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Highlights

- Co-crystallization can enhance the solubility and/or permeability of drugs
- The positions of the FDA and EMA on pharmaceutical co-crystals are very different
- Co-crystallization does not appear to affect the drugs pharmacological activity
- Co-crystals and physical mixtures can show very different biological properties

Can pharmaceutical co-crystals provide an opportunity to modify the biological properties of drugs?

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Poorly soluble and/or permeable molecules jeopardize the discovery and development of innovative medicines. Pharmaceutical co-crystals, formed by an active pharmaceutical substance (API) and a co-crystal former, can show enhanced dissolution and permeation values compared with those of the parent crystalline pure phases. It is currently assumed that co-crystallization with pharmaceutical excipients does not affect the pharmacological activity of an API or, indeed, might even improve physical properties such as solubility and permeability. However, as we highlight here, the biological behavior of co-crystals can differ drastically with respect to that of their parent physical mixtures.

Teaser: The pharmacological activity of APIs is not affected by co-crystallization, but how can we be sure that this is true? Permeation studies suggest that pharmaceutical co-crystals and their parent physical mixtures can generate totally different biological effects.

Keywords

Pharmaceutical co-crystals; Drug permeability; Bioavailability; Physicochemical properties

1. The discovery of new active molecules alone cannot suffice to develop innovative medicines

Poorly soluble molecules constitute approximately 40% of approved drugs and nearly 90% of developmental pipeline drugs [1]; moreover, several marketed drugs are also characterized by low permeability [2]. Given that both solubility and permeability correlate with bioavailability, most of the poorly soluble, poorly permeable drugs jeopardize the discovery and development of innovative medicines. Therefore, it is crucial for a pharmaceutical company to select a 'research and development' (R&D) pipeline allowing both the design of successful drugs and the resolution of issues related to their bioavailability [3]. Currently, the most successful market strategies appear to be not only the extensive testing of new APIs, but also the development of improved formulations of existing drugs [1].

Based on the fact that the solubility of a solid form depends on the energy lattice (i.e., the higher the energy, the lower the solubility), the use of amorphous phases could, in principle, overcome the drug solubility problem. However, two main disadvantages are associated with amorphous solids: (i) their stability is lower compared with that of crystals; and (ii) their excess of free energy often induces the formation of stable crystals [4]. As a consequence, competition in the field of generics appears low for already approved amorphous products [1].

1. Pharmaceutical co-crystals: one of the most exciting areas of emerging pharmaceutical research

Crystal engineering of pharmaceutical solids could be useful for optimizing the solubility and permeability of drugs, and co-crystallization appears to be show promise in this context [5]. The term 'co-crystal' originates from 'a composite crystal' and is still a subject of topical debate. There are numerous definitions [6], two of which summarize the main points of view: (i) co-crystals are multicomponent systems where two or more compounds are present, generally in a stoichiometric ratio, both in an ionic or neutral form [7,8]; and (ii) co-crystals are homogeneous crystalline solids containing stoichiometric amounts of discrete neutral molecular species that are solids under ambient conditions (all solids containing ions, including complex transition-metal ions, are excluded) [9]. According to the second definition, ions as co-crystal components are excluded. The dividing line between salts and co-crystals can be distinguished by the location of the proton between an acid and a base, taking into account that salt formation requires a difference of almost 3 pKa units between acid-base conjugates [6].

Pharmaceutical co-crystals, formed by an API and a co-crystal former [10], may show solubility and dissolution properties that are similar to those of amorphous compounds (i.e., higher than the parent crystalline pure phases) [11]. For this reason, the use of co-crystallized drug substances in formulations is one of the most exciting areas of emerging pharmaceutical research. Combining an API with a pharmaceutically acceptable agent has become an increasingly attractive route for developing new pharmaceutical products with enhanced physicochemical properties. Indeed, co-crystallization with pharmaceutical excipients does not appear to affect the pharmacological activity of an API, but can improve its physical properties, such as solubility, dissolution rate, permeability, stability, and compaction behavior [5,6,12]; in fact, in co-crystals, the molecular components are held together essentially via 'nonbinding' interactions (*H*-bonds, van der Waals forces, hydrophobic

interactions, etc.) without altering the covalent bond structure of the APIs [13], thus retaining their safety and therapeutic properties.

