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Research Paper

Bioavailability of indomethacin-saccharin cocrystals

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Abstract

Objectives Pharmaceutical cocrystals are new solid forms with physicochemical properties that appear promising for drug product development. However, the in-vivo bioavailability of cocrystals has rarely been addressed. The cocrystal of indomethacin (IND), a Biopharmaceutical Classification System class II drug, with saccharin (SAC) has been shown to have higher solubility than IND at all pH. In this study, we aimed to evaluate the in-vitro dissolution and in-vivo bioavailability of IND–SAC cocrystals in comparison with IND in a physical mixture and the marketed product Indomee®.

Methods Scale-up of the cocrystals was undertaken using cooling batch crystallisation without seeding. The chemical and physical purity of the up-scaled material was verified using high-performance liquid chromatography, differential scanning calorimetry and powder X-ray diffraction. The IND–SAC cocrystals and IND plus SAC were mixed with lactose and the formulations were placed into gelatin capsules. In-vitro dissolution studies were then performed using the rotating basket dissolution method. The intrinsic dissolution rate of IND and IND–SAC cocrystals was also determined. Finally, a bioavailability study for the formulations was conducted in beagle dogs. The plasma samples were analysed using high-performance liquid chromatography and the pharmacokinetic data were analysed using standard methodologies.

Key findings The bulk cocrystals (i.e. scaled-up material) were chemically and physically pure. The in-vitro dissolution rate of the cocrystals was higher than that of IND and similar to that of Indomee® at pH 7.4 and pH 1.2. The in-vivo bioavailability of the IND–SAC cocrystals in dogs was significantly higher (ANOVA, $P < 0.05$) than that of IND but not significantly different from Indomee® (ANOVA, $P > 0.05$).

Conclusions The study indicates that the improved aqueous solubility of the cocrystals leads to improved bioavailability of IND. Thus, the cocrystals are a viable alternative solid form that can improve the dissolution rate and bioavailability of poorly soluble drugs.

Keywords bioavailability; cocrystals; dissolution; indomethacin; salts

Introduction

Solid dosage forms such as tablets and capsules are by far the preferred drug delivery systems. The therapeutic efficacy of solid dosage forms is dependent on the bioavailability of the drug, which, in turn, is determined by its solubility and dissolution rate at the site of absorption. In particular, Biopharmaceutical Classification System (BCS) class II drugs, which permeate membranes well but are poorly soluble, often show dissolution-limited bioavailability.[1] The solubility and dissolution rate primarily depend on the solid-state form of the active pharmaceutical ingredients (APIs). Whilst numerous formulation strategies have been considered for improving dissolution rate and bioavailability, solid-form change has been the most important in product development.[2,3] The quest for solid forms of an API with optimal properties is thus a persistent activity within drug development groups.[4]

In this respect, crystalline forms are preferred over amorphous phases for stability and processing reasons. Preparation of a salt form of an API is a fundamental and widely employed approach. However, this strategy relies on sufficient ionisation of the components, which potentially renders the technology unsuitable for neutral or weakly acidic or basic

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compounds.[5] Furthermore, the solubility or dissolution advantage offered by metastable polymorphs is often insignificant and is associated with a risk of phase transformation during the life cycle of the product.[6,7]

Pharmaceutical cocrystals, a recent addition to the class of crystalline solids, are generating increasing interest, and offer an alternative means of improving the physicochemical properties of an API.[8–10] Cocrystallisation offers several key advantages: (1) cocrystals are crystalline with definite stoichiometry, leading to better solid-state stability and more predictable physical properties and performance than amorphous solids; (2) cocrystal design involves altering hydrogen-bonding motifs rather than making or breaking covalent bonds, thus retaining the safety and pharmacological profiles of the drug molecule; (3) cocrystals of all types of APIs (weakly acidic or basic or non-ionisable) can in principle be prepared, in contrast to salt formation technology; (4) greater diversity is possible with cocrystal solid forms because of the availability of numerous coformers (food additives, preservatives, pharmaceutical excipients, and other APIs); (5) cocrystals offer patenting opportunities because they are new solid forms of APIs; (6) cocrystals can be generated using green production technologies such as grinding.

Cocrystals are homogeneous crystalline materials comprising two or more components in a definite stoichiometric ratio.[11–14] Defining the precise nature of the components (solid, liquid, gas and/or neutral or ionic) has been a topic of active discussion.[13] From the pharmaceutical properties and functionality perspective, we argue that the components are restricted to solids at room temperature. Cocrystals that are formed between an API and a cocrystal former are called pharmaceutical cocrystals. Pharmaceutical cocrystallisation has been reported to improve the physicochemical properties of several APIs, including carbamazepine, theophylline, itraconazole, norfloxacin, indomethacin, etc.[8–15] A recent article reviewed several cocrystals from the point of view of their physical properties.[10]

