Polymorphs, Co-crystals, Salts and their significance

1 Polymorphs

Any drug is composed of two components. The first is the actual Active Pharmaceutical Ingredient (API), which is the central ingredient. The second component is known as an excipient. This refers to the substance present along with the API in the final drug. These are used to formulate the active agent in order to stabilize, provide bulk and size, reduce bitterness etc. If the drug is solid (tablet, granules etc.), the excipients will be solid. If it is in syrup form, then the excipient will be the liquid that has been used. Thus, excipients are the inactive or inert substances present inside a drug while the API is the chemically active substance, which is meant to produce the desired effect in the body once taken by an individual.

An active pharmaceutical ingredient is defined by The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) as

"*any substance or mixture of substances intended to be used in the manufacture of a drug product and that, when used in the production of a drug, becomes an active ingredient in the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease or to affect the structure and function of the body*."

The purpose of APIs according to the FDA is to cause 'pharmacological activity or other direct effects in the diagnosis, cure, mitigation, treatment or prevention of disease or to affect the structure and function of the human body'.

Solid state modifications of Active Pharmaceutical ingredients (APIs) (Figure 1) provide a means to alter their physical properties and certain biological properties thereby optimizing the desired therapeutic potential of the drug. It is well appreciated that solid forms of APIs offer significant advantages over other forms and therefore is generally more desired in pharmaceutical industry. However, the solid forms obtained from initial drug discovery efforts often suffer from limitations with respect to their physical and chemical properties such as solubility, melting point, chemical interaction, stability, bioavailability etc. Data shows that about 40% of the molecules which are identified as possible drug candidates suffer from one or more of these limitations. Significantly, it is estimated that nearly 80–90% of drug candidates which become part of the R&D pipeline have low solubility problem. Hence, inspite of attractive pharmacological actions, these agents can not demonstrate therapeutic benefits in patients. This is alarming and is one of the main reasons for the high failure rate of drug candidates in clinical trials and the rising cost of drug development (1).

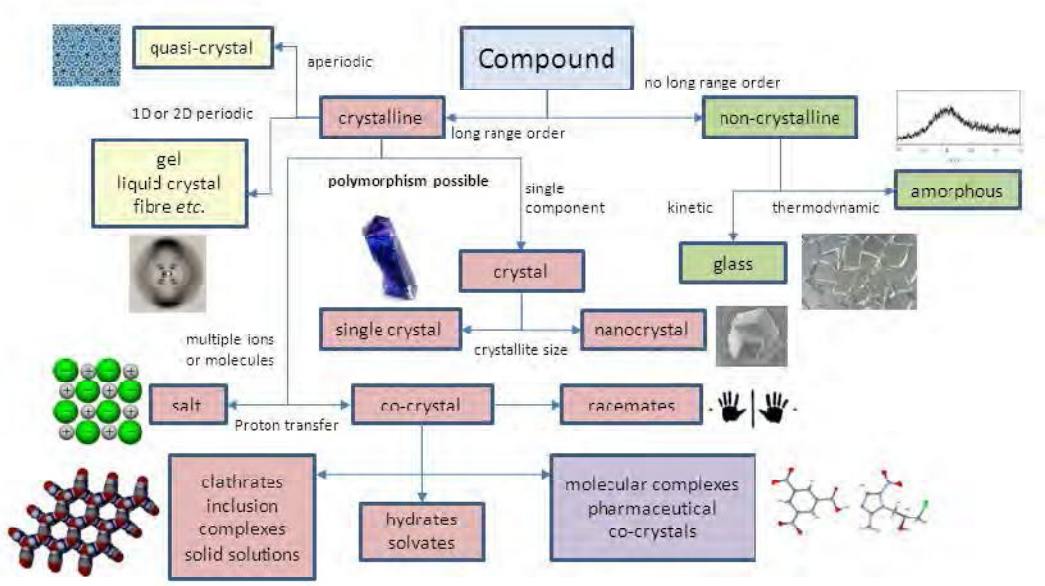


Figure 1: Different solid forms of an API[adapted from (2)]

Other challenges in developing solid medicines arise due to either lack of efficacy, possible side effects etc. Jean-Paul Garnier, former CEO of GlaxoSmithKline had once stated, “About 50 % of drug candidates that

enter clinical trials fail due to efficacy and safety concerns, and the remaining 40% fizzle due to patent concerns and issues like solubility and drug interaction" (3, 4). Therefore, efforts to try and manipulate the desired physicochemical properties of such initially identified drug candidates has gained momentum during drug development. Such improvements in physicochemical properties of many chemical substances can be achieved by altering the physical forms of a given compound through the formation of alternate polymorphs, solvates and/or hydrates, salts, co-crystals etc. Some of the physicochemical properties that can be altered/ improved upon, through preparation of these alternate forms are summarized in Table 1.

Table 1: Properties that can be altered by choosing different polymorphic forms, solvates/hydrates, co-crystals and salt forms of a compound. [adapted from (5)]

- Thermodynamic properties:
  - Melting and sublimation temperature and vapour pressure;
  - Enthalpy, entropy and heat capacity;
  - Free energy, chemical potential and solubility.
- Kinetic properties:
  - Dissolution rate;
  - Rates of solid state reactions;
  - Physical/chemical stability;
  - Rate of nucleation/crystal growth
- Packing properties:
  - Molar volume and density;
  - Conductivity, electrical and thermal;
  - Refractive index
  - Particle morphology
  - Hygroscopic nature
  - Colour
- Surface properties
  - Surface free energy
  - Interfacial tension
  - Habit
- Mechanical properties:
  - Hardness
  - Tensile strength
  - Compactibility and tableting
  - Handling, filtration, flow and blending
    - Cleavage
- Biological properties
  - Pharmacokinetics
  - Bioavailability

Developing alternative forms of a compound such as polymorphs, co-crystals and salts therefore provide opportunities to overcome many of these problems and have become invaluable tools in drug discovery and development.

Chemical entities can exist in multiple physical forms. Crystallization is the spontaneous arrangement of the particles in to a repetitive orderly array, i.e., regular geometric pattern (6). For solid substances, the term pure crystalline implies an ideal crystal in which the structural units, the unit cells, are repeated regularly and indefinitely in three dimensional spaces. Crystalline substances, including drug substances can either exist in only one crystalline form or may exist as more than one polymorphs and/or solvates. The term polymorph has been derived from a Greek word "poly", which means "many", and "morph" implying "form". Hence, polymorphism refers to existence of different structural forms of a chemical substance. The phenomenon of polymorphism was first observed towards the end of the eighteenth century by Klaproth (7) who was working with the aragonite and calcite solid phases of calcium carbonate; however, credit for recognizing this incredible property of solid substances is often ascribed to Mitscherlich (8) for his work on isomorphous metallic sulfates. Crystalline polymorphs have the same chemical composition but differ in their internal crystal structures and, therefore, in their physical and certain consequent biological properties. The different crystal structure in polymorphs arises is a result of their crystallization in different crystal packing arrangements and/or different conformations, when crystallizing out from various solvents. The occurrence of polymorphism is quite common among organic molecules (9). Polymorphism in crystalline solid can therefore be also defined as the phenomenon wherein different substances exist with the same chemical composition but having different lattice structure and/or different molecular composition (10).

