International Journal of Pharmaceutics 381 (2009) 153–159

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Investigation of preparation parameters to improve the dissolution of poorly water-soluble meloxicam

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ARTICLE INFO

Article history:
Received 27 November 2008
Received in revised form 2 March 2009
Accepted 10 July 2009
Available online 17 July 2009

Keywords:
Nanosuspension
Meloxicam
Emulsion–diffusion method
High-pressure homogenization
Sonication
X-ray powder diffraction

ABSTRACT

The rate of dissolution of drugs remains one of the most challenging aspects in formulation development of poorly water-soluble drugs. The meloxicam, a low molecular analgetic for oral administration, exhibits a slow dissolution. To improve the dissolution rate, the drug was formulated in a nanosuspension by using an emulsion–diffusion method, high-pressure homogenization or sonication. Optimization of the technological parameters (organic solvents, stabilizers, homogenization procedure and recovery of particles) allowed the formation of nanosuspensions with a particle size of 200–900 nm. SEM imaging confirmed the nanosized drug particles. Use of an SMCR method on the XRPD patterns of the nanosuspensions revealed the crystalline form of the drug and the strong interaction between meloxicam and the stabilizer. The rate of dissolution of the dried meloxicam nanosuspension was enhanced (90% in 5 min), relative to that of raw meloxicam (15% in 5 min), mainly due to the formation of nanosized particles. These results indicate the suitability of formulation procedure for preparation of nanosized poorly water-soluble drug with significantly improved *in vitro* dissolution rate, and thus possibly enhance fast onset of therapeutic drug effect.

1. Introduction

The dissolution properties of a drug and its release from a dosage form have a basic impact on its bioavailability. Solving solubility problems is a major challenge for the pharmaceutical industry with developments of new pharmaceutical products, since nearly half of the active substances being identified through the new paradigm in high-throughput screening are either insoluble or poorly soluble in water (Patravale et al., 2004). The rate of dissolution of a drug is a function of its intrinsic solubility and its particle size. Studies with poorly soluble drugs have demonstrated that particle-size reduction to the sub-micron range can lead to an increase in dissolution rate and higher bioavailability (Leuner and Dressmann, 2002; Rabinow, 2004; Patravale et al., 2004; Kesisoglon et al., 2007).

Over the last 10 years, nanoparticle engineering has been developed and reported for pharmaceutical applications. Nanosuspensions are sub-micron colloidal dispersions of solid drug particles in a liquid phase (Möschwitzer et al., 2004). The different methods used for the preparation of nanosuspensions can be divided into two main categories: "top-down" methods, where the raw material is subsequently broken down by using milling methods until nanosized particles are produced; and "bottom-up" approaches, where nanosuspensions are built up from dissolved drug molecules (Müller and Akkar, 2004; Kocbek et al., 2006). The nanosuspension engineering processes currently used are precipitation, pearl milling and high-pressure homogenization, either in water or in mixtures of water and water-miscible liquids or non-aqueous media (Liversidge and Conzentino, 1995; Peters et al., 2000; Trotta et al., 2001; Debuigne et al., 2001; Hecq et al., 2005). Furthermore, the formulation of nanosuspensions can increase the amorphous fraction in the particles or even create completely amorphous particles.

Meloxicam, a nonsteroidal anti-inflammatory and analgetic drug (NSAID), is an enolic acid oxicam derivative (Hanft et al., 2001; Fahmy, 2006). It is frequently used to treat rheumatoid arthritis, osteoarthritis and other joint diseases (Hanft et al., 2001). Besides its main therapeutic application as an anti-inflammatory and strong analgetic agent, it is also emerging as a promising drug for the treatment of Alzheimer's disease and cancer (Goldman et al., 1998). Meloxicam is a relatively well-permeable drug, with a permeability coefficient determined on the Caco-2 cell model from an apical to a basolateral site of $P_{A\ to\ B} = 17.6 \pm 1.3 \times 10^{-6}$ cm/s, and it has low solubility and a low dissolution rate, which are limiting factors for its absorption rate (bioavailability 89% after its dissolution (Del Tacca et al., 2002)). Its maximum peak plasma concentration is reached 3–7 h following the administration of an oral suspension, and after

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doi:10.1016/j.ijpharm.2009.07.009

5–9 h for tablets (Liang et al., 2000; Hanft et al., 2001). To achieve adequate pharmacodynamic properties such as rapid onset of the drug effect, fast dissolution is important for this type of drug.