1. Careful analysis of the factors influencing the solubility of co-crystals is necessary

The enhanced solubility of drugs in co-crystals is achieved mainly because of a decrease in lattice energy and an increase in solvent affinity. In particular, solubility is controlled by the lattice when the co-crystal components show little resistance to solvation; by contrast, the aqueous solubility of co-crystals is controlled by solvation in the case of drugs with relatively high hydrophobicity. It has often been observed that the melting point of co-crystals is an unreliable parameter to use to predict their aqueous solubility, suggesting that solubility is often limited by solvation but not by lattice energy [14]. Occasionally, co-crystal solubility has been correlated with that of its co-former [11], indicating that the solubility of pure co-former is proportional to the decrease in the solvation barrier of the co-crystals; however, such a correlation has not always been found [15]. In conclusion, even if universal indicators of co-crystal solubility would be useful, each case requires a careful analysis of the factors that influence the solubility [14]. Moreover, it is important to take into account that, in biorelevant media, the change in solubility of the co-crystal can be different; in particular, a co-crystal can display higher, equal, or lower solubility than the constituent drug, depending on the concentration of the media constituents [16].

1. Co-crystallization can significantly affect the bioavailability of drugs

Co-crystallization effects on pharmacokinetics have been studied for more than 20 APIs [17], 80% of which belong to class II (low solubility, high permeability) according to the Biopharmaceutics Classification System (BCS) [18]. In general, solubility enhancements obtained by API co-crystallization increase their bioavailability after oral administration, even if this is not a systematic phenomenon; indeed, few cases have been reported [11] in which co-crystallization increases the solubility of APIs but at the same time does not affect or even reduce their bioavailability. It is well known that co-crystallization can impact area under concentration (AUC) of APIs from a tenfold decrease to 30-fold increase [12], even if the most significant increases have been obtained with the support of suitable formulations [19–21].

1. Co-crystallization can influence the permeability of drugs

Several pharmaceutical co-crystals (about 60) have been analyzed so far, and many of these have been shown to improve the API bioavailability [11]. Conversely, few studies have reported their ability to modulate the permeability of APIs: indeed, only the co-crystals of three drugs belonging to BCS class III (good aqueous solubility and poor permeability) or class IV (poor aqueous solubility and poor permeability) have been studied for this purpose. These studies revealed the ability of co-crystals to increase the product in terms of its solubility and permeability, parameters presumed to predict their overall bioavailability [22–24]. Interestingly, the data, obtained by measuring skin permeation [22] or by using dialysis [23] or silicone [24] membranes, highlight that co-crystallization offers the opportunity to improve the aqueous solubility and permeability of APIs simultaneously without changing their molecular structure. The behavior of co-crystals has been attributed to not only the hydrophobic nature of the co-formers, but also to drug–co-former interactions in the crystalline lattice. Thus, it can be hypothesized that the molecules constituting the co-crystal can retain, in solution, some of the interactions formed in the solid state.

1. Co-crystallization can improve the solubility and permeability of APIs without changing their structure: should they be considered as new chemical entities or simply physical mixtures?

Owing to the possibility of delivering insoluble drugs and enhancing their oral bioavailability, studies of the co-crystallization of APIs have seen a significant increase over the past decade. According to the ISI Web of Knowledge [25], 1970 papers with the word *cocrystal** (or *co-crystal**) in the title have been published since 2006, of which 711 deal with pharmaceutical co-crystals (Figure 1). Moreover, the most recent studies concerning API permeability modulation have suggested interesting applications *in vivo* and, as a consequence, over the past decade there has also been a rise in the number of patents on pharmaceutical co-crystals, which are characterized by the required features of novelty, non-obviousness/inventiveness, and utility. Both the US Food and Drug Administration (US FDA) and the European Medicines Agency (EMA) have delivered position documents regarding pharmaceutical co-crystals, attesting to a growing interest in their use for drug products and providing guidance to industry [12].

In co-crystals, the API and another substance are in intimate contact at the molecular level. Therefore, a question to address is whether a co-crystal should be defined as a physical mixture or as a new chemical entity (NCE) that requires as much safety and toxicology testing as any other such entity [26,27].

