CHAPTER 1

NEW DRUG SUBSTANCES

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Introduction

Most drugs currently marketed are selected after several years of investigations and trials. Generally, in the selection of pharmaceutical solids, the combined efforts of chemists, biologists, molecular biologists, pharmacologists, toxicologists, statisticians, physicians, pharmacists, pharmaceutical scientists, and engineers are needed. Now-a-days team effort and well-defined strategies right from the beginning of project initiation to the drug reaching the market are executed. This consumes several years of hard work and enormous amount of capital. Occasionally, lot of the efforts is wasted because of improper planning and wrong methodologies. Thus, drug discovery is a challenging process.

Drug development can be simply classified into synthetic compounds, molecular modifications, semi-synthetic compounds and natural products. Although plant based therapy has been in practice in eastern countries like India and China for several centuries, isolation of active ingredient from these well known plants is novel. There is evidence that this kind of *natural product therapy* in western countries also existed. As a whole, this field is not progressing much because a high rate of failures is reported with some of the active compounds tested. *Synthetic compounds* have been in medical practice for only 70 years. At the end of Second World War, with a lot of casualties reported in the war and also because of several diseases like plague afflicting western countries and with several accidental discoveries, synthetic chemicals were introduced into medicine. Quickly these compounds proved to be very successful as therapeutic agents. The other area is the modification of the synthetic compounds. Some of the recent introductions into the synthetic chemistry with the advent of high-throughput screening are highly potent molecules. However, these molecules suffer from several disadvantages including very low solubility, poor permeability, toxicity etc. *Chemical modifications* such as salt formation, prodrugs etc. were found to be helpful in reducing the disadvantages. Finally, *semi-synthetic compounds:* This class of compounds includes antibiotics like semi-synthetic penicillins and anti-cancer molecules like flavopiridol. These are synthetic modifications in a fermented or a plant derived compound. Drugs obtained as microbial end products have been in the market for several years. The most famous of these molecules is penicillin and its derivatives. Currently several penicillins are in the market for the treatment of various cancers, fungal diseases, bacterial diseases and viral diseases. Plant-derived products are currently a fashion in the pharmaceutical research. These products existed in ancient India for thousands of years. Till allopathy was introduced, pharmacists in the Indian subcontinent used these products to treat the people. As mentioned before, Ayurveda is very sophisticated and only very efficient practitioner or pharmacist was able to use its therapy rightly. Current knowledge about Ayurveda is mostly drawn from relatively later writings, primarily the Charaka Samhita (approximately

1500 BC), the Ashtang Hridayam (approximately 500 AD), and the Sushrua Samhita (300-400 AD). Active leads are generally extracted from well known plant drugs.

New Drug Substances

Any chemical substance with new therapeutic value could be called a “New Drug Substance”. As mentioned previously it could be a synthetic compound, natural product or a semi-synthetic compound. According to the FDA, *any drug that is recognized among experts, qualified by scientific training and experience, as being safe and effective under the conditions recommended for its use is termed a “new drug*”. Several definitions of new drug substances could be found in the literature.

Lead Identification and Optimization

Lead identification and optimization is an important aspect of new drug substance development. A “lead compound” is the basic structure that elicits some pharmacological action against the target disease. In earlier times, leads were identified by random synthesis of a series of molecules, their pharmacological activity determined and the structure-activity relationship (SAR) established for this series of molecules. The lead would be modified as per the pharmacological results. The goal is to enhance the potency and to obtain better therapeutic agents of this series of compounds. However, this used to be a tedious process in drug discovery. With advanced technologies such as high-throughput synthesis and screening techniques, innovations were introduced into the lead identification and optimization. The molecular targets for a disease generally are proteins, may be enzymes, receptors or structural proteins. Since proteins are important targets for a disease either in drug discovery or diagnosis, pharmaceutical companies are currently investing hugely on protein targeted drug design and discovery. The target proteins are isolated to pure state and the crystal structure determined. Lead is then identified by predictions based on *in-silico* techniques and optimized. These molecules could be synthesized and screened.

The bottom line in the current drug discovery process is the rapid and accurate lead optimization. This requires tremendous expertize in medicinal chemistry, synthetic chemistry, formulation technology, bioscreening and pharmacology. Currently, experts use proprietary and third party tools and QSAR (Quantitative Structure Activity Relationship) modeling for relating the key calculated molecular descriptors (physicochemical, topological, structural, ADME-related (ADME stands for Absorption, distribution, metabolism and elimination) and others) with specific biological activity in assessing lead optimization

techniques. Because of the enormous database currently available, a single small unit at one location will not be able to handle such a decision. Several third party tools such as commercial outsourcing facilities could clearly decipher their know-how of *in silico* lead optimization techniques. The resulting outcome is an efficient drug discovery process.