The aim of our research work was therefore to investigate the feasibility of preparation of a meloxicam nanosuspension in order to achieve fast dissolution, which would presumably yield quick onset of the peak plasma concentration. Rapid entry of the drug into the blood stream is especially beneficial in the treatment of acute pain with meloxicam. The novelty of this work was the study of the effects of different preparation conditions, added stabilizers and drying methods on formulated nanosuspensions with meloxicam and to investigate the possibility to change its physico-chemical properties and improve its dissolution rate.

2. Materials and methods

2.1.1. Materials

Meloxicam (4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2 H-benzothiazine-3-car-boxamide-1,1-dioxide) was obtained from EGIS Ltd., (Budapest, Hungary). Lutrol F68 (Poloxamer 188) and polyvinylpyrrolidone (PVP) K-25 were from BASF (Ludwigshafen, Germany); Tween 80 (polysorbate 80) and benzyl alcohol were supplied by Fluka. Butyl lactate, ethyl acetate and triacetin were from Merck (Darmstadt, Germany); and trehalose dihydrate was from Quadrant Holdings (Cambridge, England). Other laboratory chemicals used, such as ethanol, sodium hydroxide and potassium dihydrogen phosphate were purchased from Sigma–Aldrich (Steinheim, Germany) and were of analytical grade.

2.2. Solubility testing of raw meloxicam

The solubility of meloxicam in water and in aqueous solutions of different stabilizers was determined by addition of an excess of the drug to the solvent, after which the mixture was stirred on a magnetic stirrer at 25 °C for 24 h, then filtered (cut-off 0.45 μm, Minisart SRP 25, Sartorius, Germany), and the content of dissolved drug was analysed spectrophotometrically at 362 nm (PerkinElmer, Lambda 20 spectrophotometer, Germany). Each sample was analysed in triplicate.

The apparent solubility of meloxicam was determined in the partly water-miscible solvents benzyl alcohol, butyl lactate, ethyl acetate and triacetin, in order to prepare a nanosuspension by using emulsion–diffusion method. Briefly, 1 mg of the drug was weighed and the 0.5 ml aliquots of a solvent were gradually added until the meloxicam was judged by visual inspection to have completely dissolved.

2.3. Preliminary experiments for nanosuspension formulation

In order to select the appropriate stabilizer, preliminary experiments with 0.2% and 0.5% (w/v) of various stabilizers were performed. Firstly, a solution of meloxicam (5 mg) in benzyl alcohol (2.25 ml) or ethyl acetate (5 ml) was mixed with an aqueous solution of the stabilizer in a ratio of 1:7 (v/v) using high-speed homogenization, with an Ultra Turrax (UT) T25 (Janke & Kunkel, IKA Labortechnik, Germany) for 3 min at 8000 rpm and for 5 min at 24,000 rpm so as to obtain a coarse emulsion. In the second step, 50 ml of water was added to dilute the emulsion, resulting in the precipitation of drug particles. This process was carried out with the UT for 5 min at 8000 rpm.

2.4. Preparation of nanosuspensions

Meloxicam nanosuspensions were prepared by the emulsion–diffusion method, using the partially water-miscible organic solvent ethyl acetate or benzyl alcohol, with high-pressure homogenization (APV-2000, Invensys, Denmark) or sonication (amplitude 30%, 500 W Model, Cole-Palmer Instrument Co., UK). Before the final applied composition, the stabilizers, homogenization types and drying methods were tested to reach the most appropriate samples for further investigations. After the preliminary experiments the following methods were used (Table 1).

Table 1
The process-parameters of sample preparation.

Parameters	SPD-NS	SPD-REF	LIO-NS	LIO-REF
Meloxicam	20 mg	20 mg	20 mg	20 mg
Ethyl acetate	20 ml	–	–	–
Benzyl alcohol	–	–	9 ml	–
Tween 80	140 ml	140 ml	–	–
Poloxamer 188	–	–	63 ml	63 ml
Homogenization	UT	Magnetic	UT	Magnetic
Procedure	HPH	Stirrer	US	Stirrer
Redispersant	Trehalose	Trehalose	Trehalose	Trehalose
Lyoprotectant	6 g	6 g	6 g	6 g
Drying method	Spray-drying	Spray-drying	Lyophylization	Lyophylization

20 mg of meloxicam was dissolved in 20 ml of ethyl acetate, and the solution was poured under stirring at 13500 rpm with the UT into 140 ml of a 0.5% aqueous solution of Tween 80, followed by high-pressure homogenization (HPH) at 800 bar for 5 min, dilution with 160 ml of water and further homogenization for 5 min. Immediately after preparation, 6 g of trehalose was dissolved in the nanosuspension and the sample was spray-dried, using a Büchi Mini Dryer B-290 equipped with a Dehumidifier B-296 (Switzerland), at an inlet air temperature of 160 °C and an outlet temperature of 80 °C. The aspiration rate of the drying air was set to the maximum, which is about 38 m^3/h. This spray-dried nanosuspension is referred as SPD-NS in the following text.