The FDA has elected to classify co-crystals as dissociable 'API–excipient' molecular complexes, where the co-former is the excipient. In its interpretation, the FDA has taken the position that a co-crystal can be treated as a drug product intermediate and not as a new active substance [27,28]. To gain product approval, the FDA requires the applicant to address two issues [12]: (i) APIs and excipients must completely dissociate before reaching the pharmacologically active site; and (ii) APIs and excipients must be in a neutral state (i.e., not interacting through ionic bonds). Use of the ΔpKa rule was suggested as a way to satisfy the second criterion.

The EMA published a reflection paper summarizing its position on the subject of pharmaceutical co-crystals, in that: (i) co-crystals have to be considered eligible for generic drug product applications in the same way as salts, solvates, and amorphous solids would be; and (ii) co-cyrstals cannot be considered as new active substances (NAS) unless they demonstrate different safety and efficacy profiles. Moreover, according to EMA reflection, co-crystals and salts share many conceptual similarities and, for this reason, similar principles for documentation should also be applied [12]. In line with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), in particular the quality guideline ICH-Q11 [29], commonly available chemicals used as co-formers in the co-crystal manufacture would be considered as reagents [30]. However, currently, no issue concerning pharmaceutical co-crystals is specifically addressed in ICH-Q guidelines [31]. The positions of the FDA and EMA on pharmaceutical co-crystals are very different and, therefore, there is a need for studies on new co-crystallized drug candidates to verify whether new advantages can be realized by their co-crystallization with appropriate co-former reactants. Currently, it can be concluded that 'in the absence of more stringent regulations, there should be only the usual drug development obstacles to overcome to receive the approval of a new drug product' [27]. Recently, Entresto™, a pharmaceutical co-crystal comprising monosodium sacubitril, disodium valsartan, and water, was approved by the FDA to treat chronic heart failure, whereas the pharmaceutical co-crystal

ertugliflozin–L-pyroglutamic acid is in late-stage clinical development for the treatment of diabetes [12].

1. Is it true that the pharmacological activity of APIs is not affected by co-crystallization?

The different positions taken by the FDA and the EMA on pharmaceutical co-crystals show how it is not yet clear whether a co-crystal should be defined as a physical mixture or as a NCE. To clarify these aspects, the properties (dissolution profile, permeation across intestinal cell monolayers, and oral bioavailability) of the poorly aqueous soluble API indomethacin and those of its co-crystals obtained with 2-hydroxy-4-methylpyridine (co-crystal 1), 2-methoxy-5-nitroaniline (co-crystal 2), and saccharin (co-crystal 3) were recently analyzed (Figure 2) [32]. The relevant novelty of this study was the analysis of permeation across an *in vitro* intestinal barrier model of the API, its co-crystals and parent physical mixtures. In general, Caco-2 cells or the parallel artificial membrane permeability assay (PAMPA) are frequently used to test drugs. The Caco-2 model is a monolayer of cells grown on a filter separating two stacked microwell plates and the permeability of the cells is determined after introduction of the drug on one side of the filter [5]. Given that changes in growth characteristics (monolayers/multilayers) are known to occur in the Caco-2 cell line, because of the loss of contact inhibition and polarization in transformed cells, human normal colonic epithelial NCM460 cells, an immortalized, nontransformed cell line, were used for the permeation analysis of indomethacin and its co-crystals. These cells are neither of tumour origin nor transfected and, therefore, they more closely retain the physiological characteristics of the normal human colon compared with pathologically or experimentally transformed cell lines. In addition, the transepithelial electrical resistance (TEER) developed by the NCM460 cells is within the range reported for intact sheets of human colonic mucosa [32].

Qualitative concordance was found between the API dissolution patterns in the 200 mM phosphate buffer and bioavailability: dissolution and bioavailability profiles of pure γ-indomethacin were similar to those of its physical mixtures with the co-crystallizing molecules; by contrast, both indomethacin solubility and its bioavailability were significantly increased by co-crystals 1 and 3, and slightly decreased by co-crystal 2.

