Use of calcined Mg–Al–hydrotalcite to enhance the stability of celecoxib in the amorphous form

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Abstract

The use of compound in amorphous forms is a promising approach to improve solubility of poorly water-soluble drugs. However, during storage, amorphous state can spontaneously transform to the lower energy crystalline form and this is a limiting factor for a large commercial use of drugs.

In this paper, calcined hydrotalcite was employed to support amorphous celecoxib and several preparations at different weight drug/crystal ratio were prepared. Solubility of celecoxib from the prepared systems was evaluated and its physical stability during storage at different conditions was examined as well. The results show that HTlc-calc can be used as a support of amorphous celecoxib with consequent improvement of drug solubility and physical stability.

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1. Introduction

Many drugs are poorly water soluble and therefore their solubility represents the limiting factor for their bioavailability. Many technical approaches have been studied in order to increase drug bioavailability by improving their dissolution rate, such as micronization [1], solid dispersions [2], deposition of drugs on specific carrier [3] or in insoluble polymers [4], and/or enhancing their solubility through the use of amorphous or polymorphic forms [5]. Amorphous form and solid dispersions are not physically stable, in fact, during storage, the formation of drug stable crystals often occurs [6]. Here, the use of an inorganic powder soluble in gastric fluid, namely the calcined Mg–Al–hydrotalcite, has been investigated as a carrier for celecoxib, a poorly water-soluble drug, belonging to class 2 of the biopharmaceutical classification system [7].

Hydrotalcite is an anionic layered mixed hydroxide clay, containing exchangeable anions. This kind of clay has many practical applications and is used as such or after calcination [8]. The general formula of synthetic hydrotalcite-like compound is \[ \text{M}^{II}_{x-1} \text{M}^{III}_x (\text{OH})_{2x} \text{A}_{n-x}^{x-} \text{m S} \] where \( \text{M}^{II} \) is a divalent metal cation, usually Mg, \( \text{M}^{III} \) is a trivalent metal cation, usually Al, \( \text{A}^{x-} \) is an exchangeable inorganic or organic anion which compensates for the positive charge of the layer and \( m \) are the moles of solvent S, usually water, co-intercalated per mole of compound. The Mg–Al–hydrotalcite (HTlc) is biocompatible [8] and is used, inter alia, in pharmaceutical applications as an ingredient in sustained-release pharmaceuticals containing nifedipine [9], as stabilizing pharmaceutical compositions [10,11], as a component to prepare aluminium magnesium salts of antipyretic, analgesic and anti-inflammatory drugs [12] and, more recently, as a matrix for controlled release of drugs...
and for improving dissolution of intercalated drugs in acid environment [15]. By calcination, HTlc originates a homogeneous mixture of oxides in very small size crystals (HTlc-calc) soluble at pH < 4. This property distinguishes this compound from other inorganic materials, such as kaolin [16], montmorillonite [17], Florite® [18] and silica aerogel [3].

In this paper, the feasibility of HTlc-calc to reduce the crystallinity of poorly soluble drugs and improve their apparent solubility was investigated. Celecoxib (Fig. 1), chosen as a model drug, is an anti-inflammatory compound, that inhibits selectively cyclooxygenase-2-enzymes. It is well absorbed through the gastrointestinal tract, but its very low solubility in aqueous media (ca. 3 μg/ml in pH 1.2 aqueous solution) [19] represents the rate-limiting factor for its absorption from solid dosage forms. As the amorphous is in a higher energy state than the crystalline form providing thus improvement of drug solubility [5], crystalline celecoxib was transformed accordingly.

In the present study, celecoxib was deposited on HTlc-calc by solvent evaporation in order to induce its amorphization and to evaluate its solubility. Finally, as amorphous celecoxib is metastable and converts spontaneously during storage in a crystalline form [20,21], its physical state stability, when deposited onto hydrotalcite, was determined.

2. Materials and methods

2.1. Materials

Celecoxib was extracted from Celebrex®, a Searle Farmaceutici (Milano, Italy) formulation containing celecoxib (200 mg/capsule) as below reported.

Other chemicals and solvents were of reagent grade and used without further purification.

2.2. Methodology

2.2.1. Extraction and purification of celecoxib from Celebrex®

Pure and well-crystallized celecoxib was obtained by treating the content of Celebrex® capsules with cool ethanol and by washing many times the solid, collected by filtration, with water. The powder obtained was recrystallized by ethanol/isopropanol 1:1 (m.p. 160.5 °C).