Alternatively, meloxicam (20 mg) dissolved in 9 ml of benzyl alcohol was poured into 63 ml of 0.5% Poloxamer 188 aqueous solution, and sonicated for 3 min. The emulsion was diluted with 200 ml of water and further sonicated for 3 min. Prior to lyophilization, the nanosuspension with dissolved trehalose (6 g) was quickly frozen in liquid nitrogen and lyophilized (Crist Beta 1–8 K, Germany) at 0.570 mbar for 24 h at room temperature. This lyophilized nanosuspension is referred as LIO-NS in following text.

Reference samples were prepared using the same compositions, but instead of high-pressure homogenization or sonication, they were only stirred using a magnetic stirrer. Reference samples were transformed into dry products either by spray-drying (SPD-REF) or by lyophilization (LIO-REF) using trehalose (6 g) as a redispersant or lyoprotectant. In absence of trehalose the dried sample was agglomerated.

2.5. Particle-size analysis

The particle sizes of the nanosuspensions were determined by photon correlation spectroscopy, using a Zetasizer 3000 (Malvern Instruments, Worcestershire, UK). This technique yields the mean particle diameter and the range of the particle-size distribution (polydispersity index, PI). All the data presented are the mean values of the results on three independent samples produced under identical conditions.

To compare the size and the size distribution of the raw meloxicam, the reference samples and the nanosuspensions, the samples were dispersed in water, using an ultrasonic bath for 2 min. The sonication was used to obtain the size of individual particles. The volume particle-size distribution was measured by laser diffraction (Mastersizer S, Malvern Instruments Ltd, UK) using the following parameters: 300RF lens; small volume dispersion unit (1000 rpm); true density of meloxicam 1.565 g/cm^3 (AccuPyc

1330, Micromeritics, USA); 1.596 was used as refractive index for dispersed particles and 1.330 for dispersion medium.

Laser diffractometry yields the volume size distribution, with particle measurement in the size range 0.1–2000 μm. The reported particle-size distribution typically includes Dv10, Dv50 and Dv90, which are the percentages of particles below the given size.

2.6. Scanning electron microscopy

The surface morphology of the raw drug and formulated powder samples was visualized by scanning electron microscopy (SEM). Samples were fixed onto a metallic stub with double-sided conductive tape (diameter 12 mm, Oxon, Oxford Instruments, UK). A Supra 35 VP (Oberkochen, Zeiss, Germany) scanning electron microscope was used with an acceleration voltage of 1.00 kV and a secondary detector.

2.7. Determination of drug content in powder samples

After the emulsion–diffusion method and drying procedure some amount of meloxicam will be lost. Therefore meloxicam content in the dried samples was determined by dissolving 100 mg of dried sample (e.g. containing meloxicam, Poloxamer 188 and trehalose) in 100 ml of phosphate buffer solution (pH 7.4 ± 0.1), stirring the solution on a magnetic stirrer (400 rpm) at room temperature for 24 h, filtering and analysing spectrophotometrically at 362 nm. Each sample was prepared and analysed in triplicate.

2.8. Studies of meloxicam dissolution

The dissolution of different powder samples, containing the same amount of drug (3 mg), was determined according to the Eur. Ph. 6th Ed. paddle method (Erweka DT 6, Germany). 900 ml of phosphate buffer solution (pH 7.4 ± 0.1) at 37 ± 0.5 °C was used as a dissolution medium and the rotation speed of the paddles was 100 rpm. At predetermined time intervals, 7 ml samples were withdrawn and immediately filtered (cut-off 0.2 μm, Minisart SRP 25, Sartorius, Germany) and the amount of dissolved drug was determined spectrophotometrically. Withdrawn samples were replaced with 7 ml of fresh medium.

