# Indomethacin Co-Crystals and Their Parent Mixtures: Does the Intestinal Barrier Recognize Them Differently?

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Ⓢ Supporting Information

**ABSTRACT:** Co-crystals are crystalline complexes of two or more molecules bound together in crystal lattices through noncovalent interactions. The solubility and dissolution properties of co-crystals can allow to increase the bioavailability of poorly water-soluble active pharmaceutical ingredients (APIs). It is currently believed that the co-crystallization strategy should not induce changes on the pharmacological profile of the APIs, even if it is not yet clear whether a co-crystal would be defined as a physical mixture or as a new chemical entity. In order to clarify these aspects, we chose indomethacin as guest poorly aqueous soluble molecule and compared its properties with those of its co-crystals obtained with 2-hydroxy-4-methylpyridine (co-crystal **1**), 2-methoxy-5-nitroaniline (co-crystal **2**), and saccharine (co-crystal **3**). In particular, we performed a systematic comparison among indomethacin, its co-crystals, and their parent physical mixtures by evaluating via HPLC analysis the API dissolution profile, its ability to permeate across intestinal cell monolayers (NCM460), and its oral bioavailability in rat. The indomethacin dissolution profile was not altered by the presence of co-crystallizing agents as physical mixtures, whereas significant changes were observed by the dissolution of the co-crystals. Furthermore, there was a qualitative concordance between the API dissolution patterns and the relative oral bioavailabilities in rats. Co-crystal **1** induced a drastic decrease of the transepithelial electrical resistance (TEER) value of NCM460 cell monolayers, whereas its parent mixture did not evidence any effect. The saccharin–indomethacin mixture induced a drastic decrease of the TEER value of monolayers, whereas its parent co-crystal **3** did not induce any effects on their integrity, being anyway able to increase the permeation of indomethacin. Taken together, these results demonstrate for the first time different effects induced by co-crystals and their parent physical mixtures on a biologic system, findings that could raise serious concerns about the use of co-crystal strategy to improve API bioavailability without performing appropriate investigations.

**KEYWORDS:** co-crystals, indomethacin, saccharin, drug permeation, NCM 460 cells, bioavailability
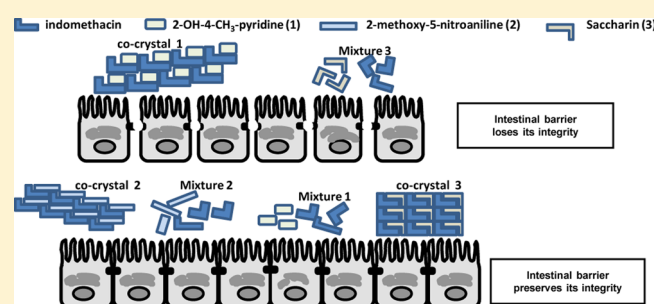
## ■ INTRODUCTION

The therapeutic efficacy of a pharmaceutical formulation depends on its bioavailability, i.e., the absorption extent and rate of the active pharmaceutical ingredient (API) into the bloodstream following its administration. The bioavailability of a solid pharmaceutical formulation may depend in turn on the dissolution profile of its components, in particular of the API. In the case of highly lipophilic API, and therefore of a compound poorly soluble in water but capable of effectively permeating through biological membranes (Biopharmaceutical Classification System (BCS) class II), the dissolution process is the limiting factor of its absorption; in this case the bioavailability is highly dependent on both dissolution rate and maximum amount dissolved of the APIs themselves.[1]

The solid state form of an API is determinant in influencing its solubility and dissolution rate. In general, the amorphous phases are easier to solubilize than crystalline solids, and, among them, the metastable polymorphs can offer solubility or dissolution advantages with respect to stable ones.[2,3]

The crystal engineering of pharmaceutical solids may be very useful to optimize the API stability and bioavailability, and the co-crystals seem to be promising in this context.[4] A co-crystal can be considered as a crystalline complex of two or more molecules bound together in the crystal lattice through noncovalent interactions, often including hydrogen bonding. Pharmaceutical co-crystals are obtained by an API and a co-crystal former.[5] It is known that the solubility and dissolution properties of co-crystals can be similar to those of amorphous compounds, i.e., higher than the parent crystalline pure phases.

A

62 As a consequence, pharmaceutical co-crystals give the
63 opportunities to increase bioavailability of APIs showing, at
64 the same time, the stability of their stable crystalline forms.[6,7]

65 Currently, several APIs are known to improve their solubility
66 profile and bioavailability when co-crystallized.[7−9] These APIs
67 include the anticonvulsant carbamazepine,[10] nonsteroidal anti-
68 inflammatory drugs such as indomethacin and meloxicam,[11,12]
69 the flavonoid quercetin,[13] and other molecules employed as
70 model drugs.[14−17]

71 It is currently believed that the co-crystallization strategy
72 should not induce changes in the pharmacological profile of the
73 APIs. Indeed, co-crystal design requires changes in crystal
74 structures that essentially alter hydrogen bonding motifs rather
75 than covalent bonds of the API, thus retaining its safety and
76 therapeutic properties.[11,18] On the other hand, the regulatory
77 status regarding the use of co-crystals in pharmaceutical
78 products appears still unsettled. In particular, it is not yet
79 clear whether a co-crystal would be defined as a physical
80 mixture (enabling its classification within current compendial
81 guidelines) or as a new chemical entity requiring full safety and
82 toxicology testing.[7,8] In this context, FDA has taken the
83 position that a co-crystal may be treated as a drug product
84 intermediate.[8]

85 In this study we evaluated the properties of (i) indomethacin,
86 chosen as guest molecule poorly soluble in aqueous environ-
87 ment,[11] (ii) its two new co-crystals with 2-hydroxy-4-
88 methylpyridine in its keto form (co-crystal 1) and 2-methoxy-
89 5-nitroaniline (co-crystal 2), and (iii) a previously described
90 indomethacin−saccharine co-crystal[11,19] (co-crystal 3). The
91 schematic representation of indomethacin and the coformers is
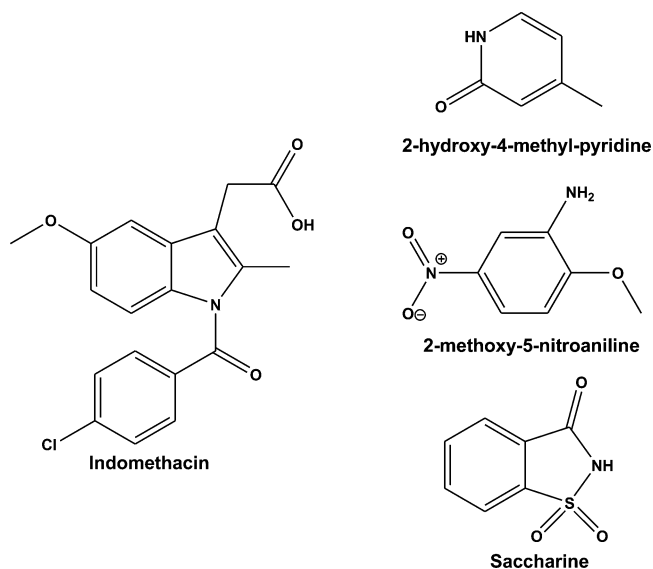92 reported in Figure 1. In particular, the dissolution, the



**Figure 1.** Schematic representation of indomethacin and the coformers 2-hydroxy-4-methylpyridine in its keto form, 2-methoxy-5-nitroaniline, and saccharine in co-crystals 1, 2, and 3, respectively.

