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Research paper

Exploring the use of spray congealing to produce solid dispersions with enhanced indomethacin bioavailability: *In vitro* characterization and *in vivo* study

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ABSTRACT

The current study proposes an original oral delivery system for the bioavailability enhancement of indomethacin (IND), a BCS class II drug, with the aim to overcome the common limitations of amorphous solid dispersion. In fact, the potential risk of drug re-crystallization is a serious concern for the stability of amorphous systems and represents, despite the great bioavailability, one of the primary causes of their limited clinical applications. IND-loaded microparticles (MPs) were prepared by spray congealing using oral-approved excipients (Gelucire 50/13 and the recently marketed Gelucire 48/16). MPs were characterized regarding particle size, morphology, drug content and IND solid state; moreover, they were tested *in vitro* for IND solubility and dissolution rate. Solid state characterization indicated that IND was present into the MPs in the amorphous form. The best formulation showed a considerable enhancement in drug dissolution rate and 31-fold higher drug solubility than pure γ-IND. The oral administration of MPs showed 2.5-times increased bioavailability *in vivo* compared to either pure γ-IND or its physical mixture with unloaded MPs. Notably, the formulation was stable after 18 months with no changes in IND solid state and dissolution performance. This study offers a valid approach to enhance IND oral bioavailability by conversion into the amorphous form by spray congealed MPs, which have great potential for industrial application due to their characteristics of high encapsulation efficiency, no-toxicity, low-cost, prolonged stability and the use of a simple and easily scaled-up manufacturing technology.

1. Introduction

The preparation of Solid Dispersion (SD) is one of the most commonly used approach to improve the biopharmaceutical properties of drugs belonging to class II of the Biopharmaceutical Classification System (BCS) [1–3]. In SD the drug is incorporated in an inert hydrophilic carrier in the solid state. The success of SD lies in the potential to (i) decrease the drug particle size even up to molecular level (ii) modify the drug solid state from a thermodynamically stable form to a high-energy one and (iii) improve drug particles wettability by the aid of the hydrophilic excipient. However, various problems limit SD industrial applications. Specifically, the low reproducibility and consistency in the quality of SD often lead to variations in the bioavailability [4]. In addition, due to the intrinsic characteristics of SD, the physico-chemical instability of the dosage form during manufacturing and storage is probably the main issue that still need to be totally addressed [5]. For the aforementioned reasons, managing the commercial production of SD-based products is more challenging than traditional products containing drugs in their most stable solid form [6]. The methods for the preparation of SD can be categorized in three types: solvent-based methods (e.g. spray drying, freeze-drying), melting-based methods (e.g. hot melt extrusion) and the recently introduced mechanochemical activation, based on high energy milling techniques (e.g. cryo-milling) [7]. An important reason of concerns in view of an industrial application of SD is the toxicity related with the use of organic solvents. On the other hand, the melting-based methods also presents some drawbacks, such as need of multiple downstream processes and, thus, higher production costs. The mechanochemical activation leads to products with unfavourable handling characteristics (i.e. poor flowability). Therefore, there is increasing need of simple, inexpensive technologies for the

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production of stable SD.

Indomethacin (IND) is a non-steroidal anti-inflammatory drug, belonging to BCS class II, used for the treatment of rheumatoid arthritis and other chronic inflammatory diseases. Despite its long time and widespread use [8], IND can cause severe gastrointestinal complications, increased blood pressure and decreased kidney function [9], and the risk of developing these adverse effects increases in the case of high doses and prolonged treatments. Additionally, the poor water solubility of IND represents a major limitation for its oral bioavailability. Many attempts to increase IND solubility and dissolution rate using different formulation strategies have been reported [9–12], with the ultimate objective of reducing the daily dose and/or administration frequency.

This research proposes an original oral delivery system for the bioavailability enhancement of IND, which can address the multiple demands, desirable for a successful SD, of reproducible *in vitro* and *in vivo* performance, easily scaled-up manufacturing technology, non-toxic and low-cost formulation and prolonged stability. Specifically, spray congealing technology was used for the manufacturing of IND-containing SD in the form of microparticles (MPs). Spray congealing is a technology commonly used for the encapsulations of nutrients and drugs mainly in the food and veterinary industries. The process is based on the atomization of a fluid, consisting in a solution or suspension of a drug in a molten carrier, and on the subsequent solidification of the "spray". The result consists in solid, highly spherical MPs with very good flowing properties and ready-to-use [13]. A commercially available mixtures of mono, di and triglycerides with PEG esters of fatty acids, called "Gelucires" were chosen as hydrophilic low-melting temperature carriers. Gelucires are a family of vehicle including different excipients, all Generally Recognized as Safe (GRAS) and oral-approved, wherein Gelucire 50/13 is probably the most studied for preparing matrix system such as particles, granules [14], minitablets [15] and solid dispersions [16] and successfully used in spray congealing [17]. In this study Gelucire 48/16, a new excipient recently marketed, was evaluated as suitable carrier to prepare MPs by means of spray congealing. Hence, the initial focus of our research was to explore the potential of Gelucire 48/16 for the dissolution enhancement of IND and compare it to Gelucire 50/13. Being SD complex binary systems where each component might influence the behaviour of the other, the physicochemical properties of both components may determine the overall performance of the final formulation [18]. Therefore, a detailed solid state characterization was performed to understand the physicochemical properties of IND-loaded MPs after manufacturing and during storage. *In vitro* investigation on the MPs biopharmaceutical properties and *in vivo* study on rats after oral administration were performed to assess the benefits of the proposed formulation on oral bioavailability. Finally, the long-term stability of the best formulation for more than a year was evaluated.

2. Material and methods

2.1. Materials

Gelucire 50/13 and Gelucire 48/16 were kindly supplied from Gattefossè (Milan, Italy). γ-Indomethacin (γ-IND), 9-phenyl-carbazole, phosphoric acid and absolute ethanol were obtained from Sigma Aldrich (Steinheim, Germany). Methanol and water were high performance liquid chromatography (HPLC) grade from Sigma Aldrich (Milan, Italy). All other reagents and solvents were of analytical grade (Sigma-Aldrich). Male Sprague – Dawley rats were provided by Charles-River (Milan, Italy).

2.2. Preparation of IND-loaded MPs

MPs were produced by spray congealing using an external-mix two-fluid atomizer, called Wide Pneumatic Nozzle (WPN). Initially, the excipients of the formulation (Gelucire 50/13 and Gelucire 48/16 in different ratio) were heated up to a temperature 5 °C above their melting point. IND (10% w/w) was added to the molten carrier and magnetically stirred until complete solubilization, and then loaded into the feeding tank. The temperature of the feeding tank of the nozzle and the inlet air pressure were set at 65 °C and 3.5 bar, respectively. The atomized molten droplets hardened during the fall into a cylindrical cooling chamber, which was held at room temperature and the MPs were collected from the bottom of the cooling chamber. Three different drug-loaded formulations (MPs A, MPs B, MPs C) were produced (Table 1). For comparison purposes, physical mixes of γ-IND and excipients in the same weight ratio as the loaded MPs were prepared by mixing 10% of γ-IND with 90% of unloaded MPs.