2.9. X-ray powder diffraction analysis

The physical state of meloxicam in the different samples was evaluated by X-ray powder diffraction (XRPD). Diffraction patterns were analysed with a Miniflex II X-ray Diffractometer (Rigaku Co. Tokyo, Japan), where the tube anode was Cu with Kα = 15,405 Å. The pattern was collected with a tube voltage of 30 kV and a tube current of 15 mA of in step scan mode (4°/min). The instrument was calibrated by using Si. A chemometric method was used to evaluate the X-ray results. The self-modelling curve resolution (SMCR) method is a chemometric procedure used for two- and three-component systems to deconvolve raw spectroscopic data and to obtain an analytical solution in band form, which provides a clearer interpretation. A computer program involving the use of SMCR and multivariate curve resolution (MCR) methods was employed to analyse the XRPD data and to study the interactions between meloxicam and the stabilizer in the formulated nanosuspensions.

Table 2
Solubilities of meloxicam in different solvents, and solubilities of organic solvents in water.

Solvent	Meloxicam solubility (μg/ml)[a]	Solubility of solvent in water (%, w/w)
Water	4.4 ± 0.7	–
Benzyl alcohol	2220	3.5[c]
Butyl lactate	500	7.7[b]
Ethyl acetate	1000	8[c]
Triacetin	220	7.1[b]
0.5% Tween 80	9.0 ± 0.14	–
0.5% Poloxamer 188	6.3 ± 0.16	–
0.5% PVP K-25	4.0 ± 0.90	–
0.5% Tween 80–PVP K-25 (1:1)	7.6 ± 0.30	–
0.5% Tween 80–Poloxamer 188 (1:1)	8.2 ± 0.14	–

[a] Present experimental data.
[b] Data from Trotta et al.
[c] Data from Yeo et al. (2003).

3. Results and discussion

3.1. Preliminary selection of the stabilizer and solvent for meloxicam nanosuspension preparation

The solubility of meloxicam in water, four different organic solvents and aqueous solutions of stabilizers, and the solubility of selected organic solvents in water, are presented in Table 2. A drug which is a suitable candidate for nanosuspension preparation by solvent diffusion method should be very poorly soluble in water ($<10^{-3}$ to 10^{-4} mol/l) and well-soluble in the selected organic solvent (Kocbek et al., 2006). Meloxicam is very poorly water-soluble (4.4 μg/ml, i.e 1.2×10^{-8} mol/l), and significantly better soluble in organic solvents, as determined in this study. Furthermore, the water solubility of the organic solvent is a determining factor that affects the precipitation process. Meloxicam is well soluble in ethyl acetate and benzyl alcohol, which are both partially water miscible and were therefore selected for the preparation of nanosuspensions. Ethyl acetate has higher water solubility than benzyl alcohol,

Table 3
Effects of solvents, stabilizers and their concentrations on the nanosuspension particle size produced by the emulsion–diffusion method with rotor–stator homogenization.

Stabilizer concentration	0.2%		0.5%	
	Size (nm)*	PI	Size (nm)*	PI
Ethyl acetate				
Tween 80	864.6 ± 117.90	0.414 ± 0.33	788 ± 17.30	0.732 ± 0.42
Poloxamer 188	704.1 ± 14.60	1	595.5 ± 21.90	0.541 ± 0.38
PVP K-25	Crystallization	–	Crystallization	–
Tween 80-Poloxamer 188 (1:1)	599.3 ± 30.40	0.730 ± 0.43	799 ± 45.60	0.304 ± 0.28
Tween 80-PVP K-25 (1:1)	705.7 ± 41.20	0.452 ± 0.50	895.8 ± 99.10	0.577 ± 0.39
Benzyl alcohol				
Tween 80	406 ± 11.30	0.823 ± 0.10	294.5 ± 28.30	0.495 ± 0.20
Poloxamer 188	300.7 ± 3.30	0.363 ± 0.17	292.9 ± 2.80	0.188 ± 0.16
PVP K-25	604.9 ± 57.40	0.578 ± 0.22	605.6 ± 116.90	1
Tween 80–Poloxamer 188 (1:1)	430.3 ± 6.50	0.502 ± 0.34	416.4 ± 13.90	1
Tween 80–PVP K-25 (1:1)	509.6 ± 18.40	0.898 ± 0.18	382.1 ± 38.70	0.655 ± 0.38

* Measured by Zetasizer 3000.

