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Research Article

INFLUENCE OF STABILIZERS IN MELOXICAM NANOCRYSTAL FORMATION AND ITS APPLICATION ON SUSPENSION ORAL DOSAGE FORM

Magdalena Yuni Kristanti [1,2*)], Rachmat Mauludin[2], Heni Rachmawati[2]

[1]PT.Kalbe Farma, Tbk,
Jl. MH Thamrin Blok A3-1
Kawasan Industri Delta
Silicon, Lippo Cikarang
Bekasi 17550
[2]School of Pharmacy,
Bandung Institute of
Technology, Ganesha10
Bandung 40132

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*Corresponding author
Magdalena Yuni Kristanti

Email :
magdalena.yunik@gmail.co.id

ABSTRACT

Meloxicam is a non steroid anti inflammatory drug that is classified as Biopharmaceutics Classification System (BCS) class II. Meloxicam is poorly soluble in water, therefore its solubility would be the rate limiting step for drug absorption. This study was conducted to improve meloxicam solubility using nanotechnology approach. Meloxicam nanocrystal was prepared using high pressure homogenization technique. Several stabilizers were investigated for suitable nanocrystal production. Formulation of suspension on the meloxicam nanocrystal was developed. Short physical stability was performed to assess the potential use of the stabilizer. Nanocrystal containing 10% meloxicam and 5% PVP K25 was formed faster with better physical stability compared to other stabilizers (xanthan gum, HPMC 2910 type 603 dan 645). Meloxicam nanocyrstal suspension containing meloxicam nanocrystal with stabilizer 5% or 10% of PVP K25 showed excellent particle size stability (with particle size 466.6nm and 486.9nm) and dissolution rate compared to reference product (without nanonization). Particle size and dissolution rate of meloxicam nanocrystal suspensions (containing 5% or 10% of PVP K25) were stable after storage for 30 days at room temperature. Kinetic solubility of meloxicam nanocrystal was three times higher than that of meloxicam. According to XRD profile, there was no differences in crystallinity between meloxicam and meloxicam nanocrystal.

Key words: meloxicam, high pressure homogenizer, nanocrystal, dissolution rate, kinetic solubility

INTRODUCTION

Numbers of new drugs were developed and become challenge for pharmacy scientist to develop products with good bioavailability because of those poor solubility. Some of the traditional approaches could overcome such solubility factors but resulting bioavailability problems. In this respect, nanoparticle technology could become promising solution. The major advantages of this technology are its general applicability to most drugs and its simplicity (Mauludin, *et al.,* 2008).

Meloxicam [4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2H -1,2-benzothiazine- 3-carboxamide -1,1-dioxide], a nonsteroidal anti-inflammatory drug (NSAID), is an enolic oxicam derivative that included in BCS class II. Meloxicam has a low solubility in aqueous media and low dissolution rate, which are limiting step to its absorption (Ambrus, *et al*., 2009). It has been used for rheumatoid arthritis, osteoarthritis treatment. The anti-inflamation mechanism is by inhibiting cyclooxygenase (most of cyclooxygenase 2) and then inhibiting prostaglandin formation.

Several techniques have been reported to enhance the meloxicam dissolution, such as nanosizing by emulsion-diffusion method (Ambrus, *et al.,* 2009), nanosizing using co-grinding for nasal powder (Kürti, *et al.,* 2011), hot-melted method with premiling meloxicam (Nassab, *et al.,* 2006), meloxicam-cyclodextrin complex (Naidu, *et al.,* 2006). All these reports have been established to improve meloxicam solubility in solid dosage form where physical particle interaction leading to particle agglomeration is diminished. But, it is a challenge to develop meloxicam nanoparticle for suspension dosage form with maintaining particle size while enhancing dissolution rate.