Despite the current interest, a very limited number of animal bioavailability studies for cocrystalline forms of APIs have been reported to date. In one of the earliest studies, the bioavailability of 1 : 1 2-[4-(4-chloro-2-fluorphenoxy)phenyl] pyrimidine-4-carboxamide-glutaric acid cocrystals was three times greater than that of the parent compound when administered orally to dogs in the form of powders in a capsule.[16] In another study, a significant increase in the bioavailability of 1 : 1 AMG 517-sorbic acid cocrystals was reported compared to that of the pure drug in a suspension formulation. In both cases, the cocrystals were unstable in water or fasted state simulated intestinal fluid.[17] In a further study, the pharmacokinetic parameters (AUC, C_{max} and T_{max}) of a carbamazepine-saccharin cocrystal (particle size < 53 μm, lactose, 200 mg dose, capsule) were similar to those of the marketed product (Tegretol®).[18] Any general conclusions on the improved in-vivo performance of cocrystals based on the very limited literature would be premature and more studies are needed to better understand the in-vivo behaviour of cocrystals and the correlations between in-vivo and in-vitro data.

Indomethacin (IND) is a non-steroidal anti-inflammatory drug (NSAID) that is widely prescribed for patients with moderate to severe rheumatoid arthritis, ankylosing spondylitis, osteoarthritis or acute gouty arthritis. Indomethacin is a weakly acidic compound with pKa 4.5 and it is generally classified as a BCS II drug, based on solubility criteria applied to the entire pH range of 1.2 to 7.4. However, this classification is arguable if one considers its solubility at pH 7.4.[19] The poor solubility of IND is claimed to be responsible not only for its low and erratic oral bioavailability but also for gastric irritation associated with the drug. In fact, IND has been a classic model compound for demonstrating the ability of various strategies to improve solubility and dissolution rates.[20–22]

In a recent study, cocrystallisation was studied as a means of improving the dissolution rate of IND.[23] It was shown using powder dissolution studies that cocrystals of IND with saccharin (SAC) (IND–SAC cocrystals) were more soluble and had higher dissolution rates than IND at pH 7.4 but they were unstable at this pH. The solubility behaviour and solution stability of IND–SAC cocrystals in water as a function of pH have also been investigated in a different study.[24] The IND–SAC cocrystals showed higher solubility than IND at all pH (i.e. unstable and transformed to IND). We were interested in investigating whether this improvement in solubility and dissolution rate of the cocrystals resulted in improved in-vivo bioavailability.

This paper presents an evaluation of the bioavailability of IND–SAC cocrystals in comparison with IND in a physical mixture and the marketed product Indomee® in beagle dogs. Scale-up, formulation and dissolution studies of IND–SAC cocrystals are also discussed. To our knowledge, this is the first report on the in-vivo studies of this new class of solids of indomethacin: its cocrystals.

Materials and Methods

Materials

Indomethacin (γ-form; thermodynamically stable) was ordered from Sigma Co. Ltd, USA, and was used as received for the entire study. Other chemicals and solvents were obtained from different commercial suppliers. Hard gelatin capsules (No. 4) were gifted from Suheung Capsule Co. Ltd, South Korea. Indomee® 25 mg hard gelatin capsules (Merck Sharp & Dohme, Sweden) were purchased from Apoteket AB, Sweden. Distilled water was used as required.

Scale up of IND–SAC cocrystalline material

Small-scale preparation of pure cocrystals

The solvent evaporation method was used as described elsewhere.[23] Briefly, a mixture of 0.01 M IND and 0.01 M SAC was dissolved in 200 ml of ethyl acetate and heated to aid dissolution. The solution was left at room temperature to evaporate. The crystals thus formed were filtered and dried. The cocrystal physical purity was confirmed by powder X-ray diffraction (PXRD; both experimental and simulated PXRD patterns from single X-ray diffraction). These crystals were used as reference material to verify the purity of the scaled-up batch.

Large-scale cooling batch crystallisation

The crystallisation apparatus set-up consisted of a 500 ml water-jacketed glass vessel (DURAN® reaction vessel), a reflux column, an overhead stirrer, and a Teflon shaft and

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blade. The vessel was connected to a water circulator with heating and cooling capabilities. A temperature sensor attached to the circulator was immersed in the glass vessel to monitor the temperature of the contents. IND–SAC is known to be a congruently saturating system in ethyl acetate.[25] The experimental conditions were chosen based on the solubility of IND–SAC cocrystals in ethyl acetate at different temperatures in preliminary experiments (Supporting Information, Figure S1). Equimolar quantities of IND (6.16 g, 0.132 M) and SAC (12.03 g, 0.132 M) were added to the crystalliser. A quantity of 300 ml of ethyl acetate was added to the solids. The mixture was heated to 70°C and refluxed for 1 h to aid complete dissolution. The temperature was progressively decreased in 10°C/h decrements with 10 min waiting time for every 10°C decrease, in order for the contents to attain equilibrium. The contents were constantly stirred at 100 rpm and no seeds were added. The temperature was lowered to about 10°C and maintained for 3 h before the suspension was emptied into a Büchner funnel. The isolated solids were rinsed with cold ethanol and dried in a desiccator (0% relative humidity, 25°C) for 24 h. The cocrystalline material was characterised using high-performance liquid chromatography (HPLC), differential scanning calorimetry (DSC) and PXRD to verify the chemical and physical purity. The powder was then used for the in-vitro and in-vivo studies.