The design of molecules in the current *in silico* techniques is based on knowledge of biochemistry, the understanding of interaction of ligands with proteins, affinity generating structure elements (AE) substructures involved in interaction with target proteins, chemistry-perception and the characterization of molecules. The basic precept in such a design is different characterizations of molecules leads to different molecular representation spaces and the same set of molecules could have vastly different distributions in its various representation spaces. When the target knowledge is zero, diverse libraries are generated using *in silico* techniques. If pharmacophore is known focused libraries are developed. In this case pharmacophore based libraries are generated. A structure-based design is used if protein X-ray crystallographic study has given a known target structure. Random drug-like libraries involve expanding the corporate compound collection by suitable acquisitions, and enumerating virtual libraries and choosing diverse sets using, for example, Genetic Algorithms (GA). Pharmacophore based libraries can also be developed by traditional medicinal chemistry skills.

The other important area in lead discovery and optimization is proteomics. Proteomics is the study of proteins and its application in several scientific areas including drug discovery and development. Proteins are important targets of drug discovery. In several disease states, the protein expression is altered. This is one of the reasons for the evolution of proteomic techniques. The identification, characterization and quantification of all proteins involved in a particular pathway, organelle, cell, tissue, organ or organism that can be studied in concert to provide accurate and comprehensive data about that system. When scientists can accurately and dependably identify and understand the activity of these protein systems, the underlying characteristics of disease and wellness will be clearly deciphered. The same principle holds true for protein alteration expressions in disease state models. Thus, proteomics has the potential to revolutionize the development of innovative clinical diagnostics and pharmaceutical therapeutics. For example, a specific configuration of proteins in liver tissue could define a particular tumor, or a successful regression of that tumor, in response to therapy and thus amenably, this is the underlying top principle in the role of recent therapy discovery.

The techniques in proteomics fathom from the identification of thousands of proteins in a particular model system, to the detailed analysis of the 3D structure, possible modifications/isoforms, and function of a single protein. All these factors

are very contributing to the drug discovery in all its stages. The stages include target identification, target validation, drug design, lead optimization, and pre-clinical and clinical development. Currently high throughput proteomics is aiding this process of drug development. High-throughput proteomics are able to identify potentially hundreds to thousands of protein expression changes in model systems following perturbation by drug treatment or disease. This lends itself particularly well to target identification in drug discovery process. However, this data analysis and validation of potential protein targets is a time-consuming and labor-intensive process. Identification of proteins is only beginning to assume importance in rapid drug discovery process. Identification of appropriate protein in a disease state as well as a suitable molecule with thorough binding or targeting properties to the protein of interest is not only time consuming but also may be very costly. In these situations, the best alternative is to use the existing database of proteins and drug molecules of interest and proceed with drug discovery process. Several biochemical methods are currently in place in the identification of the proteins that are altered during disease state. The most common method used in biochemistry labs is two-dimensional gel electrophoresis. It is very effective at identifying protein expression changes in a system. Currently high throughput techniques are used in proteomic technologies. In a recent technique, each protein is terminally tagged and digested, and then only the terminal peptides are isolated and sequenced, allowing for rapid identification of an entire proteome. This technique is termed as protein sequence tags (PST).

Apart from the lead optimization using binding of ligands to the proteins of interest, parallel synthesis techniques is a part in the lead optimization process. A range of innovative methods using computer software is currently used in speeding up solution phase synthesis that further accelerates lead optimization. Synthesis of batches of 100 compounds at one time using stem-reflux or stem-cool stirrer hotplates or MTP blocks is reported. Purification is a processing step in the synthesis. Current high throughput purification methods include simple “lollipop” technique, membrane technology to separate aqueous and organic phases, the use of resin based scavenging agents, parallel centrifugation, parallel solvent blow-down and parallel cartridge based chromatography. As per one report, because of the introduction of these techniques there has been a 10-fold increase in assay productivity since 1995. The other methods use biocatalytic and chemoenzymatic techniques. The technique worth mentioning to describe is solid phase synthesis and its modifications.