Table 1
Composition, drug loading (DL) and encapsulation efficiency (EE) values of IND-loaded MPs.

Sample	Costituents (%, w/w)			DL (%, w/w ± SD)	EE (%)
	Gelucire 50/13	Gelucire 48/16	IND		
MPs A	90	–	10	10.73 ± 0.14	107.26
MPs B	45	45	10	9.91 ± 0.22	99.06
MPs C	27	63	10	10.06 ± 0.20	100.61

2.3. IND-loaded MPs characterization

Morphological analysis. Shape and surface morphology of IND and MPs were observed by Scanning Electron Microscopy (SEM). Samples were fixed on the sample holder with double-sided adhesive tape, sputter coated with Au/Pd under argon atmosphere performed using a vacuum evaporator (Edwards, Crawley UK) and examined by means of a scanning electron microscope (ESEM Quanta-200) operating at 20,0 kV accelerating voltage.

Particle size analysis. Size distribution of MPs was evaluated by sieve analysis using a vibrating shaker (Octagon Digital, Endecotts, London UK) and a set of six sieves ranging from 75 to 500 μm (Scientific Instrument, Milan, Italy).

Determination of drug content. IND content was determined by dissolving 20 mg of MPs accurately weighed in 50 mL of ethanol. The solution was shaken for 24 h at 25 °C. Finally, the solution was filtered, diluted with the same solvent, and the drug content was assayed spectrophotometrically (UV2 Spectrometer, Unicam) at 320 nm. Each formulation was analysed in triplicate and the mean ± SD was reported.

Differential scanning calorimetry (DSC) studies. DSC analysis were performed using a Perkin Elmer DSC 6 (Perkin Elmer, Beaconsfield UK) with nitrogen as purge gas (20 mL/min). The instrument was calibrated with indium and lead for temperature, and with indium for the measurements of the enthalpy. Samples of pure IND, unloaded MPs and IND-loaded MPs, weighing 8–9 mg, were placed in an aluminium pan and heated from 25 to 220 °C at a scanning rate of 10 °C/min.

Fourier transform-infrared spectra (FT-IR) analysis. Studies of infrared spectra of pure drug, unloaded MPs and IND-loaded MPs were conducted with an IR spectrophotometer (Jasco FT-IR A-200) using the KBr disc method. The samples were mixed with KBr and compressed into tablet (10 mm in diameter and 1 mm in thickness) using an hydraulic press (Perkin Elmer, Norwalk USA). The scanning range was 650–4000 cm^{-1} and the resolution was 1 cm^{-1}.

Hot Stage Microscopy (HSM) analysis. Physical changes in the samples during heating were monitored by HSM studies using a hot stage apparatus (Mettler-Toledo S.p.A., Novate Milanese, Italy) mounted on Nikon Eclipse E400 optical microscope connected to a Nikon Digital Net Camera DN100 for the image acquisition. The samples were equilibrated 25 °C for 1 min and then heated at a scanning rate of 10 °C/min in the 25–200 °C range of temperature. The magnification was set at 10x.

Powder X-Ray Diffraction (PXRD) analysis. Single components, MPs and corresponding physical mixtures were studied by X-ray powder diffraction technique using a Philips X'Pert powder diffractometer equipped with a graphite monochromator in the diffracted beam. CuKα radiation was used (40 mA, 40 kV). The spectra had been obtained in the 3°–35° 2θ range using a 0.05° step and a 0.216°/s speed.

2.4. Solubility and dissolution studies

Solubility measurements of pure IND and of IND-loaded MPs were performed in 10 mL of phosphate buffer (0.2 M, pH 5.8) at 25 °C. The samples were magnetically stirred for 48 h, equilibrated for 2 h and the suspensions were then centrifuged at 10.000 rpm for 10 min. The supernatant was filtered through a 0.20 μm membrane filter. Finally, the filtrates were suitably diluted with the same solvent and analysed at 266 nm by UV–Visible spectrophotometer. The study was performed in triplicate.

A dissolution paddle apparatus (Pharmatest, Steinheim, Germany) was used with a stirring rate of 50 rpm. The dissolution medium (phosphate buffer 0.2 M, pH 5.8) was maintained at a temperature of 37 °C. Samples of IND, physical mixtures and IND-loaded MPs (size fraction 100–150 μm) containing a suitable amount of IND for sink conditions (C < 0.2 Cs) were added to 500 mL of dissolution medium. The aqueous solution was filtered and continuously pumped (12.5 mL/min) to a flow cell in a spectrophotometer (UV2 Spectrometer, Unicam) and absorbance values were recorded at 266 nm. The dissolution tests were performed in triplicate. Dissolution profiles were individually compared using the "similarity factor, f2", which could be defined as follows:

$$f_2 = 50 * \log\left\{1 + \left[\frac{1}{n} * \sum_{t=1}^{n} (R_t - T_t)^2\right]^{-0.5} * 100\right\}$$

where n is the sample number, Rt and Tt are the reference assay and test assay at time point t, respectively.

2.5. In vivo studies

HPLC Analysis. The IND quantification for bioavailability studies was performed by HPLC. The chromatographic apparatus consisted of a modular system (model LC-10 AD VD pump and model SPD- 10A VP variable wavelength UV − vis detector; Shimadzu, Kyoto, Japan) and an injection valve with 20 μL sample loop (model 7725; Rheodyne, IDEX, Torrance, CA, USA). Separation was performed at room temperature on a reverse phase column Hypersil BDS C-18, 5U, equipped with a guard column packed with the same Hypersil material (Alltech Italia Srl BV, Milan, Italy). Data acquisition and processing were accomplished with a personal computer using CLASS-VP Software, version 7.2.1 (Shimadzu Italia, Milan, Italy). The detector was set at 319 nm. The mobile phase consisted of a mixture of methanol and 0.2% phosphoric acid (75:25 v/v). The flow rate was 1 mL/min. The compound 9-phenyl-carbazole was used as internal standard in extraction procedures of IND from rat blood (see below). The retention times for IND and 9-phenyl-carbazole were 3.9 and 14.7 min, respectively.

The chromatographic precision for each compound was evaluated by repeated analysis (n = 6) of the same samples. For IND and 9-phenyl-carbazole dissolved in mobile phase the values were obtained for 50 μM (0.018 mg/mL and 0.012 mg/mL, respectively) solutions and were represented by the relative standard deviation (RSD) values ranging between 0.61% and 0.72%, respectively.

The efficacy of IND extraction from blood samples was determined by recovery experiments, comparing the peak areas extracted from 10 μM (3.6 μg/mL) blood test samples at 4° C with those obtained by injection of an equivalent concentration of the drug dissolved in their mobile phase. The average recovery ± SD of IND from rat blood resulted 85.8 ± 3.6%. The concentrations of this compound were therefore referred to as peak area ratio with respect to the internal standard 9-phenyl-carbazole. The precision of the method based on peak area ratio, calculated for 10 μM (3.6 μg/mL) solutions, was represented by RSD values of 0.91%. The calibration of IND was performed by using nine different concentrations in whole blood at 4 °C ranging from 2 μM (0.72 μg/mL) to 80 μM (28.6 μg/mL) and expressed as peak area ratios of the compounds to the internal standard *versus* concentration. The calibration curve resulted linear (n = 9, r = 0.992, P < 0.0001).