Polymorphism in molecular crystals is generally divided into two categories: conformational and packing polymorphism. In the former, conformationally flexible molecules adopt more than one conformation in the solid state (11).

Examples of drugs exhibit conformational polymorphism are the anti-depressant venlafaxine hydrochloride (Effexor) wherein the orientation of a methoxy group is opposite to each other (12) and the antipsychotic olanzapine (Zyprexa) (13), wherein one of the two conformers the piperazinyl ring is in a chair conformation while in the other it acquires a boat conformation. Packing polymorphism arises from different possible packing arrangements of conformationally rigid molecules. An example of packing polymorphism is the case of 3-Acetylcoumarin, wherein the Form-A is arranged in head-to-head stacking, while the Form-B is arranged in a head-to-tail stacking (14). Another form of polymorphism exists in cases of solids wherein the molecules exist in different configurations such as geometric isomers and tautomers, and this phenomenon is known as configurational polymorphism. Desmotropy refers to the phenomenon of crystallization of molecules in two different tautomeric forms (15) and is used to describe alternate tautomeric forms (16) or tautomerizational polymorphism (17). A well-known example of a desmotropic system is the tetrazole-containing antihypertensive drug irbesartan (Avapro) (18); its two phases are stable in the crystalline state, but the tautomers exist as equilibrium mixtures in solution.

Polymorphism has also been considered to be of two types of systems, viz. monotropic systems and enantiotropic systems. In the monotropic system, only one polymorphic form is stable at the temperatures below the melting point of the solid drug. However, in an enantiotropic system, no such phenomenon is observed, although one or more forms may be more stable than others irrespective of any noticeable temperature pattern. Among such polymorphs, there exists a temperature at which two or more such forms have the same free energy, and this temperature is termed as the transition temperature (19, 20).

1.1 Hydrates and Solvates

Crystalline solids that involve the inclusion or incorporation of solvent molecules in the crystal lattice are known as solvates (14), pseudopolymorphs (21), or solvatomorphs (22), although the first term is

widely accepted. Solvates, in which the solvent molecule is water, are known as hydrates. Water's small size and ability to serve as both a hydrogen bond donor and acceptor make it likely to be incorporated in many locations within the lattice either as space fillers or as a stabilizing force. Generally removal departure of this incorporated water will eventually lead to the collapse of the crystal structure (23). The ease with which hydrates are formed can be appreciated from the fact that the Cambridge Structural Database (CSD) contains over 500,000 entries of organic and organo-metallic crystal structures, of which 70,484 structures contain water in one form or another. (24). Desiraju (25) has investigated a test-set of 411 hydrate crystal structures and concluded that although manifold factors play a role in hydrate formation, hydrate formation is more likely to happen when the host molecule shows an imbalance between the number of hydrogen bond donor and acceptor groups. Gorbitz and Hersleth (26) have discussed the increase in recent years in the occurrence of solvated crystal forms, including hydrates and mixed solvates (i.e., water and an organic solvent), and concluded that this increase is due to the increasing complexity of modern molecules and their reduced packing efficiency. Infantes and Motherwell (27) investigated 1516 organic hydrate structures of the CSD with a view to classifying water clusters. They found that the majority of structures (61%) have distinct finite chain motifs, and of these usually only two water molecules hydrogen bond to each other, representing the whole chain. Infinite chain motifs at almost 20% of the samples are the second most common pattern. It has generally found that hydrate formation is most frequent when charged groups are present. Haynes et al (28, 29) have found that presence of doubly charged counter ions increase the possibility of water incorporation even more, with the proportion forming hydrates greater than 50%. According to their findings, the only triply charged counter ion (PO_4^{3-}) studied, forms hydrates as the only known crystal forms. The authors also found out that in the $-NH^+$ containing subgroup of pharmaceutical salts, hydrate formation was found to decrease with increasing ion size in the halide series, while secondary and tertiary amine salts tend to form fewer hydrates. Based on all the studies, it is

possible to do a qualitative prediction of the likelihood of hydrate formation in salts.

Hydrates can be generally classified into two categories, the stoichiometric ones with a fixed ratio of water to host compound, and the non-stoichiometric ones, in which the water contents can reversibly change due to changing outer conditions without a major change of the crystal structure. Although it is difficult to determine from the crystal structure whether a hydrated crystal form is stoichiometric or non-stoichiometric, certain structural characteristics are indicative of a tendency to belong to one group or the other. In stoichiometric hydrates, water molecules are situated in isolated sites as a component of the molecular network and may form hydrogen bonds either to the host molecule or to other independent water molecules. For these compounds water cannot be easily removed from the positions it occupies and its removal results in the destruction of the crystal architecture. One example of such a hydrate is the monoclinic 5-azauracil monohydrate (Figure 2) (30). The water forms hydrogen bonds only to the host molecules, acting both as donor and as an acceptor, while the next crystal plane is shifted so that the water molecules are embedded in a matrix of host molecules.

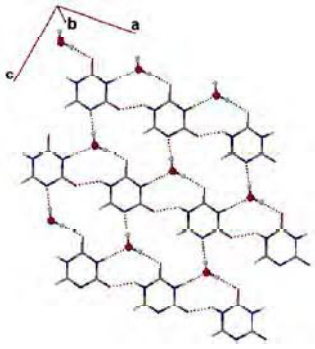


Figure 2: Crystal packing in 5-azauracil monohydrate in which the water molecules occupy isolated sites (41)

The water can also be located in discrete pockets forming clusters of different size depending on the number of water molecules involved. One example is a dipyridylbis(urea) compound, which incorporates one molecule

of water per 6 molecules of host (31). The water molecules are located in discrete cavities enclosed by host molecules.