Thus, in tandem with in silico techniques, synthesis of compounds is also initiated. New techniques are leading to the rapid synthesis of compounds. Solid-phase organic synthesis (SPOS) is an important tool in the field of synthetic

chemistry. The basics of this synthetic process for the generation of new chemicals were adopted from solid-phase peptide synthesis. In this method, the substrates are attached to a solid support (polystyrene, polyethylene glycol, cellulose, controlled pore glass, etc.). The reaction is achieved in this state and subsequently the reactants and the products are detached from the support by using specific techniques. The purification of this mixture of products is achieved using several separation and analytical techniques and a library of chemicals is generated. Most commonly used and one of the earliest supports is polystyrene cross-linked with divinylbenzene (PS-DVB). Because of its hydrophobicity and steric hindrances, PS-DVB does not provide environment that is solution like. Thus, currently several new supports are being investigated. The new supports are aimed at achieving enhanced product isolation, compound purity and would also support solution-phase synthesis resulting in faster reaction rates through rapid diffusion and reaction mobility. Cross-linking of SPOS with polyethylene glycol (PEG) was the major addition to the art of solid-phase polymer synthesis. The spacer PEG determined the properties of the solid-phase support. This increased the hydrophilicity and conferred flowing solvent-like properties along with mechanical and physico-chemical properties more ideal compared to that of SPOS. Several factors like bead size, nature of the polymer, the lipophilicity, its porosity all affect the effectiveness of the synthesis. Basically, with optimum properties, a resin behaves like a micro-reactor. The reactions are rapid and selective in this micro-reactor.

Now-a-days pharmaceutics scientists play a major role in lead optimization. Once leads are procured after synthesis, pharmacological activity is quickly determined. This is generally accomplished in pharmacology labs. Usually this information is obtained with NCEs (new chemical entities) solubilized in DMSO (dimethylsulfoxide) or other similar easy formulations either in cell culture or small animal models. Without pharmacological or toxicological evaluation, the complete activity or safety of a lead, as per the regulatory agencies demands is not established. Thus, its pharmacological activity is first investigated. Further, it reaches pharmaceutical technology group for toxicological and pharmacological evaluation. As a first step, the preformulators prepare a dosage form to be used for preclinical toxicological studies. A lowest and highest possible dose will be incorporated into the formulation and administered into the animal. The maximum toxicological dose will be identified. Subsequently, this formulation will be tested for its activity in animal models. Dose-dependency of the activity is the first priority. Based on the pharmacology and toxicology results, few compounds will be selected from the library of leads. These compounds will be further developed. Scientists from exploratory pharmaceutics then take up the challenging issue of the formulation development.

Once a compound is obtained in pure form, the solid-state characterization becomes important. At the end of synthesis and purification, solid drug substances display a wide and unpredictable solid-state properties. Any change in these forms is not a big issue after synthesis. The project could be dropped at a later date. However, the appearance of these crystals during processing and upon storage of the final product would be an important issue. As per New Drug Application (NDA) guidelines, a new drug application should contain information on solid-state properties of a drug particularly, when bioavailability is an issue. Appropriate analytical procedures should be used to detect various solid-state forms such as polymorphs, hydrates, desolvated solvates and amorphous forms, as part of regulatory requirements.

Procurement of New Drug Substances

Modern drugs are either synthesized, extracted or semi-synthetic. However, a systematic drug development is valid yet for only synthetic compounds. These compounds could be synthesized using the very routine laboratory techniques or with the help of modern high throughput techniques. Once the lead for a disease is identified and it is optimized its synthesis in large quantities is initiated. High throughput synthetic techniques are recently introduced into the field of synthetic chemistry. The identification of new leads and the optimization of the synthetic techniques saw a new growth in this area. Lately, several new procedures developed have resulted in enhanced productivity of pharmaceutical industry. The recent innovations in synthetic chemistry such as solid/liquid phase synthetic techniques have reduced reaction times and often result in improved yields compared to solution state synthesis. These polymer-based synthetic techniques are able to generate large library of diverse chemicals in a rapid and parallel manner. In addition, the current screening techniques are very sophisticated and often times very efficient thereby making the drug discovery process very productive.

Molecular Optimization

Once the pharmacology and toxicology are determined, final formulation development, clinical trial and market potential formulation development are subsequently pursued. However, the molecules that are lately being synthesized are very poorly water-soluble. As such, the formulation development is a big issue with these molecules. Thus, it is currently a routine practice in big pharmaceutical companies to develop the formulations for these types of molecules and then proceed to the next step of toxicological evaluations. This may be time consuming. However, the studies may become leads to quick

formulation development for such poorly insoluble molecules that may enter the lab later. In any case either of the methods depending on the convenience could be preceded.