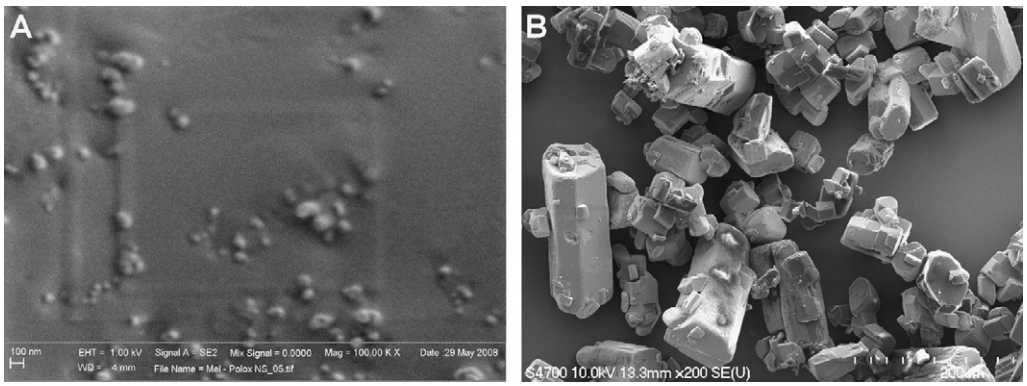

Fig. 1. (A) SEM image of meloxicam nanosuspension prepared with benzyl alcohol as organic solvent and dried at room temperature (scale bar: 100 nm). (B) SEM image of raw meloxicam (scale bar: 200 μm).

but benzyl alcohol is a better solvent for meloxicam. The solubility of meloxicam in triacetin and butyl lactate is very low, and are therefore less appropriate for the preparation of nanosuspensions.

The influence of different stabilizers on the formulation of the nanosuspension was investigated, using only UT to homogenize the dispersion. The concentration of stabilizer employed to stabilize an emulsion has marked effect on the particle size and the PI value of the nanosuspension (Table 3). The higher concentration of Tween 80 or Poloxamer 188 resulted in a smaller average particle size using either of the organic solvents. PVP K-25 did not demonstrate any concentration-dependent effect on the particle size using benzyl alcohol, and a nanosuspension was not formed using ethyl acetate. The particle size in the nanosuspensions stabilized with 0.5% Tween 80 or Poloxamer 188 using benzyl alcohol is significantly smaller (~290 nm) than for any other combination of stabilizer and organic solvent. For the further nanosuspension formulation, 0.5% Tween 80 or Poloxamer 188 in combination with benzyl alcohol or ethyl acetate was chosen.

3.2. Influence of homogenization and drying method on particle size

The important features in the preparation of a nanosuspension are the manner of diluting the emulsion (the amount of water added and the speed of stirring), the precipitation of nanosized particles, and the recovery of the dried particles (Patil and Pandit, 2007). As in the preliminary experiments, nanosuspensions stabilized with Poloxamer 188 or Tween 80 were prepared using either ethyl acetate or benzyl alcohol.

The use of ultrasonication as homogenization method and ethyl acetate as organic solvent did not yield a nanosuspension, but HPH enabled nanoparticle formation. The average particle size of the resulting nanosuspension stabilized with Poloxamer 188 was 923.4 ± 14.0 nm, and that of the nanosuspension stabilized with Tween 80 was 773.4 ± 16.1 nm. When benzyl alcohol was used to formulate a nanosuspension, smaller particles were obtained with sonication in presence of Poloxamer 188 as a stabilizer (274.4 ± 32.7 nm). However, the smallest particles were obtained with Tween 80 (165.0 ± 16.4 nm). Nanosuspensions were successfully formulated by sonication, when benzyl alcohol was used, therefore HPH was not necessary for preparation of nanosuspensions.

The particle size in nanosuspensions prepared from ethyl acetate/water emulsions and stabilized with Poloxamer 188 was more than 1 μm (1039 ± 76.6 nm) after spray-drying, and this combination was therefore not chosen for further examinations. However, the SPD-NS prepared with ethyl acetate as organic solvent and Tween 80 as stabilizer resulted in smaller particles (847.9 ± 54.7 nm). Spray-drying was chosen to prepare a dry product, since Tween 80 is liquid at room temperature and therefore inappropriate for lyophilization. On the other hand, it was impossible to spray-dry nanosuspensions containing benzyl alcohol, and these samples were therefore lyophilized. The particle size in LIO-NS prepared from the benzyl alcohol/water emulsion stabilized with Poloxamer 188 was 327.3 ± 17.8 nm.

Table 4 lists the particle-size distribution of raw meloxicam, the dried nanosuspensions and their references measured by laser diffractometry. The results clearly show that the particle size in the nanosuspensions is indeed in the nanometer range (460–530 nm), whereas the reference samples have particle sizes in the micrometer range (40–60 μm). Spray-drying or lyophilization of the meloxicam dispersion in the solution of the stabilizer (reference sample) did not result in nanosized particles, even though their size was decreased by about 50%. This was presumably due to the presence of the surface-active agent.