2.2.2. Preparation of quenched amorphous celecoxib

Celecoxib (200 mg) was kept at 170 °C for a few minutes to melt, then was cooled rapidly by using dry CO₂. Solidified celecoxib was pulverized using a mortar and pestle, then stored in a desiccator at room temperature and at a reduced pressure for 24 h [22] and immediately investigated.

2.2.3. Preparation of HTlc-calc

HTlc-CO₃, obtained by co-precipitation from a “homogeneous solution” of Mg(II) and Al(III) hydroxycarbonate [23], was calcined at 500 °C for 12 h.

2.2.4. Preparation of evaporated celecoxib/HTlc-calc 1/1, 1/2 and 1/4 complexes

HTlc-calc (200 mg) was added to an absolute ethanolic solution (10 ml) of celecoxib (200 mg), and then the suspension was dried in vacuo. The recovered solid (celecoxib/HTlc-calc 1/1 w/w) was pulverized, stored in a desiccator at room temperature and at a reduced pressure for 24 h and then characterized by DSC and X-ray powder diffraction.

Other preparations of the two components in different molar ratios (celecoxib/HTlc-calc 1/2, 1/4 w/w) were prepared accordingly.

2.2.5. Preparation of celecoxib and HTlc-calc physical mixture

The physical mixture was prepared by lightly grinding crystalline celecoxib and HTlc-calc (1/1 weight ratio) using a small mortar and pestle.

2.2.6. X-ray powder diffraction

The X-ray powder diffraction (XRPD) patterns were taken by a computer controlled PW 1710 Philips diffractometer (Lelyweg, Netherlands), using the Ni-filtered Cu Kα radiation (40 kV, 30 mA) by the stepwise scanning procedure (step size 2θ = 0.03°, time per step = 1 s).

2.2.7. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were recorded in KBr dispersion on a Jasco model FT/IR-410, 420 Herschel series (Jasco Corporation Tokyo, Japan).

2.2.8. Thermal analysis

Differential scanning calorimetry (DSC) analyses were performed using an automatic thermal analyser (Mettler Toledo DSC821®). Temperature calibrations were performed using indium as a standard. Holed aluminium pans were employed in the experiments for all samples and an empty pan, prepared in the same way, was used as a reference. Samples of 3–6 mg were weighted directly into the aluminium pans and the thermal analyses were conducted at a heating rate of 5 °C/min from 25 to 200 °C.

Fig. 1. Chemical structure of celecoxib, 4-{5-(4-methylphenyl)-3-(trifluoro- methyl)-1H-pyrazol-1-yl}benzenesulphonamide.
2.2.9. Surface analysis

The N₂ adsorption–desorption isotherms were determined by nitrogen adsorption at −196 °C using a Micrometrics ASAP 2010 apparatus. The preparation of samples was made by degassing the materials at 350 °C, overnight. The specific surface area was calculated by the Brunauer, Emmett and Teller (B.E.T.) theory.

2.2.10. Solubility studies

The solubilities of celecoxib from the evaporated complexes, the celecoxib/HTlc-calc physical mixture, the crystalline and quenched celecoxib were determined in the following way: excess amounts of each sample, in a manner that each preparation corresponded to 9 mg of free celecoxib, were placed in series of closed flat-bottomed glass vessels containing 50 ml of gastric fluid maintained at 37 ± 0.5 °C. The vessels were stirred at 37 °C and 100 rpm for 24 h using an orbital incubator (Gallencamp Incubator Type INR 2000, Leicestershire, UK). At appropriate time intervals, 4 ml samples were withdrawn and centrifuged. The drug concentration was determined with a UV spectrophotometer (Jasco V-250, Tokyo, Japan) at λ_max = 248.8 nm. Tests were made in triplicate, the results were registered as an average and the error was expressed as standard deviation.

2.2.11. Stability of amorphous state

Evaporated complexes (cel/HTlc-calc 1/1, 1/2 and 1/4) and quenched celecoxib were kept in two different storage conditions: (a) at room temperature in a desiccator in the presence of CaCl₂ for 90 days; (b) at 40 °C and 75% of relative humidity for 30 days. The presence of the crystalline form was evaluated by X-ray diffraction at the end of the experiment. Only samples stored at 40 °C and 75% of relative humidity were analysed by X-ray after 2 and 7 days.

3. Results and discussion

After purification and characterization of celecoxib, the evaporated celecoxib/HTlc-calc complexes were characterized for their physico-chemical properties and compared with the crystalline form, the amorphous celecoxib, obtained by quenching, and the celecoxib/HTlc-calc physical mixture.