Suspension is binary system consist of colloidal particles in a dispersion medium. These particles show Brownian motion and hence collide with each other frequently. The stability of colloids is thus determined by the interaction between the particles during such a collision. There are two basic interactions: one being attractive and the other repulsive. When attraction dominates, the particles will adhere with each other and finally the entire dispersion may coalesce. Van der Waals forces are the primary source of attraction between colloidal particles (Rowe, 2009). Smaller particles show more energic motion, and probability to coalesce is higher. Particles stability obtained by surrounding colloidal particles with electrical double layer (electric stabilization), polymer adsorbed on the particle surface (steric stabilization). This stabilizations are providing a long range repulsion between the particles (Rowe, 2009). Particle size stability in nanocyrstal formulation is major factor to maintain solubility and dissolution enhancement. Therefore, investigation on potential stabilizers was studied with focusing on the process efficiency and the particle size with narrow distribution size.

Meloxicam nanocrystal was prepared using high pressure homogenization technique. Several stabilizers providing both steric and electric stabilization on the surface of nanocrystal were evaluated for suitable meloxicam nanocrystal production. Meloxicam nanocrystal would be formulated to oral suspension dosage form in order to improve its dissolution rate, and expected to achieve fast onset of the peak plasma concentration.

MATERIAL AND METHODS

Meloxicam was obtained from Changzhou Xinhua, China. PVP K25 from Danochemo, hydroxypropyl methylcellulose (HPMC 2910) type 603 and type 645 were from Shin Etsu, Japan, polysorbate 80 was from Shino Japan Chemical, Japan. Sodium lauryl sulfate (SLS) was from Cognis Indonesia. Xanthan gum was from Danisco, France. Sorbitol Sol 70% was from Roquette, France. Ethyl paraben was from Iporg. Saccharin was from Keifeng Xinhua Fine Chem, China. Sucralose® was from Guangzhou, China. Citric acid was from Tate and Lyle, Australia. And poloxamer 188 was from BASF.

Preparation of meloxicam nanocrystal

Meloxicam and the stabilizer were first dispersed in an aqueous media in the volume scale of 500mL using Turbomixer (Euro ST PB IKA® Werke) and Ultra Turax (T25 IKA® Werke) and the suspension was milled with high pressure homogenizer (Panda Plus 2000, GEA Niro Soavy, Italy). Fine suspension then processed to premilling step at 200bar and 500bar (two cycle each), continued with main milling step at 1000bar for 20cycle. Samples were taken at cycle number of 5, 10, 15 and 20. Nanocrystal characterization include particle size, particle distribution and zeta potential.

Meloxicam nanocrystal development

Preliminary experiment

In order to select the appropriate stabilizer, preliminary experiment using polysorbate 80 (1%), sodium lauryl sulfate (0.25%), xanthan gum (0.20%), PVP K25 (5%), HPMC 2910 type 603 and type 645 (5%) were performed. A solution of stabilizer in aqueous media (500mL) was mixed with meloxicam using Turbomixer and Ultraturax UT 25 for 3min. Prospective stabilizer showed good dispersion stability and sedimentation.

Study of polymer stabilizer to meloxicam nanocrystal characteristics

Meloxicam nanocrystal (containing meloxicam 10% in aqueous media) was develop using xanthan gum (0,20%), PVP K25 (5%), HPMC 2910 type 603 and type 645 (5%). Characterization of particle size were observed after main milling step at cycle number 0, 5, 10, 15 and 20. Prospective stabilizer with good particle size stability and most efficient process resulting nanoparticle was choosed.

Study of polymer concentration and addition of surfactant to meloxicam nanocrystal characteristics

The choosen stabilizer was Meloxicam nanocrystal (containing meloxicam 10% in aqueous media) was develop using xanthan gum (0,2%), PVP K25 (5%), HPMC 2910 type 603 and type 645 (5%). Characterization of particle size were observed after main milling step at cycle number 0, 5, 10, 15 and 20.

Prospective stabilizer with good particle size stability and most efficient process resulting nanoparticle was choosed.

Characterization of nanocrystal

Particle size and zeta potential determination

The particle sizes of the nanocyrstal were determined by photon correlation spectroscopy, using a Particle Size Analyzer Delsa Nano C (Beckman Coulter). Results of this technique were performe as mean particle diameter and the range of the particle-size distribution (polydispersity index, PI). Zeta potentials were determined using the same instrument Hemholtz-Smoluchowski equation.

