



Combination of Solvent Displacement and Wet Ball Milling Techniques for Size Reduction of Celecoxib

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ABSTRACT

Background: The objective of the present study was to investigate the combinative effect of ball milling and solvent displacement method on size reduction of Celecoxib particles. Celecoxib is a poorly water soluble cyclooxygenase 2 inhibitor which has a wide range of therapeutic applicability.

Methods: Microparticles were developed via solvent displacement method followed by planetary ball milling. In order to obtain an optimized size and size distribution of Celecoxib microparticles various factors were evaluated; the role of solvent type, type and concentration of stabilizer, milling effect, and the effect of milling duration were the most important factors studied during the present investigation.

Results: All the obtained formulations were in micron range that the smallest particles had the size of 1.76 μm and the formulation containing the largest particles was of 8.30 μm by volume mean diameter. Both solvent displacement and milling methods are common and potential approaches in order to formulate micron scaled particles.

Conclusion: The combination of these two methods generates a synergistic effect which leads to smaller particle size and a narrow size distribution. Celecoxib microparticles have the potential to use as promising delivery systems to treat various disease and malignancies.

Introduction

The problem of insufficient and variable bioavailability after oral administration of poorly water soluble drugs is often a major difficulty with the formulation of such compounds. When administering drugs classified in Class II of the Biopharmaceutical Classification System (BCS), a high dose is needed to attain the minimum effective therapeutic concentration in blood. Therefore, in formulating these drug substances, enhancing rate and extent of dissolution is an important issue.^{1,2} Celecoxib, a highly selective cyclooxygenase-2 inhibitor, is a potent treating agent for osteoarthritis and rheumatoid arthritis; it has also been widely used for relieving acute pain.³ Recently, several investigations have confirmed the potential of Celecoxib as a chemopreventive agent; it has been reported that Celecoxib can be effectively involved in the treatment of various malignancies, such as non-small cell lung cancer,⁴⁻⁶ breast,⁷⁻¹² prostate,¹³⁻¹⁵ colon,^{9,16-18} bladder,¹⁹⁻²¹ and UV-induced skin^{22,23} carcinogenesis. Moreover, recent reports have indicated that Celecoxib can reduce expression of

the retinal vascular endothelial growth factor in diabetic patients.²⁴ As a Class II BSC drug, Celecoxib suffers from poor and variable bioavailability.²⁵ The problem of side effects associated with high oral doses of Celecoxib is negligible; yet it becomes more significant when considering recent reports of Celecoxib-induced cardiovascular disease.²⁶

Size reduction is an efficient technique for improving the therapeutic applicability of non-soluble drugs. Micro- and nanoparticulate systems are promising controlled drug delivery devices mainly because of their small size, which enhances their surface-to-volume ratio. This enhanced surface area increases solubility, which in turn leads to a reduction in the administered dose and, consequently, the reduction of side effects and toxicity; this will enhance patient compliance and convenience.²⁷⁻²⁹

The solvent displacement method (also known as interfacial deposition) is a conventional, fast, and reproducible technique used to produce micro- and nanoparticles (Figure 1). With this method, the formation of microparticles occurs

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spontaneously following the addition of water miscible organic solvent to an aqueous phase.

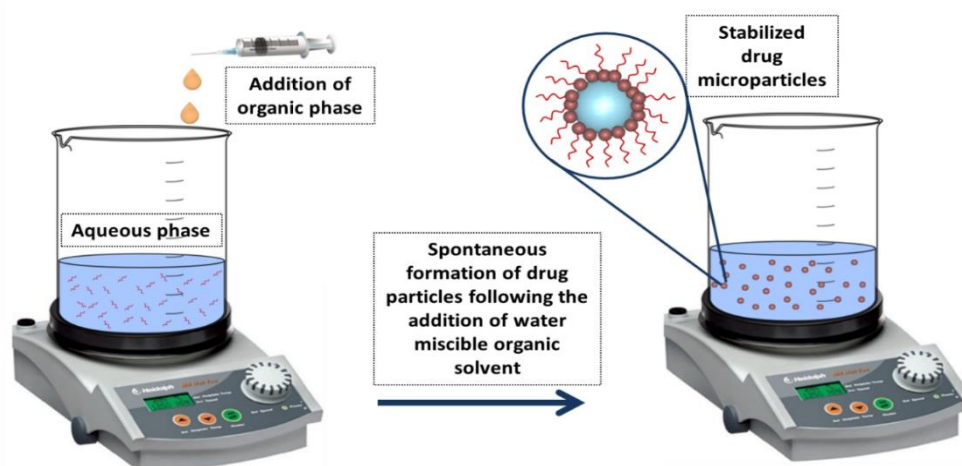


Figure 1. Schematic view of spontaneous formation of drug microparticles via solvent displacement method.