Wherever applied, the powders were ground using a simple mortar and pestle and the physical purity was tested.

Chemical and physical characterisation of the scaled-up material

High-performance liquid chromatography

The HPLC analysis was conducted at room temperature with a flow rate of 1 ml/min. IND was detected at 320 nm. The mobile phase consisted of 25 : 75 phosphoric acid (0.2% w/v) and methanol, and was degassed for 30 min before use. The HPLC system used for verifying the chemical purity and solubility of the scaled-up material at different temperatures was a series 200 binary LC pump and a 200 UV-vis detector from Perkin-Elmer (Wellesley, MA) and C18 column (Dalco Chrometch, 5 μm, 150 mm × 4.6 mm). For the in-vitro dissolution studies, a Shimadzu LC-10AD (Japan) system, with a solvent delivery pump, controller, Waters 486 UV detector and C-18 column (Waters μ Bondapak, 5 μm, 3.9 × 300 mm), was used at room temperature.

Differential scanning calorimetry

The thermal behaviour of the materials was studied using a Thermal Advantage DSC Q1000 (TA Instrument) equipped with a refrigerated cooling system. The instrument had been calibrated for temperature and enthalpy using indium. The standard DSC method was used to determine the melting temperature and the heat of fusion of the samples. The sample (1–3 mg) was accurately weighed into non-hermetic aluminium pans and crimped. Triplicate samples were scanned from 25 to 160°C at a heating rate of 10°C/min under a continuous nitrogen purge (50 ml/min).

Powder X-ray diffraction

PXRD patterns were recorded using a Siemens D5000 powder diffractometer equipped with a CuKα radiation (1.540 56 Å) source. The tube voltage and amperage were set at 40 kV and 40 mA, respectively. The divergence slit and antiscattering slit settings were variable for illumination of the 20 mm sample. The samples were packed in a standard holder with minimal preferred orientation effects (top-fill method). The sample stage was spun at 30 rpm and samples were scanned between 5 and 40° in 2θ with a step size of 0.02° and 3.2 steps/s. The instrument had previously been calibrated using a silicon standard.

Microscopy

The morphology and size distribution of the relevant samples were examined by scanning electron microscopy (SEM; JSM-6300, Jeol Ltd, Japan). Samples were coated with gold and palladium using a vacuum evaporator and were examined using an SEM at 15 kV accelerating voltage.

The quantitative information on the size distribution of the particles was determined from SEM images using ITPro 3.03 image analysis software (Sometech Inc., Korea). A number of particles (up to 30) were observed and the average length of the diagonal line was reported.

Formulations

Hard gelatine capsules (No. 4) were filled with lactose-based formulations: (1) IND (F-IND); (2) the physical mixture of IND and SAC, geometrically and gently mixed in a mortar (PhyMix); the (3) unground; (4) ground IND–SAC cocrystals, as detailed in Table 1. Lactose was used as filler. Prior to filling the capsules, the formulation ingredients were geometrically mixed with a mortar and spatula and passed twice through a 450-μm sieve to ensure proper mixing. Indomee® capsules are a marketed formulation of indomethacin, lactose monohydrate, gelatin, lecithin, magnesium stearate and silicon dioxide etc.

Table 1 Formulation details for IND, the physical mixture of IND and SAC (PhyMix) and the IND–SAC cocrystals (unground and ground) used in the study

	Indomethacin (F-IND)	IND and SAC (PhyMix)	IND–SAC cocrystal (unground)	IND–SAC cocrystal (ground)	Indomee®
Indomethacin (IND)	25	25	25	25	25
Saccharin (SAC)	–	12.8	12.8	12.8	–
Lactose	120	120	120	120	212
Mg.stearate (other excipients)	–	–	–	–	not disclosed
Total weight	25	157.8	157.8	157.8	237
Weight of final capsule	65	197.8	197.8	197.8	285

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In-vitro dissolution study

The in-vitro dissolution study was performed in a dissolution bath (Vankel VK7000, Cary, NC) following USP method I (basket method). The capsules were placed in a basket, which was lowered into a vessel filled with 900 ml of dissolution medium or buffer and rotated at 100 rpm. The test was performed at 37 ± 0.5°C. The buffers used were in accordance with the USP recommendation for gastrointestinal tract formulations: 0.1 M HCl (pH 1.2 with or without Tween 80 (0.5%) and phosphate buffer (pH 7.4). At each sampling time, 2 ml of the solution was withdrawn and filtered through a 0.45 μm filter. The dissolution medium was then replaced by 2 ml buffer to maintain a constant volume. The filtrate was analysed by HPLC. Three capsules were analysed for each formulation.

Intrinsic dissolution rates for IND and the IND–SAC cocrystal ground powder were determined. In addition, the intrinsic dissolution rate of unground material was measured to verify the effect of the altered surface properties of the particles due to grinding. The powders were packed into a stationary disc (0.5 cm^2 surface area, Distek Inc., NJ, USA) and compressed at a pressure of 1500 psi for 1 min. The concentration of IND in the phosphate buffer and HCl media were determined using a UV spectrometer (Shimadzu mini 1240) at 320 nm and HPLC. The rest of the method was similar to that described for in-vitro dissolution. Each sample was tested in triplicate.