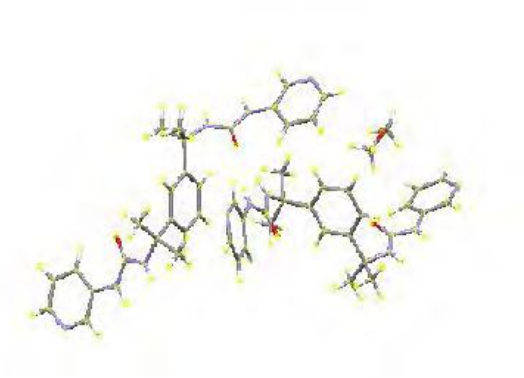


Figure 3: dipyridylbis(urea) hydrate in which one molecule of water present per 6 molecules of host

Channel hydrates represent another large group of structurally related hydrate crystal forms. They incorporate water in channels or networks of channels throughout the crystal. Depending on the extent of hydrogen bonding and the size of the channels, these hydrates can again be either stoichiometric or non-stoichiometric. Thymine is such an example which forms a monohydrate (32) with the water molecules located in such channels along the c-axis. Perrier and Byrn investigated the dehydration behaviour of thymine monohydrate and found that the dehydration proceeds along these channels (33), accompanied with the destruction of the mother crystal into a multi-crystalline powder. Other examples of channel hydrates formation are in caffeine 0.8 hydrate (34) and theophylline monohydrate (35). Another example of a channel hydrate is given in the co-crystal of pamoic acid and piperazine, which forms a trihydrate (36). Interestingly, it was found, two of the water molecules are located in helical channels in the structure surrounded by three host helices. The remaining water molecule links the host helices and is situated in isolated sites. The helical water can easily leave the crystal lattice through the channels without the overall structure to collapse, as was shown by the authors through drying and rehydrating. This is a characteristic feature of a non-stoichiometric hydrate. Larger channels are more likely to be a structural feature of non-

stoichiometric hydrates, as the water can diffuse more easily through these channels without the general structure changing. Cephalexin dehydrate (37) shows such large channels along the a-axis. The water molecules are mostly disordered and Stephenson et al. (38) proved that this hydrate behaves as a non-stoichiometric material. Saha and Nangia (39) have described another case of channel hydrate formed by dibromophloroglucinol. It has been postulated that depending on the relative humidity, loosely bound water can get in (hydration) or out (dehydration) of the channels, which may lead to expansion or contraction of the crystal lattice, respectively. In contrast, there are also channel (or variable) hydrates, such as the antitumor drug topotecan hydrochloride (Hycamtin) that can accommodate additional guest water molecules with a minimal effect on the cell dimensions of the crystal lattice (40).

Several well-known pharmaceutical products are available in which the drug substance is present as a hydrate such as alendronate sodium trihydrate (Fosamax), amoxicillin trihydrate (Amoxil), atorvastatin calcium trihydrate (Lipitor), and pantoprazole sodium sesquihydrate (Protonix), to name a few. Conversely, there are cases in which a hydrate exists but the anhydrate has been chosen because of improved physical properties, processability, and/or stability. Examples of anhydrate commercial drug products include mometasone furoate (Elocon), pazopanib hydrochloride (Votrient), and sertraline hydrochloride (Zoloft), although each of the drugs exists as hydrates also.

When a solvent other than water is present in the crystal lattice, they are called solvates. Solvates, like hydrates, can contain either a stoichiometric or a non-stoichiometric amount of solvent in the crystal lattice. Generally, desolvation of a stoichiometric solvate results in either a disordered non-crystalline state or a different crystalline form (41). In non-stoichiometric solvates, solvent molecules are accommodated in the structure to fill intermolecular voids. Variations in pressure, temperature, and/or humidity may result in solvent exchange, loss, or uptake in case of non-stoichiometric solvates. Solvates other than hydrates are not normally selected for development owing to risk of desolvation, toxicity concerns with organic

solvents. Nonetheless, there are marketed drug products that contain solvates such as darunavir ethanolate (Prezista), indinavir sulfate ethanolate (Crixivan), warfarin sodium isopropanol solvate (Coumadin) and dapagliflozin propanediol monohydrate (Forxiga). Solvates can also be polymorphic, as exemplified by olanzapine (13) and nitrofurantoin (42), which has three dihydrate and two monohydrate forms, respectively. In other cases, solvates can have different stoichiometries in their crystal lattices, which may result in varying packing arrangements.

Polymorphism and solid-state solvation are common among chiral drugs also. Polymorphism has been observed among individual enantiomers for example, of carvoxime (43) and nitrendipine (44) as well as by racemates, for example, mandelic acid (45). The existence of polymorphism of a chiral drug will not only affect its pharmaceutically relevant properties, but can also result in inter-conversion between the different types of racemates, for e.g. as seen in sodium ibuprofen (46). Certain chiral drugs can also form solvates. In chiral systems, in certain cases, the racemic compound and the corresponding enantiomer undergo different degrees of solvation under given conditions. For example, enantiomeric histidine hydrochloride forms a monohydrate when crystallized from water, whereas racemic histidine hydrochloride forms a dehydrate (47). Similarly, diastereomeric pairs also form solvates with different degrees of solvation that can affect their individual solubilities.

1.2 Preparation of polymorphs and solvates/hydrates

Several techniques have been developed for the preparation of polymorphs and solvates/hydrates. However, it is difficult to identify one or two standardized processes for obtaining these crystal forms, nor is it possible to select one over the other. This is primarily due to the inherent unpredictability in the formation as well as which form will be obtained, if any. The more common and widely used techniques are enumerated in the following table 2.

Table 2: Methods of preparation of polymorphs/solvates/hydrates

Sl. No.	Methods of preparing polymorphs
1.	Crystallization from a single or mixed solvents
2.	Thermal activation of the solid substrates
3.	Crystallization from the melt
4.	Desolvation/dehydration of solvates/hydrates by heat or by re-slurrying
5.	Crystallization in nano-confined structures
6.	Seeding/pseudoseeding
7.	Solution mediated polymorphic transformation/slurry conversion method
8.	Solid-state polymorphic transformation
9.	Mechanical activation of the solid substance
10.	Crystallization in a capillary tube
11.	Exposure to vapor at high or low humidity
12.	Exposure to organic solvents
13.	Directed crystallization on molecular substrates
14.	Crystallization in the presence of tailor-made additives
15.	Laser induced crystallization
16.	Crystallization from a supercritical fluid

Some of the more prevalent techniques among those above, are discussed briefly below:

1.2.1 Crystallization from a single or mixed solvent

One of the most commonly used techniques involves crystallizing the substances from a single or mixture of solvents via either cooling crystallization, or evaporation, or by addition of suitable anti-solvents. Selection of appropriate solvents as well as anti-solvents, wherever necessary, is challenging and is one of the most crucial steps. Several investigators have tried to classify solvents based on their various properties in order to come up with some criterion for selecting solvents for crystallization. Gu et al., for example, have summarized the

physicochemical properties of 96 solvents based on solvent parameters such as hydrogen-bond acceptor/donor propensity, polarity/dipolarity, dipole moment, dielectric constant, etc. (48). Additionally, heating and cooling rates, crystallization temperature, evaporation rate, the degree of supersaturation, the rate of agitation, pH of the media, length of time, physical intervention like scratching etc. are some of the variables which can affect the crystallization process and thus, whether a crystal will be formed and if so, the nature of the crystal formed.