Kinetic solubility of meloxicam

Solubility studies were performed with roller mixer. Vials contained meloxicam 0.1% in aqueous media were sealed and shaken at 45rpm for 3days in room temperature condition. After 3days, suspensions were filtered twice through PALL® 0.2µm filters (Pall Europe Limited, England, 0.293m). An filtrat from each vial was withdrawn assayed by high performance liquid chromatography to evaluate the amount of drug dissolved.

Morphology of nanocrystal

Morphology of nanocrystals determination were conducted using light microscopy and scanning electron microscope. Light microscopy was performed using Nikon Polarizing Microscope (Eclipse E501 Pol) with 400x magnification.

The surface morphology of the raw drug and nanocrystal were visualized by scanning electron microscopy (SEM). Samples were fixed onto a metallic holder and coated with gold to have a perfect image. Scanning electron microscope was used with an acceleration voltage of 10kV and a secondary detector.

Determination of drug content

Meloxicam content in nanocrystal and suspension were determined using high performance liquid chromatography (with isocratic elution system) using ultraviolet detector at 360nm and C18 column (United States, 2011).

Crystalinity evaluation

Crystalinity evaluation of meloxicam nanocrystal was evaluated using X-ray powder diffraction (XRD) and differential scanning calorimetry (DSC). The crystalinity state of meloxicam in raw material and meloxicam nanocrystal was evaluated by X-ray diffraction (PW17170 BASED, Philips) using Cu anode tube and the pattern was collected with a tube voltage of 40kV and a tube current of 30mA.

Endotermic or Exotermic profile of raw material and meloxicam nanocrystal was determined by Thermal Analysis (Shimadzu, 60-series). Sample analyzed with heating speed 10ºC/minute until 300ºC.

Meloxicam nanocrystal suspension formulation

Meloxicam nanocrystals using the most prospective stabilizer were formulated to oral suspension with concentration of 7.5mg meloxicam in 5mL suspension. Suspension base contained ethyl paraben, saccharin, xanthan gum, sorbitol sol and citric acid. Meloxicam nanocrystal suspension were store until 30 days at room temperature (30°C RH 75%) and particle size and dissolution profile were observed.

Determination of *in vitro* dissolution

Samples contained 7.5mg of meloxicam were put in to dissolution flask (900mL of phosphate buffer solution (pH 7.4±0.1) at 37±0.5°C) and then using paddle method with rotation speed of the paddles was 25rpm. At predetermined time intervals (after 5, 10, 15, 30, and 45min), 7mL samples were withdrawn and the amount of dissolved drug was determined using ultraviolet spectrophotometry method at wavelength 360nm (Rachmawati*, et al.,* 2009).

RESULT AND DISCUSSION

Preliminary stabilizer screening

The influence of different stabilizer on meloxicam dispersion was investigated using Turbomixer and Ultra Turax. Prospective stabilizers are the one that have a wetting property for meloxicam (so meloxicam shall easily dispersed in to aqueous media) and have a stabilizing properties for meloxicam dispersion to stay homogenously for a certain time.

Table I. Meloxicam nanocrystal characteristic of several polymer stabilizer

Parameters	Stabilizer			
	Xanthan gum 0,25%	PVP K25 5%	HPMC 2910 type 603 5%	HPMC 2910 type 645 5%
Particle size				
Mean±SD (nm)	204.90±30.18	196.60±12.03	184.90±24.63	175.37±34.96
Polydispersity Index				
Mean±SD (%)	-0.500±-0.038	-0.390±-0.077	-0.368±-0.034	-0.369±-0.038
Zeta potensial (mV)	-14.49	-10.99	-0.43	-0.48

Table II. Physical properties of meloxicam nanocrystals

Parameters	Stabilizer			
	PVP K25 5%	PVP K25 10%	PVP 5%-Polys 1%	PVP-Polx 1%
Particle size				
Mean±SD (nm)	523.7±32,61	478.95±17.47	835.60±183.99	906.60±93.62
Polydispersity Index				
Mean±SD (%)	-0.452±-0.028	-0.681±-0.002	-1.873±-1.338	-2.292±-2.617
Zeta potensial (mV)	-3.98	-1.27	-2.64	-1.53

According to the results, polysorbate 80 and SLS showed very good wetting properties but very poor sedimentation stability (tends to form *cake*).