In-vivo study

Pharmacokinetic study in beagle dogs

The pharmacokinetic data of the IND–SAC cocrystals were compared with those of PhyMix and the commercial product, Indomee®, in beagle dogs. All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals at Chungnam National University (approval number 2008–2028). A randomised three-period crossover design was used to administer one capsule containing 25 mg of drug (based on the weight of IND) to six healthy beagle dogs weighing 9–11 kg. The washout period between administrations was 2 weeks. The dogs were fasted overnight before the experiments and food was reoffered 4 h post dosing. At each time interval, 4 ml of blood was withdrawn from the jugular vein, immediately centrifuged for 10 min at 4000 rpm and stored at –20°C until the assay.

Determination of indomethacin in dog plasma

The HPLC method, after Nam *et al*. but with slight modifications, was used to determine the plasma concentrations of IND.[26] The Waters™ 2690 alliance analytical HPLC system included an auto-sampler and a Waters™ 996 photodiode array UV detector (Waters, Milford, MA, USA). Separations were performed at room temperature on a C18 column (X-Terra™ RP18, 5 μm, 4.6 × 250 mm, Waters, MA, USA) using a mobile phase of acetonitrile and 10 mM acetate buffer (pH 4.0) in a ratio of 70 : 30 (v/v) pumped at a flow rate of 1.0 ml/min. The signal was detected at 268 nm.

Plasma samples were prepared by liquid–liquid extraction. The plasma sample (0.5 ml) was added to a clean test-tube with 0.5 ml of 20 mM phosphate buffer (pH 3.0), 0.5 ml of internal standard solution (mefenamic acid 10 μg/ml in acetonitrile) and 1 ml of acetonitrile. The mixture was vortex-mixed for 5 min and centrifuged for 10 min at 4000 rpm. A 2 ml sample of the supernatant was transferred to a new test-tube and 6 ml ethyl acetate was added. The mixture was vortex-mixed for 10 min and centrifuged for 10 min at 4000 rpm. The 5 ml upper organic layer was transferred to a new test-tube and evaporated under a stream of nitrogen at 40°C. The dried residue was reconstituted in 0.1 ml of the mobile phase and a 20 μl aliquot was injected into the HPLC system.

Pharmacokinetic data analysis

A non-compartmental pharmacokinetic method was employed to determine the pharmacokinetic parameters of IND. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated using BA Calc 2002 software (from the National Institute of Food and Drug Safety Evaluation of Korea for Pharmacokinetic Studies). The maximum plasma concentration of drug (C_{max}) and the time to reach maximum plasma concentrations (T_{max}) were obtained directly from the plasma data. A one-way analysis of variance (ANOVA) test followed by Turkey's HSD test was performed to demonstrate statistical significance. The SPSS for Windows standard version 14.0 (SPSS, Chicago, IL, USA) was used for this purpose. $P < 0.05$ was considered statistically significant.

Results and Discussion

Scale-up of IND–SAC cocrystals

IND–SAC cocrystals, discovered through solution-based methods, have previously been thoroughly characterised in terms of crystal structure.[23] In the cocrystal structure, IND forms an acid dimer synthon and SAC forms an imide dimer synthon. These two dimers interact, through weak N–H·O hydrogen bonds, resulting in a unique interaction pattern (Supplementary Information, Scheme 1).

The primary objective of the crystallisation work was to develop a reasonable crystallisation process to generate pure cocrystals of IND–SAC on a 10-g scale. A systematic discussion of the importance of kinetic and thermodynamic aspects of the scale-up of cocrystals is outside the scope of this article, but recent studies on the scale-up of carbamazepine-nicotinamide cocrystals address these topics.[27,28] The IND–SAC cocrystals were up-scaled using the non-seeded cooling batch crystallisation method. Some cocrystals had already appeared below 50°C but cooling of the ethyl acetate solution was maintained down to 10°C in order to get good yield. The cooling rate and rotation speed of the stirrer were optimised to 10°C/h and 100 rpm. Under these processing conditions, the production of IND–SAC cocrystals was consistently up-scaled to 8–10 g per batch, with yields of up to 70%. The crystals from each batch were de-aggregated by gentle grinding using mortar and pestle, and the resulting material from four batches was combined for the latter part of the study.