1.2.2 Thermal activation of the solid substrates

Any two polymorphs can be either monotropic or enantiotropic. A polymorph is monotropic when it is stable over the entire temperature range. In the case of enantiotropic system, one form is stable below the transition temperature, and the other form is stable above the transition temperature.

In an enantiotropic system, the form which is metastable at room temperature can be obtained by heating the thermodynamically stable form above the transition temperature. On the other hand, in a monotropic system, the stable form at room temperature can be obtained by heating the metastable form at any temperature. The rate of transformation can be facilitated by heating the metastable form at high temperature. Starting with the stable form, it is impossible to obtain the metastable form by thermal activation method in a monotropic system. For example, flufenamic acid form I which is metastable form at room temperature can be obtained easily by heating flufenamic acid form III above 105 °C (49).

1.2.3 Crystallization from the melt

Crystallization from the melt is similar to crystallization from amorphous material. Since amorphous is thermodynamically unstable, amorphous materials tend to crystallize quickly. Depending on the external stress applied to the melt, crystallization from the melt generates different

polymorphic forms with different kinetics and mechanism (50, 51). It is reported that crystallization from the melt is the only method to generate metastable forms II (or ß) and III (or γ) of nifedipine, (50) as well as the metastable form III of acetaminophen (52).

1.2.4 Crystallization in nano-confined structures

In this process, crystals are grown in nano-sized pores in order to reverse the relative thermodynamic stability between polymorphs (53). This technique is often used to improve the physical stability of the metastable polymorph for e.g. in case of acetaminophen form III (54).

1.2.5 Seeding

Another commonly used technique is seeding which facilitates the crystallization process via heterogeneous or secondary nucleation. Seed crystals can exactly be of the same form as that of interest, a pseudo-seed that is structurally compatible to the desired form but not exactly the same, or one that is not related to the form of interest. Seeding with a desired polymorphic form is a commonly used technique for controlling polymorphic form during industrial crystallization. Pseudo-seeding is often used when the seed of interest is not available (55). It is usually expected that the desired polymorphic form will crystallize out depending on the seed being used although sometimes undesired forms have also been obtained.

1.2.6 Solution mediated polymorphic transformation/slurry conversion method

Solution mediated polymorphic transformation (SMPT) is a fast, easy, and reliable method to obtain the stable polymorph. In this process, the metastable form is first dissolved followed by nucleation/ growth of another form which is more stable than the previous form. The thermodynamic driving force for SMPT is the solubility difference between polymorphs. The kinetic factors govern the rate of solution-mediated polymorphic transformation. Additionally, particle size can also play an important role in determining the rate of SMPT. When

specific solvates/hydrates are needed, solvent-free crystals can be slurried in the given solvent for an extend period of time.

Using some of the techniques described above, attempts have been made and are continuing to be made to alter the physico-chemical properties of drug substances by preparing alternate polymorphic forms. Thus, attempts have been made to improve the aqueous solubility of the anti-diabetes drug Gliclazide, 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-tosylurea (56). Three polymorphs namely, Form-I, II and III and an amorphous powder were produced from different solvents and solvent mixtures. However, there was no remarkable difference in their aqueous solubility. Similarly, the polymorphic behavior of deflazacort (DEF), a glucocorticoid discovered >40 years ago, has been investigated in details (57). Using different methods described above, three different polymorphic forms of the drug was prepared: a crystalline (DEF-1); a hydrated X-ray amorphous (DEF-t-bw) and an anhydrous amorphous phase (DEF-g) obtained by manually grinding DEF-1. After studying the *in vitro* and *in vivo* dissolution rates and the stability of the different forms, the authors concluded that deflazacort (DEF) is a glucorticoid with low tendency to exhibit different crystalline forms and that DEF-t-bw has no advantages over DEF-1 in terms of solubility, dissolution rates and solid-state stability.

The transformation between the two known crystal forms of (3aRS, 4RS, 7RS, 7aSR)-2-(Tricyclo[3.3.1.13,7]decan-1-yl)-4,5,6,7-tetrahydro-4,7-eposyisoindoline-1,3-dione (SU2162) - a novel anti-cancer compound has also been studied (58). It was found that both the two known crystal forms are interchangeable and the transformation can be carried out by dissolving into different organic solvents such as acetone and ethyl acetate. It was concluded that organic solvents have significant influences on the two crystal forms. Crystal form I showed better thermal stability than crystal form II.

The feasibility of applying ultraviolet-visible and short-wave near-infrared diffuse reflectance spectroscopy (UV–vis–SWNIR DRS) coupled

with chemometrics in changing the crystal forms of drug polymorphs was investigated by studying three polymorphs and one mixed crystal of cimetidine (84). Three polymorphic forms (A, B and D) and a mixed crystal (M1) of cimetidine, prepared using different crystallization conditions, were characterized by microscopy, X-ray powder diffraction (XRPD) and infrared spectroscopy (IR), whereby the Form B was found to be the most suitable pharmaceutically.

2 Co-crystals

Aakeröy and Salmon (60) defines co-crystal as a homogeneous crystalline solid that contains stoichiometric amounts of discrete neutral molecular species that are solids under ambient conditions. This definition excludes solvatomorphs from being termed co-crystals, as the solvent of crystallization (i.e., water or an organic solvent) would be a liquid under ordinary conditions and not a solid. Co-crystals vary in their physical properties such as habit, bulk density, solubility, compressability, friability, melting point, hygroscopicity and dissolution rate.

According to Aakeröy, co-crystal design is usually targeted at crystalline, structurally homogeneous materials in which the two or more component molecules are present in a well-defined stoichiometric ratio. This also excludes non-stoichiometric inclusion compounds such as urea channel clathrates and solid solutions from being considered as co-crystals (61).
A major point of contention and research is the distinction between a salt form and a co-crystal. The most basic distinction between a salt and a co-crystal is that a proton is completely transferred in a salt, and not transferred at all in a co-crystal.