3.3. SEM analysis

The SEM image revealed nanosized particles (around 200 nm) of meloxicam (Fig. 1A) in contrast to microsized particles (around 86 μm) of raw meloxicam (Fig. 1B). Nanosuspension shown on Fig. 1A did not contain trehalose, since meloxicam nanoparticles were not visible in the SEM images of nanosuspensions dried in the presence of trehalose

3.4. In vitro dissolution of nanosized meloxicam

In order to ascertain whether the goal of improving the rate of dissolution of meloxicam is achieved, the results of *in vitro* dissolution of different meloxicam samples are shown in Fig. 2. The rate of dissolution of raw meloxicam, with particles in the micrometer size range, was very low: only 10% of the drug was dissolved in the first 10 min; formulation of the SPD-REF samples of meloxicam doubled the dissolution rate. In the case of the LIO-REF sample,

Table 4
Particle size distribution after dispersion of raw meloxicam or meloxicam in dried nanosuspension or reference samples in water.

Samples	d (μm)*		
	10%	50%	90%
Raw meloxicam	24.80 ± 2.94	85.39 ± 6.63	237.92 ± 28.44
SPD-NS	0.140 ± 0.09	0.460 ± 0.23	2.71 ± 0.91
SPD-REF	5.62 ± 3.18	42 ± 6.21	50 ± 4.37
LIO-NS	0.168 ± 0.04	0.530 ± 0.11	3.6 ± 1.26
LIO-REF	22.83 ± 2.15	59.17 ± 3.44	68.87 ± 1.06

* Measured by Mastersizer S.

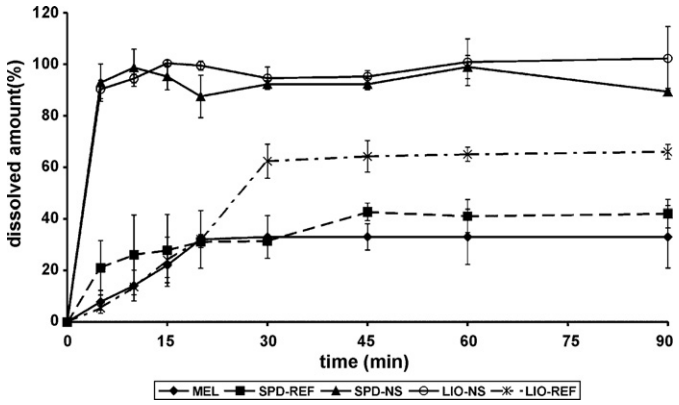

Fig. 2. Dissolution profiles for raw meloxicam (MEL), spray-dried reference sample (SPD-REF), spray-dried nanosuspension (SPD-NS), lyophilized nanosuspension (LIO-NS) and lyophilized reference sample (LIO-REF).

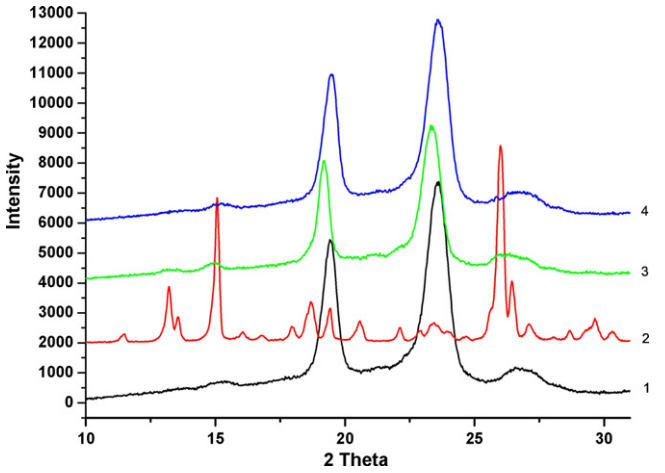

Fig. 3. XRPD patterns of Poloxamer 188 (1), meloxicam (2), lyophylized product (3) and physical mixture (4) of meloxicam and Poloxamer 188 in the same ratio as in LIO-NS.

60% of the meloxicam was dissolved after 60 min. The formulation of meloxicam nanosuspensions significantly improved the dissolution rate, since almost 100% of the drug was dissolved in the first 10 min (Fig. 2). Besides the increase in surface area due to the formation of nanosized drug particles, the surface-active agents may have contributed to the increase in dissolution rate due to the improved wettability of the drug.