No additional peaks corresponding to degradation products of IND or SAC or impurities were observed in the HPLC chromatogram (Supporting Information, Figure S2), demonstrating the chemical stability of the cocrystal components

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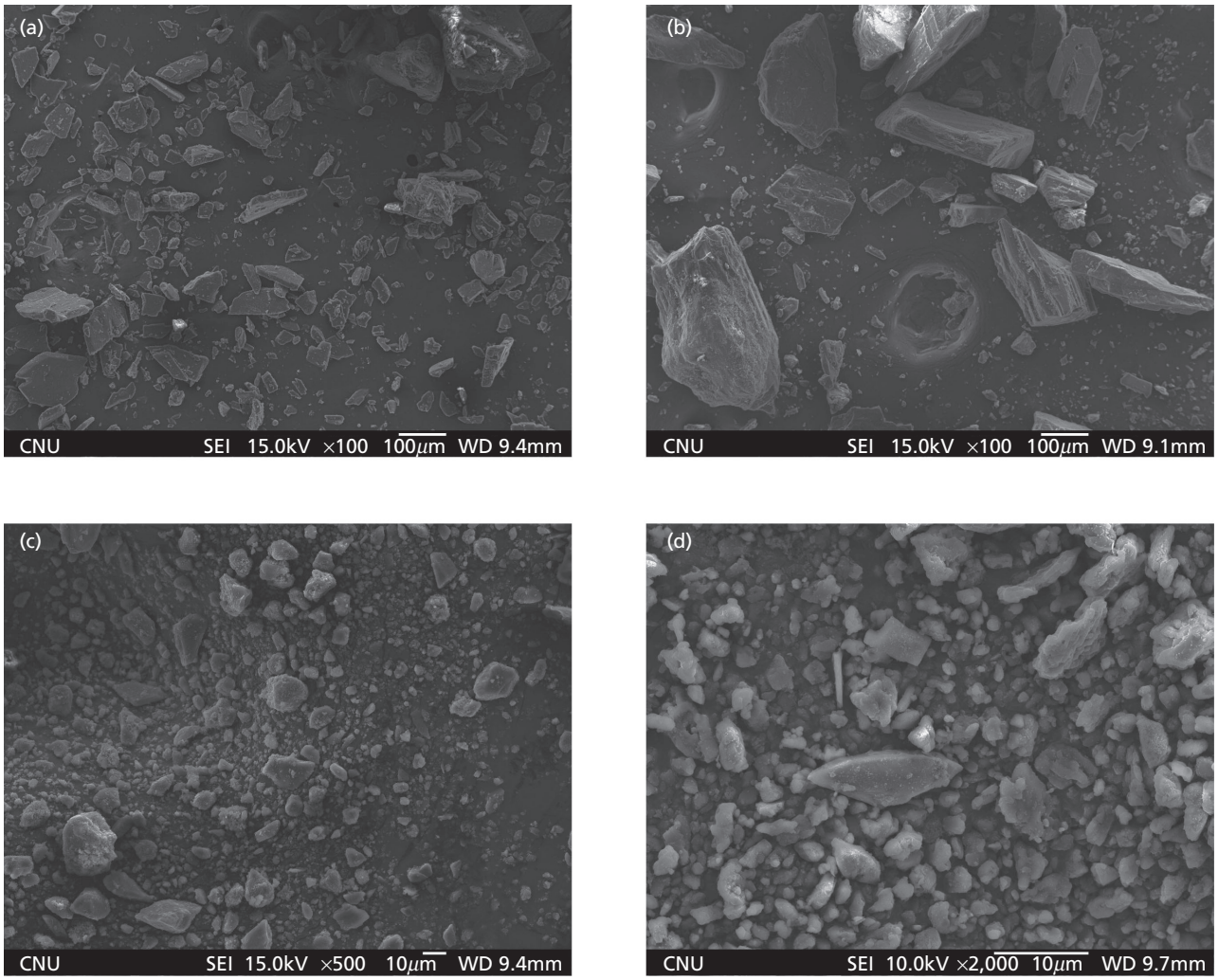

Figure 1 SEM micrographs of (a) IND, (b) IND–SAC cocrystals (unground), (c) IND–SAC cocrystals (ground) and (d) Indomee® (powder from the capsule).

during crystallisation. The physical purity of the scaled-up material was compared with that of cocrystals ('pure crystals') generated on a small scale using the solvent evaporation method. The DSC thermogram and PXRD patterns for the small-scale crystals were identical to previously published data (Supporting Information, Figure S3 and S4).[23] Moreover, the PXRD pattern of the small-scale crystals matched perfectly with that of the simulated PXRD of single crystals. The overlaid DSC thermograms and PXRD patterns for the scaled-up material and for crystals from solution crystallisation ('pure crystals') are shown in Figures S3 and S4. It is clear from the DSC and PXRD data that the scaled-up cocrystalline material was as pure as crystals generated from small-scale solution crystallisation (in other words the scaled-up material is highly pure). This cocrystalline material was used in its unground and ground forms (ground using mortar and pestle) in the in-vitro study and in the ground form in the in-vivo study. The PXRD of the ground powder was essentially similar to that of the unground material, confirming that there had been no considerable form changes (data not shown).

Table 2 Particle size data for selected samples

Sample name	Particle size range (length of diagonal line in μm)
Indomethacin (IND)	57 ± 16
IND–SAC cocrystal (unground)	170 ± 48
IND–SAC cocrystal (ground)	22 ± 7
Indomee® (particles of the formulation)	17 ± 3

SAC, saccharin.
Size determined from SEM images using ITPro 3.03 image analysis software (Sometech Inc., Korea). The variation (±) indicates standard deviation ($n = 30$ particles).