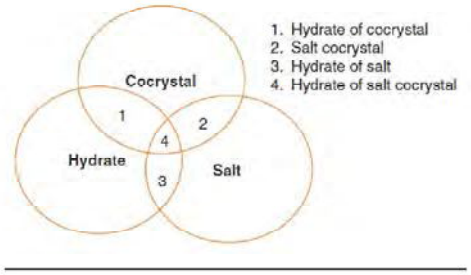


Figure 4: Relationship among co-crystal, hydrate and salt. (62)

For acidic and basic coformers, it is possible to estimate the degree of transference by considering the difference in pKa values, wherein ΔpKa greater than 3 would be characteristic of a salt, and ΔpKa less than 2 would be characteristic of a co-crystal. It is difficult to predict beforehand the outcome of a co-crystallization system where the ΔpKa value lies between 2 and 3, as either a salt or a co-crystal could be obtained (63). THE USFDA has also accepted this classification for co-crystals.

The ability to form co-crystals has given a new tool in the solid-state modification, and the pharmaceutical industry is making serious efforts in improving its feasibility and utility. The formation of API co-crystals offers a new method to modify the physical and chemical properties of drugs without changing their chemical nature. Co-crystallization has provided pharmaceutical industry advantages in at least two ways as compared to salt formation:

(1) According to the concept of co-crystallization, all types of molecules can form co-crystals, including weakly ionizable and non-ionizable APIs, which is considered to be a better method in optimization of the physical properties because salt formation is either limited or has no scope at all in such APIs (64);

(2) In case of salt formation due to toxicological reasons only 12 or so acidic or basic counter counter-ions are explored in a typical API salt screen, whereas in case of co-crystal screening there are large number of potential co-crystal co-formers which are free from toxicological constraints. The US Food and Drug Administration maintains a list of

substances (e.g., FDA's GRAS list–a list of substances "generally recognized as safe") which numbers in the thousands and can be used as potential co-formers for co-crystal formations.

Polymorphism is also widely prevalent in co-crystals. Thus, five polymorphic forms have been reported for Furosemide :Nicotinamide 1:1 co-crystal (65), and three each for Barbituric acid–Urea 1:1 co-crystal (66), Pimelic acid–4,4′-Bipyridineco-crystal (67) and Ethenzamide–Gentisic acid1:1 co-crystal (68).

2.1 Crystal habit modifiers

The synthesis of inorganic and organic materials with a specific size and morphology has recently received much attention in the material science research area. Of the two, morphology control or morphogenesis is more important for the chemical industry. Many routes have been reported to control the crystal growth and eventually modify the morphology of the crystals. In one such technique of crystal-habit modification, crystals are grown in the presence of naturally occurring soluble additives, which usually adsorb or bind to the crystal faces and influence the crystal growth or morphology.

The crystal-habit modifiers may be of a very diverse nature, such as multivalent cations, complexes, surface active agents, soluble polymers, biologically active macromolecules, fine particles of sparingly soluble salts, and so on. These crystal modifiers often adsorb selectively on to different crystal faces and retard their growth rates, thereby influencing the final morphology of the crystals. The use of crystal modifiers involves use of inorganic or organic additives to control the nucleation, growth, and alignment of inorganic and organic crystals.

2.2 Crystal habit modifications

The morphology of a crystal depends on the growth rates of the different crystallographic faces. Some faces grow very fast and have little or no effect

on the growth form; while slow growing faces have more influence. The growth of a given face is governed by the crystal structure and defects on one hand and by the environmental conditions on the other (69).

The crystals may grow rapidly, or be stunted, in one direction; thus an elongated growth of a prismatic habit gives a needle shaped crystal (acicular habit) and a stunted growth gives a flat plate-like crystal (platy or flaky habit). The relative growths of the faces of a crystal can be altered and often controlled by a number of factors. Rapid crystallization, produced by the sudden cooling or seeding of a supersaturated solution, may result in the formation of needle crystals. The growth of a crystal may be stunted in certain directions due to presence of impurities in the crystallizing solution. It is well known that change of solvent often changes the crystal habit.

Using water-soluble polymers as crystal modifiers for controlled crystallization has been widely used for controlling and designing the architectures of inorganic materials. Investigators have used different double hydrophilic block copolymers, such as poly(ethylene glycol)-block-poly(methacrylic acid), to control the morphology of a number of inorganic salts, namely, $CaCO_3$, $BaCO_3$, $CdCO_3$, $MnCO_3$, $PbCO_3$. Investigators have also used poly(vinyl alcohol) (PVA), agar, gelatin, and pectin-based gel matrices to control the morphology of inorganic crystals such as PbI_2, AgI, $Ag_2Cr_2O_7$, $PbSO_4$, $PbCl_2$, and so forth. However, use of these polymers for crystal habit modifications of organic molecules has not been so widely reported.

Non-ionic triblock copolymer surfactants such as hydrophilic poly(ethylene oxide) block and of the hydrophobic poly(propylene oxide) block - Pluronics L44NF (BASF), and Pluronics P-123 (BASF) were used as additives to study their effect on the crystal habit of 5,6-dimethyl-1H-benzotria-zole (5,6-DMBTA), a compound useful as inhibitor of copper corrosion. (70). The optical microscope images of 5,6-DMBTA crystals obtained in the absence of any non-ionic surfactant depict a needle or acicular habit. In the presence of the three different non-ionic surfactants, two different crystal habits were obtained: shorter needles (Figs. 5b, c, and e) and platelets (Fig. 5d). This

indicates that non-ionic block copolymers affect the growth of the individual crystal faces of 5,6-DMBTA differently.

However, X-ray diffraction, differential scanning calorimetry, and thermal gravimetric analysis showed that none of the surfactant was incorporated into the crystal lattice.

