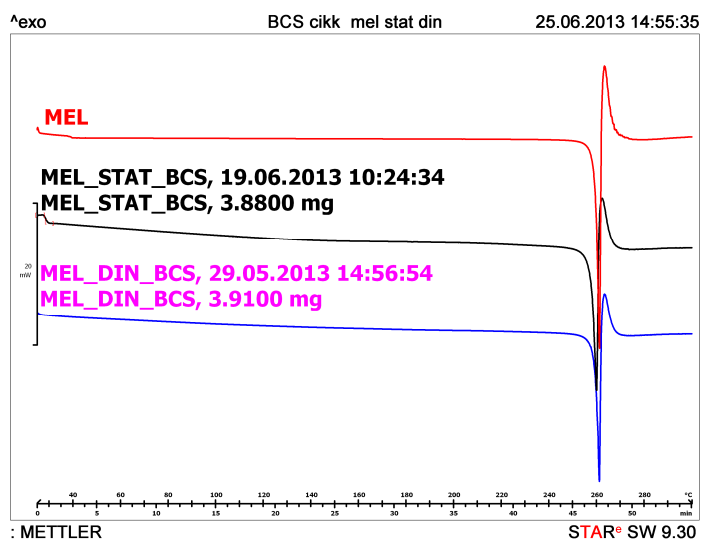


Fig. 7 DSC curves of raw material and of the products

3.2.5 FT-IR

To determine any decomposition occurred during the sonication process, FT-IR spectroscopy was carried out. This proved that no disintegration took place in the samples (Fig. 8). The characteristic bands of MEL were seen in all of the curves of the raw MEL and sonicated products, which appeared at 3289.76, 1550.04, 1530.36, 1346.73, 1265.88, 1184.90 cm^{-1} , respectively, denoting stretching vibration of, -NH, the thiazole ring (together with 1184.90 cm^{-1}), the amide II band of -CO-NH-C, the asymmetry stretching vibration of sulfone and amide III band of -CO-NH-C [FT-IR értékelés].

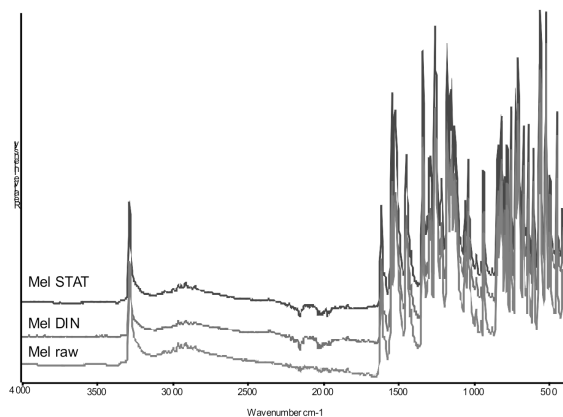


Fig. 8 FT-IR curves of the raw MEL and sonicated products

4. Conclusions

The top-down method can be applied to decrease the particle size and change the crystal habit of the drugs.

This study has compared by two-level factorial design plan the process parameters of the static and the dynamic sonication methods, based on their particle size reduction effects. Long sonication time, high amplitude, high temperature and low concentration of MEL had important role at sonication procedures. Both disintegration methods with optimized process parameters, involving a change in crystal habit, may decrease the MEL particle size to the micrometer range with presence of PVP as an additive (Table 5).

Table 5. Optimized parameters of the static and dynamic sonication

	Vol. (ml)	Pos. of son.	Pump (rpm)	Conc (mg/10ml)	Temp. (°C)	Amp. (%)	Time (min.)	D0.1 (µm)	D0.5 (µm)	D0.9 (µm)
Stat.	25	0.25	-	20	36	70	30	1.51	10.16	19.53
Dyn.	100	-	50	20	36	70	30	2,20	14,60	35,02

The examination of the dried products showed, that in both cases during the sonication the crystallinity of MEL was retained and the process did not cause chemical degradation.

The static method is applicable for preparation of preclinical sample with reduced particle size of drug candidate, for which the small sample volume is sufficient.

The dynamic sonication is suitable for scale-up, larger volume of samples could be used by this method. It has possibility for standardization and this is important for the industry.

These two methods are appropriate for producing of intermediate (suspension) and (after drying) dried product for additional pharmaceutical formulations.

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