In Vivo Administration of Indomethacin: Intravenous Infusion. Male Sprague Dawley rats (200–250 g; n = 4), received a femoral intravenous infusion (0.2 mL/min for 5 min) of 0.90 mg/mL indomethacin dissolved in a medium constituted by 20% (v/v) DMSO and 80% (v/v) physiologic solution. At the end of infusion and at fixed time points within 24 h, blood samples (300 μL) were collected and inserted in heparinized test tubes that were centrifuged at 4 °C for 15 min at 1,500 × g; 100 μL of plasma were then withdrawn and immediately quenched in 300 μL of ethanol (4 °C); 100 μL of internal standard (100 μM 9-phenyl-carbazole dissolved in ethanol) was then added. After centrifugation at 13.000 × g for 10 min, 400 μL aliquots were reduced to dryness under a nitrogen stream and stored at − 20° C until analysis. The samples were dissolved in 150 μL of mobile phase (methanol and 0.2% phosphoric acid 75:25 v/v), and, after centrifugation, 10 μL was injected into the HPLC system for IND assay. All the values obtained were the mean of four independent experiments. The *in vivo* half-life of IND in the blood was calculated by nonlinear regression (exponential decay) of concentration values in the time range within 24 h after infusion and confirmed by linear regression of the log concentration values *versus* time. The area under the concentration-time curve (AUC) value was calculated by the trapezoidal method within 24 h, the remaining area was determined as the ratio between the indomethacin concentration detected at 24 h and the elimination constant (k_{el}), that was obtained from the slope of the semilogarithmic (-slope · 2.3). All the calculations were performed by using the computer program Graph Pad Prism.

In Vivo Administration: Oral Administration of Indomethacin. Powders constituted by γ-IND, or its physical mixture with unloaded MPs C or by IND-loaded MPs C were mixed with palatable food in order to induce their oral assumption by male Sprague Dawley rats (200–250 g; n = 4/group) fasted for 24 h. The dose of orally administered IND was 2 mg for each type of powders. At the end of administration at fixed time points within 8 h, blood samples (300 μL) were collected, then extracted and analyzed as above described.

All *in vivo* experiments were performed in accordance with the European Communities Council Directive of September 2010 (2010/63/EU), a revision of Directive 86/609/EEC, the Declaration of Helsinki, and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Bethesda, Maryland, USA). The protocol of all the *in vivo* experiments was approved by the Local Ethics Committee (University of Ferrara, Ferrara, Italy). Efforts were made to reduce the number of the animals and their suffering.

The AUC values referred to each orally administered treatment were calculated by the trapezoidal method within 8 h, the remaining area was determined as the ratio between the indomethacin concentration detected at 8 h and the elimination constant (k_{el}). The absolute bioavailability values of IND, referred to the oral administered samples, were obtained as the ratio between their oral AUC values and AUC of the intravenous administration of the drug, normalized with respect to their doses, according to the following equation [19]:

$$F = \frac{AUC_{oral}}{AUC_{IV}} \cdot \frac{dose_{IV}}{dose_{oral}}$$

All the calculations were performed by using the computer program Graph Pad Prism.

2.6. Stability studies

After 18 months from production, the physical stability of the IND loaded into the MPs was assessed by FT-IR analysis and by measuring the drug content. The solid state properties of the MPs were studied by means of X-ray powder diffraction analysis. Moreover, dissolution studies were performed to examine possible changes in the biopharmaceutical properties of the MPs.

2.7. Statistical analysis

One-way analysis of variance (ANOVA) followed by the Bonferroni posthoc test (GraphPadPrism, GraphPad software Inc., CA, USA) was used. For the data of solubility studies the level of significance was set at the probabilities of $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. For the data of AUC values obtained by oral administrations $p < 0.001$ was considered statistically significant.

3. Results and discussion

3.1. Production of IND-loaded MPs: morphology, particle size and drug loading

Spray congealing (SC) technology allows the preparation of solid microparticles (MPs) by solidification of the atomized molten fluid along a cooling chamber. Preliminary SC experiments showed that it was not possible to obtain solid MPs using Gelucire 48/16 as the only carrier; in fact after atomization, the droplets could not harden during the fall into the cooling chamber, kept at room temperature. However, a binary system of Gelucire 48/16 and Gelucire 50/13 was identified as suitable for SC production. Different weight ratios between the carriers were tested (Table 1) and the formulation with the highest possible amount of Gelucire 48/16 was MPs C, containing 70% w/w of Gelucire 48/16. It is also important to highlight that in the first step of the SC process, IND completely solubilized in the molten Gelucire forming a bright yellow fluid. IND amount was selected as 10% w/w. Considering that common dosage forms of IND are capsules containing 25 or 50 mg of API, our dosage form would therefore result in a capsule weighting 250–500 mg, which is largely below the upper limit for the mass of a tablet or capsule (about 1 g) [4,20]. Therefore, our approach is feasible by using a drug loading of 10% for this particular API.

All MPs exhibited an experimental drug loading (DL) similar to the theoretical one (10% w/w), hence the encapsulation efficiency (EE) was very close to 100%. Notably, excellent EE values are usually obtained with SC technology [21], representing one of the major advantages of this method.

Fig. 1a shows an unimodal Gaussian particle size distribution for all formulations. More than 90% of MPs presented average diameter between 75 and 500 μm, with minor differences regarding the prevalent size fraction, which was 250–355 μm for MPs A and 150–250 μm for MPs B and MPs C. SEM images of IND and particles (MPs A and MPs C) are reported in Fig. 1b (on the left: low mag. and on the right: high mag.). SEM analysis revealed the successful formation of spherical MPs; the particle surface of MPs A showed some needle-like crystals, which were absent in MPs C.

3.2. Solid state characterization of IND-loaded MPs

IND is known to have a complex solid phase behaviour which include an amorphous form and at least 4 polymorphic forms [22]. Notably, the fact that IND solubilized in melted Gelucire might indicate the formation of MPs as solid solutions, with the drug molecularly dispersed into the carrier. Otherwise, the drug might have undergo recrystallization during the solidification step, leading to the formation of solid dispersions. To have a clear and precise vision of the physicochemical properties of the loaded drug and understand their influence on the biopharmaceutical properties of the final formulation, a detailed solid state characterization was carried out. In order to gain information about the original polymorphic form of IND, a DSC cycle (Fig. 2a) was performed by a first heating step followed by a cooling step and then a second heating step. In the first step of the cycle, a single sharp melting endotherm was observed at 161.2 °C (onset = 157.5 °C) with heat of fusion of 119.11 J/g. During the cooling step, no event correlated to IND crystallization was detected, suggesting the conversion in the amorphous form. Indeed, in the following re-heating step the amorphous IND exhibited a *Tg* at 40.7 °C followed by recrystallization at 101.6 °C (onset temperature and ΔH were 89.1 °C and −66.23 J/g, respectively). In addition, two melting endothermic peaks were observed at 151.8 °C (onset = 147.4 °C) and 158.5 °C (onset = 156.2 °C). Those results were in accordance with the literature [22,23] and revealed that original IND was the stable γ-form. Fig. 2b shows the DSC results of the unloaded and IND-loaded MPs C. The thermogram of *un*-MPs C displayed a broad endothermic event characterized by a small pre-transition and a main transition at 45.9 °C (onset = 40.8 °C), indicating the melting of the carrier. The DSC profile of the particles containing 10% of IND (MPs C) is very similar to the unloaded one, with a broad endothermic peak corresponding to the carrier melting, showing that the presence of IND had no effect on the carrier melting temperature and suggesting the crystallization of Gelucire during MPs solidification in the original crystalline form. Nevertheless, in the DCS curve of MPs C the melting peak of the drug was absent, and the same behaviour was noted for formulations A and B (data not shown). The disappearance of the IND melting peak suggests the conversion of the drug in the amorphous form after the spray congealing process. However, also the dissolution of the drug crystals into the molten carrier during the DSC analysis may cause the melting peak disappearance, as already noticed in the case of Gelucire 50/13 as carrier [24,25].