93 permeation across NCM460 cell monolayers employed as an
94 *in vitro* model of human intestinal epithelial barrier, and the
95 bioavailability after oral administration to rats of indomethacin,
96 its co-crystals, and their parent mixtures (1, 2 and 3,
97 respectively) have been investigated. Overall, the results
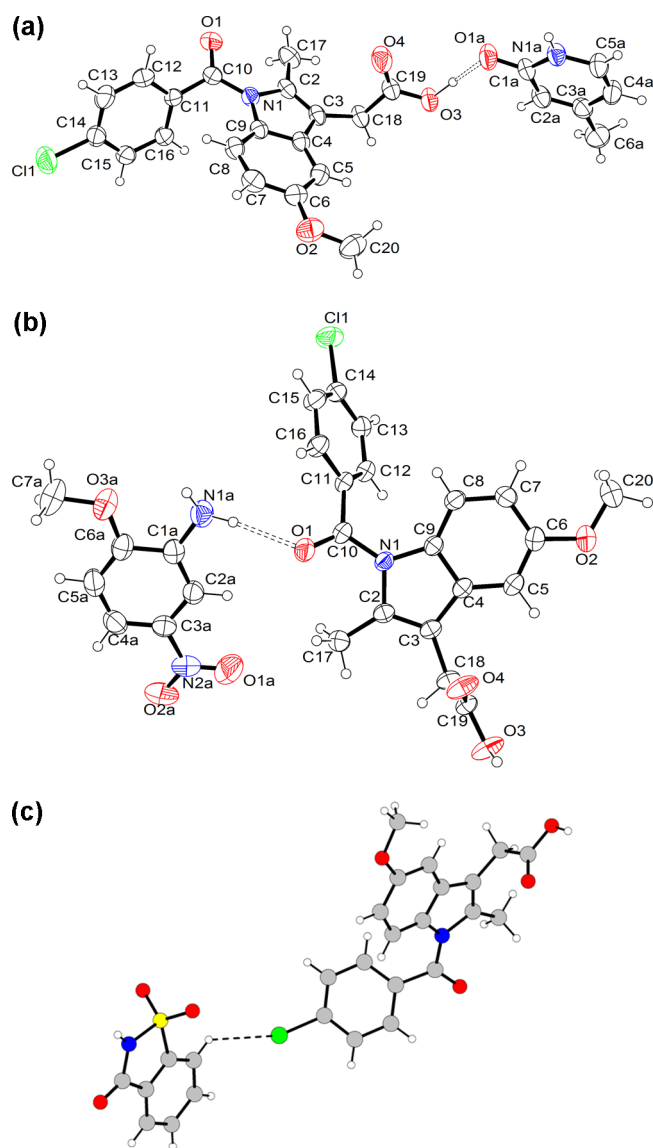98 indicate, for the first time, that strongly different effects on

99 the integrity of intestinal cell monolayers can be derived by the
100 dissolution of co-crystals or their parent mixtures.

## ■ MATERIALS AND METHODS

101
**Materials and Reagents.** γ-Indomethacin, 2-hydroxy-4-
102 methylpyridine, 2-methoxy-5-nitroaniline, saccharine, metha-
103 nol, acetonitrile, ethyl acetate, isoamyl acetate, and water were
104 of high performance liquid chromatography (HPLC) grade
105 from Sigma-Aldrich (Milan, Italy). All other reagents and
106 solvents were of analytical grade (Sigma-Aldrich). NCM-460
107 cells were kindly provided by Dr. Antonio Strillacci, University
108 of Bologna, Italy. The male Sprague−Dawley rats were
109 provided by Charles-River (Milan, Italy).

110
**Synthesis of Adducts.** Two new co-crystals containing the
111 indomethacin API have been synthesized and characterized by
112 X-ray crystallography: co-crystal 1, γ-indomethacin and 2-
113 hydroxy-4-methylpyridine 1:1; co-crystal 2, γ-indomethacin and
114 2-methoxy-5-nitroaniline 1:1. Two other co-crystals have been
115 synthesized and characterized but not used in the present work
116 because of their poor reproducibility: co-crystal a, γ-
117 indomethacin and 4-nitropyridine N-oxide monohydrate
118 1:1:1; co-crystal b, γ-indomethacin and pyridine N-oxide 1:1.
119 Details of the X-ray crystallographic analysis for all four crystals
120 are reported in Table S1 in the Supporting Information.
121 Equimolar quantities of indomethacin and the co-crystal
122 partner were dissolved in the minimum quantity of isoamyl
123 acetate and left for slow evaporation at room temperature.
124 Crystals were observed after a few days. Co-crystal 3,
125 containing saccharine as the coformer, has been obtained by
126 solvent slow evaporation of an equimolar saccharine/γ-
127 indomethacin solution prepared according to ref 19. The
128 phase and composition of the co-crystals 1, 2, and 3 have been
129 checked by X-ray powder crystallography, comparing the
130 experimental spectra with those calculated from the single-
131 crystal crystallography structures (Figures S1−S3 in the
132 Supporting Information).

133
**Experimental: X-ray.** The crystallographic data for the four
134 co-crystals 1, 2, a, and b were collected on a Nonius Kappa
135 CCD diffractometer at room temperature using graphite-
136 monochromated Mo Kα radiation ($\lambda$ = 0.71073 Å). Data sets
137 were integrated with the Denzo-SMN package[20] and corrected
138 for Lorentz-polarization effects. The structures were solved by
139 direct methods with the SIR97 suite of programs,[21] and
140 refinement was performed on $F^2$ by full-matrix least-squares
141 methods with all non-hydrogen atoms anisotropic. The N/O−
142 H atoms were found in the difference Fourier map and refined
143 isotropically; all other hydrogen atoms were included on
144 calculated positions, riding on their carrier atoms. All
145 calculations were performed using SHELXL-97[22] implemented
146 in the WINGX system of programs.[23] The ORTEPIII[24]
147 diagrams of co-crystals 1 and 2 are shown in Figure 2. Powder
148 diffraction spectra for co-crystals 1, 2, and 3 were recorded, at
149 room temperature, on a Bruker D-8 Advance diffractometer
150 with graphite monochromatized Cu Kα radiation ($\lambda$ = 1.5406
151 Å). The data were recorded at 2θ steps of 0.02° with 1 s/step.
152 Crystallographic data for the structural analysis of the four new
153 compounds have been deposited at the Cambridge Crystallo-
154 graphic Data Center, 12 Union Road, Cambridge, CB2 1EZ,
155 U.K., and are available free of charge from the Director on
156 request quoting the deposition number CCDC 1005832−
157 1005835 for 1, 2, a, and b, respectively.

158
**Differential Scanning Calorimetry (DSC).** Thermal
159 analyses on the samples were performed on a PerkinElmer

**Figure 2.** (a) ORTEPIII view and atom numbering scheme for co-crystals **1**. (b) ORTEPIII view and atom numbering scheme for co-crystals **2**. (c) Indomethacin−saccharine complex **3** (from ref 19). Thermal ellipsoids are drawn at the 40% probability level. Hydrogen bonds are drawn as dashed lines.

differential scanning calorimeter DSC7; temperature and heat calibration were done using indium and zinc standards. The samples (4−6 mg) were put in nonhermetic aluminum pans and scanned at a heating rate of 10 °C/min in the 30−300 °C range under a continuous purged dry nitrogen atmosphere. The data were collected in triplicate for each sample.