The SEM micrographs and particle size data for selected samples are shown in Figure 1 and Table 2, respectively. IND particles were of the order of 57 μm, whilst IND–SAC crystals (unground) were irregular prisms in of around 170 μm (Figure 1a and 1b). The ground cocrystals and Indomee®

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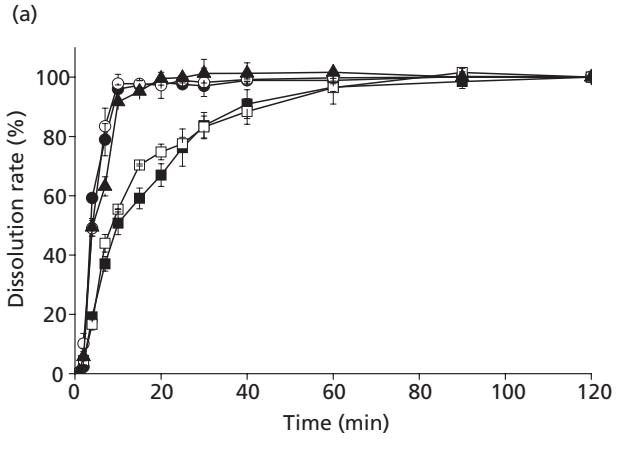

Figure 2 (a) In-vitro dissolution profiles for different formulations: ●, IND–SAC cocrystal (ground); ○, INC-SAC cocrystal (unground); ■, PhyMix; □, F-IND; ▲, Indomee®. (b) Intrinsic dissolution profiles for IND (□) and IND–SAC cocrystals (ground) (●) in phosphate buffer, pH 7.4. Triplicate samples were analysed and error bars show standard deviation.

(particles of formulation *not* primary drug particles) were of a similar size (Figure 1c and 1d; Table 2).

Formulation and in-vitro dissolution study

The objectives of the formulation work were to identify a simple, relevant formulation that would facilitate filling the hard gelatin capsules and would not influence the performance characteristics of IND in either stable or cocrystalline form. The details of the formulations utilised in the in-vitro and in-vivo studies are listed in Table 1. A simple formulation of unground and ground IND–SAC cocrystals with lactose (as filler) that allowed easy filling of the hard capsules was chosen for further study. The PhyMix and Indomee® were also studied.

The in-vitro dissolution profiles for F-IND, PhyMix, IND–SAC cocrystals (unground and ground) and Indomee® in phosphate buffer (pH = 7.4) are shown in Figure 2a. Unground and ground IND–SAC cocrystals had reached steady state, with 100% release, in less than 20 min (Figure 2a). However, no difference in dissolution rate was observed between the unground and ground cocrystals, signifying that there was no effect of particle size on dissolution in buffer at pH 7.4. This may have been because the IND–SAC cocrystals were so soluble at this pH that any effect of particle size (or surface energy changes) on the dissolution rate was negligible.[23,24] It has been reported that carbamazepine–saccharin and magesterol acetate–saccharin cocrystals with a smaller particle size distribution dissolved faster than larger cocrystals in a different dissolution medium.[18,29] On the other hand, the dissolution rate of F-IND and PhyMix was slower, resulting in delayed complete release of the drug. This demonstrates that the increased dissolution rates of IND–SAC cocrystals compared to F-IND or PhyMix were the result of its increased solubility.[24] Indomee® had a similar dissolution profile to that of the IND–SAC cocrystals. This is expected, as the marketed formulation is optimised and contains a different additive compared to the IND–SAC cocrystal simple formulation. The intrinsic dissolution profiles for IND and IND–SAC cocrystals at pH 7.4 are shown in Figure 2b. The intrinsic dissolution rate of IND–SAC cocrystals in phosphate buffer at pH 7.4 was 3.6 times higher than that of IND. The intrinsic dissolution rate of the ground and unground cocrystals was not significantly different (ANOVA, $P > 0.05$) (Supporting Information, Figure S5). This implies that the differences in the surface energy of the ground and unground particles (if such differences exist) did not have a considerable effect on the wetting of the pellets.

The in-vitro dissolution profiles for F-IND, PhyMix, IND–SAC cocrystals (unground and ground) and Indomee® in 0.1 M HCl (pH 1.2 with 0.5% Tween 80) are shown in Figure 3a. IND was not completely released from any formulation, even after 240 min. The dissolution rate of the unground IND–SAC cocrystals was similar to those of F-IND and PhyMix, but was much slower than that of the ground IND–SAC cocrystals in this medium. Here, the IND–SAC cocrystal particle size has a prominent effect at pH 1.2, the cocrystals showing less solubility.[24] The dissolution rate of Indomee® was similar to that of the ground IND–SAC cocrystals but the percentage release was slightly higher, for the reasons indicated above. The intrinsic dissolution profiles for IND and IND–SAC cocrystals (ground) in the same medium are shown in Figure 3b. The intrinsic dissolution rate of the ground IND–SAC cocrystals was 1.7 times higher than that of IND at pH 1.2, correlating well with the higher solubility of IND–SAC cocrystals than IND.[24] However, it was much slower compared to intrinsic dissolution at pH 7.4 because of its lower solubility at pH 1.2. The pellets from the intrinsic dissolution tests in both buffer media were intact and no solid-state form changes had taken place (Figure S6, Supporting Information).