HSM study was then performed on original drug, MPs A and MPs C (Fig. 3) to get more information on the solid state and physicochemical properties of the drug-loaded MPs. The study confirmed the melting of IND between 160 and 165 °C. Afterwards, the sample was cooled to 25 °C, and interestingly the solidification occurred without evident recrystallization of the drug. In the case of MPs A, the particle started to melt at 60 °C and the melting was complete at 65 °C, in agreement with the melting point of Gelucire 50/13. Although small crystals were observed immediately after carrier melting, they completely disappeared as soon as the temperature was above 80 °C. Differently, MPs C melted at a lower temperature (from 50 to 55 °C) and no crystal was observed thereafter. The results suggested the absence of IND crystals into the MPs, and thus the presence of molecularly dispersed or amorphous IND was hypothesized. However, as also noticed in the DSC study, another possible explanation consists in the progressive melting of IND in the molten Gelucire during analysis, and the consequent lack of clearly visible crystals.

FT-IR analysis was performed to investigate the possible interactions between drug and carrier as well as to gain other information regarding IND physical state inside the MPs. Fig. 4a reported, as example, the spectra of MPs C, Phy mix C, *un*-MP C and pure drug. IND spectrum showed sharp bands at 1716.3, 1691.3, 1613.2, 1588.1 and 1599.6 cm^{-1}, characteristics of the γ form [22,26], confirming that the raw drug was in the stable polymorphic form. Unloaded particles showed bands typical of Gelucire: 3100–3600 cm^{-1} (broad, stretching of free OH groups), 1738.5 cm^{-1} (stretching C = O group), 1469.5 cm^{-1} (C–H deformation of alkyl group), 1113.7 cm^{-1} (–C–O stretching), and 963.3 cm^{-1} (double band, characteristic of the polyethylene glycol groups) [24,27,28]. MPs and Phy mix present FT-IR spectra similar to the unloaded particles but with some extra band that can be ascribed to the presence of drug, although with lower intensity due to the limited content of drug in the sample (10%). To allow a better analysis of the results, the spectra of MPs and Phy mix were compared in the region between the wavenumbers 1800–1500 cm^{-1}, specific for the carbonyl stretching. As visible in Fig. 4b, the carrier presents only one band at

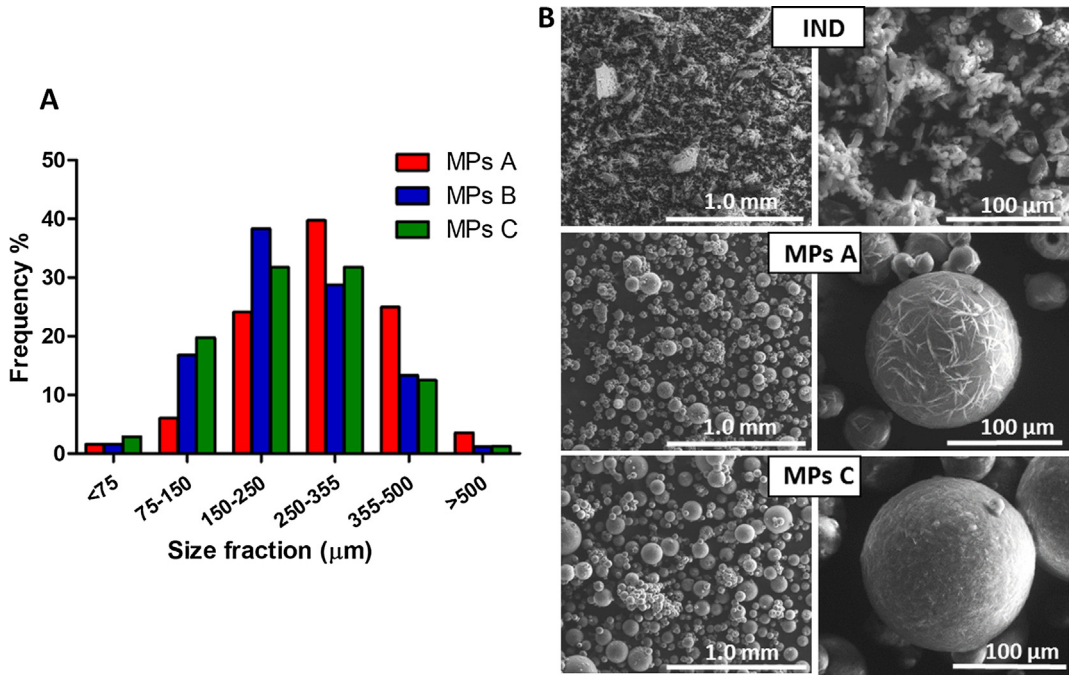

Fig. 1. Particle size distribution of MPs A, MPs B and MPs C (A), SEM images of pure IND, MPs A and MPs C after preparation (B).

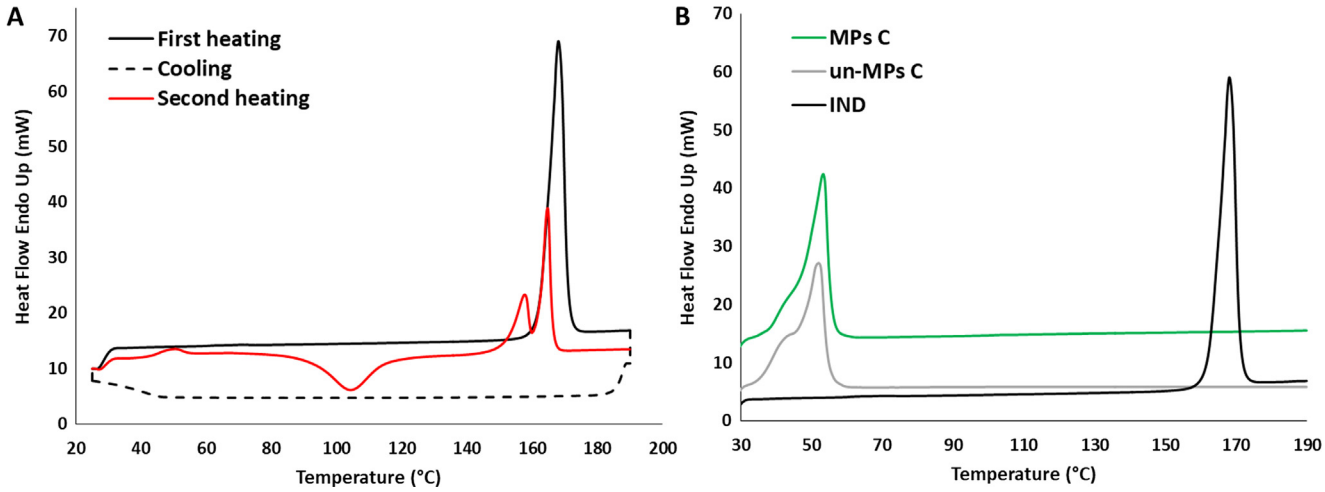

Fig. 2. DSC cycle of IND (A) and DSC profiles of MPs C, unloaded MPs C (un-MPs C) and original IND (B).