**HPLC Analysis.** The quantification of the indomethacin 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-phenylcarbazole was employed as internal standard in extraction procedures of indomethacin from rat blood (see below). The retention times for indomethacin and 9-phenylcarbazole were 4.0 and 13.5 min, respectively.

The chromatographic precision for each compound was evaluated by repeated analysis ($n = 6$) of the same samples (100 μM). For indomethacin and 9-phenylcarbazole (employed as internal standard in the extraction procedures) dissolved in aqueous phase the values were obtained for 100 μM (0.036 mg/mL) solutions and were represented by the relative standard deviation (RSD) values ranging between 0.63% and 0.74%, respectively.

The calibration curves of indomethacin dissolved in phosphate buffer 200 mM and in PBS 10 mM were linear over the ranges of 50 μM (0.018 mg/mL) to 1500 μM (0.54 mg/mL) and 2 μM (0.00072 mg/mL) to 500 μM (0.18 mg/mL), respectively ($n = 8$, $r > 0.997$, $P < 0,0001$). The limit of quantification for indomethacin was 625 nM (224 ng/mL, 2.24 ng injected) with a signal-to-noise ratio of 10, whereas the limit of detection was 188 nM (67 ng/mL, 0.67 ng injected) with a signal-to-noise ratio of 3.

A preliminary analysis performed with 100 μM solutions showed that hydroxy-4-methylpyridine, 2-methoxy-5-nitroaniline, and saccharin did not interfere with the indomethacin and 9-phenylcarbazone retention times.

**Dissolution Studies.** For the dissolution studies, the samples were micronized and sieved using stainless steel standard-mesh sieves (mesh size 106 μm). In each experiment, the solid powders were added to 12 mL of phosphate buffer 200 mM and incubated at 37 °C under gentle shaking (100 rpm) in a water bath. The amounts of sieved samples added to the buffer solution were 57.6 mg of indomethacin; 75.2 mg of co-crystal **1**; 84.7 mg of co-crystal **2**; 86.9 mg of co-crystal **3**; 57.6 mg of indomethacin mixed with 17.6 mg of 2-hydroxy-4-methylpyridine, 27.1 mg of 2-methoxy-5-nitroaniline, or 29.3 mg of saccharin for mixtures **1**, **2**, or **3**, respectively. Aliquots (200 μL) were withdrawn from the resulting slurry at fixed time intervals and filtered through regenerated cellulose filters (0.45 μm). The resulting filtered samples were diluted 1:10 in water, and then 10 μL was injected into the HPLC system in order to quantify the indomethacin concentrations.

Dissolution experiments were conducted also in PBS 10 mM at 37 °C with the same procedure as described above, with the only difference that the filtered samples obtained from the slurry of mixture **3** were not diluted 1:10, but directly injected into the HPLC system. All the values obtained were the mean of three independent experiments.

**Cell Culture.** The NCM460 cell line was grown in DMEM + Glutamax supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 g/mL streptomycin at 37 °C in a humidified atmosphere of 95%, with 5% of $CO_2$. For maximum viability, NCM460 cells were subcultured in fresh and spent growth medium in 1:1 ratio. All cell culture reagents were provided by Invitrogen (Life Technologies, Milan, Italy).

**Differentiation of NCM460 Cells to Polarized Monolayers.** Differentiation to NCM460 cell monolayers was performed modifying the method reported by Dalpiaz and co-workers.[25] Briefly, after two passages, confluent NCM460 cells were seeded at a density of $10^5$ cells/mL in a 1:1 ratio of fresh and spent culture medium in 12-well Millicell inserts

(Millipore, Milan, Italy) consisting of 1.0 $\mu$m pore size polyethylene terephthalate (PET) filter membranes, whose surface was 1.12 cm$^2$. Filters were presoaked for 24 h with fresh culture medium, and then the upper compartment (apical, A) received 400 $\mu$L of the diluted cells, whereas the lower (basolateral, B) received 2 mL of the medium in the absence of cells. Half volume of the culture medium was replaced every 2 days with fresh medium to each of the apical and basolateral compartments. The integrity of the cell monolayers was monitored by measuring the transepithelial electrical resistance (TEER) by means of a voltmeter (Millicell-ERS; Millipore, Milan, Italy). The measured resistance value was multiplied by the area of the filter to obtain an absolute value of TEER, expressed as $\Omega \cdot$cm$^2$. The background resistance of blank inserts not plated with cells was around 35 $\Omega \cdot$cm$^2$ and was deducted from each value. The homogeneity and integrity of the cell monolayer were also monitored by phase contrast microscopy. Based on these parameters, cell monolayers reached confluence and epithelial polarization after 6 days, and monolayers with TEER stable value around 180 $\Omega \cdot$cm$^2$ were used for permeation studies. At this time, the medium was replaced with low serum fresh medium (1% FBS) in both the apical and basal compartments.

**Permeation Studies across Cell Monolayers.** For permeation studies, inserts were washed twice with prewarmed PBS buffer in the apical (A, 400 $\mu$L) and basolateral (B, 2 mL) compartments, and then PBS buffer containing 5 mM glucose at 37 °C was added to the apical compartment. The sieved powders (mesh size 106 $\mu$m) were added to the apical compartments in the following amounts: 1.92 mg of indomethacin; 2.5 mg of co-crystal **1**; 2.8 mg of co-crystal **2**; 2.9 of mg co-crystal **3**; 1.92 mg of indomethacin mixed with 0.59 mg of 2-hydroxy-4-methylpyridine, or 0.90 mg of 2-methoxy-5-nitroaniline, or 0.98 mg of saccharin for mixtures **1**, **2**, or **3**, respectively. During permeation experiments, Millicell inserts loaded with the powders were continuously swirled on an orbital shaker (100 rpm; model 711/CT, ASAL, Cernusco, Milan, Italy) at 37 °C. At programmed time points the inserts were removed and transferred into the subsequent wells containing fresh PBS, and then basolateral PBS was harvested, filtered through regenerated cellulose filters (0.45 $\mu$m), and injected (10 $\mu$L) into the HPLC system for the determination of the concentration of indomethacin.

At the end of incubation the apical slurries were withdrawn, filtered, and injected into the HPLC system (10 $\mu$L) after 1:10 dilution, with the exception of the apical sample of the mixture **3**, which was directly injected after filtration, without dilution. After the withdrawal of apical samples, 400 $\mu$L of PBS was inserted in the apical compartments and TEER measurements were performed.

Permeation experiments were also conducted using cell-free inserts in the same conditions described above. All the values obtained were the mean of three independent experiments.

Apparent permeability coefficients ($P_{app}$) of indomethacin were calculated according to the following equation:[26−28]

$$P_{app} = \frac{\frac{dc}{dt}V_r}{S_A C} \tag{1}$$

where $P_{app}$ is the apparent permeability coefficient in cm/min; $dc/dt$ is the flux of drug across the filters, calculated as the linearly regressed slope through linear data; $V_r$ is the volume in the receiving compartment (basolateral = 2 mL); $S_A$ is the

diffusion area (1.13 cm$^2$); and $C$ is the compound concentration in the donor chamber (apical) detected at 60 min and chosen as approximate apical concentration.