Prior to the development of formulations, the first criterion is the physical characterization and optimization of the molecule. This helps in the formulation development process. A physical pharmacist develops a formulation. The short-term stability of this molecule is determined. This ensures that the formulation would be stable during the course of preclinical toxicological evaluations. If the molecule is not stable, a different formulation is attempted till a stable short-term formulation is developed. The other problem that may be encountered is the low bioavailability of the molecule being tested with the formulation developed. In these circumstances, desired concentration of the drug in the plasma is not obtained to elicit its biological activity. Definitely when biological activity is not elicited, toxicological manifestations are also not observed. In any of these situations, the best solution is the development of a parenteral intravenous formulation. This helps in determining direct toxic dose of a drug in the model of interest. Generally this formulation is a solution. A suitable solvent in which the drug dissolves should be identified. The other possibility is the development of a suitable formulation to enhance the bioavailability of the drug. Thus, if there is any problem with the drug substance, although demonstrating good pharmacological property, the formulations used in the early toxicology or mechanistic pharmacology investigations a intravenous dosage form is preferred. In this case the drug has to be soluble in water or any other suitable solvent. On the other hand, if any of the techniques and methods do not assist in the investigations, the best alternative is to modify the drug to change its physico-chemical properties or select an optimum physical form with suitable properties. These modifications could result in salts, prodrugs, suitable physical forms (solvates, polymorphs, hydrates) or even new analogs may emerge from the modification efforts. Thus, the investigations into the molecular modifications of a drug entity to make it optimum to demonstrate pharmacological and therapeutic activity would be of interest and innovation for drug development scientists. These modifications to the original molecule can be called as molecular optimization.

Although the above modifications are the likely possibilities, the very commonly tested derivatizations are salts and prodrugs. Salt formation results in the removal of an acidic or basic group from a molecule and thereby enhances the dissolution of this counter ion in water and thus likely enhances the bioavailability. For instance, ephedrine hydrochloride is formed by the addition of a proton to form an ionized drug molecule that is then neutralized with a counter ion (Ephedrine hydrochloride is prepared by addition of a proton to the basic secondary nitrogen atom on ephedrine resulting in a protonated drug molecule

that is neutralized with a chloride ion). In general, organic salts are more water-soluble than the corresponding un-ionized molecules, and hence, offer a simple means of increasing dissolution rates and possible improvement in the bioavailability. Ample literature is available with regard to the prodrugs. Along with salt formation, prodrug synthesis is also one of the techniques to alter the physico-chemical properties of a drug substance to enhance the formulation and biopharmaceutic developments. Uptil today, most prodrugs are esters or amides designed to increase lipophilicity. One of the first investigated prodrugs is a morphine analog. These prodrugs are synthesized to enhance the brain permeation of morphine and other CNS (central nervous system) agents. The main characteristics of prodrugs include the rate of hydrolysis, formulation stability, bioavailability and tissue permeation. These are discussed in detail elsewhere in this textbook. Each of the techniques for molecular optimization; selection of suitable physical form, selection of suitable solid form, suitable salt form and development of prodrugs is discussed below.

Selection of a suitable solid form

Solid state of the drug is also an important aspect of molecular optimization. Once it is established that a molecule is a promising candidate for future investigations, its synthesis procedure in large quantities will be developed. This step is called bulk drug synthesis. In most instances, bulk synthesis of a chemical entity is developed in parallel with preformulatory investigations. Generally, a drug candidate is not thoroughly characterized during this stage. However, if synthesis steps are achieved by the end of all preformulation studies and the bulk synthesis yields a different tougher solid substance (a polymorph, a hydrate, a clathrate etc.) and if the bioavailability of this substance is different from the already investigated physical form of the drug, it is likely that all the preclinical toxicological studies have to be repeated with the new physical drug substance or a new bulk synthetic process for this molecule has to be developed. Thus, synthesis procedure for a suitable solid form is important. There are several other reasons why a suitable solid form has to be selected. The selection of a suitable solid form of the drug substance is a vital step in pharmaceutical development. Given that a drug substance can exist in multiple solid forms, including polymorphs, hydrates, solvates, salts, co-crystals, and amorphous, and that each of them have different properties that can affect bioavailability, solubility, dissolution rate, stability and manufacturability, the appropriate evaluation and comparison of the properties of the solid forms in a rigorous and effective manner requires extensive expertise, knowledge and a good methodology Thus, solid form of the new chemical entity has to be thoroughly investigated.