1720–1750 cm^{-1} with no other bands in this region, thus the extra bands present in Phy mix and MPs samples can be ascribed to IND. Interestingly, various differences were detected between the two samples. Notably, the specific bands positions of IND in the Phy mix C corresponded to the original IND crystalline form γ, and the same bands were observed for Phy mix A and B (data not shown). The bands of IND were observed at different wavenumbers in case of all MPs formulations (Fig. 4b for MPs C, data not shown for MPs A and MPs B). The differences in the carbonyl stretching region depend on the hydrogen-bonding of the carboxylic acid and amide carbonyl group of IND, which can have different arrangements in the various drug solid forms [29]. The bands detected in the MPs in this region (at wavenumbers of 1681, 1591 and 1610 cm^{-1}) were similar to the characteristic signals of the amorphous form, whereas the band at 1649 cm^{-1} is characteristic α-form [30]. Additionally, the strong band (at 1734 cm^{-1} and 1735 cm^{-1} for α and amorphous forms, respectively) assigned to the non-hydrogen bonded carboxylic acid was missing. Therefore, these data provided a clear indication that spray congealing process modified the IND solid state, but further analysis were needed to confirm the solid state form of IND in our system.

PXRD analysis were thus performed; the results are shown in Fig. 5. The diffractogram of IND showed main peaks at 11.6, 17.1, 19.7, 21.9, 26.7 and 29.4 of 2θ, confirming that the raw drug was in the crystalline γ form. The XRD spectra of both formulations A and C physical mixtures showed, besides the two main peaks at 19.3 and 23.5 of 2θ typical of Gelucires [16,25], all the characteristics peaks of γ IND, although less intense due to the small amount (10% w/w) of the drug. On the contrary, the diffractograms of either MPs A and MPs C showed only the peaks correspondent to the carrier, and no distinct peak attributable to IND was detected. These results indicated a loss of IND crystallinity into the spray-congealed MPs.

Gelucire 50/13 has been used as carrier for different poorly water soluble APIs with formation of either *solid solutions* of the drug molecularly dispersed within the carrier, as in the case of ursolic acid [16] or *solid dispersions* with crystalline drug molecules, such as for piroxicam [31], carbamazepine [24] and praziquantel [25]. The formation of one of the other system depends on the solubility of the drug into the Gelucire at the molten state. In the present study, the solid state

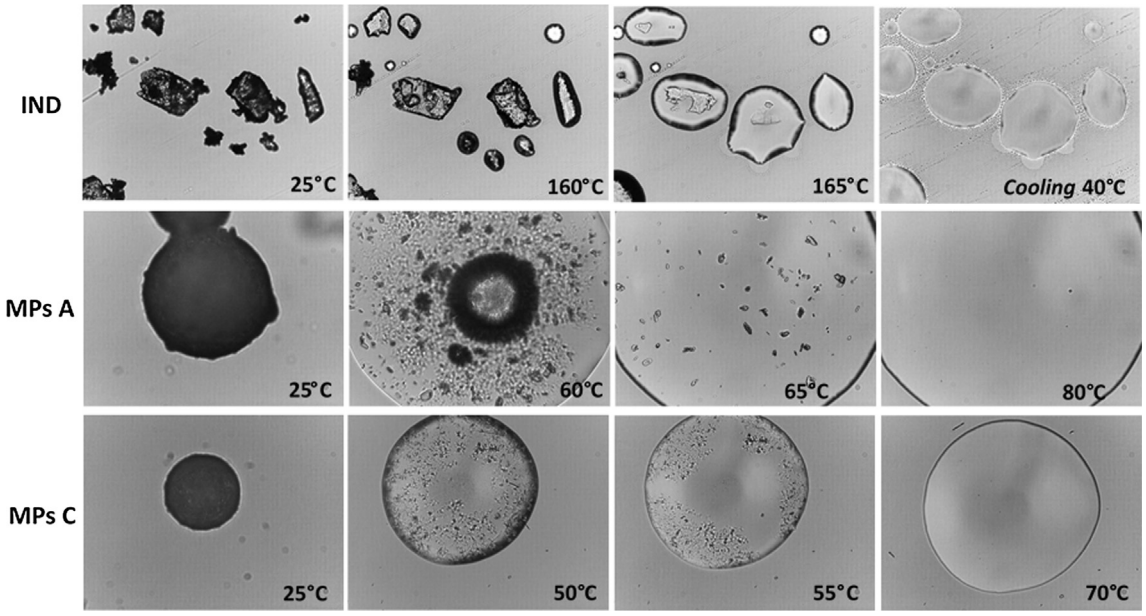

Fig. 3. HSM images of pure IND, MPs A and MPs C during heating. For all images the magnification was set at 20x.

characterization data suggested the presence of non-crystalline IND in crystalline carrier Gelucire. IND-loaded MPs could be thus considered a solid solution, defined as a system in which one solid component is (at least partially) dissolved in the other solid component, resulting in a one-phase system [4]. On the other hand, the experimental results do not exclude that API molecules may exist as separated amorphous phase or may be present in an intimate mixture with the crystalline carrier in which the degree of contact may vary from fully miscible (solid solution) to partly separated domains of the drug [32].

3.3. *In vitro* dissolution and solubility studies

IND (pKa 4.5) can be considered practically insoluble in simulated gastric fluid (pH 1.2) and slightly soluble in simulated intestinal fluid (pH 7.4) [33]. Since IND solubility increases with the raising of the pH, the drug absorption is facilitated in intestinal environment, where the pH is higher compared to the gastric fluid. However, intestinal pH values weakly acidic are not uncommon, especially in fed conditions, where the average pH is reported to be around 5.8 [34], thus leading to a difficult drug dissolution. For this reason, in the present research the dissolution and solubility studies were performed in weakly acidic