3.5. Structural analysis of meloxicam in nanosuspension (XRPD)

The XRPD patterns of meloxicam, Poloxamer 188, LIO-NS and a physical mixture containing meloxicam and Poloxamer 188 in a ratio of 1:35 are presented in Fig. 3. For the XRPD investigation, we used LIO-NS without trehalose. The XRPD patterns of these samples were compared to those of the raw material in order to investigate the crystalline form of meloxicam in the final nanosuspension. For the LIO-NS sample, the 2 theta characteristics of Poloxamer 188 were a little shifted, to 19.18 and 23.3. Addition of the characteristic peaks of meloxicam and Poloxamer 188 was observed. Despite the small quantity of meloxicam (ratio meloxicam:Poloxamer 188, 1:35), the crystalline form of meloxicam is presumable, and because of the overlap of the characteristic values, additional statistical analysis was carried out to evaluate the XRPD patterns (Fiala, 1980).

Lawton and Sylvestre (1971) introduced the SMCR method for two-component data, using minimal constraints, i.e. non-negativity for the concentrations and for the intensities. Borgen and Knowalski (1985) and Borgen et al. (1986) extended the Lawton and Sylvestre method to three-component systems, but the published descriptions were rather difficult to understand, and accordingly researchers turned to the application of more constraints in an attempt to obtain a possibly unique solution instead of the band one. This method is known as MCR (de Juan and Tauler, 2006). The method of Borgen et al. was recently revisited by Rajkó and István (2005) and Rajkó (2006) who give a clearer interpretation, with the use of computational geometry tools to find inner and outer polygons.

To investigate the interactions and evaluate the XRPD data, chemometric method SMCR was used. As we have successfully used SMCR method to reveal XRPD measurements previously, we only mention the results obtained by SMCR, and for the theoretical details of the chemometric methods the readers can turn to the published papers (Reisi Nassab et al., 2006; Bashiri-Shahroodi et al., 2008). Borgen plot has a strong relationship to PCA (principal component analysis) plots, however in this case special 1-norm normalization (all element of a vector is divided by the sum of the elements, thus the sum of the elements of the normalized vector turns into 1) was applied. In this Borgen plot figure, if the points are very close to each other then the properties of the two features

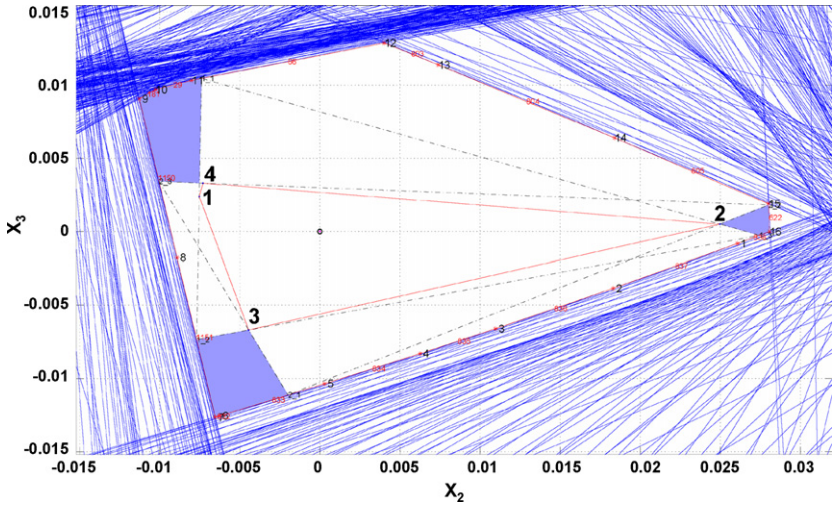

Fig. 4. Borgen plot of the transformed diffractograms. There are four points in or on the inner polygon according to the four samples featuring in Fig. 3 (1: Poloxamer 188; 2: meloxicam; 3: LIO-NS and 4: physical mixture).