**Statistical Analysis about Permeation Studies.** Statistical comparisons between apparent permeability coefficients or between apical concentrations of indomethacin were performed by one way ANOVA followed by Dunnett's post-test; statistical comparisons between transepithelial electrical resistance before and after incubation with the sieved samples was performed by one way ANOVA followed by Bonferroni post-test. $P < 0.001$ was considered statistically significant. All the calculations were performed by using the computer program Graph Pad Prism (GraphPad Software Incorporated, La Jolla, CA, USA), which was employed also for the linear regression of the cumulative amounts of the compounds in the basolateral compartments of the Millicell systems. The quality of fit was determined by evaluating the correlation coefficients ($r$) and $P$ values.

***In Vivo* Administration of Indomethacin: Intravenous Infusion.** Male Sprague−Dawley rats (200−250 g) kept fasting since 24 h received a femoral intravenous infusion of 0.90 mg/mL indomethacin dissolved in a medium constituted by 20% (v/v) DMSO and 80% (v/v) physiologic solution, with a rate of 0.2 mL/min for 5 min. Four rats were employed for femoral intravenous infusions. At the end of infusion and at fixed time points within 24 h, blood samples (300 $\mu$L) were collected and inserted in heparinized test tubes, which were centrifuged at 4 °C for 15 min at 1500$g$; 100 $\mu$L of plasma was then withdrawn and immediately quenched in 300 $\mu$L of ethanol (4 °C); 100 $\mu$L of internal standard (100 $\mu$M 9-phenylcarbazole dissolved in ethanol) was then added. After centrifugation at 13000$g$ for 10 min, 400 $\mu$L aliquots were reduced to dryness under a nitrogen stream and stored at −20 °C until analysis. The samples were dissolved in 150 $\mu$L of mobile phase (methanol and 0.2 phosphoric acid 75:25 v/v), and, after centrifugation, 10 $\mu$L was injected into the HPLC system for indomethacin assay. All the values obtained were the mean of four independent experiments.

The efficacy of indomethacin extraction from blood samples was determined by recovery experiments, comparing the peak areas extracted from 10 $\mu$M (3.58 $\mu$g/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 indomethacin from rat blood resulted 87.4 ± 3.9%. The concentrations of this compound were therefore referred to as peak area ratio with respect to the internal standard 9-phenylcarbazole. The precision of the method based on peak area ratio, calculated for 10 $\mu$M (3.6 $\mu$g/mL) solutions, was represented by RSD values of 0.93% . The calibration of indomethacin was performed by employing eight different concentrations in whole blood at 4 °C ranging from 2 $\mu$M (0.72 $\mu$g/mL) to 50 $\mu$M (18.0 $\mu$g/mL) and expressed as peak area ratios of the compounds to the internal standard versus concentration. The calibration curve resulted as linear ($n = 8$, $r = 0.990$, $P < 0.0001$). The accuracy of the extraction method was determined with respect to the calibration curve and was described by relative errors comprised between −2.63% and 0.24%.

The *in vivo* half-life of indomethacin 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, and the remaining area was determined as the ratio between the indomethacin concentration detected at 24 h and the elimination constant ($k_{el}$), which was obtained from the slope of the semilogarithmic plot (−slope × 2.3). All the calculations were performed by using the computer program Graph Pad Prism.

***In Vivo* Administration: Oral Administration of Indomethacin, Its Co-Crystals, and Its Parent Mixtures.** The sieved powders were mixed with palatable food in order to induce their oral assumption by male Sprague−Dawley rats (200−250 g) kept fasting since 24 h. The following doses were administered: 0.90 mg of indomethacin; 1.18 mg of co-crystal **1**; 1.32 mg of co-crystal **2**; 1.36 mg of co-crystal **3**; 0.90 mg of indomethacin mixed with 0.28 mg of 2-hydroxy-4-methylpyridine, or 0.42 mg of 2-methoxy-5-nitroaniline, or 0.46 mg of saccharin for mixtures **1**, **2**, or **3**, respectively. Four rats/group were employed for the oral administration experiments. At the end of administration and at fixed time points within 24 h, blood samples (300 $\mu$L) were collected, then extracted, and analyzed as described above. All the concentration values obtained for indomethacin were the mean of four independent experiments. The AUC values referred to each orally administered treatment were calculated as described above. The absolute bioavailability values of indomethacin, referred to the oral administered samples, were obtained as the ratio between their oral AUC values and the AUC of the intravenous administration of the drug. All the calculations were performed by using the computer program Graph Pad Prism.

**Statistical Analysis about *in Vivo* Administration of Indomethacin.** Statistical comparisons between absolute bioavailability values were performed by one way ANOVA followed by Dunnett's post-test. $P < 0.001$ was considered statistically significant. All the calculations were performed by using the computer program Graph Pad Prism.

# ■ RESULTS

**Indomethacin Co-Crystals.** Indomethacin was co-crystallized with two model molecules, 2-hydroxy-4-methylpyridine in its keto form (co-crystal **1**) and 2-methoxy-5-nitroaniline (co-crystal **2**). Their chemical structures along with that of the previously reported[11,19] indomethacin−saccharin co-crystal (co-crystal **3**) are reported in Figure 1. The X-ray three-dimensional structures for the three co-crystals are shown in Figure 2, which evidences that the covalent bonds of each single molecule are not altered in the co-crystallized structures and shows the main hydrogen bonding interactions between indomethacin and co-crystallizing agents (dashed lines). In **1**, the pyridine derivatives are linked in dimers by N1A−H···O1A hydrogen bonds (Table S3 of the Supporting Information) and each dimer, in turn, is linked on both sides to two indomethacin molecules through O3−H···O1A hydrogen bonds involving the indomethacin carboxylic group (Figure 2a). In **2**, the indomethacin molecules are coupled in dimeric units by O3−H···O4 hydrogen bonds, as found in the crystal lattice of the pure $\gamma$-indomethacin crystal.[29] The dimers link the coformer molecules through the O1 chetonic oxygen, forming N−H···O interactions of medium strength (Figure 2b). In **3**, the indomethacin and saccharin molecules form in the crystal carboxylic acid and imide centrosymmetric dimers, respectively, resembling the arrangements found in the pure crystals.[29,30] The different dimers interact via a number of weak C−H···O/Cl interactions; the most relevant of them is shown in Figure 2c

(C···Cl distance: 3.54 Å). More details about the crystal structures and the packing arrangements can be found in the Supporting Information.

**DSC Analysis.** The DSC traces and thermal data for $\gamma$-indomethacin and its co-crystals are presented in Figure 3.
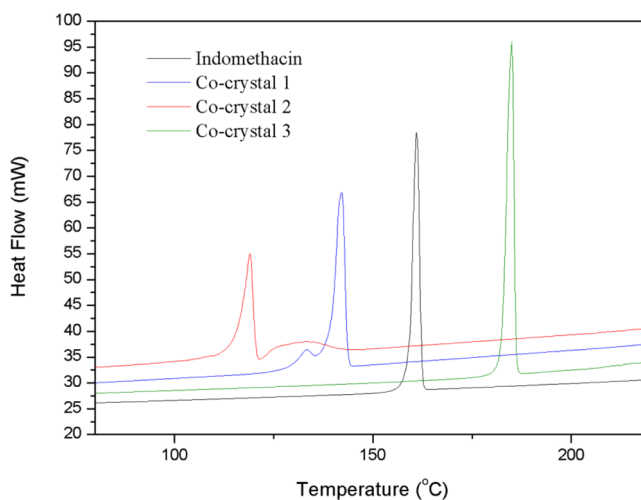
**Figure 3.** DSC thermograms for indomethacin and its co-crystals.