Mechanical disruption is a common size reduction technique; planetary ball mill is an efficient mechanical method that reduces the size of particles to micron range via very strong centrifugal and pulverization forces.³⁰

The combination of these two methods improves the size characteristics of prepared particles and generates more monodisperse systems.

The present study investigated the effect of a combination of solvent displacement and planetary ball mill techniques on size reduction of Celecoxib particles.

Materials and methods

Materials

Celecoxib was kindly provided by Zahravi Pharmaceutical Co. (Iran). Soy phosphatidylcholine (SPC 98%) was supplied from Lipoid (Germany). Ethyl Alcohol 96 % (v/v), 1, 4 Dioxane, Tween 80, HPMC and Poloxamer 407 were purchased from JATA Co. (Iran), DAEJUNG chemicals and materials Co. (Korea), Merck Chemicals (Germany), Samsung Fine

Chemicals Co. (Korea), and Sigma Aldrich (USA), respectively.

Methods

Preparation of microparticles

Solvent displacement method

Microparticles of Celecoxib produced via solvent displacement technique at room temperature. Drug dissolved in an appropriate amount of semipolar organic solvent was injected to magnetically stirred (1700rpm) aqueous solution. Drug particles spontaneously formed after the addition of organic phase.

Wet ball milling method

The resulting suspension was wet milled using a planetary ball mill (PM 100 RESTCH, Germany) equipped with a zirconia milling chamber volume of 50 mL. The effect of grinding, milling duration, solvent type, and type and concentration of surfactant were studied. A summary of the 10 different experiments is given in Table 1.

Table 1. Composition of various formulations.

Formulation	Drug (w/v)	% Solvent or E) ^a	(D Lecithin (w/v)	% Tween80 (w/v)	% HPMC ^b (w/v)	% Poloxamer (w/v)	407 SLS ^c (w/v)	% Milling time(h)
F1	5	D	1	-	-	-	-	-
F2	5	D	1	-	-	-	-	4
F3	5	E	1	-	-	-	-	-
F4	5	E	1	-	-	-	-	4
F5	5	E	-	0.1	-	0.1	-	4
F6	5	E	-	-	-	0.1	-	4
F7	5	E	-	-	2.5	1	-	-
F8	5	E	-	-	2.5	1	-	4
F9	2.5	-	-	-	2	-	2	20
F10	2.5	-	-	-	2	-	2	4

^aDioxane/Ethanol

^bHydroxy Propyl Methyl Cellulose

^cSodium Lauryl Sulfate

Particle size measurement

The size and size distribution of the processed particles were determined using a laser diffraction particle size analyzer (SALD2101, Shimadzu, Japan). An appropriate concentration of freshly produced microparticles in distilled water was prepared. After the transmission of mentioned dispersion to the cell of analyzer measuring was carried out. Before the analysis was begun distilled water was used as blank to eliminate the peaks by water itself. The size of particles was evaluated by volume mean diameter and the span value which indicates the size distribution pattern of particles was calculated using the following equation:

$$\text{Span} = \frac{D_{90\%} - D_{10\%}}{D_{50\%}} \quad \text{Eq.(1)}$$

Where D90%, D10% and D50% are the equivalent volume diameters at 90, 10 and 50% cumulative volumes, respectively (21).

X-ray powder diffraction

The crystalline structure of pure Celecoxib was studied by X-ray diffraction (XRD) (D5000, Siemens diffractometer, Germany) equipped with a nickel-filtered CuK α radiation. The X-ray tube voltage was set to 40 KV and amperage at 20mA. The scanning rate was 2°/min over a 2 θ range of 2-70° and with an interval of 0.02°.

Results

Preparation of microparticles

Data describing the size and size distribution pattern of the tested formulations is summarized in Table 2.

Table 2. Size characteristics of formulations.