The polymeric stabilizers (xanthan gum, PVP K25, HPMC 2910 type 603 and type 645) show good stabilizer properties for meloxicam with there good wetting properties and sedimentation stability after 1day. Furthermore, four stabilizers were chosen for meloxicam nanocrystal preparation using high pressure homogenizer.

Effect of polymer stabilizer to meloxicam nanocrystal characteristics

Table I presents particle size reduction profile during the process and performace stabilizer to reduce the size of the particles. Mean diameter of meloxicam using four stabilizer relatively almost the same, with size range from 175nm until 205nm. Formula meloxicam nanocrystal using PVP K25 showed fastest particle size reduction ability compare to other stabilizer with good particle size distribution (PI : -0.390±-0.077 %). According to nanocurcumin study (Rachmawati, *et al.,* 2009), the higher the molecular weight of polymers (in this case is Xanthan gum, HPMCs), the slower was the decrease in particle size with increasing number of cycles. For that reason, PVP with lower molecular weight (Rowe, 2009) shows faster size reduction.

Formula meloxicam nanocrystal using PVP K25 showed good stability after five days storage in room temperature. According to Shi (2002), bulky and complex moleculs give good physical stability properties. PVP K25 have long and branches molecul structure, it shows good steric stabilizing properties. For the further meloxicam nanocrystal formulation, PVP K25 5% was chosen.

Study of polymer concentration and addition of surfactant to meloxicam nanocrystal characteristics

The average particle size of the resulting nanocrystal stabilized with PVP K25 5% was 523.7±32.61nm, and that of the nanosuspension stabilized with PVP 10% was 478.95±17.47nm. When surfactants were used to formulate a nanocrystal, bigger particles were observed. Poloxamer 188 (formula PVP 5%-Polx 1%) and polysorbate 80 (formula PVP 5%-Polys 1%) addition to PVP K25 system disturb the stabilizers ability to form small particles (Table II).

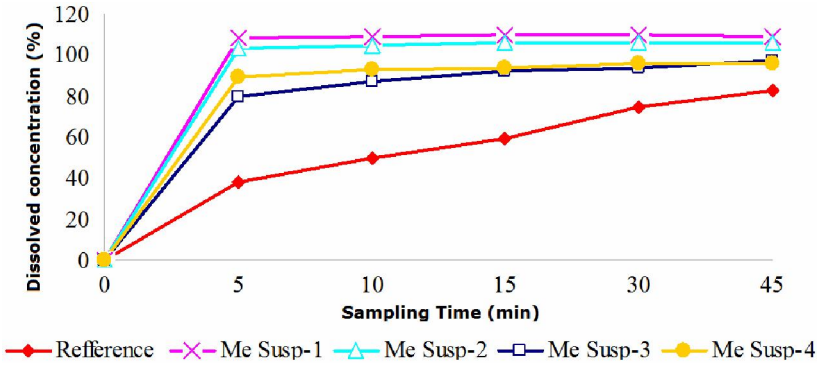

Figure 1. Initial dissolution profiles of meloxicam nanocrystal suspension

Table III. Particle size analysis of Meloxicam nanocrystal suspensions at initial and after 30 days observation in room temperature condition

Parameters	Formula			
	Me Susp-1	Me Susp-2	Me Susp-3	Me Susp-4
Initial				
Particle size				
Mean ±SD (nm)	278.05±5.16	297.60±10.18	626±23.05	347.55±38.25
Polydispersity Index				
Mean±SD (%)	0.936±0.057	1.037±0.008	1.996±0.001	1.115±0.055
After 30 days				
Particle size				
Mean ±SD (nm)	278.05±5.16	297.60±10.18	626±23.05	347.55±38.25
Polydispersity Index				
Mean ±SD (%)	0.936±0.057	1.037±0.008	1.996±0.001	1.115±0.055