Indomethacin showed a single melting transition with $T_{max}$ = 160.8 °C and enthalpy ($\Delta H_f$) = 38.42 kJ/mol (Figure 3 and Table 1). The DSC thermogram for co-crystals **1**, **2**, and **3**

**Table 1. Melting Points and Enthalpy Values for $\gamma$-Indomethacin and Its Co-Crystals[a]**

| compound | melting point (°C) | $\Delta H$ (kJ/mol) |
| --- | --- | --- |
| $\gamma$-indomethacin | 160.8 ± 0.8 | 38.42 ± 0.11 |
| co-crystal **1** | 141.7 ± 0.3 | 66.16 ± 0.14 |
| co-crystal **2** | 118.9 ± 0.3 | 55.75 ± 1.05 |
| co-crystal **3** | 184.6 ± 0.4 | 77.46 ± 0.81 |

[a]Data are reported as mean ± SD of three independent experiments.

showed marked endothermic transitions attributed to the melting transition at $T_{max}$ = 141.7, 118.9, and 184.6 °C, respectively (Figure 3 and Table 1). The related $\Delta H_f$ values were 66.16 kJ/mol for co-crystal **1**, 55.75 kJ/mol for co-crystal **2**, and 77.46 kJ/mol for co-crystal **3**.

**Dissolution Studies.** *Co-Crystals Can Significantly Change the Dissolution Profiles of Indomethacin.* Figure 4A reports a comparison between the dissolution profiles in 200 mM phosphate buffer at 37 °C of $\gamma$-indomethacin, as free drug, co-crystallized, or mixed in the parent mixtures. The saturation concentration of free $\gamma$-indomethacin, reached after about 2 h of its incubation in the buffer, was about 1.8 mg/mL. The dissolution profile of $\gamma$-indomethacin was not altered by the presence of the co-crystallizing agents when mixed with the drug. Differently, the co-crystallized powders induced significant changes of indomethacin dissolution profiles. In particular, the co-crystal **1** and the co-crystal **2** induced an increase up to three times and a 50% decrease of $\gamma$-indomethacin saturation concentration, respectively, without affecting the dissolution rate. Finally, the co-crystal **3** induced not only a significant enhancement of the saturation concentration of $\gamma$-indomethacin (up to four times) but, differently from the other co-crystals,
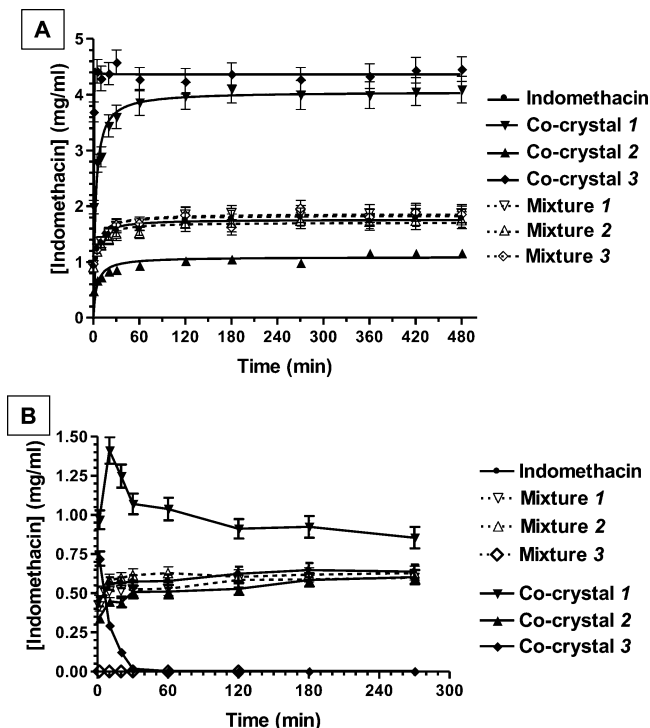
**Figure 4.** Solubility and dissolution profiles in phosphate buffer 200 mM (A) and PBS 10 mM (B) at 37 °C for γ-indomethacin as free drug, or co-crystallized, or mixed in the parent mixtures. Data are reported as the mean ± SD of three independent experiments.

medium adopted for dissolution studies. The indomethacin permeation profiles, expressed by the cumulative concentrations in the receiving basolateral compartments, are reported in Figure 5. The linear profiles indicate constant permeation
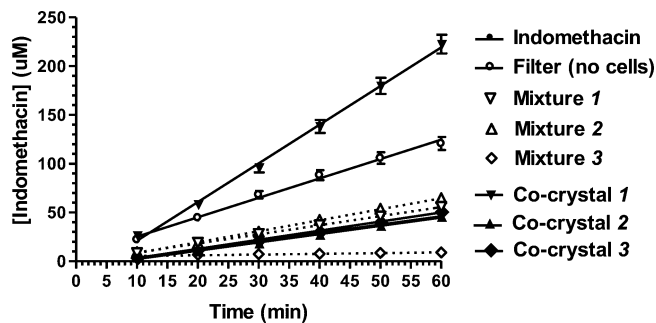


**Figure 5.** Permeation kinetics of indomethacin after introduction in the "Millicell" apical compartments of powders constituted by free γ-indomethacin, its co-crystals, or the parent mixtures of γ-indomethacin with co-crystallizing agents. The permeations were analyzed across monolayers obtained by NCM460 cells. The permeation of free γ-indomethacin was analyzed across the Millicell filters alone (filter) or coated by monolayers. The cumulative amounts in the basolateral receiving compartments were linear within 60 min ($r \geq 0.97$, $P \leq 0.001$). The resulting slopes of the linear fits were used for the calculation of permeability coefficients ($P_{app}$). All data are reported as mean ± SD of three independent experiments.

also an increase of the drug dissolution rate, 30 min being the time necessary to reach the saturation conditions.

The indomethacin solubility and dissolution profiles represented in Figure 4A drastically changed when the powders were incubated in PBS 10 mM, the medium employed for the drug permeation studies across NCM460 cell monolayers (see below). In particular, under these experimental conditions, the saturation concentration of free γ-indomethacin was reduced of about 50% when compared to that measured when it was dissolved in 200 mM phosphate buffer (Figure 4B); the mixtures **1** and **2** showed dissolution profiles similar to that of free γ-indomethacin, whereas the mixture **3** induced a drastic decrease of the saturation concentration of the drug, showing a mean ± SD value of 0.0012 ± 0.0004 mg/mL (about 0.2% of the saturation concentration of the free drug). The co-crystal **1** was instead associated with an increase of indomethacin saturation concentration. Differently from the results obtained under 200 mM phosphate buffer conditions (see above), the co-crystal **2** led to an indomethacin dissolution profile similar to those displayed by the free drug. Finally, the co-crystal **3** induced a very fast dissolution of indomethacin that was, however, followed by a sudden precipitation of the drug; this event was completed within 60 min, showing indomethacin concentration values about 0.003 mg/mL.