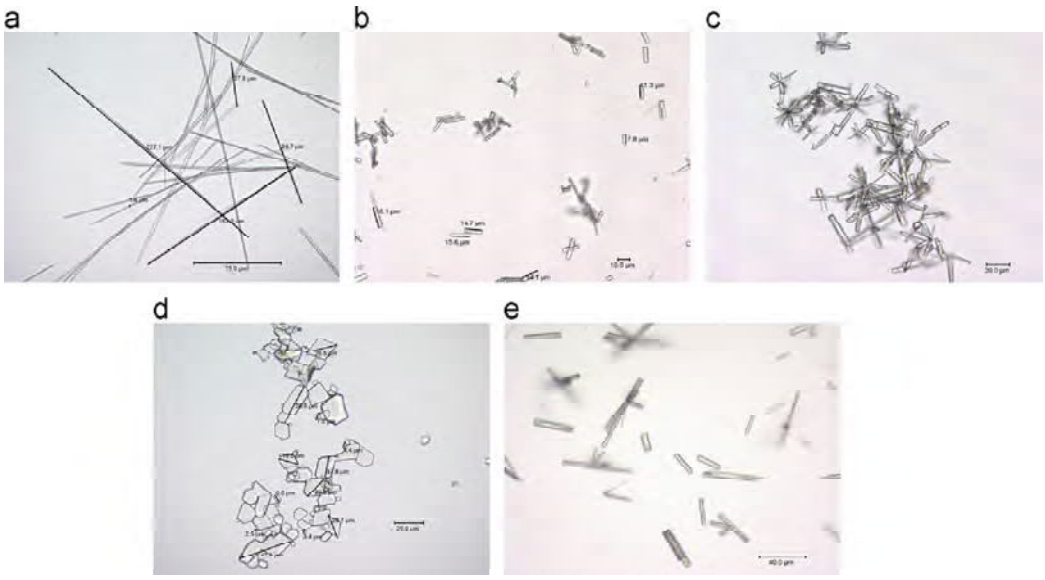


Figure 5: Optical microscopic images of 5,6-DMBTA crystals obtained by recrystallization with (a) the absence of additive, (b) Pluronics F-127, (c) Pluronics L44 NF, (d) Pluronics P-123, and (e) poly(ethylene oxide). Magnification is 40X [70].

Daisuke Iohara et. al. describes the use of hydroxybutyl-β-cyclodextrin (HB-β-CD) or 2,6-di-O-methyl-β-cyclodextrin (DM-β-CD) for changing the crystal habit of acetyl salicylic acid. The habitat changed to needle crystals that elongated along the crystallographic b-axis, Figure 6 (71).

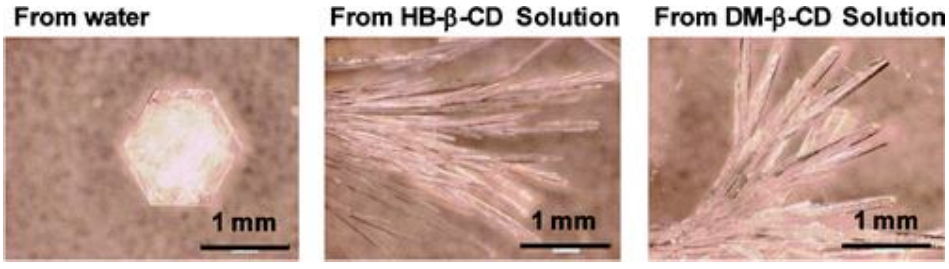


Figure 6: Crystals obtained in absence and presence of HB-β-CD and DM-β-CD [71].

Powerful ultrasound has been used for cooling crystallization to control and modify the particle size and crystal habit of an active pharmaceutical ingredient, Phenacetin (72). The crystal habit of re-crystallized Phenacetin is modified substantially and shows an elliptic shape. Re-crystallized Phenacetin was also found to show an enhanced dissolution rate compared with the original sample.

The crystal habit of Nifedipine polymorph (Nif) was modified by the use of Polysorbate-80 (T-80) (73). Various concentration of Polysorbate-80 was used. The dissolution rate order of the re-crystallized Nif habits was in the order of: Nif-D (Nif with 0.6 % v/v T-80) >Nif-C (Nif with 0.4 % v/v T-80) >Nif-B (Nif with 0.2 % v/v T-80) >Nif-A (plain Nif). Although such attempts to modify the crystal morphology of organic compounds using a few GRAS materials have been reported, use of different double hydrophilic polymers such as polyethylene glycols (PEG), gel matrices such as polyvinyl alcohols (PVA) & polyvinyl pyrrolidones have not been reported for modification of crystal habits.

3 Salts

A salt refers to a multi-component system where protons are transferred from acid to base in the ionic state (74).

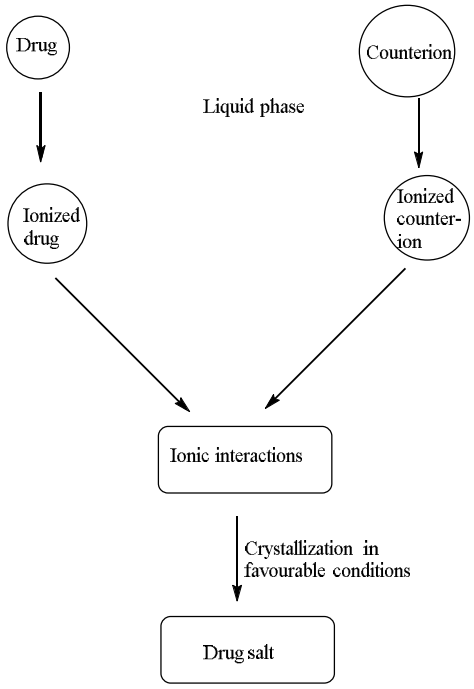


Figure 7: Diagrammatic representation of salt formation**.**

In case an API is ionizable, preparation of its salts using pharmaceutically acceptable acids or bases is a common strategy to modulate its various physical properties such as solubility or dissolution rate, to increase chemical stability, to improve bioavailability or to enhance manufacturability. It is estimated that more than 50% drugs approved are administered as salts. The selection strategy for a new drug candidate in the salt form involves the selection of chemical forms of salts, and the selection of physical forms of salts.

The various advantages of salt formation are provided below [adapted from (75)]

- Altered solubility or dissolution rate of drugs;
- Controlled release dosage forms;
- Targeted drug delivery of dosage forms;
- Improved thermal stability;
- Improved hydrolytic stability;
- Improved photostability;
- Reduced hygroscopicity;
- Improved permeability;

- Improved organoleptic properties (e.g. better taste or reduced bitterness);
- Improved drug efficacy [the pharmacological actions of counterions can mitigate side effects. Thus, for e.g. dimenhydrinate (salt of diphenyl hydramine and 8-chloro theophylline) reduces the soporific effect of diphenyl hydramine because theophylline acts as a stimulant;
- Reduced pain of injection (e.g. the morpholine and N-methyl glucamine salts of cephalosporins);
- Altered melting points which may result in possibility of developing different formulations e.g. oral to topical and vice versa;
- Ease of handling, purification or processability;
- Improved compactibility;
- Extended patent protection;
- Possibility to resolve and or separate chiral isomers as well as mixtures.

Till now, several strategies have been employed to conduct salt selection, such as in-situ salt screening technique for ranking the solubility of salts, the multi-tier approach developed by Morris et al. (76).

The choice of salt former (counter-ion) depends on several factors. One of the primary criterions is the possibility of formation of crystalline substance. Crystalline salts afford a means of purification and removal of unwanted impurities. For a solid dosage form, crystalline products are preferred.