Because of the various reasons mentioned above, it is definitely very important to know the various types of solid forms a drug could exist in. This would be of future help in sorting out any problem that might have arised during the storage conditions of the formulations. The first step in this investigation is to obtain various physical forms by the recrystallization of the drug from various solvents. These solvents include water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethylacetate, hexane and mixtures, if appropriate. Cooling hot saturated solutions or partly evaporating clear saturated solutions could also obtain new crystal forms. Crystal habit and the internal structure of a drug could affect bulk and physicochemical properties, which range from flowability to chemical stability. Two terms are described in defining a crystal. One *habit* and the other, *crystal structure*. Both of them are separate. The description of the outer appearance of a crystal is the habit and the molecular arrangement within the solid is termed the internal structure. The crystal habits could be platy, equant (massive); needle (acicular); bladed; tabular and prismatic. A single internal structure can have several different habits, depending on the environment for growing crystals. A change in the internal structure alters the crystal habit. However, chemical changes such as salt formation would lead to a change in the internal structure and the external habit. It has to be noted that various crystal structures, habit as well as internal structure, exist for a single molecule. In addition, the physical, physico-chemical, physiological and the pharmacological properties of these individual polymorphs are different. Thus, a drug substance's visual appearance and its microscopic view are to be thoroughly investigated to avoid any future problems associated with the clinical substance to reduce the expenditure invested by a pharmaceutical company on a single chemical entity. The internal structure could classify a drug into either a crystalline or an amorphous solid. Crystals are characterized by repetitious spacing of constituent atoms or molecules in three-dimensional array, whereas amorphous forms have atoms or molecules randomly placed in a liquid. Amorphous forms are typically prepared by techniques like rapid precipitation, lyophilization, or rapid cooling of liquid melts. Solubilities of amorphous solids are higher than crystalline forms because of the higher thermodynamic energy of amorphous forms than corresponding crystalline forms. The major problem associated with the existence of different physical forms for a single drug is the transition of one physical form to the other upon storage or during processing. Generally, amorphous solids revert to more stable crystalline forms during formulation development or storage.

Crystal form of drug substances influences the physical, chemical and mechanical properties of drugs. Therefore, solid-state properties of drugs and the excipients are to be done to obtain consistent product performance. As mentioned before, the first aspect investigated is the physical nature of a new chemical entity. This ensures the commonness of the New Chemical Identity

(NCI) used for various purposes. This includes synthesis and formulation development. Immediately after it is received, a pharmaceutical scientist looks the NCI under a microscope. This will give an indication of the physical form of the drug. The drug substances as looked under a polarized microscope are either isotropic or anisotropic. Isotropic substances have single refractive index. Amorphous drugs like supercooled glasses and noncrystalline solid organic compounds, or substances with cubic crystal lattices, such as sodium chloride, are isotropic material. Under cross-polarized filters, these isotropic substances do not transmit light, and they appear black. Substances with more than one refractive index are anisotropic and appear bright with brilliant colors (birefringence) against the black polarized background. The differences in the refractive indices and the crystal thicknesses result in the different colors of a crystal. Anisotropic substances have either two (uniaxial) or three principle refractive indices (biaxial). Most drug substances are biaxial, corresponding to either orthorhombic, monoclinic or triclinic crystal system. Only a well-trained crystallographer can identify the crystal nature of a biaxial system or a drug substance. One refractive index should be enough to describe a crystal structure. However, proper orientation and exposure of crystals under a microscope along with its crystallographic axes is required to define a crystal properly. Orientation also affects the crystal identification under the microscope. This requires good training. However, regular scientists could investigate the routine microscopic investigations such as crystal habit and observe transitions induced by heat or solvents. With the presence of organic solvents or water in a crystal, there is always a question to a pharmaceutical scientist to define the characteristic feature of a drug substance. The presence of water or organic solvent either resulting during synthetic steps or during formulation development or storage affects the function of the pharmaceutical formulation effects the properties of new drug substances. When solvent molecules exist in a crystal lattice and form molecular adducts, the substance is called a solvate. If the solvent is water, the molecular adducts are called as hydrates. Hydrates are very common with most of the pharmaceutical formulations because of the omnipresence of water in all the pharmaceutical formulations. Desolvated solvate is a crystal from which the solvate is removed intentionally or unintentionally and the crystal retains its solvate structure. However, this is not always the case. Some times crystals are more rigid than the other forms of drugs.

A drug's bioavailability, formulation development, solubility changes and stability depend on the physical structure of the drug. As such, polymorphism could be defined as the ability of a compound to crystallize as more than one distinct crystalline species with different internal lattices. As mentioned before, this sometimes makes things complicated for new drug development. The well-known example is the existence of chloramphenicol palmitate as three different