**Permeation Studies.** *Co-Crystals Can Induce Different Effects on Cell Monolayers with Respect to Their Parent Mixtures.* The PBS was used as incubation medium for the permeation studies of indomethacin across an *in vitro* model of human intestinal wall, i.e., NCM460 cell monolayers.[31] In order to simulate an oral administration, the powders of γ-indomethacin, its co-crystals, or the parent mixtures were introduced in the apical compartment of the "Millicell" systems with the same ratio between solid powders and incubation

conditions during the analysis time period (60 min) for all samples. The straight line related to mixture **3** ($r = 0.970$, $P = 0.001$) was characterized by indomethacin concentration values strongly lower than those of the straight lines of the other powders ($r \geq 0.998$, $P < 0.0001$). The apparent permeability coefficients ($P_{app}$) of indomethacin (Table 2) have been calculated on the basis of the resulting slopes of the linear fits and the indomethacin concentrations detected in the apical compartments after 1 h of incubation of the powders (Table 2), chosen as approximate apical concentrations. These latter values appeared essentially in line with those obtained from dissolution studies of indomethacin powders in 10 mM PBS (Figure 4B). Indeed, the drug concentrations obtained from the powders constituted by γ-indomethacin, mixtures **1** and **2**, and co-crystal **2** were not statistically dissimilar among them (about 1000 μM, $P > 0.05$). On the other hand, mixture **3** induced a drastic reduction of indomethacin concentration ($P < 0.001$), showing values close to the drug limit of quantification. Furthermore, the co-crystal **1** induced an increase of indomethacin concentration of about three times with respect to the free drug powder ($P < 0.001$), whereas the co-crystal **3** significantly reduced the γ-indomethacin concentration ($P < 0.001$), even if in a less drastic manner than detected from dissolution experiments in 10 mM PBS in the absence of cells (Figure 4B). Indeed, as reported in Table 2, the apical concentration of indomethacin dissolved at 60 min from co-crystal **3** was about 440 μM, with a pH value of 5.5, whereas in dissolution experiments (without cells) it was about 10 μM with a pH value of 3.2. The same pH values were registered at 60 min for dissolution of mixture **3** in the presence and in the absence of cells, respectively. These data, therefore, confirm the aptitude of co-crystal **3** to enhance the indomethacin dissolution pattern with respect to its parent physical mixture. Indeed, a less acid pH value induced by the presence of cells allowed co-crystal **3** to enhance the amounts of indomethacin

**Table 2. Data Related to Indomethacin *in Vitro* Dissolution and Permeation Studies and *in Vivo* Oral Bioavailability[a]**

| powder | permeation condition | solubility in PBS 10 mM at 60 min (μM) | apical concns at 60 min (μM) | $P_{app}$ (×10⁻⁵ cm/min) | TEER (Ω·cm²) 0 min | TEER (Ω·cm²) 60 min | absolute bioavailability (%) |
|---|---|---|---|---|---|---|---|
| γ-indomethacin | cells | 1612 ± 84 | 1010 ± 40 | 150 ± 6 | 181 ± 10 | 163 ± 8 | 23.5 ± 0.8 |
| mixture **1** | cells | 1475 ± 90 | 989 ± 42 | 165 ± 7 | 181 ± 9 | 158 ± 7 | 25.7 ± 1.6 |
| co-crystal **1** | cells | 2900 ± 138* | 2708 ± 84* | 301 ± 14* | 185 ± 11 | 21 ± 1** | 37.7 ± 1.5*** |
| mixture **2** | cells | 1755 ± 96 | 1047 ± 50 | 147 ± 10 | 183 ± 10 | 152 ± 7 | 24.4 ± 1.2 |
| co-crystal **2** | cells | 1426 ± 88 | 1013 ± 38 | 145 ± 6 | 177 ± 9 | 159 ± 8 | 20.1 ± 0.9*** |
| mixture **3** | cells | 2.3 ± 0.3* | 3.2 ± 0.1* | | 180 ± 10 | 36 ± 2** | 24.8 ± 0.9 |
| co-crystal **3** | cells | 10.4 ± 0.8* | 442 ± 18* | 374 ± 16* | 183 ± 10 | 164 ± 8 | 33.6 ± 0.6*** |
| γ-indomethacin | filter | | 1015 ± 45 | 429 ± 18* | | | |

[a]Permeation studies were performed by using "Millicell" filters alone (filter) or coated by NCM460 cell monolayers (cells). Indomethacin was introduced in the donor compartment as sieved powder of γ-indomethacin, or its co-crystals, or the parent mixtures. The apical concentrations detected at the end of incubation were employed for the calculation of the apparent permeation coefficients ($P_{app}$). Permeation studies were performed after cell cultures reached the confluence using parallel sets of "Millicell" well plates with similar TEER values (TEER 0 min). The TEER values were measured again at the end of incubation (TEER 60 min). All data related to permeation studies are reported as the mean ± SD of three independent experiments. Bioavailability data were obtained after oral administration to rats of the powders and are reported as the mean ± SD of four independent experiments. *$P < 0.001$ versus γ-indomethacin corresponding value. **$P < 0.001$ versus TEER at "time 0" (0 min). ***$P < 0.001$ versus absolute bioavailability of γ-indomethacin.

dissolved in the medium. The dissolution of all other powders analyzed appeared slightly influenced by the presence of the cells (Table 2). The pH values of dissolution media of these powders at 60 min ranged from 7.0 to 7.5 both in the presence and in the absence of cells. The slope of the permeation profile (Figure 5) and the apical concentration of indomethacin related to mixture **3** (Table 2) appeared too low to obtain a reliable $P_{app}$ value of indomethacin dissolved from this sample.

A comparison of the $P_{app}$ values of γ-indomethacin (Table 2) obtained in the presence ($150 \times 10^{-5} \pm 6 \times 10^{-5}$ cm/min) and in the absence ($429 \times 10^{-5} \pm 18 \times 10^{-5}$ cm/min) of NCM460 cell monolayers indicated a significantly lower permeation of the drug in the presence of cells ($P < 0.001$), confirming the validity of the monolayer as an *in vitro* model of a physiologic barrier. This behavior appeared in agreement with the transepithelial electrical resistance (TEER) values (about 180 Ω·cm²) attributed to the monolayers before their incubation with the powders (Table 2). The apparent coefficient values across the monolayer of indomethacin dissolved from mixtures **1** and **2** and co-crystal **2** did not significantly differ from the $P_{app}$ obtained for the free γ-indomethacin (Table 2, $P > 0.05$). On the other hand, the co-crystal **1** induced a consistent increase of indomethacin permeation ($P_{app} = 301 \times 10^{-5} \pm 11 \times 10^{-5}$ cm/min, $P < 0.001$). This phenomenon was associated with the ability of this co-crystal to impair the tight junctions among the cells of the monolayer. This ability was evidenced by the drastic reduction of TEER values ($P < 0.001$) measured after 60 min of incubation with this sample ($21 \pm 1$ Ω·cm²) in comparison to the value obtained before incubation ($185 \pm 11$). Moreover, monitoring the monolayer after its incubation with co-crystal **1** by phase contrast microscopy evidenced a complete separation of the cells (data not shown). It is remarkable that mixture **1** incubation altered neither the monolayer integrity, as monitored by phase contrast microscopy (data not shown), nor the TEER value (before incubation = $181 \pm 9$ Ω·cm²; after incubation = $158 \pm 7$ Ω·cm²; $P > 0.05$). The same profile has been also registered for the powder of free γ-indomethacin, the mixture **2**, and the co-crystals **2** and **3**. It is interesting to observe that the mixture **3** induced a cell monolayer fragmentation, as monitored by phase contrast microscopy (data not shown) and indicated by the TEER value (before incubation = $180 \pm 10$ Ω·cm²; after incubation = $36 \pm 2$ Ω·

cm²; $P < 0.001$). The co-crystal **3** has been characterized by a $P_{app}$ value of $374 \times 10^{-5} \pm 16 \times 10^{-5}$ cm/min, significantly higher with respect to that obtained with the powder of free γ-indomethacin ($P < 0.001$). Interestingly, the permeation enhancement induced by co-crystal **3** was not related to the monolayer fragmentation (data not shown).