Formulation	Cumulative percent (undersize) ^a			VMD ^b Span	
	D _{10%} (μm)	D _{50%} (μm)	D _{90%} (μm)	(μm)	(μm)
F1	1.79	8.30	17.19	8.30	1.85
F2	1.97	5.31	14.65	5.21	2.39
F3	1.65	8.22	23.06	7.20	2.61
F4	1.16	5.72	14.52	4.94	2.33
F5	0.67	1.97	4.33	1.76	1.85
F6	0.82	5.656	24.93	5.06	4.26
F7	1.76	8.40	30.73	7.90	3.45
F8	0.50	1.96	11.25	2.17	5.39
F9	1.08	4.76	16.30	4.45	3.18
F10	0.88	3.77	11.33	3.49	2.77

a: Equivalent volume diameters at 10 (D_{10%}), 50 (D_{50%}) and 90% (D_{90%}) cumulative volume. ^bVolume median diameter.

Clearly, the particle size of all formulations is distributed in the micrometer size range (from 1.76 μm to 8.30 μm), which is an appropriate size for most drug delivery purposes. Formulation F5 with size and span values of 1.76 and 1.85, respectively, was the most acceptable formulation with the smallest particle size

and narrowest size distribution.

Size comparisons of formulations F1 and F2, F3 and F4, and F7 and F8 illustrate the differences in average particle size prior to and after milling. The results indicate the combinative effect of grinding and the solvent displacement method. Obviously, grinding subsequent to solvent removal greatly affects the disruption of drug particles to smaller sizes, and it modifies the size distribution pattern.

Although ethanol caused smaller particles (comparison of F1 and F3, and F2 and F4), a review of the results revealed that solvent type (either as a drug solvent medium added to a non-solvent for the production of drug particles via solvent displacement (F1, F3, and F7) or as a milling media) did not significantly influence size.

The presence of Tween 80 (F5) caused a drastic decrease in size and provided much uniform size distribution compared to the identical formulation without it (F6). Longer milling times (20 h) had substantial impacts on size, but increased the size distribution (comparison of formulations F9 and F10). The change in surfactant type (poloxamer407, SLS, and lecithin), concentration, and the addition of polymer (HPMC) to avoid drug recrystallization did not effectively help the milling procedure for size reduction.

X-ray powder diffraction

The solid state properties of Celecoxib were studied via XRD technique. The resultant XRD pattern (Figure 2) illustrates the semi-crystalline structure of Celecoxib, which may explain the unsatisfactory size reduction of Celecoxib particles during the milling procedure.

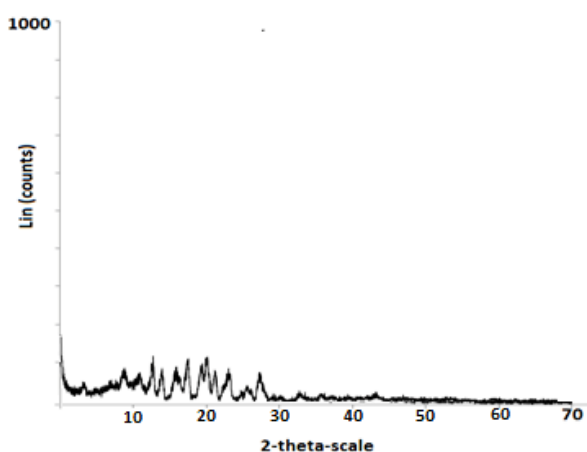


Figure 2. The X-Ray diffraction patterns of Celecoxib.

Discussion

Celecoxib is a widely used treating agent for acute pain and rheumatoid arthritis. It is also FDA approved for the treatment of familial adenomatous polyyps.³ Various investigations have illustrated the potential efficiency of Celecoxib in the treatment and prevention of several

malignant and premalignant tissues.³¹ As a Class II drug of the BCS, Celecoxib requires a high oral dose to attain the therapeutic concentration in plasma. Micronization effectively enhances the rate and extent of dissolution and improves the bioavailability of the orally administered drug. Furthermore, it enhances the ability of the drug to target the site of the malignancy. Celecoxib reportedly has the ability to reduce the expression of diabetes-induced vascular endothelial growth factor; the tight endothelium of retinoid blood vessels is a limitation which the micronization of drug particles can overcome.²⁴

To prepare microparticles of Celecoxib, the solvent displacement method, milling technique, and a combination of the two were used in this study.³² The rotation of the milling beaker can be best described as a lunar motion; just like the moon rotating simultaneously around the earth and the sun, the milling beaker rotates around not only the planetary axis, but also the sun axis. This motion results in two separate rotational paths of a single chamber. Figure 3 describes the lunar motion generated during a planetary ball mill process. The collision of milling media with drug crystals generates the high shear force needed to fracture drug crystals into micro-scaled particles.