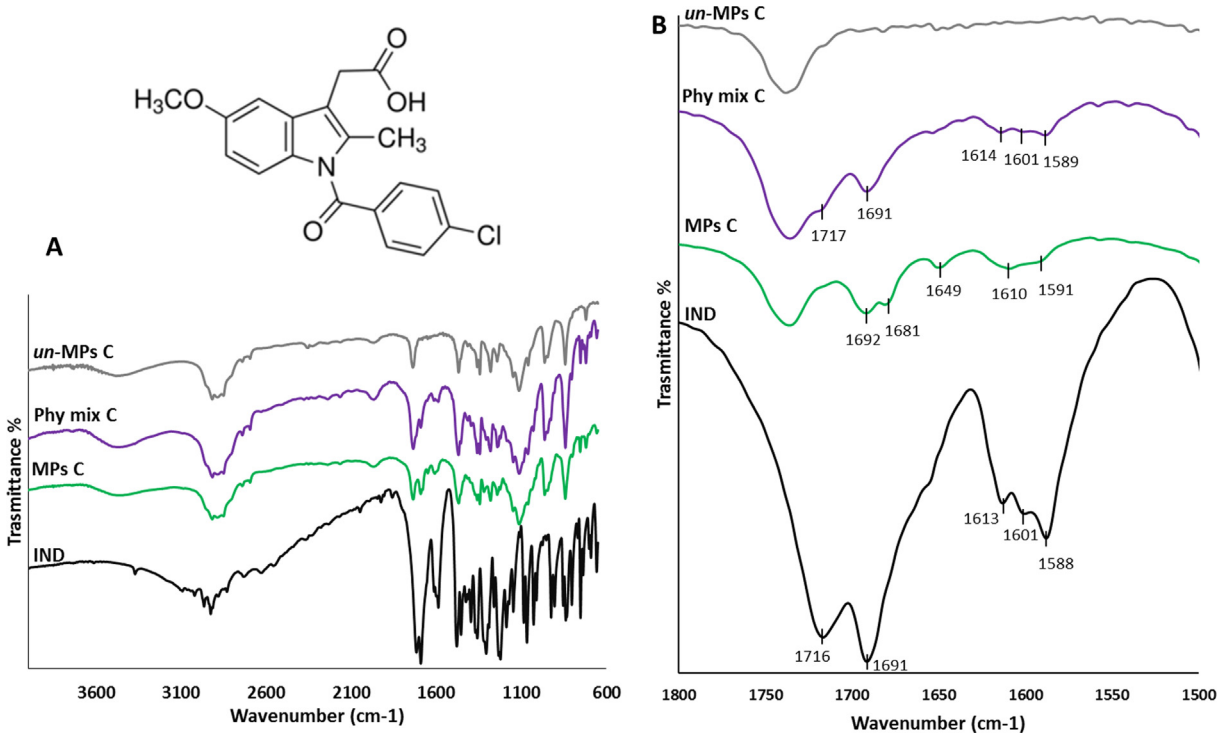

Fig. 4. FT-IR spectra of IND, MPs C, Phy mix C and *un*-MPs C in the spectral region between 4000 and 400 cm^{-1} (A) and focus on the carbonyl stretching region 1800–1500 cm^{-1} (B).

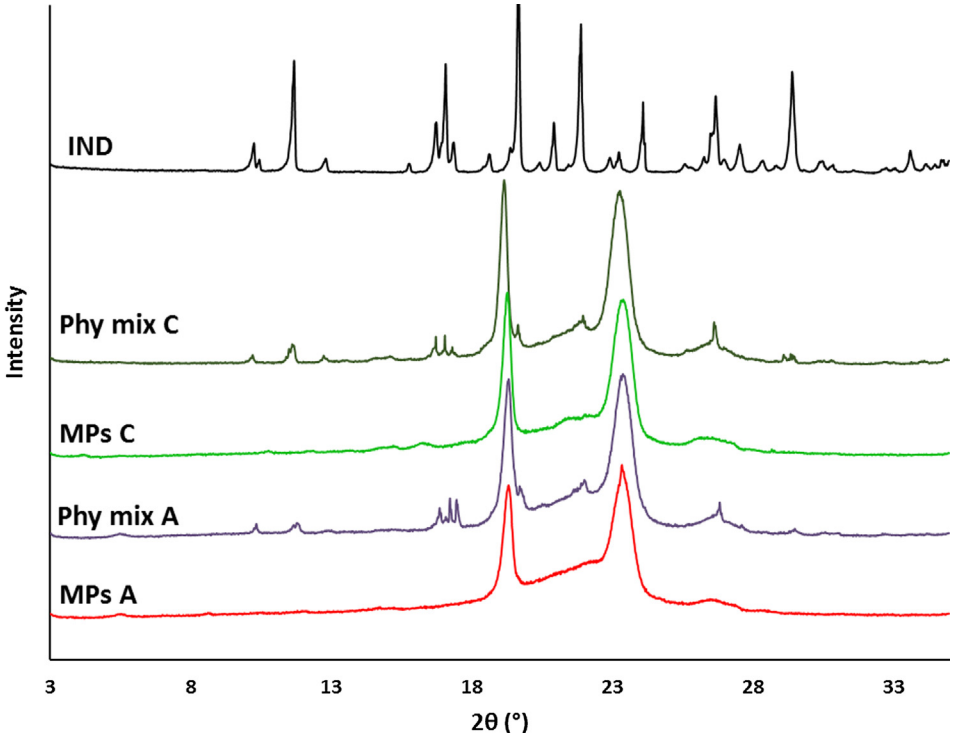

Fig. 5. Powder X-Ray diffractograms of raw IND, IND-loaded MPs and the correspondent physical mixture (formulations A and C).

buffer (pH 5.8) rather than at neutral pH (i.e. pH 6.8 or 7.4), in order to simulate the least favourable (and more challenging) intestinal condition.

In vitro dissolution profiles are reported in Fig. 6a. Dissolution profiles were compared using the "similarity factor, f_2". IND powder showed the slowest dissolution rate, with 53% of drug dissolved within 30 min. Phy mix A, B and C improved IND dissolution rate ($f2$ = 39.3, 39.2 and 36.3 for Phy mix A, B and C respect to IND, respectively), with an effect more evident in the beginning of the test, probably attributed to the improvement of wettability [35]. MPs A considerably enhanced IND dissolution rate, leading to 80% of drug dissolution within 30 min. The highest dissolution rate was achieved by MPs B and MPs C, with 89 and 87% of drug dissolved in the first 30 min, respectively. Specifically, the dissolution performance of MPs resulted different from both the correspondent physical mixtures (f_2 = 26.7, 20.3 and 22.7 for formulation A, B and C, respectively) and IND with f_2 values lower than 20 (f_2 = 17.1, 12.8 and 13.6 for MPs A, B and C, respectively). The dissolution profiles of the different MPs formulations resulted not significantly different ($f_2 \geq 50$) indicating no substantial influence of the Gelucire 50/13 and 48/16 ratio on IND dissolution profiles. Overall, the significantly different dissolution profiles given by the physical mixtures compared to those given by the MPs could be correlated to different mechanisms. In the case of physical mixtures, the improvement of wettability of IND and the solubilisation of the drug by Gelucire at the diffusion layer [36] are likely to be the main mechanisms involved. Moreover, the surface active properties of Gelucire may influence both drug dissolution rate and solubility by formation of micelles. In the case of spray congealed-MPs, we suppose that additional mechanisms are