Unexpected results were obtained from permeation studies: the γ-indomethacin crystals appeared able to maintain the integrity of the monolayer characterized by TEER values of around 180 $\Omega \cdot cm^2$. Incubation with co-crystal 3 maintained the integrity of the monolayer as well as the retention of its normal TEER value, inducing, at the same time, an increase in indomethacin permeation across the NCM460 cells with respect to pure γ-indomethacin crystals. Conversely, the parent physical mixture of indomethacin and saccharin induced a drastic decrease in the TEER value of the monolayer, whose cells appeared to completely lose their mutual contacts (Figures 2 and 3). Co-crystal 1 caused a drastic decrease in the TEER value of the monolayer, whose cells appeared completely separated. Also unexpectedly, incubation with the parent physical mixture 1 did not induce any changes in the monolayer integrity, as evidenced by the unaffected TEER value (Figures 2 and 3). Finally, no relevant differences between γ-indomethacin and co-crystal 2 or mixture 2 were observed (Figures 2 and 3) [32]. Currently, we do not know of any other published investigation showing or highlighting this peculiar behavior of drug co-crystals across cell monolayers mimicking biological barriers.

1. Concluding remarks

An up-to-date analysis has brought to light an intriguing aspect of the biological properties of pharmaceutical co-crystals, which can be drastically different from those of their parent physical mixtures. This result suggests that the molecules constituting the co-crystal can retain, in solution, some of the intermolecular interactions formed in the solid state. If this is the case, the resulting supramolecular complexes, although transient, could interact with proteins by means of mechanisms different from those of the molecules resulting from pure crystal dissolution. Indeed, the protein affinity of APIs strongly depends on specific and concerted weak interactions. Therefore, further investigations would appear necessary to evaluate potential new applications as well as the potentially damaging effects of pharmaceutical co-crystals.

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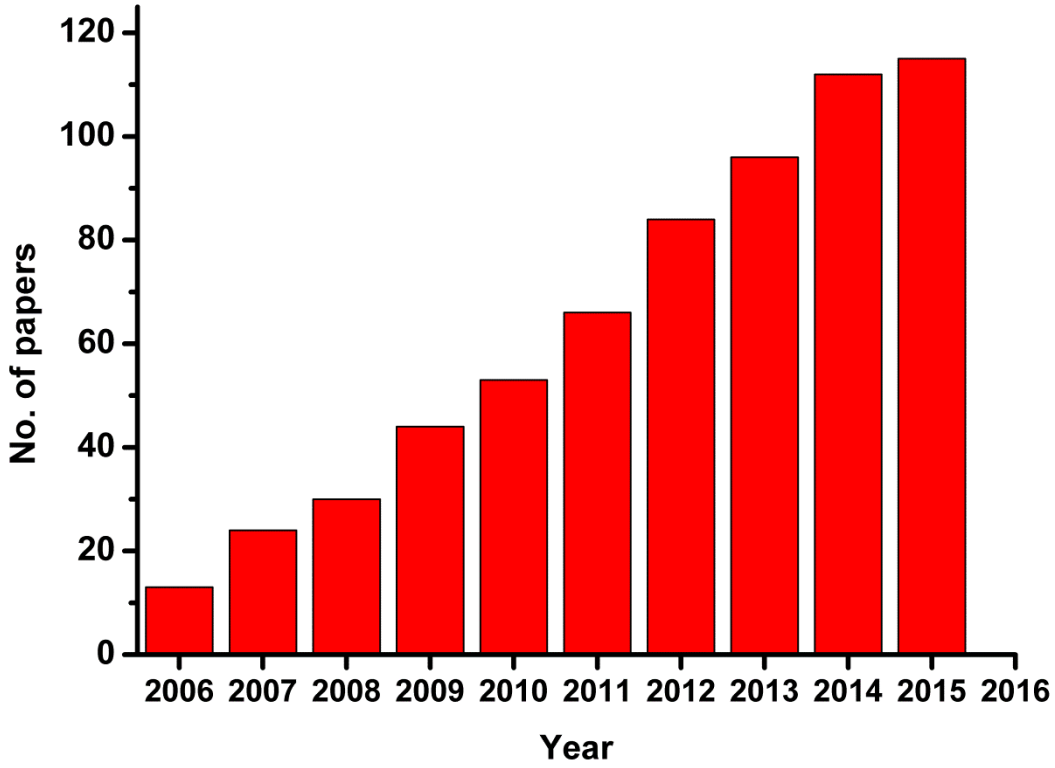
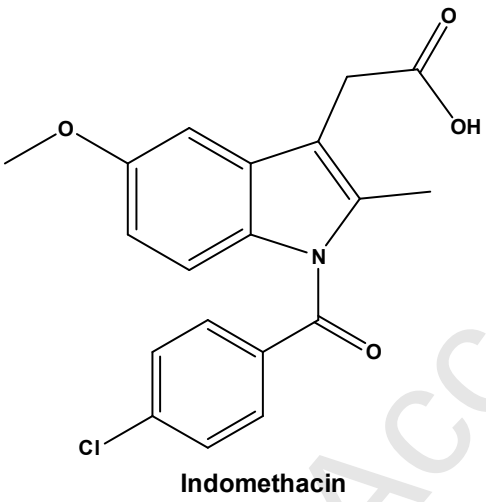
Figure 1 Increase in the number of papers published on pharmaceutical co-crystals, expressed as the number of entries per publication year, for the period 2006–2015. Source: ISI Web of Knowledge, August 2016.