crystalline forms and one amorphous form. It was found that three formulations demonstrated different bioavailabilities suggesting this to be a key phenomenon in chloramphenicol development. The other example is the anticonvulsant drug carbamazepine. This drug exists in solid state as three polymorphic anhydrous forms and as a dihydrate. It is practically insoluble in water and is marketed as a tablet. Wet granulation is the technique that is used in the development of granules for such a drug to be used for tablet compression. Examples of other drugs that are known to exist as polymorphs are mebendezole, theophylline, dihydroepiandrosterone, and tenoxicam. Several properties such as melting point, density, hardness, crystal shape, optical properties and vapor pressure are influenced by the physical states of a drug. Some of these properties can be used in investigating polymorphic nature of a drug. Because of all these variations, a suitable solid state of a drug should be identified very early on. Techniques such as microscopy, fusion methods, differential scanning calorimetry, infrared spectroscopy, x-ray diffraction, scanning electron microscopy, thermogravimetric analysis and dissolution/solubility studies are used in the assay of the physical forms of drugs. A specific technique should be fine to investigate the physical nature. However, it is always advisable to use several alternative techniques to perfectly confirm the physical nature of drugs so as to reduce the cost of formulation development and as such, drug development process. Some of these techniques were in place for over several years. Inspiteof the availability of a lot of information on these techniques, new techniques are always investigated to improvise the formulation development process with new chemical entities. Differential scanning calorimetry (DSC) is the best technique for detecting solvates. This is because of the heat change involved in the desolvation, esp. for hydrates. However, DSC alone does not indicate the existence of solvates. The analytical data obtained from nuclear magnetic resonance spectroscopy (NMR) and thermogravimetric analysis (TGA) indicates the existence of solvates. DSC then becomes good technique for analyzing solvates and determining the percentage of the solvates present. General protocols can be followed when deciding an optimum physical form for the drug substance. As indicated previously different physical forms are investigated very early on, they are characterized and also known to the pharmaceutical scientist. A decision tree for polymorphic forms and hydrates (solvates can be developed). These decision trees are generally based on physical form consistency, the dissolution, critical relative humidity values, and transition points. Consistency of the API solid state under the influence of environment destabilizing factors such as exposure time, ultraviolet light, pH, moisture, temperature, and pharmaceutical processing operations like milling, compression and compaction should be investigated so as to select optimum physical form. There are a set of rules for selection of suitable solid form. These are described below.

The first choice of API for a solid drug formulation is the anhydrate of active substance (free acid, free base or neutral compound). Anhydrates together with salts form the majority of all drug formulations. If the anhydrate for some reason is not suitable (e.g. it is little soluble, unstable, has complicated polymorphism etc.), then possible hydrates are monitored. The hydrate is most frequent a solvate containing water molecules in its crystal structure. Water molecules can be incorporated in the structure in a stoichiometric manner (stoichiometric hydrates) or non-stoichiometrically (non-stoichiometric hydrates), For the formulation stable stoichiometric hydrates in a lower stage of hydration are chosen in which water molecules are bound to molecules of the active substance by H-bonds. The dehydration of a stoichiometric hydrate often results in the collapse of the crystal structure and the origin of an amorphous phase. Non-stoichiometric hydrates are not suitable for the formulation because the water content in them changes with the partial pressure of water vapour in the ambience and with temperature a thus they difficult to define. In non-stoichiometric hydrates, water is not bound very firmly, it rather fills present cavities in the structure without forming Hbridges.The dehydration of non-stoichiometric hydrates does not result in the origin of an amorphous phase but a crystalline anhydrate originates. Other solvates (with the exception of ethanol solvates) are not used for the formulation but can be used as important precursors. For instance polymorhs which are otherwise difficult to attain can be obtained by their desolvation. Stability is another important issue when a hydrate is selected as the final molecular form. The stability of the system anhydrate/hydrate depends on the ambient relative humidity. Many active substances form hydrates, often in a various degree of hydration and stability. If the hydrate is the more stable in the system anhydrate/hydrate then the hydrate has all available reliable proton donors and acceptors better satiated compared to anhydrate (Etters rule). For instance ergot alkaloid tergurid exists as an anhydrate, a twothird hydrate and a monohydrate, and the stable phase is the monohydrate Formulations from hydrates are not very frequent and represent only several per cent of the total number of APIs (e.g. chloral hydrate, levofloxacin hemihydrate, terpin hydrate and others). The reason is their thermal instability and possibility of the potential dehydration during drying. Of excipients, much used is the lactose monohydrate.

Selection of a suitable polymorphic form also is some times very important. Compounds can exist in different crystalline forms. Among pharmaceutical molecules, the most frequent case is dimorphism (existence of two different polymorphs). A well-known example is the patent litigation between pharmaceutical companies Glaxo and Novopharm over two polymorphs of ranitidine hydrochloride, which decreases the production of stomach acid, or the problems of the company Abbot Laboratories concerning two polymorphs of

ritonavir – an inhibitor of HIV-protease. Since polymorphs differ by their crystal structures, they differ by their properties, of which solubility and dissolution rate are the most important. A typical ratio of solubility of two polymorphs is less than two, but there are exceptions, e.g. polymorphs of premafloxacin I/III or polymorphs of chloramphenicol A/B have this ratio larger than 10. Thus it can happen that a less soluble polymorph does not even reach the minimum medicinal concentration in blood.