***In Vivo* Administration of Indomethacin: Its Oral Bioavailability Is Modulated by Co-Crystallization.** After intravenous infusion of 0.90 mg indomethacin, the drug concentration in the rat bloodstream was $13.2 \pm 1.4$ μg/mL. This value decreased during time with an apparent first order kinetic (Figure 6A) confirmed by the linearity of the semilogarithmic plot reported in the inset of Figure 6A ($n = 8$, $r = 0.983$, $P < 0.0001$), showing a half-life value of $8.94 \pm 0.38$ h.

The rat blood indomethacin concentrations within 24 h after its intravenous infusion (iv) as free drug or the oral administration of 0.90 mg of indomethacin as sieved powders of free γ-drug, its co-crystals, and the parent mixtures are reported in Figure 6B. In order to better compare among them the results, Figure 6C reports a section of Figure 6B, focused on the profiles obtained within 8 h after of the oral administration of the powders. It can be observed that the free γ-indomethacin powder induced a concentration peak in the rat bloodstream of about 5 μg/mL 2 h after the administration. A similar profile was obtained with mixtures **1** and **2**, whereas mixture **3** was characterized by a profile showing a peak concentration of about 3.5 μg/mL 2 h after its administration.

The co-crystal **1** induced a concentration peak in the rat bloodstream of about 5 μg/mL 30 min after the administration, the same time required for co-crystal **3** to induce a concentration peak of about 3 μg/mL. The co-crystal **2** profile was instead characterized by a peak lower than 2 μg/mL obtained between 1 and 2 h after the administration. In general, the profiles of the co-crystals appeared characterized by a decrease of indomethacin blood concentration within 4 h after their administration with a rate lower than those observed following free γ-indomethacin and the parent mixtures administrations.

The AUC values of the profiles reported in Figure 6B were employed for the calculation of absolute bioavailabilities ($F$) of the solid formulations, reported in Table 2. In particular the
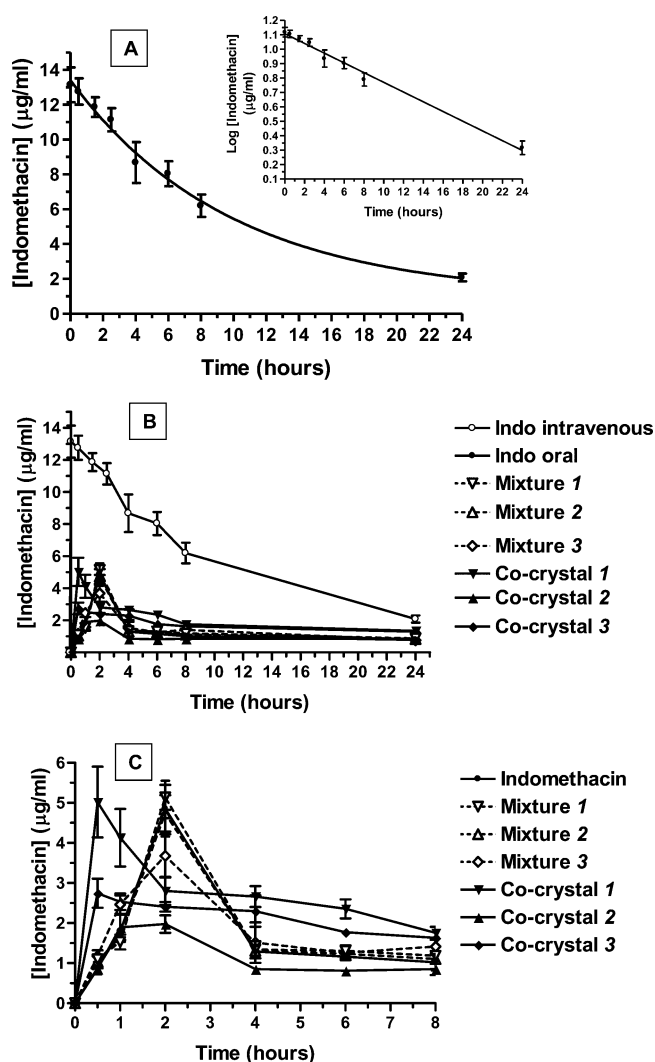
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**Figure 6.** (A) Elimination profile of indomethacin after 0.90 mg infusion to rats. The elimination followed an apparent first order kinetic, confirmed by the semilogarithmic plot reported in the inset ($n = 8$, $r = 0.983$, $P < 0.0001$). The half-life of indomethacin was calculated to be $8.94 \pm 0.38$ h. (B) Blood indomethacin concentrations ($\mu g/mL$) after intravenous infusion (iv) or oral administration of 0.90 mg dose to rats within 24 h. The oral formulations were constituted by the sieved powders of free $\gamma$-indomethacin, its co-crystals, and the parent mixtures. (C) Detailed blood indomethacin concentrations ($\mu g/mL$) after oral administration of 0.90 mg dose to rats within 8 h. All data reported in the figure are expressed as the mean $\pm$ SD of four independent experiments.

free $\gamma$-indomethacin powder was characterized by an $F$ value of $23.5 \pm 0.8\%$, not statistically dissimilar ($P > 0.05$) from those of mixtures 1, 2, and 3. The co-crystals 1 and 3 were characterized by significantly higher ($P < 0.001$) $F$ values than free $\gamma$-indomethacin ($37.7 \pm 1.5\%$ and $33.58 \pm 0.59\%$, respectively), while, co-crystal 2 induced a relatively small, but significant ($P < 0.001$), decrease of bioavailability with respect to the powder of the free drug ($F$ value = $20.1 \pm 0.9\%$).

## ■ DISCUSSION

Co-crystallization, by enhancing BCS class II API solubility, has been proposed as a new strategy to increase drug bioavailability.[9−17] However, experimental evidence about the effects of the co-crystals on the permeation of APIs across intestinal barriers and on intestinal epithelial barrier integrity is not reported in the literature. These studies should be relevant as the disruption of intestinal epithelial tight junctions has two undesirable consequences: unwanted substances such as endotoxins are allowed into the body, and depolarization of cells may be promoted.[32,33] Moreover, the regulatory status regarding the use of co-crystals in pharmaceutical products appears still unsettled, it being necessary to clarify whether the co-crystal would be defined as a physical mixture or as a new chemical entity requiring full safety and toxicology testing.[7,8] In the attempt to contribute to clarify these aspects, we have chosen indomethacin as a model BCS class II API and compared its properties with those of three of its co-crystals along with their parent physical mixtures. To our knowledge, this type of study is absolutely novel, being not only focused on a systematic comparison among the behavior of powders constituted by the pure API, its co-crystals, and their parent mixtures, but also involving the analysis of the API permeation across an *in vitro* intestinal barrier model.

The dissolution studies were first performed in a 200 mM phosphate buffer, pH 7.4. The relatively high ionic strength of this buffer was necessary to maintain the stability of the pH value during the dissolution processes.[19] As already described, we have observed that the dissolution profile of indomethacin was not altered by the presence of the co-crystallizing agents in the form of physical mixtures with the API, whereas significant changes were observed by the dissolution of the co-crystals. This behavior is consistent with the different crystal packing forces due to the different intermolecular interaction patterns of the considered crystals as described in Results. These differences are reflected by the different thermal behavior of the indomethacin and its co-crystals. In particular, the enthalpy data for $\gamma$-indomethacin and co-crystal 3 differ by about 39 kJ/mol, as previously reported;[19] in this case the higher lattice energy of 3 is related to a solubility higher than that of the pure indomethacin, similarly as it happens with co-crystal 1. It is known[34] that the ideal solubility depends on the melting temperature and enthalpy of the solute; such behavior, however, only applies to specific cases, such as polymorphs. On the contrary, melting point, along with related enthalpy values, "has often been shown to be a poor parameter to judge aqueous solubilities of co-crystals",[35] indicating that the co-crystal solubility is dependent on more than a single factor.