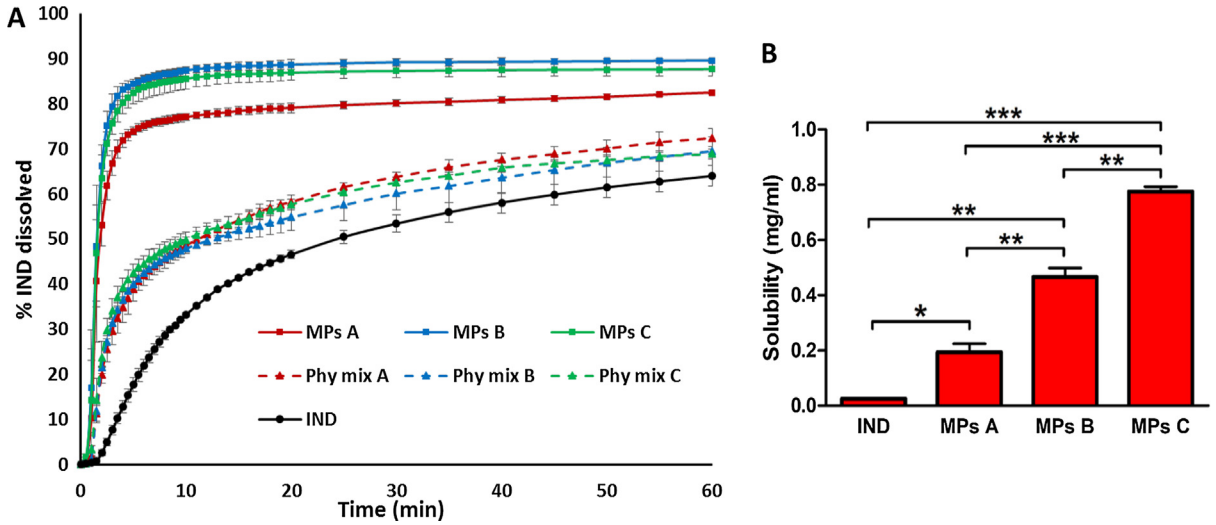

Fig. 6. (a) Dissolution profiles of IND, MPs and physical mixtures (Phy mix) in phosphate buffer pH 5.8 and (b) 48 h equilibrium solubility of IND and MPs in phosphate buffer pH 5.8. Data represent mean ± S.D. (n = 3), and the level of significance was set at the probabilities of $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

involved. The presence of the drug embedded in the hydrophilic excipient can further improve the wetting and dissolution. Qi et al. suggested that, during dissolution, this excipient is subjected to swelling (hydration) and formation of a liquid crystalline phase. This process can facilitate the wetting of the drug particles embedded in the microspheres and maximise the surface area via prevention of aggregation [31]. In addition, the loss of crystallinity of IND in Gelucire-based system, as demonstrated by solid state results, surely represents an advantage in terms of dissolution rate. It is well known that the absence of drug crystals improve dissolution rate as the energy normally required to break up the ordered crystalline structure is no longer a limitation.

In addition to the dissolution rate, the change in IND solid state as well as the formation of micelles can both determine an enhancement in drug solubility, which was evaluated in phosphate buffer (pH 5.8). As expected, the solubility of IND from MPs A, MPs B and MPs C (Fig. 6b) was 0.194 ± 0.044, 0.466 ± 0.045 and 0.775 ± 0.025 mg/mL, respectively. Compared to free IND (0.025 ± 0.002 mg/mL), the solubility of IND formulated into MPs A, B and C was approximately 4-, 19-, and 31-fold higher, respectively. Specifically, the enhancement of solubility with increasing amount of Gelucire 48/16 in the formulation indicates a better solubilisation ability of this excipient. MPs C were therefore selected for oral administration studies.

3.4. In vivo bioavailability studies

After intravenous (IV) infusion of 0.90 mg IND, the drug concentration in the rat bloodstream was 13.43 ± 0.9 μg/mL. This value decreased during time with an apparent first order kinetic (Fig. 7) confirmed by the linearity of the semilogarithmic plot reported in the inset of Fig. 7 (n = 8, r = 0.996, P < 0.0001), showing an half-life value of 8.84 ± 0.31 h. These data are in good agreement with those obtained by previous studies on IND pharmacokinetics [37].

Fig. 8 reported the rat blood IND concentrations within 8 h the oral administration of 2.0 mg of drug (about 8 mg/kg) as powders of free γ-IND, its physical mixture with unloaded MPs C (Phy mix C), or IND-loaded MPs C (MPs C). It can be observed that the free γ-IND powder induced a concentration peak in the rat bloodstream of about 10 μg/mL two hours after the administration (T_{max}) and a similar profile was obtained with the Phy mix C. These data appear in agreement with those obtained by previous studies, indicating IND peak concentrations in bloodstream of rats of around 30 μg/mL two hours after the administration of 22.5 mg/kg of IND suspended in methyl cellulose [38]. In addition, it is reported that the oral administration of 0.9 mg IND (about 3.6 mg/kg) as solid powder to rats induces peak concentrations

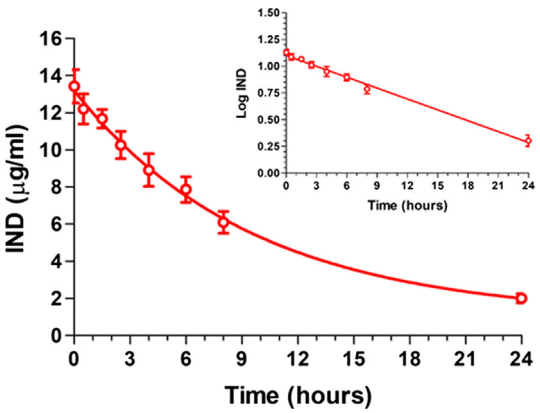

Fig. 7. Elimination profile of indomethacin after 0.90 mg IV infusion to rats. The elimination followed an apparent first order kinetic, confirmed by the semilogarithmic plot reported in the inset (n = 8, r = 0.996, P < 0.0001). The half-life of IND was calculated to be 8.84 ± 0.31 h. All data are expressed as the mean ± SD of four independent experiments.

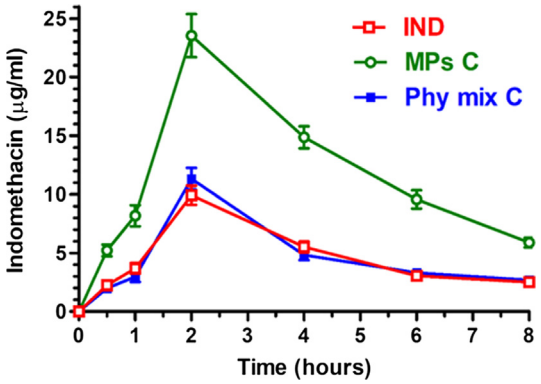

Fig. 8. Blood IND concentrations (μg/mL) obtained by oral administration of 2.0 mg dose to rats within 8 h. The formulations were constituted by the powders of free γ-indomethacin (IND), its physical mixture with unloaded MPs C (Phy mix C), or IND-loaded MPs C (MPs C). All data are expressed as the mean ± SD of four independent experiments.

in the bloodstream near to 5 μg/mL, with a corresponding T_{max} value of two hours [37].

On the other hand, the profile resulting from loaded MPs C showed a peak concentration of about 24 μg/mL two hours after its administration (Fig. 8).