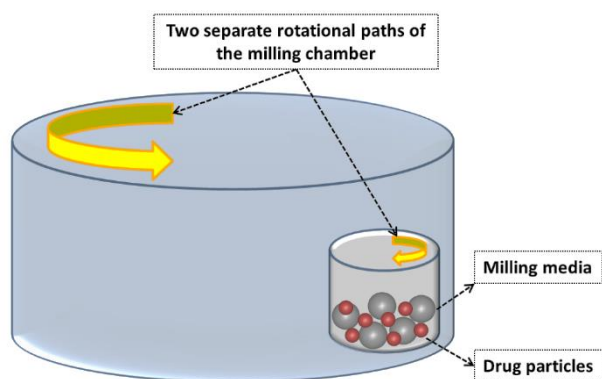


Figure 3. A scheme of the movement of a milling beaker during the planetary ball mill process.

Although it is generally known that planetary ball milling is an appropriate approach to producing microparticles,³³ the solid state characteristics of drug substances significantly affect the size reduction process. It has been reported that the size of materials with a very low enthalpy value and a weak crystalline structure is unlikely to be reduced by media milling.³⁴ On the other hand, XRD experiments (Figure 2) proved the low crystalline structure of Celecoxib, which makes it an appropriate option upon which to apply a combination of milling and other size reduction methods to obtain formulations with optimized size characteristics.

Because the type of stabilizer and its provided barrier on the surface of particles dictate particle stability, various types of stabilizers were evaluated for their stabilizing effect. Commonly, there are two types of

stabilization: 1) steric stabilization, and 2) electrostatic stabilization. The first occurs via absorption of polymeric stabilizers to the surface of microparticles, and the second is achieved by charged stabilizers that adsorb electrostatically on the surface of drug particles. In pulmonary delivery, it is well known that the inhalable particle size is a narrow range of 2 to 5 micrometers. Moreover, it is known that there are few surfactants available for pulmonary delivery. Lecithin, a phospholipid, is an important part of the natural complex of pulmonary surfactant;³⁵ therefore, it can be used to stabilize pulmonary formulations. Despite its safety, lecithin could not properly stabilize formulations, and the particle sizes of formulations F1 to F4 are over the inhalable range.

Owing to it having the smallest particle size and span value, formulation F5 is the most acceptable formulation for all drug delivery purposes, including retinal, pulmonary, and even transdermal drug delivery. Moreover, Tween 80 is a non-ionic surfactant which can be safely used in preparing systemic or local drug delivery systems.

Formulations F9 and F10 are prepared via milling without the initial solvent displacement step. A comparison of the sizes of formulation F9 and formulations F1 and F3 showed that solvent displacement is obviously not as efficient as milling in reducing size; however, the span values illustrate the wider size distribution range of formulations prepared via milling. This indicates that a combination of these two methods can lead to the production of formulations with a smaller particle size and a narrow size distribution.

The smaller particle size of F10 compared to F9 illustrates the effect of milling duration on particle size; the longer the milling time is, the smaller the particle size will be. There is, however, a limitation: enhancing the time a drug is exposed to the high shear forces generated by milling media increases the probability of the destruction of drug particles and/or particle-particle fusion, because of the high temperatures associated with mechanical forces during the milling procedure.

Conclusion

Celecoxib microparticles were successfully prepared via a two-step method comprised of a solvent displacement method and a planetary ball mill process. To achieve an optimized microparticle system, many factors must be considered. As well as the type and concentration of surfactant, the nature and crystalline structure of the active pharmaceutical agent are very important factors affecting the process; compounds such as Celecoxib, which have soft crystalline structures, show little reduction in particle size during milling. Thus, it seems that a combination of the solvent displacement and milling methods will lead to optimized formulations with desirable size characteristics.

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Conflict of interests

The authors claim that there is no conflict of interest.

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