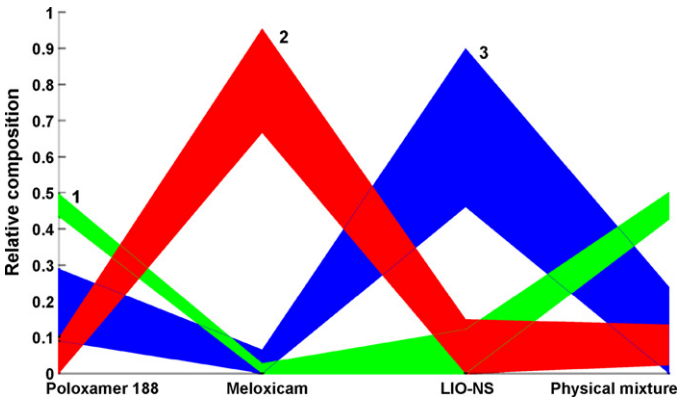

Fig. 5. Compositions of the samples given by using the self-modelling curve resolution method. Component 1 (green band) represents both Poloxamer 188 and the physical mixture, meloxicam is component 2 (red band), and a new crystalline phase of LIO-NS is component 3 (blue band). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

represented by the points are very similar. The Borgen plot (Fig. 4) of the transformed diffractograms revealed that points 1 and 4 of the inner polygon are very close to each other, because of the large amount of Poloxamer 188 in the physical mixture, and its characteristic peaks are nearly the same as in the case of pure Poloxamer 188.

Fig. 5 depicts the composition profiles of the analysed samples. Component 1 (green band) represents both Poloxamer 188 and the physical mixture. Component 2 (red band) relates to meloxicam, and a new crystalline phase of LIO-NS is component 3 (blue band). Examination of the red band of meloxicam suggests that LIO-NS contains less of the original crystalline form of meloxicam in than the physical mixture, thought the ratio used (1:35) was the same. To clarify the situation, MCR was used in the hope that the "unique" solution could be interpreted. Table 5 shows the relative concentrations.

The relative concentrations in Fig. 5 and Table 5 differ because different normalizations were used for the diffractograms in the SMCR and the MCR.

The unique solution given by MCR is chemically interpretable, because without any further constraints, zero concentrations appear in the correct places: component 1 is Poloxamer 188, and the meloxicam content is now zero; component 2 is meloxicam, and the Poloxamer 188 content is zero; while component 3 is the new crystalline structure formed by Poloxamer 188 and meloxicam, and the relative concentration of component 3 in physical mixture is zero. Supposing the new crystalline form is evident because composition of LIO-NS is markedly different from the composition of the physical mixture. A new component appeared which is different from both Poloxamer 188 and meloxicam. Based on the results in Table 5, LIO-NS contains mainly component 3, but the physical mixture does not contain component 3 at all. In addition LIO-NS contains only 0.58% and 0.20% of components 1 and 2, respectively, while physical mixture contains 98.47% of component 1, and 1.53% of component 2. Component 3 is definitely a new crystalline form composed from Poloxamer 188 and meloxicam.

Table 5
Compositions of the samples given by using the multivariate curve resolution method.

	Component 1	Component 2	Component 3
Poloxamer 188	57,513	0	5,735
Meloxicam	0	30,647	18
LIO-NS	262	89	45,196
Physical mixture	57,367	889	0

The quantity of meloxicam in the physical mixture and in the LIO-NS is small, but the image reveals that this content is in a crystalline state. The interaction between the drug and the stabilizer in the physical mixture and in the resulting nanosized particles did not produce any significantly different pattern in the X-ray diffractogram, because of the overlapping of the characteristic values. The chemometric method, however, demonstrated the presence of a new crystalline phase, which can contain nanometer-sized meloxicam particles, contributing to the fast dissolution of this meloxicam.

4. Conclusion

This study has shown that the emulsion–diffusion method can be used to formulate a meloxicam nanosuspension. Careful selection of homogenization procedure and stabilizer are critical, firstly to achieve stabilization during controlled crystallization and secondly to increase the wettability of hydrophobic drug in dissolution medium. Nanosized meloxicam dissolved significantly faster than raw micro-sized drug particles. The new crystalline surface, formed during preparation of nanosuspension, exhibited an interaction with a stabilizer used as was determined by X-ray powder diffraction and evaluation of its results chemometricaly (self-modelling curve resolution method). Moreover, the fact that the physical mixture of the drug and stabilizer did not significantly improve the dissolution of the drug suggests that the increased dissolution rate for the nanosuspension is primarily due to the reduction of the particle size. These findings indicate the suitability of formulation procedure for preparation of nanosized poorly water-soluble drug with significantly improved *in vitro* dissolution rate, and thus possibly enhance fast onset of therapeutic drug effect.

Acknowledgement

This research work was supported by the Hungarian-Slovenian Bilateral Intergovernmental Cooperation Programme (HU-SLO-7/2006).

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