The choice of salt is also governed largely by the acidity or basicity of the ionizable group, the safety of the counter-ion and the drug indications. Toxicological and pharmacological implications of the selected salt forming ion must also be considered, as well as the effects on the parent drug. Salt formers can be subdivided into a number of categories depending upon their functionality and purpose. Some of the most frequently used examples are listed in the following Table 3.

Table 3: Classification of Salt formers

Salt formers	**Examples**
Anions	
Inorganic acids	Hydrochloride, Hydrobromide, Sulfate, Nitrate, Phosphate
Sulfonic acids	Mesylate, Esylate, Isethionate, Tosylate, Napsylate, Besylate
Carboxylic acids	Acetate, Propionate, Maleate, Benzoate, Salicylate, Fumarate
Anionic amino acids	Glutamate, Aspartate
Hydroxy acids	Citrate, Lactate, Succinate, Tartrate, Glycolate
Fatty acids	Hexanoate, Octanoate, Decanoate, Oleate, Stearate
Acids for insoluble salts	Pamoate, Resinate
Cations	
Metallic	Sodium, Potassium, Calcium, Magnesium, Zinc
Organic amines	Triethylamine, Ethanolamine, Triethanolamine, Meglumine, Ethylene diamine, Choline
Cationic amino acids	Arginine, Lysine, Histidine
Bases for insoluble salts	Procaine, Benzathiazine.

An integrated approach to the selection of optimal salt form for a new drug candidate is shown in figure 8 below:

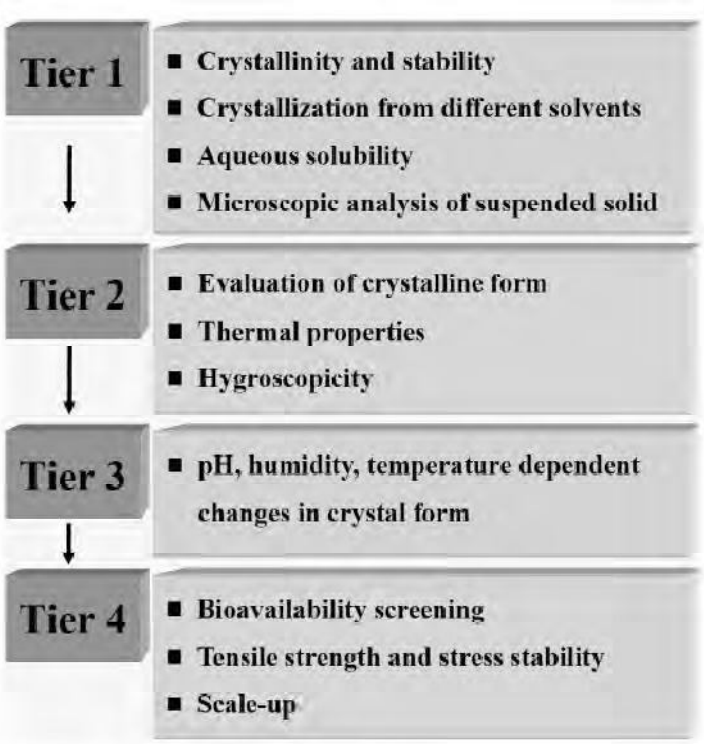


Figure 8: Flow chart of multi-tier approach for the selection of pharmaceutical salts (77).

A unique technique using a microfluidic platform comprised of multi-wells to screen pharmaceutical salts has been developed for salt screening (78). In this method, solutions of the parent compounds and salt formers are mixed on-chip in a combinatorial fashion in arrays of nanolitre wells. Nucleation and growth of salt crystals is induced by diffusive and/or convective mixing of the two solutions in a variety of solvents. Crystals are visualized using bright field polarized light microscopy, followed by the on-chip analyses using Raman spectroscopy to identify different salts.

The presence of ions strongly influences the physicochemical properties of the crystals of formed salts, including solubility, dissolution rate, hygroscopicity, crystallinity, crystal habit, stability, etc. (79).

Generally a solvent can influence the solubility of a salt in the following ways:

(i) increasing solubility of non-ionized species;

(ii) decreasing protonation; and

(iii) decreasing solubility of salt formed.

Like their parent compounds, pharmaceutical salts may also exist in several polymorphic, solvated and/or hydrated forms. For example, ranitidine hydrochloride, has been found to have two polymorphic forms and tautomerism was considered as the main reason of structural differences in the solid state of ranitidine hydrochloride.

An example of a salt form that is highly polymorphic and prone to solvate formation is sertraline hydrochloric acid (HCl), which has been found to have 28 forms, including 17 polymorphs, 4 solvates, 6 hydrates and the amorphous solid. It has been suggested that differences in salt former can have profound effects on the number of polymorphs and solvates that can be found in the corresponding salts (77).

As the properties of an ionic drug salt can be modified simply by switching the counter-ion used to produce the salt, this characteristic has been utilised by pharmaceutical companies to generate different formulations that can be used to treat different indications and alter the drug's pharmacokinetics when using the same active ingredient. Altering certain properties of a compound such as log P (partition coefficient), pKa (dissociation constant) and melting point by salt formation influences the behaviour of the parent drug in the body through alterations in drug solubility, dissolution and stability. For example, the type of counter-ion used to prepare an ionic drug salt will affect the log P value of the administered active. Log P, which is a measure of the relative affinity between a hydrophobic and hydrophilic vehicle, dictates the drug's absorption and distribution within the body. This effect of variation of salt counter-ion on log P and thereby impact on membrane absorption has been illustrated using Ibuprofen as a test system (table 4) (80).

Table 4: Molecular Wt. (MW), partition coefficient (logP) and steady state flux values of ibuprofen ionic drug salts

Counter-ion	MW	Log P	Flux (µg/cm/h)
Sodium	22.99	0.92	3.09
Ethylamine	45.08	0.967	5.42
Ethylenediamine	60.10	1.11	15.31

Diethylamine	73.14	1.12	7.91
Triethylamine	101.20	1.18	48.4

An increase in log P for ibuprofen salts correlates well with an increase in the molecular weight of the counter-ion employed to form the drug salt, and this chemical property has been shown to alter the rate of absorption (flux) across biological membranes.