Meloxicam nanocrystal using PVP K25 5% and 10% showed good particle size stability after 14 days, with average particle size was 547.05±27.08nm and 497.75±0.35nm. Formula PVP K25 5%-Polx 1% and PVP K25 5%-Polys 1% showed slight increase in particle size. According to Ostwald Ripening theory, small particles have a large free energy and the thermodynamic unstable. To decrease their free energy, nanocrystals tend to reduce interaction with water via flocculation, aggregation or crystal growth (Verma, *et al.,* 2004). According to the result, PVP K25 have a good stabilizing properties to prevent aggregation through steric stabilization mechanism. PVP K25 formed adsorbed layer on dispersed particles and minimizing interaction between nanoparticles (Shi, 2002).

Meloxicam nanocrystal suspension formulation

Meloxicam nanocrystal that has been developed is applied on the basis of the suspension to get meloxicam nanocrystal suspensions Me Susp-1 (containing PVP K25 5%), Me Susp-2 (containing PVP K25 10%), Me Susp-3 (containing PVP K25 5%-Polys 1%), Me Susp-4 (containing PVP K25 5%-Polx 1%). Observations were conducted on the particle size and dissolution rate.

Particle size analysis

Meloxicam particle size evaluation in suspension (Table III). The particle size in the suspension were smaller than the nanocrystal because of some small particles dissolve in media base suspension (Khanam, 2007).

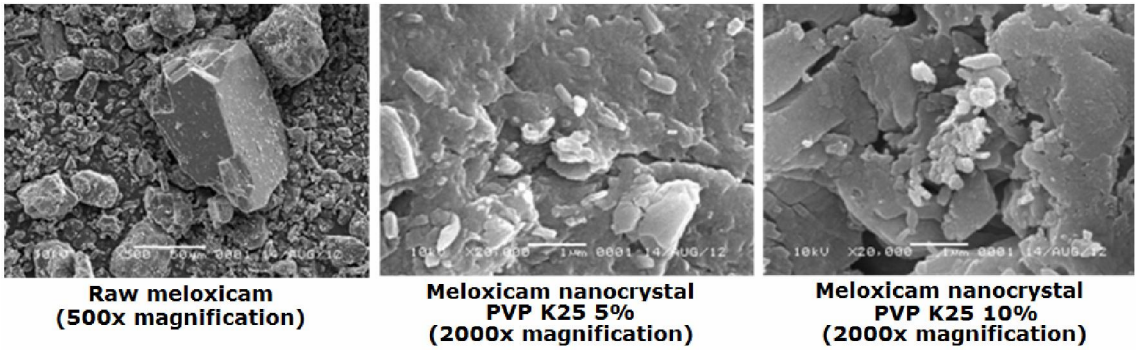

Figure 2. Scanning Electron Microscopy image of non nanosozing meloxicam (A1, A2) and meloxicam nanocrystal with PVP K25 5% (B1) dan PVP K25 10% (B2)