Amorphous compound are also some times preferred over crystalline compounds because of the advantages they offer for this drug. Amorphous forms are thermodynamically metastable which results from the disordering of their inner structure on molecular level. Compared to ordered crystalline phases, amorphates have better molecular mobility which results in a better dissolution profile and thus a better oral bioavailability. On the other hand this is compensated by lower chemical and physical stability (shorter expiration) and by greater demands on production and storing (e.g. protecting atmosphere). Current formulations from amorphous phases include asthma medicine, e.g. zafirlukast (Accolate, Astra-Zeneca), quinapril hydrochloride (Accupro, Accupril, Pfizer), anti-fungal drug itraconazole (Sporanox, Janssen-Cilag) or nonsteroidal anti-inflammatory drug indomethacin (Indocin, Merck). In solid drug formulations, amorphates are stabilized by suitable excipients (e.g. PVP, trehalose, sorbitol,etc.). Another type of molecular optimization leads to the formation of cocrystals. Cocrystals are at present the most dynamically developing group of solid pharmaceutical substances. The definition of the term “pharmaceutical cocrystal” is still under discussion, but essentially it is a multi-component compound that is formed between a molecular or ionic API and a cocrystal former that is a solid under ambient conditions.

Selection of Suitable Salt Form

Salt selection is the important step in the development process initiated very early on. Before even toxicology studies are initiated. This is because if in toxicology studies it is determined that the salt form that was used was not appropriate; the entire studies should be repeated. Toxicology studies are generally time consuming and expensive. But what is the need to prepare salts. The unionized (free) form of weak acids and bases may not be ideal molecular forms for development. Very early on salts can be synthesized and tested. Analog selection was based on the in vitro activity, however, today a blend of developmental issues such as salt formation are surfacing. Thus, salt screening is performed very early on. Salt selection affects several physical properties of the API. Salts have different physical properties than their free forms. Selection explores whether a particular salt might have properties that are more

appropriate than the parent. Improving oral absorption by increasing the dissolution is one main reason for prepare salts. It is important that a suitable physical form of a particular salt form has to be selected. More details about the salt formation are described in another chapter of this book. However, the minute details of molecular optimization with this technique will be discussed here. Very early on permeability, solubility (Cs) and pKa, the intrinsic properties of the molecules are determined. The major dependent variables, absorption and consistency of the API, can be manipulated, optimized and balanced in salt selection. For a given acid or base form of a drug different salts will be synthesized and then a suitable salt form for further development will be selected. This is called salt selection. A general protocol for the salt selection (optimization) has been recently proposed. It is called as a three-tier system (2) and is described as below:

Tier 1: Hygroscopicity

Tier 2: Thermal analysis and x-ray diffraction

Tier 3: Accelerated solid-state stability

Tier 1 eliminates any form with excess moisture sorption desorption characteristics. Only good candidates progress to the next step. In the second tier, changes in the crystal structure are examined under extremes of moistures condition using any of the techniques previously described for the physical forms which include thermal analysis or x-ray diffraction. This is to detect desolvation or aqueous phase transformation problems. Aqueous solubility is also determined at this stage to address dissolution problems. The best candidates for formulation and manufacturing are considered here and the only good candidates proceed to tier-3. In this tier, accelerated thermal and photostability testing is carried. This is a time consuming process. Drug-excipient compatibility with commonly used excipients is also performed at this stage. If tier 3 eliminates all the salts synthesized, newer salts can be considered. The entire salt screening process can be reevaluated. Although these are the general rules that have been proposed, deviations are also possible and optimization of molecule for salt development should be carefully performed. For example, the HCl salt of ranitidine due to its hygroscopicity would not have been selected in this three tier approach, however, it is the most successful salt in the market. This emphasizes a need for prioritizing the salt selection process so that a wide range of development issues should be considered and addressed as early as possible. If a hydrochloric acid has much better absorption properties than the free base but is hygroscopic, it is better to deal with this problem. Otherwise, bioavailability may be compromised when more stress is given to the consistency of the physical form. The free base/acid forms are generally not preferred in this three tier

approach unless all alternatives have failed despite its potentially favourable dissolution in gastric fluids and its sensitivity to particle size reduction with a reactive sink.