3.1. X-ray diffraction analysis

The XRD patterns of crystalline, quenched, evaporated complexes (different ratios) and celecoxib HTlc-calc physical mixture are shown in Fig. 2. HTlc-calc has no crystalline structure and has no peaks, the celecoxib/HTlc-calc physical mixture showed the characteristic diffraction peaks of crystalline celecoxib [20] and the evaporated complexes at the three different weight ratios under examination showed the absence of diffraction peaks. These data indicate that the drug crystallinity did not change in the physical mixture, while the amorphous form for the drug is present in the evaporated complexes.

3.2. Differential scanning calorimetry

DSC thermograms of crystalline, quenched, evaporated complexes at the different weight ratios and the celecoxib physical mixture are reported in Fig. 3.

Crystalline celecoxib (Fig. 3a) showed an endothermic peak at 163.4 °C (ΔH = −93 J/g) due to drug melting. In contrast, quenched celecoxib (Fig. 3b) showed an exothermic peak at 100.3 °C (ΔH = 51 J/g), due to its recrystallization, and an endothermic melting peak at 165.4 °C (ΔH = −78 J/g). Melting of celecoxib could be observed in the physical mixture (Fig. 3d) at 164.1 °C (ΔH = −85 J/g of drug). On the other hand, the evaporated complex celecoxib/HTlc-calc 1/1 (Fig. 3e) thermogram shows an exothermic peak of crystallization at 102.2 °C (ΔH = 17.9 J/g of drug) and two endothermic peaks due to the fusion of two different polymorphs at 151 °C (ΔH = −12.9 J/g of drug) and 163 °C (ΔH = −33 J/g of drug). The exothermic crystallization peak and the low fusion enthalpy change are proofs for celecoxib crystallinity decrease as confirmed by the XRD diffraction pattern. In the other evaporated complexes, the crystallization peak was not observed while the peaks relative to melting points were observed at 150.8 °C (ΔH = −12.9 J/g of drug) and at 162.8 °C (ΔH = −32.6 J/g of drug), at 151.9 °C (ΔH = −7.4 J/g of drug) and at 162.9 °C (ΔH = −7.4 J/g of drug) for the evaporated complexes cel/HTlc-calc 1/2 (Fig. 3f) and 1/4 (Fig. 3g), respectively. Besides, cel/HTlc-calc systems show a lower tendency to crystallize than amorphous celecoxib. At last,
whereas 1/1 and 1/2 weight ratio cel/HTlc-calc complexes showed the same ΔH fusion values, the 1/4 showed a lower ΔH fusion value due because of the reduced presence of the crystalline form.

### 3.3. Fourier transform infrared spectroscopy

Crystalline celecoxib (Fig. 4a) and physical mixture (Fig. 4d) FT-IR spectra (Fig. 4) showed peaks at 3335 and 3230 cm⁻¹ due to the stretching of NH₂ and at 1348 and 1166 cm⁻¹ due to S=O symmetric and asymmetric stretchings. In quenched celecoxib spectrum (Fig. 4b), NH₂ stretching was more diffused and broadened while the S=O stretchings were observed at lower frequencies (1339 and 1148 cm⁻¹) and had lower intensity, probably due to hydrogen intermolecular bonds [20]. In evaporated celecoxib/HTlc-calc complexes, NH₂ stretching peaks were observable only when the weight ratio was 1/1 (Fig. 4e), in all other compositions (Figs. 4f and g) these peaks were no more detectable. To confirm this observation, IR spectra of physical mixtures of celecoxib/HTlc-calc with 1/2 and 1/4 were performed (data not reported) and both revealed the presence of NH₂ stretching peaks. The disappearance of these peaks in 1/2 and 1/4 evaporated mixtures could be due to the greatly disordered arrangement of celecoxib when the inorganic content is high. The 1/1 preparation showed peaks relative at S=O stretching less resolved and at 1346 and 1166 cm⁻¹, higher frequencies in comparison to those observed for the quenched form. In other preparations, the peak relative to asymmetric stretching of S=O gradually decreased in intensity and the one relative to symmetric stretching was detectable at higher frequencies (1372 and 1380 cm⁻¹ for 1/2 and 1/4 complexes, respectively) and in a very broadened form. These effects may be attributable to the formation of interactions between NH₂ and SO₂ of celecoxib and HTlc-calc.

### 3.4. Surface analysis

The specific surface area of crystalline celecoxib, HTlc-calc and evaporated celecoxib/HTlc-calc system 1/4 was also characterized. The calculated B.E.T. surface areas of these samples were, 4.1, 29.3 and 28.3 m²/g, respectively.