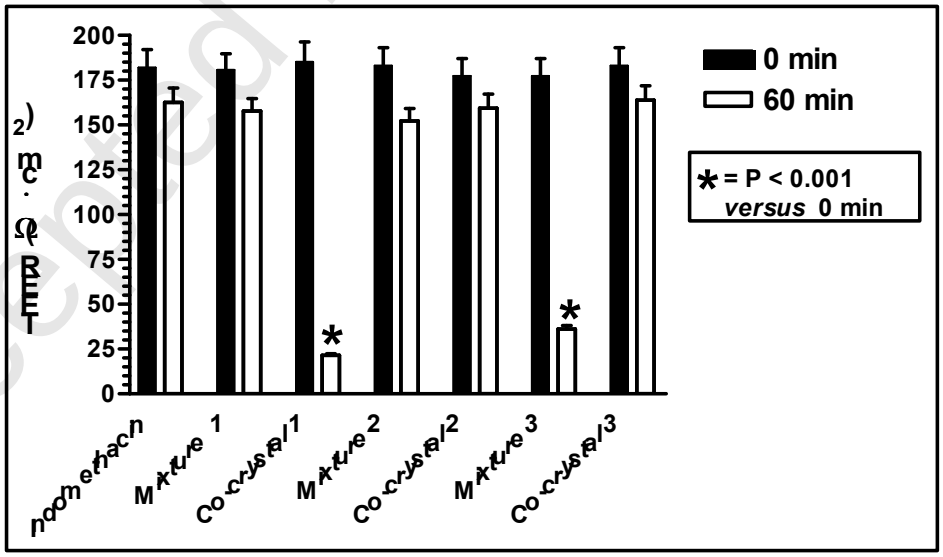
Figure 2 Chemical formulas of indomethacin and the co-formers 2-hydroxy-4-methylpyridine (in its keto form), 2-methoxy-5- nitroaniline, and saccharin in co-crystals or physical mixtures 1, 2, and 3, respectively [32]. The inset details the transepithelial electrical resistance (TEER) values of NCM460 monolayers before and after 1 h of incubation with indomethacin, its co-crystals, or the parent physical mixtures [32].

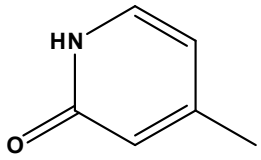
Figure 3 Effects on NCM460 monolayers after 1 h of incubation with indomethacin co-crystals or their parent physical mixtures [32]. Co-crystal 1 induced the cells to lose completely their mutual contacts, so that they appeared completely separated; the incubation with the parent physical mixture 1 did not induce any changes in the integrity of the monolayer, as also observed for co-crystal 2 and its parent physical mixture. Physical mixture 3 induced the cells to lose completely their mutual contacts, so that they appeared completely separated; incubation with the parent co-crystal 3 maintained the integrity of the monolayer.





Indomethacin





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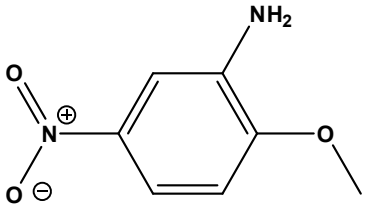
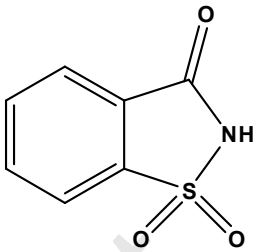


2-methoxy-5-nitroaniline (2)



Saccharin (3)