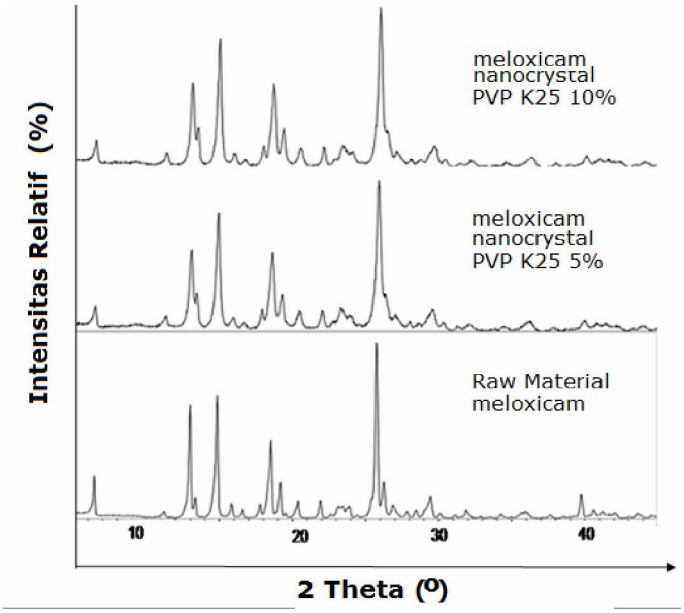

Figure 3. XRD profiles of raw meloxicam, and meloxicam nanocrystal

The average particle size of the resulting nanocrystal suspension stabilized with PVP K25 5% was 278.05±5.16nm, and that of the nanosuspension stabilized with PVP 10% was 297.60±10.18nm. Average particle size of the resulting nanocrystal suspension stabilized with PVP K25 5% and Polysorbate 80 1% was 626±23.05nm, and that of the nanosuspension stabilized with PVP K25 5% and Poloxamer 188 1% was 347.55±38.25nm. In this study, meloxicam nanocrystal suspension using PVP K25 5% and 10% showed excellent particle size stability after 30 days storage at room temperature with average particle size was 380.40±14.57nm and 398.15±1.63nm. Xanthan gum participate in the prevention of nanocrystal agglomeration beside of stabilization by PVP K25. According to research of Iron nanoparticles (Comba and Sethi, 2009), xanthan gum protected the particles and gives the distance between the particles, thereby reducing the attractive forces between particles.

In vitro dissolution profile determination

Figure 1 presents meloxicam nanocrystal dissolution profiles compared to meloxicam suspension (without nanonization). The rate of

dissolution of raw meloxicam, with particles in the micrometer size range, was very low: only 50% of the drug was dissolved in the first 10min; formulation of Me Susp-1 and Me Susp-2 samples of meloxicam doubled the dissolution rate (reaching 109% and 105% after 10min). From the experimental results, particle size reduction can increase dissolution rate. According to the Noyes-Whitney quation (Suman, 2009), high solute concentration versus time (dX/dt) is proportional to the surface area (A) and the surface area (A) is inversely proportional to particle size (D) by the formula $A=6/(Dx\rho)$, with D as the diameter of the particle. According to Prandtl equation, the diffusion layer thickness (h) of very small particles decreases while h is inversely proportional to the rate of dissolution so that the dissolution rate increases (Mauludin, 2008). Stability dissolution rate remained stable as xanthan gum can slow the increase in particle size. The rate of dissolution of Me Susp-1 and Me Susp-2 was still high, with dissolved concentration within 96.2% and 94.01% after first 10min.

Kinetic solubility analysis

Kinetic solubility meloxicam nanocrystal increased to 3-fold (28.41%) when compared to with the raw material (9.04%). According to Ostwald-Freundlich equation and the Kelvin-Gibbs, nanosuspensi solubility will increase when compared to microsuspension (Müller, 2004; Buckton, 1992).

Morphology of meloxicam nanocrystal

Morphology of meloxicam nanocrystal was obsereved using an optical microscope with a magnification of 400x and Scanning Electron Microscopy (SEM) with a magnification of 500x and 20000x (Figure 2). Results are shown in the optical microscope and SEM ranges similar to those analysed using Particle Size Analyzer.

Crystallinity evaluation

Crystallinity evaluation of raw material and meloxicam nanocrystal were presented at figure 3 using X-ray diffraction method (XRD). XRD profile between raw material and meloxicam nanocrystal were similar. This is show that nanosizing process using high pressure homogenizer did not influence meloxicam crystallinity behavior.

There was slight shifting of endotermic peak (with DSC analysis) from meloxicam nanocrystal compared to raw material melting point peak. This is presumably due to the influence of PVP K25 which has a low melting point. These examinations show that the increase of dissolution rate is caused by particle size reduction and not due to changes in crystal properties.

CONCLUSION

High pressure homogenization methods was succeded to produce meloxicam nanocrystals with particle size in range of 523.7 until 478.95nm. PVP K25 5% and 10% was the most suitable stabilizer for nanocrystal. Meloxicam nanocrystal shown better solubility than raw micro-sized particles. Meloxicam nanocrystal suspension shown higher dissolution profile compare to meloxicam suspension without nanonization.

Pharmacokinetic study suggest to conduct *in vitro* and *in vivo* correlation of meloxicam nanocrystal suspension.

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