### 3.5. Solubility studies

In Table 1, solubility (mg/L) values for crystalline celecoxib, quenched celecoxib and celecoxib from physical mixture and evaporated systems in gastric fluid are reported. In the case of the evaporated complex cel/HTlc-calc 1/1, no increase of drug concentration was observed in comparison to crystalline celecoxib. The evaporated mixtures at 1/2 and 1/4 weight ratio showed a gradual increase of drug solubility (4.51 and 5.22 mg/L, respectively).

The solubility of celecoxib from 1/4 complex was almost similar to that of quenched celecoxib (5.97 mg/L).
Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Celecoxib concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystalline celecoxib</td>
<td>3.42 ± 0.49</td>
</tr>
<tr>
<td>Celecoxib/HTlc-calc 1/1</td>
<td>3.55 ± 0.24</td>
</tr>
<tr>
<td>Celecoxib/HTlc-calc 1/2</td>
<td>4.51 ± 0.34</td>
</tr>
<tr>
<td>Celecoxib/HTlc-calc 1/4</td>
<td>5.2 ± 0.56</td>
</tr>
<tr>
<td>Quenched celecoxib</td>
<td>5.97 ± 0.48</td>
</tr>
<tr>
<td>Celecoxib/HTlc-calc 1/1 complex</td>
<td>3.22 ± 0.19</td>
</tr>
</tbody>
</table>

Data are means ± SD, n = 3
interactions, between the inorganic matrix and the drug, is present in the highest concentration, probably because of celecoxib. This process does not happen when HTlc-calc and then the nucleation and the subsequent crystallization promotes the dissolution of the drug on the solid surface in the presence of humidity, the adsorption of water vapour in the presence of high HTlc-calc amount. Maybe that in an increase of the amorphous form stability was achieved, for example, in 1/4 systems decreases the tendency of celecoxib to crystallize because of the presence of interactions established between celecoxib and HTlc-calc, as shown by FT-IR, improving hence the stability of amorphous form during storage. According to these findings, the use of high concentration of HTlc-calc into the complexes appears to be very useful to enhance the solubility of poorly water-soluble drugs. The celecoxib/HTlc-calc systems have also other advantages:

(1) quite low ratio drug/excipient,
(2) easy preparation,
(3) use of ethanol as a solvent, with consequent reduction of toxicity issues and ecological and economic problems related to the use of other more toxic solvents.

4. Conclusions

Amorphous celecoxib was prepared by deposition on HTlc-calc at three different weight ratios. These samples and the quenched amorphous form showed no significant difference in powder X-ray diffraction patterns, confirming the presence of the drug in an amorphous form, but differed by DSC and IR spectra. This could be attributed to the differences, within the amorphous forms, of dissimilar intermolecular interactions (celecoxib/celecoxib and celecoxib/HTlc-calc). DSC spectra showed that the presence of HTlc-calc in 1/4 systems decreases the tendency of celecoxib to crystallize because of the presence of interactions established between celecoxib and HTlc-calc, as shown by FT-IR, improving hence the stability of amorphous form during storage. According to these findings, the use of high concentration of HTlc-calc into the complexes appears to be very useful to enhance the solubility of poorly water-soluble drugs. The celecoxib/HTlc-calc systems have also other advantages:

The X-ray diffraction patterns of quenched celecoxib and evaporated systems 1/1 and 1/4, stored at different conditions of temperature and humidity, are reported in Figs. 5–7, respectively. XRPD of 1/2 complex was almost the same of 1/1 system (data not reported).

When samples were stored at room temperature and at anhydrous conditions, quenched celecoxib did not conserve its amorphous status and peaks of the crystalline drug could be seen in its XR diffraction pathway. On the other hand, the amorphous form of the evaporated systems, especially in the case of 1/4 weight ratio, was maintained longer. In fact powder X-ray diffraction patterns of quenched celecoxib, performed after 90 days of storing, showed the characteristic peaks of the crystalline drug. Moreover these peaks were not present in X-ray diffraction patterns of evaporated complexes confirming thus their amorphous form stabilization.

When complexes and amorphous samples were stored at 40 °C and 75% relative humidity, the presence of the crystalline form could be revealed after 2 days in all samples, with the exception of the system 1/4. In this case the amorphous state was maintained for over 30 days. It means that an increase of the amorphous form stability was achieved in the presence of high HTlc-calc amount. Maybe that in the presence of humidity, the adsorption of water vapour promotes the dissolution of the drug on the solid surface and then the nucleation and the subsequent crystallization of celecoxib. This process does not happen when HTlc-calc is present in the highest concentration, probably because interactions, between the inorganic matrix and the drug, prevent celecoxib crystallization. Besides HTlc-calc interacts with water molecules and reconstructs its layered structure on the base on the memory effect [24], thus subtracting water which is no more disposable for drug dissolution and nucleation.

References