Prodrugs

Prodrugs are one way to optimize the molecular properties of new drug substances. Basically, they come under structural modification for molecular optimization. There are several reasons for the development of prodrugs. A separate chapter is devoted for prodrugs in this book and is thus not discussed here in detail. A brief overview for pharmaceutics student will be presented here. Often NCEs have adequate biological activity but do not have the required physical properties to become a drug. For orally administered drugs, the compounds need to dissolve and then permeate across the gastrointestinal tract. For intravenous drugs, the compound must have adequate solubility in the vehicle as well as in the blood for the reasons of safety. Prodrugs are one of the methods to solve safety issues and property design problems and should be considered very early on in the drug discovery stages. Poor membrane permeability, poor solubility, and poor dissolution are the problems that can be addressed using prodrug approach. Prodrug design has also been used to reduce the toxicity. Lets discuss absorption.

The three factors that inhibit the passage of a drug molecule through biological membranes are:

(a) Charge,

(b) water of hydration and

(c) molecular size.

To enhance the permeability, now scientists are exploring prodrugs to exploit membrane transporters. There are several membrare transporters on the membranes which can be utilized to enhance the permeability of molecule. For instances, nucleoside prodrugs can be synthesized for a given drug to aid its transport across the membrane using nucleoside transporters. Similarly, peptide transporters can be utilized. Recently, some of the structural requirements of the plasma membrane peptide transporter, PEPT1 has been elucidated. Thus, prodrugs which utilize this transporter can be synthesized with improved absorption. The antiviral agent valcyclovir is a prodrug of acyclovir. It has been observed that the H-bonding of guanidine molecule of valcyclovir may enhance its PEPT1 absorption. Reducing ionization using prodrug strategy can also increase the absorption of the drug. For instance, most factor Xa inhibitors used for preventing the activation of thrombin and blood clots have utilized a highly

charged group either guanidine or amidine group. These groups however have reduced oral bioavailability because of the charge. One strategy to over come this bioavailability is to have prodrugs with reduced charge. Once they reach systemic circulation, they convert back to the original drugs. Water of hydration can be reduced with prodrugs to enhance the oral bioavailability. Impact of strong H-bonds between NCE polar groups and water provides barrier for absorption. By converting the original drug to a lipophilic prodrug, the water barrier can be removed and thereby enhancing the oral absorption. As the molecular size increases, the permeability decreases. A prodrug with reduced molecular size can be synthesized to enhance its permeability.

Using prodrugs for solubility enhancement can take atleast two different pathways:

(a) increasing water solubility,

(b) decreasing crystal packing.

Fosphenytoin is an injectable prodrug of phenytoin. It is freely soluble and rapidly cleaved back to phenytoin upon injection. The aqueous solubility of the parent drug is 20-25 mcg/ml while that of the prodrug is 88000 mcg/ml. Such enhancement in solubility can also decrease the pain at site of the injection. Parecoxib sodium is a prodrug of valdecoxib with enhanced solubility. The increase in the solubility is because of the disruption of H-bonding and crystal packing as well as increasing pKa. Prodrugs can increase the dissolution of the parent drug. Fosamprevanir is a prodrug of amprevanir with enhanced dissolution and then bioavailability. Amprevanir is formulated with large amounts of excipients for optimum dissolution and bioavailability. This made 8 capsules to be administered twice or thrice a day with 1200 mg dosing.

Conclusions

New drug research is currently in a good swing. New methods are being innovated and placed. The trend for the past 100 years in pharmaceutical therapy is synthetic molecules. Their clinical testing, pharmaceutical testing and the synthesis procedure were all slower and thus the process consumed several years before the drug entered the market. On the other hand, currently these processes have become high throughput i.e., high-speed processes. The older techniques were very robust and history has proved that they are effective. However, the new high-throughput screening techniques are still in the development and transitional state. Before the total introduction of these techniques into drug research, it would take several years for continuous and robust development of methods in this area. Some of these techniques are

currently fruitful and some are promising for further considerations. However, the goal of this chapter is to introduce facts about the discovery of new chemical entities. In addition several other areas are being introduced into new drug discovery process. These include microbial and plant products.

Questions for Practice and Exercise

1. What constitutes the body of team involved in the selection of pharmaceutical solids? Briefly, elucidate the role of each specialist in such a selection process.
2. Explain about a new drug substance.
3. Explain the different solid-state characterization techniques used in new drug substance discovery.
4. What are the different types of solid states of drugs?
5. How do you perform the molecular optimization of an API?
6. How is salt screening used in the molecular optimization of an API?
7. How is a suitable solid form for a drug substance selected?
8. Explain structural modification as a tool for molecular optimization of an API.

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