The presence of SAC in the physical mixture did not have any influence on the dissolution rate of IND at pH 7.4 or 1.2 (Figures 2 and 3). Moreover, the PhyMix (reference material) was confirmed to have a dissolution rate not significantly different (ANOVA, $P > 0.05$) from PhyMix prepared with ground IND (particle size of about 20 μm) (Supporting Information, Figure S7).

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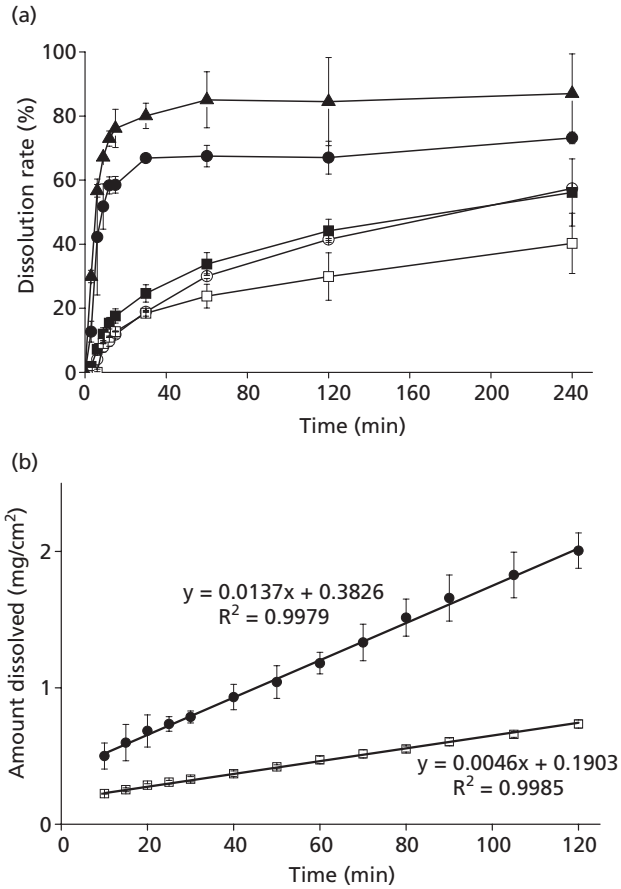

Figure 3 (a) In-vitro dissolution profiles for different formulations: ●, IND–SAC cocrystal (ground); ○, INC-SAC cocrystal (unground); ■, PhyMix; □, F-IND; ▲, Indomee®. (b) Intrinsic dissolution profiles for IND (□) and IND–SAC cocrystals (ground) (●) in 0.1 M HCl, pH 1.2 with Tween 80. Triplicate samples were analysed and error bars show standard deviation.

In-vivo studies in beagle dogs

The in-vivo studies were conducted in beagle dogs, which are the commonly used animal model for pharmacokinetic studies of new APIs, polymorphs or salts.[30,31] PhyMix, IND–SAC cocrystals (ground) and Indomee® were selected for this study. A capsule containing 25 mg of the drug (based on the weight of IND) was orally administered to beagle dogs weighing 9–11 kg. The mean pharmacokinetic parameters calculated from this study are summarised in Table 3 and the corresponding plasma concentration profiles for selected samples are presented in Figure 4. In this study, larger inter-subject variations in the pharmacokinetic parameters were observed for the cocrystals and Indomee® than for PhyMix; these could have been the result of physiological, weight and metabolic variations in the dogs.[32]

The AUC and C_{max} for IND–SAC cocrystals were consistently higher than those for PhyMix (ANOVA, $P < 0.05$). This result confirms that the IND–SAC cocrystals offer significantly improved in-vivo exposure in dogs compared to IND. Understandably, the improved bioavailability correlates well with the higher aqueous solubility and dissolution rate of the cocrystal. Interestingly, these pharmacokinetic advantages over the stable form of IND in dogs occurred despite the instability of the cocrystals in water or at all pH. The higher drug concentration achieved by the cocrystals compared to the stable form indicates improved pharmacokinetics.[23] Indeed, similar results have been reported for AMG 517-sorbic acid cocrystals, which are also unstable under physiological conditions but sustain higher concentrations of AMG 517 for more than 2.5 h.[17] The use of polymers and surfactants has been shown to improve the stability of cocrystals in solution.[33,34] Such formulation strategies are of great interest in capturing the full potential of cocrystals, and are the subject of ongoing research in our group.

The AUC, T_{max} and C_{max} for IND–SAC were not significantly different from those for Indomee®, confirming that the bioavailability of these formulations is equivalent. In an earlier study, carbamazepine cocrystals were also shown to have similar bioavailability to that of a marketed product.[18] Indomee® is a highly optimised formulation that has been marketed for a long time and hence would be expected to have good bioavailability.