A qualitative concordance between the API dissolution patterns in the 200 mM phosphate buffer and its absorption in the rat bloodstream after the oral administration of the powders has been observed. In particular, dissolution (Figure 4A) and bioavailability (Figure 6C) profiles of pure $\gamma$-indomethacin were similar to those of its physical mixtures with the co-crystallizing molecules; on the other hand either indomethacin solubility or its bioavailability was significantly increased by co-crystals 1 and 3, and weakly decreased by co-crystal 2. It is worth noting that, to allow a direct comparison among the powder constituted by the free drug, its co-crystals, and the parent mixtures, we intentionally decided to not follow the suitable formulation strategies suggested to improve the plasma levels of APIs orally administered as co-crystals.[9] This can explain the relatively weak, although significant, changes of bioavailability induced by the co-crystallization observed in the present study. However, the accordance between dissolution and bioavailability profiles described above is in line with literature data concerning pharmaceutical co-crystals.[10−17] Since this correlation does not provide any information on

the effective role of the co-crystals in influencing the API absorption mechanisms across the intestinal barrier, we decided to perform permeation experiments across NCM460 cell monolayers. These studies are absolutely innovative for systems involving pharmaceutical co-crystals and their parent mixtures.

Various cell monolayer models that mimic the human intestinal epithelial barrier have been developed, providing ideal systems for the rapid *in vitro* assessment of the intestinal absorption of drug candidates. We have chosen the human normal colonic epithelial NCM460 cells, their being an immortalized, non-transformed cell line, derived from primary cells of the normal human transverse colonic mucosa.[29] As these cells are not of tumor origin nor transfected ones, they retain more closely the physiological characteristics of the normal human colon compared to the pathologically or experimentally transformed cell lines. In this context, it is worth noting that TEER developed by the NCM460 cells are within the range reported for intact sheets of human colonic mucosa.[36,37] Furthermore, the lipophilic nature of indomethacin enables the molecule to diffuse quickly and to get absorbed completely through the intestinal membrane after oral ingestion, resulting as almost equally permeable in the colon and small intestine.[38,39]

The permeation studies were performed by glucose-enriched PBS as dissolution medium of the indomethacin powder, the concentration of 200 mM phosphate buffer being too high to allow cell survival. Moreover, PBS represented the simplest medium in which to dissolve indomethacin from its powders, in order to study the permeation properties across NCM460 cells in the absence of other interfering substances. It is indeed known that simulated intestinal buffers can induce TEER changes of the monolayers and have inhibitory activity toward efflux transporters expressed on the cell membranes.[40] As described above, the dissolution profiles in PBS of indomethacin showed some marked differences with respect to the patterns obtained in 200 mM phosphate buffer, attributable to the PBS relatively weak buffering power.

The suspensions obtained by the introduction of the solid powders containing indomethacin in the apical compartments of the "Millicell" systems allowed us to simulate an oral administration. The $\gamma$-indomethacin crystals appeared able to maintain the integrity of the monolayer characterized by TEER values around 180 $\Omega \cdot cm^2$. Moreover, a comparison of the permeability values obtained in the absence and in the presence of cell monolayer validated the ability of this preparation to behave as a physiologic barrier. We have instead observed that the physical mixture of indomethacin and saccharin induced a drastic decrease of the TEER value of the monolayer, whose cells appeared to lose completely their mutual contacts after 1 h of incubation. Surprisingly, the incubation with the parent co-crystal **3** allowed the integrity of the monolayer as well as of its TEER value to be maintained, and induced an increase of indomethacin permeation across the NCM460 cells, with respect to $\gamma$-indomethacin crystals. These results appear unexpected, it being currently believed that the co-crystallization strategy should influence the dissolution properties of an API, without inducing changes on its pharmacological profile.[11,18] Conversely, the opposite effects observed on NCM460 cells using mixture **3** and co-crystal **3** indicate that APIs can have highly different biological behavior dependent on the type of powder (a physical mixture or a co-crystal) from which they are dissolved. A similar aspect was also observed for co-crystal **1** and its parent mixture **1**. In particular we have observed that co-crystal **1** induced a drastic decrease of the TEER value of the monolayer, whose cells appeared completely separated after 1 h of incubation. The permeation profile of indomethacin dissolved from co-crystal **1** showed indeed the highest values with respect to all other cases, probably due to both the loss of the barrier effect of the monolayer and the increased dissolution of the API. Surprisingly, the incubation with the parent physical mixture **1** did not induce any changes on the monolayer integrity, as evidenced by unaffected TEER value after 1 h of incubation. Moreover, the permeation profile of indomethacin dissolved from this mixture was the same as that obtained with solid $\gamma$-indomethacin. The phenomenon of the different biological behavior of the powders does not, however, amount to a systematic rule. Indeed, no significant differences between $\gamma$-indomethacin and co-crystal **2** or mixture **2** were observed, as far as the integrity of the monolayer and API permeation profile is concerned.

Several previous studies described API gastrointestinal absorption by using cell lines.[41,42] To the best of our knowledge, however, none of them have been performed with the employment of co-crystals. At present, on the basis of our results we would venture to guess that the molecules constituting the co-crystal in some way could retain in solution some of the interactions formed in the solid state. If it be so, the resulting molecular aggregations, although transient, could interact with the proteins responsible for drug transport and for the integrity of the cellular layers with different mechanisms with respect to the molecules coming from pure crystals' dissolution. It is worth noting that the affinity of APIs for proteins is induced by specific and concerted weak interactions. Thus, the different dissolution mechanisms at molecular level of an API, dissolving from a co-crystal or from its parent physical mixture, may have important different effects on the proteins of biological systems.

## ■ CONCLUSIONS

To the best of our knowledge this is the first study demonstrating different effects induced by co-crystals and their parent physical mixtures on a biologic system, findings that could raise serious concerns about the use of co-crystal strategy to improve API bioavailability without performing appropriate investigations. In this case co-crystal **1** was found to induce a drastic decrease of the TEER value of NCM460 cell monolayers, whereas its parent mixture did not evidence any effect. On the other hand, the physical mixture of saccharin and indomethacin was able to induce a drastic decrease of the TEER value of NCM460 monolayers, whereas its parent co-crystal **3** did not evidence any effect on the integrity of the monolayers, being anyway able to increase the permeation of indomethacin across the monolayers. On the basis of the present experimental data we can only hypothesize the reason(s) for these phenomena, but it is clearly evidenced that the biological effects of a co-crystal and its parent mixture can be drastically different, even if this is not to be taken as a general rule. Indeed, any difference was registered by our permeation measurements between co-crystal **2** and its parent physical mixture on NCM460 cell monolayer.

Our results seem to open new perspectives about the application of pharmaceutical products containing co-crystals. New and appropriate investigations appear therefore necessary in order to evaluate the potential new applications and the potential damaging effects of pharmaceutical co-crystals.

## ■ ASSOCIATED CONTENT

### ⓈSupporting Information

Experimental details and crystallographic analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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