The reasons behind the selection of a certain drug counter-ion may vary. In some circumstances, it is necessary to prepare specific counter-ions to resolve specific problems with the parent drug. For example, the analgesic propoxyphene was initially formulated as a hydrochloride salt and co-administered with aspirin. However, when propoxyphene hydrochloride came into close contact with aspirin, the compound became unstable. Propoxyphene therefore was reformulated as the napsylate salt, which showed no indication of instability (81). Another example is perindopril, an angiotensin-converting enzyme (ACE) inhibitor. It is available as a salt of tert-butylamine (erbumine), which is freely soluble in water and in ethanol (96%), and has a shelf-life of approximately two years. However, the draw back with this formulation is that it requires special packaging in countries with high temperature and relative humidity. On the other hand, it has been reported that the arginine salt, in comparison with the erbumine salt, is more stable and led to a 50% increase in shelf-life even in high temperature and humidity, an important step for distribution of this drug in the developing world (82). The safety profile of the parent drug can also be altered by switching an ionic salt counter-ion; for example the acute oral toxicity of propoxyphene was halved when prepared as the napsylate salt rather than the hydrochloride salt (83).

An appreciation of how the nature of a counter-ion can influence the properties of a drug allows one to assess how an agent may behave in a particular set of circumstances. For example, it is unlikely that a drug that is highly soluble in water and rapidly absorbed and excreted will be affected by the switching of one small molecular weight drug salt counter-ion to another. However, the use of large counter-ions (for example xinafoate) will

alter the permeation of the therapeutic agent across a biological membrane, as well as the drug's solubility and dissolution, and, thus, could affect its duration of action, onset of action and clearance rate.

Selecting an ideal salt form is a very essential step to guarantee a successful development of a highly safe and efficient drug product. A well designed screening plan is required to meet the essential and desirable criteria that set the standard for salt screening. Furthermore, the selection processes of the salt must also measure the regulatory and marketing considerations to balance the drug's physicochemical and biopharmaceutical properties against commercial considerations.

4 Characterization of polymorphs, co-crystals and salts of active pharmaceutical ingredients:

The various forms of API are evaluated in developmental studies from three aspects:

(1) characterization of the API;

(2) determination of the thermodynamic relationship of various forms of the existing API; and

(3) finding of the most stable form.

X-ray powder diffraction (XRPD) is the most convenient way to determine crystal forms, since different crystal forms produce different diffraction patterns. Although a relatively large amount of API is usually required for detailed investigation, the sample is reusable.

Other spectroscopic methods, including infrared, Raman, and near-infrared spectroscopy, may also differentiate crystal forms (84). Terahertz spectroscopy is a relatively new technology in this field, and is another option for investigating different forms of APIs (85). Other techniques which are commonly used for characterizing solid forms include solid-state fluorescence spectra (86), solid-state NMR (87) etc.

Thermal analysis is a very sensitive method for evaluating solid forms and can directly provide thermodynamic information (88). Also, due to the small sample size, thermal analysis is suitable for characterization in early stages of development. Such methods include thermogravimetry, differential

thermal analysis (DTA) and differential scanning calorimetry (DSC) (89). If multiple crystal forms are identified, their stability order must be determined to identify the most stable form at the storage temperature and processing temperature during manufacture of the formulation.

5 Aim of the research work

Based on the discussions above it is evident that polymorphic forms and/or salts of an API has huge impact on the physico-chemical, pharmaceutical as well as therapeutic properties of an API. Several important molecules having excellent biological properties could not be developed because of their poor physico-chemical properties and inability to identify a suitable form which can be developed. Hence the aim of the present research work is to

- Identify compounds which have been shelved due to their poor physico-chemical properties making them unsuitable for further development;
- Use different techniques to develop alternate polymorphic forms, salts/co-crystal forms of these compounds;
- Develop alternate processes which are scalable, whenever required;
- Evaluate the impact of the new forms/salts/co-crystals on the biological properties of the compounds.

There is a significant need to pursue such strategies for improving the quality of several existing drugs in order to meet the unmet medical needs. Such strategies can provide significant opportunities for developing better drugs quickly and with much lesser expenses that developing completely new drugs. Such a strategy will be particularly beneficial for countries like India who have a very wide variety of patient population and where the need for cost effective therapies are acutely felt. Such an approach can provide therapies particularly suitable for the Indian population.

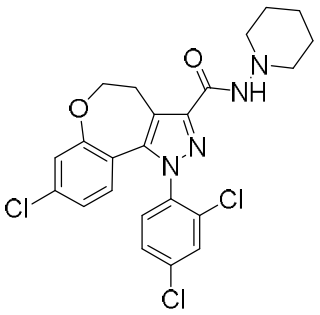
6 Scope of the present work and approaches taken

Two drugs were identified from the internal research pipeline which has been shelved since they could not be scaled up and also due to limitations in their physico-chemical or pharmaceutical properties. A third drug selected is an approved drug with reported problems both in its manufacturing and in the market. The patent space for the process of manufacture of this drug is also very crowded requiring development of a non-infringing process for preparing the compound. After identifying these molecules, efforts were directed to overcome these challenges through suitable chemical modifications and include development of suitable processes for preparing the compounds. The results of these efforts are reported in the thesis.

7 The thesis is divided into the following chapters

7.1 Chapter 2

This chapter describe the work done in improving the pharmaceutical properties of a novel cannabinoid receptor antagonist, 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide of the following structure:



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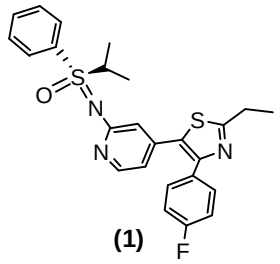
7.2 Chapter 3

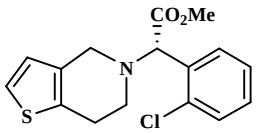
This chapter describes the work done in improving the stability and pharmaceutical properties of a novel p38 MAP kinase inhibitor of the following formula, developed in-house.



(1)

7.3 Chapter 4

This chapter describes the work done on a known anti-platelet agent, Clopidogrel, which is available commercially as the bisulfate salt, having the brand name Plavix. Chemically it is Methyl α- 5(4,5,6,7-tetrahydro(3,2-c) thienopyridyl) (2-chlorophenyl)-acetate having the formula (1) andis an anti-aggregatory and antithrombotic drug for the treatment and prevention of peripheral vascular, cerebrovascular, and coronary artery diseases.



(1)

A novel and non-infringing process for preparing the compound of formula (1) has been developed. Also, work done in identifying a novel organic salt of the compound (1) is described.

7.4 Chapter 5

This chapter describes the studies conducted on the use of crystal modifiers to change the morphology of the crystalline form of compounds. For this study the crystalline compounds obtained in chapters 2 and 4 were taken. The crystal modifiers which have been used are various polymers. Specifically, the following polymers have been tried:

- PEG 200;
- PEG 300;
- PEG 4000;
- Polyvinyl alcohol (PVA);

- Polyvinyl pyrrolidone K-30 (PVP K-30)

The work done in modifying the crystal morphologies of the selected compounds are described in this chapter.

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