Conclusions

This study presents an evaluation of the bioavailability of IND–SAC cocrystals, which were characterised previously and proven to have higher solubility than IND. IND–SAC cocrystals were scaled-up successfully in a chemically and physically pure form. The IND–SAC cocrystals showed a better in-vitro dissolution rate and in-vivo bioavailability than IND, which correlates well with their improved solubility. Moreover, the in-vivo performance of the simply mixed IND–SAC cocrystals with lactose was similar to that of the marketed product. An additional improvement in the bioavailability of IND–SAC cocrystals may be possible through formulation approaches and by controlling the transformation of the cocrystals at physiological pH. An understanding of solubility and solid-state stability of cocrystals at physiological conditions is crucial for fully exploiting their potential in drug development. Finally, the study demonstrates that cocrystals provide an alternative platform for formulation exploration in the search for the best possible drug products.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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Table 3 Pharmacokinetic parameters after oral administration of various IND formulations

	PhyMix	IND–SAC cocrystal (ground)	Indomee®
AUC0-∞ (μg h/ml)	7.58 ± 3.04	14.83 ± 7.46	16.46 ± 2.28
AUC_{0-24} (μg h/ml)	5.98 ± 2.74	14.52 ± 6.78	16.45 ± 3.41
C_{max} (μg/ml)	1.01 ± 0.40	6.31 ± 3.26	8.02 ± 4.22
T_{max} (h)	2.83 ± 1.03	1.00 ± 1.01	1.53 ± 1.34

Pharmacokinetic parameters were significantly different between ground IND–SAC and PhyMix; Indomee and PhyMix (ANOVA, $P < 0.05$). Whilst they were not significantly different between ground IND–SAC cocrystals and Indomee® (ANOVA, $P > 0.05$). The variation (±) indicates standard deviation in the value.

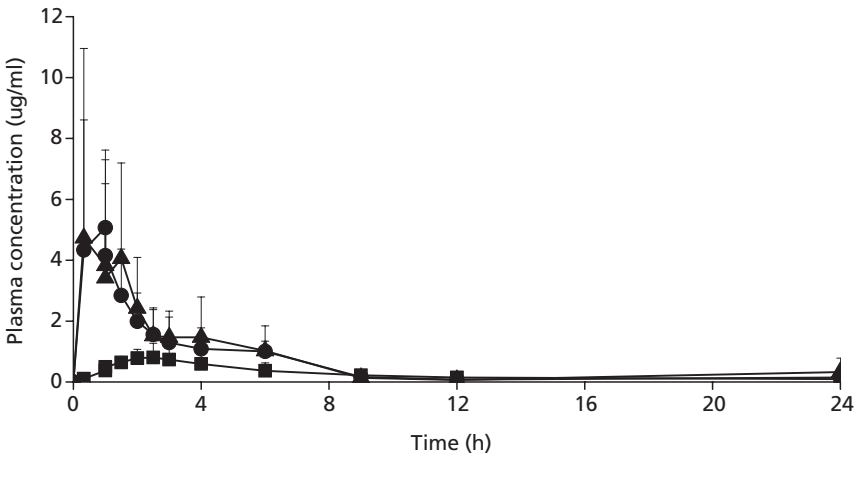

Figure 4 Plasma concentration-versus time profiles for indomethacin after oral administration of various formulations: ●, IND–SAC cocrystal (ground); ■, PhyMix; ▲, Indomee®. Error bars show standard deviation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Scheme 1 The crystal structure of IND–SAC cocrystal and its components.
Figure S1 Plot of solubility of IND–SAC cocrystal versus temperature.
Figure S2 HPLC chromatograms for IND–SAC Cocrystals.
Figure S3 DSC thermograms for IND–SAC cocrystals prepared using, (a) small-scale solvent evaporation method ('pure crystals'), [$T_{max} = 183.8 \pm 0.2°C$, $\Delta H_f = 153.2 \pm 13.3$ J/g], (b) cooling batch crystallization (scale-up material) [$T_{max} = 184.3 \pm 0.4°C$, $\Delta H_f = 147.1 \pm 8.9$ J/g]. T_{max} = peak melting, ΔH_f = heat of fusion.
Figure S4 PXRD patterns for IND–SAC cocrystals prepared using (a) solvent evaporation method ('pure crystals') and (b) cooling batch crystallization (scale-up material).
Figure S5 Intrinsic dissolution profiles for unground and ground IND–SAC cocrystal in pH = 7.4 buffer.
Figure S6 PXRD patterns take on the pellets after the intrinsic dissolution study; (a) IND, (b) IND–SAC cocrystal, (c) after intrinsic dissolution test in phosphate buffer, pH 7.4 (after 45 min) and (d) after intrinsic dissolution test in 0.1 M HCl, pH 1.2 with Tween 80 (after 120 min).
Figure S7 In-vitro dissolution profiles for PhyMix in 0.1 M HCl, pH 1.2 with Tween 80. Particle size range = PhyMix (57.2 ± 16.4 μm) and PhyMix-ground (19.8 ± 4.7 μm).

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