As reported in Table 2, it can be observed that the AUC value obtained by the oral administration of free γ-IND (70.55 ± 2.26 μg·mL^{-1}·h) was not significantly different (P > 0.05) from the AUC value obtained by the physical mixture administration (76.99 ± 2.26 μg·mL^{-1}·h), whereas a significant difference (P < 0.001) was detected between the AUC values obtained by the oral administration of free γ-IND and MPs C.

In particular, the AUC value of the microparticulate formulation was 174.3 ± 5.8 μg·mL^{-1}·h, about 2.5 times higher than the AUC value of free γ-IND. These data indicate that the formulation of IND into Gelucire-based MPs allows to sensibly increase the amounts of IND absorbed in the bloodstream. Interestingly, no significant effect was observed on the absorption rate of IND in the bloodstream, being 2 h the T_{max} values for all the samples tested. A similar behaviour was previously registered with IND formulations constituted by self-emulsifying systems [38].

The ability of the MPs C to increase *in vivo* IND bioavailability was therefore attributable to the microparticulate formulation and not to its excipients, being the AUC value of the physical mixture not significantly different to that of free γ-IND. Although the physical mixtures led to a small enhancement of IND *in vitro* dissolution rate (Fig. 6), the improvement of the drug wettability promoted by the presence of Gelucire did not induce a significant effect on the drug absorption in the bloodstream. This can be explained by considering that the effect of increased wettability might be induced either way by other components of the GIT, such as bile acid salts, released by the gall bladder for the

Table 2
AUC values obtained by intravenous (IV) infusion of 0.9 mg indomethacin (IND IV) or by the oral administration of 2 mg of indomethacin (IND oral), its physical mixture with unloaded MPs C (Phy Mix C), or encapsulated in MPs C (MPs C). All the AUC values are reported as the mean ± SD of four independent experiments. The absolute bioavailability values were calculated by the AUC data normalized with respect to their IND doses. $^{*}p > 0.05$ *versus* IND (oral); $^{**}p < 0.001$ *versus* IND (oral).

Formulation	IND dose	AUC (μg·mL^{-1}·h)	Absolute Bioavailability (F)
IND (IV)	0.9 mg	165.06 ± 6.2	–
IND (oral)	2.0 mg	70.55 ± 2.26	19.20%
Phy mix C	2.0 mg	76.99 ± 2.26*	20.98%
MPs C	2.0 mg	174.3 ± 5.8**	47.51%

emulsification of hydrophobic compounds during digestion [39].

The AUC values of the profiles reported in Figs. 7 and 8 were used for the calculation of absolute bioavailability (F) values of the solid formulations, which are reported in Table 2. The F values were calculated by the AUC data normalized with respect to their IND doses. In this case the IND dose for the IV administration was 0.9 mg for each rat (about 3.6 mg/Kg), being the maximum amount allowing to obtain solubilized IND in 1 mL of the medium constituted by 20% (v/v) DMSO and 80% (v/v) physiologic solution. The IND dose for the oral administration was 2 mg for each rat (about 8 mg/kg), a value included between 1.85 mg/kg and 22.5 mg/kg, a range normally used for oral bioavailability studies of this drug [19,37,38,40].

The F values of γ-IND in the free form, or mixed with unloaded MPs C, were about the 20%, in accordance with previous studies [37]. According to these studies, we evidenced that an approach of co-crystallization of IND can induced the increase of both water solubility and oral bioavailability of this drug. As an example, the co-crystallization of IND with saccharin or 2-hydroxy-4-methyl-pyridine induced a drug bioavailability increase from the 23% to the 34% or 38%, respectively [37]. Unfortunately, 2-hydroxy-4-methyl-pyridine is characterized by acute toxicity for our body.

According to the measurements here reported, the oral administration of the loaded MPs C allowed to obtain a F value of 47.51%, about 2.5 times higher than that obtained with the free γ-IND, indicating the ability of the MPs to sensibly increase the oral IND bioavailability. It is important to remark that this bioavailability enhancement was obtained with a formulation characterized by high biocompatibility, being Gelucire recognized as Safe (GRAS) and oral-approved.

3.5. Stability studies

No statistical change in drug content was found for all samples ($p > 0.05$) after 18 months of storage (data not shown), indicating that all the formulations were physically stable with no loss or degradation of the drug during storage. Moreover, the FT-IR analysis of MPs C (Fig. 1 of the supplementary material) showed all the characteristic peaks correspondent to IND and excipient kept unchanged, suggesting the absence of interaction between carrier and drugs during long-term storage. IND solid state in MPs A and MPs C after 18 months was characterized by XRPD. As shown in Fig. 2 SI, the diffractograms showed no evident change in the pattern compared to the zero time samples, therefore suggesting the stability of the IND amorphous form. Additionally, the dissolution profile of the formulation C (Fig. 3 SI) resulted unchanged after 18 months storage, thus confirming the stability of the pharmaceutical performance of this formulation.

The stability of the drug in the amorphous form represent a major challenge in the development of solid dispersions. A number of factors, such as molecular mobility, thermodynamic properties, environmental stress, preparation methods, and storage conditions contribute to determine the stability of the drug amorphous form [41]. Changings in polymorphic form of IND in dispersion with hydrophilic carrier have been previously reported. Recently, Van Duong et al. reported the study of semicrystalline dispersions of IND in PEG where the crystallization of the drug was observed at different times, depending on the drug loading [29]. The SD with 10% of IND, the same drug loading of our system, showed the longest time for IND recrystallization compared to the SD with higher drug loadings. In our study, the SD showed no trace of recrystallization for at least 18 months. In case of low drug loadings, the amount of drug is generally insufficient to affect the carrier crystallization during solidification from the melt [29,42]. Thus, during solidification of the MPs, the dispersions exhibits instant crystallization of Gelucire (Fig. 2b) with amorphous or molecularly dispersed drug entrapped in the ordered crystalline matrix (Fig. 5). The mobility of IND molecules would be thus extremely low in the highly viscous crystalline Gelucire matrix. Therefore, we hypothesize that the crystallization of IND in the SD was prevented because of the lack of molecular mobility required for nucleation and crystal growth.

4. Conclusions

In this work, spray congealing technology has been explored to produce solid dispersion with enhanced oral indomethacin bioavailability. Spray congealing enabled the preparation of MPs with encapsulation efficiency values closer to 100%. The MPs were spherical and free flowing, thus ready-to-use for tableting or capsule filling. The new excipient Gelucire 48/16 showed great potential for the bioavailability enhancement of IND. Specifically, the formulation with 30% Gelucire 50/13 and 70% Gelucire 48/16 (MPs C) led to an important increase in drug solubility and a considerable enhancement of drug dissolution rate compared with the pure drug. *In vivo* pharmacokinetic studies indicate that MPs C allows to significantly increase (about 2.5 times) the oral bioavailability of the drug. The bioavailability enhancement was mainly due to the conversion of IND into the amorphous form, as confirmed by solid state characterization, which was maintained during storage.

Overall, the low-cost and easily scaled-up spray congealing technology allowed to produce MPs with consistent and reproducible *in vitro* and *in vivo* performances as well as ideal technological properties. Thus, spray congealing is a promising approach for the industrial production of stable SD with amorphous IND dispersed in crystalline Gelucire.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2019.03.020.

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